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(54) **COMPOSITIONS AND METHODS FOR TTR GENE EDITING AND TREATING ATTR AMYLOIDOSIS**

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(73) Assignee: **Intellia Therapeutics, Inc.**, Cambridge, MA (US)

(21) Appl. No.: **16/828,573**

(22) Filed: **Mar. 24, 2020**

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(52) **U.S. Cl.**

CPC ***C12N 15/113*** (2013.01); ***C12N 9/22*** (2013.01); *A61K 48/00* (2013.01); *C12N 2310/20* (2017.05); *C12N 2800/80* (2013.01); ***C12N 15/102*** (2013.01)

(57) **ABSTRACT**

Compositions and methods for editing, e.g., introducing double-stranded breaks, within the TTR gene are provided. Compositions and methods for treating subjects having amyloidosis associated with transthyretin (ATTR), are provided.

Specification includes a Sequence Listing.

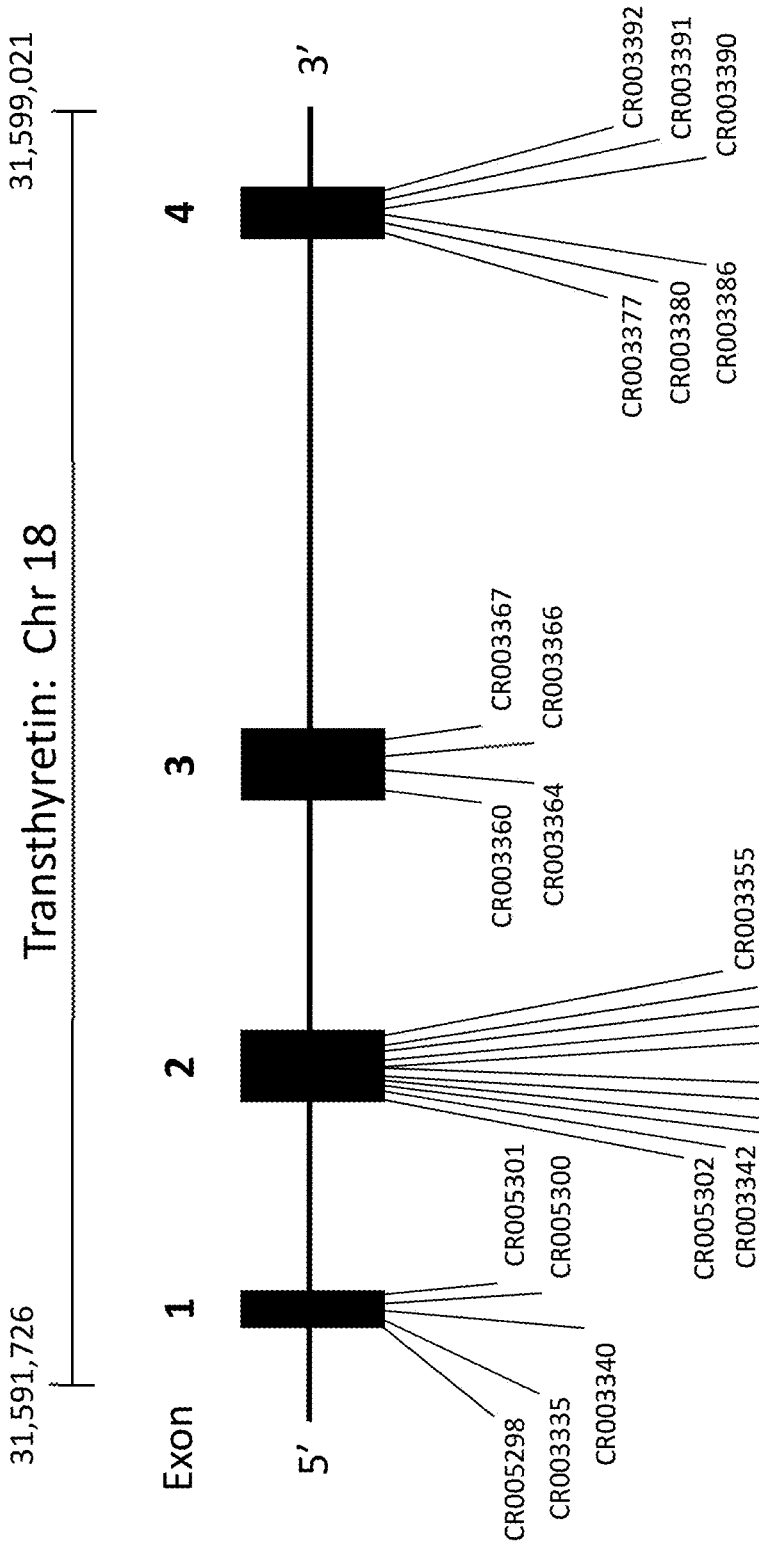

31,591,726
Transthyretin: Chr 18
31,599,021
Exon 1 2 3 4
5′
3′
CR005298
CR003335
CR003340
CR005301
CR005300
CR005302
CR003342
CR005303
CR003344
CR003345
CR003346
CR003347
CR003350
CR003351
CR005304
CR003355
CR003360
CR003364
CR003367
CR003366
CR003377
CR003380
CR003386
CR003392
CR003391
CR003390

FIG. 1

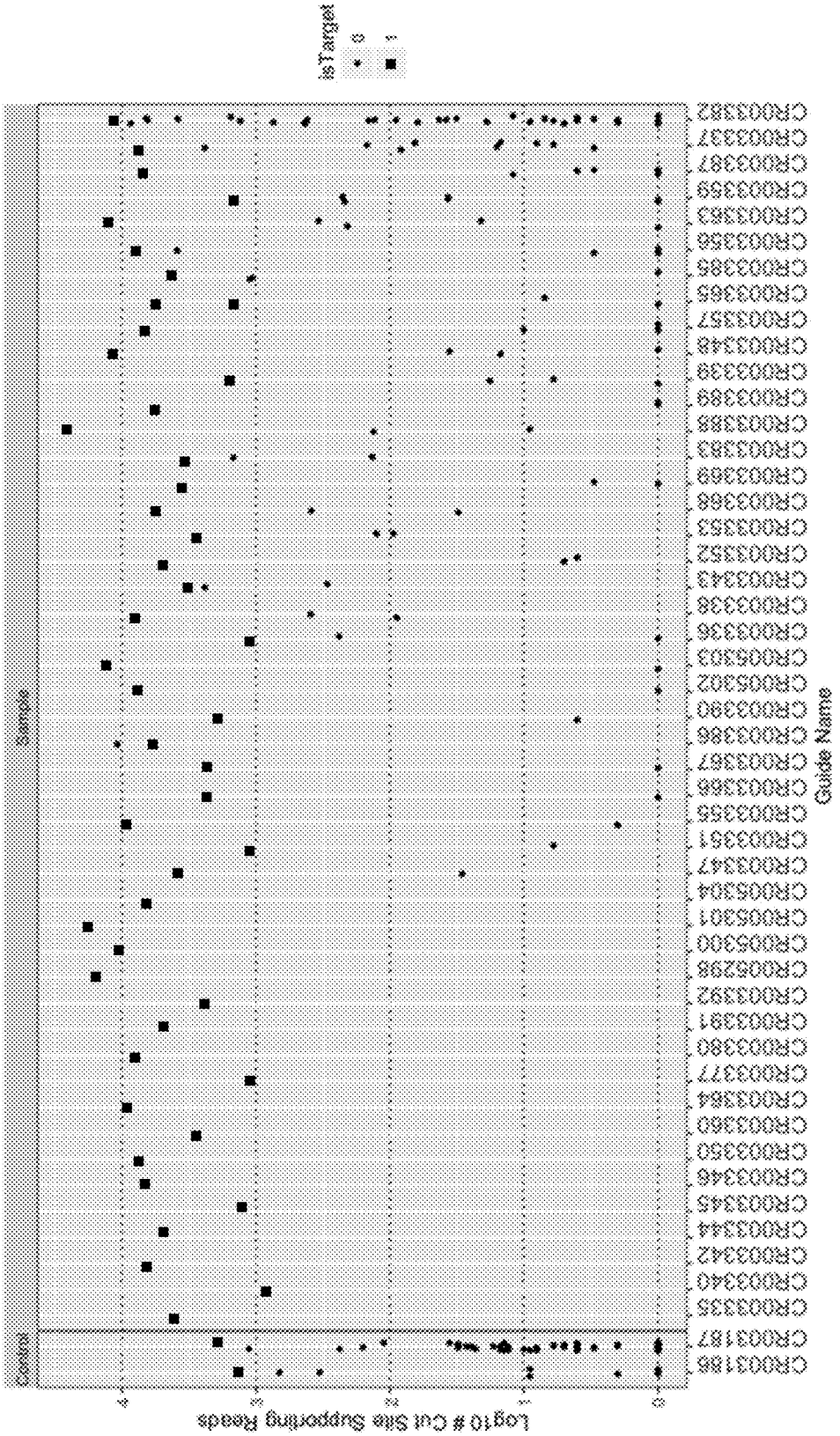

isTarget
0
1
Control
Sense
Log10 # Cut Site Supporting Reads
Guide Name

FIG. 2

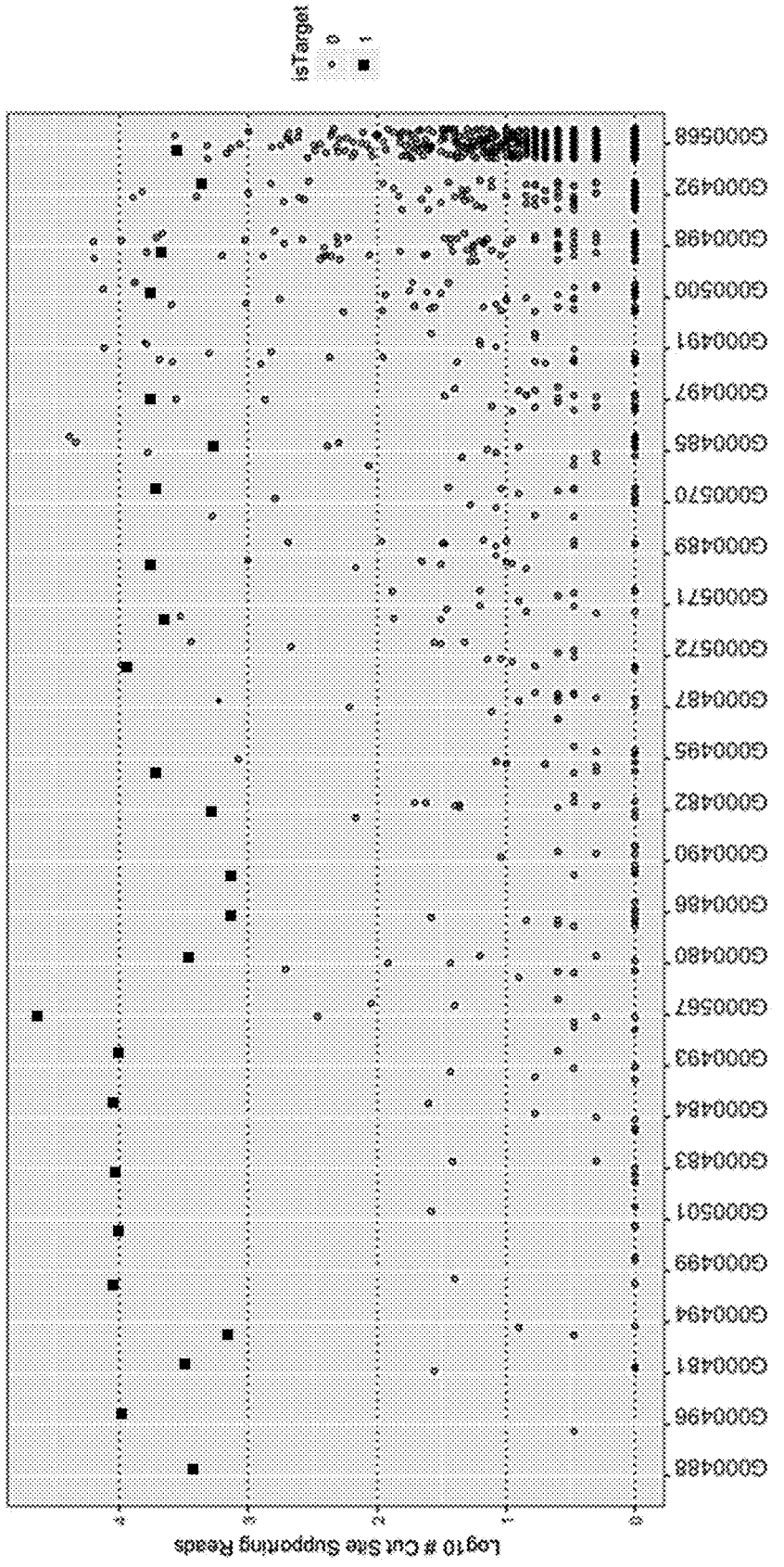

isTarget
0
1
GOOO568
GOOO492
GOOO498
GOOO500
GOOO491
GOOO497
GOOO485
GOOO570
GOOO489
GOOO571
GOOO572
GOOO487
GOOO495
GOOO482
GOOO490
GOOO486
GOOO480
GOOO567
GOOO493
GOOO484
GOOO483
GOOO501
GOOO499
GOOO494
GOOO481
GOOO496
GOOO488
-4
-3
-2
-1
0
Log10 # Cut Site Supporting Reads

FIG. 3

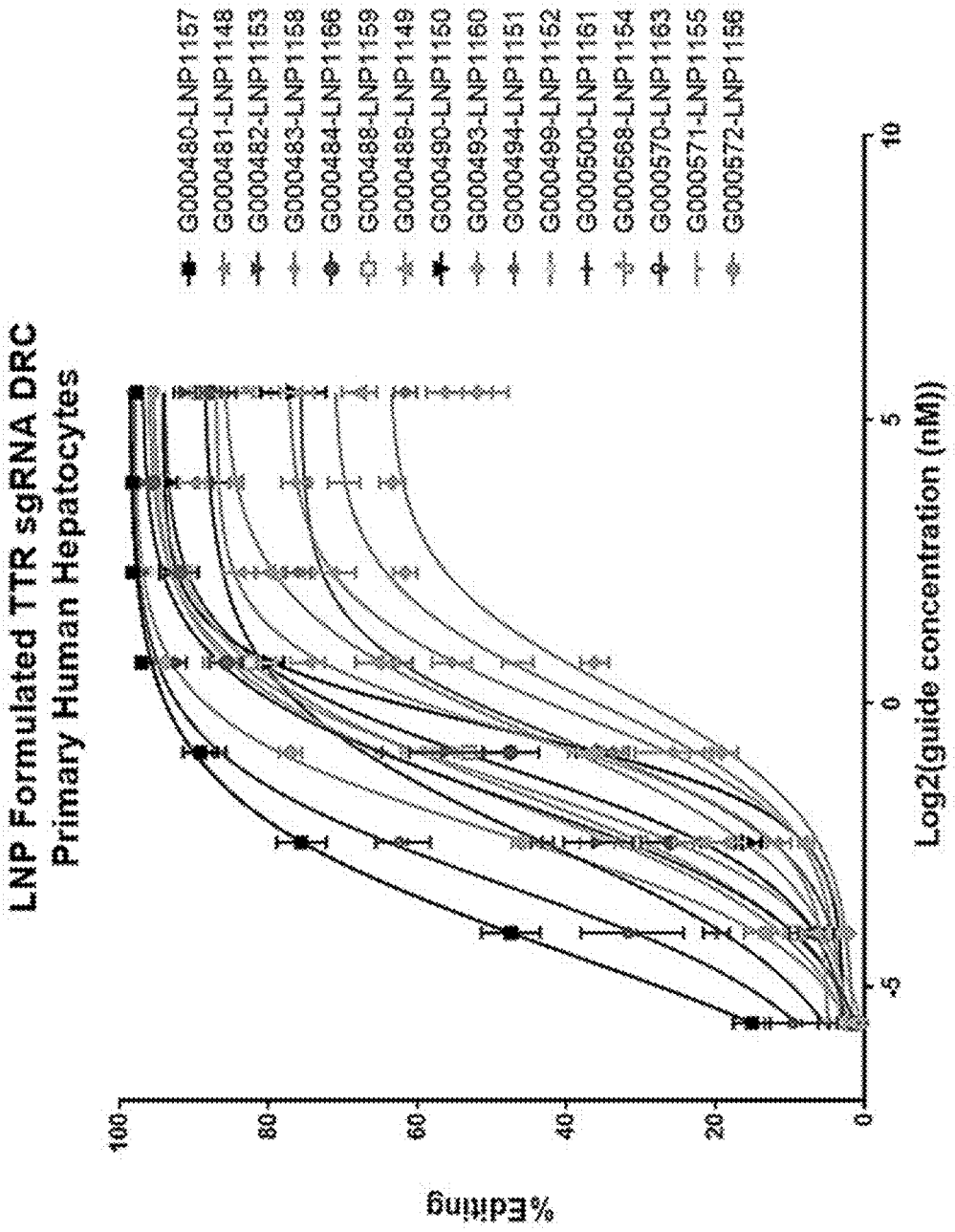

LNP Formulated TTR sgRNA DRC
Primary Human Hepatocytes
G000480-LNP1157
G000481-LNP1148
G000482-LNP1153
G000483-LNP1158
G000484-LNP1166
G000488-LNP1159
G000489-LNP1149
G000490-LNP1150
G000493-LNP1160
G000494-LNP1151
G000499-LNP1152
G000500-LNP1161
G000568-LNP1154
G000570-LNP1163
G000571-LNP1155
G000572-LNP1156
%Editing
100
80
60
40
20
0
-5
0
5
10
Log2(guide concentration (nM))

FIG. 4

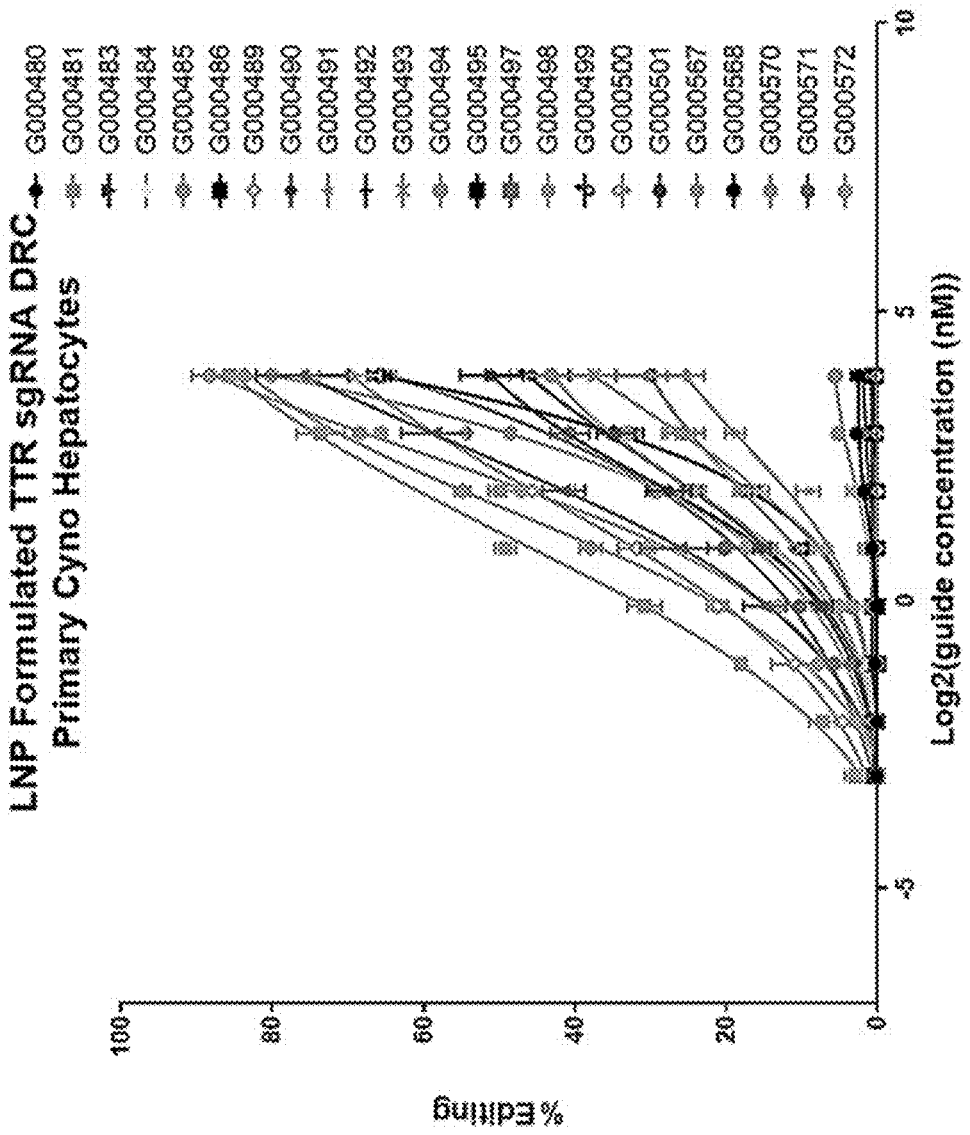

LNP Formulated TTR sgRNA DRC
Primary Cyno Hepatocytes
G000480
G000481
G000483
G000484
G000485
G000486
G000489
G000490
G000491
G000492
G000493
G000494
G000495
G000497
G000498
G000499
G000500
G000501
G000567
G000568
G000570
G000571
G000572
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 5

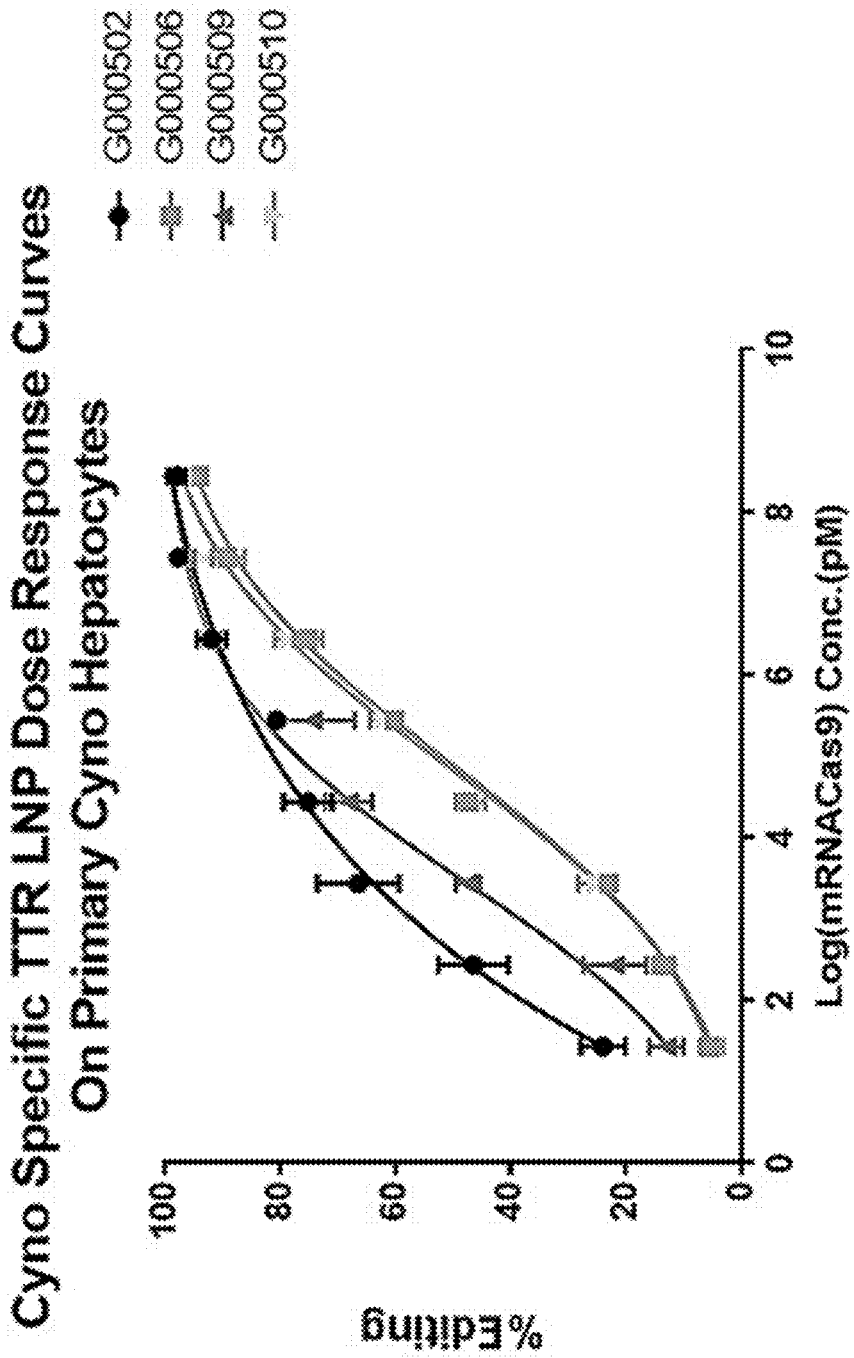

Cyno Specific TTR LNP Dose Response Curves
On Primary Cyno Hepatocytes
G000502
G000506
G000509
G000510
%Editing
100
80
60
40
20
0
0
2
4
6
8
10
Log(mRNACas9) Conc.(pM)

FIG. 6

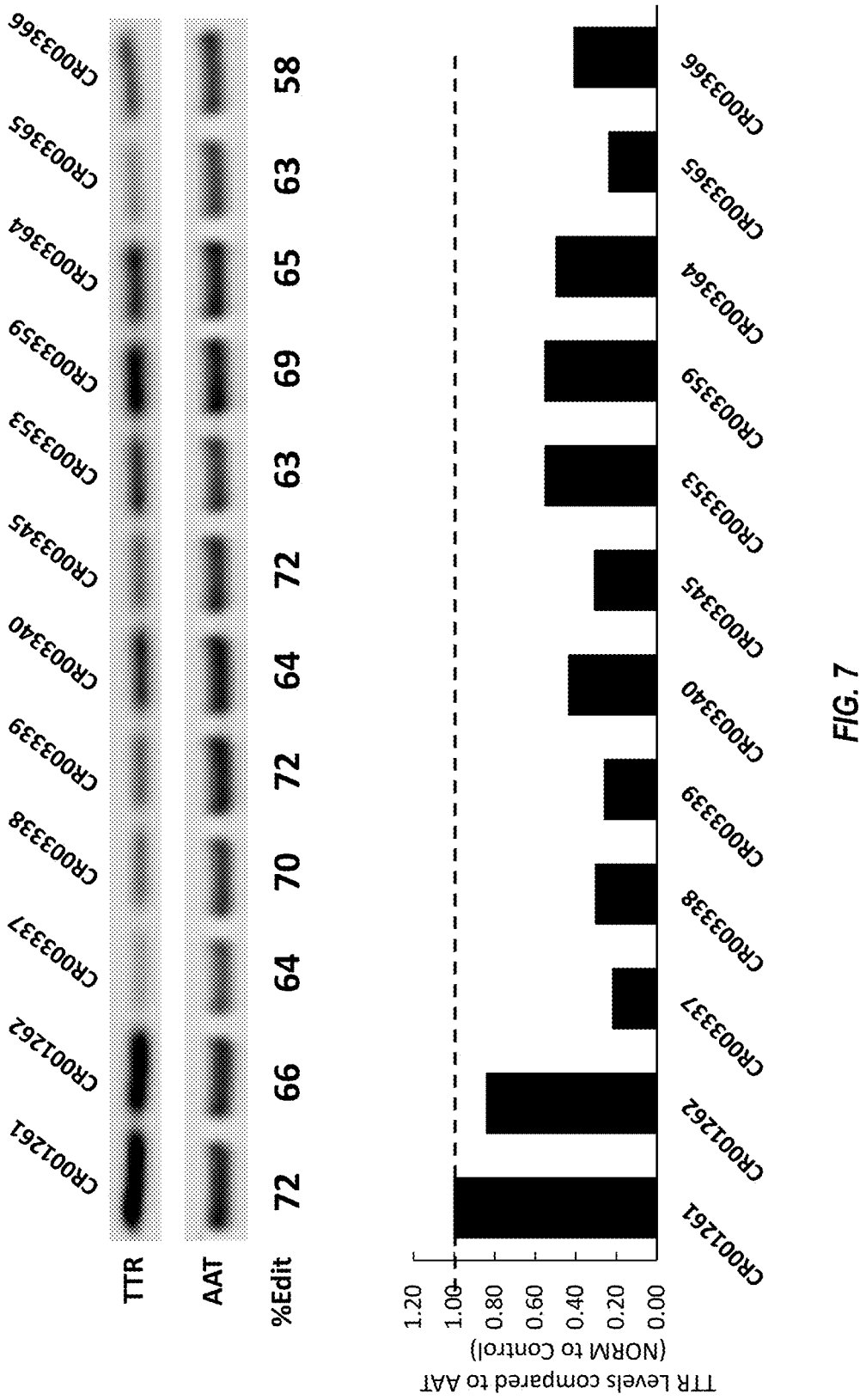

CR001261
CR001262
CR003337
CR003338
CR003339
CR003340
CR003345
CR003353
CR003359
CR003364
CR003365
CR003366
%Edit
72
66
64
70
72
64
72
63
69
65
63
58
AAT
TTR
TTR Levels compared to AAT
(NORM to Control)
0.00
0.20
0.40
0.60
0.80
1.00
1.20

FIG. 7

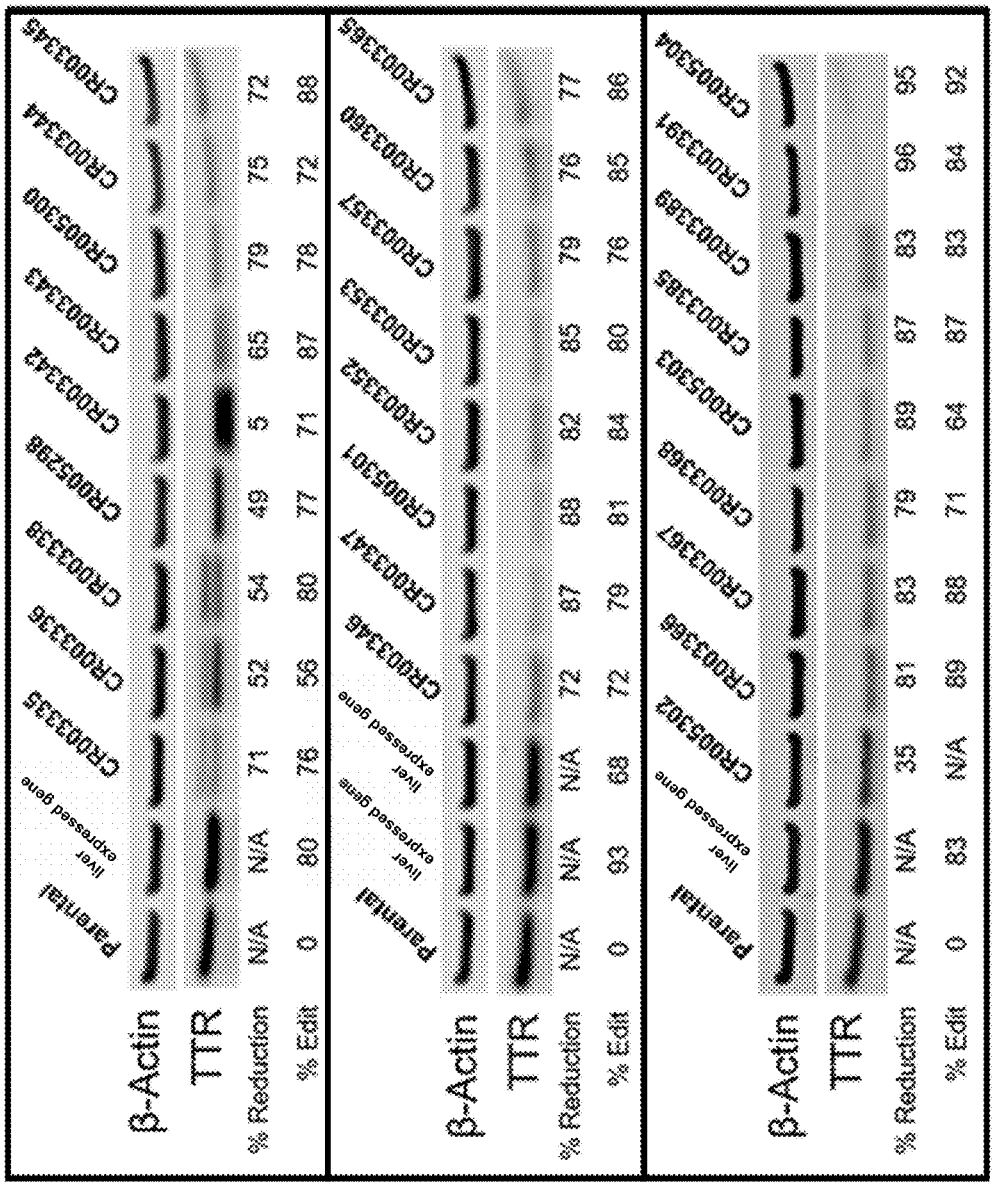

β-Actin
TTR
% Reduction
% Edit
Parental
Liver expressed gene
CR003335
CR003336
CR003339
CR005298
CR003342
CR005300
CR003344
CR003345
N/A 0
N/A 80
71 76
52 56
54 80
49 77
5 71
65 87
79 78
75 72
72 88
β-Actin
TTR
% Reduction
% Edit
Parental
Liver expressed gene
CR003346
CR003347
CR005301
CR003352
CR003353
CR003357
CR003360
CR003361
N/A 0
N/A 93
N/A 68
72 72
87 79
88 81
82 84
85 80
79 76
76 85
77 86
β-Actin
TTR
% Reduction
% Edit
Parental
Liver expressed gene
CR005302
CR003366
CR003367
CR003368
CR005303
CR003385
CR003389
CR003391
CR005304
N/A 0
N/A 83
35 N/A
81 89
83 88
79 71
89 64
87 87
83 83
96 84
95 92

FIG. 8

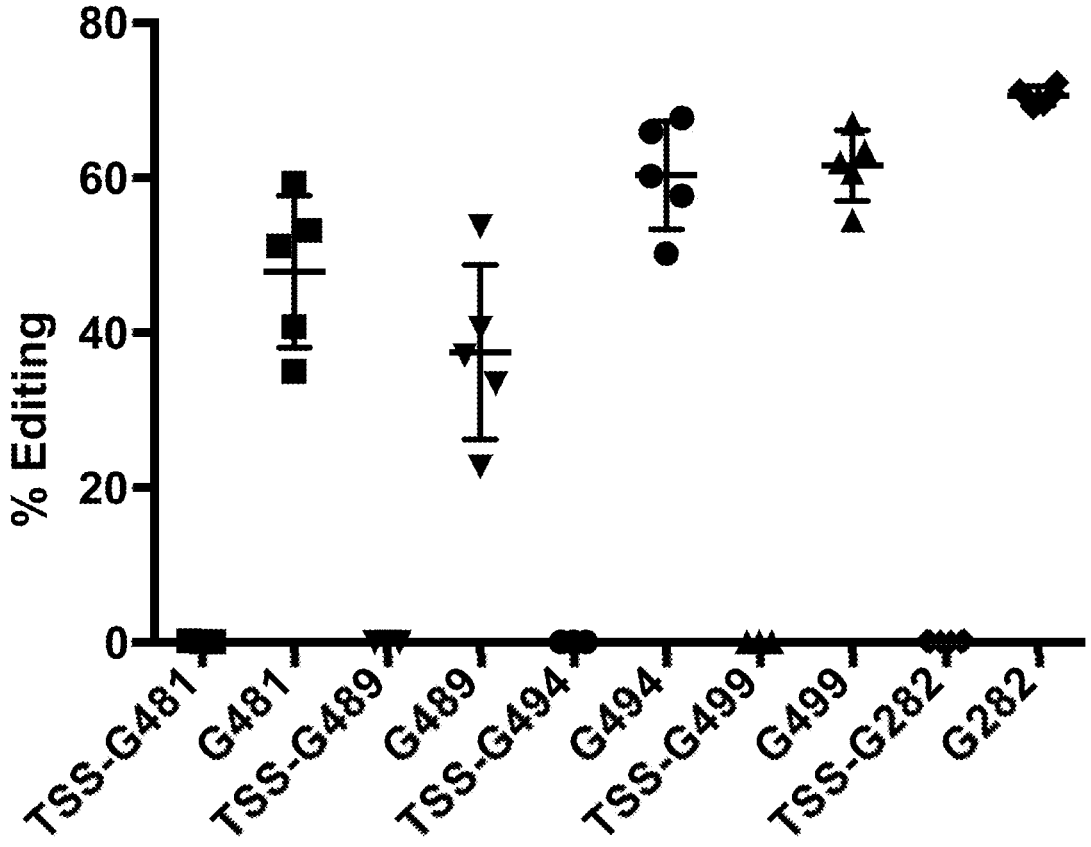

80
60
40
20
0
% Editing
TSS-G481
G481
TSS-G489
G489
TSS-G494
G494
TSS-G499
G499
TSS-G282
G282

FIG. 9

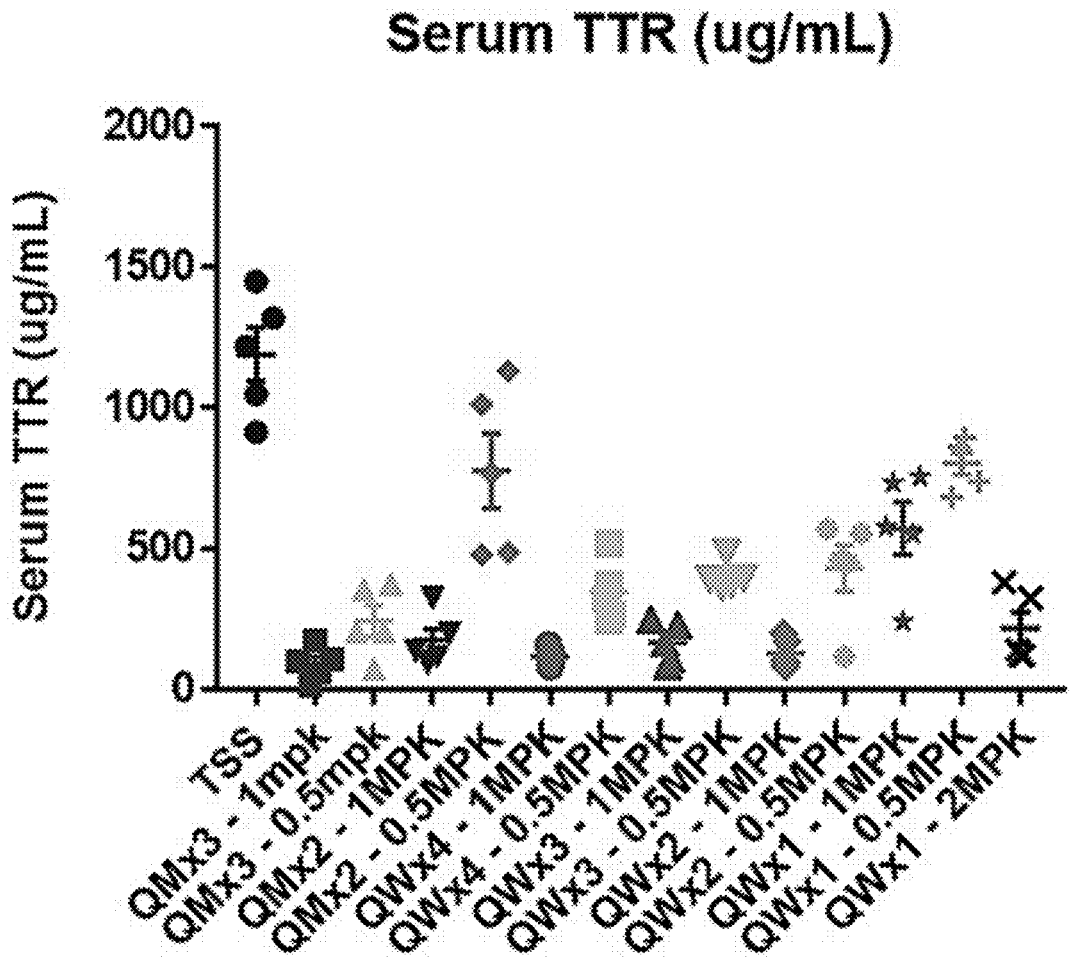

Serum TTR (ug/mL)
Serum TTR (ug/mL)
2000
1500
1000
500
0
TSS
QMx3 - 1mpk
QMx3 - 0.5mpk
QMx2 - 1MPK
QMx2 - 0.5MPK
QWx4 - 1MPK
QWx4 - 0.5MPK
QWx3 - 1MPK
QWx3 - 0.5MPK
QWx2 - 1MPK
QWx2 - 0.5MPK
QWx1 - 1MPK
QWx1 - 0.5MPK
QWx1 - 2MPK

FIG. 10A

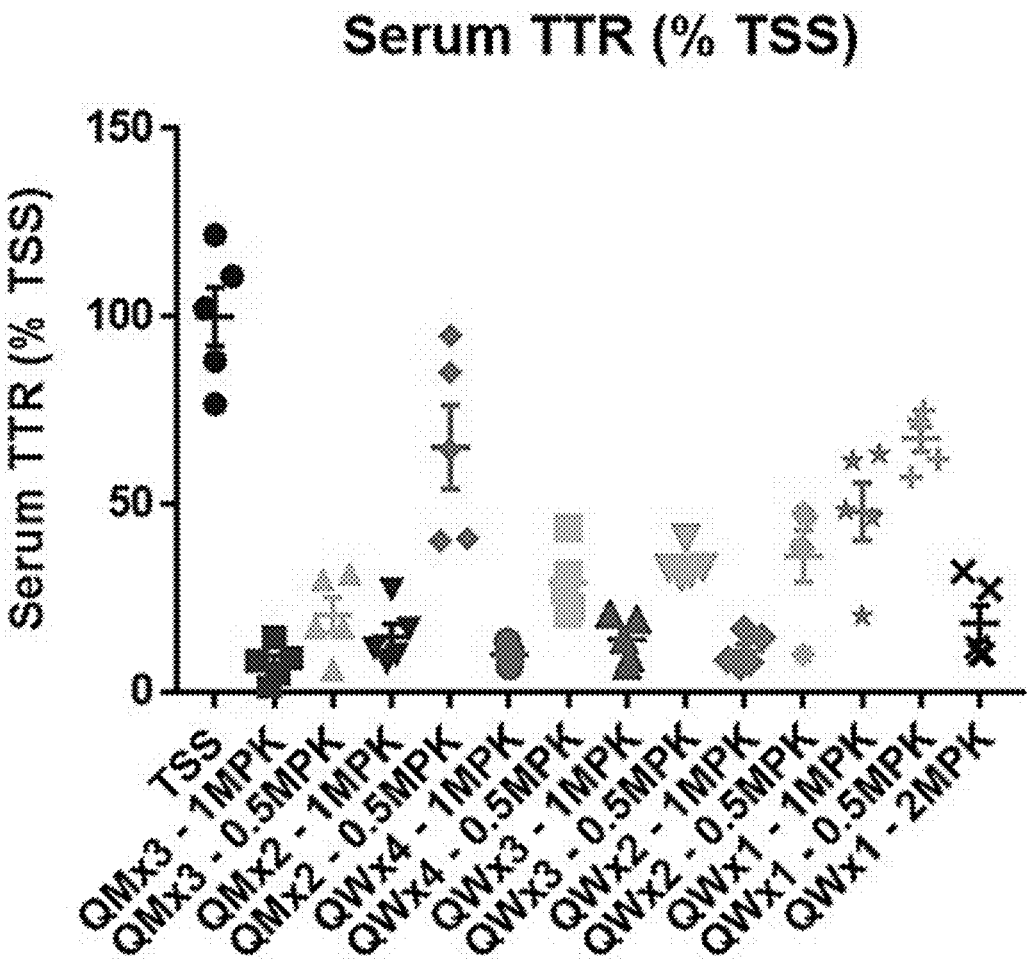

Serum TTR (% TSS)
Serum TTR (% TSS)
150
100
50
0
TSS
QMx3 - 1MPK
QMx3 - 0.5MPK
QMx2 - 1MPK
QMx2 - 0.5MPK
QWx4 - 1MPK
QWx4 - 0.5MPK
QWx3 - 1MPK
QWx3 - 0.5MPK
QWx2 - 1MPK
QWx2 - 0.5MPK
QWx1 - 1MPK
QWx1 - 0.5MPK
QWx1 - 2MPK

FIG. 10B

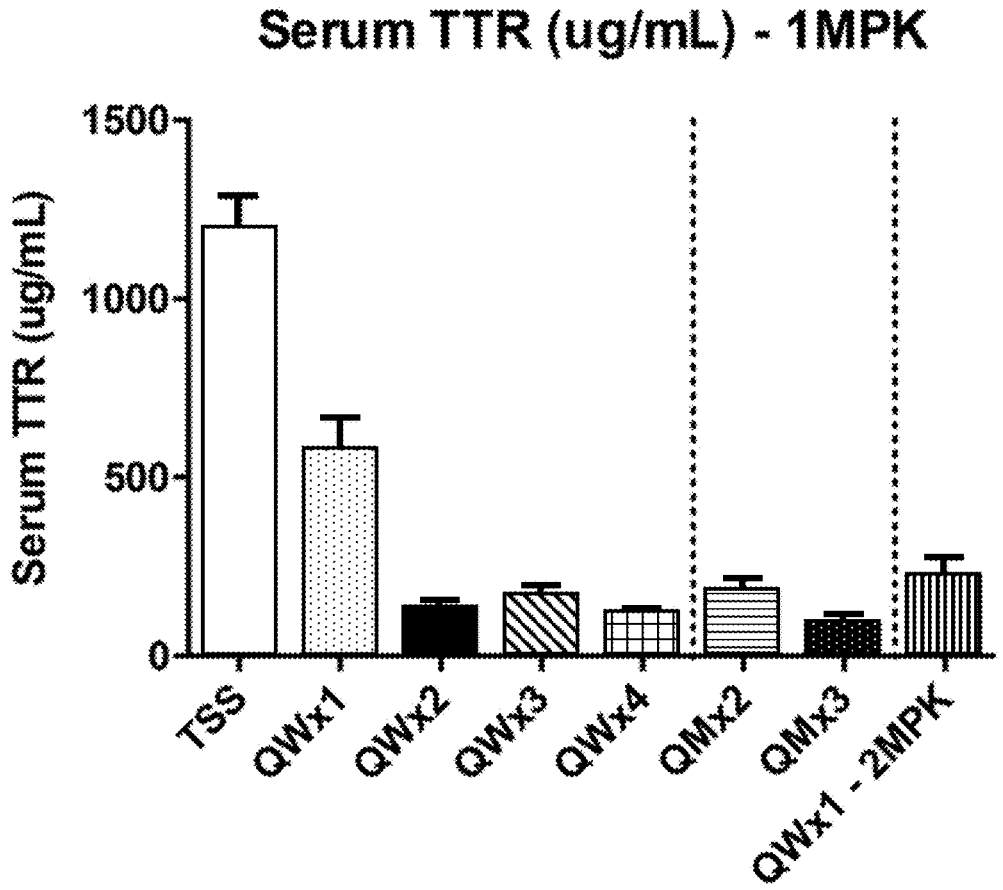

Serum TTR (ug/mL) - 1MPK
Serum TTR (ug/mL)
1500
1000
500
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 11A

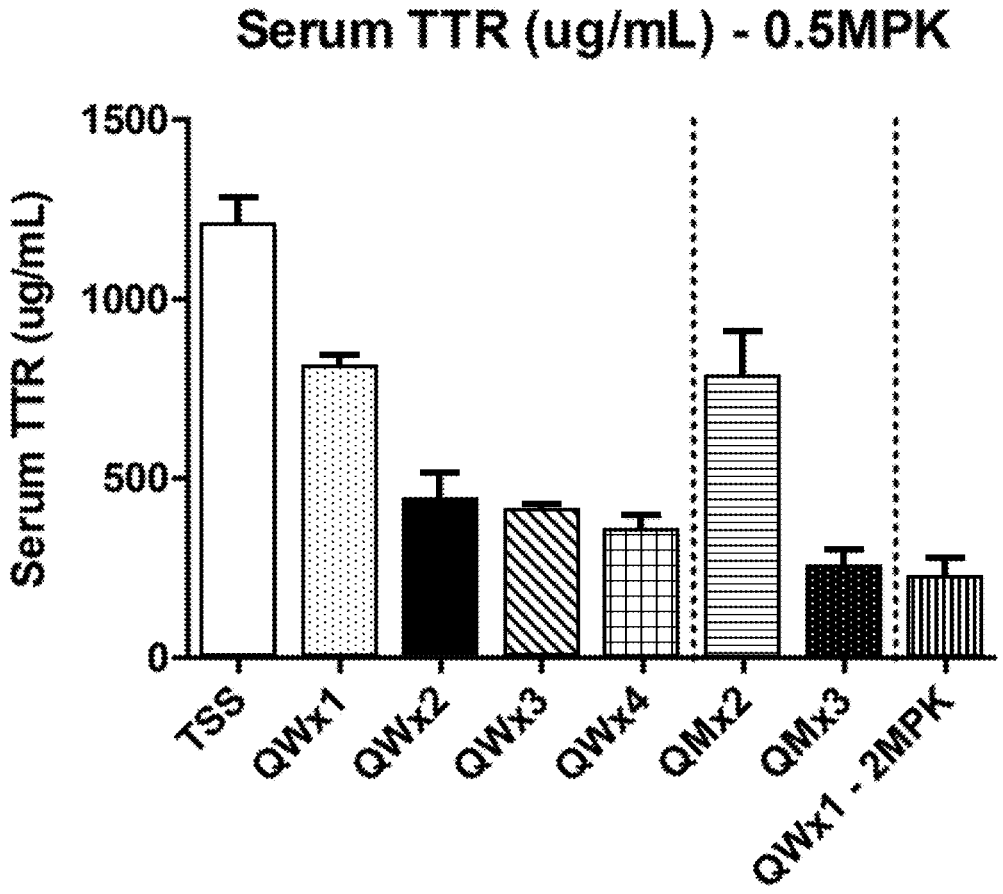

Serum TTR (ug/mL) - 0.5MPK
Serum TTR (ug/mL)
1500
1000
500
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 11B

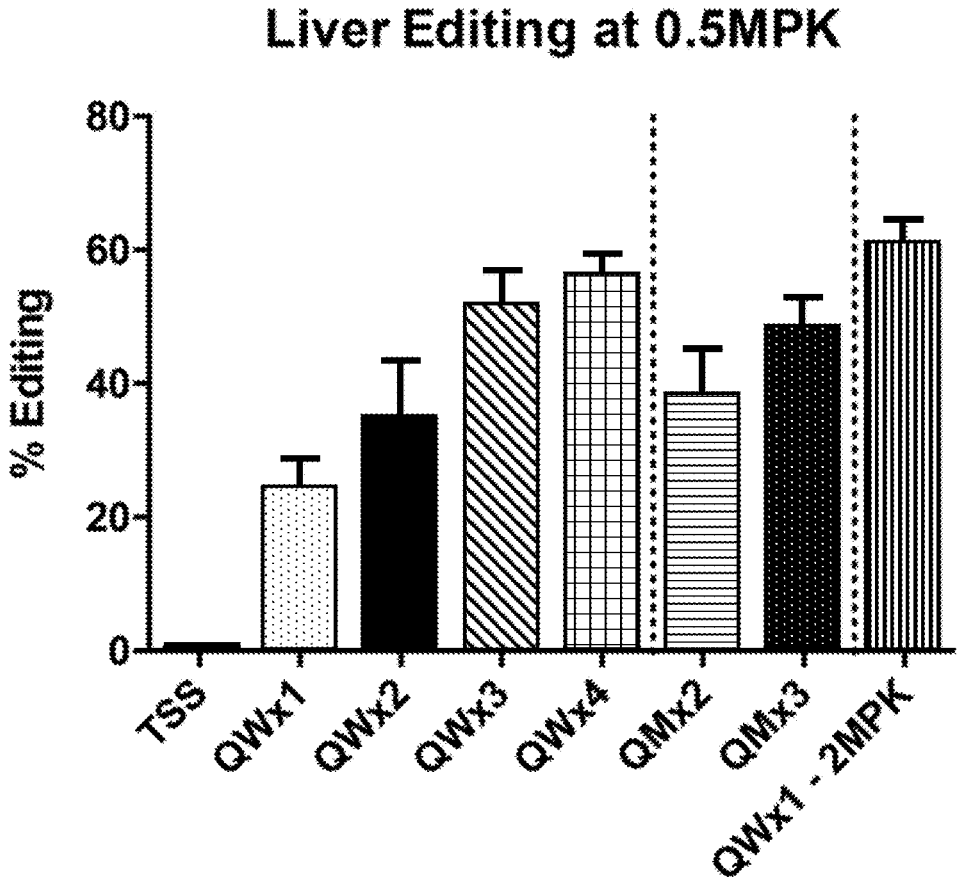

Liver Editing at 0.5MPK
% Editing
80
60
40
20
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 12A

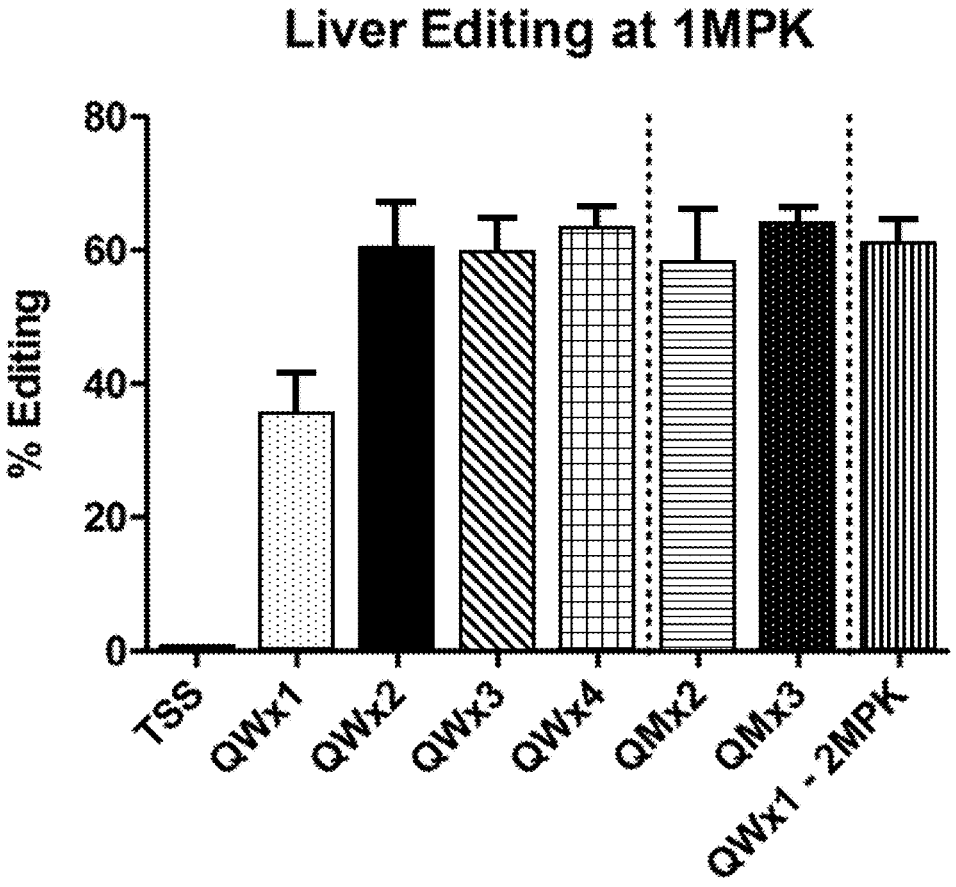

Liver Editing at 1MPK
% Editing
80
60
40
20
0
TSS
QWx1
QWx2
QWx3
QWx4
QMx2
QMx3
QWx1 - 2MPK

FIG. 12B

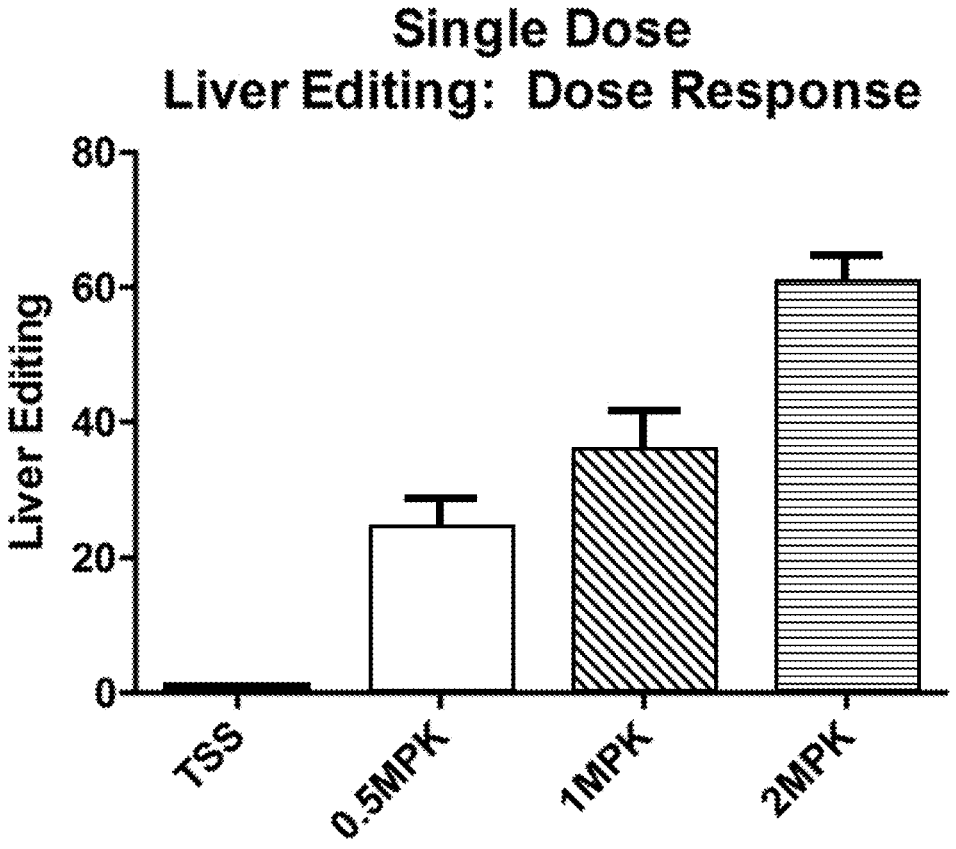

Single Dose
Liver Editing: Dose Response
Liver Editing
80
60
40
20
0
TSS
0.5MPK
1MPK
2MPK

FIG. 12C

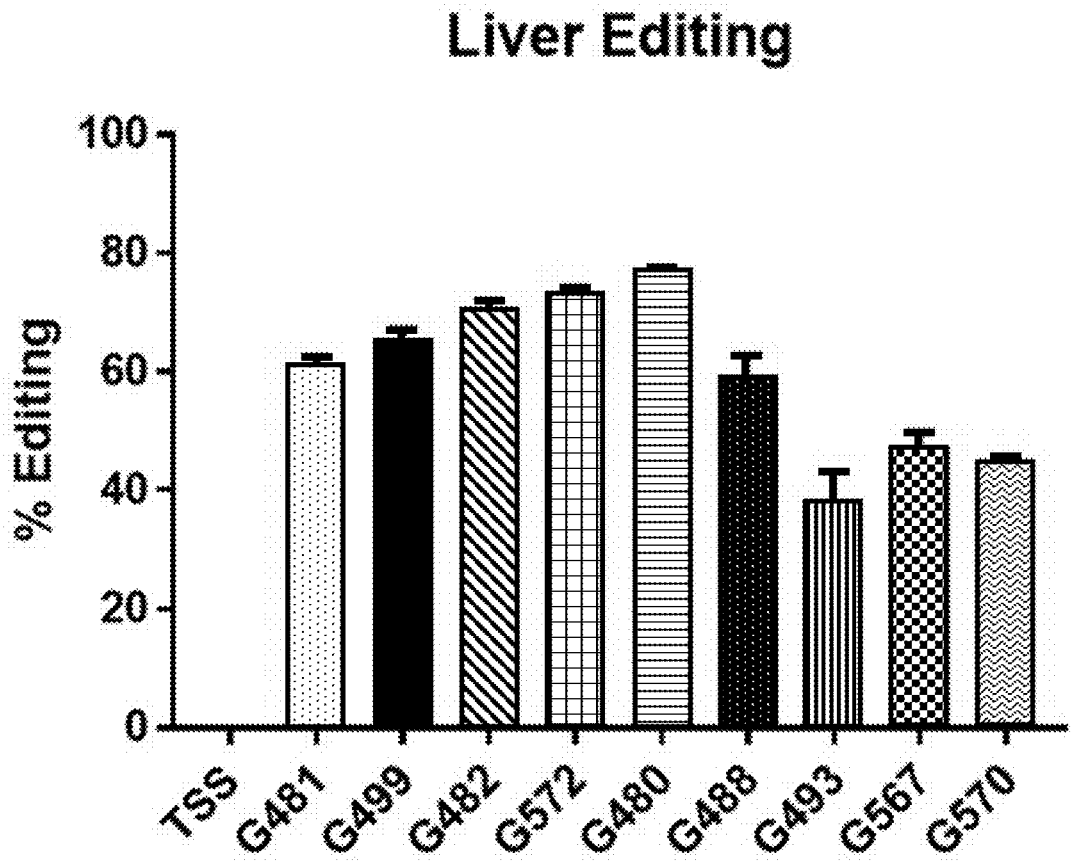

Liver Editing
% Editing
100
80
60
40
20
0
TSS
G481
G499
G482
G572
G480
G488
G493
G567
G570

FIG. 13

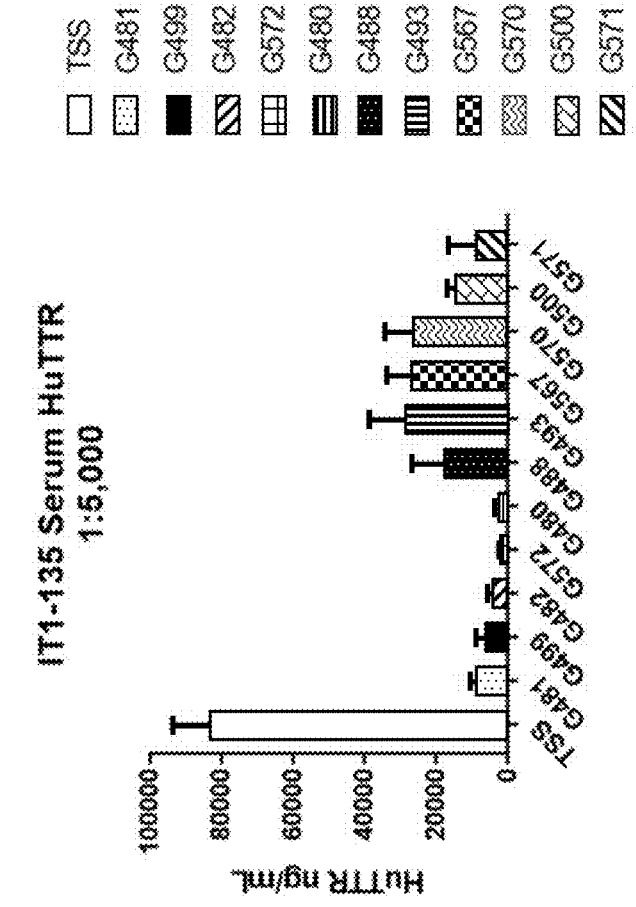


FIG. 14B

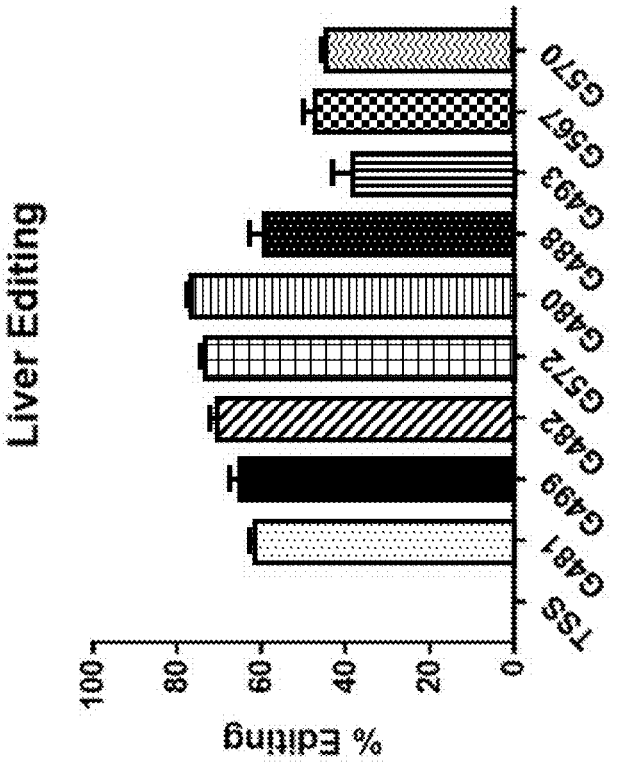


FIG. 14A

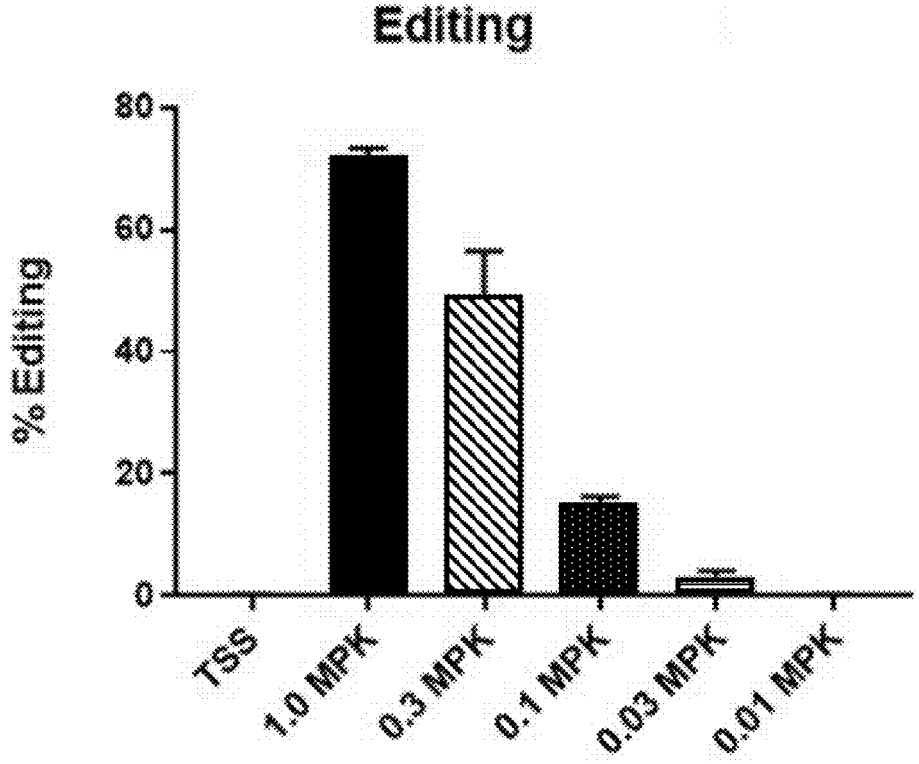

Editing
% Editing
80
60
40
20
0
TSS
1.0 MPK
0.3 MPK
0.1 MPK
0.03 MPK
0.01 MPK

FIG. 15A

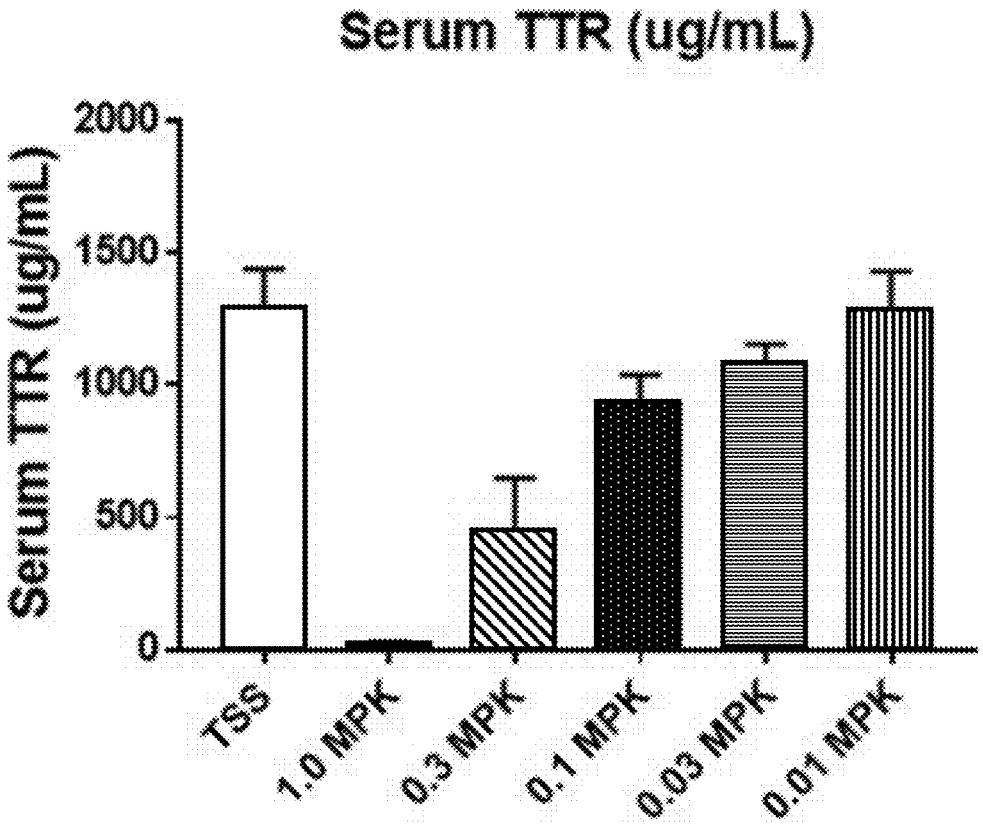

Serum TTR (ug/mL)
Serum TTR (ug/mL)
2000
1500
1000
500
0
TSS
1.0 MPK
0.3 MPK
0.1 MPK
0.03 MPK
0.01 MPK

FIG. 15B

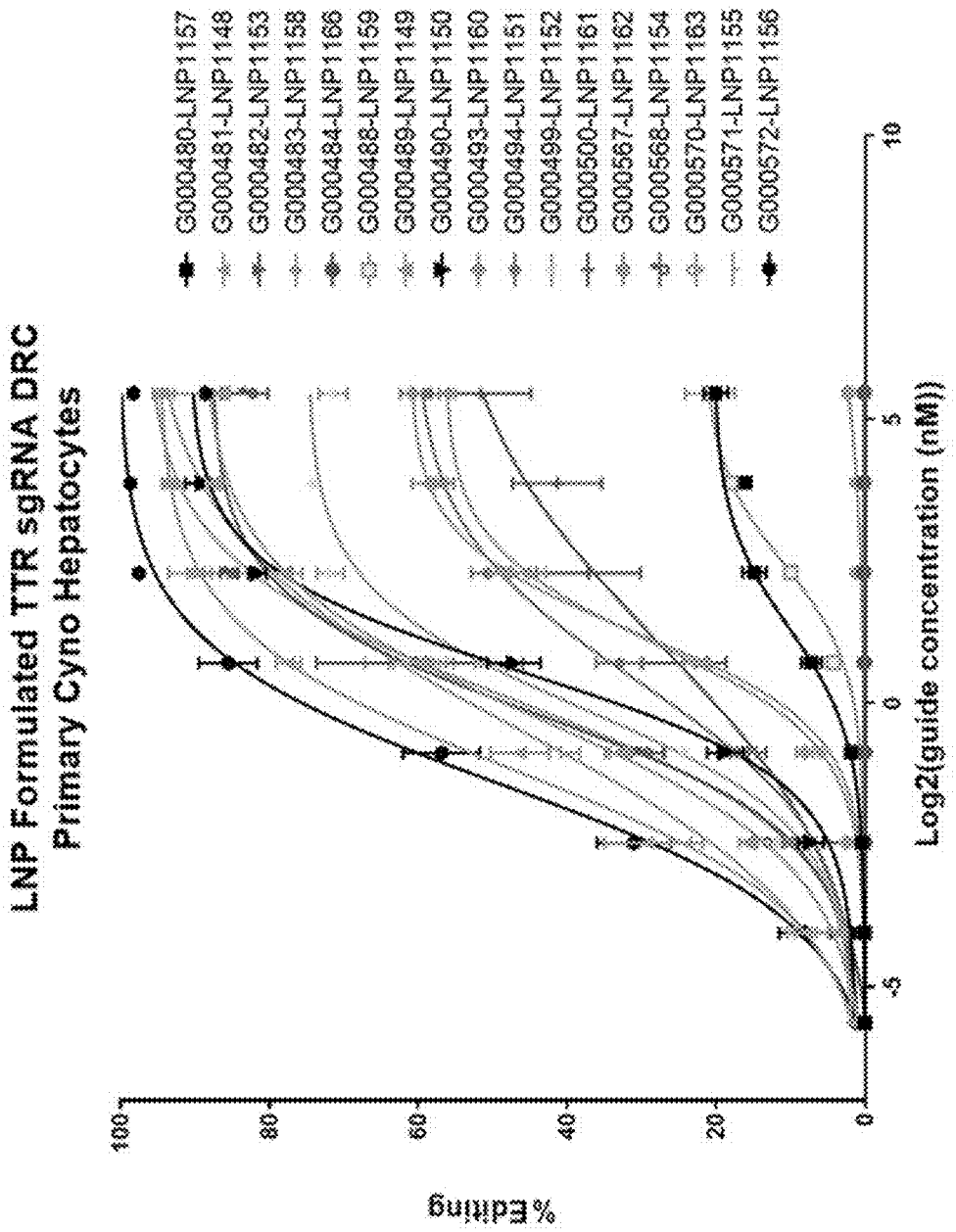

LNP Formulated TTR sgRNA DRC
Primary Cyno Hepatocytes
G000480-LNP1157
G000481-LNP1148
G000482-LNP1153
G000483-LNP1158
G000484-LNP1166
G000488-LNP1159
G000489-LNP1149
G000490-LNP1150
G000493-LNP1160
G000494-LNP1151
G000499-LNP1152
G000500-LNP1161
G000567-LNP1162
G000568-LNP1154
G000570-LNP1163
G000571-LNP1155
G000572-LNP1156
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 16

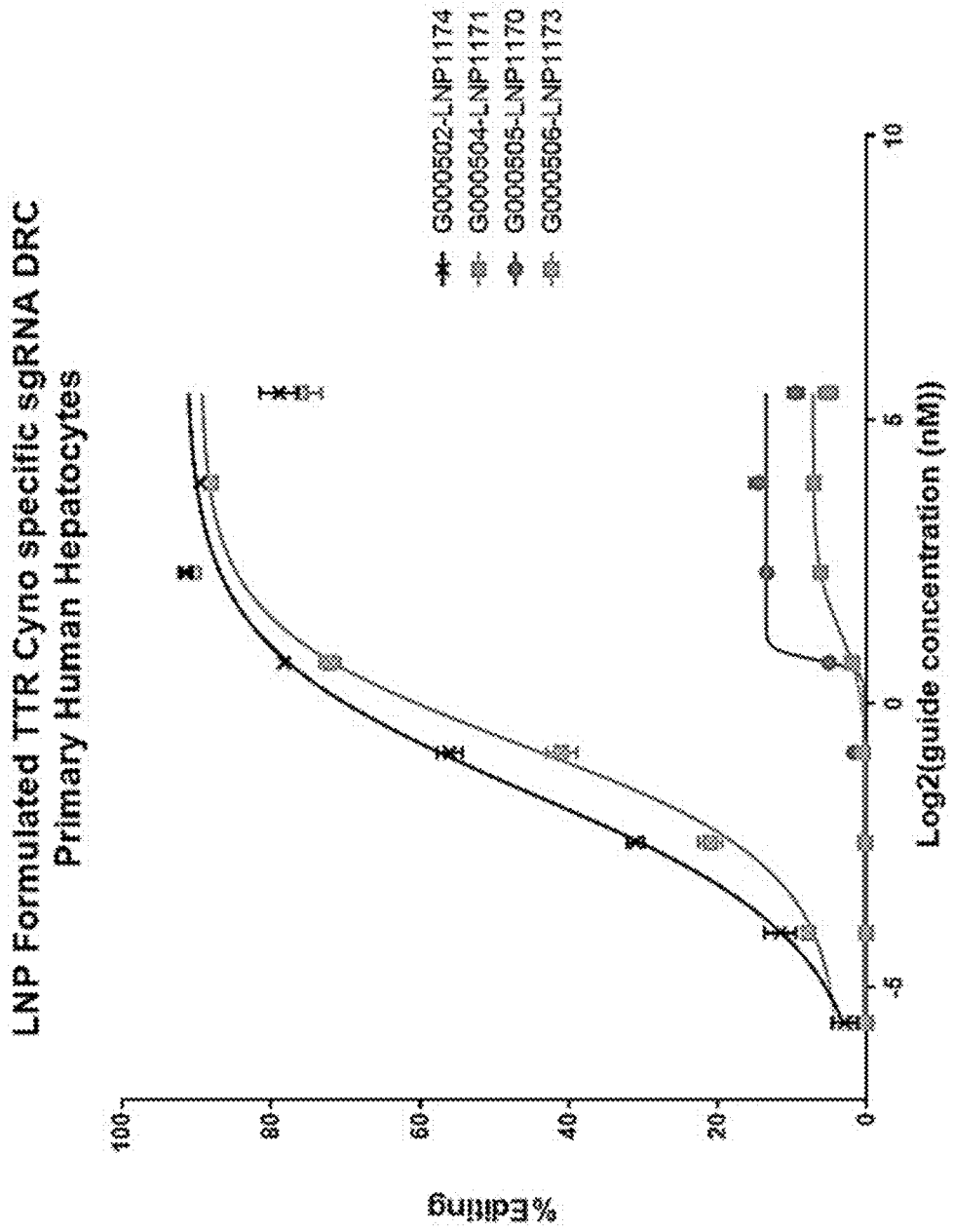

LNP Formulated TTR Cyno specific sgRNA DRC
Primary Human Hepatocytes
G000502-LNP1174
G000504-LNP1171
G000505-LNP1170
G000506-LNP1173
%Editing
100
80
60
40
20
0
-5
0
5
10
Log2(guide concentration (nM))

FIG. 17

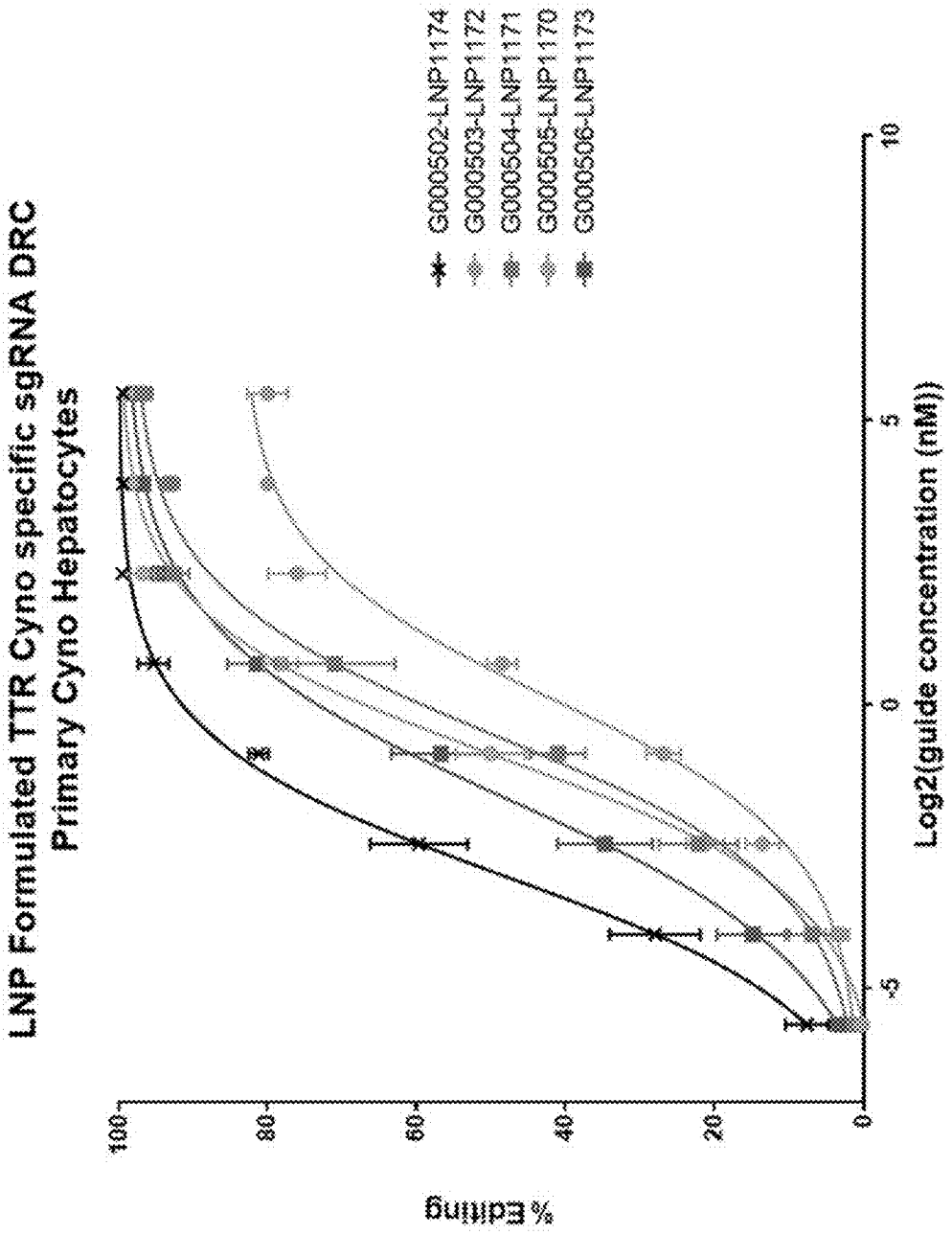

LNP Formulated TTR Cyno specific sgRNA DRC
Primary Cyno Hepatocytes
G000502-LNP1174
G000503-LNP1172
G000504-LNP1171
G000505-LNP1170
G000506-LNP1173
% Editing
0
20
40
60
80
100
-5
0
5
10
Log2(guide concentration (nM))

FIG. 18

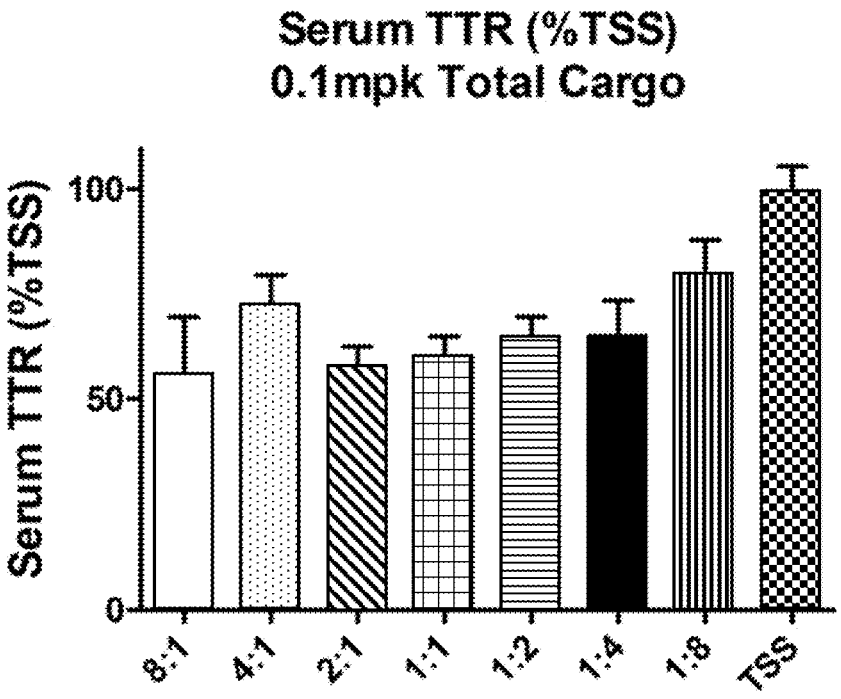


FIG. 19A

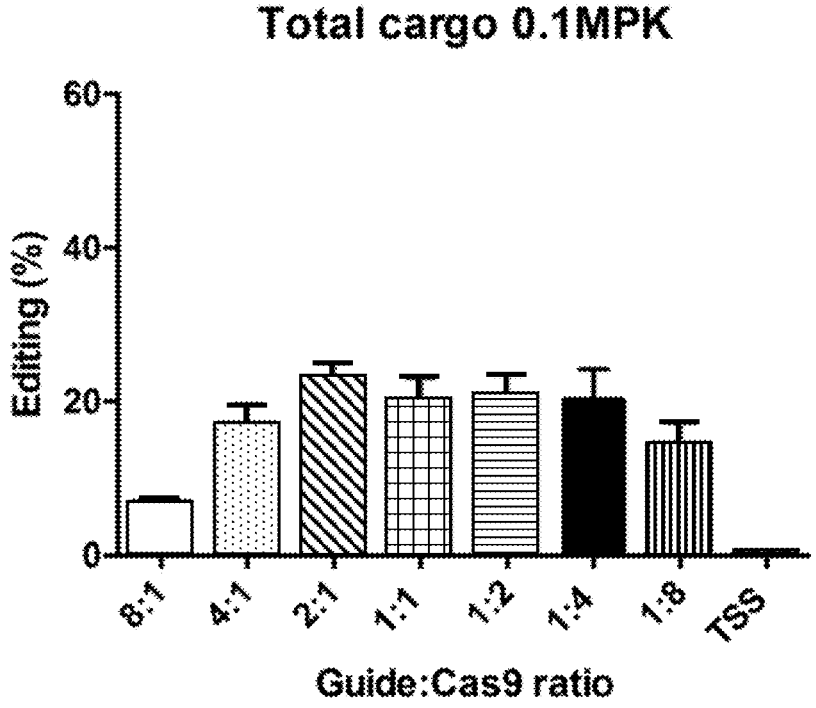


FIG. 19B

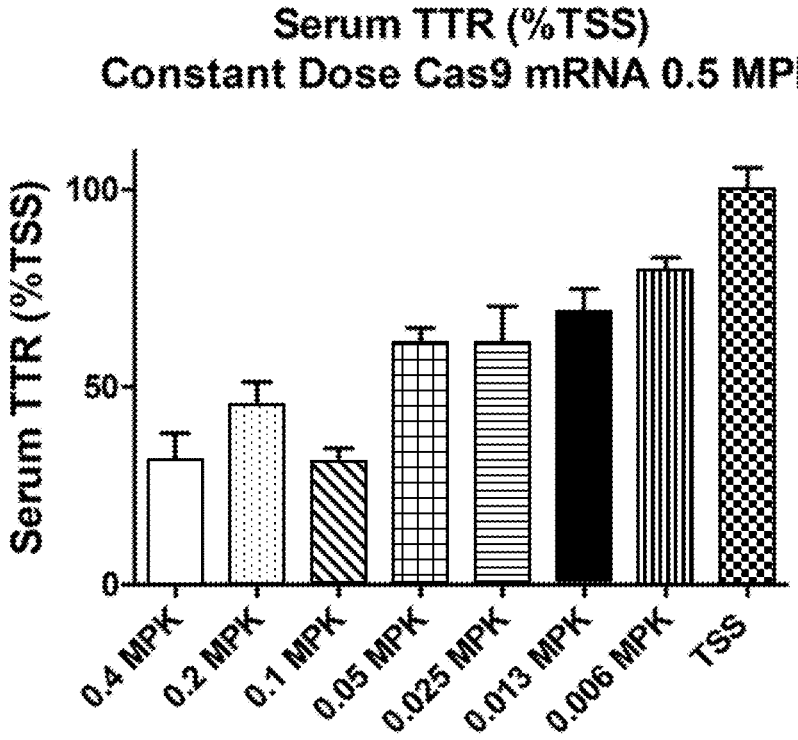


FIG. 19C

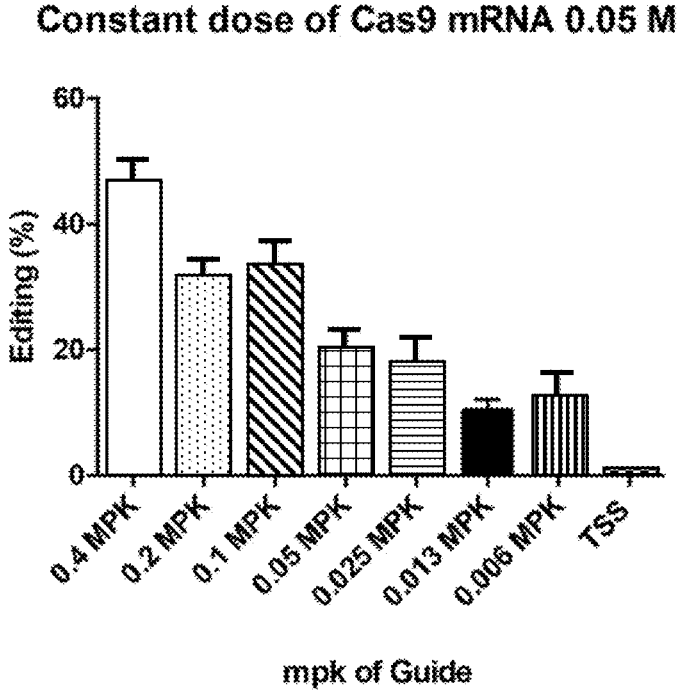


FIG. 19D

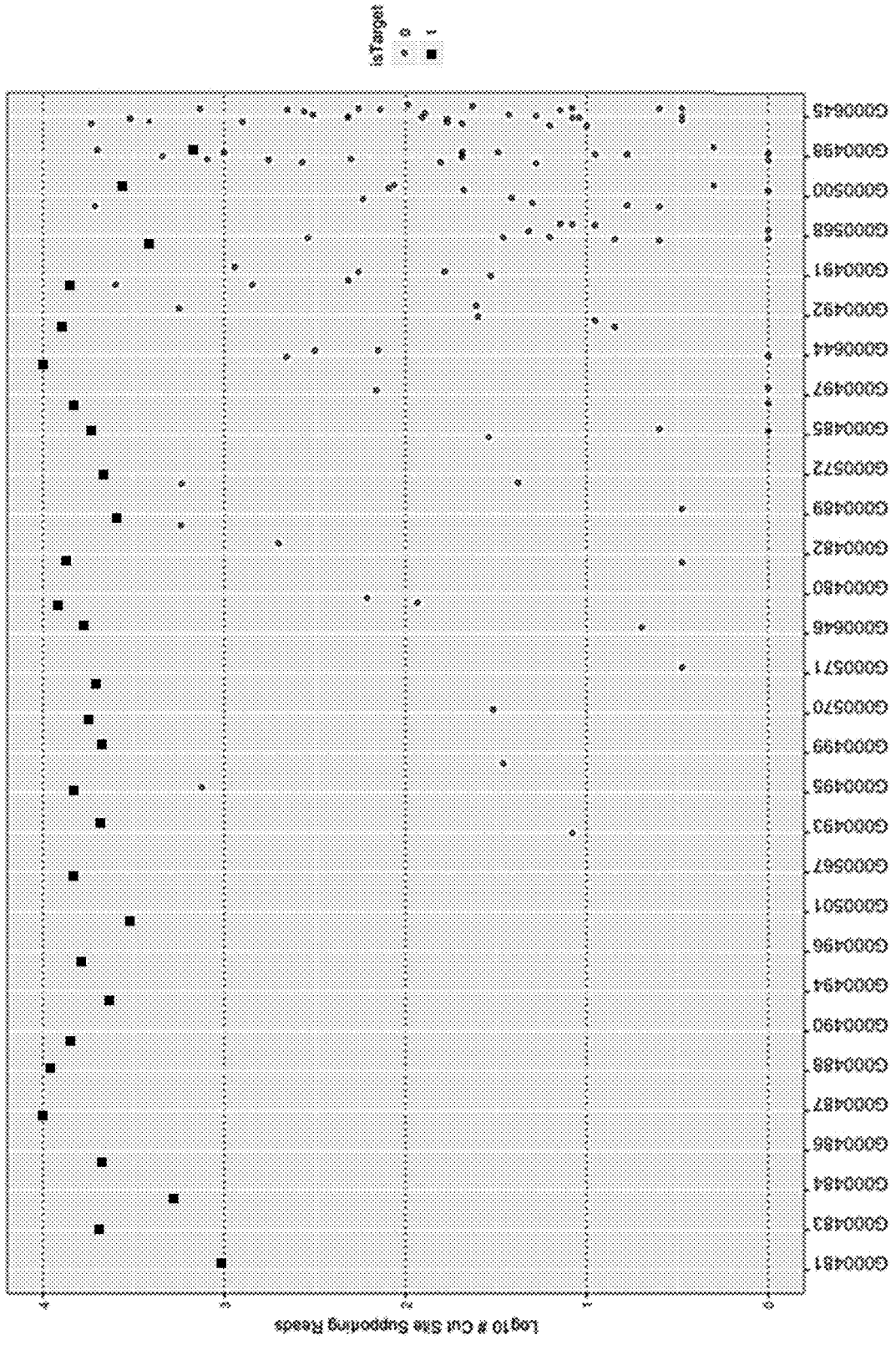

isTarget
log10 # Cut Site Supporting Reads
-4
-3
-2
-1
0
G0006451
G0004981
G0004834
G0004863
G0004864
G0004866
G0004867
G0004868
G0004890
G0004894
G0004896
G0005011
G0005671
G0004893
G0004895
G0004899
G0005701
G0004870
G0005711
G0006461
G0004880
G0004882
G0004889
G0005721
G0004885
G0004897
G0006441
G0004892
G0004891
G0005681
G0005001
G0004998
G0006451

FIG. 20

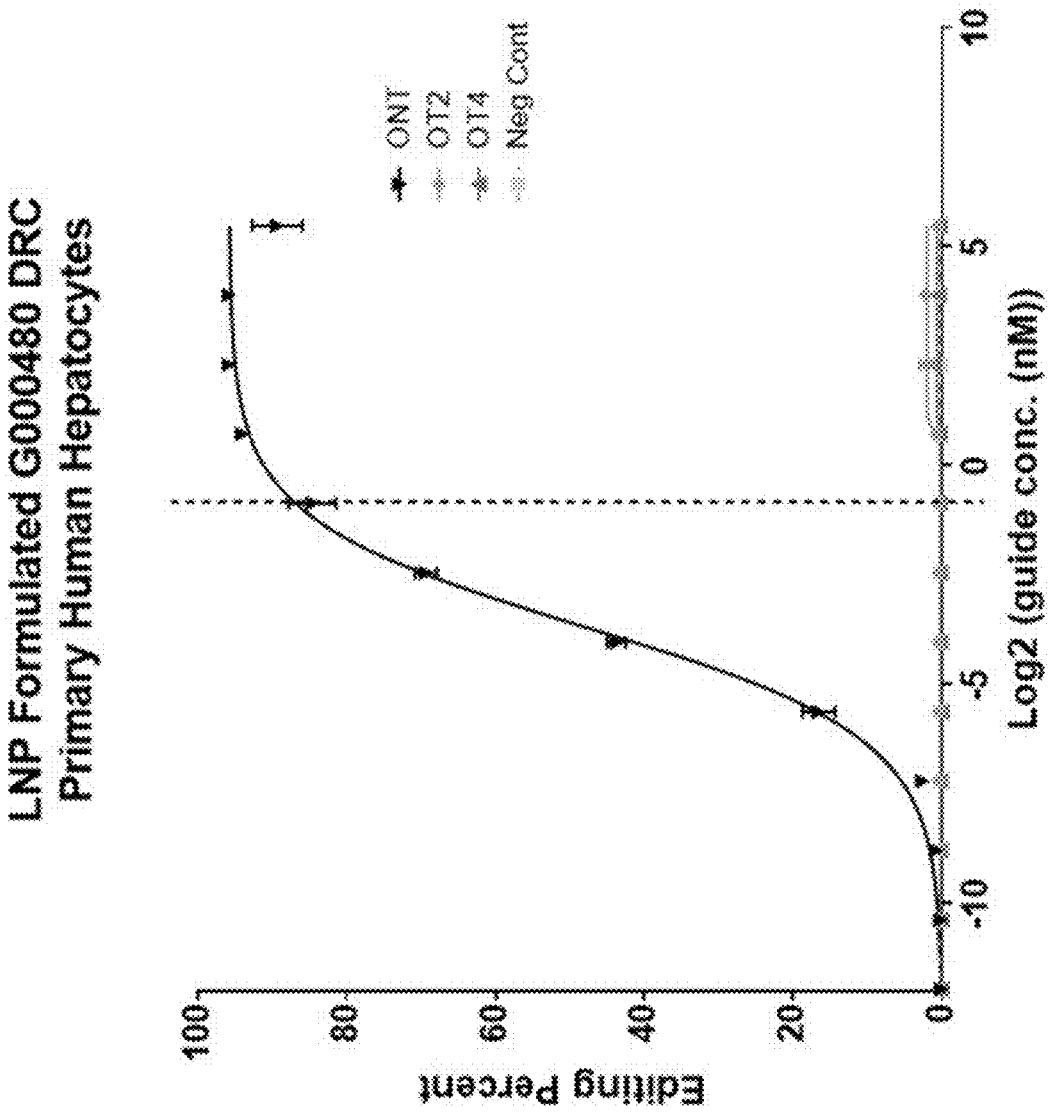

LNP Formulated G000480 DRC
Primary Human Hepatocytes
ONT
OT2
OT4
Neg Cont
Editing Percent
0
20
40
60
80
100
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 21A

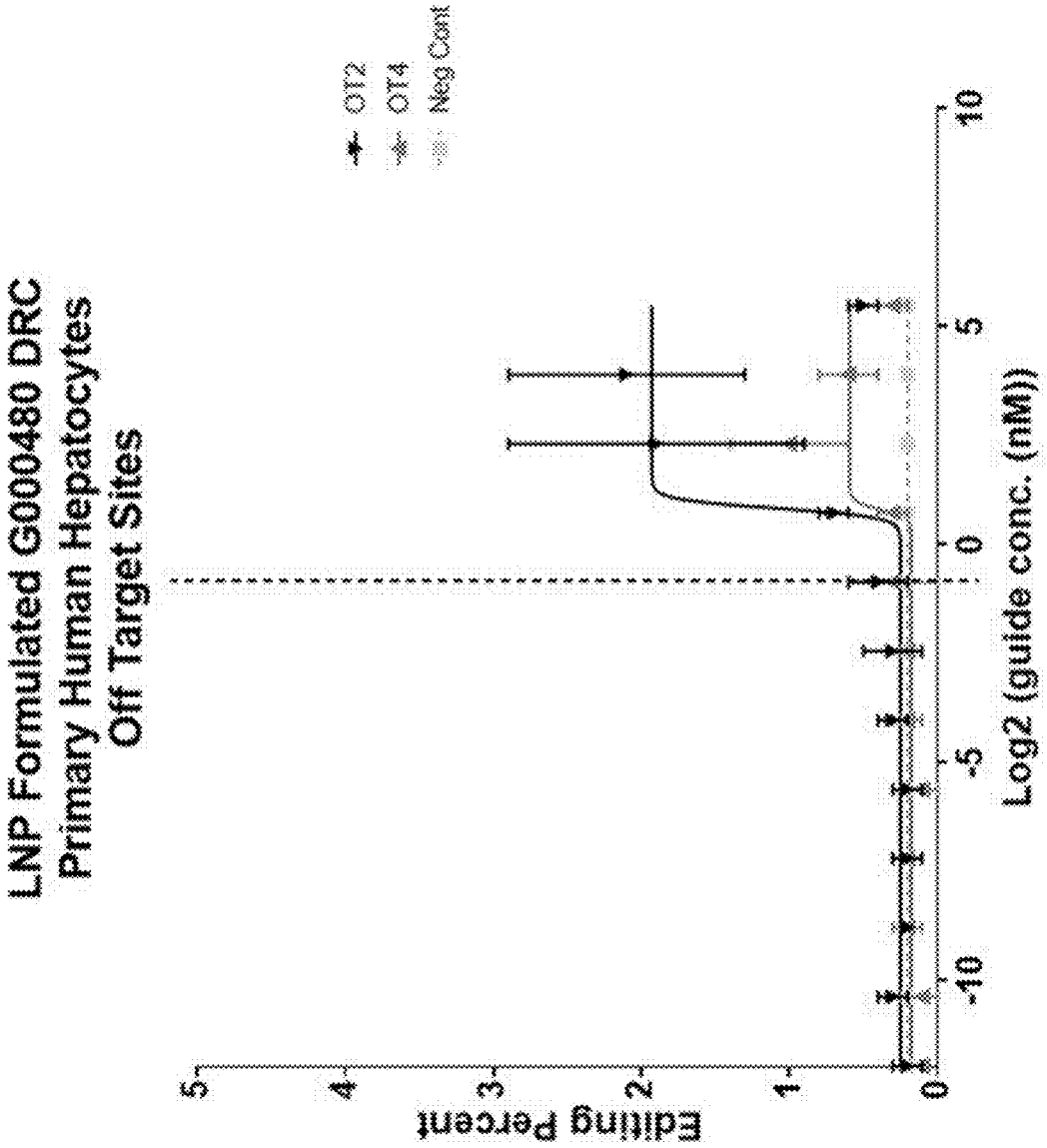

LNP Formulated G000480 DRC
Primary Human Hepatocytes
Off Target Sites
OT2
OT4
Neg Cont
Editing Percent
0
1
2
3
4
5
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 21B

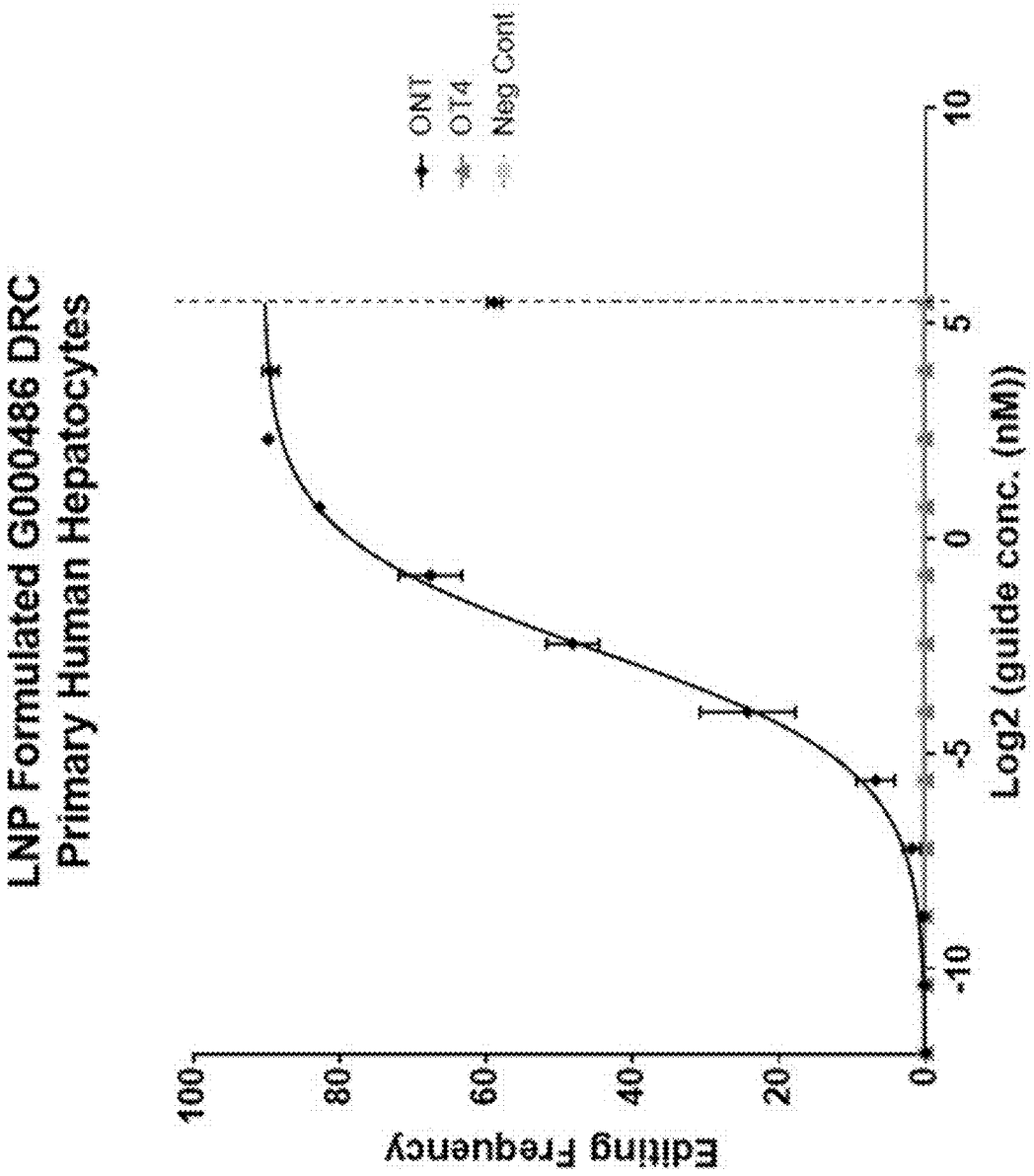

LNP Formulated G000486 DRC
Primary Human Hepatocytes
ONT
OT4
Neg Cont
Editing Frequency
100
80
60
40
20
0
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 22A

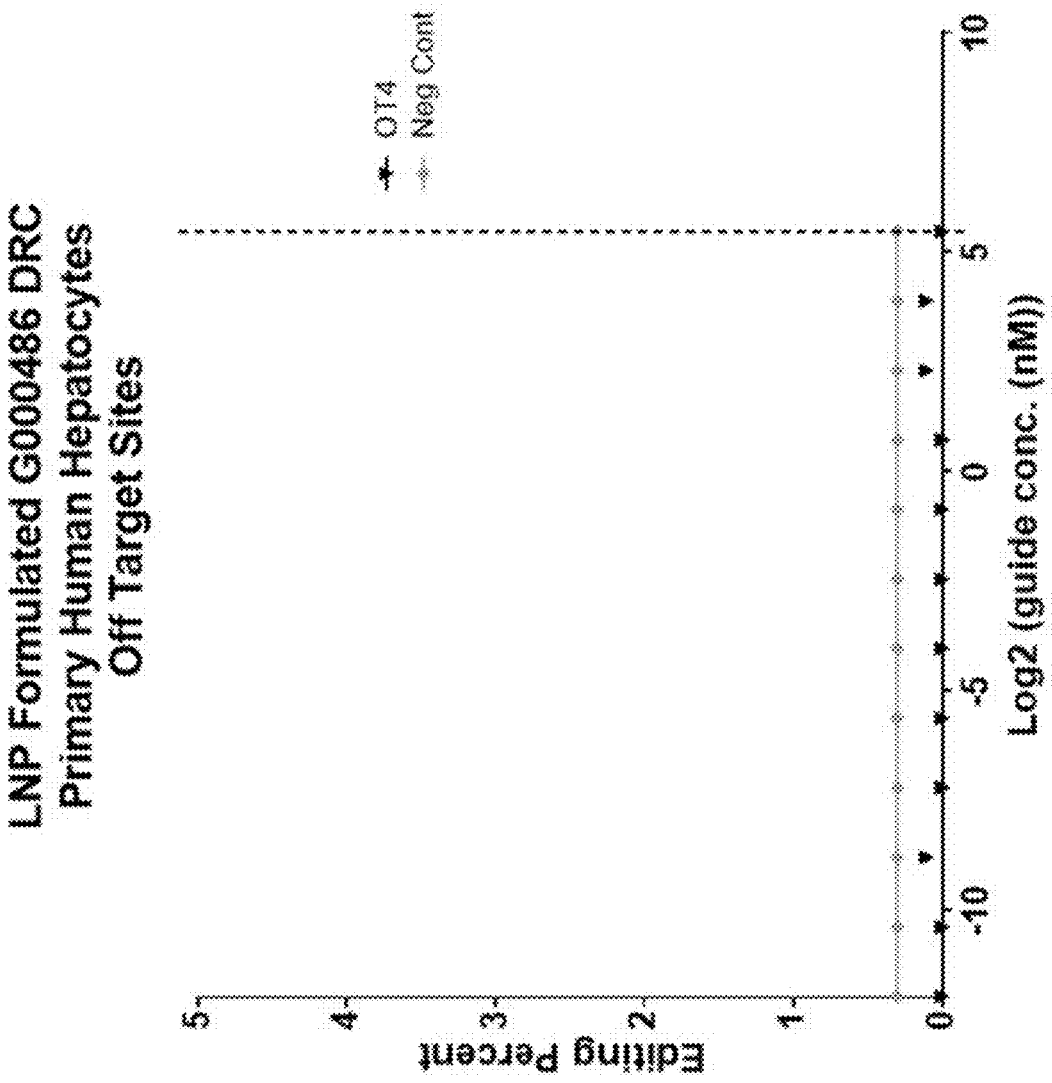

LNP Formulated G000486 DRC
Primary Human Hepatocytes
Off Target Sites
OT4
Neg Cont
Editing Percent
0
1
2
3
4
5
-10
-5
0
5
10
Log2 (guide conc. (nM))

FIG. 22B

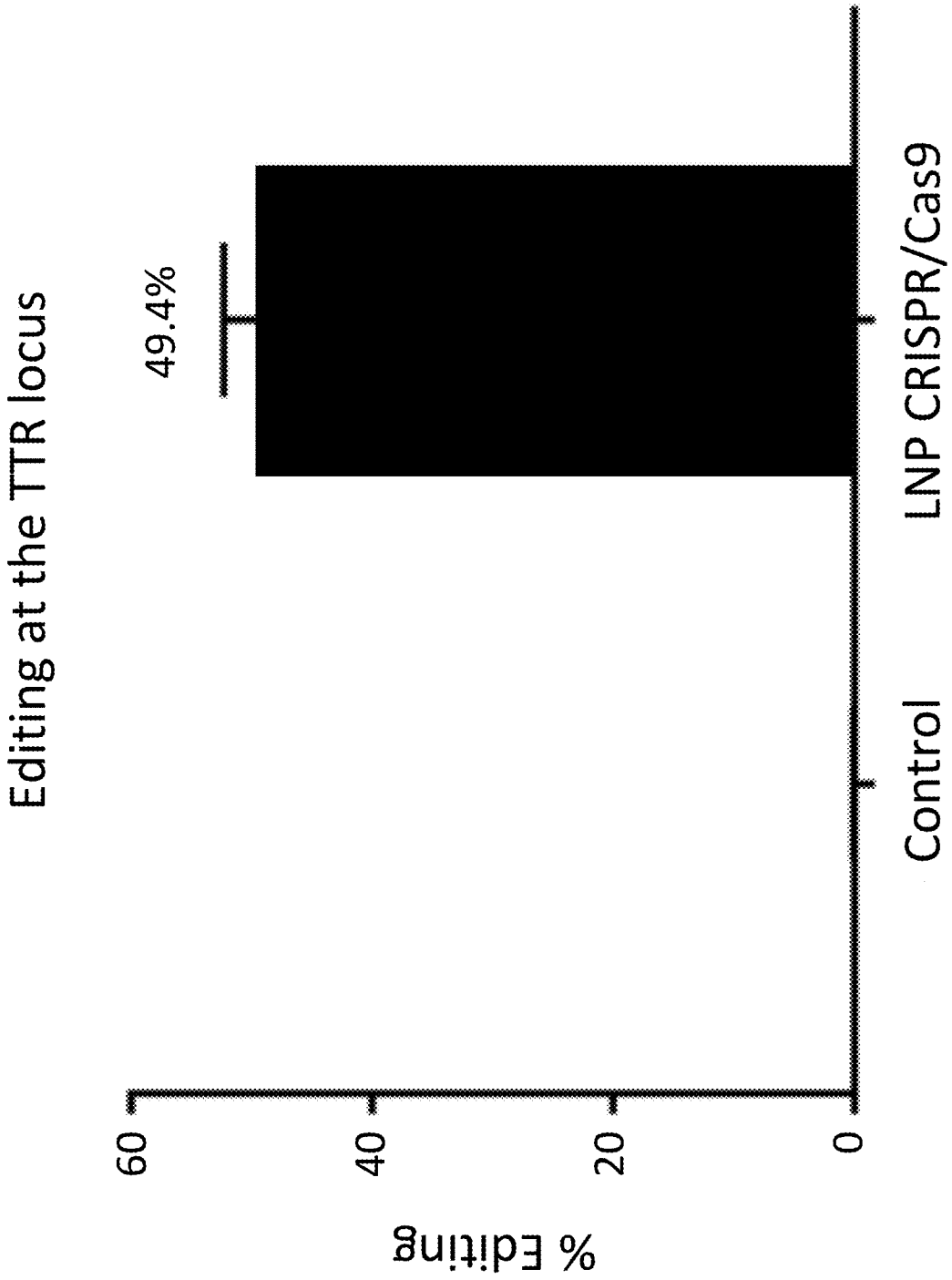

Editing at the TTR locus
49.4%
% Editing
60
40
20
0
Control
LNP CRISPR/Cas9

FIG. 23A

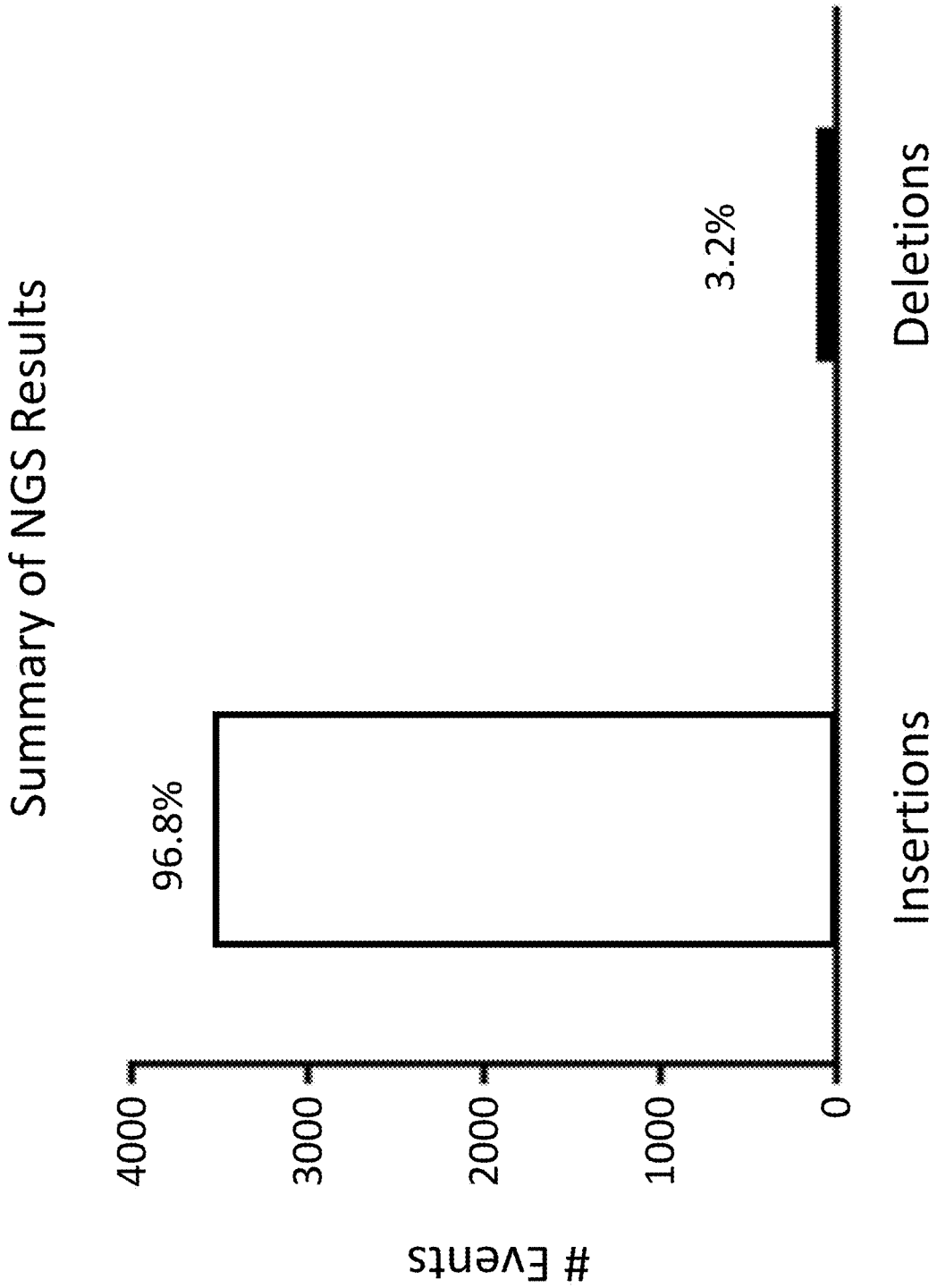

Summary of NGS Results
Events
4000
3000
2000
1000
0
96.8%
3.2%
Insertions
Deletions

FIG. 23B

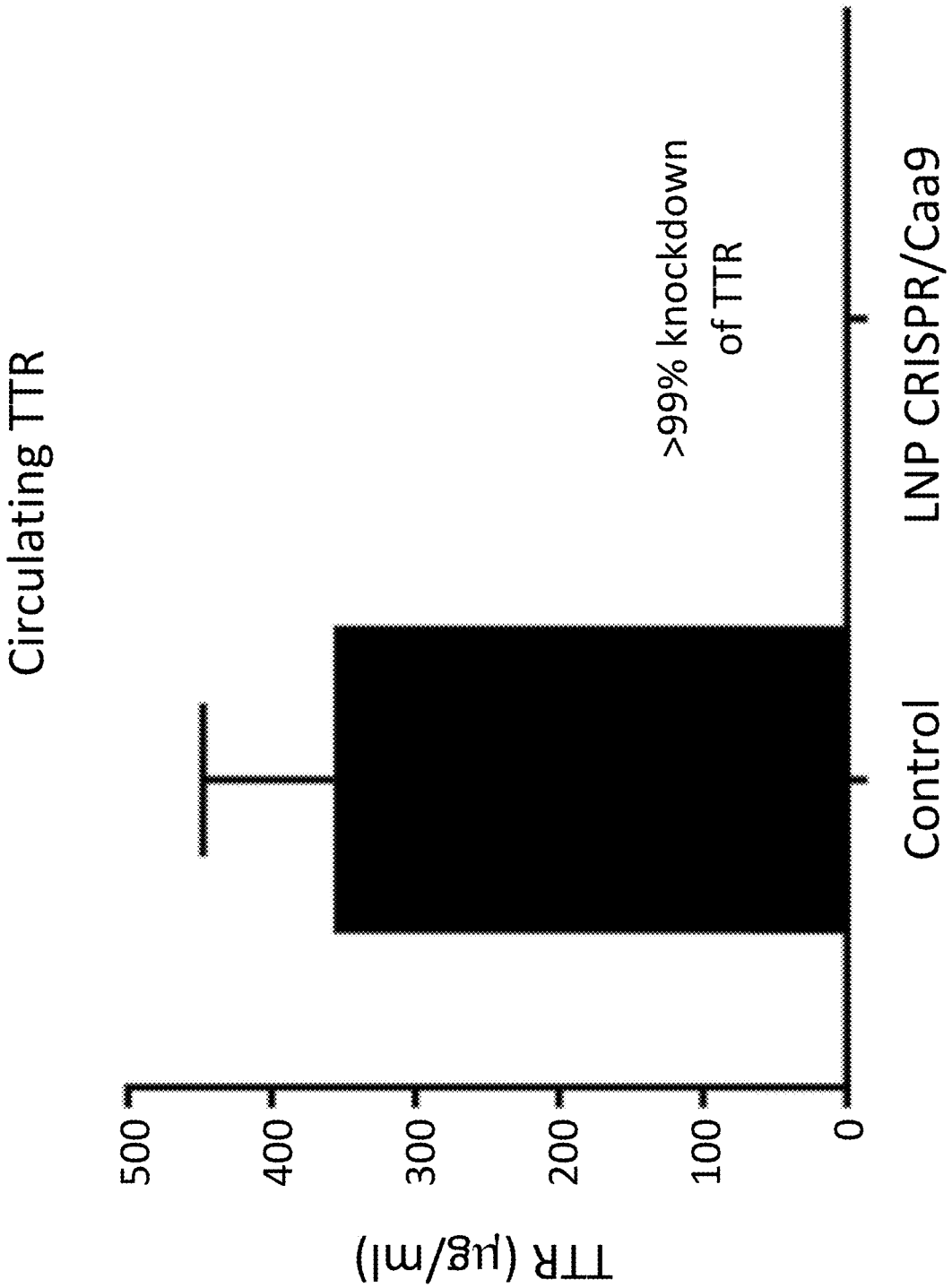

Circulating TTR
>99% knockdown of TTR
TTR (μg/ml)
500
400
300
200
100
0
Control
LNP CRISPR/Caa9

FIG. 24A

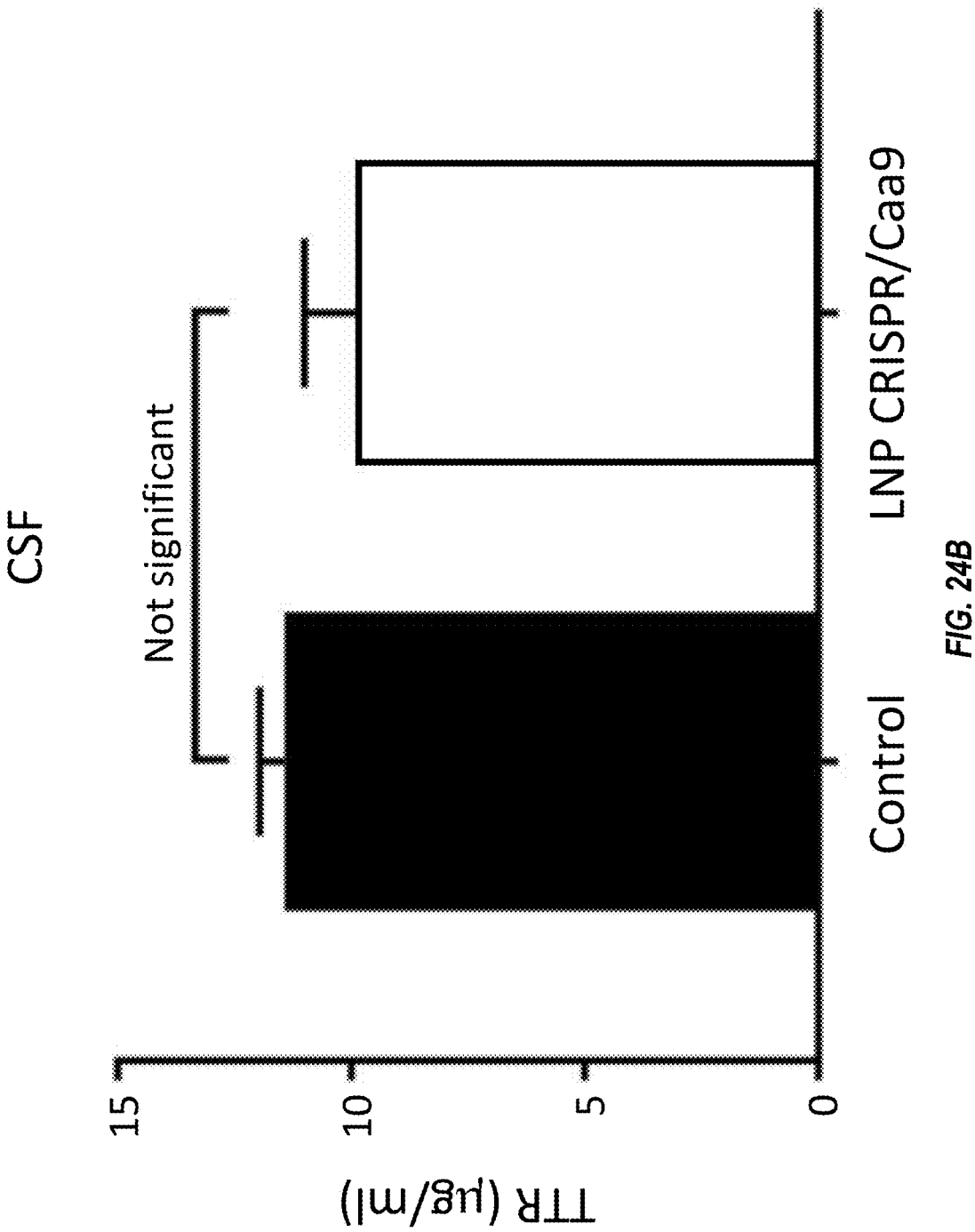

CSF
Not significant
LNP CRISPR/Caa9
Control
TTR (μg/ml)
15
10
5
0

FIG. 24B

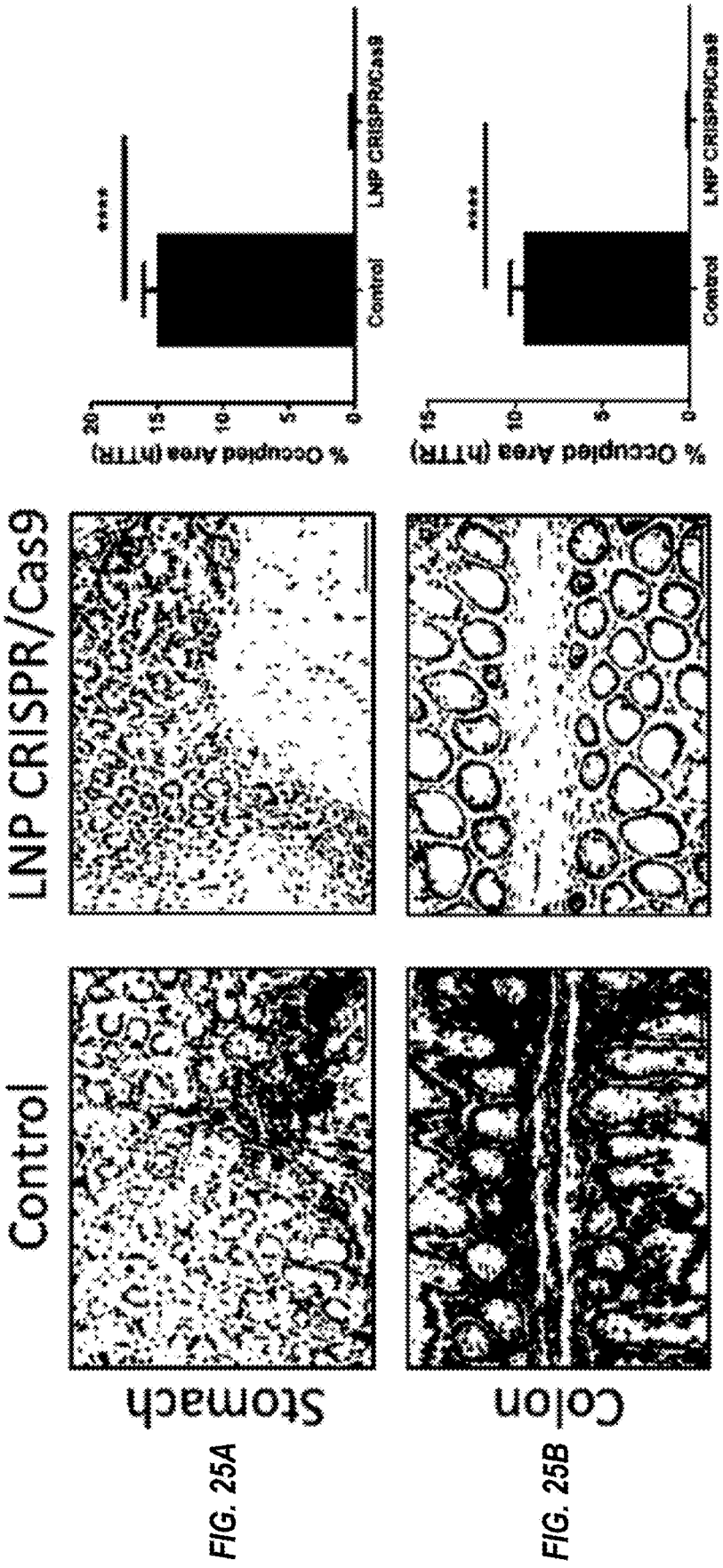


FIG. 25A

FIG. 25B

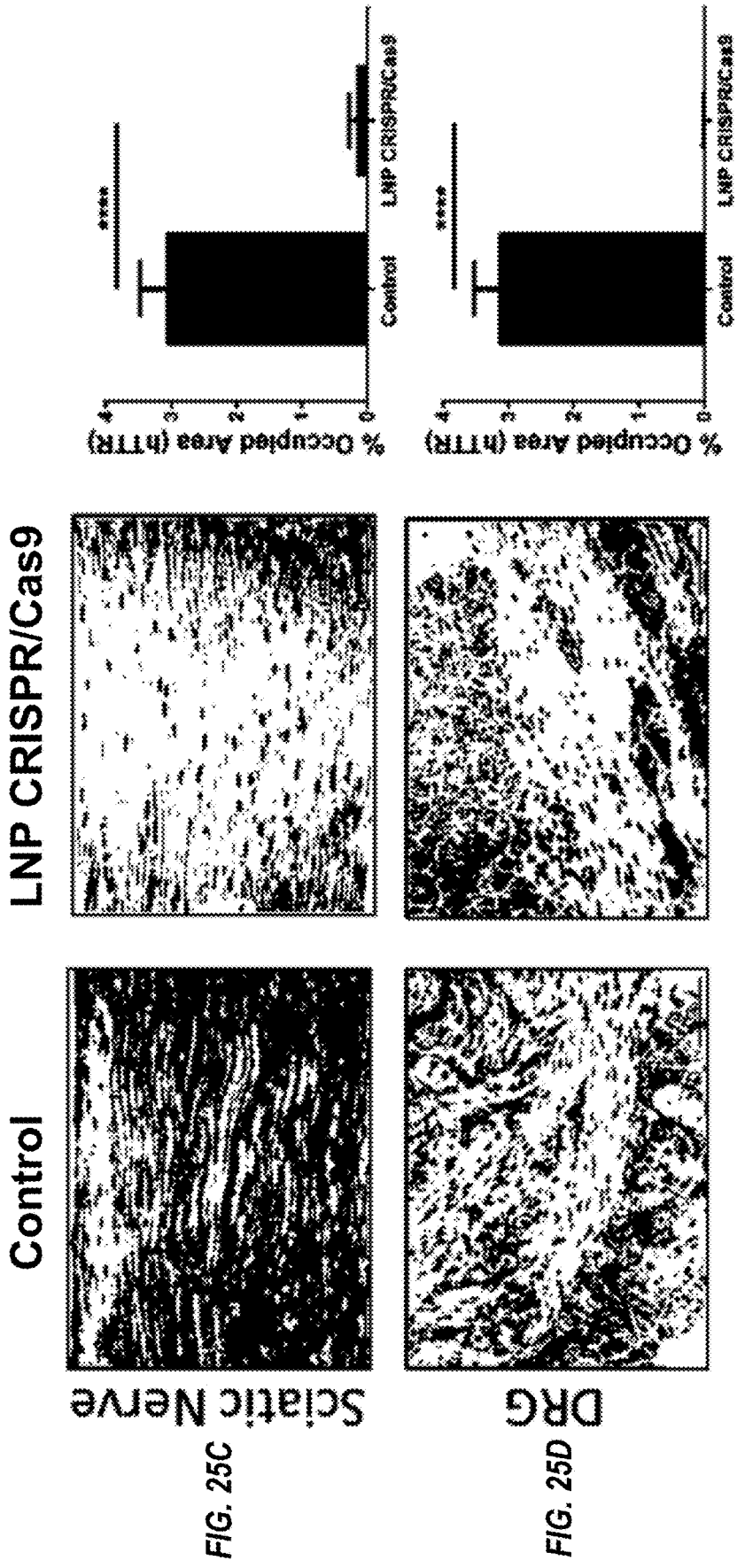


FIG. 25C

FIG. 25D

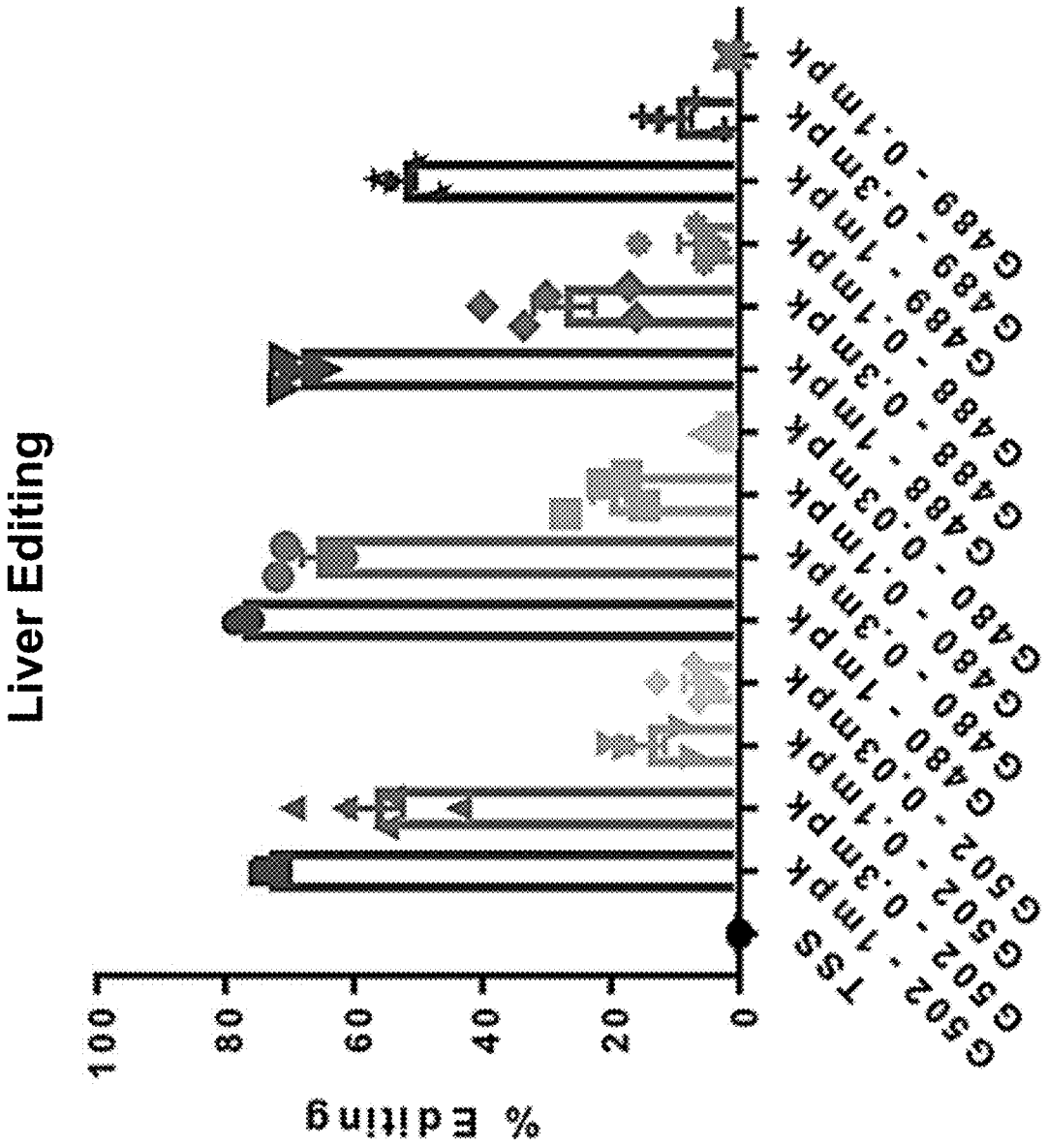

Liver Editing
% Editing
100
80
60
40
20
0
TSS - 1m pk
G502 - 0.3m pk
G502 - 0.1m pk
G502 - 0.03m pk
G502 - 1m pk
G480 - 0.3m pk
G480 - 0.1m pk
G480 - 0.03m pk
G480 - 1m pk
G488 - 0.3m pk
G488 - 0.1m pk
G488 - 0.03m pk
G488 - 1m pk
G489 - 0.1m pk
G489 - 0.3m pk
G489 - 1m pk

FIG. 26A

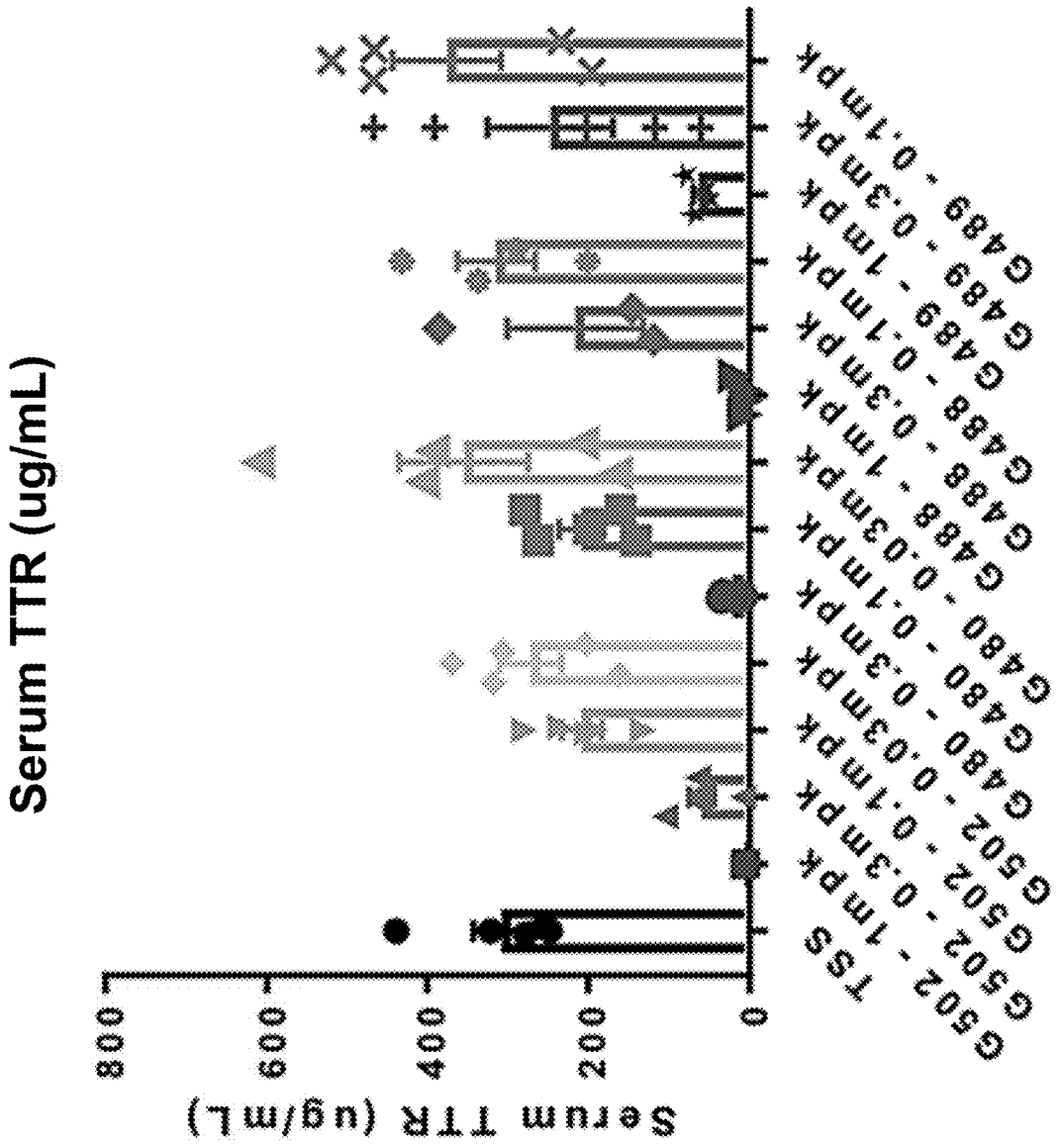

Serum TTR (ug/mL)
800
600
400
200
0
TSS - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk
G502 - 0.03mpk
G480 - 0.3mpk
G480 - 0.1mpk
G480 - 0.03mpk
G488 - 1mpk
G488 - 0.3mpk
G488 - 0.1mpk
G489 - 1mpk
G489 - 0.3mpk
G489 - 0.1mpk

FIG. 26B

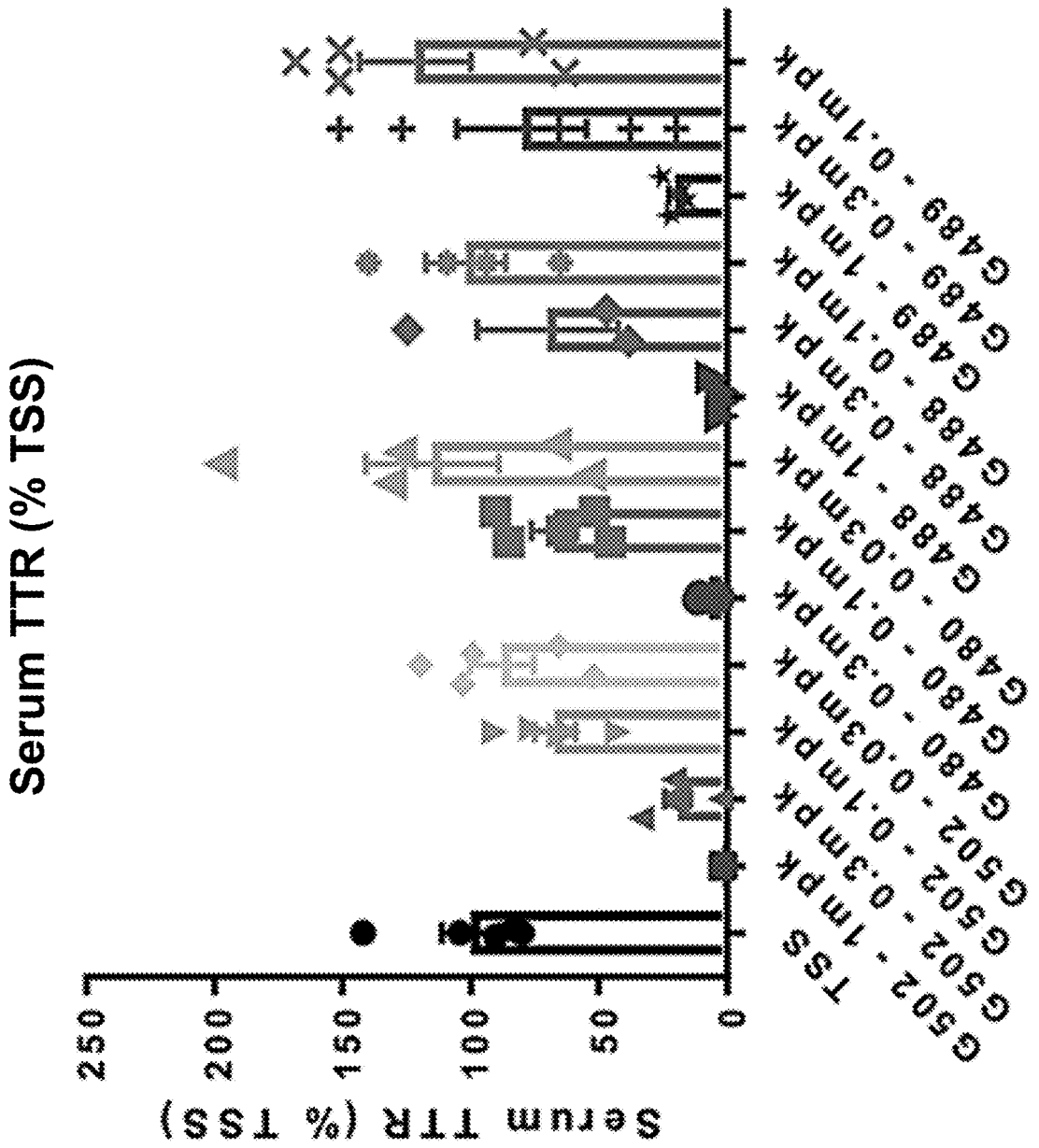

Serum TTR (% TSS)
250
200
150
100
50
0
TSS - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk
G502 - 0.03mpk
G480 - 0.3mpk
G480 - 0.1mpk
G480 - 0.03mpk
G488 - 1mpk
G488 - 0.3mpk
G488 - 0.1mpk
G489 - 1mpk
G489 - 0.3mpk
G489 - 0.1mpk

FIG. 26C

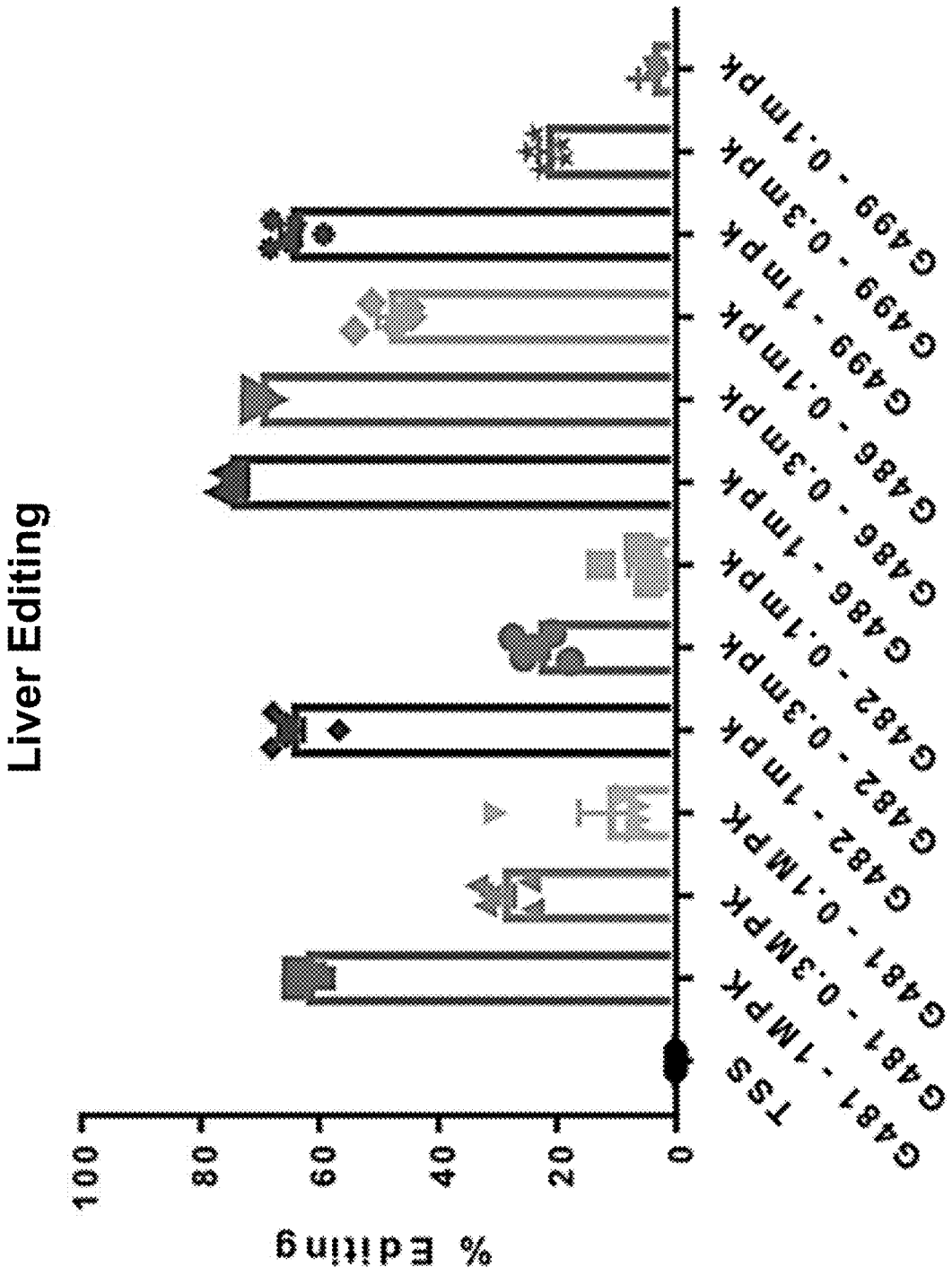

Liver Editing
% Editing
0
20
40
60
80
100
TSS
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G482 - 1MPK
G482 - 0.3MPK
G482 - 0.1MPK
G486 - 1MPK
G486 - 0.3MPK
G486 - 0.1MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

FIG. 27A

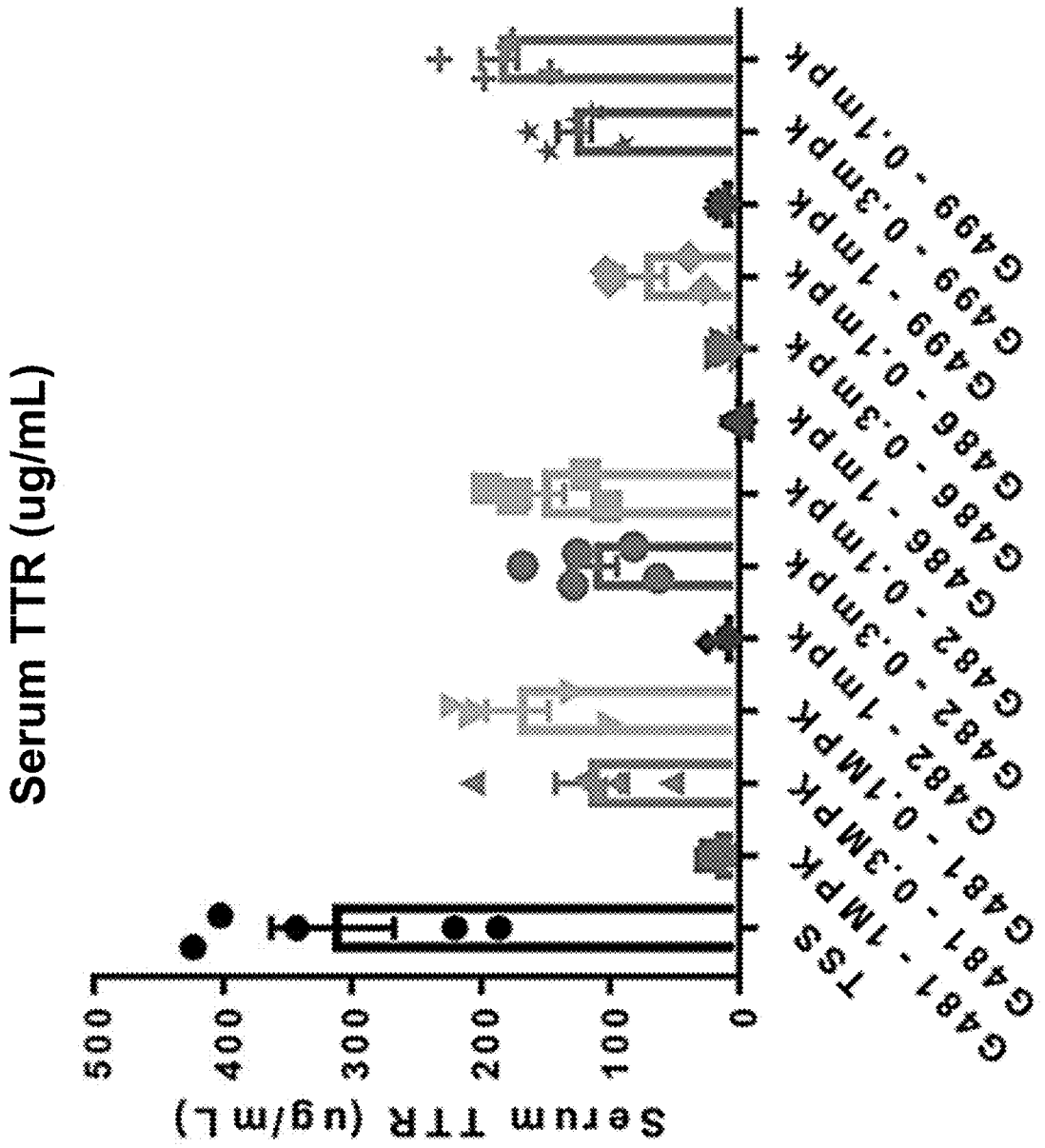

Serum TTR (ug/mL)
500
400
300
200
100
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G481 - 0.1MPK
G482 - 1MPK
G482 - 0.3MPK
G482 - 0.1MPK
G486 - 1MPK
G486 - 0.3MPK
G486 - 0.1MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

FIG. 27B

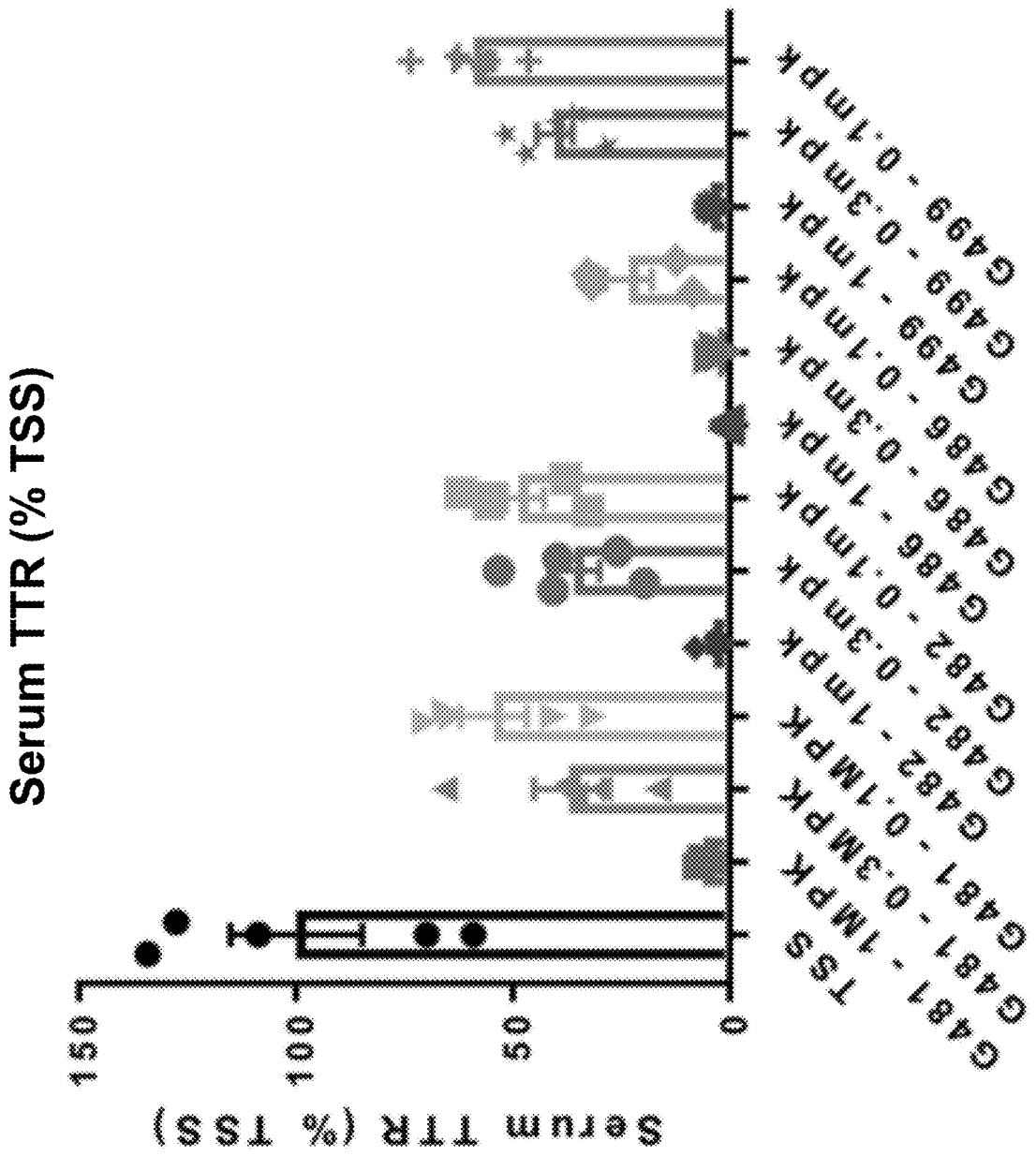

Serum TTR (% TSS)
150
100
50
0
TSS
G481 - 1MPK
G481 - 0.3MPK
G482 - 1MPK
G482 - 0.3MPK
G486 - 1MPK
G486 - 0.3MPK
G499 - 1MPK
G499 - 0.3MPK
G499 - 0.1MPK

FIG. 27C

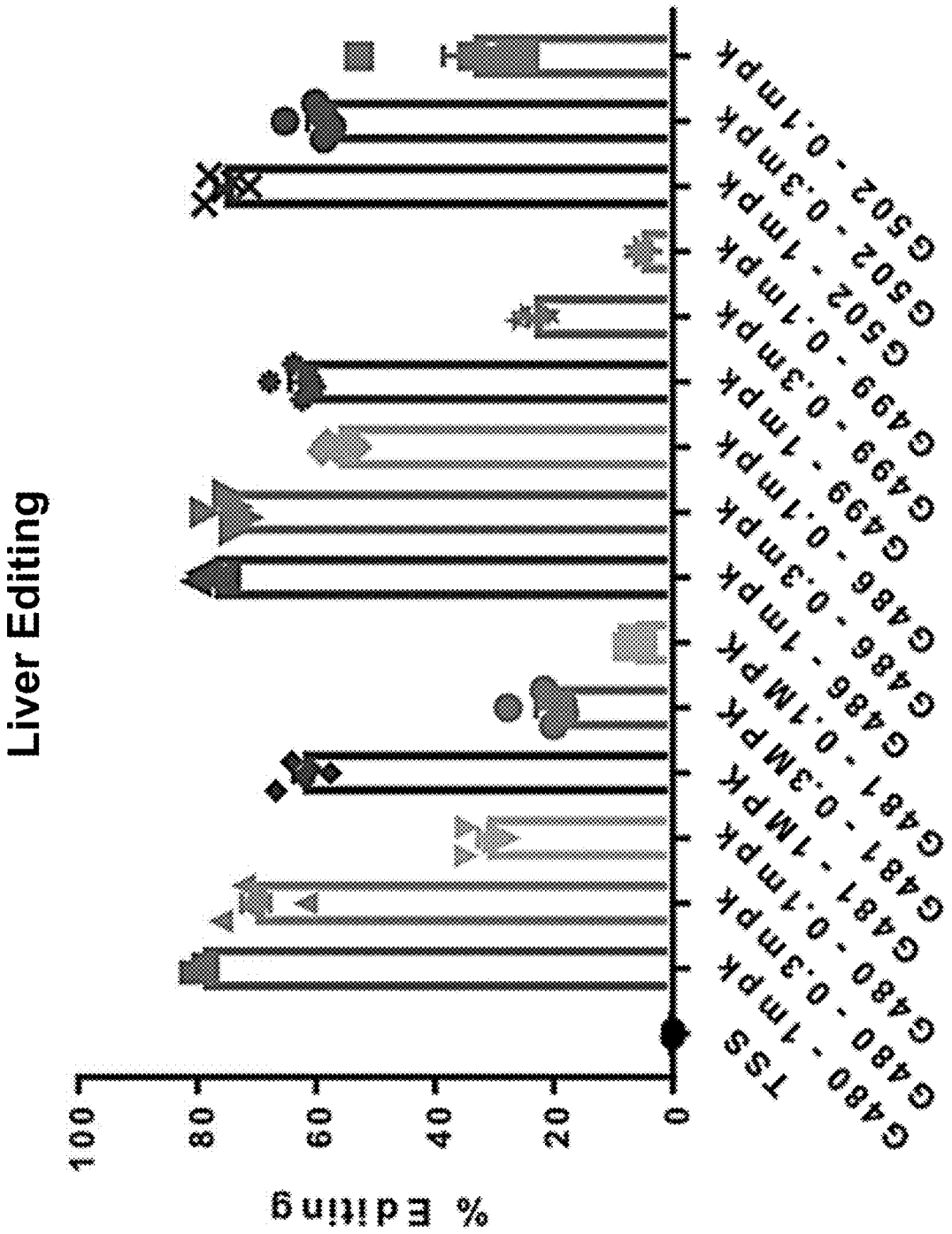

Liver Editing
% Editing
100
80
60
40
20
0
TSS - 1mpk
G480 - 0.3mpk
G480 - 1mpk
G481 - 0.1mpk
G481 - 1MPK
G481 - 0.3MPK
G486 - 0.1MPK
G486 - 1mpk
G486 - 0.3mpk
G499 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G502 - 0.1mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

FIG. 28A

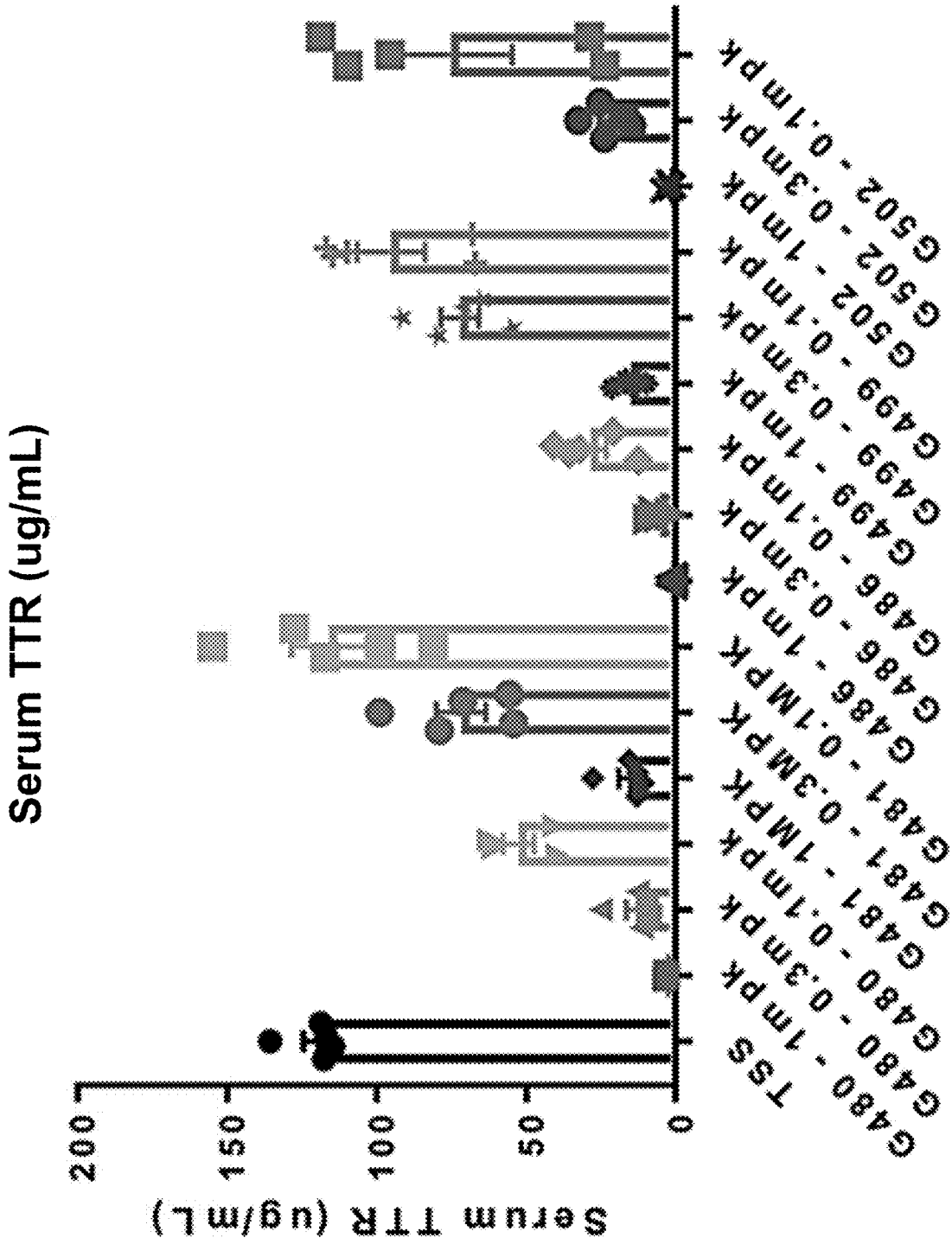

Serum TTR (ug/mL)
200
150
100
50
0
Serum TTR (ug/mL)
TSS - 1m pk
G480 - 0.3m pk
G480 - 0.1m pk
G481 - 1M pk
G481 - 0.3M pk
G481 - 0.1M pk
G486 - 1m pk
G486 - 0.3m pk
G486 - 0.1m pk
G499 - 1m pk
G499 - 0.3m pk
G499 - 0.1m pk
G502 - 1m pk
G502 - 0.3m pk
G502 - 0.1m pk

FIG. 28B

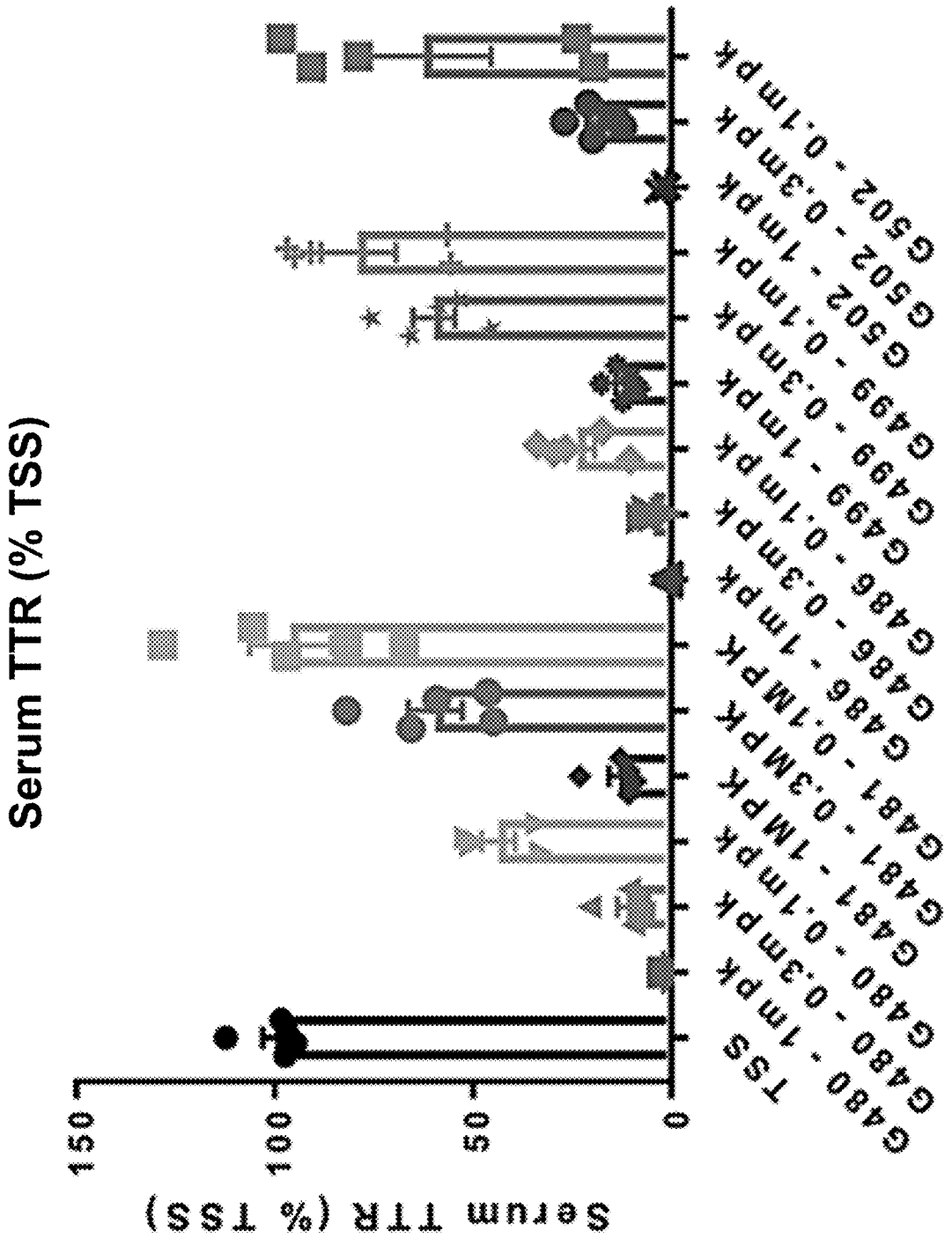

Serum TTR (% TSS)
150
100
50
0
TSS - 1mpk
G480 - 0.3mpk
G480 - 1mpk
G481 - 0.1mpk
G481 - 1Mpk
G481 - 0.3Mpk
G486 - 0.1Mpk
G486 - 1mpk
G486 - 0.3mpk
G499 - 0.1mpk
G499 - 1mpk
G499 - 0.3mpk
G502 - 0.1mpk
G502 - 1mpk
G502 - 0.3mpk
G502 - 0.1mpk

FIG. 28C

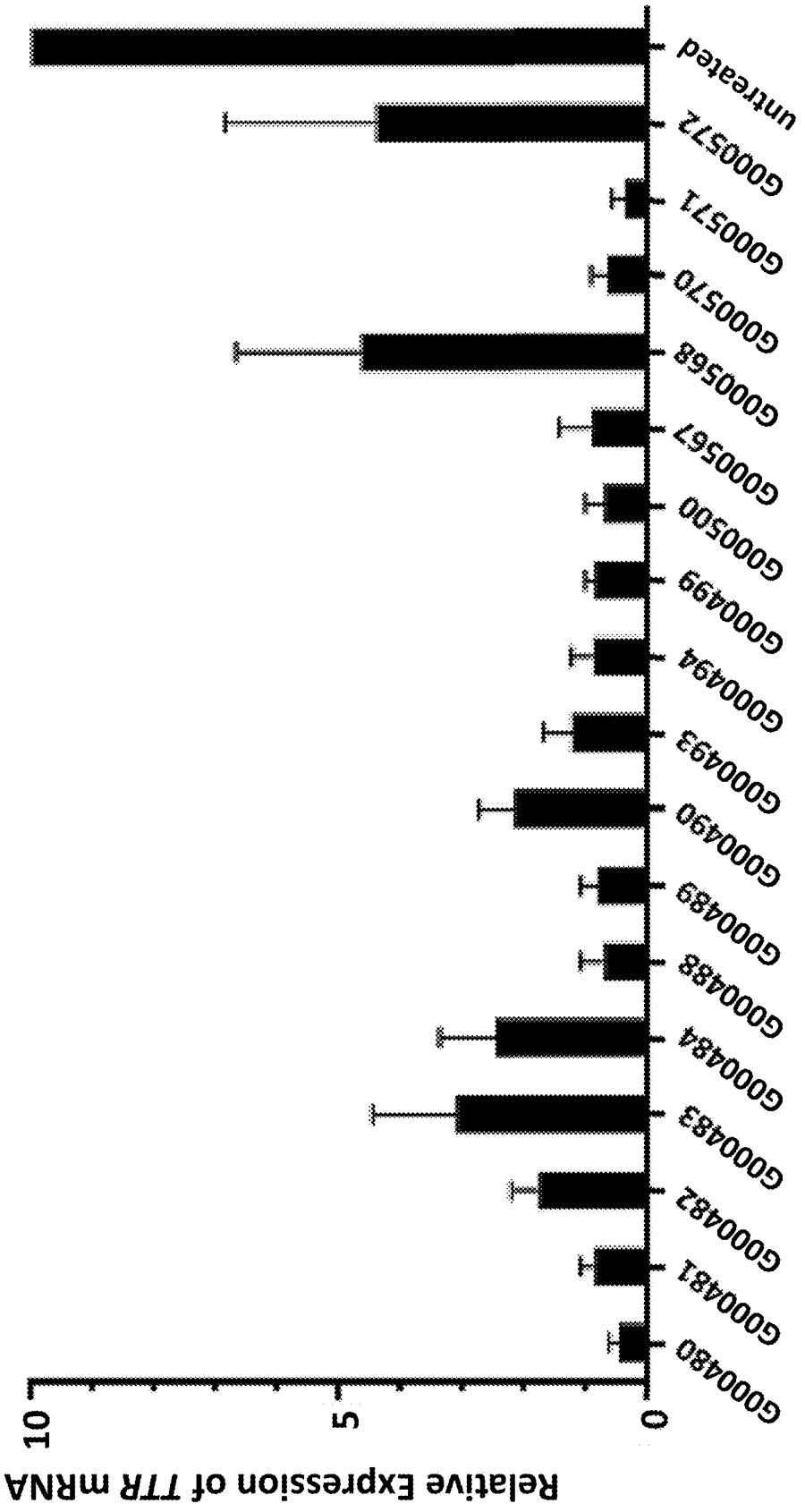

Relative Expression of TTR mRNA
10
5
0
untreated
G000572
G000571
G000570
G000568
G000567
G000500
G000499
G000494
G000493
G000490
G000489
G000488
G000484
G000483
G000482
G000481
G000480

FIG. 29

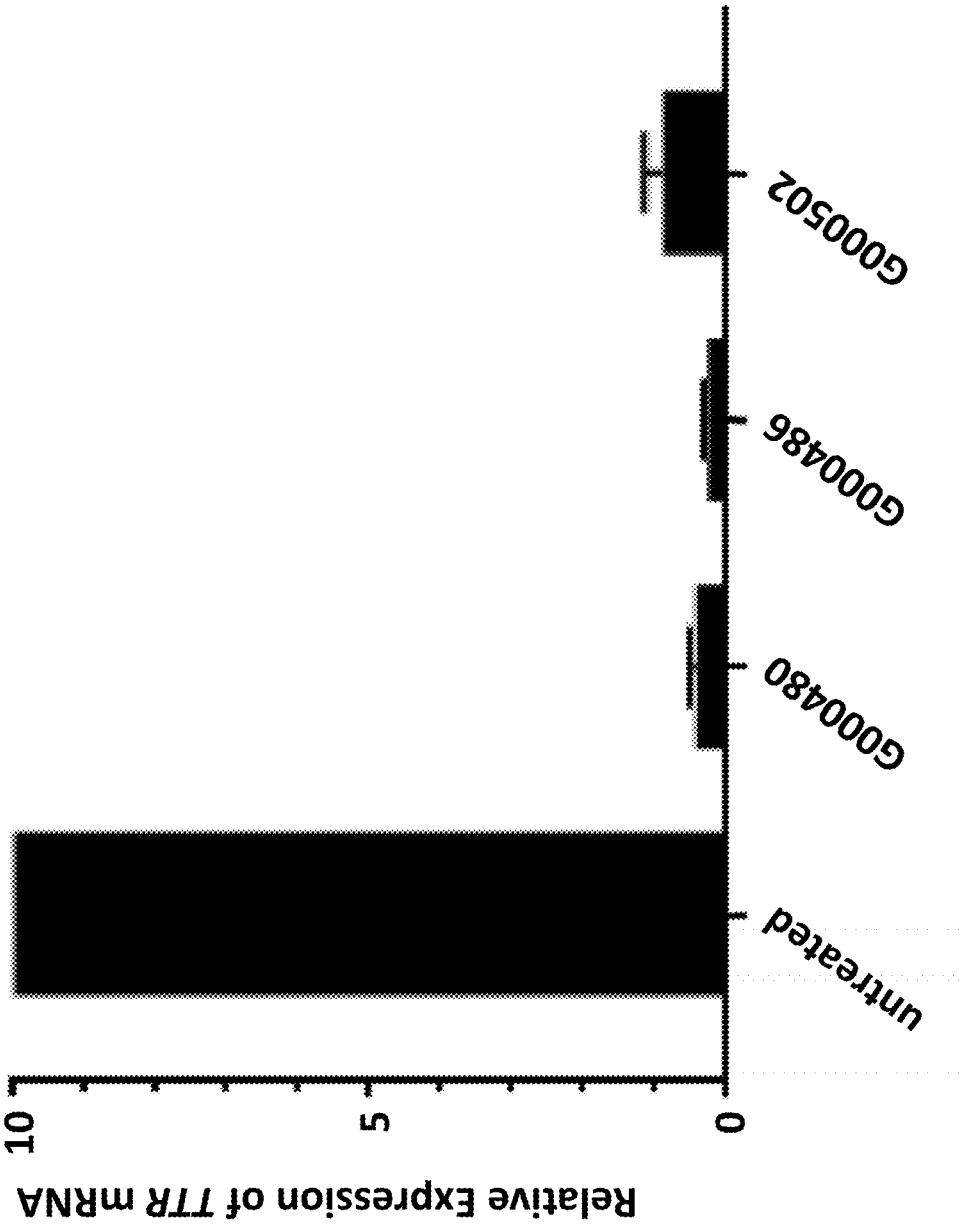

Relative Expression of TTR mRNA
10
5
0
untreated
G000480
G000486
G000502

FIG. 30

COMPOSITIONS AND METHODS FOR TTR GENE EDITING AND TREATING ATTR AMYLOIDOSIS

[0001] This application is a Continuation of International Application No. PCT/US2018/053382, which was filed on Sep. 28, 2018, which claims the benefit of priority to U.S. Provisional Application No. 62/556,236, which was filed on Sep. 29, 2017, and U.S. Provisional Application No. 62/671,902, which was filed on May 15, 2018, the contents of each of which are incorporated by reference in their entirety.

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Mar. 23, 2020, is named 2020-03-23_01155-0013-US_ST25.txt and is 417,533 bytes in size.

[0003] Transthyretin (TTR) is a protein produced by the TTR gene that normally functions to transport retinol and thyroxine throughout the body. TTR is predominantly synthesized in the liver, with small fractions being produced in the choroid plexus and retina. TTR normally circulates as a soluble tetrameric protein in the blood.

[0004] Pathogenic variants of TTR, which may disrupt tetramer stability, can be encoded by mutant alleles of the TTR gene. Mutant TTR may result in misfolded TTR, which may generate amyloids (i.e., aggregates of misfolded TTR protein). In some cases, pathogenic variants of TTR can lead to amyloidosis, or disease resulting from build-up of amyloids. For example, misfolded TTR monomers can polymerize into amyloid fibrils within tissues, such as the peripheral nerves, heart, and gastrointestinal tract. Amyloid plaques can also comprise wild-type TTR that has deposited on misfolded TTR.

[0005] Misfolding and deposition of wild-type TTR has also been observed in males aged 60 or more and is associated with heart rhythm problems, heart failure, and carpal tunnel.

[0006] Amyloidosis characterized by deposition of TTR may be referred to as "ATTR," "TTR-related amyloidosis," "TTR amyloidosis," or "ATTR amyloidosis," "ATTR familial amyloidosis" (when associated with a genetic mutation in a family), or "ATTRwt" or "wild-type ATTR" (when arising from misfolding and deposition of wild-type TTR).

[0007] ATTR can present with a wide spectrum of symptoms, and patients with different classes of ATTR may have different characteristics and prognoses. Some classes of ATTR include familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and wild-type TTR amyloidosis (wt-TTR amyloidosis). FAP commonly presents with sensorimotor neuropathy, while FAC and wt-TTR amyloidosis commonly present with congestive heart failure. FAP and FAC are usually associated with a genetic mutation in the TTR gene, and more than 100 different mutations in the TTR gene have been associated with ATTR. In contrast, wt-TTR amyloidosis is associated with aging and not with a genetic mutation in TTR. It is estimated that approximately 50,000 patients worldwide may be affected by FAP and FAC.

[0008] While more than 100 mutations in TTR are associated with ATTR, certain mutations have been more closely associated with neuropathy and/or cardiomyopathy. For example, mutations at T60 of TTR are associated with both cardiomyopathy and neuropathy; mutations at V30 are more associated with neuropathy; and mutations at V122 are more associated with cardiomyopathy.

[0009] A range of treatment approaches have been studied for treatment of ATTR, but there are no approved drugs that stop disease progression and improve quality of life. While liver transplant has been studied for treatment of ATTR, its use is declining as it involves significant risk and disease progression sometimes continues after transplantation. Small molecule stabilizers, such as diflunisal and tafamidis, appear to slow ATTR progression, but these agents do not halt disease progression.

[0010] Approaches using small interfering RNA (siRNA) knockdown, antisense knockdown, or a monoclonal antibody targeting amyloid fibrils for destruction are also currently being investigated, but while results on short-term suppression of TTR expression show encouraging preliminary data, a need exists for treatments that can produce long-lasting suppression of TTR.

[0011] Accordingly, the following embodiments are provided. In some embodiments, the present invention provides compositions and methods using a guide RNA with an RNA-guided DNA binding agent such as the CRISPR/Cas system to substantially reduce or knockout expression of the TTR gene, thereby substantially reducing or eliminating the production of TTR protein associated with ATTR. The substantial reduction or elimination of the production of TTR protein associated with ATTR through alteration of the TTR gene can be a long-term reduction or elimination.

SUMMARY

[0012] Embodiment 1 is a method of inducing a double-stranded break (DSB) within the TTR gene, comprising delivering a composition to a cell, wherein the composition comprises

a. a guide RNA comprising a guide sequence selected from SEQ ID NOs: 5-82;

b. a guide RNA comprising at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide RNA comprising a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[0013] Embodiment 2 is a method of modifying the TTR gene comprising delivering a composition to a cell, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[0014] Embodiment 3 is a method of treating amyloidosis associated with TTR (ATTR), comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,

thereby treating ATTR.

[0015] Embodiment 4 is a method of reducing TTR serum concentration, comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:
a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,
thereby reducing TTR serum concentration.

[0016] Embodiment 5 is a method for reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:
a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82,
thereby reducing accumulation of amyloids or amyloid fibrils.

[0017] Embodiment 6 is a composition comprising a guide RNA comprising:
a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[0018] Embodiment 7 is a composition comprising a vector encoding a guide RNA, wherein the guide RNA comprises:
a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

[0019] Embodiment 8 is the composition of embodiment 6 or 7, for use in inducing a double-stranded break (DSB) within the TTR gene in a cell or subject.

[0020] Embodiment 9 is the composition of embodiment 6 or 7, for use in modifying the TTR gene in a cell or subject.

[0021] Embodiment 10 is the composition of embodiment 6 or 7, for use in treating amyloidosis associated with TTR (ATTR) in a subject.

[0022] Embodiment 11 is the composition of embodiment 6 or 7, for use in reducing TTR serum concentration in a subject.

[0023] Embodiment 12 is the composition of embodiment 6 or 7, for use in reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

[0024] Embodiment 13 is the method of any one of embodiments 1-5 or the composition for use of any one of embodiments 8-12, wherein the composition reduces serum TTR levels.

[0025] Embodiment 14 is the method or composition for use of embodiment 13, wherein the serum TTR levels are reduced by at least 50% as compared to serum TTR levels before administration of the composition.

[0026] Embodiment 15 is the method or composition for use of embodiment 13, wherein the serum TTR levels are reduced by 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, 95-98%, 98-99%, or 99-100% as compared to serum TTR levels before administration of the composition.

[0027] Embodiment 16 is the method or composition for use of any one of embodiments 1-5 or 8-15, wherein the composition results in editing of the TTR gene.

[0028] Embodiment 17 is the method or composition for use of embodiment 16, wherein the editing is calculated as a percentage of the population that is edited (percent editing).

[0029] Embodiment 18 is the method or composition for use of embodiment 17, wherein the percent editing is between 30 and 99% of the population.

[0030] Embodiment 19 is the method or composition for use of embodiment 17, wherein the percent editing is between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the population.

[0031] Embodiment 20 is the method of any one of embodiments 1-5 or the composition for use of any one of embodiments 8-19, wherein the composition reduces amyloid deposition in at least one tissue.

[0032] Embodiment 21 is the method or composition for use of embodiment 20, wherein the at least one tissue comprises one or more of stomach, colon, sciatic nerve, or dorsal root ganglion.

[0033] Embodiment 22 is the method or composition for use of embodiment 20 or 21, wherein amyloid deposition is measured 8 weeks after administration of the composition.

[0034] Embodiment 23 is the method or composition for use of any one of embodiments 20-22, wherein amyloid deposition is compared to a negative control or a level measured before administration of the composition.

[0035] Embodiment 24 is the method or composition for use of any one of embodiments 20-23, wherein amyloid deposition is measured in a biopsy sample and/or by immunostaining.

[0036] Embodiment 25 is the method or composition for use of any one of embodiments 20-24, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen in a negative control.

[0037] Embodiment 26 is the method or composition for use of any one of embodiments 20-25, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen before administration of the composition.

[0038] Embodiment 27 is the method or composition for use of any one of embodiments 1-5 or 8-26, wherein the composition is administered or delivered at least two times.

[0039] Embodiment 28 is the method or composition for use of embodiment 27, wherein the composition is administered or delivered at least three times.

[0040] Embodiment 29 is the method or composition for use of embodiment 27, wherein the composition is administered or delivered at least four times.

[0041] Embodiment 30 is the method or composition for use of embodiment 27, wherein the composition is administered or delivered up to five, six, seven, eight, nine, or ten times.

[0042] Embodiment 31 is the method or composition for use of any one of embodiments 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days.

[0043] Embodiment 32 is the method or composition for use of any one of embodiments 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks.

[0044] Embodiment 33 is the method or composition for use of any one of embodiments 27-30, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 months.

[0045] Embodiment 34 is the method or composition of any one of the preceding embodiments, wherein the guide sequence is selected from SEQ ID NOs: 5-82.

[0046] Embodiment 35 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is at least partially complementary to a target sequence present in the human TTR gene.

[0047] Embodiment 36 is the method or composition of embodiment 35, wherein the target sequence is in exon 1, 2, 3, or 4 of the human TTR gene.

[0048] Embodiment 37 is the method or composition of embodiment 35, wherein the target sequence is in exon 1 of the human TTR gene.

[0049] Embodiment 38 is the method or composition of embodiment 35, wherein the target sequence is in exon 2 of the human TTR gene.

[0050] Embodiment 39 is the method or composition of embodiment 35, wherein the target sequence is in exon 3 of the human TTR gene.

[0051] Embodiment 40 is the method or composition of embodiment 35, wherein the target sequence is in exon 4 of the human TTR gene.

[0052] Embodiment 41 is the method or composition of any one of embodiments 1-40, wherein the guide sequence is complementary to a target sequence in the positive strand of TTR.

[0053] Embodiment 42 is the method or composition of any one of embodiments 1-40, wherein the guide sequence is complementary to a target sequence in the negative strand of TTR.

[0054] Embodiment 43 is the method or composition of any one of embodiments 1-40, wherein the first guide sequence is complementary to a first target sequence in the positive strand of the TTR gene, and wherein the composition further comprises a second guide sequence that is complementary to a second target sequence in the negative strand of the TTR gene.

[0055] Embodiment 44 is the method or composition of any one of the preceding embodiments, wherein the guide RNA comprises a crRNA that comprises the guide sequence and further comprises a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end.

[0056] Embodiment 45 is the method or composition of any one of the preceding embodiments, wherein the guide RNA is a dual guide (dgRNA).

[0057] Embodiment 46 is the method or composition of embodiment 45, wherein the dual guide RNA comprises a crRNA comprising a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end, and a trRNA.

[0058] Embodiment 47 is the method or composition of any one of embodiments 1-43, wherein the guide RNA is a single guide (sgRNA).

[0059] Embodiment 48 is the method or composition of embodiment 47, wherein the sgRNA comprises a guide sequence that has the pattern of SEQ ID NO: 3.

[0060] Embodiment 49 is the method or composition of embodiment 47, wherein the sgRNA comprises the sequence of SEQ ID NO: 3.

[0061] Embodiment 50 is the method or composition of embodiment 48 or 49, wherein each N in SEQ ID NO: 3 is any natural or non-natural nucleotide, wherein the N's form the guide sequence, and the guide sequence targets Cas9 to the TTR gene.

[0062] Embodiment 51 is the method or composition of any one of embodiments 47-50, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-82 and the nucleotides of SEQ ID NO: 126.

[0063] Embodiment 52 is the method or composition of any one of embodiments 47-51, wherein the sgRNA comprises a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID Nos: 87-124.

[0064] Embodiment 53 is the method or composition of embodiment 47, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-124.

[0065] Embodiment 54 is the method or composition of any one of the preceding embodiments, wherein the guide RNA comprises at least one modification.

[0066] Embodiment 55 is the method or composition of embodiment 54, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

[0067] Embodiment 56 is the method or composition of embodiment 54 or 55, wherein the at least one modification includes a phosphorothioate (PS) bond between nucleotides.

[0068] Embodiment 57 is the method or composition of any one of embodiments 54-56, wherein the at least one modification includes a 2'-fluoro (2'-F) modified nucleotide.

[0069] Embodiment 58 is the method or composition of any one of embodiments 54-57, wherein the at least one modification includes a modification at one or more of the first five nucleotides at the 5' end.

[0070] Embodiment 59 is the method or composition of any one of embodiments 54-58, wherein the at least one modification includes a modification at one or more of the last five nucleotides at the 3' end.

[0071] Embodiment 60 is the method or composition of any one of embodiments 54-59, wherein the at least one modification includes PS bonds between the first four nucleotides.

[0072] Embodiment 61 is the method or composition of any one of embodiments 54-60, wherein the at least one modification includes PS bonds between the last four nucleotides.

[0073] Embodiment 62 is the method or composition of any one of embodiments 54-61, wherein the at least one

modification includes 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

[0074] Embodiment 63 is the method or composition of any one of embodiments 54-62, wherein the at least one modification includes 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

[0075] Embodiment 64 is the method or composition of any one of embodiments 54-63, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

[0076] Embodiment 65 is the method or composition of any one of embodiments 1-64, wherein the composition further comprises a pharmaceutically acceptable excipient.

[0077] Embodiment 66 is the method or composition of any one of embodiments 1-65, wherein the guide RNA is associated with a lipid nanoparticle (LNP).

[0078] Embodiment 67 is the method or composition of embodiment 66, wherein the LNP comprises a CCD lipid.

[0079] Embodiment 68 is the method or composition of embodiment 67, wherein the CCD lipid is Lipid a or Lipid B.

[0080] Embodiment 69 is the method or composition of embodiment 66-68, wherein the LNP comprises a neutral lipid.

[0081] Embodiment 70 is the method or composition of embodiment 69, wherein the neutral lipid is DSPC

[0082] Embodiment 71 is the method or composition of any one of embodiments 66-70, wherein the LNP comprises a helper lipid.

[0083] Embodiment 72 is the method or composition of embodiment 71, wherein the helper lipid is cholesterol.

[0084] Embodiment 73 is the method or composition of any one of embodiments 66-72, wherein the LNP comprises a stealth lipid.

[0085] Embodiment 74 is the method or composition of embodiment 73, wherein the stealth lipid is PEG2k-DMG.

[0086] Embodiment 75 is the method or composition of any one of the preceding embodiments, wherein the composition further comprises an RNA-guided DNA binding agent.

[0087] Embodiment 76 is the method or composition of any one of the preceding embodiments, wherein the composition further comprises an mRNA that encodes an RNA-guided DNA binding agent.

[0088] Embodiment 77 is the method or composition of embodiment 75 or 76, wherein the RNA-guided DNA binding agent is a Cas cleavase.

[0089] Embodiment 78 is the method or composition of embodiment 77, wherein the RNA-guided DNA binding agent is Cas9.

[0090] Embodiment 79 is the method or composition of any one of embodiments 75-78, wherein the RNA-guided DNA binding agent is modified.

[0091] Embodiment 80 is the method or composition of any one of embodiments 75-79, wherein the RNA-guided DNA binding agent is a nickase.

[0092] Embodiment 81 is the method or composition of embodiment 79 or 80, wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

[0093] Embodiment 82 is the method or composition of any one of embodiments 75-81, wherein the RNA-guided DNA binding agent is a Cas from a Type-II CRISPR/Cas system.

[0094] Embodiment 83 is the method or composition of any one of the preceding embodiments, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

[0095] Embodiment 84 is the method or composition for use of any one of embodiments 1-5 or 8-83, wherein the composition reduces or prevents amyloids or amyloid fibrils comprising TTR.

[0096] Embodiment 85 is the method or composition for use of embodiment 84, wherein the amyloids or amyloid fibrils are in the nerves, heart, or gastrointestinal track.

[0097] Embodiment 86 is the method or composition for use of any one of embodiments 1-5 or 8-83, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the TTR gene.

[0098] Embodiment 87 is the method or composition for use of embodiment 86, wherein NHEJ leads to a deletion or insertion of a nucleotide(s) during repair of a DSB in the TTR gene.

[0099] Embodiment 88 is the method or composition for use of embodiment 87, wherein the deletion or insertion of a nucleotide(s) induces a frame shift or nonsense mutation in the TTR gene.

[0100] Embodiment 89 is the method or composition for use of embodiment 87, wherein a frame shift or nonsense mutation is induced in the TTR gene of at least 50% of liver cells.

[0101] Embodiment 90 is the method or composition for use of embodiment 89, wherein a frame shift or nonsense mutation is induced in the TTR gene of 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100% of liver cells.

[0102] Embodiment 91 is the method or composition for use of any one of embodiments 87-90, wherein a deletion or insertion of a nucleotide(s) occurs in the TTR gene at least 50-fold or more than in off-target sites.

[0103] Embodiment 92 is the method or composition for use of embodiment 91, wherein the deletion or insertion of a nucleotide(s) occurs in the TTR gene 50-fold to 150-fold, 150-fold to 500-fold, 500-fold to 1500-fold, 1500-fold to 5000-fold, 5000-fold to 15000-fold, 15000-fold to 30000-fold, or 30000-fold to 60000-fold more than in off-target sites.

[0104] Embodiment 93 is the method or composition for use of any one of embodiments 87-92, wherein the deletion or insertion of a nucleotide(s) occurs at less than or equal to 3, 2, 1, or 0 off-target site(s) in primary human hepatocytes, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[0105] Embodiment 94 is the method or composition for use of embodiment 93, wherein the deletion or insertion of a nucleotide(s) occurs at a number of off-target sites in primary human hepatocytes that is less than the number of off-target sites at which a deletion or insertion of a nucleotide(s) occurs in Cas9-overexpressing cells, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[0106] Embodiment 95 is the method or composition for use of embodiment 94, wherein the Cas9-overexpressing cells are HEK293 cells stably expressing Cas9.

[0107] Embodiment 96 is the method or composition for use of any one of embodiments 93-95, wherein the number

of off-target sites in primary human hepatocytes is determined by analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA and the guide RNA, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[0108] Embodiment 97 is the method or composition for use of any one of embodiments 93-95, wherein the number of off-target sites in primary human hepatocytes is determined by an oligonucleotide insertion assay comprising analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

[0109] Embodiment 98 is the method or composition of any one of embodiments 1-43 or 47-97, wherein the sequence of the guide RNA is:

[0110] a) SEQ ID NO: 92 or 104;

[0111] b) SEQ ID NO: 87, 89, 96, or 113;

[0112] c) SEQ ID NO: 100, 102, 106, 111, or 112; or

[0113] d) SEQ ID NO: 88, 90, 91, 93, 94, 95, 97, 101, 103, 108, or 109,

optionally wherein the guide RNA does not produce indels at off-target site(s) that occur in a protein coding region in the genome of primary human hepatocytes.

[0114] Embodiment 99 is the method or composition for use of any one of embodiments 1-5 or 8-98, wherein administering the composition reduces levels of TTR in the subject.

[0115] Embodiment 100 is the method or composition for use of embodiment 99, wherein the levels of TTR are reduced by at least 50%.

[0116] Embodiment 101 is the method or composition for use of embodiment 100, wherein the levels of TTR are reduced by 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100%.

[0117] Embodiment 102 is the method or composition for use of embodiment 100 or 101, wherein the levels of TTR are measured in serum, plasma, blood, cerebral spinal fluid, or sputum.

[0118] Embodiment 103 is the method or composition for use of embodiment 100 or 101, wherein the levels of TTR are measured in liver, choroid plexus, and/or retina.

[0119] Embodiment 104 is the method or composition for use of any one of embodiments 99-103, wherein the levels of TTR are measured via enzyme-linked immunosorbent assay (ELISA).

[0120] Embodiment 105 is the method or composition for use of any one of embodiments 1-5 or 8-104, wherein the subject has ATTR.

[0121] Embodiment 106 is the method or composition for use of any one of embodiments 1-5 or 8-105, wherein the subject is human.

[0122] Embodiment 107 is the method or composition for use of embodiment 105 or 106, wherein the subject has ATTRwt.

[0123] Embodiment 108 is the method or composition for use of embodiment 105 or 106, wherein the subject has hereditary ATTR.

[0124] Embodiment 109 is the method or composition for use of any one of embodiments 1-5, 8-106, or 108, wherein the subject has a family history of ATTR.

[0125] Embodiment 110 is the method or composition for use of any one of embodiments 1-5, 8-106, or 108-109, wherein the subject has familial amyloid polyneuropathy.

[0126] Embodiment 111 is the method or composition for use of any one of embodiments 1-5 or 8-110, wherein the subject has only or predominantly nerve symptoms of ATTR.

[0127] Embodiment 112 is the method or composition for use of any one of embodiments 1-5 or 8-110, wherein the subject has familial amyloid cardiomyopathy.

[0128] Embodiment 113 is the method or composition for use of any one of embodiments 1-5, 8-109, or 112, wherein the subject has only or predominantly cardiac symptoms of ATTR.

[0129] Embodiment 114 is the method or composition for use of any one of embodiments 1-5 or 8-113, wherein the subject expresses TTR having a V30 mutation.

[0130] Embodiment 115 is the method or composition for use of embodiment 114, wherein the V30 mutation is V30A, V30G, V30L, or V30M.

[0131] Embodiment 116 is the method or composition for use of embodiment any one of embodiments 1-5 or 8-113, wherein the subject expresses TTR having a T60 mutation.

[0132] Embodiment 117 is the method or composition for use of embodiment 116, wherein the T60 mutation is T60A.

[0133] Embodiment 118 is the method or composition for use of embodiment any one of embodiments 1-5 or 8-113, wherein the subject expresses TTR having a V122 mutation.

[0134] Embodiment 119 is the method or composition for use of embodiment 118, wherein the V122 mutation is V122A, V122I, or V122(−).

[0135] Embodiment 120 is the method or composition for use of any one of embodiments 1-5 or 8-119, wherein the subject expresses wild-type TTR.

[0136] Embodiment 121 is the method or composition for use of any one of embodiments 1-5, 8-107, or 120, wherein the subject does not express TTR having a V30, T60, or V122 mutation.

[0137] Embodiment 122 is the method or composition for use of any one of embodiments 1-5, 8-107, or 120-121, wherein the subject does not express TTR having a pathological mutation.

[0138] Embodiment 123 is the method or composition for use of embodiment 121, wherein the subject is homozygous for wild-type TTR.

[0139] Embodiment 124 is the method or composition for use of any one of embodiments 1-5 or 8-123, wherein after administration the subject has an improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy.

[0140] Embodiment 125 is the method or composition for use of embodiment 124, wherein the improvement, stabilization, or slowing of change in sensory neuropathy is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

[0141] Embodiment 126 is the method or composition for use of any one of embodiments 1-5 or 8-125, wherein the subject has an improvement, stabilization, or slowing of change in symptoms of congestive heart failure.

[0142] Embodiment 127 is the method or composition for use of embodiment 126, wherein the improvement, stabilization, or slowing of change in congestive heart failure is measured using cardiac biomarker tests, lung function tests, chest x-rays, or electrocardiography.

[0143] Embodiment 128 is the method or composition for use of any one of embodiments 1-5 or 8-127, wherein the composition or pharmaceutical formulation is administered via a viral vector.

[0144] Embodiment 129 is the method or composition for use of any one of embodiments 1-5 or 8-127, wherein the composition or pharmaceutical formulation is administered via lipid nanoparticles.

[0145] Embodiment 130 is the method or composition for use of any one of embodiments 1-5 or 8-129, wherein the subject is tested for specific mutations in the TTR gene before administering the composition or formulation.

[0146] Embodiment 131 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 5.

[0147] Embodiment 132 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 6.

[0148] Embodiment 133 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 7.

[0149] Embodiment 134 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 8.

[0150] Embodiment 135 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 9.

[0151] Embodiment 136 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 10.

[0152] Embodiment 137 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 11.

[0153] Embodiment 138 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 12.

[0154] Embodiment 139 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 13.

[0155] Embodiment 140 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 14.

[0156] Embodiment 141 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 15.

[0157] Embodiment 142 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 16.

[0158] Embodiment 143 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 17.

[0159] Embodiment 144 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 18.

[0160] Embodiment 145 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 19.

[0161] Embodiment 146 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 20.

[0162] Embodiment 147 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 21.

[0163] Embodiment 148 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 22.

[0164] Embodiment 149 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 23.

[0165] Embodiment 150 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 24.

[0166] Embodiment 151 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 25.

[0167] Embodiment 152 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 26.

[0168] Embodiment 153 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 27.

[0169] Embodiment 154 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 28.

[0170] Embodiment 155 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 29.

[0171] Embodiment 156 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 30.

[0172] Embodiment 157 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 31.

[0173] Embodiment 158 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 32.

[0174] Embodiment 159 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 33.

[0175] Embodiment 160 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 34.

[0176] Embodiment 161 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 35.

[0177] Embodiment 162 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 36.

[0178] Embodiment 163 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 37.

[0179] Embodiment 164 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 38.

[0180] Embodiment 165 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 39.

[0181] Embodiment 166 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 40.

[0182] Embodiment 167 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 41.

[0183] Embodiment 168 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 42.

[0184] Embodiment 169 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 43.

[0185] Embodiment 170 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 44.

[0186] Embodiment 171 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 45.

[0187] Embodiment 172 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 46.

[0188] Embodiment 173 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 47.

[0189] Embodiment 174 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 48.

[0190] Embodiment 175 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 49.

[0191] Embodiment 176 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 50.

[0192] Embodiment 177 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 51.

[0193] Embodiment 178 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 52.

[0194] Embodiment 179 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 53.

[0195] Embodiment 180 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 54.

[0196] Embodiment 181 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 55.

[0197] Embodiment 182 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 56.

[0198] Embodiment 183 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 57.

[0199] Embodiment 184 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 58.

[0200] Embodiment 185 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 59.

[0201] Embodiment 186 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 60.

[0202] Embodiment 187 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 61.

[0203] Embodiment 188 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 62.

[0204] Embodiment 189 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 63.

[0205] Embodiment 190 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 64.

[0206] Embodiment 191 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 65.

[0207] Embodiment 192 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 66.

[0208] Embodiment 193 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 67.

[0209] Embodiment 194 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 68.

[0210] Embodiment 195 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 69.

[0211] Embodiment 196 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 70.

[0212] Embodiment 197 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 71.

[0213] Embodiment 198 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 72.

[0214] Embodiment 199 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 73.

[0215] Embodiment 200 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 74.

[0216] Embodiment 201 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 75.

[0217] Embodiment 202 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 76.

[0218] Embodiment 203 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 77.

[0219] Embodiment 204 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 78.

[0220] Embodiment 205 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 79.

[0221] Embodiment 206 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 80.

[0222] Embodiment 207 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 81.

[0223] Embodiment 208 is the method or composition of any one of embodiments 1-130, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 82.

[0224] Embodiment 209 is a use of a composition or formulation of any of embodiments 6-208 for the preparation of a medicament for treating a human subject having ATTR.

[0225] Also disclosed is the use of a composition or formulation of any of the foregoing embodiments for the preparation of a medicament for treating a human subject

having ATTR. Also disclosed are any of the foregoing compositions or formulations for use in treating ATTR or for use in modifying (e.g., forming an indel in, or forming a frameshift or nonsense mutation in) a TTR gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[0226] FIG. **1** shows a schematic of chromosome 18 with the regions of the TTR gene that are targeted by the guide sequences provided in Table 1.

[0227] FIG. **2** shows off-target analysis in HEK293_Cas9 cells of certain dual guide RNAs targeting TTR. The on-target site is designated by a filled square for each dual guide RNA tested, whereas closed circles represent a potential off-target site.

[0228] FIG. **3** shows off-target analysis in HEK_Cas9 cells of certain single guide RNAs targeting TTR. The on-target site is designated by a filled square for each single guide RNA tested, whereas open circles represent a potential off-target site.

[0229] FIG. **4** shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary human hepatocytes.

[0230] FIG. **5** shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary cyno hepatocytes.

[0231] FIG. **6** shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary cyno hepatocytes.

[0232] FIG. **7** shows percent editing (% edit) of TTR and reduction of secreted TTR following administration of the guide in HUH7 cells sequences provided on the x-axis. The values are normalized to the amount of alpha-1-antitrypsin (AAT) protein.

[0233] FIG. **8** shows western blot analysis of intracellular TTR following administration of targeted guides (listed in Table 1) in HUH7 cells.

[0234] FIG. **9** shows percentage liver editing of TTR observed following administration of LNP formulations to mice with humanized (G481-G499) or murine (G282) TTR. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and 3.

[0235] FIGS. **10**A-B show serum TTR levels observed following the dosing regimens indicated on the horizontal axis as μg/ml (FIG. **10**A) or percentage of TSS control (FIG. **10**B). MPK=mg/kg throughout.

[0236] FIGS. **11**A-B show serum TTR levels observed following the dosing regimens indicated on the horizontal axis for 1 mg/kg (FIG. **11**A) or 0.5 mg/kg dosages (FIG. **11**B). Data for a single 2 mg/kg dose is included as the right column in both panels.

[0237] FIGS. **12**A-B show percentage liver editing observed following the dosing regimens indicated on the horizontal axis for 1 mg/kg (FIG. **12**A) or 0.5 mg/kg dosages (FIG. **12**B). FIG. **12**C shows percentage liver editing observed following a single dose at 0.5, 1, or 2 mg/kg.

[0238] FIG. **13** shows percent liver editing observed following administration of LNP formulations to mice humanized with respect to the TTR gene. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and

[0239] FIGS. **14**A-B show that there is correlation between liver editing (FIG. **14**A) and serum human TTR levels (FIG. **14**B) following administration of LNP formulations to mice humanized with respect to the TTR gene. Note: the first three '0's in each Guide ID is omitted from the Figure, for example "G481" is "G000481" in Tables 2 and 3.

[0240] FIGS. **15**A-B show that there is a dose response with respect to percent editing (FIG. **15**A) and serum TTR levels (FIG. **15**B) in wild type mice following administration of LNP formulations comprising guide G502, which is cross homologous between mouse and cyno.

[0241] FIG. **16** shows dose response curves of lipid nanoparticle formulated human TTR specific sgRNAs on primary cyno hepatocytes.

[0242] FIG. **17** shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary human hepatocytes.

[0243] FIG. **18** shows dose response curves of lipid nanoparticle formulated cyno TTR specific sgRNAs on primary cyno hepatocytes.

[0244] FIGS. **19**A-D show serum TTR (% TSS; FIGS. **19**A and **19**C) and editing results following dosing of LNP formulations at the indicated ratios and amounts (FIGS. **19**B and **19**D).

[0245] FIG. **20** shows off-target analysis of certain single guide RNAs in Primary Human Hepatocytes (PHH) targeting TTR. In the graph, filled squares represent the identification of the on-target cut site, while open circles represent the identification of potential off-target sites.

[0246] FIGS. **21**A-B show percent editing on-target (ONT, FIG. **21**A) and at two off-target sites (OT2 and OT4) in primary human hepatocytes following administration of lipid nanoparticle formulated G000480. FIG. **21**B is a re-scaled version of the OT2, OT4, and negative control (Neg Cont) data in FIG. **21**A.

[0247] FIGS. **22**A-B show percent editing on-target (ONT, FIG. **22**A) and at an off-target site (OT4) in primary human hepatocytes following administration of lipid nanoparticle formulated G000486. FIG. **22**B is a re-scaled version of the OT4 and negative control (Neg Cont) data in FIG. **22**A.

[0248] FIGS. **23**A-B show percent editing (FIG. **23**A) and number of insertion and deletion events at the TTR locus (FIG. **23**B). FIG. **23**A shows percent editing at the TTR locus in control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) groups. FIG. **23**B shows the number of insertion and deletion events at the TTR locus when editing was observed in the treatment group of FIG. **23**A.

[0249] FIGS. **24**A-B show TTR levels in circulating serum (FIG. **24**A) and cerebrospinal fluid (CSF) (FIG. **24**B), respectively, in μg/mL for control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) groups. Treatment resulted in >99% knockdown of TTR levels in serum.

[0250] FIGS. **25**A-D show immunohistochemistry images with staining for TTR in stomach (FIG. **25**A), colon (FIG. **25**B), sciatic nerve (FIG. **25**C), and dorsal root ganglion (DRG) (FIG. **25**D) from control and treatment (dosed with lipid nanoparticle formulated TTR specific sgRNA) mice. At right, bar graphs show reduction in TTR staining 8 weeks after treatment in treated mice as measured by percent occupied area for each tissue type.

[0251] FIGS. **26**A-C show liver TTR editing (FIG. **26**A) and serum TTR results (in μg/mL (FIG. **26**B) and as percentage of TSS-treated control (FIG. **26**C)), respectively, from humanized TTR mice dosed with LNP formulations

across a range of doses with guides G000480, G000488, G000489 and G000502 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide.

[0252] FIGS. **27**A-C show liver TTR editing (FIG. **27**A) and serum TTR results (in μg/mL (FIG. **27**B) and as percentage of TSS-treated control (FIG. **27**C)), respectively, from humanized TTR mice dosed with LNP formulations across a range of doses with guides G000481, G000482, G000486 and G000499 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide.

[0253] FIGS. **28**A-C show liver TTR editing (FIG. **28**A) and serum TTR results (in μg/mL (FIG. **28**B) and as percentage of TSS-treated control (FIG. **28**C)), respectively, from humanized TTR mice dosed with LNP formulations across a range of doses with guides G000480, G000481, G000486, G000499 and G000502 and containing Cas9 mRNA (SEQ ID NO: 1) in a 1:2 ratio by weight to the guide.

[0254] FIG. **29** shows relative expression of TTR mRNA in primary human hepatocytes (PHH) after treatment with LNPs comprising Cas9 mRNA and a gRNA as indicated, as compared to negative (untreated) controls.

[0255] FIG. **30** shows relative expression of TTR mRNA in primary human hepatocytes (PHH) after treatment with LNPs comprising Cas9 mRNA and a gRNA as indicated, as compared to negative (untreated) controls.

DETAILED DESCRIPTION

[0256] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention will be described in conjunction with the illustrated embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the invention as defined by the appended claims.

[0257] Before describing the present teachings in detail, it is to be understood that the disclosure is not limited to specific compositions or process steps, as such may vary. It should be noted that, as used in this specification and the appended claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a conjugate" includes a plurality of conjugates and reference to "a cell" includes a plurality of cells and the like.

[0258] Numeric ranges are inclusive of the numbers defining the range. Measured and measurable values are understood to be approximate, taking into account significant digits and the error associated with the measurement. Also, the use of "comprise", "comprises", "comprising", "contain", "contains", "containing", "include", "includes", and "including" are not intended to be limiting. It is to be understood that both the foregoing general description and detailed description are exemplary and explanatory only and are not restrictive of the teachings.

[0259] Unless specifically noted in the above specification, embodiments in the specification that recite "comprising" various components are also contemplated as "consisting of" or "consisting essentially of" the recited components; embodiments in the specification that recite "consisting of" various components are also contemplated as "comprising" or "consisting essentially of" the recited components; and embodiments in the specification that recite "consisting essentially of" various components are also contemplated as "consisting of" or "comprising" the recited components (this interchangeability does not apply to the use of these terms in the claims). The term "or" is used in an inclusive sense, i.e., equivalent to "and/or," unless the context clearly indicates otherwise.

[0260] The section headings used herein are for organizational purposes only and are not to be construed as limiting the desired subject matter in any way. In the event that any material incorporated by reference contradicts any term defined in this specification or any other express content of this specification, this specification controls. While the present teachings are described in conjunction with various embodiments, it is not intended that the present teachings be limited to such embodiments. On the contrary, the present teachings encompass various alternatives, modifications, and equivalents, as will be appreciated by those of skill in the art.

I. Definitions

[0261] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[0262] "Polynucleotide" and "nucleic acid" are used herein to refer to a multimeric compound comprising nucleosides or nucleoside analogs which have nitrogenous heterocyclic bases or base analogs linked together along a backbone, including conventional RNA, DNA, mixed RNA-DNA, and polymers that are analogs thereof. A nucleic acid "backbone" can be made up of a variety of linkages, including one or more of sugar-phosphodiester linkages, peptide-nucleic acid bonds ("peptide nucleic acids" or PNA; PCT No. WO 95/32305), phosphorothioate linkages, methylphosphonate linkages, or combinations thereof. Sugar moieties of a nucleic acid can be ribose, deoxyribose, or similar compounds with substitutions, e.g., 2' methoxy or 2' halide substitutions. Nitrogenous bases can be conventional bases (A, G, C, T, U), analogs thereof (e.g., modified uridines such as 5-methoxyuridine, pseudouridine, or N1-methylpseudouridine, or others); inosine; derivatives of purines or pyrimidines (e.g., N^4-methyl deoxyguanosine, deaza- or aza-purines, deaza- or aza-pyrimidines, pyrimidine bases with substituent groups at the 5 or 6 position (e.g., 5-methylcytosine), purine bases with a substituent at the 2, 6, or 8 positions, 2-amino-6-methylaminopurine, O^6-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O^4-alkyl-pyrimidines; U.S. Pat. No. 5,378,825 and PCT No. WO 93/13121). For general discussion see *The Biochemistry of the Nucleic Acids* 5-36, Adams et al., ed., 11th ed., 1992). Nucleic acids can include one or more "abasic" residues where the backbone includes no nitrogenous base for position(s) of the polymer (U.S. Pat. No. 5,585,481). A nucleic acid can comprise only conventional RNA or DNA sugars, bases and linkages, or can include both conventional components and substitutions (e.g., conventional bases with 2' methoxy linkages, or polymers containing both conventional bases and one or more base analogs). Nucleic acid includes "locked nucleic acid" (LNA), an analogue containing one or more LNA nucleotide monomers with a bicyclic furanose unit locked in an RNA mimicking sugar conformation, which enhance hybridization affinity toward complementary RNA and DNA sequences (Vester and Wengel, 2004, *Biochemistry* 43(42): 13233-41). RNA and DNA have different sugar moieties and

can differ by the presence of uracil or analogs thereof in RNA and thymine or analogs thereof in DNA.

[0263] "Guide RNA", "gRNA", and "guide" are used herein interchangeably to refer to either a crRNA (also known as CRISPR RNA), or the combination of a crRNA and a trRNA (also known as tracrRNA). The crRNA and trRNA may be associated as a single RNA molecule (single guide RNA, sgRNA) or in two separate RNA molecules (dual guide RNA, dgRNA). "Guide RNA" or "gRNA" refers to each type. The trRNA may be a naturally-occurring sequence, or a trRNA sequence with modifications or variations compared to naturally-occurring sequences.

[0264] As used herein, a "guide sequence" refers to a sequence within a guide RNA that is complementary to a target sequence and functions to direct a guide RNA to a target sequence for binding or modification (e.g., cleavage) by an RNA-guided DNA binding agent. A "guide sequence" may also be referred to as a "targeting sequence," or a "spacer sequence." A guide sequence can be 20 base pairs in length, e.g., in the case of *Streptococcus pyogenes* (i.e., Spy Cas9) and related Cas9 homologs/orthologs. Shorter or longer sequences can also be used as guides, e.g., 15-, 16-, 17-, 18-, 19-, 21-, 22-, 23-, 24-, or 25-nucleotides in length. For example, in some embodiments, the guide sequence comprises at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82. In some embodiments, the target sequence is in a gene or on a chromosome, for example, and is complementary to the guide sequence. In some embodiments, the degree of complementarity or identity between a guide sequence and its corresponding target sequence may be about 75%, 80%, 85%, 88%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. For example, in some embodiments, the guide sequence comprises a sequence with about 75%, 80%, 85%, 88%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82. In some embodiments, the guide sequence and the target region may be 100% complementary or identical. In other embodiments, the guide sequence and the target region may contain at least one mismatch. For example, the guide sequence and the target sequence may contain 1, 2, 3, or 4 mismatches, where the total length of the target sequence is at least 17, 18, 19, 20 or more base pairs. In some embodiments, the guide sequence and the target region may contain 1-4 mismatches where the guide sequence comprises at least 17, 18, 19, 20 or more nucleotides. In some embodiments, the guide sequence and the target region may contain 1, 2, 3, or 4 mismatches where the guide sequence comprises 20 nucleotides.

[0265] Target sequences for Cas proteins include both the positive and negative strands of genomic DNA (i.e., the sequence given and the sequence's reverse compliment), as a nucleic acid substrate for a Cas protein is a double stranded nucleic acid. Accordingly, where a guide sequence is said to be "complementary to a target sequence", it is to be understood that the guide sequence may direct a guide RNA to bind to the reverse complement of a target sequence. Thus, in some embodiments, where the guide sequence binds the reverse complement of a target sequence, the guide sequence is identical to certain nucleotides of the target sequence (e.g., the target sequence not including the PAM) except for the substitution of U for T in the guide sequence.

[0266] As used herein, an "RNA-guided DNA binding agent" means a polypeptide or complex of polypeptides having RNA and DNA binding activity, or a DNA-binding subunit of such a complex, wherein the DNA binding activity is sequence-specific and depends on the sequence of the RNA. Exemplary RNA-guided DNA binding agents include Cas cleavases/nickases and inactivated forms thereof ("dCas DNA binding agents"). "Cas nuclease", also called "Cas protein", as used herein, encompasses Cas cleavases, Cas nickases, and dCas DNA binding agents. Cas cleavases/nickases and dCas DNA binding agents include a Csm or Cmr complex of a type III CRISPR system, the Cas10, Csm1, or Cmr2 subunit thereof, a Cascade complex of a type I CRISPR system, the Cas3 subunit thereof, and Class 2 Cas nucleases. As used herein, a "Class 2 Cas nuclease" is a single-chain polypeptide with RNA-guided DNA binding activity, such as a Cas9 nuclease or a Cpf1 nuclease. Class 2 Cas nucleases include Class 2 Cas cleavases and Class 2 Cas nickases (e.g., H840A, D10A, or N863A variants), which further have RNA-guided DNA cleavases or nickase activity, and Class 2 dCas DNA binding agents, in which cleavase/nickase activity is inactivated. Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, C2c3, HF Cas9 (e.g., N497A, R661A, Q695A, Q926A variants), HypaCas9 (e.g., N692A, M694A, Q695A, H698A variants), eSPCas9(1.0) (e.g, K810A, K1003A, R1060A variants), and eSPCas9(1.1) (e.g., K848A, K1003A, R1060A variants) proteins and modifications thereof. Cpf1 protein, Zetsche et al., *Cell,* 163: 1-13 (2015), is homologous to Cas9, and contains a RuvC-like nuclease domain. Cpf1 sequences of Zetsche are incorporated by reference in their entirety. See, e.g., Zetsche, Tables S1 and S3. "Cas9" encompasses Spy Cas9, the variants of Cas9 listed herein, and equivalents thereof. See, e.g., Makarova et al., *Nat Rev Microbiol,* 13(11): 722-36 (2015); Shmakov et al., *Molecular Cell,* 60:385-397 (2015).

[0267] "Modified uridine" is used herein to refer to a nucleoside other than thymidine with the same hydrogen bond acceptors as uridine and one or more structural differences from uridine. In some embodiments, a modified uridine is a substituted uridine, i.e., a uridine in which one or more non-proton substituents (e.g., alkoxy, such as methoxy) takes the place of a proton. In some embodiments, a modified uridine is pseudouridine. In some embodiments, a modified uridine is a substituted pseudouridine, i.e., a pseudouridine in which one or more non-proton substituents (e.g., alkyl, such as methyl) takes the place of a proton. In some embodiments, a modified uridine is any of a substituted uridine, pseudouridine, or a substituted pseudouridine.

[0268] "Uridine position" as used herein refers to a position in a polynucleotide occupied by a uridine or a modified uridine. Thus, for example, a polynucleotide in which "100% of the uridine positions are modified uridines" contains a modified uridine at every position that would be a uridine in a conventional RNA (where all bases are standard A, U, C, or G bases) of the same sequence. Unless otherwise indicated, a U in a polynucleotide sequence of a sequence table or sequence listing in, or accompanying, this disclosure can be a uridine or a modified uridine.

[0269] As used herein, a first sequence is considered to "comprise a sequence with at least X % identity to" a second sequence if an alignment of the first sequence to the second sequence shows that X % or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because

an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenosine for all of thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine or modified guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and parameter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server is generally appropriate.

[0270] "mRNA" is used herein to refer to a polynucleotide that is not DNA and comprises an open reading frame that can be translated into a polypeptide (i.e., can serve as a substrate for translation by a ribosome and amino-acylated tRNAs). mRNA can comprise a phosphate-sugar backbone including ribose residues or analogs thereof, e.g., 2'-methoxy ribose residues. In some embodiments, the sugars of an mRNA phosphate-sugar backbone consist essentially of ribose residues, 2'-methoxy ribose residues, or a combination thereof. In general, mRNAs do not contain a substantial quantity of thymidine residues (e.g., 0 residues or fewer than 30, 20, 10, 5, 4, 3, or 2 thymidine residues; or less than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1% thymidine content). An mRNA can contain modified uridines at some or all of its uridine positions.

[0271] As used herein, the "minimum uridine content" of a given open reading frame (ORF) is the uridine content of an ORF that (a) uses a minimal uridine codon at every position and (b) encodes the same amino acid sequence as the given ORF. The minimal uridine codon(s) for a given amino acid is the codon(s) with the fewest uridines (usually 0 or 1 except for a codon for phenylalanine, where the minimal uridine codon has 2 uridines). Modified uridine residues are considered equivalent to uridines for the purpose of evaluating minimum uridine content.

[0272] As used herein, the "minimum uridine dinucleotide content" of a given open reading frame (ORF) is the lowest possible uridine dinucleotide (UU) content of an ORF that (a) uses a minimal uridine codon (as discussed above) at every position and (b) encodes the same amino acid sequence as the given ORF. The uridine dinucleotide (UU) content can be expressed in absolute terms as the enumeration of UU dinucleotides in an ORF or on a rate basis as the percentage of positions occupied by the uridines of uridine dinucleotides (for example, AUUAU would have a uridine dinucleotide content of 40% because 2 of 5 positions are occupied by the uridines of a uridine dinucleotide). Modified uridine residues are considered equivalent to uridines for the purpose of evaluating minimum uridine dinucleotide content.

[0273] As used herein, "TTR" refers to transthyretin, which is the gene product of a TTR gene.

[0274] As used herein, "amyloid" refers to abnormal aggregates of proteins or peptides that are normally soluble. Amyloids are insoluble, and amyloids can create proteinaceous deposits in organs and tissues. Proteins or peptides in amyloids may be misfolded into a form that allows many copies of the protein to stick together to form fibrils. While some forms of amyloid may have normal functions in the human body, "amyloids" as used herein refers to abnormal or pathologic aggregates of protein. Amyloids may comprise a single protein or peptide, such as TTR, or they may comprise multiple proteins or peptides, such as TTR and additional proteins.

[0275] As used herein, "amyloid fibrils" refers to insoluble fibers of amyloid that are resistant to degradation. Amyloid fibrils can produce symptoms based on the specific protein or peptide and the tissue and cell type in which it has aggregated.

[0276] As used herein, "amyloidosis" refers to a disease characterized by symptoms caused by deposition of amyloid or amyloid fibrils. Amyloidosis can affect numerous organs including the heart, kidney, liver, spleen, nervous system, and digestive track.

[0277] As used herein, "ATTR," "TTR-related amyloidosis," "TTR amyloidosis," "ATTR amyloidosis," or "amyloidosis associated with TTR" refers to amyloidosis associated with deposition of TTR.

[0278] As used herein, "familial amyloid cardiomyopathy" or "FAC" refers to a hereditary transthyretin amyloidosis (ATTR) characterized primarily by restrictive cardiomyopathy. Congestive heart failure is common in FAC. Average age of onset is approximately 60-70 years of age, with an estimated life expectancy of 4-5 years after diagnosis.

[0279] As used herein, "familial amyloid polyneuropathy" or "FAP" refers to a hereditary transthyretin amyloidosis (ATTR) characterized primarily by sensorimotor neuropathy. Autonomic neuropathy is common in FAP. While neuropathy is a primary feature, symptoms of FAP may also include cachexia, renal failure, and cardiac disease. Average age of onset of FAP is approximately 30-50 years of age, with an estimated life expectancy of 5-15 after diagnosis.

[0280] As used herein, "wild-type ATTR" and "ATTRwt" refer to ATTR not associated with a pathological TTR mutation such as T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(−). ATTRwt has also been referred to as senile systemic amyloidosis. Onset typically occurs in men aged 60 or higher with the most common symptoms being congestive heart failure and abnormal heart rhythm such as atrial fibrillation. Additional symptoms include consequences of poor heart function such as shortness of breath, fatigue, dizziness, swelling (especially in the legs), nausea, angina, disrupted sleep, and weight loss. A history of carpal tunnel syndrome indicates increased risk for ATTRwt and may in some cases be indicative of early-stage disease. ATTRwt generally leads to decreasing heart function over time but can have a better prognosis than hereditary ATTR because wild-type TTR deposits accumulate more slowly. Existing treatments are similar to other forms of ATTR (other than liver transplantation) and are generally directed

to supporting or improving heart function, ranging from diuretics and limited fluid and salt intake to anticoagulants, and in severe cases, heart transplants. Nonetheless, like FAC, ATTRwt can result in death from heart failure, sometimes within 3-5 years of diagnosis.

[0281] Guide sequences useful in the guide RNA compositions and methods described herein are shown in Table 1 and throughout the application.

[0282] As used herein, "hereditary ATTR" refers to ATTR that is associated with a mutation in the sequence of the TTR gene. Known mutations in the TTR gene associated with ATTR include those resulting in TTR with substitutions of T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(−).

[0283] As used herein, "indels" refer to insertion/deletion mutations consisting of a number of nucleotides that are either inserted or deleted at the site of double-stranded breaks (DSBs) in a target nucleic acid.

[0284] As used herein, "knockdown" refers to a decrease in expression of a particular gene product (e.g., protein, mRNA, or both). Knockdown of a protein can be measured either by detecting protein secreted by tissue or population of cells (e.g., in serum or cell media) or by detecting total cellular amount of the protein from a tissue or cell population of interest. Methods for measuring knockdown of mRNA are known, and include sequencing of mRNA isolated from a tissue or cell population of interest. In some embodiments, "knockdown" may refer to some loss of expression of a particular gene product, for example a decrease in the amount of mRNA transcribed or a decrease in the amount of protein expressed or secreted by a population of cells (including in vivo populations such as those found in tissues).

[0285] As used herein, "knockout" refers to a loss of expression of a particular protein in a cell. Knockout can be measured either by detecting the amount of protein secretion from a tissue or population of cells (e.g., in serum or cell media) or by detecting total cellular amount of a protein a tissue or a population of cells. In some embodiments, the methods of the disclosure "knockout" TTR in one or more cells (e.g., in a population of cells including in vivo populations such as those found in tissues). In some embodiments, a knockout is not the formation of mutant TTR protein, for example, created by indels, but rather the complete loss of expression of TTR protein in a cell.

[0286] As used herein, "mutant TTR" refers to a gene product of TTR (i.e., the TTR protein) having a change in the amino acid sequence of TTR compared to the wildtype amino acid sequence of TTR. The human wild-type TTR sequence is available at NCBI Gene ID: 7276; Ensembl: Ensembl: ENSG00000118271. Mutants forms of TTR associated with ATTR, e.g., in humans, include T60A, V30M, V30A, V30G, V30L, V122I, V122A, or V122(−).

[0287] As used herein, "mutant TTR" or "mutant TTR allele" refers to a TTR sequence having a change in the nucleotide sequence of TTR compared to the wildtype sequence (NCBI Gene ID: 7276; Ensembl: ENSG00000118271).

[0288] As used herein, "ribonucleoprotein" (RNP) or "RNP complex" refers to a guide RNA together with an RNA-guided DNA binding agent, such as a Cas nuclease, e.g., a Cas cleavase, Cas nickase, or dCas DNA binding agent (e.g., Cas9). In some embodiments, the guide RNA guides the RNA-guided DNA binding agent such as Cas9 to a target sequence, and the guide RNA hybridizes with and the agent binds to the target sequence; in cases where the agent is a cleavase or nickase, binding can be followed by cleaving or nicking.

[0289] As used herein, a "target sequence" refers to a sequence of nucleic acid in a target gene that has complementarity to the guide sequence of the gRNA. The interaction of the target sequence and the guide sequence directs an RNA-guided DNA binding agent to bind, and potentially nick or cleave (depending on the activity of the agent), within the target sequence.

[0290] As used herein, "treatment" refers to any administration or application of a therapeutic for disease or disorder in a subject, and includes inhibiting the disease, arresting its development, relieving one or more symptoms of the disease, curing the disease, or preventing reoccurrence of one or more symptoms of the disease. For example, treatment of ATTR may comprise alleviating symptoms of ATTR.

[0291] "Modified uridine" is used herein to refer to a nucleoside other than thymidine with the same hydrogen bond acceptors as uridine and one or more structural differences from uridine. In some embodiments, a modified uridine is a substituted uridine, i.e., a uridine in which one or more non-proton substituents (e.g., alkoxy, such as methoxy) takes the place of a proton. In some embodiments, a modified uridine is pseudouridine. In some embodiments, a modified uridine is a substituted pseudouridine, i.e., a pseudouridine in which one or more non-proton substituents (e.g., alkyl, such as methyl) takes the place of a proton, e.g., N1-methyl pseudouridine. In some embodiments, a modified uridine is any of a substituted uridine, pseudouridine, or a substituted pseudouridine.

[0292] As used herein, a first sequence is considered to "comprise a sequence with at least X % identity to" a second sequence if an alignment of the first sequence to the second sequence shows that X % or more of the positions of the second sequence in its entirety are matched by the first sequence. For example, the sequence AAGA comprises a sequence with 100% identity to the sequence AAG because an alignment would give 100% identity in that there are matches to all three positions of the second sequence. The differences between RNA and DNA (generally the exchange of uridine for thymidine or vice versa) and the presence of nucleoside analogs such as modified uridines do not contribute to differences in identity or complementarity among polynucleotides as long as the relevant nucleotides (such as thymidine, uridine, or modified uridine) have the same complement (e.g., adenosine for all of thymidine, uridine, or modified uridine; another example is cytosine and 5-methylcytosine, both of which have guanosine as a complement). Thus, for example, the sequence 5'-AXG where X is any modified uridine, such as pseudouridine, N1-methyl pseudouridine, or 5-methoxyuridine, is considered 100% identical to AUG in that both are perfectly complementary to the same sequence (5'-CAU). Exemplary alignment algorithms are the Smith-Waterman and Needleman-Wunsch algorithms, which are well-known in the art. One skilled in the art will understand what choice of algorithm and param-

eter settings are appropriate for a given pair of sequences to be aligned; for sequences of generally similar length and expected identity >50% for amino acids or >75% for nucleotides, the Needleman-Wunsch algorithm with default settings of the Needleman-Wunsch algorithm interface provided by the EBI at the www.ebi.ac.uk web server are generally appropriate.

[0293] The term "about" or "approximately" means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined.

II. Compositions

[0294] A. Compositions Comprising Guide RNA (gRNAs)

[0295] Provided herein are compositions useful for editing the TTR gene, e.g., using a guide RNA with an RNA-guided DNA binding agent (e.g., a CRISPR/Cas system). The compositions may be administered to subjects having wild-type or non-wild type TTR gene sequences, such as, for example, subjects with ATTR, which may be ATTR wt or a hereditary or familial form of ATTR. Guide sequences targeting the TTR gene are shown in Table 1 at SEQ ID Nos: 5-82.

TABLE 1

TTR targeted guide sequences, nomenclature, chromosomal coordinates, and sequence.

SEQ ID No.	Guide ID	Description	Species	Chromosomal Location	Guide Sequences*
5	CR003335	TTR (Exon 1)	Human	chr18:31591917-31591937	CUGCUCCUCCUCUGCCUUGC
6	CR003336	TTR (Exon 1)	Human	chr18:31591922-31591942	CCUCCUCUGCCUUGCUGGAC
7	CR003337	TTR (Exon 1)	Human	chr18:31591925-31591945	CCAGUCCAGCAAGGCAGAGG
8	CR003338	TTR (Exon 1)	Human	chr18:31591928-31591948	AUACCAGUCCAGCAAGGCAG
9	CR003339	TTR (Exon 1)	Human	chr18:31591934-31591954	ACACAAAUACCAGUCCAGCA
10	CR003340	TTR (Exon 1)	Human	chr18:31591937-31591957	UGGACUGGUAUUUGUGUCUG
11	CR003341	TTR (Exon 1)	Human	chr18:31591941-31591961	CUGGUAUUUGUGUCUGAGGC
12	CR003342	TTR (Exon 2)	Human	chr18:31592880-31592900	CUUCUCUACACCCAGGGCAC
13	CR003343	TTR (Exon 2)	Human	chr18:31592902-31592922	CAGAGGACACUUGGAUUCAC
14	CR003344	TTR (Exon 2)	Human	chr18:31592911-31592931	UUUGACCAUCAGAGGACACU
15	CR003345	TTR (Exon 2)	Human	chr18:31592919-31592939	UCUAGAACUUUGACCAUCAG
16	CR003346	TTR (Exon 2)	Human	chr18:31592928-31592948	AAAGUUCUAGAUGCUGUCCG
17	CR003347	TTR (Exon 2)	Human	chr18:31592948-31592968	CAUUGAUGGCAGGACUGCCU
18	CR003348	TTR (Exon 2)	Human	chr18:31592948-31592968	AGGCAGUCCUGCCAUCAAUG
19	CR003349	TTR (Exon 2)	Human	chr18:31592958-31592978	UGCACGGCCACAUUGAUGGC
20	CR003350	TTR (Exon 2)	Human	chr18:31592962-31592982	CACAUGCACGGCCACAUUGA
21	CR003351	TTR (Exon 2)	Human	chr18:31592974-31592994	AGCCUUUCUGAACACAUGCA
22	CR003352	TTR (Exon 2)	Human	chr18:31593006-31593006	GAAAGGCUGCUGAUGACACC

TABLE 1-continued

TTR targeted guide sequences, nomenclature, chromosomal coordinates, and sequence.

SEQ ID No.	Guide ID	De-scription	Species	Chromosomal Location	Guide Sequences*
23	CR003353	TTR (Exon 2)	Human	chr18:31592987-31593007	AAAGGCUGCUGAUGACACCU
24	CR003354	TTR (Exon 2)	Human	chr18:31593003-31593023	ACCUGGGAGCCAUUUGCCUC
25	CR003355	TTR (Exon 2)	Human	chr18:31593007-31593027	CCCAGAGGCAAAUGGCUCCC
26	CR003356	TTR (Exon 2)	Human	chr18:31593015-31593035	GCAACUUACCCAGAGGCAAA
27	CR003357	TTR (Exon 2)	Human	chr18:31593022-31593042	UUCUUUGGCAACUUACCCAG
28	CR003358	TTR (Exon 3)	Human	chr18:31595127-31595147	AUGCAGCUCUCCAGACUCAC
29	CR003359	TTR (Exon 3)	Human	chr18:31595126-31595146	AGUGAGUCUGGAGAGCUGCA
30	CR003360	TTR (Exon 3)	Human	chr18:31595127-31595147	GUGAGUCUGGAGAGCUGCAU
31	CR003361	TTR (Exon 3)	Human	chr18:31595140-31595160	GCUGCAUGGGCUCACAACUG
32	CR003362	TTR (Exon 3)	Human	chr18:31595143-31595163	GCAUGGGCUCACAACUGAGG
33	CR003363	TTR (Exon 3)	Human	chr18:31595156-31595176	ACUGAGGAGGAAUUUGUAGA
34	CR003364	TTR (Exon 3)	Human	chr18:31595157-31595177	CUGAGGAGGAAUUUGUAGAA
35	CR003365	TTR (Exon 3)	Human	chr18:31595170-31595190	UGUAGAAGGGAUAUACAAAG
36	CR003366	TTR (Exon 3)	Human	chr18:31595193-31595213	AAAUAGACACCAAAUCUUAC
37	CR003367	TTR (Exon 3)	Human	chr18:31595197-31595217	AGACACCAAAUCUUACUGGA
38	CR003368	TTR (Exon 3)	Human	chr18:31595205-31595225	AAGUGCCUUCCAGUAAGAUU
39	CR003369	TTR (Exon 3)	Human	chr18:31595235-31595255	CUCUGCAUGCUCAUGGAAUG
40	CR003370	TTR (Exon 3)	Human	chr18:31595236-31595256	CCUCUGCAUGCUCAUGGAAU
41	CR003371	TTR (Exon 3)	Human	chr18:31595237-31595257	ACCUCUGCAUGCUCAUGGAA
42	CR003372	TTR (Exon 3)	Human	chr18:31595242-31595262	UACUCACCUCUGCAUGCUCA
43	CR003373	TTR (Exon 4)	Human	chr18:31598570-31598590	GUAUUCACAGCCAACGACUC
44	CR003374	TTR (Exon 4)	Human	chr18:31598583-31598603	GCGGCGGGGGCCGGAGUCGU
45	CR003375	TTR (Exon 4)	Human	chr18:31598592-31598612	AAUGGUGUAGCGGCGGGGGC

TABLE 1-continued

TTR targeted guide sequences, nomenclature, chromosomal coordinates, and sequence.

SEQ ID No.	Guide ID	De-scription	Species	Chromosomal Location	Guide Sequences*
46	CR003376	TTR (Exon 4)	Human	chr18:31598596-31598616	CGGCAAUGGUGUAGCGGCGG
47	CR003377	TTR (Exon 4)	Human	chr18:31598597-31598617	GCGGCAAUGGUGUAGCGGCG
48	CR003378	TTR (Exon 4)	Human	chr18:31598598-31598618	GGCGGCAAUGGUGUAGCGGC
49	CR003379	TTR (Exon 4)	Human	chr18:31598599-31598619	GGGCGGCAAUGGUGUAGCGG
50	CR003380	TTR (Exon 4)	Human	chr18:31598602-31598622	GCAGGGCGGCAAUGGUGUAG
51	CR003381	TTR (Exon 4)	Human	chr18:31598610-31598630	GGGGCUCAGCAGGGCGGCAA
52	CR003382	TTR (Exon 4)	Human	chr18:31598616-31598636	GGAGUAGGGGCUCAGCAGGG
53	CR003383	TTR (Exon 4)	Human	chr18:31598619-31598639	AUAGGAGUAGGGGCUCAGCA
54	CR003384	TTR (Exon 4)	Human	chr18:31598620-31598640	AAUAGGAGUAGGGGCUCAGC
55	CR003385	TTR (Exon 4)	Human	chr18:31598626-31598646	CCCCUACUCCUAUUCCACCA
56	CR003386	TTR (Exon 4)	Human	chr18:31598629-31598649	CCGUGGUGGAAUAGGAGUAG
57	CR003387	TTR (Exon 4)	Human	chr18:31598630-31598650	GCCGUGGUGGAAUAGGAGUA
58	CR003388	TTR (Exon 4)	Human	chr18:31598637-31598657	GACGACAGCCGUGGUGGAAU
59	CR003389	TTR (Exon 4)	Human	chr18:31598643-31598663	AUUGGUGACGACAGCCGUGG
60	CR003390	TTR (Exon 4)	Human	chr18:31598646-31598666	GGGAUUGGUGACGACAGCCG
61	CR003391	TTR (Exon 4)	Human	chr18:31598647-31598667	GGCUGUCGUCACCAAUCCCA
62	CR003392	TTR (Exon 4)	Human	chr18:31598661-31598681	AGUCCCUCAUUCCUUGGGAU
63	CR005298	TTR (Exon 1)	Human	chr18:31591883-31591903	UCCACUCAUUCUUGGCAGGA
64	CR005299	TTR (Exon 4)	Human	chr18:31598631-31598651	AGCCGUGGUGGAAUAGGAGU
65	CR005300	TTR (Exon 1)	Human	chr18:31591967-31591987	UCACAGAAACACUCACCGUA
66	CR005301	TTR (Exon 1)	Human	chr18:31591968-31591988	GUCACAGAAACACUCACCGU
67	CR005302	TTR (Exon 2)	Human	chr18:31592874-31592894	ACGUGUCUUCUCUACACCCA
68	CR005303	TTR (Exon 2)	Human	chr18:31592903-31592923	UGAAUCCAAGUGUCCUCUGA

TABLE 1-continued

TTR targeted guide sequences, nomenclature, chromosomal coordinates, and sequence.

SEQ ID No.	Guide ID	De-scription	Species	Chromosomal Location	Guide Sequences*
69	CR005304	TTR (Exon 2)	Human	chr18:31592969-31592989	GGCCGUGCAUGUGUUCAGAA
70	CR005305	TTR (Exon 3)	Human	chr18:31595114-31595134	UAUAGGAAAACCAGUGAGUC
71	CR005306	TTR (Exon 3)	Human	chr18:31595204-31595224	AAAUCUUACUGGAAGGCACU
72	CR005307	TTR (Exon 4)	Human	chr18:31598548-31598568	UGUCUGUCUUCUCUCAUAGG
73	CR000689	TTR	Cyno	chr18:50681533-50681553	ACACAAAUACCAGUCCAGCG
74	CR005364	TTR	Cyno	chr18:50680481-50680501	AAAGGCUGCUGAUGAGACCU
75	CR005365	TTR	Cyno	chr18:50680520-50680540	CAUUGACAGCAGGACUGCCU
76	CR005366	TTR	Cyno	chr18:50681539-50681559	AUACCAGUCCAGCGAGGCAG
77	CR005367	TTR	Cyno	chr18:50681542-50681562	CCAGUCCAGCGAGGCAGAGG
78	CR005368	TTR	Cyno	chr18:50681545-50681565	CCUCCUCUGCCUCGCUGGAC
79	CR005369	TTR	Cyno	chr18:50680540-50680560	AAAGUUCUAGAUGCCGUCCG
80	CR005370	TTR	Cyno	chr18:50680594-50680614	ACUUGUCUUCUCUAUACCCA
81	CR005371	TTR	Cyno	chr18:50678216-50678236	AAGUGACUUCCAGUAAGAUU
82	CR005372	TTR	Cyno	chr18:50680482-50680502	AAAAGGCUGCUGAUGAGACC

[0296] Each of the Guide Sequences above may further comprise additional nucleotides to form a crRNA, e.g., with the following exemplary nucleotide sequence following the Guide Sequence at its 3' end: GUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 126). In the case of a sgRNA, the above Guide Sequences may further comprise additional nucleotides to form a sgRNA, e.g., with the following exemplary nucleotide sequence following the 3' end of the Guide Sequence: GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 125) in 5' to 3' orientation.

[0297] In some embodiments, the sgRNA is modified. In some embodiments, the sgRNA comprises the modification pattern shown below in SEQ ID NO: 3, where N is any natural or non-natural nucleotide, and where the totality of the N's comprise a guide sequence as described herein and the modified sgRNA comprises the following sequence: mN*mN*mN*GUUUUAGAmGmCmUmAmGmAmAmAmU mAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAm AmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmGmCmU*mU*mU*mU (SEQ ID NO: 3), where "N" may be any natural or non-natural nucleotide. For example, encompassed herein is SEQ ID NO: 3, where the N's are replaced with any of the guide sequences disclosed herein. The modifications remain as shown in SEQ ID NO: 3 despite the substitution of N's for the nucleotides of a guide. That is, although the nucleotides of the guide replace the "N's", the first three nucleotides are 2'OMe modified and there are phosphorothioate linkages between the first and second nucleotides, the second and third nucleotides and the third and fourth nucleotides.

[0298] In some embodiments, any one of the sequences recited in Table 2 is encompassed.

TABLE 2

TTR targeted sgRNA sequences

SEQ ID No.	Guide ID	Target and Description	Species	Sequence
87	G000480	TTR sgRNA modified sequence	Human	mA*mA*mA*GGCUGCUGAUGACACCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
88	G000481	TTR sgRNA modified sequence	Human	mU*mC*mU*AGAACUUUGACCAUCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
89	G000482	TTR sgRNA modified sequence	Human	mU*mG*mU*AGAAGGGAUAUACAAAGG UUUUAGAmGmCmUmAmGmAmAmAmUm AmGmCAAGUUAAAAUAAGGCUAGUCCG UUAUCAmAmCmUmUmGmAmAmAmAmA mGmUmGmGmCmAmCmCmGmAmGmUmC mGmGmUmGmCmU*mU*mU*mU
90	G000483	TTR sgRNA modified sequence	Human	mU*mC*mC*ACUCAUUCUUGGCAGGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
91	G000484	TTR sgRNA modified sequence	Human	mA*mG*mA*CACCAAAUCUUACUGGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
92	G000485	TTR sgRNA modified sequence	Human	mC*mC*mU*CCUCUGCCUUGCUGGACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
93	G000486	TTR sgRNA modified sequence	Human	mA*mC*mA*CAAAUACCAGUCCAGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
94	G000487	TTR sgRNA modified sequence	Human	mU*mU*mC*UUUGGCAACUUACCCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
95	G000488	TTR sgRNA modified sequence	Human	mA*mA*mA*GUUCUAGAUGCUGUCCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
96	G000489	TTR sgRNA modified sequence	Human	mU*mU*mU*GACCAUCAGAGGACACUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

TABLE 2-continued

TTR targeted sgRNA sequences

SEQ ID No.	Guide ID	Target and Description	Species	Sequence
97	G000490	TTR sgRNA modified sequence	Human	mA*mA*mA*UAGACACCAAAUCUUACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
98	G000491	TTR sgRNA modified sequence	Human	mA*mU*mA*CCAGUCCAGCAAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
99	G000492	TTR sgRNA modified sequence	Human	mC*mU*mU*CUCUACACCCAGGGCACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
100	G000493	TTR sgRNA modified sequence	Human	mA*mA*mG*UGCCUUCCAGUAAGAUUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
101	G000494	TTR sgRNA modified sequence	Human	mG*mU*mG*AGUCUGGAGAGCUGCAUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
102	G000495	TTR sgRNA modified sequence	Human	mC*mA*mG*AGGACACUUGGAUUCACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
103	G000496	TTR sgRNA modified sequence	Human	mG*mG*mC*CGUGCAUGUGUUCAGAAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
104	G000497	TTR sgRNA modified sequence	Human	mC*mU*mG*CUCCUCCUCUGCCUUGCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
105	G000498	TTR sgRNA modified sequence	Human	mA*mG*mU*GAGUCUGGAGAGCUGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
106	G000499	TTR sgRNA modified sequence	Human	mU*mG*mA*AUCCAAGUGUCCUCUGAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
107	G000500	TTR sgRNA	Human	mC*mC*mA*GUCCAGCAAGGCAGAGGGU UUUAGAmGmCmUmAmGmAmAmAmUmA

TABLE 2-continued

TTR targeted sgRNA sequences

SEQ ID No.	Guide ID	Target and Description	Species	Sequence
		modified sequence		mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
108	G000501	TTR sgRNA modified sequence	Human	mU*mC*mA*CAGAAACACUCACCGUAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
109	G000567	TTR sgRNA modified sequence	Human	mG*mA*mA*AGGCUGCUGAUGACACCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
110	G000568	TTR sgRNA modified sequence	Human	mG*mG*mC*UGUCGUCACCAAUCCCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
111	G000570	TTR sgRNA modified sequence	Human	mC*mA*mU*UGAUGGCAGGACUGCCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
112	G000571	TTR sgRNA modified sequence	Human	mG*mU*mC*ACAGAAACACUCACCGUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
113	G000572	TTR sgRNA modified sequence	Human	mC*mC*mC*CUACUCCUAUUCCACCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
114	G000502	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mC*mA*CAAAUACCAGUCCAGCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
115	G000503	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*AGGCUGCUGAUGAGACCGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
116	G000504	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*GGCUGCUGAUGAGACCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

TABLE 2-continued

TTR targeted sgRNA sequences

SEQ ID No.	Guide ID	Target and Description	Species	Sequence
117	G000505	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mA*mU*UGACAGCAGGACUGCCUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
118	G000506	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mU*mA*CCAGUCCAGCGAGGCAGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
119	G000507	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mC*mA*GUCCAGCGAGGCAGAGGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
120	G000508	TTR Cyno specific sgRNA modified sequence	Cyno	mC*mC*mU*CCUCUGCCUCGCUGGACGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
121	G000509	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mA*GUUCUAGAUGCCGUCCGGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
122	G000510	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mC*mU*UGUCUUCUCUAUACCCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
123	G000511	TTR Cyno specific sgRNA modified sequence	Cyno	mA*mA*mG*UGACUUCCAGUAAGAUUGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU
124	G000282	TTR	Mouse	mU*mU*mA*CAGCCACGUCUACAGCAGU UUUAGAmGmCmUmAmGmAmAmAmUmA mGmCAAGUUAAAAUAAGGCUAGUCCGU UAUCAmAmCmUmUmGmAmAmAmAm GmUmGmGmCmAmCmCmGmAmGmUmCm GmGmUmGmCmU*mU*mU*mU

* = PS linkage; 'm' = 2'-O-Me nucleotide

[0299] An alignment mapping of the Guide IDs with the corresponding sgRNA IDs as well as homology to the cyno genome and cyno matched guide IDs are provided in Table 3.

TABLE 3

TTR targeted guide sequence ID mapping and Cyno Homology

Description	Human Dual Guide ID	Human Single Guide ID	Number Mismatches to Cyno Genome	Cyno Matched dgRNA ID	Cyno Matched sgRNA ID
TTR	CR003335	G000497	1		
TTR	CR003336	G000485	1	CR005368	G000508
TTR	CR003337	G000500	1	CR005367	G000507
TTR	CR003338	G000491	1	CR005366	G000506
TTR	CR003339	G000486	1	CR000689	G000502
TTR	CR003340		0		
TTR	CR003341		0		
TTR	CR003342	G000492	no PAM in cyno		
TTR	CR003343	G000495	no PAM in cyno		
TTR	CR003344	G000489	0		
TTR	CR003345	G000481	0		
TTR	CR003346	G000488	1	CR005369	G000509
TTR	CR003347	G000570	2	CR005365	G000505
TTR	CR003348		2		
TTR	CR003349		>3		
TTR	CR003350		no PAM in cyno		
TTR	CR003351		no PAM in cyno		
TTR	CR003352	G000567	2	CR005372	G000503
TTR	CR003353	G000480	1	CR005364	G000504
TTR	CR003354		1		
TTR	CR003355		1		
TTR	CR003356		3		
TTR	CR003357	G000487	>3		
TTR	CR003358		0		
TTR	CR003359	G000498	0		
TTR	CR003360	G000494	0		
TTR	CR003361		0		
TTR	CR003362		0		
TTR	CR003363		0		
TTR	CR003364		0		
TTR	CR003365	G000482	0		
TTR	CR003366	G000490	0		
TTR	CR003367	G000484	no PAM in cyno		
TTR	CR003368	G000493	1	CR005371	G000511
TTR	CR003369		0		
TTR	CR003370		0		
TTR	CR003371		0		
TTR	CR003372		0		
TTR	CR003373		1		
TTR	CR003374		2		
TTR	CR003375		2		
TTR	CR003376		2		
TTR	CR003377		2		
TTR	CR003378		2		
TTR	CR003379		2		
TTR	CR003380		1		
TTR	CR003381		1		
TTR	CR003382		0		
TTR	CR003383		0		
TTR	CR003384		0		
TTR	CR003385	G000572	0		
TTR	CR003386		0		
TTR	CR003387		0		
TTR	CR003388		0		
TTR	CR003389	G000569	0		
TTR	CR003390		0		
TTR	CR003391	G000568	0		
TTR	CR003392		0		
TTR	CR005298	G000483	1		
TTR	CR005299		0		
TTR	CR005300	G000501	no PAM in cyno		
TTR	CR005301	G000571	0		
TTR	CR005302		2	CR005370	G000510
TTR	CR005303	G000499	0		
TTR	CR005304	G000496	>3		
TTR	CR005305		0		
TTR	CR005306		1		
TTR	CR005307		0		

[0300] In some embodiments, the invention provides a composition comprising one or more guide RNA (gRNA) comprising guide sequences that direct an RNA-guided DNA binding agent, which can be a nuclease (e.g., a Cas nuclease such as Cas9), to a target DNA sequence in TTR. The gRNA may comprise a crRNA comprising a guide sequence shown in Table 1. The gRNA may comprise a crRNA comprising 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the gRNA comprises a crRNA comprising a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to at least 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the gRNA comprises a crRNA comprising a sequence with about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a guide sequence shown in Table 1. The gRNA may further comprise a trRNA. In each composition and method embodiment described herein, the crRNA and trRNA may be associated as a single RNA (sgRNA), or may be on separate RNAs (dgRNA). In the context of sgRNAs, the crRNA and trRNA components may be covalently linked, e.g., via a phosphodiester bond or other covalent bond.

[0301] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise two RNA molecules as a "dual guide RNA" or "dgRNA". The dgRNA comprises a first RNA molecule comprising a crRNA comprising, e.g., a guide sequence shown in Table 1, and a second RNA molecule comprising a trRNA. The first and second RNA molecules may not be covalently linked, but may form a RNA duplex via the base pairing between portions of the crRNA and the trRNA.

[0302] In each of the composition, use, and method embodiments described herein, the guide RNA may comprise a single RNA molecule as a "single guide RNA" or "sgRNA". The sgRNA may comprise a crRNA (or a portion thereof) comprising a guide sequence shown in Table 1 covalently linked to a trRNA. The sgRNA may comprise 17, 18, 19, or 20 contiguous nucleotides of a guide sequence shown in Table 1. In some embodiments, the crRNA and the trRNA are covalently linked via a linker. In some embodiments, the sgRNA forms a stem-loop structure via the base pairing between portions of the crRNA and the trRNA. In some embodiments, the crRNA and the trRNA are covalently linked via one or more bonds that are not a phosphodiester bond.

[0303] In some embodiments, the trRNA may comprise all or a portion of a trRNA sequence derived from a naturally-occurring CRISPR/Cas system. In some embodiments, the

trRNA comprises a truncated or modified wild type trRNA. The length of the trRNA depends on the CRISPR/Cas system used. In some embodiments, the trRNA comprises or consists of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more than 100 nucleotides. In some embodiments, the trRNA may comprise certain secondary structures, such as, for example, one or more hairpin or stem-loop structures, or one or more bulge structures.

[0304] In some embodiments, the invention provides a composition comprising one or more guide RNAs comprising a guide sequence of any one of SEQ ID NOs: 5-82.

[0305] In one aspect, the invention provides a composition comprising a gRNA that comprises a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 5-82.

[0306] In other embodiments, the composition comprises at least one, e.g., at least two gRNA's comprising guide sequences selected from any two or more of the guide sequences of SEQ ID NOs: 5-82. In some embodiments, the composition comprises at least two gRNA's that each comprise a guide sequence at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID NOs: 5-82.

[0307] In some embodiments, the gRNA is a sgRNA comprising any one of the sequences shown in Table 2 (SEQ ID Nos. 87-124). In some embodiments, the gRNA is a sgRNA comprising any one of the sequences shown in Table 2 (SEQ ID Nos. 87-124, but without the modifications as shown (i.e., unmodified SEQ ID Nos. 87-124). In some embodiments, the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID Nos. 87-124. In some embodiments, the sgRNA comprises a sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to any of the nucleic acids of SEQ ID Nos. 87-124, but without the modifications as shown (i.e., unmodified SEQ ID Nos. 87-124). In some embodiments, the sgRNA comprises any one of the guide sequences shown in Table 1 in place of the guide sequences shown in the sgRNA sequences of Table 2 at SEQ ID Nos: 87-124, with or without the modifications.

[0308] The guide RNA compositions of the present invention are designed to recognize (e.g., hybridize to) a target sequence in the TTR gene. For example, the TTR target sequence may be recognized and cleaved by a provided Cas cleavase comprising a guide RNA. In some embodiments, an RNA-guided DNA binding agent, such as a Cas cleavase, may be directed by a guide RNA to a target sequence of the TTR gene, where the guide sequence of the guide RNA hybridizes with the target sequence and the RNA-guided DNA binding agent, such as a Cas cleavase, cleaves the target sequence.

[0309] In some embodiments, the selection of the one or more guide RNAs is determined based on target sequences within the TTR gene.

[0310] Without being bound by any particular theory, mutations (e.g., frameshift mutations resulting from indels occurring as a result of a nuclease-mediated DSB) in certain regions of the gene may be less tolerable than mutations in other regions of the gene, thus the location of a DSB is an important factor in the amount or type of protein knockdown that may result. In some embodiments, a gRNA complementary or having complementarity to a target sequence within TTR is used to direct the RNA-guided DNA binding agent to a particular location in the TTR gene. In some embodiments, gRNAs are designed to have guide sequences that are complementary or have complementarity to target sequences in exon 1, exon 2, exon 3, or exon 4 of TTR.

[0311] In some embodiments, the guide sequence is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a target sequence present in the human TTR gene. In some embodiments, the target sequence may be complementary to the guide sequence of the guide RNA. In some embodiments, the degree of complementarity or identity between a guide sequence of a guide RNA and its corresponding target sequence may be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the target sequence and the guide sequence of the gRNA may be 100% complementary or identical. In other embodiments, the target sequence and the guide sequence of the gRNA may contain at least one mismatch. For example, the target sequence and the guide sequence of the gRNA may contain 1, 2, 3, or 4 mismatches, where the total length of the guide sequence is 20. In some embodiments, the target sequence and the guide sequence of the gRNA may contain 1-4 mismatches where the guide sequence is 20 nucleotides.

[0312] In some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered.

[0313] In some embodiments, the RNA-guided DNA-binding agent is a Class 2 Cas nuclease. In some embodiments, the RNA-guided DNA-binding agent has cleavase activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease, such as a Class 2 Cas nuclease (which may be, e.g., a Cas nuclease of Type II, V, or VI). Class 2 Cas nucleases include, for example, Cas9, Cpf1, C2c1, C2c2, and C2c3 proteins and modifications thereof. Examples of Cas9 nucleases include those of the type II CRISPR systems of *S. pyogenes, S. aureus*, and other prokaryotes (see, e.g., the list in the next paragraph), and modified (e.g., engineered or mutant) versions thereof. See, e.g., US2016/0312198 A1; US 2016/0312199 A1. Other examples of Cas nucleases include a Csm or Cmr complex of a type III CRISPR system or the Cas10, Csm1, or Cmr2 subunit thereof; and a Cascade complex of a type I CRISPR system, or the Cas3 subunit thereof. In some embodiments, the Cas nuclease may be from a Type-IIA, Type-IIB, or Type-IIC system. For discussion of various CRISPR systems and Cas nucleases see, e.g., Makarova et al., Nat. Rev. Microbiol. 9:467-477 (2011); Makarova et al., Nat. Rev. Microbiol, 13: 722-36 (2015); Shmakov et al., Molecular Cell, 60:385-397 (2015).

[0314] Non-limiting exemplary species that the Cas nuclease can be derived from include *Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus* sp., *Staphylococcus aureus, Listeria innocua, Lactobacillus gasseri, Francisella novicida, Wolinella succinogenes, Sutterella wadsworthensis, Gammaproteobacterium, Neisseria meningitidis, Campylobacter jejuni, Pasteurella multocida, Fibrobacter succinogene, Rhodospirillum rubrum, Nocardiopsis*

dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Lactobacillus buchneri, Treponema denticola, Microscilla marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas sp., *Crocosphaera watsonii, Cyanothece* sp., *Microcystis aeruginosa, Synechococcus* sp., *Acetohalobium arabaticum, Ammonifex degensii, Caldicelulosiruptor becscii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter* sp., *Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc* sp., *Arthrospira maxima, Arthrospira platensis, Arthrospira* sp., *Lyngbya* sp., *Microcoleus chthonoplastes, Oscillatoria* sp., *Petrotoga mobilis, Thermosipho africanus, Streptococcus pasteurianus, Neisseria cinerea, Campylobacter lari, Parvibaculum lavamentivorans, Corynebacterium diphtheria, Acidaminococcus* sp., *Lachnospiraceae* bacterium ND2006, and *Acaryochloris marina.*

[0315] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Neisseria meningitidis*. In some embodiments, the Cas nuclease is the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Acidaminococcus* sp. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Lachnospiraceae bacterium* ND2006. In further embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella tularensis, Lachnospiraceae bacterium, Butyrivibrio proteoclasticus, Peregrinibacteria bacterium, Parcubacteria bacterium, Smithella, Acidaminococcus, Candidatus Methanoplasma termitum, Eubacterium eligens, Moraxella bovoculi, Leptospira inadai, Porphyromonas crevioricanis, Prevotella disiens*, or *Porphyromonas macacae*. In certain embodiments, the Cas nuclease is a Cpf1 nuclease from an *Acidaminococcus* or Lachnospiraceae.

[0316] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 nuclease comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 nuclease is a wild type Cas9. In some embodiments, the Cas9 is capable of inducing a double strand break in target DNA. In certain embodiments, the Cas nuclease may cleave dsDNA, it may cleave one strand of dsDNA, or it may not have DNA cleavase or nickase activity. An exemplary Cas9 amino acid sequence is provided as SEQ ID NO: 203. An exemplary Cas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 204. An exemplary Cas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 210.

[0317] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as FokI. In some embodiments, a Cas nuclease may be a modified nuclease.

[0318] In other embodiments, the Cas nuclease may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

[0319] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a "nick." In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. See, e.g., U.S. Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain. An exemplary Cas9 nickase amino acid sequence is provided as SEQ ID NO: 206. An exemplary Cas9 nickase mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 207. An exemplary Cas9 nickase mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 211.

[0320] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclease domain. For example, the agent protein may be modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[0321] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015) *Cell* October 22:163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB—A0Q7Q2 (CPF1_FRATN)).

[0322] In some embodiments, an mRNA encoding a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, use of double nicking may improve specificity and reduce off-target effects. In some embodiments, a nickase is used together with two separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[0323] In some embodiments, the RNA-guided DNA-binding agent lacks cleavase and nickase activity. In some embodiments, the RNA-guided DNA-binding agent comprises a dCas DNA-binding polypeptide. A dCas polypeptide has DNA-binding activity while essentially lacking catalytic (cleavase/nickase) activity. In some embodiments, the dCas polypeptide is a dCas9 polypeptide. In some embodiments, the RNA-guided DNA-binding agent lacking cleavase and nickase activity or the dCas DNA-binding polypeptide is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which its endonucleolytic active sites are inactivated, e.g., by one or more alterations (e.g., point mutations) in its catalytic domains. See, e.g., US 2014/0186958 A1; US 2015/0166980 A1. An exemplary dCas9 amino acid sequence is provided as SEQ ID NO: 208. An exemplary dCas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 209. An exemplary dCas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 212.

[0324] In some embodiments, the RNA-guided DNA-binding agent comprises one or more heterologous functional domains (e.g., is or comprises a fusion polypeptide).

[0325] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent into the nucleus of a cell. For example, the heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be linked at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. It may also be inserted within the RNA-guided DNA binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (e.g., two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two SV40 NLS sequences linked at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a monopartite sequence, such as, e.g., the SV40 NLS, PKKKRKV (SEQ ID NO: 274) or PKKKRRV (SEQ ID NO: 275). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 276). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 274) NLS may be linked at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site.

[0326] In some embodiments, the heterologous functional domain may be capable of modifying the intracellular half-life of the RNA-guided DNA binding agent. In some embodiments, the half-life of the RNA-guided DNA binding agent may be increased. In some embodiments, the half-life of the RNA-guided DNA-binding agent may be reduced. In some embodiments, the heterologous functional domain may be capable of increasing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may be capable of reducing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may act as a signal peptide for protein degradation. In some embodiments, the protein degradation may be mediated by proteolytic enzymes, such as, for example, proteasomes, lysosomal proteases, or calpain proteases. In some embodiments, the heterologous functional domain may comprise a PEST sequence. In some embodiments, the RNA-guided DNA-binding agent may be modified by addition of ubiquitin or a polyubiquitin chain. In some embodiments, the ubiquitin may be a ubiquitin-like protein (UBL). Non-limiting examples of ubiquitin-like proteins include small ubiquitin-like modifier (SUMO), ubiquitin cross-reactive protein (UCRP, also known as interferon-stimulated gene-15 (ISG15)), ubiquitin-related modifier-1 (URM1), neuronal-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8, also called Rub 1 in *S. cerevisiae*), human leukocyte antigen F-associated (FAT10), autophagy-8 (ATG8) and -12 (ATG12), Fau ubiquitin-like protein (FUB1), membrane-anchored UBL (MUB), ubiquitin fold-modifier-1 (UFM1), and ubiquitin-like protein-5 (UBLS).

[0327] In some embodiments, the heterologous functional domain may be a marker domain. Non-limiting examples of marker domains include fluorescent proteins, purification tags, epitope tags, and reporter gene sequences. In some embodiments, the marker domain may be a fluorescent protein. Non-limiting examples of suitable fluorescent proteins include green fluorescent proteins (e.g., GFP, GFP-2, tagGFP, turboGFP, sfGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreen1), yellow fluorescent proteins (e.g., YFP, EYFP, Citrine, Venus, YPet, PhiYFP, ZsYellowl), blue fluorescent proteins (e.g., EBFP, EBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire), cyan fluorescent proteins (e.g., ECFP, Cerulean, CyPet, AmCyanl, Midoriishi-Cyan), red fluorescent proteins (e.g., mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRedl, AsRed2, eqFP611, mRasberry, mStrawberry, Jred), and orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato) or any other suitable fluorescent protein. In other embodiments, the marker domain may

be a purification tag and/or an epitope tag. Non-limiting exemplary tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein (MBP), thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AUS, E, ECS, E2, FLAG, HA, nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, 6×His, 8×His, biotin carboxyl carrier protein (BCCP), poly-His, and calmodulin. Non-limiting exemplary reporter genes include glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, or fluorescent proteins.

[0328] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to a specific organelle, cell type, tissue, or organ. In some embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to mitochondria.

[0329] In further embodiments, the heterologous functional domain may be an effector domain. When the RNA-guided DNA-binding agent is directed to its target sequence, e.g., when a Cas nuclease is directed to a target sequence by a gRNA, the effector domain may modify or affect the target sequence. In some embodiments, the effector domain may be chosen from a nucleic acid binding domain, a nuclease domain (e.g., a non-Cas nuclease domain), an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. In some embodiments, the heterologous functional domain is a nuclease, such as a FokI nuclease. See, e.g., U.S. Pat. No. 9,023,649. In some embodiments, the heterologous functional domain is a transcriptional activator or repressor. See, e.g., Qi et al., "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression," Cell 152:1173-83 (2013); Perez-Pinera et al., "RNA-guided gene activation by CRISPR-Cas9-based transcription factors," Nat. Methods 10:973-6 (2013); *Mali* et al., "CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," Nat. Biotechnol. 31:833-8 (2013); Gilbert et al., "CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes," Cell 154:442-51 (2013). As such, the RNA-guided DNA-binding agent essentially becomes a transcription factor that can be directed to bind a desired target sequence using a guide RNA.

[0330] B. Modified gRNAs and mRNAs

[0331] In some embodiments, the gRNA is chemically modified. A gRNA comprising one or more modified nucleosides or nucleotides is called a "modified" gRNA or "chemically modified" gRNA, to describe the presence of one or more non-naturally and/or naturally occurring components or configurations that are used instead of or in addition to the canonical A, G, C, and U residues. In some embodiments, a modified gRNA is synthesized with a non-canonical nucleoside or nucleotide, is here called "modified." Modified nucleosides and nucleotides can include one or more of: (i) alteration, e.g., replacement, of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens in the phosphodiester backbone linkage (an exemplary backbone modification); (ii) alteration, e.g., replacement, of a constituent of the ribose sugar, e.g., of the 2' hydroxyl on the ribose sugar (an exemplary sugar modification); (iii) wholesale replacement of the phosphate moiety with "dephospho" linkers (an exemplary backbone modification); (iv) modification or replacement of a naturally occurring nucleobase, including with a non-canonical nucleobase (an exemplary base modification); (v) replacement or modification of the ribose-phosphate backbone (an exemplary backbone modification); (vi) modification of the 3' end or 5' end of the oligonucleotide, e.g., removal, modification or replacement of a terminal phosphate group or conjugation of a moiety, cap or linker (such 3' or 5' cap modifications may comprise a sugar and/or backbone modification); and (vii) modification or replacement of the sugar (an exemplary sugar modification).

[0332] As noted above, in some embodiments, a composition or formulation disclosed herein comprises an mRNA comprising an open reading frame (ORF) encoding an RNA-guided DNA binding agent, such as a Cas nuclease as described herein. In some embodiments, an mRNA comprising an ORF encoding an RNA-guided DNA binding agent, such as a Cas nuclease, is provided, used, or administered. In some embodiments, the ORF encoding an RNA-guided DNA nuclease is a "modified RNA-guided DNA binding agent ORF" or simply a "modified ORF," which is used as shorthand to indicate that the ORF is modified in one or more of the following ways: (1) the modified ORF has a uridine content ranging from its minimum uridine content to 150% of the minimum uridine content; (2) the modified ORF has a uridine dinucleotide content ranging from its minimum uridine dinucleotide content to 150% of the minimum uridine dinucleotide content; (3) the modified ORF has at least 90% identity to any one of SEQ ID NOs: 201, 204, 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266; (4) the modified ORF consists of a set of codons of which at least 75% of the codons are codons listed in the Table 3A of Minimal Uridine Codons; or (5) the modified ORF comprises at least one modified uridine. In some embodiments, the modified ORF is modified in at least two, three, or four of the foregoing ways. In some embodiments, the modified ORF comprises at least one modified uridine and is modified in at least one, two, three, or all of (1)-(4) above.

TABLE 3A

of Minimal Uridine Codons

	Amino Acid	Minimal uridine codon
A	Alanine	GCA or GCC or GCG
G	Glycine	GGA or GGC or GGG
V	Valine	GUC or GUA or GUG
D	Aspartic acid	GAC
E	Glutamic acid	GAA or GAG
I	Isoleucine	AUC or AUA
T	Threonine	ACA or ACC or ACG
N	Asparagine	AAC
K	Lysine	AAG or AAA
S	Serine	AGC
R	Arginine	AGA or AGG
L	Leucine	CUG or CUA or CUC
P	Proline	CCG or CCA or CCC
H	Histidine	CAC
Q	Glutamine	CAG or CAA
F	Phenylalanine	UUC
Y	Tyrosine	UAC
C	Cysteine	UGC
W	Tryptophan	UGG
M	Methionine	AUG

[0333] In any of the foregoing embodiments, the modified ORF may consist of a set of codons of which at least 75%,

80%, 85%, 90%, 95%, 98%, 99%, or 100% of the codons are codons listed in Table 3A showing Minimal Uridine Codons.

[0334] In any of the foregoing embodiments, the modified ORF may comprise a sequence with at least 90%, 95%, 98%, 99%, or 100% identity to any one of SEQ ID NO: 201, 204, 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[0335] In any of the foregoing embodiments, the modified ORF may have a uridine content ranging from its minimum uridine content to 150%, 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of the minimum uridine content.

[0336] In any of the foregoing embodiments, the modified ORF may have a uridine dinucleotide content ranging from its minimum uridine dinucleotide content to 150%, 145%, 140%, 135%, 130%, 125%, 120%, 115%, 110%, 105%, 104%, 103%, 102%, or 101% of the minimum uridine dinucleotide content.

[0337] In any of the foregoing embodiments, the modified ORF may comprise a modified uridine at least at one, a plurality of, or all uridine positions. In some embodiments, the modified uridine is a uridine modified at the 5 position, e.g., with a halogen, methyl, or ethyl. In some embodiments, the modified uridine is a pseudouridine modified at the 1 position, e.g., with a halogen, methyl, or ethyl. The modified uridine can be, for example, pseudouridine, N1-methyl-pseudouridine, 5-methoxyuridine, 5-iodouridine, or a combination thereof. In some embodiments, the modified uridine is 5-methoxyuridine. In some embodiments, the modified uridine is 5-iodouridine. In some embodiments, the modified uridine is pseudouridine. In some embodiments, the modified uridine is N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of N1-methyl pseudouridine and 5-methoxyuridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and N1-methyl-pseudouridine. In some embodiments, the modified uridine is a combination of pseudouridine and 5-iodouridine. In some embodiments, the modified uridine is a combination of 5-iodouridine and 5-methoxyuridine.

[0338] In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the uridine positions in an mRNA according to the disclosure are modified uridines. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are modified uridines, e.g., 5-methoxyuridine, 5-iodouridine, N1-methyl pseudouridine, pseudouridine, or a combination thereof. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are 5-methoxyuridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are N1-methyl pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are 5-iodouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are 5-methoxyuridine, and the remainder are N1-methyl pseudouridine. In some embodiments, 10%-25%, 15-25%, 25-35%, 35-45%, 45-55%, 55-65%, 65-75%, 75-85%, 85-95%, or 90-100% of the uridine positions in an mRNA according to the disclosure are 5-iodouridine, and the remainder are N1-methyl pseudouridine.

[0339] In some embodiments, the mRNA comprises at least one UTR from an expressed mammalian mRNA, such as a constitutively expressed mRNA. An mRNA is considered constitutively expressed in a mammal if it is continually transcribed in at least one tissue of a healthy adult mammal. In some embodiments, the mRNA comprises a 5' UTR, 3' UTR, or 5' and 3' UTRs from an expressed mammalian RNA, such as a constitutively expressed mammalian mRNA. Actin mRNA is an example of a constitutively expressed mRNA.

[0340] In some embodiments, the mRNA comprises at least one UTR from Hydroxysteroid 17-Beta Dehydrogenase 4 (HSD17B4 or HSD), e.g., a 5' UTR from HSD. In some embodiments, the mRNA comprises at least one UTR from a globin mRNA, for example, human alpha globin (HBA) mRNA, human beta globin (HBB) mRNA, or *Xenopus laevis* beta globin (XBG) mRNA. In some embodiments, the mRNA comprises a 5' UTR, 3' UTR, or 5' and 3' UTRs from a globin mRNA, such as HBA, HBB, or XBG. In some embodiments, the mRNA comprises a 5' UTR from bovine growth hormone, cytomegalovirus (CMV), mouse Hba-a1, HSD, an albumin gene, HBA, HBB, or XBG. In some embodiments, the mRNA comprises a 3' UTR from bovine growth hormone, cytomegalovirus, mouse Hba-a1, HSD, an albumin gene, HBA, HBB, or XBG. In some embodiments, the mRNA comprises 5' and 3' UTRs from bovine growth hormone, cytomegalovirus, mouse Hba-a1, HSD, an albumin gene, HBA, HBB, XBG, heat shock protein 90 (Hsp90), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta-actin, alpha-tubulin, tumor protein (p53), or epidermal growth factor receptor (EGFR).

[0341] In some embodiments, the mRNA comprises 5' and 3' UTRs that are from the same source, e.g., a constitutively expressed mRNA such as actin, albumin, or a globin such as HBA, HBB, or XBG.

[0342] In some embodiments, the mRNA does not comprise a 5' UTR, e.g., there are no additional nucleotides between the 5' cap and the start codon. In some embodiments, the mRNA comprises a Kozak sequence (described below) between the 5' cap and the start codon, but does not have any additional 5' UTR. In some embodiments, the mRNA does not comprise a 3' UTR, e.g., there are no additional nucleotides between the stop codon and the poly-A tail.

[0343] In some embodiments, the mRNA comprises a Kozak sequence. The Kozak sequence can affect translation initiation and the overall yield of a polypeptide translated from an mRNA. A Kozak sequence includes a methionine codon that can function as the start codon. A minimal Kozak sequence is NNNRUGN wherein at least one of the following is true: the first N is A or G and the second N is G. In the context of a nucleotide sequence, R means a purine (A or G). In some embodiments, the Kozak sequence is

RNNRUGN, NNNRUGG, RNNRUGG, RNNAUGN, NNNAUGG, or RNNAUGG. In some embodiments, the Kozak sequence is rccRUGg with zero mismatches or with up to one or two mismatches to positions in lowercase. In some embodiments, the Kozak sequence is rccAUGg with zero mismatches or with up to one or two mismatches to positions in lowercase. In some embodiments, the Kozak sequence is gccRccAUGG (SEQ ID NO: 277) with zero mismatches or with up to one, two, or three mismatches to positions in lowercase. In some embodiments, the Kozak sequence is gccAccAUG with zero mismatches or with up to one, two, three, or four mismatches to positions in lowercase. In some embodiments, the Kozak sequence is GCCACCAUG. In some embodiments, the Kozak sequence is gccgccRccAUGG (SEQ ID NO: 278) with zero mismatches or with up to one, two, three, or four mismatches to positions in lowercase.

[0344] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 1, optionally wherein the ORF of SEQ ID NO: 1 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[0345] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 244, optionally wherein the ORF of SEQ ID NO: 244 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[0346] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 256, optionally wherein the ORF of SEQ ID NO: 256 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[0347] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 257, optionally wherein the ORF of SEQ ID NO: 257 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[0348] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 257, optionally wherein the ORF of SEQ ID NO: 258 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[0349] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 259, optionally wherein the ORF of SEQ ID NO: 259 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

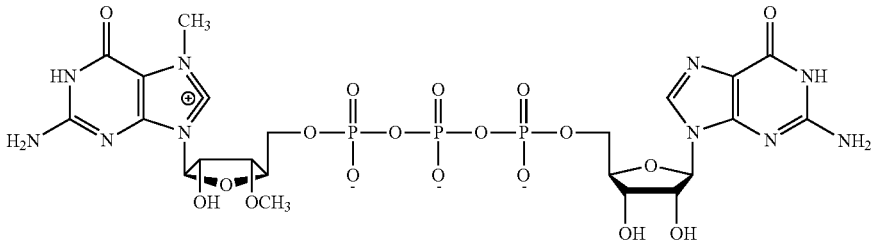
[0350] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 260, optionally wherein the ORF of SEQ ID NO: 260 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

[0351] In some embodiments, the mRNA comprising an ORF encoding an RNA-guided DNA binding agent comprises a sequence having at least 90% identity to SEQ ID NO: 261, optionally wherein the ORF of SEQ ID NO: 261 (i.e., SEQ ID NO: 204) is substituted with an alternative ORF of any one of SEQ ID NO: 210, 214, 215, 223, 224, 250, 252, 254, 265, or 266.

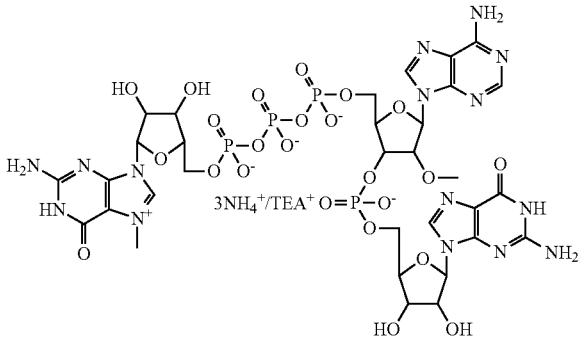
[0352] In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, or 256-261 is 95%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, or 256-261 is 98%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, or 256-261 is 99%. In some embodiments, the degree of identity to the optionally substituted sequences of SEQ ID NOs 243, 244, or 256-261 is 100%.

[0353] In some embodiments, an mRNA disclosed herein comprises a 5' cap, such as a Cap0, Cap1, or Cap2. A 5' cap is generally a 7-methylguanine ribonucleotide (which may be further modified, as discussed below e.g. with respect to ARCA) linked through a 5'-triphosphate to the 5' position of the first nucleotide of the 5'-to-3' chain of the mRNA, i.e., the first cap-proximal nucleotide. In Cap0, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-hydroxyl. In Cap1, the riboses of the first and second transcribed nucleotides of the mRNA comprise a 2'-methoxy and a 2'-hydroxyl, respectively. In Cap2, the riboses of the first and second cap-proximal nucleotides of the mRNA both comprise a 2'-methoxy. See, e.g., Katibah et al. (2014) *Proc Natl Acad Sci USA* 111(33):12025-30; Abbas et al. (2017) *Proc Natl Acad Sci USA* 114(11):E2106-E2115. Most endogenous higher eukaryotic mRNAs, including mammalian mRNAs such as human mRNAs, comprise Cap1 or Cap2. Cap0 and other cap structures differing from Cap1 and Cap2 may be immunogenic in mammals, such as humans, due to recognition as "non-self" by components of the innate immune system such as IFIT-1 and IFIT-5, which can result in elevated cytokine levels including type I interferon. Components of the innate immune system such as IFIT-1 and IFIT-5 may also compete with eIF4E for binding of an mRNA with a cap other than Cap1 or Cap2, potentially inhibiting translation of the mRNA.

[0354] A cap can be included co-transcriptionally. For example, ARCA (anti-reverse cap analog; Thermo Fisher Scientific Cat. No. AM8045) is a cap analog comprising a 7-methylguanine 3'-methoxy-5'-triphosphate linked to the 5' position of a guanine ribonucleotide which can be incorporated in vitro into a transcript at initiation. ARCA results in a Cap0 cap in which the 2' position of the first cap-proximal nucleotide is hydroxyl. See, e.g., Stepinski et al., (2001) "Synthesis and properties of mRNAs containing the novel 'anti-reverse' cap analogs 7-methyl(3'-O-methyl)GpppG and 7-methyl(3'deoxy)GpppG," RNA 7: 1486-1495. The ARCA structure is shown below.



[0355] CleanCap™ AG (m7G(5')ppp(5')(2'OMeA)pG; TriLink Biotechnologies Cat. No. N-7113) or CleanCap™ GG (m7G(5')ppp(5')(2'OMeG)pG; TriLink Biotechnologies Cat. No. N-7133) can be used to provide a Cap1 structure co-transcriptionally. 3'-O-methylated versions of CleanCap™ AG and CleanCap™ GG are also available from TriLink Biotechnologies as Cat. Nos. N-7413 and N-7433, respectively. The CleanCap™ AG structure is shown below.



[0356] Alternatively, a cap can be added to an RNA post-transcriptionally. For example, Vaccinia capping enzyme is commercially available (New England Biolabs Cat. No. M2080S) and has RNA triphosphatase and guanylyltransferase activities, provided by its D1 subunit, and guanine methyltransferase, provided by its D12 subunit. As such, it can add a 7-methylguanine to an RNA, so as to give Cap0, in the presence of S-adenosyl methionine and GTP. See, e.g., Guo, P. and Moss, B. (1990) *Proc. Natl. Acad. Sci. USA* 87, 4023-4027; Mao, X. and Shuman, S. (1994) *J. Biol. Chem.* 269, 24472-24479. For additional discussion of caps and capping approaches, see, e.g., WO2017/053297 and Ishikawa et al., *Nucl. Acids. Symp. Ser.* (2009) No. 53, 129-130.

[0357] In some embodiments, the mRNA further comprises a poly-adenylated (poly-A) tail. In some embodiments, the poly-A tail comprises at least 20, 30, 40, 50, 60, 70, 80, 90, or 100 adenines, optionally up to 300 adenines. In some embodiments, the poly-A tail comprises 95, 96, 97, 98, 99, or 100 adenine nucleotides. In some instances, the poly-A tail is "interrupted" with one or more non-adenine nucleotide "anchors" at one or more locations within the poly-A tail. The poly-A tails may comprise at least 8 consecutive adenine nucleotides, but also comprise one or more non-adenine nucleotide. As used herein, "non-adenine nucleotides" refer to any natural or non-natural nucleotides that do not comprise adenine. Guanine, thymine, and cytosine nucleotides are exemplary non-adenine nucleotides. Thus, the poly-A tails on the mRNA described herein may comprise consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA binding agent or a sequence of interest. In some instances, the poly-A tails on mRNA comprise non-consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA binding agent or a sequence of interest, wherein non-adenine nucleotides interrupt the adenine nucleotides at regular or irregularly spaced intervals.

[0358] In some embodiments, the one or more non-adenine nucleotides are positioned to interrupt the consecutive adenine nucleotides so that a poly(A) binding protein can bind to a stretch of consecutive adenine nucleotides. In some embodiments, one or more non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-100 consecutive adenine nucleotides. In some embodiments, the non-adenine nucleotide is after one, two, three, four, five, six, or seven adenine nucleotides and is followed by at least 8 consecutive adenine nucleotides.

[0359] The poly-A tail may comprise one sequence of consecutive adenine nucleotides followed by one or more non-adenine nucleotides, optionally followed by additional adenine nucleotides.

[0360] In some embodiments, the poly-A tail comprises or contains one non-adenine nucleotide or one consecutive stretch of 2-10 non-adenine nucleotides. In some embodiments, the non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some instances, the one or more non-adenine nucleotides are located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotides are located after at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 consecutive adenine nucleotides.

[0361] In some embodiments, the non-adenine nucleotide is guanine, cytosine, or thymine. In some instances, the non-adenine nucleotide is a guanine nucleotide. In some embodiments, the non-adenine nucleotide is a cytosine nucleotide. In some embodiments, the non-adenine nucleotide is a thymine nucleotide. In some instances, where more than one non-adenine nucleotide is present, the non-adenine nucleotide may be selected from: a) guanine and thymine nucleotides; b) guanine and cytosine nucleotides; c) thymine and cytosine nucleotides; or d) guanine, thymine and cytosine nucleotides. An exemplary poly-A tail comprising non-adenine nucleotides is provided as SEQ ID NO: 4.

[0362] In some embodiments, the mRNA further comprises a poly-adenylated (poly-A) tail. In some instances, the poly-A tail is "interrupted" with one or more non-adenine nucleotide "anchors" at one or more locations within the poly-A tail. The poly-A tails may comprise at least 8 consecutive adenine nucleotides, but also comprise one or more non-adenine nucleotide. As used herein, "non-adenine nucleotides" refer to any natural or non-natural nucleotides that do not comprise adenine. Guanine, thymine, and cytosine nucleotides are exemplary non-adenine nucleotides. Thus, the poly-A tails on the mRNA described herein may comprise consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA-binding agent or a sequence of interest. In some instances, the poly-A tails on mRNA comprise non-consecutive adenine nucleotides located 3' to nucleotides encoding an RNA-guided DNA-binding agent or a sequence of interest, wherein non-adenine nucleotides interrupt the adenine nucleotides at regular or irregularly spaced intervals.

[0363] In some embodiments, the one or more non-adenine nucleotides are positioned to interrupt the consecutive adenine nucleotides so that a poly(A) binding protein can bind to a stretch of consecutive adenine nucleotides. In some embodiments, one or more non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotide is located after at least 8-100 consecutive adenine nucleotides. In some embodiments, the non-adenine nucleotide is after one, two, three, four, five, six, or seven adenine nucleotides and is followed by at least 8 consecutive adenine nucleotides.

[0364] The poly-A tail of the present invention may comprise one sequence of consecutive adenine nucleotides followed by one or more non-adenine nucleotides, optionally followed by additional adenine nucleotides.

[0365] In some embodiments, the poly-A tail comprises or contains one non-adenine nucleotide or one consecutive stretch of 2-10 non-adenine nucleotides. In some embodiments, the non-adenine nucleotide(s) is located after at least 8, 9, 10, 11, or 12 consecutive adenine nucleotides. In some instances, the one or more non-adenine nucleotides are located after at least 8-50 consecutive adenine nucleotides. In some embodiments, the one or more non-adenine nucleotides are located after at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 consecutive adenine nucleotides.

[0366] In some embodiments, the non-adenine nucleotide is guanine, cytosine, or thymine. In some instances, the non-adenine nucleotide is a guanine nucleotide. In some embodiments, the non-adenine nucleotide is a cytosine nucleotide. In some embodiments, the non-adenine nucleotide is a thymine nucleotide. In some instances, where more than one non-adenine nucleotide is present, the non-adenine nucleotide may be selected from: a) guanine and thymine nucleotides; b) guanine and cytosine nucleotides; c) thymine and cytosine nucleotides; or d) guanine, thymine and cytosine nucleotides. An exemplary poly-A tail comprising non-adenine nucleotides is provided as SEQ ID NO: 4:

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAA.

[0367] Chemical modifications such as those listed above can be combined to provide modified gRNAs and/or mRNAs comprising nucleosides and nucleotides (collectively "residues") that can have two, three, four, or more modifications. For example, a modified residue can have a modified sugar and a modified nucleobase. In some embodiments, every base of a gRNA is modified, e.g., all bases have a modified phosphate group, such as a phosphorothioate group. In certain embodiments, all, or substantially all, of the phosphate groups of an gRNA molecule are replaced with phosphorothioate groups. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 5' end of the RNA. In some embodiments, modified gRNAs comprise at least one modified residue at or near the 3' end of the RNA.

[0368] In some embodiments, the gRNA comprises one, two, three or more modified residues. In some embodiments, at least 5% (e.g., at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%) of the positions in a modified gRNA are modified nucleosides or nucleotides.

[0369] Unmodified nucleic acids can be prone to degradation by, e.g., intracellular nucleases or those found in serum. For example, nucleases can hydrolyze nucleic acid phosphodiester bonds. Accordingly, in one aspect the gRNAs described herein can contain one or more modified nucleosides or nucleotides, e.g., to introduce stability toward intracellular or serum-based nucleases. In some embodiments, the modified gRNA molecules described herein can exhibit a reduced innate immune response when introduced into a population of cells, both in vivo and ex vivo. The term "innate immune response" includes a cellular response to exogenous nucleic acids, including single stranded nucleic acids, which involves the induction of cytokine expression and release, particularly the interferons, and cell death.

[0370] In some embodiments of a backbone modification, the phosphate group of a modified residue can be modified by replacing one or more of the oxygens with a different substituent. Further, the modified residue, e.g., modified residue present in a modified nucleic acid, can include the wholesale replacement of an unmodified phosphate moiety with a modified phosphate group as described herein. In some embodiments, the backbone modification of the phosphate backbone can include alterations that result in either an uncharged linker or a charged linker with unsymmetrical charge distribution.

[0371] Examples of modified phosphate groups include, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. The phosphorous atom in an unmodified phosphate group is achiral. However, replacement of one of the non-bridging oxygens with one of the above atoms or groups of atoms can render the phosphorous atom chiral. The stereogenic phosphorous atom can possess either the "R" configuration (herein Rp) or the "S" configuration (herein Sp). The backbone can also be modified by replacement of a bridging

oxygen, (i.e., the oxygen that links the phosphate to the nucleoside), with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylenephosphonates). The replacement can occur at either linking oxygen or at both of the linking oxygens.

[0372] The phosphate group can be replaced by non-phosphorus containing connectors in certain backbone modifications. In some embodiments, the charged phosphate group can be replaced by a neutral moiety. Examples of moieties which can replace the phosphate group can include, without limitation, e.g., methyl phosphonate, hydroxylamino, siloxane, carbonate, carboxymethyl, carbamate, amide, thioether, ethylene oxide linker, sulfonate, sulfonamide, thioformacetal, formacetal, oxime, methyleneimino, methylenemethylimino, methylenehydrazo, methylenedimethylhydrazo and methyleneoxymethylimino.

[0373] Scaffolds that can mimic nucleic acids can also be constructed wherein the phosphate linker and ribose sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates. Such modifications may comprise backbone and sugar modifications. In some embodiments, the nucleobases can be tethered by a surrogate backbone. Examples can include, without limitation, the morpholino, cyclobutyl, pyrrolidine and peptide nucleic acid (PNA) nucleoside surrogates.

[0374] The modified nucleosides and modified nucleotides can include one or more modifications to the sugar group, i.e. at sugar modification. For example, the 2' hydroxyl group (OH) can be modified, e.g. replaced with a number of different "oxy" or "deoxy" substituents. In some embodiments, modifications to the 2' hydroxyl group can enhance the stability of the nucleic acid since the hydroxyl can no longer be deprotonated to form a 2'-alkoxide ion.

[0375] Examples of 2' hydroxyl group modifications can include alkoxy or aryloxy (OR, wherein "R" can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or a sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$ wherein R can be, e.g., H or optionally substituted alkyl, and n can be an integer from 0 to 20 (e.g., from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20). In some embodiments, the 2' hydroxyl group modification can be 2'-O-Me. In some embodiments, the 2' hydroxyl group modification can be a 2'-fluoro modification, which replaces the 2' hydroxyl group with a fluoride. In some embodiments, the 2' hydroxyl group modification can include "locked" nucleic acids (LNA) in which the 2' hydroxyl can be connected, e.g., by a C_{1-6} alkylene or C_{1-6} heteroalkylene bridge, to the 4' carbon of the same ribose sugar, where exemplary bridges can include methylene, propylene, ether, or amino bridges; O-amino (wherein amino can be, e.g., NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino) and aminoalkoxy, $O(CH_2)_n$-amino, (wherein amino can be, e.g., NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroarylamino, ethylenediamine, or polyamino). In some embodiments, the 2' hydroxyl group modification can included "unlocked" nucleic acids (UNA) in which the ribose ring lacks the C2'-C3' bond. In some embodiments, the 2' hydroxyl group modification can include the methoxyethyl group (MOE), ($OCH_2CH_2OCH_3$, e.g., a PEG derivative).

[0376] "Deoxy" 2' modifications can include hydrogen (i.e. deoxyribose sugars, e.g., at the overhang portions of partially dsRNA); halo (e.g., bromo, chloro, fluoro, or iodo); amino (wherein amino can be, e.g., NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, diheteroarylamino, or amino acid); $NH(CH_2CH_2NH)_nCH_2CH_2$— amino (wherein amino can be, e.g., as described herein), —NHC(O)R (wherein R can be, e.g., alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar), cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; and alkyl, cycloalkyl, aryl, alkenyl and alkynyl, which may be optionally substituted with e.g., an amino as described herein.

[0377] The sugar modification can comprise a sugar group which may also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, a modified nucleic acid can include nucleotides containing e.g., arabinose, as the sugar. The modified nucleic acids can also include abasic sugars. These abasic sugars can also be further modified at one or more of the constituent sugar atoms. The modified nucleic acids can also include one or more sugars that are in the L form, e.g. L-nucleosides.

[0378] The modified nucleosides and modified nucleotides described herein, which can be incorporated into a modified nucleic acid, can include a modified base, also called a nucleobase. Examples of nucleobases include, but are not limited to, adenine (A), guanine (G), cytosine (C), and uracil (U). These nucleobases can be modified or wholly replaced to provide modified residues that can be incorporated into modified nucleic acids. The nucleobase of the nucleotide can be independently selected from a purine, a pyrimidine, a purine analog, or pyrimidine analog. In some embodiments, the nucleobase can include, for example, naturally-occurring and synthetic derivatives of a base.

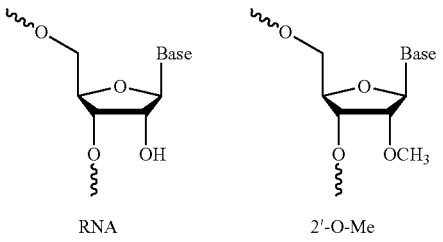
[0379] In embodiments employing a dual guide RNA, each of the crRNA and the tracr RNA can contain modifications. Such modifications may be at one or both ends of the crRNA and/or tracr RNA. In embodiments comprising an sgRNA, one or more residues at one or both ends of the sgRNA may be chemically modified, or the entire sgRNA may be chemically modified. Certain embodiments comprise a 5' end modification. Certain embodiments comprise a 3' end modification. In certain embodiments, one or more or all of the nucleotides in single stranded overhang of a guide RNA molecule are deoxynucleotides.

[0380] In some embodiments, the guide RNAs disclosed herein comprise one of the modification patterns disclosed in U.S. 62/431,756, filed Dec. 8, 2016, titled "Chemically Modified Guide RNAs," the contents of which are hereby incorporated by reference in their entirety.

[0381] In some embodiments, the invention comprises a gRNA comprising one or more modifications. In some embodiments, the modification comprises a 2'-O-methyl (2'-O-Me) modified nucleotide. In some embodiments, the modification comprises a phosphorothioate (PS) bond between nucleotides.

[0382] The terms "mA," "mC," "mU," or "mG" may be used to denote a nucleotide that has been modified with 2'-O-Me.

[0383] Modification of 2'-O-methyl can be depicted as follows:

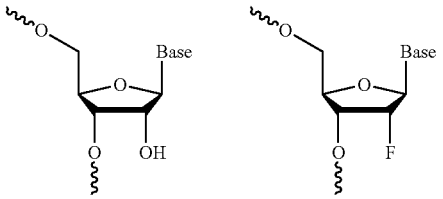


RNA 2′-O-Me

[0384] Another chemical modification that has been shown to influence nucleotide sugar rings is halogen substitution. For example, 2'-fluoro (2'-F) substitution on nucleotide sugar rings can increase oligonucleotide binding affinity and nuclease stability.

[0385] In this application, the terms "fA," "fC," "fU," or "fG" may be used to denote a nucleotide that has been substituted with 2'-F.

[0386] Substitution of 2'-F can be depicted as follows:



RNA
Natural composition of RNA

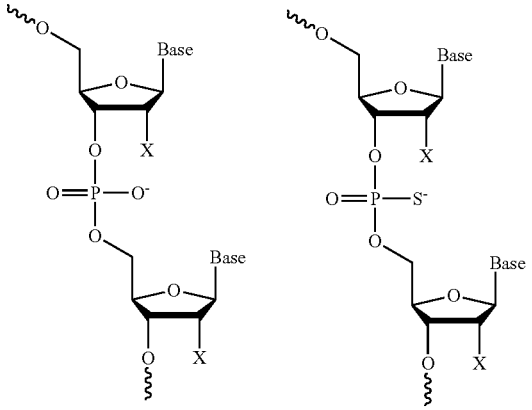
2′F-RNA
2′F substitution

[0387] Phosphorothioate (PS) linkage or bond refers to a bond where a sulfur is substituted for one nonbridging phosphate oxygen in a phosphodiester linkage, for example in the bonds between nucleotides bases. When phosphorothioates are used to generate oligonucleotides, the modified oligonucleotides may also be referred to as S-oligos.

[0388] A "*" may be used to depict a PS modification. In this application, the terms A*, C*, U*, or G* may be used to denote a nucleotide that is linked to the next (e.g., 3') nucleotide with a PS bond.

[0389] In this application, the terms "mA*," "mC*," "mU*," or "mG*" may be used to denote a nucleotide that has been substituted with 2'-O-Me and that is linked to the next (e.g., 3') nucleotide with a PS bond.

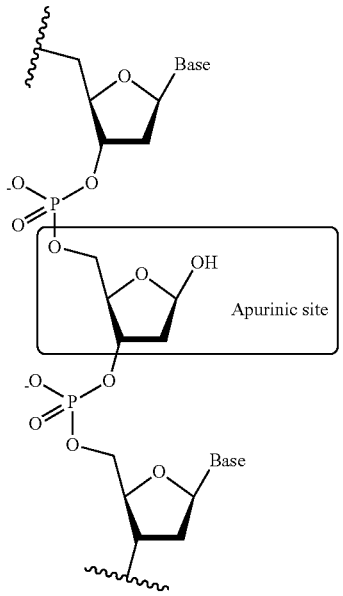
[0390] The diagram below shows the substitution of S— into a nonbridging phosphate oxygen, generating a PS bond in lieu of a phosphodiester bond:



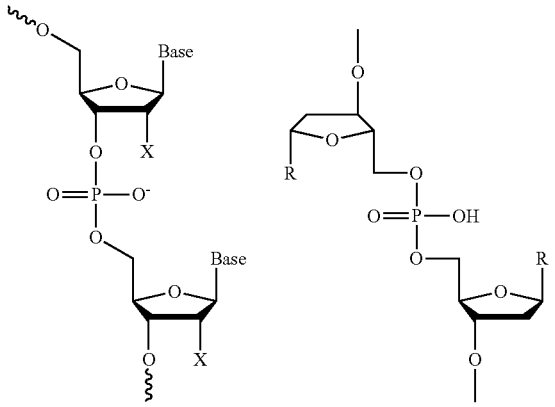
Phosphodiester
Natural phosphodiester linkage of RNA

Phosphorothioate (PS)
Modified phosphorothioate (PS) bond

[0391] Abasic nucleotides refer to those which lack nitrogenous bases. The figure below depicts an oligonucleotide with an abasic (also known as apurinic) site that lacks a base:



[0392] Inverted bases refer to those with linkages that are inverted from the normal 5' to 3' linkage (i.e., either a 5' to 5' linkage or a 3' to 3' linkage). For example:



[0393] An abasic nucleotide can be attached with an inverted linkage. For example, an abasic nucleotide may be attached to the terminal 5' nucleotide via a 5' to 5' linkage, or an abasic nucleotide may be attached to the terminal 3' nucleotide via a 3' to 3' linkage. An inverted abasic nucleotide at either the terminal 5' or 3' nucleotide may also be called an inverted abasic end cap.

[0394] In some embodiments, one or more of the first three, four, or five nucleotides at the 5' terminus, and one or more of the last three, four, or five nucleotides at the 3' terminus are modified. In some embodiments, the modification is a 2'-O-Me, 2'-F, inverted abasic nucleotide, PS bond, or other nucleotide modification well known in the art to increase stability and/or performance.

[0395] In some embodiments, the first four nucleotides at the 5' terminus, and the last four nucleotides at the 3' terminus are linked with phosphorothioate (PS) bonds.

[0396] In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-O-methyl (2'-O-Me) modified nucleotide. In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise a 2'-fluoro (2'-F) modified nucleotide. In some embodiments, the first three nucleotides at the 5' terminus, and the last three nucleotides at the 3' terminus comprise an inverted abasic nucleotide.

[0397] In some embodiments, the guide RNA comprises a modified sgRNA. In some embodiments, the sgRNA comprises the modification pattern shown in SEQ ID No: 3, where N is any natural or non-natural nucleotide, and where the totality of the N's comprise a guide sequence that directs a nuclease to a target sequence.

[0398] In some embodiments, the guide RNA comprises a sgRNA shown in any one of SEQ ID No: 87-124. In some embodiments, the guide RNA comprises a sgRNA comprising any one of the guide sequences of SEQ ID No: 5-82 and the nucleotides of SEQ ID No: 125, wherein the nucleotides of SEQ ID No: 125 are on the 3' end of the guide sequence, and wherein the guide sequence may be modified as shown in SEQ ID No: 3.

[0399] C. Ribonucleoprotein Complex

[0400] In some embodiments, a composition is encompassed comprising one or more gRNAs comprising one or more guide sequences from Table 1 or one or more sgRNAs from Table 2 and an RNA-guided DNA binding agent, e.g., a nuclease, such as a Cas nuclease, such as Cas9. In some embodiments, the encoded RNA-guided DNA-binding agent has cleavase activity, which can also be referred to as double-strand endonuclease activity. In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nuclease. Examples of Cas9 nucleases include those of the type II CRISPR systems of *S. pyogenes, S. aureus*, and other prokaryotes (see, e.g., the list in the next paragraph), and modified (e.g., engineered or mutant) versions thereof. See, e.g., US2016/0312198 A1; US 2016/0312199 A1. Other examples of Cas nucleases include a Csm or Cmr complex of a type III CRISPR system or the Cas10, Csm1, or Cmr2 subunit thereof; and a Cascade complex of a type I CRISPR system, or the Cas3 subunit thereof. In some embodiments, the Cas nuclease may be from a Type-IIA, Type-IIB, or Type-IIC system. For discussion of various CRISPR systems and Cas nucleases see, e.g., Makarova et al., Nat. Rev. Microbiol. 9:467-477 (2011); Makarova et al., Nat. Rev. Microbiol, 13: 722-36 (2015); Shmakov et al., Molecular Cell, 60:385-397 (2015).

[0401] Non-limiting exemplary species that the Cas nuclease can be derived from include *Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus* sp., *Staphylococcus aureus, Listeria innocua, Lactobacillus gasseri, Francisella novicida, Wolinella succinogenes, Sutterella wadsworthensis, Gammaproteobacterium, Neisseria meningitidis, Campylobacter jejuni, Pasteurella multocida, Fibrobacter succinogene, Rhodospirillum rubrum, Nocardiopsis dassonvillei, Streptomyces pristinaespiralis, Streptomyces viridochromogenes, Streptomyces viridochromogenes, Streptosporangium roseum, Streptosporangium roseum, Alicyclobacillus acidocaldarius, Bacillus pseudomycoides, Bacillus selenitireducens, Exiguobacterium sibiricum, Lactobacillus delbrueckii, Lactobacillus salivarius, Lactobacillus buchneri, Treponema denticola, Microscilla marina, Burkholderiales bacterium, Polaromonas naphthalenivorans, Polaromonas* sp., *Crocosphaera watsonii, Cyanothece* sp., *Microcystis aeruginosa, Synechococcus* sp., *Acetohalobium arabaticum, Ammonifex degensii, Caldicelulosiruptor becscii, Candidatus Desulforudis, Clostridium botulinum, Clostridium difficile, Finegoldia magna, Natranaerobius thermophilus, Pelotomaculum thermopropionicum, Acidithiobacillus caldus, Acidithiobacillus ferrooxidans, Allochromatium vinosum, Marinobacter* sp., *Nitrosococcus halophilus, Nitrosococcus watsoni, Pseudoalteromonas haloplanktis, Ktedonobacter racemifer, Methanohalobium evestigatum, Anabaena variabilis, Nodularia spumigena, Nostoc* sp., *Arthrospira maxima, Arthrospira platensis, Arthrospira* sp., *Lyngbya* sp., *Microcoleus chthonoplastes, Oscillatoria* sp., *Petrotoga mobilis, Thermosipho africanus, Streptococcus pasteurianus, Neisseria cinerea, Campylobacter lari, Parvibaculum lavamentivorans, Corynebacterium diphtheria, Acidaminococcus* sp., *Lachnospiraceae bacterium* ND2006, and *Acaryochloris marina.*

[0402] In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus pyogenes*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Streptococcus thermophilus*. In some embodiments, the Cas nuclease is the Cas9 nuclease from *Neisseria meningitidis*. In some embodiments, the Cas nuclease is the Cas9 nuclease is from *Staphylococcus aureus*. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella novicida*. In some embodiments, the Cas nuclease is the Cpf1 nuclease

from *Acidaminococcus* sp. In some embodiments, the Cas nuclease is the Cpf1 nuclease from *Lachnospiraceae bacterium* ND2006. In further embodiments, the Cas nuclease is the Cpf1 nuclease from *Francisella tularensis, Lachnospiraceae bacterium, Butyrivibrio proteoclasticus, Peregrinibacteria bacterium, Parcubacteria bacterium, Smithella, Acidaminococcus, Candidatus Methanoplasma termitum, Eubacterium eligens, Moraxella bovoculi, Leptospira inadai, Porphyromonas crevioricanis, Prevotella disiens*, or *Porphyromonas macacae*. In certain embodiments, the Cas nuclease is a Cpf1 nuclease from an *Acidaminococcus* or Lachnospiraceae.

[0403] In some embodiments, the gRNA together with an RNA-guided DNA binding agent is called a ribonucleoprotein complex (RNP). In some embodiments, the RNA-guided DNA binding agent is a Cas nuclease. In some embodiments, the gRNA together with a Cas nuclease is called a Cas RNP. In some embodiments, the RNP comprises Type-I, Type-II, or Type-III components. In some embodiments, the Cas nuclease is the Cas9 protein from the Type-II CRISPR/Cas system. In some embodiment, the gRNA together with Cas9 is called a Cas9 RNP.

[0404] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 protein comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 protein is a wild type Cas9. In each of the composition, use, and method embodiments, the Cas induces a double strand break in target DNA.

[0405] Wild type Cas9 has two nuclease domains: RuvC and HNH. The RuvC domain cleaves the non-target DNA strand, and the HNH domain cleaves the target strand of DNA. In some embodiments, the Cas9 nuclease comprises more than one RuvC domain and/or more than one HNH domain. In some embodiments, the Cas9 nuclease is a wild type Cas9. In some embodiments, the Cas9 is capable of inducing a double strand break in target DNA. In certain embodiments, the Cas nuclease may cleave dsDNA, it may cleave one strand of dsDNA, or it may not have DNA cleavase or nickase activity. An exemplary Cas9 amino acid sequence is provided as SEQ ID NO: 203. An exemplary Cas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 204. An exemplary Cas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 210.

[0406] In some embodiments, chimeric Cas nucleases are used, where one domain or region of the protein is replaced by a portion of a different protein. In some embodiments, a Cas nuclease domain may be replaced with a domain from a different nuclease such as FokI. In some embodiments, a Cas nuclease may be a modified nuclease.

[0407] In other embodiments, the Cas nuclease may be from a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a component of the Cascade complex of a Type-I CRISPR/Cas system. In some embodiments, the Cas nuclease may be a Cas3 protein. In some embodiments, the Cas nuclease may be from a Type-III CRISPR/Cas system. In some embodiments, the Cas nuclease may have an RNA cleavage activity.

[0408] In some embodiments, the RNA-guided DNA-binding agent has single-strand nickase activity, i.e., can cut one DNA strand to produce a single-strand break, also known as a "nick." In some embodiments, the RNA-guided DNA-binding agent comprises a Cas nickase. A nickase is an enzyme that creates a nick in dsDNA, i.e., cuts one strand but not the other of the DNA double helix. In some embodiments, a Cas nickase is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which an endonucleolytic active site is inactivated, e.g., by one or more alterations (e.g., point mutations) in a catalytic domain. See, e.g., U.S. Pat. No. 8,889,356 for discussion of Cas nickases and exemplary catalytic domain alterations. In some embodiments, a Cas nickase such as a Cas9 nickase has an inactivated RuvC or HNH domain. An exemplary Cas9 nickase amino acid sequence is provided as SEQ ID NO: 206. An exemplary Cas9 nickase mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 207. An exemplary Cas9 nickase mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 211.

[0409] In some embodiments, the RNA-guided DNA-binding agent is modified to contain only one functional nuclease domain. For example, the agent protein may be modified such that one of the nuclease domains is mutated or fully or partially deleted to reduce its nucleic acid cleavage activity. In some embodiments, a nickase is used having a RuvC domain with reduced activity. In some embodiments, a nickase is used having an inactive RuvC domain. In some embodiments, a nickase is used having an HNH domain with reduced activity. In some embodiments, a nickase is used having an inactive HNH domain.

[0410] In some embodiments, a conserved amino acid within a Cas protein nuclease domain is substituted to reduce or alter nuclease activity. In some embodiments, a Cas nuclease may comprise an amino acid substitution in the RuvC or RuvC-like nuclease domain. Exemplary amino acid substitutions in the RuvC or RuvC-like nuclease domain include D10A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015) Cell October 22:163(3): 759-771. In some embodiments, the Cas nuclease may comprise an amino acid substitution in the HNH or HNH-like nuclease domain. Exemplary amino acid substitutions in the HNH or HNH-like nuclease domain include E762A, H840A, N863A, H983A, and D986A (based on the *S. pyogenes* Cas9 protein). See, e.g., Zetsche et al. (2015). Further exemplary amino acid substitutions include D917A, E1006A, and D1255A (based on the *Francisella novicida* U112 Cpf1 (FnCpf1) sequence (UniProtKB—A0Q7Q2 (CPF1_FRATN)).

[0411] In some embodiments, an mRNA encoding a nickase is provided in combination with a pair of guide RNAs that are complementary to the sense and antisense strands of the target sequence, respectively. In this embodiment, the guide RNAs direct the nickase to a target sequence and introduce a DSB by generating a nick on opposite strands of the target sequence (i.e., double nicking). In some embodiments, use of double nicking may improve specificity and reduce off-target effects. In some embodiments, a nickase is used together with two separate guide RNAs targeting opposite strands of DNA to produce a double nick in the target DNA. In some embodiments, a nickase is used together with two separate guide RNAs that are selected to be in close proximity to produce a double nick in the target DNA.

[0412] In some embodiments, the RNA-guided DNA-binding agent lacks cleavase and nickase activity. In some

embodiments, the RNA-guided DNA-binding agent comprises a dCas DNA-binding polypeptide. A dCas polypeptide has DNA-binding activity while essentially lacking catalytic (cleavase/nickase) activity. In some embodiments, the dCas polypeptide is a dCas9 polypeptide. In some embodiments, the RNA-guided DNA-binding agent lacking cleavase and nickase activity or the dCas DNA-binding polypeptide is a version of a Cas nuclease (e.g., a Cas nuclease discussed above) in which its endonucleolytic active sites are inactivated, e.g., by one or more alterations (e.g., point mutations) in its catalytic domains. See, e.g., US 2014/0186958 A1; US 2015/0166980 A1. An exemplary dCas9 amino acid sequence is provided as SEQ ID NO: 208. An exemplary Cas9 mRNA ORF sequence, which includes start and stop codons, is provided as SEQ ID NO: 209. An exemplary Cas9 mRNA coding sequence, suitable for inclusion in a fusion protein, is provided as SEQ ID NO: 212.

[0413] In some embodiments, the RNA-guided DNA-binding agent comprises one or more heterologous functional domains (e.g., is or comprises a fusion polypeptide).

[0414] In some embodiments, the heterologous functional domain may facilitate transport of the RNA-guided DNA-binding agent into the nucleus of a cell. For example, the heterologous functional domain may be a nuclear localization signal (NLS). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-10 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with 1-5 NLS(s). In some embodiments, the RNA-guided DNA-binding agent may be fused with one NLS. Where one NLS is used, the NLS may be linked at the N-terminus or the C-terminus of the RNA-guided DNA-binding agent sequence. In some embodiments, the RNA-guided DNA-binding agent may be fused C-terminally to at least one NLS. An NLS may also be inserted within the RNA-guided DNA binding agent sequence. In other embodiments, the RNA-guided DNA-binding agent may be fused with more than one NLS. In some embodiments, the RNA-guided DNA-binding agent may be fused with 2, 3, 4, or 5 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs. In certain circumstances, the two NLSs may be the same (e.g., two SV40 NLSs) or different. In some embodiments, the RNA-guided DNA-binding agent is fused to two SV40 NLS sequences linked at the carboxy terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with two NLSs, one linked at the N-terminus and one at the C-terminus. In some embodiments, the RNA-guided DNA-binding agent may be fused with 3 NLSs. In some embodiments, the RNA-guided DNA-binding agent may be fused with no NLS. In some embodiments, the NLS may be a monopartite sequence, such as, e.g., the SV40 NLS, PKKKRKV (SEQ ID NO: 274) or PKKKRRV (SEQ ID NO: 275). In some embodiments, the NLS may be a bipartite sequence, such as the NLS of nucleoplasmin, KRPAATKKAGQAKKKK (SEQ ID NO: 276). In a specific embodiment, a single PKKKRKV (SEQ ID NO: 274) NLS may be linked at the C-terminus of the RNA-guided DNA-binding agent. One or more linkers are optionally included at the fusion site. In some embodiments, one or more NLS(s) according to any of the foregoing embodiments are present in the RNA-guided DNA-binding agent in combination with one or more additional heterologous functional domains, such as any of the heterologous functional domains described below.

[0415] In some embodiments, the heterologous functional domain may be capable of modifying the intracellular half-life of the RNA-guided DNA binding agent. In some embodiments, the half-life of the RNA-guided DNA binding agent may be increased. In some embodiments, the half-life of the RNA-guided DNA-binding agent may be reduced. In some embodiments, the heterologous functional domain may be capable of increasing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may be capable of reducing the stability of the RNA-guided DNA-binding agent. In some embodiments, the heterologous functional domain may act as a signal peptide for protein degradation. In some embodiments, the protein degradation may be mediated by proteolytic enzymes, such as, for example, proteasomes, lysosomal proteases, or calpain proteases. In some embodiments, the heterologous functional domain may comprise a PEST sequence. In some embodiments, the RNA-guided DNA-binding agent may be modified by addition of ubiquitin or a polyubiquitin chain. In some embodiments, the ubiquitin may be a ubiquitin-like protein (UBL). Non-limiting examples of ubiquitin-like proteins include small ubiquitin-like modifier (SUMO), ubiquitin cross-reactive protein (UCRP, also known as interferon-stimulated gene-15 (ISG15)), ubiquitin-related modifier-1 (URM1), neuronal-precursor-cell-expressed developmentally downregulated protein-8 (NEDD8, also called Rub 1 in *S. cerevisiae*), human leukocyte antigen F-associated (FAT10), autophagy-8 (ATG8) and -12 (ATG12), Fau ubiquitin-like protein (FUB1), membrane-anchored UBL (MUB), ubiquitin fold-modifier-1 (UFM1), and ubiquitin-like protein-5 (UBLS).

[0416] In some embodiments, the heterologous functional domain may be a marker domain. Non-limiting examples of marker domains include fluorescent proteins, purification tags, epitope tags, and reporter gene sequences. In some embodiments, the marker domain may be a fluorescent protein. Non-limiting examples of suitable fluorescent proteins include green fluorescent proteins (e.g., GFP, GFP-2, tagGFP, turboGFP, sfGFP, EGFP, Emerald, Azami Green, Monomeric Azami Green, CopGFP, AceGFP, ZsGreen1), yellow fluorescent proteins (e.g., YFP, EYFP, Citrine, Venus, YPet, PhiYFP, ZsYellowl), blue fluorescent proteins (e.g., EBFP, EBFP2, Azurite, mKalamal, GFPuv, Sapphire, T-sapphire), cyan fluorescent proteins (e.g., ECFP, Cerulean, CyPet, AmCyanl, Midoriishi-Cyan), red fluorescent proteins (e.g., mKate, mKate2, mPlum, DsRed monomer, mCherry, mRFP1, DsRed-Express, DsRed2, DsRed-Monomer, HcRed-Tandem, HcRedl, AsRed2, eqFP611, mRasberry, mStrawberry, Jred), and orange fluorescent proteins (mOrange, mKO, Kusabira-Orange, Monomeric Kusabira-Orange, mTangerine, tdTomato) or any other suitable fluorescent protein. In other embodiments, the marker domain may be a purification tag and/or an epitope tag. Non-limiting exemplary tags include glutathione-S-transferase (GST), chitin binding protein (CBP), maltose binding protein (MBP), thioredoxin (TRX), poly(NANP), tandem affinity purification (TAP) tag, myc, AcV5, AU1, AUS, E, ECS, E2, FLAG, HA, nus, Softag 1, Softag 3, Strep, SBP, Glu-Glu, HSV, KT3, S, S1, T7, V5, VSV-G, 6×His, 8×His, biotin carboxyl carrier protein (BCCP), poly-His, and calmodulin. Non-limiting exemplary reporter genes include glutathione-S-transferase (GST), horseradish peroxidase (HRP),

chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, or fluorescent proteins.

[0417] In additional embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to a specific organelle, cell type, tissue, or organ. In some embodiments, the heterologous functional domain may target the RNA-guided DNA-binding agent to mitochondria.

[0418] In further embodiments, the heterologous functional domain may be an effector domain. When the RNA-guided DNA-binding agent is directed to its target sequence, e.g., when a Cas nuclease is directed to a target sequence by a gRNA, the effector domain may modify or affect the target sequence. In some embodiments, the effector domain may be chosen from a nucleic acid binding domain, a nuclease domain (e.g., a non-Cas nuclease domain), an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain. In some embodiments, the heterologous functional domain is a nuclease, such as a FokI nuclease. See, e.g., U.S. Pat. No. 9,023,649. In some embodiments, the heterologous functional domain is a transcriptional activator or repressor. See, e.g., Qi et al., "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression," Cell 152:1173-83 (2013); Perez-Pinera et al., "RNA-guided gene activation by CRISPR-Cas9-based transcription factors," Nat. Methods 10:973-6 (2013); *Mali* et al., "CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," Nat. Biotechnol. 31:833-8 (2013); Gilbert et al., "CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes," Cell 154:442-51 (2013). As such, the RNA-guided DNA-binding agent essentially becomes a transcription factor that can be directed to bind a desired target sequence using a guide RNA.

[0419] D. Determination of Efficacy of gRNAs

[0420] In some embodiments, the efficacy of a gRNA is determined when delivered or expressed together with other components forming an RNP. In some embodiments, the gRNA is expressed together with an RNA-guided DNA nuclease, such as a Cas protein. In some embodiments, the gRNA is delivered to or expressed in a cell line that already stably expresses an RNA-guided DNA nuclease, such as a Cas protein. In some embodiments the gRNA is delivered to a cell as part of a RNP. In some embodiments, the gRNA is delivered to a cell along with a mRNA encoding an RNA-guided DNA nuclease, such as a Cas nuclease.

[0421] As described herein, use of an RNA-guided DNA nuclease and a guide RNA disclosed herein can lead to double-stranded breaks in the DNA which can produce errors in the form of insertion/deletion (indel) mutations upon repair by cellular machinery. Many mutations due to indels alter the reading frame or introduce premature stop codons and, therefore, produce a non-functional protein.

[0422] In some embodiments, the efficacy of particular gRNAs is determined based on in vitro models. In some embodiments, the in vitro model is HEK293 cells stably expressing Cas9 (HEK293_Cas9). In some embodiments, the in vitro model is HUH7 human hepatocarcinoma cells. In some embodiments, the in vitro model is HepG2 cells. In some embodiments, the in vitro model is primary human hepatocytes. In some embodiments, the in vitro model is primary cynomolgus hepatocytes. With respect to using primary human hepatocytes, commercially available primary human hepatocytes can be used to provide greater consistency between experiments. In some embodiments, the number of off-target sites at which a deletion or insertion occurs in an in vitro model (e.g., in primary human hepatocytes) is determined, e.g., by analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA and the guide RNA. In some embodiments, such a determination comprises analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide. Exemplary procedures for such determinations are provided in the working examples below.

[0423] In some embodiments, the efficacy of particular gRNAs is determined across multiple in vitro cell models for a gRNA selection process. In some embodiments, a cell line comparison of data with selected gRNAs is performed. In some embodiments, cross screening in multiple cell models is performed.

[0424] In some embodiments, the efficacy of particular gRNAs is determined based on in vivo models. In some embodiments, the in vivo model is a rodent model. In some embodiments, the rodent model is a mouse which expresses a human TTR gene, which may be a mutant human TTR gene. In some embodiments, the in vivo model is a non-human primate, for example cynomolgus monkey.

[0425] In some embodiments, the efficacy of a guide RNA is measured by percent editing of TTR. In some embodiments, the percent editing of TTR is compared to the percent editing necessary to achieve knockdown of TTR protein, e.g., in the cell culture media in the case of an in vitro model or in serum or tissue in the case of an in vivo model.

[0426] In some embodiments, the efficacy of a guide RNA is measured by the number and/or frequency of indels at off-target sequences within the genome of the target cell type. In some embodiments, efficacious guide RNAs are provided which produce indels at off target sites at very low frequencies (e.g., <5%) in a cell population and/or relative to the frequency of indel creation at the target site. Thus, the disclosure provides for guide RNAs which do not exhibit off-target indel formation in the target cell type (e.g., a hepatocyte), or which produce a frequency of off-target indel formation of <5% in a cell population and/or relative to the frequency of indel creation at the target site. In some embodiments, the disclosure provides guide RNAs which do not exhibit any off target indel formation in the target cell type (e.g., hepatocyte). In some embodiments, guide RNAs are provided which produce indels at less than 5 off-target sites, e.g., as evaluated by one or more methods described herein. In some embodiments, guide RNAs are provided which produce indels at less than or equal to 4, 3, 2, or 1 off-target site(s) e.g., as evaluated by one or more methods described herein. In some embodiments, the off-target site(s) does not occur in a protein coding region in the target cell (e.g., hepatocyte) genome.

[0427] In some embodiments, detecting gene editing events, such as the formation of insertion/deletion ("indel") mutations and homology directed repair (HDR) events in target DNA utilize linear amplification with a tagged primer and isolating the tagged amplification products (herein after referred to as "LAM-PCR," or "Linear Amplification (LA)" method).

[0428] In some embodiments, the method comprises isolating cellular DNA from a cell that has been induced to have a double strand break (DSB) and optionally that has been

provided with an HDR template to repair the DSB; performing at least one cycle of linear amplification of the DNA with a tagged primer; isolating the linear amplification products that comprise tag, thereby discarding any amplification product that was amplified with a non-tagged primer; optionally further amplifying the isolated products; and analyzing the linear amplification products, or the further amplified products, to determine the presence or absence of an editing event such as, for example, a double strand break, an insertion, deletion, or HDR template sequence in the target DNA. In some instances, the editing event can be quantified. Quantification and the like as used herein (including in the context of HDR and non-HDR editing events such as indels) includes detecting the frequency and/or type(s) of editing events in a population.

[0429] In some embodiments, only one cycle of linear amplification is conducted.

[0430] In some instances, the tagged primer comprises a molecular barcode. In some embodiments, the tagged primer comprises a molecular barcode, and only one cycle of linear amplification is conducted.

[0431] In some embodiments, the analyzing step comprises sequencing the linear amplified products or the further amplified products. Sequencing may comprise any method known to those of skill in the art, including, next generation sequencing, and cloning the linear amplification products or further amplified products into a plasmid and sequencing the plasmid or a portion of the plasmid. In other aspects, the analyzing step comprises performing digital PCR (dPCR) or droplet digital PCR (ddPCR) on the linear amplified products or the further amplified products. In other instances, the analyzing step comprises contacting the linear amplified products or the further amplified products with a nucleic acid probe designed to identify DNA comprising HDR template sequence and detecting the probes that have bound to the linear amplified product(s) or further amplified product(s). In some embodiments, the method further comprises determining the location of the HDR template in the target DNA.

[0432] In certain embodiments, the method further comprises determining the sequence of an insertion site in the target DNA, wherein the insertion site is the location where the HDR template incorporates into the target DNA, and wherein the insertion site may include some target DNA sequence and some HDR template sequence.

[0433] In some embodiments, the linear amplification of the target DNA with a tagged primer is performed for 1-50 cycles, 1-60 cycles, 1-70 cycles, 1-80 cycles, 1-90 cycles, or 1-100 cycles.

[0434] In some embodiments, the linear amplification of the target DNA with a tagged primer comprises a denaturation step to separate DNA duplexes, an annealing step to allow primer binding, and an elongation step. In some embodiments, the linear amplification is isothermal (does not require a change in temperature). In some embodiments, the isothermal linear amplification is a loop-mediated isothermal amplification (LAMP), a strand displacement amplification (SDA), a helicase-dependent amplification, or a nicking enzyme amplification reaction.

[0435] In some embodiments, the tagged primer anneals to the target DNA at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 210, at least 220, at least 230, at least 240, at least 250, at least 260, at least 270, at least 280, at least 290, at least 300, at least 1,000, at least 5,000, or at least 10,000 nucleotides away from of the expected editing event location, e.g., the insertion, deletion, or template insertion site.

[0436] In some embodiments, the tagged primer comprises a molecular barcode. In some embodiments, the molecular barcode comprises a sequence that is not complementary to the target DNA. In some embodiments, the molecular barcode comprises 6, 8, 10, or 12 nucleotides.

[0437] In some embodiments, the tag on the primer is biotin, streptavidin, digoxigenin, a DNA sequence, or fluorescein isothiocyanate (FITC).

[0438] In some embodiments, the linear amplification product(s) are isolated using a capture reagent specific for the tag on the primer. In some embodiments, the capture reagent is on a bead, solid support, matrix, or column. In some embodiments, the isolation step comprises contacting the linear amplification product(s) with a capture reagent specific for the tag on the primer. In some embodiments, the capture reagent is biotin, streptavidin, digoxigenin, a DNA sequence, or fluorescein isothiocyanate (FITC).

[0439] In some embodiments, the tag is biotin and capture reagent is streptavidin. In some embodiments, the tag is streptavidin and the capture reagent is biotin. In some embodiments, the tag is on the 5' terminus of the primer, the 3' terminus of the primer, or internal to the primer. In some embodiments, the tag and/or the capture reagent is removed after the isolation step. In some embodiments, the tag and/or the capture reagent is not removed, and the further amplifying and analyzing steps are performed in the presence of tag and/or capture.

[0440] In some embodiments, the further amplification is non-linear. In some embodiments, the further amplification is digital PCR, qPCR, or RT-PCR. In some embodiments, the sequencing is next generation sequencing (NGS).

[0441] In some embodiments, the target DNA is genomic or mitochondrial. In some embodiments, the target DNA is genomic DNA of a prokaryotic or eukaryotic cell. In some embodiments, the target DNA is mammalian. The target DNA may be from a non-dividing cell or a dividing cell. In some embodiments, the target DNA may be from a primary cell. In some embodiments, the target DNA is from a replicating cell.

[0442] In some instances, the cellular DNA is sheared prior to linear amplification. In some embodiments, the sheared DNA has an average size between 0.5 kb and 20 kb. In some instances, the cellular DNA is sheared to an average size of 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, 4.0, 4.25, 4.5, 4.75, 5.0, 5.25, 5.5, 5.75, 6.0, 6.25, 6.5, 6.75, 7.0, 7.25, 7.5, 7.75, 8.0, 8.25, 8.5, 8.75, 9.0, 9.25, 9.5, 9.75, 10.0, 10.25, 10.5, 10.75, 11.0, 11.25, 11.5, 11.75, 12.0, 12.25, 12.5, 12.75, 13.0, 13.25, 13.5, 13.75, 14.0, 14.25, 14.5, 14.75, 15.0, 15.25, 15.5, 15.75, 16.0, 16.25, 16.5, 16.75, 17.0, 17.25, 17.5, 17.75, 18.0, 18.25, 18.5, 18.75, 19.0, 19.25, 19.5, 19.75, or 20.0 kb. In some instances, the cellular DNA is sheared to an average size of about 1.5 kb.

[0443] In some embodiments, the efficacy of a guide RNA is measured by secretion of TTR. In some embodiments, secretion of TTR is measured using an enzyme-linked immunosorbent assay (ELISA) assay with cell culture media or serum. In some embodiments, secretion of TTR is measured in the same in vitro or in vivo systems or models used

to measure editing. In some embodiments, secretion of TTR is measured in primary human hepatocytes. In some embodiments, secretion of TTR is measured in HUH7 cells. In some embodiments, secretion of TTR is measured in HepG2 cells.

[0444] ELISA assays are generally known to the skilled artisan and can be designed to determine serum TTR levels. In one exemplary embodiment, blood is collected and the serum is isolated. The total TTR serum levels may be determined using a Mouse Prealbumin (Transthyretin) ELISA Kit (Aviva Systems Biology, Cat. OKIA00111) or similar kit for measuring human TTR. If no kit is available, an ELISA can be developed using plates that are pre-coated with capture antibody specific for the TTR one is measuring. The plate is next incubated at room temperature for a period of time before washing. Enzyme-anti-TTR antibody conjugate is added and inncubated. Unbound antibody conjugate is removed and the plate washed before the addition of the chromogenic substrate solution that reactes with the enzyme. The plate is read on an appropriate plate reader at an absorbance specific for the enzyme and substrate used.

[0445] In some embodiments, the amount of TTR in cells (including those from tissue) measures efficacy of a gRNA. In some embodiments, the amount of TTR in cells is measured using western blot. In some embodiments, the cell used is HUH7 cells. In some embodiments, the cell used is a primary human hepatocyte. In some embodiments, the cell used is a primar cell obtained from an animal. In some embodiments, the amount of TTR is compared to the amount of glyceraldehyde 3-phosphate dehydrogenase GAPDH (a housekeeping gene) to control for changes in cell number.

III. LNP Formulations and Treatment of ATTR

[0446] In some embodiments, a method of inducing a double-stranded break (DSB) within the TTR gene is provided comprising administering a composition comprising a guide RNA comprising any one or more guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID Nos: 5-82 are administered to induce a DSB in the TTR gene. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0447] In some embodiments, a method of modifying the TTR gene is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID Nos: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124, are administered to modify the TTR gene. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0448] In some embodiments, a method of treating ATTR is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to treat ATTR. The guide RNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0449] In some embodiments, a method of reducing TTR serum concentration is provided comprising administering a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82 or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to reduce or prevent the accumulation of TTR in amyloids or amyloid fibrils. The gRNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0450] In some embodiments, a method of reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject is provided comprising administering a composition comprising a guide RNA comprising any one or more of the guide sequences of SEQ ID NOs: 5-82, or any one or more of the sgRNAs of SEQ ID Nos: 87-124. In some embodiments, a method of reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject is provided comprising administering a composition comprising any one or more of the sgRNAs of SEQ ID Nos: 87-113. In some embodiments, gRNAs comprising any one or more of the guide sequences of SEQ ID NOs: 5-82 or any one or more of the sgRNAs of SEQ ID Nos: 87-124 are administered to reduce or prevent the accumulation of TTR in amyloids or amyloid fibrils. The gRNAs may be administered together with an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9) or an mRNA or vector encoding an RNA-guided DNA nuclease such as a Cas nuclease (e.g., Cas9).

[0451] In some embodiments, the gRNAs comprising the guide sequences of Table 1 or one or more sgRNAs from Table 2 together with an RNA-guided DNA nuclease such as a Cas nuclease induce DSBs, and non-homologous ending joining (NHEJ) during repair leads to a mutation in the TTR gene. In some embodiments, NHEJ leads to a deletion or insertion of a nucleotide(s), which induces a frame shift or nonsense mutation in the TTR gene.

[0452] In some embodiments, administering the guide RNAs of the invention (e.g., in a composition provided herein) reduces levels (e.g., serum levels) of TTR in the subject, and therefore prevents accumulation and aggregation of TTR in amyloids or amyloid fibrils.

[0453] In some embodiments, reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject comprises reducing or preventing TTR deposition in one or more tissues of the subject, such as stomach, colon, or nervous tissue. In some embodiments, the nervous tissue comprises sciatic nerve or dorsal root ganglion. In some embodiments, TTR deposition is reduced in two, three, or four of the stomach, colon, dorsal root ganglion, and sciatic nerve. The level of deposition in a given tissue can be determined using a biopsy sample, e.g., using immunostaining. In some embodiments, reducing or preventing the accumulation of TTR in amyloids or amyloid fibrils of a subject and/or reducing or preventing TTR deposition is

inferred based on reducing serum TTR levels for a period of time. As discussed in the examples, it has been found that reducing serum TTR levels in accordance with methods and uses provided herein can result in clearance of deposited TTR from tissues such as those discussed above and in the examples, e.g., as measured 8 weeks after administration of the composition.

[0454] In some embodiments, the subject is mammalian. In some embodiments, the subject is human. In some embodiments, the subject is cow, pig, monkey, sheep, dog, cat, fish, or poultry.

[0455] In some embodiments, the use of a guide RNAs comprising any one or more of the guide sequences in Table 1 or one or more sgRNAs from Table 2 (e.g., in a composition provided herein) is provided for the preparation of a medicament for treating a human subject having ATTR.

[0456] In some embodiments, the guide RNAs, compositions, and formulations are administered intravenously. In some embodiments, the guide RNAs, compositions, and formulations are administered into the hepatic circulation.

[0457] In some embodiments, a single administration of a composition comprising a guide RNA provided herein is sufficient to knock down expression of the mutant protein. In some embodiments, a single administration of a composition comprising a guide RNA provided herein is sufficient to knock out expression of the mutant protein in a population of cells. In other embodiments, more than one administration of a composition comprising a guide RNA provided herein may be beneficial to maximize editing via cumulative effects.

[0458] For example, a composition provided herein can be administered 2, 3, 4, 5, or more times, such as 2 times. Administrations can be separated by a period of time ranging from, e.g., 1 day to 2 years, such as 1 to 7 days, 7 to 14 days, 14 days to 30 days, 30 days to 60 days, 60 days to 120 days, 120 days to 183 days, 183 days to 274 days, 274 days to 366 days, or 366 days to 2 years.

[0459] In some embodiments, a composition is administered in an effective amount in the range of 0.01 to 10 mg/kg (mpk), e.g., 0.01 to 0.1 mpk, 0.1 to 0.3 mpk, 0.3 to 0.5 mpk, 0.5 to 1 mpk, 1 to 2 mpk, 2 to 3 mpk, 3 to 5 mpk, 5 to 10 mpk, or 0.1, 0.2, 0.3, 0.5, 1, 2, 3, 5, or 10 mpk.

[0460] In some embodiments, the efficacy of treatment with the compositions of the invention is seen at 1 year, 2 years, 3 years, 4 years, 5 years, or 10 years after delivery. In some embodiments, efficacy of treatment with the compositions of the invention is assessed by measuring serum levels of TTR before and after treatment. In some embodiments, efficacy of treatment with the compositions assessed via a reduction of serum levels of TTR is seen at 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, or at 11 months.

[0461] In some embodiments, treatment slows or halts disease progression.

[0462] In some embodiments, treatment slows or halts progression of FAP. In some embodiments, treatment results in improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy or autonomic neuropathy.

[0463] In some embodiments, treatment results in improvement, stabilization, or slowing of change in symptoms of FAC. In some embodiments, treatment results in improvement, stabilization, or slowing of change symptoms of restrictive cardiomyopathy or congestive heart failure.

[0464] In some embodiments, efficacy of treatment is measured by increased survival time of the subject.

[0465] In some embodiments, efficacy of treatment is measured by improvement or slowing of progression in symptoms of sensorimotor or autonomic neuropathy. In some embodiments, efficacy of treatment is measured by an increase or a slowing of decrease in ability to move an area of the body or to feel in any area of the body. In some embodiments, efficacy of treatment is measured by improvement or a slowing of decrease in the ability to swallow; breath; use arms, hands, legs, or feet; or walk. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of neuralgia. In some embodiments, the neuralgia is characterized by pain, burning, tingling, or abnormal feeling.

[0466] In some embodiments, efficacy of treatment is measured by improvement or a slowing of increase in postural hypotension, dizziness, gastrointestinal dysmotility, bladder dysfunction, or sexual dysfunction. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of weakness. In some embodiments, efficacy of treatment is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

[0467] In some embodiments, efficacy of treatment is measured by improvement or slowing of progression of symptoms of congestive heart failure or CHF. In some embodiments, efficacy of treatment is measured by an decrease or a slowing of increase in shortness of breath, trouble breathing, fatigue, or swelling in the ankles, feet, legs, abdomen, or veins in the neck. In some embodiments, efficacy of treatment is measured by improvement or a slowing of progression of fluid buildup in the body, which may be assessed by measures such as weight gain, frequent urination, or nighttime cough. In some embodiments, efficacy of treatment is measured using cardiac biomarker tests (such as B-type natriuretic peptide [BNP] or N-terminal pro b-type natriuretic peptide [NT-proBNP]), lung function tests, chest x-rays, or electrocardiography.

[0468] A. Combination Therapy

[0469] In some embodiments, the invention comprises combination therapies comprising any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein) together with an additional therapy suitable for alleviating symptoms of ATTR.

[0470] In some embodiments, the additional therapy for ATTR is a treatment for sensorimotor or autonomic neuropathy. In some embodiments, the treatment for sensorimotor or autonomic neuropathy is a nonsteroidal anti-inflammatory drug, antidepressant, anticonvulsant medication, antiarrythmic medication, or narcotic agent. In some embodiments, the antidepressant is a tricylic agent or a serotonin-norepinephrine reuptake inhibitor. In some embodiments, the antidepressant is amitriptyline, duloxetine, or venlafaxine. In some embodiments, the anticonvulsant agent is gabapentin, pregabalin, topiramate, or carbamazepine. In some embodiments, the additional therapy for sensorimotor neuropathy is transcutaneous electrical nerve stimulation.

[0471] In some embodiments, the additional therapy for ATTR is a treatment for restrictive cardiomyopathy or

congestive heart failure (CHF). In some embodiments, the treatment for CHF is a ACE inhibitor, aldosterone antagonist, angiotensin receptor blocker, beta blocker, digoxin, diuretic, or isosorbide dinitrate/hydralazine hydrochloride. In some embodiments, the ACE inhibitor is enalapril, captopril, ramipril, perindopril, imidapril, or quinapril. In some embodiments, the aldosterone antagonist is eplerenone or spironolactone. In some embodiments, the angiotensin receptor blocker is azilsartan, cadesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, or valsartan. In some embodiments, the beta blocker is acebutolol, atenolol, bisoprolol, metoprolol, nadolol, nebivolol, or propranolol. In some embodiments, the diuretic is chlorothiazide, chlorthalidone, hydrochlorothiazide, indapamide, metolazone, bumetanide, furosemide, torsemide, amiloride, or triameterene.

[0472] In some embodiments, the combination therapy comprises any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein) together with a siRNA that targets TTR or mutant TTR. In some embodiments, the siRNA is any siRNA capable of further reducing or eliminating the expression of wild type or mutant TTR. In some embodiments, the siRNA is the drug Patisiran (ALN-TTR02) or ALN-TTRsc02. In some embodiments, the siRNA is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein). In some embodiments, the siRNA is administered on a regular basis following treatment with any of the gRNA compositions provided herein.

[0473] In some embodiments, the combination therapy comprises any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein) together with antisense nucleotide that targets TTR or mutant TTR. In some embodiments, the antisense nucleotide is any antisense nucleotide capable of further reducing or eliminating the expression of wild type or mutant TTR. In some embodiments, the antisense nucleotide is the drug Inotersen (IONS-TTRRX). In some embodiments, the antisense nucleotide is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein). In some embodiments, the antisense nucleotide is administered on a regular basis following treatment with any of the gRNA compositions provided herein.

[0474] In some embodiments, the combination therapy comprises any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein) together with a small molecule stabilizer that promotes kinetic stabilization of the correctly folded tetrameric form of TTR. In some embodiments, the small molecule stabilizer is the drug tafamidis (Vyndaqel®) or diflunisal. In some embodiments, the small molecule stabilizer is administered after any one of the gRNAs comprising any one or more of the guide sequences disclosed in Table 1 or any one or more of the sgRNAs in Table 2 (e.g., in a composition provided herein). In some embodiments, the small molecule stabilizer is administered on a regular basis following treatment with any of the gRNA compositions provided herein.

[0475] B. Delivery of gRNA Compositions

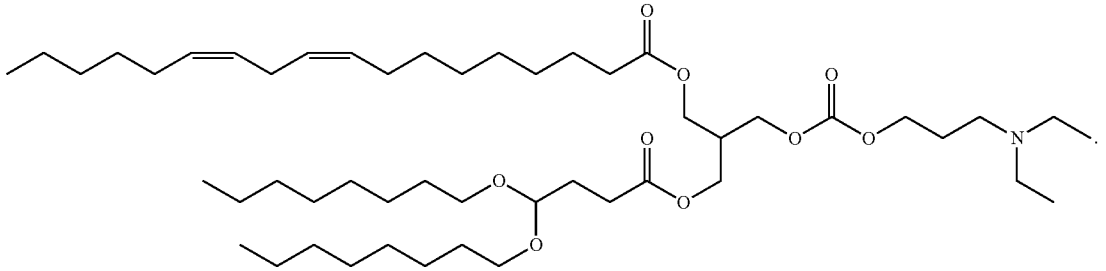
[0476] In some embodiments, the guide RNA compositions described herein, alone or encoded on one or more vectors, are formulated in or administered via a lipid nanoparticle; see e.g., PCT/US2017/024973, filed Mar. 30, 2017 entitled "LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS," the contents of which are hereby incorporated by reference in their entirety. Any lipid nanoparticle (LNP) known to those of skill in the art to be capable of delivering nucleotides to subjects may be utilized with the guide RNAs described herein, as well as either mRNA encoding an RNA-guided DNA nuclease such as Cas or Cas9, or an RNA-guided DNA nuclease such as Cas or Cas9 protein itself.

[0477] Disclosed herein are various embodiments of LNP formulations for RNAs, including CRISPR/Cas cargoes. Such LNP formulations may include (i) a CCD lipid, such as an amine lipid, (ii) a neutral lipid, (iii) a helper lipid, and (iv) a stealth lipid, such as a PEG lipid. Some embodiments of the LNP formulations include an "amine lipid", along with a helper lipid, a neutral lipid, and a stealth lipid such as a PEG lipid. By "lipid nanoparticle" is meant a particle that comprises a plurality of (i.e. more than one) lipid molecules physically associated with each other by intermolecular forces.

[0478] CCD Lipids

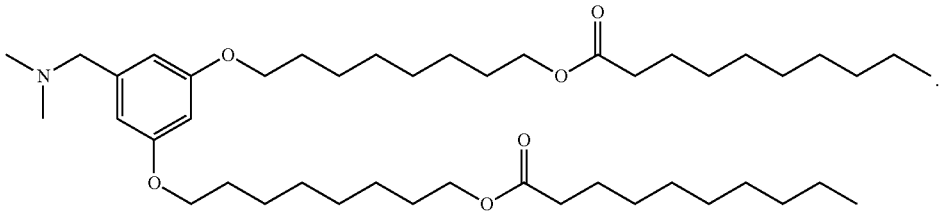
[0479] Lipid compositions for delivery of CRISPR/Cas mRNA and guide RNA components to a liver cell comprise a CCD Lipid.

[0480] In some embodiments, the CCD lipid is Lipid A, which is (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate. Lipid A can be depicted as:



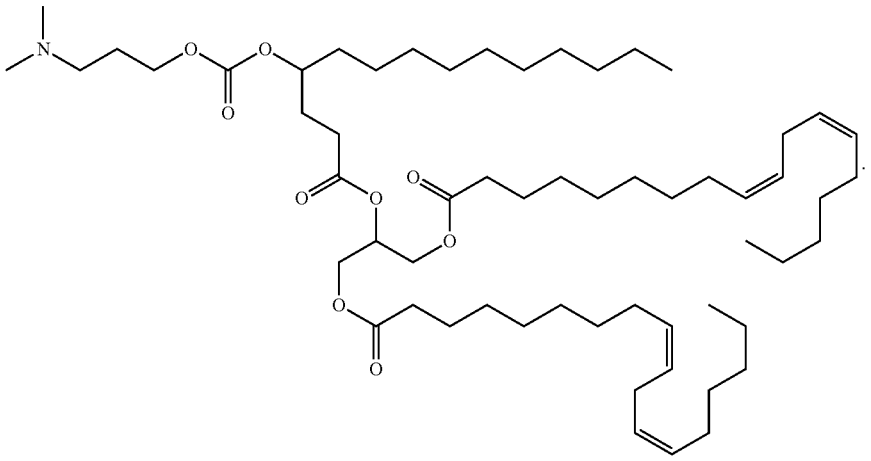
[0481] Lipid A may be synthesized according to WO2015/095340 (e.g., pp. 84-86).

[0482] In some embodiments, the CCD lipid is Lipid B, which is ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl)bis(decanoate), also called ((5-((dimethylamino)methyl)-1,3-phenylene)bis(oxy))bis(octane-8,1-diyl) bis(decanoate). Lipid B can be depicted as:



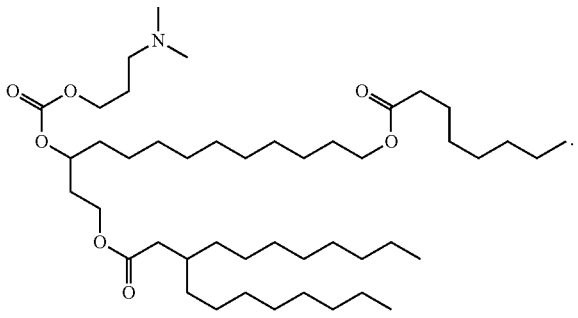
[0483] Lipid B may be synthesized according to WO2014/136086 (e.g., pp. 107-09).

[0484] In some embodiments, the CCD lipid is Lipid C, which is 2-((4-(((3-(dimethylamino)propoxy)carbonyl)oxy)hexadecanoyl)oxy)propane-1,3-diyl (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate). Lipid C can be depicted as:



[0485] In some embodiments, the CCD lipid is Lipid D, which is 3-(((3-(dimethylamino)propoxy)carbonyl)oxy)-13-(octanoyloxy)tridecyl 3-octylundecanoate.

[0486] Lipid D can be depicted as:



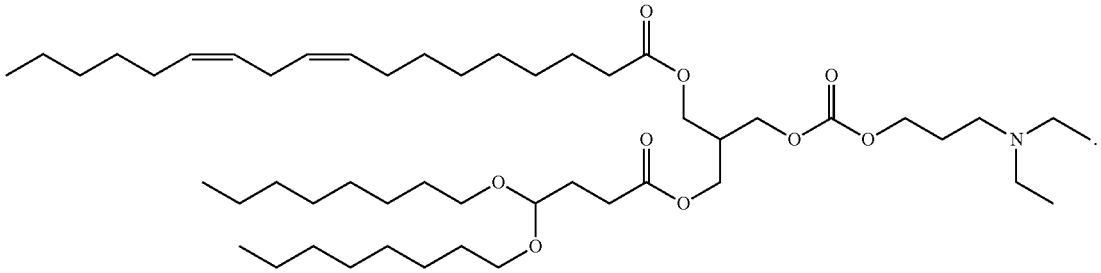
[0487] Lipid C and Lipid D may be synthesized according to WO2015/095340.

[0488] The CCD lipid can also be an equivalent to Lipid A, Lipid B, Lipid C, or Lipid D. In certain embodiments, the CCD lipid is an equivalent to Lipid A, an equivalent to Lipid B, an equivalent to Lipid C, or an equivalent to Lipid D.

[0489] Amine Lipids

[0490] In some embodiments, the LNP compositions for the delivery of biologically active agents comprise an "amine lipid", which is defined as Lipid A, Lipid B, Lipid C, Lipid D or equivalents of Lipid A (including acetal analogs of Lipid A), equivalents of Lipid B, equivalents of Lipid C, and equivalents of Lipid D.

[0491] In some embodiments, the amine lipid is Lipid A, which is (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-((((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate. Lipid A can be depicted as:



[0492] Lipid A may be synthesized according to WO2015/095340 (e.g., pp. 84-86). In certain embodiments, the amine lipid is an equivalent to Lipid A.

[0493] In certain embodiments, an amine lipid is an analog of Lipid A. In certain embodiments, a Lipid A analog is an acetal analog of Lipid A. In particular LNP compositions, the acetal analog is a C4-C12 acetal analog. In some embodiments, the acetal analog is a C5-C12 acetal analog. In additional embodiments, the acetal analog is a C5-C10 acetal analog. In further embodiments, the acetal analog is chosen from a C4, C5, C6, C7, C9, C10, C11, and C12 acetal analog.

[0494] Amine lipids suitable for use in the LNPs described herein are biodegradable in vivo. The amine lipids have low toxicity (e.g., are tolerated in animal models without adverse effect in amounts of greater than or equal to 10 mg/kg). In certain embodiments, LNPs comprising an amine lipid include those where at least 75% of the amine lipid is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days. In certain embodiments, LNPs comprising an amine lipid include those where at least 50% of the mRNA or gRNA is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days. In certain embodiments, LNPs comprising an amine lipid include those where at least 50% of the LNP is cleared from the plasma within 8, 10, 12, 24, or 48 hours, or 3, 4, 5, 6, 7, or 10 days, for example by measuring a lipid (e.g. an amine lipid), RNA (e.g. mRNA), or other component. In certain embodiments, lipid-encapsulated versus free lipid, RNA, or nucleic acid component of the LNP is measured.

[0495] Lipid clearance may be measured as described in literature. See Maier, M. A., et al. Biodegradable Lipids Enabling Rapidly Eliminated Lipid Nanoparticles for Systemic Delivery of RNAi Therapeutics. Mol. Ther. 2013, 21(8), 1570-78 ("Maier"). For example, in Maier, LNP-siRNA systems containing luciferases-targeting siRNA were administered to six- to eight-week old male C57Bl/6 mice at 0.3 mg/kg by intravenous bolus injection via the lateral tail vein. Blood, liver, and spleen samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, and 168 hours post-dose. Mice were perfused with saline before tissue collection and blood samples were processed to obtain plasma. All samples were processed and analyzed by LC-MS. Further, Maier describes a procedure for assessing toxicity after administration of LNP-siRNA formulations. For example, a luciferase-targeting siRNA was administered at 0, 1, 3, 5, and 10 mg/kg (5 animals/group) via single intravenous bolus injection at a dose volume of 5 mL/kg to male Sprague-Dawley rats. After 24 hours, about 1 mL of blood was obtained from the jugular vein of conscious animals and the serum was isolated. At 72 hours post-dose, all animals were euthanized for necropsy. Assessment of clinical signs, body weight, serum chemistry, organ weights and histopathology was performed. Although Maier describes methods for assessing siRNA-LNP formulations, these methods may be applied to assess clearance, pharmacokinetics, and toxicity of administration of LNP compositions of the present disclosure.

[0496] The amine lipids lead to an increased clearance rate. In some embodiments, the clearance rate is a lipid clearance rate, for example the rate at which an amine lipid is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is an RNA clearance rate, for example the rate at which an mRNA or a gRNA is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is the rate at which LNP is cleared from the blood, serum, or plasma. In some embodiments, the clearance rate is the rate at which LNP is cleared from a tissue, such as liver tissue or spleen tissue. In certain embodiments, a high rate of clearance rate leads to a safety profile with no substantial adverse effects. The amine lipids reduce LNP accumulation in circulation and in tissues. In some embodiments, a reduction in LNP accumulation in circulation and in tissues leads to a safety profile with no substantial adverse effects.

[0497] The amine lipids of the present disclosure may be ionizable depending upon the pH of the medium they are in. For example, in a slightly acidic medium, the amine lipids may be protonated and thus bear a positive charge. Conversely, in a slightly basic medium, such as, for example, blood where pH is approximately 7.35, the amine lipids may not be protonated and thus bear no charge. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 9. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 9. In some embodiments, the amine lipids of the present disclosure may be protonated at a pH of at least about 10.

[0498] The ability of an amine lipid to bear a charge is related to its intrinsic pKa. For example, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.8 to about 6.2. For example, the amine lipids of the present disclosure may each, independently, have a pKa in the range of from about 5.8 to about 6.5. This may be advantageous as it has been found that cationic lipids with a pKa ranging from about 5.1 to about 7.4 are effective for delivery of cargo in vivo, e.g. to the liver. Further, it has been found that cationic lipids with a pKa ranging from about 5.3 to about 6.4 are effective for delivery in vivo, e.g. to tumors. See, e.g., WO2014/136086.

[0499] Additional Lipids

[0500] "Neutral lipids" suitable for use in a lipid composition of the disclosure include, for example, a variety of neutral, uncharged or zwitterionic lipids. Examples of neutral phospholipids suitable for use in the present disclosure include, but are not limited to, 5-heptadecylbenzene-1,3-diol (resorcinol), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), pohsphocholine (DOPC), dimyristoylphosphatidylcholine (DMPC), phosphatidylcholine (PLPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DAPC), phosphatidylethanolamine (PE), egg phosphatidylcholine (EPC), dilauryloylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), 1-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoyl-2-myristoyl phosphatidylcholine (PMPC), 1-palmitoyl-2-stearoyl phosphatidylcholine (PSPC), 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DBPC), 1-stearoyl-2-palmitoyl phosphatidylcholine (SPPC), 1,2-dieicosenoyl-sn-glycero-3-phosphocholine (DEPC), palmitoyloleoyl phosphatidylcholine (POPC), lysophosphatidyl choline, dioleoyl phosphatidylethanolamine (DOPE), dilinoleoylphosphatidylcholine di stearoylphosphatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE), lysophosphatidylethanolamine and combinations thereof. In one embodiment, the neutral phospholipid may be selected from the group consisting of distearoylphosphatidylcholine (DSPC) and dimyristoyl phosphatidyl ethanolamine (DMPE). In another embodiment, the neutral phospholipid may be distearoylphosphatidylcholine (DSPC).

[0501] "Helper lipids" include steroids, sterols, and alkyl resorcinols. Helper lipids suitable for use in the present disclosure include, but are not limited to, cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate. In one embodiment, the helper lipid may be cholesterol. In one embodiment, the helper lipid may be cholesterol hemisuccinate.

[0502] "Stealth lipids" are lipids that alter the length of time the nanoparticles can exist in vivo (e.g., in the blood). Stealth lipids may assist in the formulation process by, for example, reducing particle aggregation and controlling particle size. Stealth lipids used herein may modulate pharmacokinetic properties of the LNP. Stealth lipids suitable for use in a lipid composition of the disclosure include, but are not limited to, stealth lipids having a hydrophilic head group linked to a lipid moiety. Stealth lipids suitable for use in a lipid composition of the present disclosure and information about the biochemistry of such lipids can be found in Romberg et al., Pharmaceutical Research, Vol. 25, No. 1, 2008, pg. 55-71 and Hoekstra et al., Biochimica et Biophysica Acta 1660 (2004) 41-52. Additional suitable PEG lipids are disclosed, e.g., in WO 2006/007712.

[0503] In one embodiment, the hydrophilic head group of stealth lipid comprises a polymer moiety selected from polymers based on PEG. Stealth lipids may comprise a lipid moiety. In some embodiments, the stealth lipid is a PEG lipid.

[0504] In one embodiment, a stealth lipid comprises a polymer moiety selected from polymers based on PEG (sometimes referred to as poly(ethylene oxide)), poly(oxazoline), poly(vinyl alcohol), poly(glycerol), poly(N-vinylpyrrolidone), polyaminoacids and poly[N-(2-hydroxypropyl)methacrylamide].

[0505] In one embodiment, the PEG lipid comprises a polymer moiety based on PEG (sometimes referred to as poly(ethylene oxide)).

[0506] The PEG lipid further comprises a lipid moiety. In some embodiments, the lipid moiety may be derived from diacylglycerol or diacylglycamide, including those comprising a dialkylglycerol or dialkylglycamide group having alkyl chain length independently comprising from about C4 to about C40 saturated or unsaturated carbon atoms, wherein the chain may comprise one or more functional groups such as, for example, an amide or ester. In some embodiments, the alkyl chail length comprises about C10 to C20. The dialkylglycerol or dialkylglycamide group can further comprise one or more substituted alkyl groups. The chain lengths may be symmetrical or assymetric.

[0507] Unless otherwise indicated, the term "PEG" as used herein means any polyethylene glycol or other polyalkylene ether polymer. In one embodiment, PEG is an optionally substituted linear or branched polymer of ethylene glycol or ethylene oxide. In one embodiment, PEG is unsubstituted. In one embodiment, the PEG is substituted, e.g., by one or more alkyl, alkoxy, acyl, hydroxy, or aryl groups. In one embodiment, the term includes PEG copolymers such as PEG-polyurethane or PEG-polypropylene (see, e.g., J. Milton Harris, Poly(ethylene glycol) chemistry: biotechnical and biomedical applications (1992)); in another embodiment, the term does not include PEG copolymers. In one embodiment, the PEG has a molecular weight of from about 130 to about 50,000, in a sub-embodiment, about 150 to about 30,000, in a sub-embodiment, about 150 to about 20,000, in a sub-embodiment about 150 to about 15,000, in a sub-embodiment, about 150 to about 10,000, in a sub-embodiment, about 150 to about 6,000, in a sub-embodiment, about 150 to about 5,000, in a sub-embodiment, about 150 to about 4,000, in a sub-embodiment, about 150 to about 3,000, in a sub-embodiment, about 300 to about 3,000, in a sub-embodiment, about 1,000 to about 3,000, and in a sub-embodiment, about 1,500 to about 2,500.

[0508] In certain embodiments, the PEG (e.g., conjugated to a lipid moiety or lipid, such as a stealth lipid), is a "PEG-2K," also termed "PEG 2000," which has an average molecular weight of about 2,000 daltons. PEG-2K is represented herein by the following formula (I), wherein n is 45, meaning that the number averaged degree of polymerization comprises about 45 subunits. However, other PEG embodiments known in the art may be used, including, e.g., those where the number-averaged degree of polymerization comprises about 23 subunits (n=23), and/or 68 subunits (n=68). In some embodiments, n may range from about 30 to about 60. In some embodiments, n may range from about 35 to about 55. In some embodiments, n may range from about 40 to about 50. In some embodiments, n may range from about 42 to about 48. In some embodiments, n may be 45. In some embodiments, R may be selected from H, substituted alkyl, and unsubstituted alkyl. In some embodiments, R may be unsubstituted alkyl. In some embodiments, R may be methyl.

[0509] In any of the embodiments described herein, the PEG lipid may be selected from PEG-dilauroylglycerol, PEG-dimyristoylglycerol (PEG-DMG) (catalog # GM-020 from NOF, Tokyo, Japan), PEG-dipalmitoylglycerol, PEG-di stearoylglycerol (PEG-DSPE) (catalog # DSPE-020CN, NOF, Tokyo, Japan), PEG-dilaurylglycamide, PEG-dimyristylglycamide, PEG-dipalmitoylglycamide, and PEG-di

stearoylglycamide, PEG-cholesterol (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl]carbamoyl-[omega]-methyl-poly(ethylene glycol), PEG-DMB (3,4-ditetradecoxylbenzyl-[omega]-methyl-poly(ethylene glycol)ether), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DMG) (cat. #880150P from Avanti Polar Lipids, Alabaster, Ala., USA), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSPE) (cat. #880120C from Avanti Polar Lipids, Alabaster, Ala., USA), 1,2-distearoyl-sn-glycerol, methoxypolyethylene glycol (PEG2k-DSG; GS-020, NOF Tokyo, Japan), poly(ethylene glycol)-2000-dimethacrylate (PEG2k-DMA), and 1,2-distearyloxypropyl-3-amine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSA). In one embodiment, the PEG lipid may be PEG2k-DMG. In some embodiments, the PEG lipid may be PEG2k-DSG. In one embodiment, the PEG lipid may be PEG2k-DSPE. In one embodiment, the PEG lipid may be PEG2k-DMA. In one embodiment, the PEG lipid may be PEG2k-C-DMA. In one embodiment, the PEG lipid may be compound 5027, disclosed in WO2016/010840 (paragraphs [00240] to [00244]). In one embodiment, the PEG lipid may be PEG2k-DSA. In one embodiment, the PEG lipid may be PEG2k-C11. In some embodiments, the PEG lipid may be PEG2k-C14. In some embodiments, the PEG lipid may be PEG2k-C16. In some embodiments, the PEG lipid may be PEG2k-C18.

[0510] LNP Formulations

[0511] The LNP may contain (i) an amine lipid for encapsulation and for endosomal escape, (ii) a neutral lipid for stabilization, (iii) a helper lipid, also for stabilization, and (iv) a stealth lipid, such as a PEG lipid.

[0512] In some embodiments, an LNP composition may comprise an RNA component that includes one or more of an RNA-guided DNA-binding agent, a Cas nuclease mRNA, a Class 2 Cas nuclease mRNA, a Cas9 mRNA, and a gRNA. In some embodiments, an LNP composition may include a Class 2 Cas nuclease and a gRNA as the RNA component. In certain embodiments, an LNP composition may comprise the RNA component, an amine lipid, a helper lipid, a neutral lipid, and a stealth lipid. In certain LNP compositions, the helper lipid is cholesterol. In other compositions, the neutral lipid is DSPC. In additional embodiments, the stealth lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the LNP composition comprises Lipid A or an equivalent of Lipid A; a helper lipid; a neutral lipid; a stealth lipid; and a guide RNA. In certain compositions, the amine lipid is Lipid A. In certain compositions, the amine lipid is Lipid A or an acetal analog thereof; the helper lipid is cholesterol; the neutral lipid is DSPC; and the stealth lipid is PEG2k-DMG.

[0513] In certain embodiments, lipid compositions are described according to the respective molar ratios of the component lipids in the formulation. Embodiments of the present disclosure provide lipid compositions described according to the respective molar ratios of the component lipids in the formulation. In one embodiment, the mol-% of the amine lipid may be from about 30 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 40 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 45 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 50 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 55 mol-% to about 60 mol-%. In one embodiment, the mol-% of the amine lipid may be from about 50 mol-% to about 55 mol-%. In one embodiment, the mol-% of the amine lipid may be about 50 mol-%. In one embodiment, the mol-% of the amine lipid may be about 55 mol-%. In some embodiments, the amine lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target mol-%. In some embodiments, the amine lipid mol-% of the LNP batch will be ±4 mol-%, ±3 mol-%, ±2 mol-%, ±1.5 mol-%, ±1 mol-%, ±0.5 mol-%, or ±0.25 mol-% of the target mol-%. All mol-% numbers are given as a fraction of the lipid component of the LNP compositions. In certain embodiments, LNP inter-lot variability of the amine lipid mol-% will be less than 15%, less than 10% or less than 5%.

[0514] In one embodiment, the mol-% of the neutral lipid may be from about 5 mol-% to about 15 mol-%. In one embodiment, the mol-% of the neutral lipid may be from about 7 mol-% to about 12 mol-%. In one embodiment, the mol-% of the neutral lipid may be about 9 mol-%. In some embodiments, the neutral lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target neutral lipid mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[0515] In one embodiment, the mol-% of the helper lipid may be from about 20 mol-% to about 60 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 55 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 50 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 25 mol-% to about 40 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 30 mol-% to about 50 mol-%. In one embodiment, the mol-% of the helper lipid may be from about 30 mol-% to about 40 mol-%. In one embodiment, the mol-% of the helper lipid is adjusted based on amine lipid, neutral lipid, and PEG lipid concentrations to bring the lipid component to 100 mol-%. In some embodiments, the helper mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[0516] In one embodiment, the mol-% of the PEG lipid may be from about 1 mol-% to about 10 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 10 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 8 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2 mol-% to about 4 mol-%. In one embodiment, the mol-% of the PEG lipid may be from about 2.5 mol-% to about 4 mol-%. In one embodiment, the mol-% of the PEG lipid may be about 3 mol-%. In one embodiment, the mol-% of the PEG lipid may be about 2.5 mol-%. In some embodiments, the PEG lipid mol-% of the LNP batch will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target PEG lipid mol-%. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[0517] In certain embodiments, the cargo includes an mRNA encoding an RNA-guided DNA-binding agent (e.g. a Cas nuclease, a Class 2 Cas nuclease, or Cas9), and a gRNA or a nucleic acid encoding a gRNA, or a combination of mRNA and gRNA. In one embodiment, an LNP composition may comprise a Lipid A or its equivalents. In some

aspects, the amine lipid is Lipid A. In some aspects, the amine lipid is a Lipid A equivalent, e.g. an analog of Lipid A. In certain aspects, the amine lipid is an acetal analog of Lipid A. In various embodiments, an LNP composition comprises an amine lipid, a neutral lipid, a helper lipid, and a PEG lipid. In certain embodiments, the helper lipid is cholesterol. In certain embodiments, the neutral lipid is DSPC. In specific embodiments, PEG lipid is PEG2k-DMG. In some embodiments, an LNP composition may comprise a Lipid A, a helper lipid, a neutral lipid, and a PEG lipid. In some embodiments, an LNP composition comprises an amine lipid, DSPC, cholesterol, and a PEG lipid. In some embodiments, the LNP composition comprises a PEG lipid comprising DMG. In certain embodiments, the amine lipid is selected from Lipid A, and an equivalent of Lipid A, including an acetal analog of Lipid A. In additional embodiments, an LNP composition comprises Lipid A, cholesterol, DSPC, and PEG2k-DMG.

[0518] Embodiments of the present disclosure also provide lipid compositions described according to the molar ratio between the positively charged amine groups of the amine lipid (N) and the negatively charged phosphate groups (P) of the nucleic acid to be encapsulated. This may be mathematically represented by the equation N/P. In some embodiments, an LNP composition may comprise a lipid component that comprises an amine lipid, a helper lipid, a neutral lipid, and a helper lipid; and a nucleic acid component, wherein the N/P ratio is about 3 to 10. In some embodiments, an LNP composition may comprise a lipid component that comprises an amine lipid, a helper lipid, a neutral lipid, and a helper lipid; and an RNA component, wherein the N/P ratio is about 3 to 10. In one embodiment, the N/P ratio may about 5-7. In one embodiment, the N/P ratio may about 4.5-8. In one embodiment, the N/P ratio may about 6. In one embodiment, the N/P ratio may be 6±1. In one embodiment, the N/P ratio may about 6±0.5. In some embodiments, the N/P ratio will be ±30%, ±25%, ±20%, ±15%, ±10%, ±5%, or ±2.5% of the target N/P ratio. In certain embodiments, LNP inter-lot variability will be less than 15%, less than 10% or less than 5%.

[0519] In some embodiments, the RNA component may comprise an mRNA, such as an mRNA disclosed herein, e.g., encoding a Cas nuclease. In one embodiment, RNA component may comprise a Cas9 mRNA. In some compositions comprising an mRNA encoding a Cas nuclease, the LNP further comprises a gRNA nucleic acid, such as a gRNA. In some embodiments, the RNA component comprises a Cas nuclease mRNA and a gRNA. In some embodiments, the RNA component comprises a Class 2 Cas nuclease mRNA and a gRNA.

[0520] In certain embodiments, an LNP composition may comprise an mRNA disclosed herein, e.g., encoding a Cas nuclease, such as a Class 2 Cas nuclease, an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In certain LNP compositions comprising an mRNA encoding a Cas nuclease such as a Class 2 Cas nuclease, the helper lipid is cholesterol. In other compositions comprising an mRNA encoding a Cas nuclease such as a Class 2 Cas nuclease, the neutral lipid is DSPC. In additional embodiments comprising an mRNA encoding a Cas nuclease such as a Class 2 Cas nuclease, the PEG lipid is PEG2k-DMG or PEG2k-C11. In specific compositions comprising an mRNA encoding a Cas nuclease such as a Class 2 Cas nuclease, the amine lipid is selected from Lipid A and its equivalents, such as an acetal analog of Lipid A.

[0521] In some embodiments, an LNP composition may comprise a gRNA. In certain embodiments, an LNP composition may comprise an amine lipid, a gRNA, a helper lipid, a neutral lipid, and a PEG lipid. In certain LNP compositions comprising a gRNA, the helper lipid is cholesterol. In some compositions comprising a gRNA, the neutral lipid is DSPC. In additional embodiments comprising a gRNA, the PEG lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the amine lipid is selected from Lipid A and its equivalents, such as an acetal analog of Lipid A.

[0522] In one embodiment, an LNP composition may comprise an sgRNA. In one embodiment, an LNP composition may comprise a Cas9 sgRNA. In one embodiment, an LNP composition may comprise a Cpf1 sgRNA. In some compositions comprising an sgRNA, the LNP includes an amine lipid, a helper lipid, a neutral lipid, and a PEG lipid. In certain compositions comprising an sgRNA, the helper lipid is cholesterol. In other compositions comprising an sgRNA, the neutral lipid is DSPC. In additional embodiments comprising an sgRNA, the PEG lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the amine lipid is selected from Lipid A and its equivalents, such as acetal analogs of Lipid A.

[0523] In certain embodiments, an LNP composition comprises an mRNA encoding a Cas nuclease and a gRNA, which may be an sgRNA. In one embodiment, an LNP composition may comprise an amine lipid, an mRNA encoding a Cas nuclease, a gRNA, a helper lipid, a neutral lipid, and a PEG lipid. In certain compositions comprising an mRNA encoding a Cas nuclease and a gRNA, the helper lipid is cholesterol. In some compositions comprising an mRNA encoding a Cas nuclease and a gRNA, the neutral lipid is DSPC. In additional embodiments comprising an mRNA encoding a Cas nuclease and a gRNA, the PEG lipid is PEG2k-DMG or PEG2k-C11. In certain embodiments, the amine lipid is selected from Lipid A and its equivalents, such as acetal analogs of Lipid A.

[0524] In certain embodiments, the LNP compositions include a Cas nuclease mRNA, such as a Class 2 Cas mRNA and at least one gRNA. In certain embodiments, the LNP composition includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 25:1 to about 1:25. In certain embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 10:1 to about 1:10. In certain embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease mRNA from about 8:1 to about 1:8. As measured herein, the ratios are by weight. In some embodiments, the LNP formulation includes a ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas mRNA from about 5:1 to about 1:5. In some embodiments, ratio range is about 3:1 to 1:3, about 2:1 to 1:2, about 5:1 to 1:2, about 5:1 to 1:1, about 3:1 to 1:2, about 3:1 to 1:1, about 3:1, about 2:1 to 1:1. In some embodiments, the gRNA to mRNA ratio is about 3:1 or about 2:1 In some embodiments the ratio of gRNA to Cas nuclease mRNA, such as Class 2 Cas nuclease is about 1:1. The ratio may be about 25:1, 10:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:10, or 1:25.

[0525] The LNP compositions disclosed herein may include a template nucleic acid. The template nucleic acid may be co-formulated with an mRNA encoding a Cas nuclease, such as a Class 2 Cas nuclease mRNA. In some embodiments, the template nucleic acid may be co-formulated with a guide RNA. In some embodiments, the template nucleic acid may be co-formulated with both an mRNA encoding a Cas nuclease and a guide RNA. In some embodiments, the template nucleic acid may be formulated separately from an mRNA encoding a Cas nuclease or a guide RNA. The template nucleic acid may be delivered with, or separately from the LNP compositions. In some embodiments, the template nucleic acid may be single- or double-stranded, depending on the desired repair mechanism. The template may have regions of homology to the target DNA, or to sequences adjacent to the target DNA.

[0526] In some embodiments, LNPs are formed by mixing an aqueous RNA solution with an organic solvent-based lipid solution, e.g., 100% ethanol. Suitable solutions or solvents include or may contain: water, PBS, Tris buffer, NaCl, citrate buffer, ethanol, chloroform, diethylether, cyclohexane, tetrahydrofuran, methanol, isopropanol. A pharmaceutically acceptable buffer, e.g., for in vivo administration of LNPs, may be used. In certain embodiments, a buffer is used to maintain the pH of the composition comprising LNPs at or above pH 6.5. In certain embodiments, a buffer is used to maintain the pH of the composition comprising LNPs at or above pH 7.0. In certain embodiments, the composition has a pH ranging from about 7.2 to about 7.7. In additional embodiments, the composition has a pH ranging from about 7.3 to about 7.7 or ranging from about 7.4 to about 7.6. In further embodiments, the composition has a pH of about 7.2, 7.3, 7.4, 7.5, 7.6, or 7.7. The pH of a composition may be measured with a micro pH probe. In certain embodiments, a cryoprotectant is included in the composition. Non-limiting examples of cryoprotectants include sucrose, trehalose, glycerol, DMSO, and ethylene glycol. Exemplary compositions may include up to 10% cryoprotectant, such as, for example, sucrose. In certain embodiments, the LNP composition may include about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10% cryoprotectant. In certain embodiments, the LNP composition may include about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10% sucrose. In some embodiments, the LNP composition may include a buffer. In some embodiments, the buffer may comprise a phosphate buffer (PBS), a Tris buffer, a citrate buffer, and mixtures thereof. In certain exemplary embodiments, the buffer comprises NaCl. In certain embodiments, NaCl is omitted. Exemplary amounts of NaCl may range from about 20 mM to about 45 mM. Exemplary amounts of NaCl may range from about 40 mM to about 50 mM. In some embodiments, the amount of NaCl is about 45 mM. In some embodiments, the buffer is a Tris buffer. Exemplary amounts of Tris may range from about 20 mM to about 60 mM. Exemplary amounts of Tris may range from about 40 mM to about 60 mM. In some embodiments, the amount of Tris is about 50 mM. In some embodiments, the buffer comprises NaCl and Tris. Certain exemplary embodiments of the LNP compositions contain 5% sucrose and 45 mM NaCl in Tris buffer. In other exemplary embodiments, compositions contain sucrose in an amount of about 5% w/v, about 45 mM NaCl, and about 50 mM Tris at pH 7.5. The salt, buffer, and cryoprotectant amounts may be varied such that the osmolality of the overall formulation is maintained. For example, the final osmolality may be maintained at less than 450 mOsm/L. In further embodiments, the osmolality is between 350 and 250 mOsm/L. Certain embodiments have a final osmolality of 300+/−20 mOsm/L.

[0527] In some embodiments, microfluidic mixing, T-mixing, or cross-mixing is used. In certain aspects, flow rates, junction size, junction geometry, junction shape, tube diameter, solutions, and/or RNA and lipid concentrations may be varied. LNPs or LNP compositions may be concentrated or purified, e.g., via dialysis, tangential flow filtration, or chromatography. The LNPs may be stored as a suspension, an emulsion, or a lyophilized powder, for example. In some embodiments, an LNP composition is stored at 2-8° C., in certain aspects, the LNP compositions are stored at room temperature. In additional embodiments, an LNP composition is stored frozen, for example at −20° C. or −80° C. In other embodiments, an LNP composition is stored at a temperature ranging from about 0° C. to about −80° C. Frozen LNP compositions may be thawed before use, for example on ice, at 4° C., at room temperature, or at 25° C. Frozen LNP compositions may be maintained at various temperatures, for example on ice, at 4° C., at room temperature, at 25° C., or at 37° C.

[0528] In some embodiments, an LNP composition has greater than about 80% encapsulation. In some embodiments, an LNP composition has a particle size less than about 120 nm. In some embodiments, an LNP composition has a pdi less than about 0.2. In some embodiments, at least two of these features are present. In some embodiments, each of these three features is present. Analytical methods for determining these parameters are discussed below in the general reagents and methods section.

[0529] In some embodiments, microfluidic mixing, T-mixing, or cross-mixing is used. In certain aspects, flow rates, junction size, junction geometry, junction shape, tube diameter, solutions, and/or RNA and lipid concentrations may be varied. LNPs or LNP compositions may be concentrated or purified, e.g., via dialysis or chromatography. The LNPs may be stored as a suspension, an emulsion, or a lyophilized powder, for example. In some embodiments, the LNP compositions are stored at 2-8° C., in certain aspects, the LNP compositions are stored at room temperature. In additional embodiments, the LNP composition is stored frozen, for example at −20° C. or −80° C. In other embodiments, the LNP composition is stored at a temperature ranging from 0° C. to −80° C. Frozen LNP compositions may be thawed before use, for example on ice, at room temperature, or at 25° C.

[0530] Dynamic Light Scattering ("DLS") can be used to characterize the polydispersity index ("pdi") and size of the LNPs of the present disclosure. DLS measures the scattering of light that results from subjecting a sample to a light source. PDI, as determined from DLS measurements, represents the distribution of particle size (around the mean particle size) in a population, with a perfectly uniform population having a PDI of zero. In some embodiments, the pdi may range from 0.005 to 0.75. In some embodiments, the pdi may range from 0.01 to 0.5. In some embodiments, the pdi may range from 0.02 to 0.4. In some embodiments, the pdi may range from 0.03 to 0.35. In some embodiments, the pdi may range from 0.1 to 0.35.

[0531] In some embodiments, LNPs disclosed herein have a size of 1 to 250 nm. In some embodiments, the LNPs have a size of 10 to 200 nm. In further embodiments, the LNPs have a size of 20 to 150 nm. In some embodiments, the LNPs

have a size of 50 to 150 nm. In some embodiments, the LNPs have a size of 50 to 100 nm. In some embodiments, the LNPs have a size of 50 to 120 nm. In some embodiments, the LNPs have a size of 75 to 150 nm. In some embodiments, the LNPs have a size of 30 to 200 nm. Unless indicated otherwise, all sizes referred to herein are the average sizes (diameters) of the fully formed nanoparticles, as measured by dynamic light scattering on a Malvern Zetasizer. The nanoparticle sample is diluted in phosphate buffered saline (PBS) so that the count rate is approximately 200-400 kcts. The data is presented as a weighted-average of the intensity measure. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 50% to 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 50% to 70%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 70% to 90%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 90% to 100%. In some embodiments, the LNPs are formed with an average encapsulation efficiency ranging from 75% to 95%.

[0532] In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for treating ATTR. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for reducing or preventing accumulation and aggregation of TTR in amyloids or amyloid fibrils in subjects having ATTR. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in preparing a medicament for reducing serum TTR concentration. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in treating ATTR in a subject, such as a mammal, e.g., a primate such as a human. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in reducing or preventing accumulation and aggregation of TTR in amyloids or amyloid fibrils in subjects having ATTR, such as a mammal, e.g., a primate such as a human. In some embodiments, LNPs associated with the gRNAs disclosed herein are for use in reducing serum TTR concentration in a subject, such as a mammal, e.g., a primate such as a human.

[0533] Electroporation is also a well-known means for delivery of cargo, and any electroporation methodology may be used for delivery of any one of the gRNAs disclosed herein. In some embodiments, electroporation may be used to deliver any one of the gRNAs disclosed herein and an RNA-guided DNA nuclease such as Cas9 or an mRNA encoding an RNA-guided DNA nuclease such as Cas9.

[0534] In some embodiments, the invention comprises a method for delivering any one of the gRNAs disclosed herein to an ex vivo cell, wherein the gRNA is associated with an LNP or not associated with an LNP. In some embodiments, the gRNA/LNP or gRNA is also associated with an RNA-guided DNA nuclease such as Cas9 or an mRNA encoding an RNA-guided DNA nuclease such as Cas9.

[0535] In certain embodiments, the invention comprises DNA or RNA vectors encoding any of the guide RNAs comprising any one or more of the guide sequences described herein. In some embodiments, in addition to guide RNA sequences, the vectors further comprise nucleic acids that do not encode guide RNAs. Nucleic acids that do not encode guide RNA include, but are not limited to, promoters, enhancers, regulatory sequences, and nucleic acids encoding an RNA-guided DNA nuclease, which can be a nuclease such as Cas9. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, or a crRNA and trRNA. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a sgRNA and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas nuclease, such as Cas9 or Cpf1. In some embodiments, the vector comprises one or more nucleotide sequence(s) encoding a crRNA, a trRNA, and an mRNA encoding an RNA-guided DNA nuclease, which can be a Cas protein, such as, Cas9. In one embodiment, the Cas9 is from *Streptococcus pyogenes* (i.e., Spy Cas9). In some embodiments, the nucleotide sequence encoding the crRNA, trRNA, or crRNA and trRNA (which may be a sgRNA) comprises or consists of a guide sequence flanked by all or a portion of a repeat sequence from a naturally-occurring CRISPR/Cas system. The nucleic acid comprising or consisting of the crRNA, trRNA, or crRNA and trRNA may further comprise a vector sequence wherein the vector sequence comprises or consists of nucleic acids that are not naturally found together with the crRNA, trRNA, or crRNA and trRNA.

[0536] In some embodiments, the crRNA and the trRNA are encoded by non-contiguous nucleic acids within one vector. In other embodiments, the crRNA and the trRNA may be encoded by a contiguous nucleic acid. In some embodiments, the crRNA and the trRNA are encoded by opposite strands of a single nucleic acid. In other embodiments, the crRNA and the trRNA are encoded by the same strand of a single nucleic acid.

[0537] In some embodiments, the vector may be circular. In other embodiments, the vector may be linear. In some embodiments, the vector may be enclosed in a lipid nanoparticle, liposome, non-lipid nanoparticle, or viral capsid. Non-limiting exemplary vectors include plasmids, phagemids, cosmids, artificial chromosomes, minichromosomes, transposons, viral vectors, and expression vectors.

[0538] In some embodiments, the vector may be a viral vector. In some embodiments, the viral vector may be genetically modified from its wild type counterpart. For example, the viral vector may comprise an insertion, deletion, or substitution of one or more nucleotides to facilitate cloning or such that one or more properties of the vector is changed. Such properties may include packaging capacity, transduction efficiency, immunogenicity, genome integration, replication, transcription, and translation. In some embodiments, a portion of the viral genome may be deleted such that the virus is capable of packaging exogenous sequences having a larger size. In some embodiments, the viral vector may have an enhanced transduction efficiency. In some embodiments, the immune response induced by the virus in a host may be reduced. In some embodiments, viral genes (such as, e.g., integrase) that promote integration of the viral sequence into a host genome may be mutated such that the virus becomes non-integrating. In some embodiments, the viral vector may be replication defective. In some embodiments, the viral vector may comprise exogenous transcriptional or translational control sequences to drive expression of coding sequences on the vector. In some embodiments, the virus may be helper-dependent. For example, the virus may need one or more helper virus to supply viral components (such as, e.g., viral proteins) required to amplify and package the vectors into viral particles. In such a case, one or more helper components,

including one or more vectors encoding the viral components, may be introduced into a host cell along with the vector system described herein. In other embodiments, the virus may be helper-free. For example, the virus may be capable of amplifying and packaging the vectors without any helper virus. In some embodiments, the vector system described herein may also encode the viral components required for virus amplification and packaging.

[0539] Non-limiting exemplary viral vectors include adeno-associated virus (AAV) vector, lentivirus vectors, adenovirus vectors, helper dependent adenoviral vectors (HDAd), herpes simplex virus (HSV-1) vectors, bacteriophage T4, baculovirus vectors, and retrovirus vectors. In some embodiments, the viral vector may be an AAV vector. In some embodiments, the viral vector is AAV2, AAV3, AAV3B, AAV5, AAV6, AAV6.2, AAV7, AAVrh.64R1, AAVhu.37, AAVrh.8, AAVrh.32.33, AAV8, AAV9, AAVrh10, or AAVLK03. In other embodiments, the viral vector may a lentivirus vector.

[0540] In some embodiments, the lentivirus may be non-integrating. In some embodiments, the viral vector may be an adenovirus vector. In some embodiments, the adenovirus may be a high-cloning capacity or "gutless" adenovirus, where all coding viral regions apart from the 5' and 3' inverted terminal repeats (ITRs) and the packaging signal ('I') are deleted from the virus to increase its packaging capacity. In yet other embodiments, the viral vector may be an HSV-1 vector. In some embodiments, the HSV-1-based vector is helper dependent, and in other embodiments it is helper independent. For example, an amplicon vector that retains only the packaging sequence requires a helper virus with structural components for packaging, while a 30 kb-deleted HSV-1 vector that removes non-essential viral functions does not require helper virus. In additional embodiments, the viral vector may be bacteriophage T4. In some embodiments, the bacteriophage T4 may be able to package any linear or circular DNA or RNA molecules when the head of the virus is emptied. In further embodiments, the viral vector may be a baculovirus vector. In yet further embodiments, the viral vector may be a retrovirus vector. In embodiments using AAV or lentiviral vectors, which have smaller cloning capacity, it may be necessary to use more than one vector to deliver all the components of a vector system as disclosed herein. For example, one AAV vector may contain sequences encoding an RNA-guided DNA nuclease such as a Cas nuclease, while a second AAV vector may contain one or more guide sequences.

[0541] In some embodiments, the vector may be capable of driving expression of one or more coding sequences in a cell. In some embodiments, the cell may be a prokaryotic cell, such as, e.g., a bacterial cell. In some embodiments, the cell may be a eukaryotic cell, such as, e.g., a yeast, plant, insect, or mammalian cell. In some embodiments, the eukaryotic cell may be a mammalian cell. In some embodiments, the eukaryotic cell may be a rodent cell. In some embodiments, the eukaryotic cell may be a human cell. Suitable promoters to drive expression in different types of cells are known in the art. In some embodiments, the promoter may be wild type. In other embodiments, the promoter may be modified for more efficient or efficacious expression. In yet other embodiments, the promoter may be truncated yet retain its function. For example, the promoter may have a normal size or a reduced size that is suitable for proper packaging of the vector into a virus.

[0542] In some embodiments, the vector may comprise a nucleotide sequence encoding an RNA-guided DNA nuclease such as a nuclease described herein. In some embodiments, the nuclease encoded by the vector may be a Cas protein. In some embodiments, the vector system may comprise one copy of the nucleotide sequence encoding the nuclease. In other embodiments, the vector system may comprise more than one copy of the nucleotide sequence encoding the nuclease. In some embodiments, the nucleotide sequence encoding the nuclease may be operably linked to at least one transcriptional or translational control sequence. In some embodiments, the nucleotide sequence encoding the nuclease may be operably linked to at least one promoter.

[0543] In some embodiments, the promoter may be constitutive, inducible, or tissue-specific. In some embodiments, the promoter may be a constitutive promoter. Non-limiting exemplary constitutive promoters include cytomegalovirus immediate early promoter (CMV), simian virus (SV40) promoter, adenovirus major late (MLP) promoter, Rous sarcoma virus (RSV) promoter, mouse mammary tumor virus (MMTV) promoter, phosphoglycerate kinase (PGK) promoter, elongation factor-alpha (EF1a) promoter, ubiquitin promoters, actin promoters, tubulin promoters, immunoglobulin promoters, a functional fragment thereof, or a combination of any of the foregoing. In some embodiments, the promoter may be a CMV promoter. In some embodiments, the promoter may be a truncated CMV promoter. In other embodiments, the promoter may be an EF1a promoter. In some embodiments, the promoter may be an inducible promoter. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech).

[0544] In some embodiments, the promoter may be a tissue-specific promoter, e.g., a promoter specific for expression in the liver.

[0545] The vector may further comprise a nucleotide sequence encoding the guide RNA described herein. In some embodiments, the vector comprises one copy of the guide RNA. In other embodiments, the vector comprises more than one copy of the guide RNA. In embodiments with more than one guide RNA, the guide RNAs may be non-identical such that they target different target sequences, or may be identical in that they target the same target sequence. In some embodiments where the vectors comprise more than one guide RNA, each guide RNA may have other different properties, such as activity or stability within a complex with an RNA-guided DNA nuclease, such as a Cas RNP complex. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to at least one transcriptional or translational control sequence, such as a promoter, a 3' UTR, or a 5' UTR. In one embodiment, the promoter may be a tRNA promoter, e.g., $tRNA^{Lys3}$, or a tRNA chimera. See Mefferd et al., RNA. 2015 21:1683-9; Scherer et al., Nucleic Acids Res. 2007 35: 2620-2628. In some embodiments, the promoter may be recognized by RNA polymerase III (Pol III). Non-limiting examples of Pol III promoters include U6 and H1 promoters. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human U6 promoter. In other embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human H1

promoter. In embodiments with more than one guide RNA, the promoters used to drive expression may be the same or different. In some embodiments, the nucleotide encoding the crRNA of the guide RNA and the nucleotide encoding the trRNA of the guide RNA may be provided on the same vector. In some embodiments, the nucleotide encoding the crRNA and the nucleotide encoding the trRNA may be driven by the same promoter. In some embodiments, the crRNA and trRNA may be transcribed into a single transcript. For example, the crRNA and trRNA may be processed from the single transcript to form a double-molecule guide RNA. Alternatively, the crRNA and trRNA may be transcribed into a single-molecule guide RNA (sgRNA). In other embodiments, the crRNA and the trRNA may be driven by their corresponding promoters on the same vector. In yet other embodiments, the crRNA and the trRNA may be encoded by different vectors.

[0546] In some embodiments, the nucleotide sequence encoding the guide RNA may be located on the same vector comprising the nucleotide sequence encoding an RNA-guided DNA nuclease such as a Cas nuclease. In some embodiments, expression of the guide RNA and of the RNA-guided DNA nuclease such as a Cas protein may be driven by their own corresponding promoters. In some embodiments, expression of the guide RNA may be driven by the same promoter that drives expression of the RNA-guided DNA nuclease such as a Cas protein. In some embodiments, the guide RNA and the RNA-guided DNA nuclease such as a Cas protein transcript may be contained within a single transcript. For example, the guide RNA may be within an untranslated region (UTR) of the RNA-guided DNA nuclease such as a Cas protein transcript. In some embodiments, the guide RNA may be within the 5' UTR of the transcript. In other embodiments, the guide RNA may be within the 3' UTR of the transcript. In some embodiments, the intracellular half-life of the transcript may be reduced by containing the guide RNA within its 3' UTR and thereby shortening the length of its 3' UTR. In additional embodiments, the guide RNA may be within an intron of the transcript. In some embodiments, suitable splice sites may be added at the intron within which the guide RNA is located such that the guide RNA is properly spliced out of the transcript. In some embodiments, expression of the RNA-guided DNA nuclease such as a Cas protein and the guide RNA from the same vector in close temporal proximity may facilitate more efficient formation of the CRISPR RNP complex.

[0547] In some embodiments, the compositions comprise a vector system. In some embodiments, the vector system may comprise one single vector. In other embodiments, the vector system may comprise two vectors. In additional embodiments, the vector system may comprise three vectors. When different guide RNAs are used for multiplexing, or when multiple copies of the guide RNA are used, the vector system may comprise more than three vectors.

[0548] In some embodiments, the vector system may comprise inducible promoters to start expression only after it is delivered to a target cell. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech).

[0549] In additional embodiments, the vector system may comprise tissue-specific promoters to start expression only after it is delivered into a specific tissue.

[0550] The vector may be delivered by liposome, a nanoparticle, an exosome, or a microvesicle. The vector may also be delivered by a lipid nanoparticle (LNP); see e.g., U.S. Ser. No. 62/433,228, filed Dec. 12, 2016 and entitled "LIPID NANOPARTICLE FORMULATIONS FOR CRISPR/CAS COMPONENTS," the contents of which are hereby incorporated by reference in their entirety. Any of the LNPs and LNP formulations described herein are suitable for delivery of the guides alone or together a cas nuclease or an mRNA encoding a cas nuclease. In some embodiments, an LNP composition is encompassed comprising: an RNA component and a lipid component, wherein the lipid component comprises an amine lipid, a neutral lipid, a helper lipid, and a stealth lipid; and wherein the N/P ratio is about 1-10.

[0551] In some instances, the lipid component comprises Lipid A or its acetal analog, cholesterol, DSPC, and PEG-DMG; and wherein the N/P ratio is about 1-10. In some embodiments, the lipid component comprises: about 40-60 mol-% amine lipid; about 5-15 mol-% neutral lipid; and about 1.5-10 mol-% PEG lipid, wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-10. In some embodiments, the lipid component comprises about 50-60 mol-% amine lipid; about 8-10 mol-% neutral lipid; and about 2.5-4 mol-% PEG lipid, wherein the remainder of the lipid component is helper lipid, and wherein the N/P ratio of the LNP composition is about 3-8. In some instances, the lipid component comprises: about 50-60 mol-% amine lipid; about 5-15 mol-% DSPC; and about 2.5-4 mol-% PEG lipid, wherein the remainder of the lipid component is cholesterol, and wherein the N/P ratio of the LNP composition is about 3-8. In some instances, the lipid component comprises: 48-53 mol-% Lipid A; about 8-10 mol-% DSPC; and 1.5-10 mol-% PEG lipid, wherein the remainder of the lipid component is cholesterol, and wherein the N/P ratio of the LNP composition is 3-8±0.2.

[0552] In some embodiments, the vector may be delivered systemically. In some embodiments, the vector may be delivered into the hepatic circulation.

[0553] This description and exemplary embodiments should not be taken as limiting. For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities, percentages, or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term "about," to the extent they are not already so modified. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0554] It is noted that, as used in this specification and the appended claims, the singular forms "a," "an," and "the," and any singular use of any word, include plural referents unless expressly and unequivocally limited to one referent. As used herein, the term "include" and its grammatical variants are intended to be non-limiting, such that recitation

of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items.

Examples

[0555] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

Example 1. Materials and Methods

[0556] In Vitro Transcription ("IVT") of Nuclease mRNA

[0557] Capped and polyadenylated *Streptococcus pyogenes* ("Spy") Cas9 mRNA containing N1-methyl pseudo-U was generated by in vitro transcription using a linearized plasmid DNA template and T7 RNA polymerase. Plasmid DNA containing a T7 promoter, a sequence for transcription according to SEQ ID NO: 1 or 2, and a 100 nt poly (A/T) region was linearized by incubating at 37° C. for 2 hours with XbaI with the following conditions: 200 ng/μL plasmid, 2 U/μL XbaI (NEB), and 1× reaction buffer. The XbaI was inactivated by heating the reaction at 65° C. for 20 min. The linearized plasmid was purified from enzyme and buffer salts using a silica maxi spin column (Epoch Life Sciences) and analyzed by agarose gel to confirm linearization. The IVT reaction to generate Cas9 modified mRNA was incubated at 37° C. for 4 hours in the following conditions: 50 ng/μL linearized plasmid; 2 mM each of GTP, ATP, CTP, and N1-methyl pseudo-UTP (Trilink); 10 mM ARCA (Trilink); 5 U/μL T7 RNA polymerase (NEB); 1 U/μL Murine RNase inhibitor (NEB); 0.004 U/μL Inorganic *E. coli* pyrophosphatase (NEB); and 1× reaction buffer. After the 4-hour incubation, TURBO DNase (ThermoFisher) was added to a final concentration of 0.01 U/μL, and the reaction was incubated for an additional 30 minutes to remove the DNA template. The Cas9 mRNA was purified from enzyme and nucleotides using a MegaClear Transcription Clean-up kit per the manufacturer's protocol (ThermoFisher). Alternatively, the mRNA was purified through a precipitation protocol, which in some cases was followed by HPLC-based purification. Briefly, after the DNase digestion, the mRNA was precipitated by adding 0.21× vol of a 7.5 M LiCl solution and mixing, and the precipitated mRNA was pelleted by centrifugation. Once the supernatant was removed, the mRNA was reconstituted in water. The mRNA was precipitated again using ammonium acetate and ethanol. 5M Ammonium acetate was added to the mRNA solution for a final concentration of 2M along with 2× volume of 100% EtOH. The solution was mixed and incubated at −20° C. for 15 min. The precipitated mRNA was again pelleted by centrifugation, the supernatant was removed, and the mRNA was reconstituted in water. As a final step, the mRNA was precipitated using sodium acetate and ethanol. 1/10 volume of 3 M sodium acetate (pH 5.5) was added to the solution along with 2× volume of 100% EtOH. The solution was mixed and incubated at −20° C. for 15 min. The precipitated mRNA was again pelleted by centrifugation, the supernatant was removed, the pellet was washed with 70% cold ethanol and allowed to air dry. The mRNA was reconstituted in water. For HPLC purified mRNA, after the LiCl precipitation and reconstitution, the mRNA was purified by RP-IP HPLC (see, e.g., Kariko, et al. Nucleic Acids Research, 2011, Vol. 39, No. 21 e142). The fractions chosen for pooling were combined and desalted by sodium acetate/ethanol precipitation as described above. The transcript concentration was determined by measuring the light absorbance at 260 nm (Nanodrop), and the transcript was analyzed by capillary electrophoresis by Bioanlayzer (Agilent).

[0558] When SEQ ID NOs: 1 and 2 are referred to below with respect to RNAs, it is understood that Ts should be replaced with Us (which were N1-methyl pseudouridines as described above). Cas9 mRNAs used in the Examples include a 5' cap and a 3' poly-A tail, e.g., up to 100 nts, and are identified by SEQ ID NO.

SEQ ID NO: 1: Cas9 sequence 1 for transcription.

GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGT

TGCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGA

CTGGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATA

CAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACA

GCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACA

GCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAG

AAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAA

AGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAA

GAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGA

AGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGC

TGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTG

GCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAA

CCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACAT

ACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCA

AAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCT

GATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGA

TCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACCTG

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TACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTT

CATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCA

AGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGA

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AGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGA

AACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACC

GTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCA

TCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTG

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TGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGA

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AGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTG

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GGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGA

ACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACT

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ATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAA

GTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAG

TCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACA

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ATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTT

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AGAACCTCGAG

SEQ ID NO: 2: Cas9 sequence 2 for transcription.

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AGAAAAATCTCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGCAGAA

GCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAA

TCGCATCTGCTATCTGCAAGAGATCTTTTCGAACGAAATGGCAAAGGTCG

ACGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGAC

AAGAAGCATGAACGGCATCCTATCTTTGGAAACATCGTCGACGAAGTGGC

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TAACTCCGACGTGGATAAGCTTTTCATTCAACTGGTGCAGACCTACAACC

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CCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGG

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CTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGCAAGAAAGACTGGGA

CCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTCGCATACTCCGTCC

TCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAAAGCTCAAATCCGTC

AAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTCGTTCGAGAAGAA

CCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTGAAGAAGGATC

TGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAAAATGGTCGG

AAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGCTGGC

CTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGAAA

AACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTCGTGGAG

CAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTC

AAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCGGCCT

ACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTATC

CACTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTACTT

CGATACTACTATCGATCGCAAAAGATACACGTCCACCAAGGAAGTTCTGG

ACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATC

GATCTGTCGCAGCTGGGTGGCGATGGCGGTGGATCTCCGAAAAAGAAGAG

AAAGGTGTAATGAGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCAT

GAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTC

TTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACATAAATTTCTT

TAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAG

AACCTCGAG

Human TTR Guide Design and Human TTR with Cynomolgus Monkey Homology Guide Design

[0559] Initial guide selection was performed in silico using a human reference genome (e.g., hg38) and user defined genomic regions of interest (e.g., TTR protein coding exons), for identifying PAMs in the regions of interest. For each identified PAM, analyses were performed and statistics reported. gRNA molecules were further selected and rank ordered based on a number of criteria (e.g., GC content, predicted on-target activity, and potential off-target activity).

A total of 68 guide RNAs were designed toward TTR (ENSG00000118271) targeting the protein coding regions within Exon 1, 2, 3 and 4. Of the total 68 guides, 33 were 100% homologous in cynomolgus monkey ("cyno"). In addition, for 10 of the human TTR guides which were not

perfectly homologous in cyno, "surrogate" guides were designed and made in parallel to perfectly match the corresponding cyno target sequence. These "surrogate" or "tool" guides may be screened in cyno, e.g., to approximate the activity and function of the homologous human guide sequence. Guide sequences and corresponding genomic coordinates are provided (Table 1). All of the guide RNAs were made as dual guide RNAs, and a subset of the guide sequences were made as modified single guide RNA (Table 2). Guide ID alignment across dual guide RNA (dgRNA) IDs, modified single guide RNA (sgRNA) IDs, the number of mismatches to the cyno genome as well as the cyno exact matched IDs are provided (Table 3). Where dgRNAs are used in the experiments detailed throughout the Examples, SEQ ID NO: 270 was used.

Cas9 mRNA and Guide RNA Delivery In Vitro

[0560] HEK293_Cas9 Cell Line.

[0561] The human embryonic kidney adenocarcinoma cell line HEK293 constitutively expressing Spy Cas9 ("HEK293_Cas9") was cultured in DMEM media supplemented with 10% fetal bovine serum and 500 μg/ml G418. Cells were plated at a density of 10,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with a lipoplex containing individual crRNA (25 nM), trRNA (25 nM), Lipofectamine RNAiMAX (0.3 μL/well) and OptiMem.

[0562] HUH7 Cell Line.

[0563] The human hepatocellular carcinoma cell line HUH7 (Japanese Collection of Research Bioresources Cell Bank, Cat. JCRB0403) was cultured in DMEM media supplemented with 10% fetal bovine serum. Cells were plated on at a density of 15,000 cells/well in a 96-well plate 20 hours prior to transfection. Cells were transfected with Lipofectamine MessengerMAX (ThermoFisher, Cat. LMRNA003) per the manufacturer's protocol. Cells were sequentially transfected with a lipoplex containing Spy Cas9 mRNA (100 ng), MessengerMAX (0.3 μL/well) and OptiMem followed by a separate lipoplex containing individual crRNA (25 nM), tracer RNA (25 nM), MessengerMAX (0.3 μL/well) and OptiMem.

[0564] HepG2 Cell Line.

[0565] The human hepatocellular carcinoma cell line HepG2 (American Type Culture Collection, Cat. HB-8065) was cultured in DMEM media supplemented with 10% fetal bovine serum. Cells were counted and plated on Bio-coat collagen I coated 96-well plates (ThermoFisher, Cat. 877272) at a density of 10,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine 2000 (ThermoFisher, Cat. 11668019) per the manufacturer's protocol. Cells were sequentially transfected with lipoplex containing Spy Cas9 mRNA (100 ng), Lipofectamine 2000 (0.2 μL/well) and OptiMem followed by a separate lipoplex containing individual crRNA (25 nM), tracer RNA (25 nM), Lipofectamine 2000 (0.2 μL/well) and OptiMem.

[0566] Primary Liver Hepatocytes.

[0567] Primary human liver hepatocytes (PHH) and primary cynomolgus liver hepatocytes (PCH) (Gibco) were cultured per the manufacturer's protocol (Invitrogen, protocol 11.28.2012). In brief, the cells were thawed and resuspended in hepatocyte thawing medium with supplements (Gibco, Cat. CM7000) followed by centrifugation at 100 g for 10 minutes for human and 80 g for 4 minutes for cyno. The supernatant was discarded and the pelleted cells resuspended in hepatocyte plating medium plus supplement pack (Invitrogen, Cat. A1217601 and CM3000). Cells were counted and plated on Bio-coat collagen I coated 96-well plates (ThermoFisher, Cat. 877272) at a density of 33,000 cells/well for human or 60,000 cells/well for cyno (or 65,000 cells/well when assaying effects on TTR protein, described further below). Plated cells were allowed to settle and adhere for 6 or 24 hours in a tissue culture incubator at 37° C. and 5% CO_2 atmosphere. After incubation cells were checked for monolayer formation and media was replaced with hepatocyte culture medium with serum-free supplement pack (Invitrogen, Cat. A1217601 and CM4000).

[0568] Lipofectamine RNAiMax (ThermoFisher, Cat. 13778150) based transfections were conducted as per the manufacturer's protocol. Cells were sequentially transfected with a lipoplex containing Spy Cas9 mRNA (100 ng), Lipofectamine RNAiMax (0.4 μL/well) and OptiMem followed by a separate lipoplex containing crRNA (25 nM) and tracer RNA (25 nM) or sgRNA (25 nM), Lipofectamine RNAiMax (0.4 μL/well) and OptiMem.

[0569] Ribonucleotide formation was performed prior to electroporation or transfection of Spy Cas9 protein loaded with guide RNAs (RNPs) onto cells. For dual guide (dgRNAs), individual crRNA and trRNA was pre-annealed by mixing equivalent amounts of reagent and incubating at 95° C. for 2 min and cooling to room temperature. Single guide (sgRNAs) were boiled at 95° C. for 2 min and cooling to room temperature. The boiled dgRNA or sgRNA was incubated with Spy Cas9 protein in Optimem for 10 minutes at room temperature to form a ribonucleoprotein (RNP) complex.

[0570] For RNP electroporation into primary human and cyno hepatocytes, the cells are thawed and resuspended in Lonza electroporation Primary Cell P3 buffer at a concentration of 2500 cells per μL for human hepatocytes and 3500 cells per μL for cyno hepatocytes. A volume of 20 μL of resuspended cells and 5 μL of RNP are mixed together per guide. 20μL of the mixture is placed into a Lonza electroporation plate. The cells were electroporated using the Lonza nucleofector with the preset protocol EX-147. Post electroporation, the cells are transferred into a Biocoat plate containing pre-warmed maintenance media and placed in a tissue culture incubator at 37° C. and 5% CO_2.

[0571] For RNP lipoplex transfections, cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with an RNP containing Spy Cas9 (10 nM), individual guide (10 nM), tracer RNA (10 nM), Lipofectamine RNAiMAX (1.0 μL/well) and OptiMem. RNP formation was performed as described above.

[0572] LNPs were formed either by microfluidic mixing of the lipid and RNA solutions using a Precision Nanosystems NanoAssemblr™ Benchtop Instrument, per the manufacturer's protocol, or cross-flow mixing.

[0573] LNP Formulation—NanoAssemblr

[0574] In general, the lipid nanoparticle components were dissolved in 100% ethanol with the lipid component of various molar ratios. The RNA cargos were dissolved in 25 mM citrate, 100 mM NaCl, pH 5.0, resulting in a concentration of RNA cargo of approximately 0.45 mg/mL. The LNPs were formulated with a lipid amine to RNA phosphate

(N:P) molar ratio of about 4.5 or about 6, with the ratio of mRNA to gRNA at 1:1 by weight.

[0575] The LNPs were formed by microfluidic mixing of the lipid and RNA solutions using a Precision Nanosystems NanoAssemblr™ Benchtop Instrument, according to the manufacturer's protocol. A 2:1 ratio of aqueous to organic solvent was maintained during mixing using differential flow rates. After mixing, the LNPs were collected, diluted in water (approximately 1:1 v/v), held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v) before final buffer exchange. The final buffer exchange into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS) was completed with PD-10 desalting columns (GE). If required, formulations were concentrated by centrifugation with Amicon 100 kDa centrifugal filters (Millipore). The resulting mixture was then filtered using a 0.2 μm sterile filter. The final LNP was stored at −80° C. until further use.

LNP Formulation—Cross-Flow

[0576] For LNPs prepared using the cross-flow technique, the LNPs were formed by impinging jet mixing of the lipid in ethanol with two volumes of RNA solutions and one volume of water. The lipid in ethanol is mixed through a mixing cross with the two volumes of RNA solution. A fourth stream of water is mixed with the outlet stream of the cross through an inline tee. (See WO2016010840 FIG. **2**.) The LNPs were held for 1 hour at room temperature, and further diluted with water (approximately 1:1 v/v). Diluted LNPs were concentrated using tangential flow filtration on a flat sheet cartridge (Sartorius, 100 kD MWCO) and then buffer exchanged by diafiltration into 50 mM Tris, 45 mM NaCl, 5% (w/v) sucrose, pH 7.5 (TSS). Alternatively, the final buffer exchange into TSS was completed with PD-10 desalting columns (GE). If required, formulations were concentrated by centrifugation with Amicon 100 kDa centrifugal filters (Millipore). The resulting mixture was then filtered using a 0.2 μm sterile filter. The final LNP was stored at 4° C. or −80° C. until further use.

Formulation Analytics

[0577] Dynamic Light Scattering ("DLS") is used to characterize the polydispersity index ("pdi") and size of the LNPs of the present disclosure. DLS measures the scattering of light that results from subjecting a sample to a light source. PDI, as determined from DLS measurements, represents the distribution of particle size (around the mean particle size) in a population, with a perfectly uniform population having a PDI of zero. Average particle size and polydispersity are measured by dynamic light scattering (DLS) using a Malvern Zetasizer DLS instrument. LNP samples were diluted 30× in PBS prior to being measured by DLS. Z-average diameter which is an intensity based measurement of average particle size was reported along with number average diameter and pdi. A Malvern Zetasizer instrument is also used to measure the zeta potential of the LNP. Samples are diluted 1:17 (50 uL into 800 uL) in 0.1×PBS, pH 7.4 prior to measurement.

[0578] A fluorescence-based assay (Ribogreen®, ThermoFisher Scientific) is used to determine total RNA concentration and free RNA. Encapsulation efficiency is calculated as (Total RNA−Free RNA)/Total RNA. LNP samples are diluted appropriately with 1×TE buffer containing 0.2% Triton-X 100 to determine total RNA or 1×TE buffer to determine free RNA. Standard curves are prepared by utilizing the starting RNA solution used to make the formulations and diluted in 1×TE buffer+/−0.2% Triton-X 100. Diluted RiboGreen® dye (according to the manufacturer's instructions) is then added to each of the standards and samples and allowed to incubate for approximately 10 minutes at room temperature, in the absence of light. A SpectraMax M5 Microplate Reader (Molecular Devices) is used to read the samples with excitation, auto cutoff and emission wavelengths set to 488 nm, 515 nm, and 525 nm respectively. Total RNA and free RNA are determined from the appropriate standard curves.

[0579] Encapsulation efficiency is calculated as (Total RNA−Free RNA)/Total RNA. The same procedure may be used for determining the encapsulation efficiency of a DNA-based cargo component. For single-strand DNA Oligreen Dye may be used, and for double-strand DNA, Picogreen Dye.

[0580] Typically, when preparing LNPs, encapsulation was >80%, particle size was <120 nm, and pdi was <0.2.

LNP Delivery In Vivo

[0581] Unless otherwise noted, CD-1 female mice, ranging from 6-10 weeks of age were used in each study. Animals were weighed and grouped according to body weight for preparing dosing solutions based on group average weight. LNPs were dosed via the lateral tail vein in a volume of 0.2 mL per animal (approximately 10 mL per kilogram body weight). The animals were observed at approximately 6 hours post dose for adverse effects. Body weight was measured at twenty-four hours post-administration, and animals were euthanized at various time points by exsanguination via cardiac puncture under isoflourane anesthesia. Blood was collected into serum separator tubes or into tubes containing buffered sodium citrate for plasma as described herein. For studies involving in vivo editing, liver tissue was collected from the median lobe or from three independent lobes (e.g., the right median, left median, and left lateral lobes) from each animal for DNA extraction and analysis.

Transthyretin (TTR) ELISA Analysis Used in Animal Studies

[0582] Blood was collected and the serum was isolated as indicated. The total mouse TTR serum levels were determined using a Mouse Prealbumin (Transthyretin) ELISA Kit (Aviva Systems Biology, Cat. OKIA00111); rat TTR serum levels were measured using a rat specific ELISA kit (Aviva Systems Biology catalog number OKIA00159); human TTR serum levels were measured using a human specific ELISA kit (Aviva Systems Biology catalog number OKIA00081); each according to manufacture's protocol. Briefly, sera were serial diluted with kit sample diluent to a final dilution of 10,000-fold, or 5,000-fold when measuring human TTR in mouse sera. 100 ul of the prepared standard curve or diluted serum samples were added to the ELISA plate, incubated for 30 minutes at room temperature then washed 3 times with provided wash buffer. 100 uL of detection antibody was then added to each well and incubated for 20 minutes at room temperature followed by 3 washes. 100 uL of substrate is added then incubated for 10 minutes at room temperature before the addition of 100 uL stop solution. The absorbance

of the contents was measured on the Spectramax M5 plate reader with analysis using SoftmaxPro version 7.0 software. Serum TTR levels were quantitated off the standard curve using 4 parameter logistic fit and expressed as ug/mL of serum or percent knockdown relative control (vehicle treated) animals.

Genomic DNA Isolation

[0583] Transfected cells were harvested post-transfection at 24, 48, or 72 hours. The genomic DNA was extracted from each well of a 96-well plate using 50 μL/well BuccalAmp DNA Extraction solution (Epicentre, Cat. QE09050) per manufacturer's protocol. All DNA samples were subjected to PCR and subsequent NGS analyses, as described herein.

Next-Generation Sequencing ("NGS") Analysis

[0584] To quantitatively determine the efficiency of editing at the target location in the genome, sequencing was utilized to identify the presence of insertions and deletions introduced by gene editing.

[0585] Primers were designed around the target site within the gene of interest (e.g. TTR), and the genomic area of interest was amplified.

[0586] Additional PCR was performed per the manufacturer's protocols (Illumina) to add chemistry for sequencing. The amplicons were sequenced on an Illumina MiSeq instrument. The reads were aligned to a reference genome (e.g., the human reference genome (hg38), the cynomologus reference genome (mf5), the rat reference genome (rn6), or the mouse reference genome (mm10)) after eliminating those having low quality scores. The resulting files containing the reads were mapped to the reference genome (BAM files), where reads that overlapped the target region of interest were selected and the number of wild type reads versus the number of reads which contain an insertion, substitution, or deletion was calculated.

[0587] The editing percentage (e.g., the "editing efficiency" or "percent editing" or "indel frequency") is defined as the total number of sequence reads with insertions/deletions ("indels") or substitutions over the total number of sequence reads, including wild type.

Analysis of Secreted Transthyretin ("TTR") Protein by Western Blot

[0588] Secreted levels of TTR protein in media were determined using western blotting methods. HepG2 cells were transfected as previously described with select guides from Table 1. Media changes were performed every 3 days post transfection. Six days post-transfection, the media was removed, and cells were washed once with media that did not contain fetal bovine serum (FBS). Media without serum was added to the cells and incubated at 37° C. After 4 hrs the media was removed and centrifuged to pellet any debris; cell number for each well was estimated based on the values obtained from a CTG assay on remaining cells and comparison to the plate average. After centrifugation, the media was transferred to a new plate and stored at −20° C. An acetone precipitation of the media was performed to precipitate any protein that had been secreted into the media. Four volumes of ice cold acetone were added to one volume of media. The solution was mixed well and kept at −20° C. for 90 min. The acetone:media mixture was centrifuged at 15,000×g and 4° C. for 15 min. The supernatant was discarded and the retained pellet was air dried to eliminate any residual acetone. The pellet was resuspended in 154, RIPA buffer (Boston Bio Products, Cat. BP-115) plus freshly added protease inhibitor mixture consisting of complete protease inhibitor cocktail (Sigma, Cat. 11697498001) and 1 mM DTT. Lysates were mixed with Laemmli buffer and denatured at 95° C. for 10 minutes. Western blots were run using the NuPage system on 12% Bis-Tris gels (ThermoFisher) per the manufacturer's protocol followed by wet transfer onto 0.45 μm nitrocellulose membrane (Bio-Rad, Cat. 1620115). Blots were blocked using 5% Dry Milk in TBS for 30 minutes on a lab rocker at room temperature. Blots were rinsed with TBST and probed with rabbit α-TTR monoclonal antibody (Abcam, Cat. Ab75815) at 1:1000 in TBST. Alpha-1 antitrypsin was used as a loading control (Sigma, Cat. HPA001292) at 1:1000 in TBST and incubated simultaneously with the TTR primary antibody. Blots were sealed in a bag and kept overnight at 4° C. on a lab rocker. After incubation, blots were rinsed 3 times for 5 min each in TBST and probed with secondary antibodies to Rabbit (ThermoFisher, Cat. PISA535571) at 1:25,000 in TBST for 30 min at room temperature. After incubation, blots were rinsed 3 times for 5 min each in TBST and 2 times with PBS. Blots were visualized and analyzed using a Licor Odyssey system.

Analysis of Intracellular TTR by Western Blot

[0589] The hepatocellular carcinoma cell line, HUH7, was transfected as previously described with select guides from Table 1. Six-days post-transfection, the media was removed and the cells were lysed with 50 μL/well RIPA buffer (Boston Bio Products, Cat. BP-115) plus freshly added protease inhibitor mixture consisting of complete protease inhibitor cocktail (Sigma, Cat. 11697498001), 1 mM DTT, and 250 U/ml Benzonase (EMD Millipore, Cat. 71206-3). Cells were kept on ice for 30 minutes at which time NaCl (1 M final concentration) was added. Cell lysates were thoroughly mixed and retained on ice for 30 minutes. The whole cell extracts ("WCE") were transferred to a PCR plate and centrifuged to pellet debris. A Bradford assay (Bio-Rad, Cat. 500-0001) was used to assess protein content of the lysates. The Bradford assay procedure was completed per the manufacturer's protocol. Extracts were stored at minus 20° C. prior to use. Western blots were performed to assess intracellular TTR protein levels. Lysates were mixed with Laemmli buffer and denatured at 95° C. for 10 min. Western blots were run using the NuPage system on 12% Bis-Tris gels (ThermoFisher) per the manufacturer's protocol followed by wet transfer onto 0.45 μm nitrocellulose membrane (Bio-Rad, Cat. 1620115). After transfer membranes were rinsed thoroughly with water and stained with Ponceau S solution (Boston Bio Products, Cat. ST-180) to confirm complete and even transfer. Blots were blocked using 5% Dry Milk in TBS for 30 minutes on a lab rocker at room temperature. Blots were rinsed with TBST and probed with rabbit α-TTR monoclonal antibody (Abcam, Cat. Ab75815) at 1:1000 in TBST. (3-actin was used as a loading control (ThermoFisher, Cat. AM4302) at 1:2500 in TBST and incubated simultaneously with the TTR primary antibody. Blots were sealed in a bag and kept overnight at 4° C. on a lab rocker. After incubation, blots were rinsed 3 times for 5 minutes each in TBST and probed with secondary antibodies to Mouse and Rabbit (ThermoFisher, Cat. PI35518 and PISA535571) at 1:25,000 each in TBST for 30 min at room

temperature. After incubation, blots were rinsed 3 times for 5 min each in TBST and 2 times with PBS. Blots were visualized and analyzed using a Licor Odyssey system.

Example 2. Screening of dgRNA Sequences

[0590] Cross Screening of TTR dgRNAs in Multiple Cell Types

[0591] Guides in dgRNA format targeting human TTR and the cynomologus matched sequences were delivered to HEK293_Cas9, HUH7 and HepG2 cell lines, as well as primary human hepatocytes and primary cynomolgus monkey hepatocytes as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % edit. The screening data for the guide sequences in Table 1 in all five cell lines are listed below (Table 4 through 11).

[0592] Table 4 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the TTR crRNAs in the human kidney adenocarcinoma cell line, HEK293_Cas9, which constitutively over expresses Spy Cas9 protein.

TABLE 4

TTR editing data in Hek_Cas9 cells transfected with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	26.59	4.73	4.73	0.65	21.87	4.09
CR003336	29.09	4.57	3.31	0.24	25.78	4.32
CR003337	42.72	1.72	5.24	1.62	37.48	0.70
CR003338	52.42	3.28	4.76	0.03	47.66	3.30
CR003339	56.37	4.13	49.39	3.23	6.98	0.91
CR003340	42.38	8.43	27.88	4.31	14.50	4.13
CR003341	20.04	5.26	6.73	1.86	13.31	3.41
CR003342	36.57	5.80	1.19	0.22	35.38	5.59
CR003343	24.36	1.51	4.82	0.43	19.53	1.39
CR003344	33.87	2.93	4.32	0.58	29.54	2.37
CR003345	35.02	7.05	19.00	3.58	16.01	3.48
CR003346	48.33	5.81	33.03	3.12	15.30	2.72
CR003347	21.45	5.57	0.95	0.33	20.50	5.26
CR003348	35.53	5.81	22.32	3.79	13.21	2.03
CR003349	13.19	4.46	8.03	2.81	5.16	1.66
CR003350	22.31	4.25	5.54	0.74	16.77	3.51
CR003351	49.67	3.77	28.42	1.69	21.24	2.22
CR003352	27.90	7.55	4.91	1.35	22.99	6.26
CR003353	25.03	5.16	3.71	0.75	21.32	4.42
CR003354	18.46	2.02	2.56	0.21	15.90	1.89
CR003355	30.60	2.53	6.99	0.80	23.61	1.75
CR003356	32.21	4.71	10.03	1.39	22.19	3.36
CR003357	43.23	6.71	5.38	0.87	37.85	5.88
CR003358	5.44	0.86	1.29	0.16	4.14	0.84
CR003359	37.75	7.50	18.35	3.73	19.40	3.78
CR003360	22.68	3.16	2.70	0.56	19.98	2.60
CR003361	34.45	8.97	8.66	1.66	25.78	7.32
CR003362	9.90	2.66	1.48	0.33	8.41	2.33
CR003363	31.03	10.74	14.77	4.21	16.26	6.54
CR003364	35.65	7.90	19.17	4.24	16.48	3.76
CR003365	36.43	6.20	11.83	1.88	24.61	4.45
CR003366	47.36	6.59	10.10	1.28	37.26	5.32
CR003367	47.11	15.43	28.44	9.11	18.67	6.33
CR003368	40.35	10.13	3.73	0.96	36.61	9.17
CR003369	33.10	7.26	9.06	1.12	24.04	6.16
CR003370	34.22	5.69	4.49	0.67	29.73	5.06
CR003371	25.60	8.33	3.84	1.41	21.76	6.92
CR003372	15.24	7.92	3.25	1.61	11.99	6.31
CR003373	13.55	2.40	1.31	0.21	12.25	2.19
CR003374	10.91	0.88	0.81	0.10	10.10	0.81
CR003375	11.63	3.18	0.78	0.17	10.85	3.05

TABLE 4-continued

TTR editing data in Hek_Cas9 cells transfected with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003376	28.16	4.49	1.35	0.18	26.81	4.52
CR003377	24.70	4.44	2.71	0.54	21.99	3.91
CR003378	20.97	2.67	4.49	0.49	16.48	2.18
CR003379	26.32	2.91	5.34	0.61	20.98	2.30
CR003380	47.64	5.74	3.64	0.24	44.00	5.52
CR003381	22.04	5.74	3.82	1.26	18.23	4.64
CR003382	29.95	3.13	4.46	0.45	25.49	2.73
CR003383	40.47	0.64	25.12	0.45	15.35	0.66
CR003384	17.45	1.32	1.45	0.23	16.00	1.42
CR003385	26.19	5.62	7.36	1.57	18.82	4.06
CR003386	33.12	10.65	2.94	0.63	30.18	10.03
CR003387	24.68	5.93	7.75	1.99	16.92	3.94
CR003388	19.23	4.41	1.41	0.39	17.82	4.07
CR003389	34.18	5.09	10.30	2.12	23.87	3.02
CR003390	28.02	3.77	4.31	0.25	23.71	3.61
CR003391	44.81	4.67	0.61	0.07	44.19	4.63
CR003392	21.67	7.52	0.85	0.26	20.82	7.27

[0593] Table 5 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR crRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in the human hepatocellular carcinoma cell line, HUH7.

TABLE 5

TTR editing data in HUH7 cells transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	31.95	4.50	4.62	0.83	27.57	4.08
CR003336	30.05	4.25	4.14	1.07	26.56	3.55
CR003337	55.72	3.12	8.34	0.93	48.95	2.24
CR003338	75.64	2.03	10.22	1.42	67.06	2.79
CR003339	79.97	4.73	60.55	3.94	20.13	1.02
CR003340	46.93	7.12	33.33	6.01	14.23	1.65
CR003341	20.58	5.98	7.78	1.64	13.20	4.44
CR003342	45.14	7.16	1.23	0.91	44.66	7.68
CR003343	76.13	7.04	9.58	3.49	66.97	6.10
CR003344	64.02	3.33	10.76	1.35	54.40	2.71
CR003345	72.43	2.17	41.33	0.96	32.18	1.37
CR003346	18.07	1.02	13.17	1.39	6.97	3.06
CR003347	32.16	5.50	1.64	0.42	30.79	5.11
CR003348	57.14	10.98	36.08	6.97	22.71	4.42
CR003349	14.14	4.99	9.73	3.26	4.82	1.91
CR003350	52.91	7.61	13.43	2.00	41.64	6.03
CR003351	63.51	4.61	36.87	2.49	27.49	2.14
CR003352	39.68	9.53	7.62	7.42	32.79	7.37
CR003353	69.18	4.59	7.73	2.46	62.87	3.13
CR003354	12.27	3.38	1.25	0.40	11.46	3.23
CR003355	38.83	5.31	9.40	1.81	30.31	3.56
CR003356	49.63	5.55	18.98	2.67	31.31	3.04
CR003357	36.31	5.72	6.37	1.17	30.82	4.68
CR003358	36.50	6.17	10.53	1.56	26.60	4.49
CR003359	66.75	5.84	21.73	2.30	45.97	3.93
CR003360	58.62	8.73	5.01	0.60	55.13	8.19
CR003361	28.68	6.52	6.84	1.26	22.44	5.31
CR003362	26.43	0.83	3.43	0.32	23.76	0.85
CR003363	41.01	7.16	17.83	3.32	23.78	3.97
CR003364	47.13	10.61	24.68	5.15	23.03	5.74
CR003365	60.68	5.25	17.77	1.57	43.82	3.73
CR003366	69.98	8.84	20.77	3.10	50.32	5.69
CR003367	66.29	4.48	33.62	4.14	33.48	0.51

TABLE 5-continued

TTR editing data in HUH7 cells transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003368	31.57	11.73	3.08	0.92	29.69	11.32
CR003369	24.19	6.89	7.12	2.27	17.38	4.76
CR003370	39.16	11.59	4.83	1.79	35.55	10.35
CR003371	40.47	7.68	6.07	0.89	35.65	7.01
CR003372	21.52	6.02	4.89	1.66	17.25	4.58
CR003373	27.29	4.45	3.31	0.66	25.12	4.12
CR003374	3.10	0.68	0.45	0.24	2.87	0.54
CR003375	2.38	0.22	0.26	0.14	2.25	0.12
CR003376	19.42	5.60	1.37	0.45	18.55	5.28
CR003377	34.93	5.47	5.59	0.88	29.89	4.71
CR003378	40.73	4.63	9.73	1.85	32.27	2.91
CR003379	19.18	5.17	3.38	0.77	16.48	4.32
CR003380	31.76	5.81	3.29	0.57	29.29	5.42
CR003381	99.70	0.17	1.92	0.20	99.70	0.17
CR003382	34.47	5.71	0.14	0.16	34.47	5.71
CR003383	42.89	10.14	2.14	0.56	41.19	9.67
CR003384	17.03	1.95	0.84	0.30	16.29	1.84
CR003386	69.40	19.41	0.53	0.23	69.34	19.32
CR003387	25.64	3.69	0.23	0.07	25.55	3.62
CR003388	59.48	4.29	3.88	0.68	56.45	4.45
CR003389	62.32	1.97	13.19	1.18	50.90	1.02
CR003390	18.97	4.82	3.31	0.91	16.49	3.98
CR003391	61.31	13.21	2.10	0.51	59.70	12.76
CR003392	28.37	8.58	1.93	0.73	26.98	7.94

[0594] Table 6 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR and control crRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in the human hepatocellular carcinoma cell line, HepG2.

TABLE 6

TTR editing data in HepG2 cells transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261 (control)	49.16	7.45	16.46	3.46	32.71	4.06
CR001262 (control)	63.33	5.66	59.88	4.92	3.45	0.86
CR001263 (control)	39.19	6.98	37.59	8.01	1.60	1.92
CR001264 (control)	57.09	12.14	47.47	9.25	9.61	2.89
CR003335	37.19	2.12	32.96	1.67	4.23	0.59
CR003336	31.31	5.47	30.48	5.10	0.83	0.75
CR003337	61.93	2.68	59.28	2.11	2.65	1.39
CR003338	68.00	6.09	65.40	6.78	2.60	1.17
CR003339	68.21	7.67	12.37	1.47	55.84	6.31
CR003340	37.76	6.01	6.12	1.95	31.65	4.07
CR003341	15.60	5.49	9.94	3.38	5.66	2.13
CR003342	11.06	6.71	10.78	6.69	0.28	0.03
CR003343	45.41	15.20	40.05	10.79	5.36	5.20
CR003344	33.43	6.11	29.81	5.09	3.62	1.13
CR003345	10.58	9.25	6.12	5.38	4.45	3.87
CR003346	0.13	0.05	0.07	0.02	0.05	0.03
CR003347	22.57	10.94	21.08	11.19	1.49	0.90
CR003348	38.44	10.45	17.04	5.04	21.40	5.89
CR003349	8.36	2.19	4.46	1.75	3.91	0.76
CR003350	29.60	5.17	25.16	4.56	4.44	0.67
CR003351	57.54	5.67	31.98	2.63	25.57	3.08

TABLE 6-continued

TTR editing data in HepG2 cells transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003352	44.28	8.71	39.51	7.10	4.77	1.79
CR003353	60.40	11.37	56.71	9.95	3.68	1.45
CR003354	5.36	3.94	4.84	3.41	0.53	0.71
CR003355	15.80	5.38	12.36	4.23	3.44	1.16
CR003356	9.39	1.82	5.67	1.03	3.72	0.92
CR003357	45.83	10.66	42.37	8.47	3.46	2.28
CR003358	35.93	7.34	28.66	7.76	7.27	1.77
CR003359	64.44	14.90	48.79	14.32	15.65	1.94
CR003360	41.31	12.23	38.94	10.60	2.38	1.78
CR003361	14.05	4.79	11.47	4.35	2.58	0.43
CR003362	17.44	4.34	16.50	4.86	0.94	0.52
CR003363	42.65	9.90	28.58	6.95	14.07	3.01
CR003364	51.88	7.67	31.03	2.67	20.85	5.03
CR003365	46.88	15.78	35.77	13.49	11.11	2.30
CR003366	54.69	9.10	46.20	8.98	8.49	1.11
CR003367	45.55	8.19	24.28	6.57	21.27	1.62
CR003368	51.55	8.60	48.34	9.87	3.22	1.36
CR003369	22.62	4.01	17.11	4.47	5.51	2.52
CR003370	28.51	6.94	24.88	6.17	3.62	1.45
CR003371	15.91	4.17	14.07	4.02	1.84	0.22
CR003372	14.57	2.47	12.14	2.08	2.42	0.40
CR003373	17.69	8.41	15.92	6.44	1.77	1.97
CR003374	5.43	0.53	5.12	0.62	0.31	0.36
CR003375	2.06	0.04	1.96	0.06	0.10	0.03
CR003376	14.41	3.01	14.16	2.93	0.24	0.10
CR003377	16.30	2.85	15.29	2.59	1.02	0.59
CR003378	8.16	3.83	6.82	3.43	1.34	0.61
CR003379	19.74	4.24	17.70	4.30	2.04	0.33
CR003380	17.08	2.48	14.78	1.18	2.30	1.36
CR003381	6.81	3.48	6.18	3.82	0.63	0.44
CR003382	1.73	0.14	1.58	0.12	0.15	0.03
CR003383	6.35	1.67	6.19	1.68	0.16	0.04
CR003384	3.37	0.88	3.12	0.94	0.25	0.09
CR003385	53.94	9.41	46.32	10.66	7.62	1.29
CR003386	2.71	0.76	2.15	0.77	0.56	0.53
CR003387	1.39	0.15	1.27	0.17	0.12	0.02
CR003388	9.33	4.47	7.76	4.56	1.56	0.10
CR003389	31.84	6.09	27.27	5.96	4.57	1.21
CR003390	24.88	4.96	22.44	3.41	2.44	2.25
CR003391	48.78	14.41	48.28	14.44	0.50	0.52
CR003392	14.64	5.25	14.32	4.95	0.33	0.36
CR005298	42.65	10.94	21.29	8.16	21.36	2.87
CR005299	38.61	5.57	36.32	3.99	2.30	2.11
CR005300	64.34	9.55	53.20	6.59	11.15	3.33
CR005301	37.04	5.32	33.39	3.85	3.65	1.89
CR005302	33.21	2.19	30.93	2.43	2.29	0.24
CR005303	21.63	6.05	20.55	5.80	1.08	0.25
CR005304	62.82	3.28	8.07	1.22	54.75	4.27
CR005305	13.51	3.58	12.30	3.49	1.21	0.84
CR005306	24.07	5.24	21.20	5.03	2.87	1.10
CR005307	22.03	3.86	7.70	1.35	14.33	4.15

[0595] Table 7 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR dgRNAs electroporated with Spy Cas9 protein (RNP) in primary human hepatocytes.

TABLE 7

TTR editing data in primary human hepatocytes electroporated with Spy Cas9 protein loaded with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003335	72.20	4.53	69.70	4.36	2.50	0.30
CR003336	39.17	3.04	38.43	3.20	0.70	0.17
CR003337	54.27	2.70	53.23	3.05	1.30	0.26
CR003338	83.03	4.84	80.87	4.63	2.13	0.25
CR003339	43.00	2.66	8.93	1.86	34.07	1.72
CR003340	12.03	1.55	5.60	1.32	6.50	0.53
CR003341	11.43	0.71	7.03	0.50	4.40	1.21
CR003342	32.77	3.63	31.87	3.28	0.90	0.35
CR003343	77.10	2.21	75.63	2.01	1.50	0.36
CR003344	39.40	3.86	33.30	2.52	6.10	1.31
CR003345	48.07	6.24	34.53	2.95	13.57	3.74
CR003346	35.67	1.80	20.83	1.65	14.83	1.66
CR003347	82.30	5.93	81.97	5.98	0.43	0.15
CR003348	28.53	1.79	11.30	2.46	17.27	0.86
CR003349	4.10	0.17	2.33	0.46	1.87	0.25
CR003350	28.13	3.50	22.40	2.41	5.73	1.22
CR003351	51.77	5.11	30.83	3.32	20.97	2.43
CR003352	29.83	4.18	25.63	3.67	4.30	0.56
CR003353	84.83	4.68	82.23	4.05	2.63	0.74
CR003354	2.50	0.36	2.43	0.32	0.03	0.06
CR003355	12.53	1.54	10.60	2.36	1.97	1.17
CR003356	9.97	2.68	7.80	2.01	2.23	0.85
CR003357	36.23	4.02	35.47	4.11	0.77	0.61
CR003358	5.70	1.42	4.93	1.36	0.80	0.26
CR003359	63.77	7.07	56.33	5.81	7.50	1.35
CR003360	32.23	3.09	31.67	2.97	0.63	0.31
CR003361	4.10	0.36	3.73	0.42	0.37	0.06
CR003362	7.03	1.30	6.87	1.20	0.20	0.20
CR003363	9.43	8.22	7.80	6.86	1.63	1.44
CR003364	23.30	5.20	16.93	4.96	6.53	0.55
CR003365	42.37	3.88	35.57	1.88	6.83	2.00
CR003366	34.70	3.26	31.63	2.98	3.10	1.15
CR003367	39.20	5.31	22.93	4.14	16.37	1.46
CR003368	28.47	3.29	27.63	2.90	0.80	0.66
CR003369	3.67	1.16	3.30	1.06	0.40	0.20
CR003370	15.27	1.75	14.43	1.72	0.90	0.20
CR003371	16.20	2.13	14.47	2.37	1.87	0.81
CR003372	12.17	2.69	10.47	2.63	1.77	0.12
CR003373	0.87	0.21	0.83	0.25	0.07	0.12
CR003374	0.80	0.17	0.70	0.26	0.10	0.10
CR003375	1.33	1.10	1.27	1.08	0.07	0.06
CR003376	1.90	1.06	1.87	1.00	0.03	0.06
CR003377	10.23	1.53	10.13	1.51	0.10	0.10
CR003378	4.60	1.92	3.87	1.19	0.73	0.67
CR003379	6.57	1.00	6.30	0.70	0.27	0.31
CR003380	5.37	2.57	5.27	2.54	0.10	0.10
CR003381	6.20	2.74	5.83	2.61	0.50	0.10
CR003382	8.40	2.07	8.10	1.87	0.43	0.21
CR003383	8.57	0.75	3.37	0.67	5.27	0.46
CR003384	1.87	0.67	1.73	0.57	0.23	0.12
CR003385	40.87	6.86	38.43	6.41	2.53	0.45
CR003386	4.90	1.20	4.47	1.14	0.47	0.25
CR003387	1.87	0.25	1.70	0.26	0.20	0.10
CR003388	5.70	0.40	5.47	0.40	0.27	0.12
CR003389	27.67	2.76	27.20	2.88	0.50	0.36
CR003390	15.97	3.86	15.80	3.99	0.23	0.15
CR003391	29.77	3.85	29.57	3.85	0.27	0.06
CR003392	4.13	1.21	4.00	1.15	0.17	0.06
CR005298	39.90	2.92	22.37	3.04	17.57	0.42
CR005299	8.65	0.78	8.30	0.99	0.35	0.21
CR005300	57.47	1.69	53.47	1.86	4.10	0.92
CR005301	25.37	1.65	24.00	2.26	1.60	0.82
CR005302	61.10	5.20	60.10	4.77	1.00	0.46
CR005303	53.57	8.52	53.07	8.36	0.53	0.47
CR005304	67.00	5.80	5.53	1.37	61.63	6.98
CR005305	3.83	0.78	3.53	0.61	0.40	0.17
CR005306	9.43	1.63	8.07	2.17	1.37	0.72
CR005307	8.17	1.20	5.20	0.87	3.00	0.82

[0596] Table 8 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR and control dgRNAs transfected with Spy Cas9 protein (RNP) in primary human hepatocytes.

TABLE 8

TTR editing data in primary human hepatocytes transfected with Spy Cas9 loaded with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261	32.51	1.00	12.50	0.47	20.01	0.59
CR001262	50.09	1.48	45.25	1.69	4.83	0.31
CR001263	15.25	2.41	14.83	2.37	0.42	0.10
CR001264	45.30	3.48	23.87	2.09	21.43	1.68
CR003335	51.14	4.27	49.51	4.04	1.63	0.25
CR003336	30.70	2.41	30.11	2.48	0.58	0.11
CR003337	49.43	4.75	47.54	4.49	1.88	0.47
CR003338	61.34	3.55	59.13	3.44	2.22	0.11
CR003339	45.06	9.83	8.85	1.65	36.21	8.34
CR003340	10.44	2.44	5.94	1.34	4.50	1.16
CR003341	19.66	3.67	14.64	3.31	5.02	0.37
CR003342	20.66	2.55	19.85	2.54	0.81	0.15
CR003343	43.25	4.47	41.61	4.26	1.63	0.33
CR003344	35.45	13.12	30.97	11.72	4.48	1.51
CR003345	28.90	6.33	21.00	5.23	7.91	1.81
CR003346	4.11	1.36	2.27	0.53	1.84	0.85
CR003347	66.35	4.48	66.11	4.51	0.24	0.08
CR003348	23.18	2.16	13.74	1.17	9.44	0.99
CR003349	10.83	1.57	9.00	1.41	1.83	0.32
CR003350	24.84	2.74	19.77	1.91	5.07	0.89
CR003351	40.28	1.31	23.92	0.70	16.36	0.78
CR003352	30.48	1.93	27.27	2.31	3.21	0.38
CR003353	61.54	4.13	59.38	4.04	2.16	0.11
CR003354	10.31	1.47	10.07	1.50	0.23	0.11
CR003355	19.11	0.92	17.69	0.79	1.42	0.44
CR003356	7.53	1.78	6.24	1.51	1.29	0.32
CR003357	49.35	2.53	48.45	2.54	0.90	0.13
CR003358	31.62	5.97	25.95	5.03	5.67	1.04
CR003359	59.47	6.05	50.96	5.69	8.51	0.54
CR003360	31.47	4.12	30.27	4.21	1.19	0.22
CR003361	13.08	1.48	12.52	1.45	0.56	0.18
CR003362	11.65	1.24	11.10	1.06	0.56	0.36
CR003363	27.65	2.84	21.47	2.39	6.18	0.61
CR003364	35.29	3.50	23.93	2.63	11.36	1.16
CR003365	47.78	3.67	40.24	3.12	7.54	0.72
CR003366	42.74	3.41	37.95	2.88	4.79	0.60
CR003367	31.19	4.60	16.06	2.66	15.13	1.94
CR003368	34.83	5.05	33.83	5.09	1.00	0.10
CR003369	12.98	0.26	11.67	0.21	1.31	0.11
CR003370	20.06	1.79	18.80	1.65	1.26	0.28
CR003371	18.80	2.73	17.23	2.34	1.57	0.43
CR003372	17.56	2.26	15.74	2.16	1.81	0.10
CR003373	3.64	0.29	3.44	0.30	0.19	0.07
CR003374	2.65	0.33	2.52	0.33	0.14	0.02
CR003375	5.04	0.66	4.93	0.66	0.11	0.01
CR003376	5.00	1.10	4.86	1.10	0.14	0.03
CR003377	12.77	2.00	12.45	1.84	0.31	0.18
CR003378	8.66	1.90	8.24	1.74	0.42	0.19
CR003379	16.86	2.62	16.51	2.62	0.34	0.08
CR003380	8.17	1.42	7.71	1.47	0.46	0.10
CR003381	7.15	0.73	6.88	0.67	0.27	0.07
CR003382	2.44	0.06	2.28	0.05	0.15	0.03
CR003383	4.76	0.40	4.52	0.42	0.24	0.09
CR003384	3.56	0.26	3.39	0.26	0.17	0.01
CR003385	41.15	6.06	38.15	5.59	3.00	0.48
CR003386	3.22	0.25	2.97	0.27	0.25	0.02
CR003387	1.79	0.11	1.68	0.09	0.11	0.04
CR003388	5.43	1.03	4.38	1.00	1.05	0.25
CR003389	19.87	4.39	19.19	4.52	0.68	0.24
CR003390	16.09	2.84	15.85	2.91	0.24	0.09
CR003391	34.72	8.29	34.46	8.35	0.26	0.06
CR003392	10.07	1.06	9.93	1.02	0.14	0.04
CR005298	32.07	1.02	21.12	1.02	10.95	0.15

TABLE 8-continued

TTR editing data in primary human hepatocytes transfected with Spy Cas9 loaded with dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR005299	19.37	0.61	18.79	0.51	0.58	0.13
CR005300	57.23	6.24	53.62	5.44	3.61	0.87
CR005301	31.37	3.02	29.53	2.88	1.84	0.15
CR005302	48.29	5.22	47.32	5.32	0.97	0.14
CR005303	36.45	4.83	36.06	4.72	0.39	0.12
CR005304	49.45	6.85	4.32	0.31	45.13	6.74
CR005305	7.07	1.43	6.73	1.30	0.34	0.17
CR005306	18.81	1.82	16.24	1.57	2.57	0.35
CR005307	18.73	1.68	10.18	0.92	8.55	0.88

[0597] Table 9 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR and control dgRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in primary human hepatocytes.

TABLE 9

TTR editing data in primary human hepatocytes transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR001261	32.33	4.95	5.83	1.63	26.47	3.30
CR001262	41.50	4.71	34.43	3.31	7.13	1.42
CR001263	10.23	3.61	9.40	3.20	0.90	0.44
CR001264	42.80	0.50	11.90	1.32	30.90	1.80
CR003335	36.43	2.98	33.03	2.31	3.40	0.70
CR003336	16.93	3.78	16.20	3.41	0.80	0.44
CR003337	19.30	1.57	18.10	1.44	1.23	0.15
CR003338	36.30	9.55	33.73	9.27	2.73	0.49
CR003339	36.43	1.21	2.27	0.15	34.23	1.31
CR003340	24.97	2.78	1.83	0.23	23.17	2.66
CR003341	15.83	1.38	6.80	0.53	9.07	0.81
CR003342	22.10	1.27	20.60	0.57	1.50	0.71
CR003343	55.03	0.38	52.40	0.53	2.60	0.44
CR003344	31.50	1.30	22.40	1.31	9.20	0.10
CR003345	50.65	2.90	32.30	1.56	18.45	1.20
CR003346	19.97	1.94	5.63	0.55	14.33	1.72
CR003347	41.47	3.59	41.33	3.63	0.17	0.06
CR003348	18.00	0.87	2.30	0.66	15.80	0.61
CR003349	2.57	0.81	0.90	0.35	1.70	0.46
CR003350	26.63	4.25	16.33	2.45	10.33	1.75
CR003351	26.50	1.61	10.20	0.92	16.37	0.97
CR003352	16.80	5.03	11.73	3.86	5.07	1.14
CR003353	53.73	6.01	49.50	5.82	4.43	0.75
CR003354	2.97	0.95	2.87	0.85	0.13	0.12
CR003355	12.07	2.61	10.47	2.08	1.63	0.59
CR003356	7.27	0.72	4.70	0.53	2.67	0.21
CR003357	25.93	4.55	25.30	4.22	0.63	0.35
CR003358	3.90	0.79	2.73	0.45	1.17	0.51
CR003359	32.93	4.34	25.67	3.25	7.33	1.24
CR003360	14.90	4.85	14.13	4.66	0.90	0.52
CR003361	3.53	0.60	2.73	0.55	0.87	0.15
CR003362	6.60	1.47	6.17	1.45	0.47	0.21
CR003363	16.70	1.08	11.80	0.79	4.93	0.60
CR003364	15.63	2.45	6.73	0.81	8.93	1.70
CR003365	26.90	3.05	20.23	2.02	6.67	1.16
CR003366	24.53	1.26	20.47	1.45	4.07	0.23
CR003367	37.33	1.40	14.03	0.40	23.37	1.25
CR003368	11.10	1.91	10.53	1.90	0.60	0.10
CR003369	1.60	0.46	0.90	0.20	0.70	0.36
CR003370	2.83	0.57	2.33	0.40	0.50	0.17
CR003371	3.40	0.80	2.67	0.75	0.73	0.15

TABLE 9-continued

TTR editing data in primary human hepatocytes transfected with Spy Cas9 mRNA and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003372	1.77	0.75	1.13	0.57	0.63	0.23
CR003373	1.40	0.36	1.00	0.35	0.37	0.12
CR003374	0.27	0.21	0.27	0.21	0.03	0.06
CR003375	1.27	0.64	1.23	0.58	0.03	0.06
CR003376	2.83	0.81	2.73	0.81	0.13	0.06
CR003377	17.53	6.35	16.97	6.11	0.57	0.25
CR003378	9.80	1.37	8.50	1.21	1.37	0.15
CR003379	13.20	1.18	12.00	1.05	1.27	0.15
CR003380	2.93	0.58	2.47	0.57	0.47	0.15
CR003381	4.07	1.21	3.33	0.96	0.73	0.25
CR003382	0.97	0.25	0.97	0.25	0.00	0.00
CR003383	15.70	3.22	2.07	0.35	13.70	2.82
CR003384	1.70	0.62	1.50	0.56	0.20	0.10
CR003385	36.77	0.70	33.23	0.74	3.60	0.26
CR003386	8.27	1.63	8.20	1.57	0.13	0.06
CR003387	7.87	1.58	7.80	1.64	0.03	0.06
CR003388	12.97	1.30	11.87	1.21	1.17	0.25
CR003389	44.27	1.72	41.47	1.59	2.83	0.15
CR003390	20.23	2.08	18.73	1.92	1.60	0.17
CR003391	15.47	5.87	15.20	5.72	0.30	0.10
CR003392	2.43	0.55	2.37	0.59	0.07	0.06
CR005298	15.70	2.79	4.13	0.87	11.60	2.00
CR005299	9.43	0.68	8.93	0.68	0.60	0.00
CR005300	31.53	3.44	27.60	2.77	3.97	0.76
CR005301	6.77	1.44	5.47	0.96	1.40	0.61
CR005302	34.80	7.17	33.67	7.01	1.13	0.21
CR005303	35.50	5.90	35.00	5.81	0.50	0.10
CR005304	45.27	4.71	0.83	0.15	44.47	4.57
CR005305	7.53	1.06	5.93	1.10	1.60	0.10
CR005306	9.97	0.38	7.13	0.23	2.87	0.12
CR005307	12.90	2.43	3.67	0.61	9.30	1.80

[0598] Table 10 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR dgRNAs electroporated with Spy Cas9 protein (RNP) in primary cyno hepatocytes.

TABLE 10

TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003336	8.18	1.93	8.10	1.94	0.07	0.01
CR003337	24.94	5.80	24.10	4.71	0.84	1.10
CR003338	44.94	9.99	44.89	9.97	0.05	0.01
CR003339	8.95	0.89	4.93	0.64	4.02	0.25
CR003340	12.53	2.22	7.72	0.13	4.80	2.09
CR003341	8.43	10.53	7.66	9.91	0.77	0.63
CR003344	35.72	4.67	33.81	5.29	1.91	0.61
CR003345	52.92	3.26	30.74	0.78	22.19	2.48
CR003346	1.91	0.86	1.82	0.82	0.09	0.04
CR003347	72.41	0.38	72.15	0.73	0.25	0.34
CR003352	1.25	0.20	1.16	0.21	0.09	0.01
CR003353	4.75	0.43	4.67	0.47	0.08	0.04
CR003358	20.47	0.30	19.01	0.51	1.46	0.21
CR003359	46.17	1.14	40.66	2.00	5.51	0.86
CR003360	29.47	0.63	29.05	1.00	0.42	0.37
CR003361	4.53	0.14	4.46	0.18	0.08	0.04
CR003362	4.59	0.80	4.36	0.77	0.22	0.03
CR003363	15.64	1.92	13.24	2.65	2.39	0.73
CR003364	19.62	2.54	14.27	2.72	5.35	0.17

TABLE 10-continued

TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR003365	10.31	1.81	9.33	1.80	0.97	0.01
CR003366	18.52	0.71	17.62	0.33	0.90	0.39
CR003368	18.56	3.89	18.30	3.77	0.26	0.11
CR003369	1.53	0.25	1.28	0.40	0.25	0.15
CR003370	2.52	0.64	2.40	0.63	0.12	0.01
CR003371	1.83	0.38	1.69	0.41	0.14	0.03
CR003372	2.15	0.30	1.83	0.33	0.32	0.04
CR003382	10.86	2.04	8.54	1.93	2.33	0.11
CR003383	8.86	2.30	4.31	0.69	4.55	1.61
CR003384	3.75	0.35	2.50	0.37	1.25	0.02
CR003385	30.96	1.61	26.84	2.20	4.12	0.59
CR003386	5.54	1.42	3.51	1.26	2.03	0.15
CR003387	4.72	0.03	4.55	0.08	0.17	0.11
CR003388	6.81	0.17	6.59	0.28	0.22	0.11
CR003389	18.83	4.99	18.05	4.92	0.78	0.07
CR003390	16.87	3.88	16.49	3.48	0.39	0.39
CR003391	36.44	1.09	35.73	1.37	0.71	0.28
CR003392	7.02	0.97	6.63	0.59	0.38	0.37
CR005299	13.48	2.96	13.23	2.74	0.26	0.22
CR005301	46.76	1.75	46.34	2.19	0.42	0.44
CR005302	1.34	0.19	1.26	0.19	0.08	0.00
CR005303	59.28	1.05	58.72	1.06	0.56	0.00
CR005305	11.28	0.39	11.13	0.39	0.15	0.00
CR005307	4.56	0.71	2.01	0.49	2.55	0.21

[0599] Table 11 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific TTR dgRNAs electroporated with Spy Cas9 protein (RNP) on primary cyno hepatocytes.

TABLE 11

TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and cyno specific dgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
CR000689	24.41	1.67	18.11	2.41	6.30	0.93
CR005364	27.70	0.74	0.58	0.29	27.11	0.60
CR005365	64.94	2.03	0.10	0.04	64.85	2.05
CR005366	77.00	1.17	0.33	0.27	76.67	0.99
CR005367	50.79	0.53	0.53	0.25	50.26	0.36
CR005368	27.60	2.07	0.33	0.45	27.27	2.32
CR005369	42.01	0.33	8.09	0.55	33.92	0.31
CR005370	63.52	3.21	0.59	0.33	62.93	2.88
CR005371	8.42	0.69	0.31	0.12	8.10	0.57
CR005372	17.98	1.39	0.83	0.77	17.16	0.71

Example 3. Screening of sgRNA Sequences

[0600] Cross Screening of TTR sgRNAs in Multiple Cell Types

[0601] Guides in modified sgRNA format targeting human and/or cyno TTR were delivered to primary human hepatocytes and primary cyno hepatocytes as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % edit. The screening data for the guide sequences in Table 2 in both cell lines are listed below (Table 12 through 15).

[0602] Table 12 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR sgRNAs transfected with Spy Cas9 protein (RNP) in primary human hepatocytes.

TABLE 12

TTR editing data in primary human hepatocytes transfected with Spy Cas9 protein and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000480	81.80	1.98	77.15	2.19	4.70	0.28
G000481	46.90	1.71	27.77	3.88	19.43	4.76
G000482	66.67	2.35	56.57	4.14	10.10	1.85
G000483	47.90	6.56	19.57	3.37	28.50	3.25
G000484	62.97	0.90	29.23	0.21	33.83	0.95
G000485	56.07	3.37	53.07	2.84	3.13	0.60
G000486	69.73	6.86	9.83	1.93	59.93	5.63
G000487	67.30	2.75	65.27	3.41	2.07	1.06
G000488	61.27	1.95	26.30	1.55	35.00	1.30
G000489	60.17	2.75	51.07	3.18	9.43	0.45
G000490	55.90	7.88	46.13	7.55	9.80	0.69
G000491	74.30	1.55	70.27	2.37	4.33	0.72
G000492	60.97	5.81	57.90	4.64	3.13	1.35
G000493	41.40	3.08	38.90	3.29	2.67	0.35
G000494	62.23	3.30	61.47	3.25	0.77	0.31
G000495	50.80	1.85	45.80	1.25	5.37	0.64
G000496	72.33	1.63	44.73	2.14	27.67	1.46
G000497	59.67	1.40	51.10	1.14	8.73	0.71
G000498	72.80	3.75	60.17	3.12	12.70	0.72
G000499	66.40	3.55	65.23	3.72	1.17	0.38
G000500	65.53	1.21	62.00	1.11	3.83	0.40
G000501	60.93	1.91	55.13	1.43	6.00	0.56

[0603] Table 13 shows the average and standard deviation at 12.5 nM for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR sgRNAs co-transfected with Spy Cas9 mRNA (SEQ ID NO:2) in primary human hepatocytes.

TABLE 13

TTR editing data in primary human hepatocytes transfected with Spy Cas9 mRNA and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000480	73.28	0.61	59.85	0.13	13.47	0.51
G000481	34.30	5.26	14.62	2.59	19.77	2.72
G000482	40.93	3.95	27.70	2.92	13.25	0.97
G000483	27.82	2.93	4.05	0.51	23.85	2.43
G000484	43.37	6.79	13.98	2.61	29.48	4.15
G000485	30.82	5.76	28.87	5.50	1.97	0.28
G000486	59.13	5.62	2.82	0.86	56.37	4.92
G000487	49.57	0.99	47.38	0.89	2.27	0.24
G000488	49.40	5.05	11.98	1.40	37.48	3.68
G000489	24.25	2.82	14.17	2.01	10.28	1.38
G000490	24.72	2.35	19.38	2.04	5.38	0.41
G000491	45.93	1.22	42.42	1.06	3.60	0.33
G000492	34.65	2.21	32.45	2.01	2.22	0.25
G000493	11.55	1.35	10.65	1.58	0.97	0.30
G000494	26.22	4.03	25.17	3.89	1.07	0.15
G000495	47.77	1.88	43.40	1.91	4.45	0.17
G000496	63.30	2.60	11.08	2.10	52.25	0.67
G000497	40.33	3.32	34.48	2.71	5.85	0.61
G000498	60.02	5.42	45.20	4.34	14.90	1.08
G000499	39.30	6.04	38.58	5.86	0.77	0.12
G000500	35.50	0.61	32.47	0.49	3.10	0.18
G000501	40.32	1.50	33.82	2.04	6.62	0.55

TABLE 13-continued

TTR editing data in primary human hepatocytes transfected with Spy Cas9 mRNA and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000567	27.28	7.59	17.35	4.72	10.02	2.94
G000568	43.75	5.83	43.00	5.81	0.80	0.18
G000570	68.42	3.64	68.08	3.61	0.35	0.00
G000571	20.47	3.41	14.47	2.72	6.13	0.78
G000572	55.42	8.13	41.62	6.48	13.85	1.60

[0604] Table 14 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR sgRNAs electroporated with Spy Cas9 protein (RNP) on primary cyno hepatocytes. Note that guides G000480 and G000488 have one mismatch to cyno, which may compromise their editing efficiency in cyno cells.

TABLE 14

TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and sgRNAs

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000480	10.20	0.56	9.83	0.81	0.37	0.25
G000481	69.13	8.62	33.73	2.67	35.50	11.23
G000482	75.17	2.34	55.23	2.00	20.03	0.85
G000485	22.93	0.95	22.00	0.82	1.07	0.21
G000486	79.90	0.79	11.90	0.85	68.07	0.35
G000488	9.63	0.50	5.37	0.38	4.27	0.35
G000489	67.53	1.15	53.53	1.56	14.17	0.64
G000490	61.67	0.72	54.47	1.10	7.27	1.23
G000491	66.20	1.11	64.37	0.47	1.90	0.70
G000493	50.13	0.74	48.07	1.69	2.10	0.98
G000494	81.53	0.71	79.57	0.49	2.07	0.67
G000498	91.37	1.48	68.50	1.64	22.87	1.50
G000499	83.40	0.36	82.00	0.20	1.43	0.55
G000500	45.20	3.66	42.60	3.80	2.63	0.25

[0605] Table 15 shows the average and standard deviation for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific TTR sgRNAs electroporated with Spy Cas9 protein (RNP) on primary cyno hepatocytes.

TABLE 15

TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and cyno specific sgRNAs (e.g., those having an analogous human gRNA, See Table 3)

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000502	95.10	0.96	13.97	1.69	81.27	2.60
G000503	58.53	2.40	52.07	1.68	6.50	2.46
G000504	77.17	0.96	69.73	1.29	7.53	0.57
G000505	95.53	1.06	95.50	1.01	0.10	0.10
G000506	89.43	1.36	86.90	1.64	3.07	0.42
G000507	71.17	3.22	67.03	2.39	4.60	1.65
G000508	45.63	3.01	41.57	2.95	4.17	0.91
G000509	93.03	0.81	43.60	1.30	49.73	1.76

TABLE 15-continued

TTR editing data in primary cyno hepatocytes electroporated with Spy Cas9 protein and cyno specific sgRNAs (e.g., those having an analogous human gRNA, See Table 3)

GUIDE ID	Avg % Edit	Std Dev % Edit	Avg % Insert	Std Dev % Insert	Avg % Deletion	Std Dev % Deletion
G000510	90.80	0.53	89.13	0.40	1.77	0.12
G000511	62.77	1.63	60.87	1.55	2.00	0.35

Example 4. Screening of Lipid Nanoparticle (LNP) Formulations Containing Spy Ca9 mRNA and sgRNA

[0606] Cross screening of LNP formulated TTR sgRNAs with Spy Cas9 mRNA in primary human hepatocytes and primary cyno hepatocytes.

[0607] Lipid nanoparticle formulations of modified sgRNAs targeting human TTR and the cyno matched sgRNA sequences were tested on primary human hepatocytes and primary cyno hepatocytes in a dose response curve. Primary human and cyno hepatocytes were plated as described in Example 1. Both cell lines were incubated at 37° C., 5% CO_2 for 24 hours prior to treatment with LNPs. The LNPs used in the experiments detailed in Tables 16-19 were prepared using the Nanoassemblr™ procedure, each containing the specified sgRNA and Cas9 mRNA (SEQ ID NO:2), each having Lipid. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 45:44:9:2 molar ratio, respectively, and had a N:P ratio of 4.5. LNPs were incubated in hepatocyte maintenance media containing 6% cyno serum at 37° C. for 5 minutes. Post incubation the LNPs were added onto the primary human or cyno hepatocytes in an 8 point 2-fold dose response curve starting at 100 ng mRNA. The cells were lysed 72 hours post treatment for NGS analysis as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on highest % editing at 12.5 ng mRNA input and 3.9 nM guide concentration. The dose response curve data for the guide sequences in both cell lines is shown in FIGS. **4** through **7**. The % editing at 12.5 ng mRNA input and 3.9 nM guide concentration are listed below (Table 16 through 18).

[0608] Table 16 shows the average and standard deviation at 12.5 ng of cas9 mRNA for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary human hepatocytes as dose response curves. G000570 exhibited an uncharacteristic dose response curve compared to the other sgRNAs which may be an artifact of the experiment. The data are shown graphically in FIG. **4**.

TABLE 16

TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO: 2) and sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA Avg % Edit	Std Dev % Edit
G000480	59.33	0.73
G000481	24.37	0.37

TABLE 16-continued

TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO: 2) and sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA Avg % Edit	Std Dev % Edit
G000482	19.10	2.64
G000483	7.37	0.67
G000484	16.67	1.23
G000485	14.23	2.36
G000486	61.33	2.59
G000487	17.37	0.95
G000488	44.80	3.00
G000489	16.85	0.06
G000490	10.53	1.90
G000491	31.60	2.33
G000492	15.87	0.44
G000493	7.33	0.73
G000494	6.37	1.07
G000495	23.97	1.66
G000496	30.73	3.76
G000497	15.10	3.30
G000498	24.43	1.30
G000499	16.07	1.67
G000500	23.57	2.44
G000501	32.30	2.49
G000567	48.95	1.06
G000568	54.60	3.68
G000570	88.30	1.84
G000572	55.45	1.20

[0609] Table 17 shows the average and standard deviation at 12.5 ng of mNRA and 3.9 nM guide concentration for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested TTR sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG. **5**.

TABLE 17

TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO: 2) and sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA, Avg % Edit	Std Dev % Edit
G000480	0.73	0.15
G000481	49.20	1.39
G000482	26.13	5.33
G000483	0.73	0.60
G000484	0.10	0.00
G000485	1.43	1.02
G000489	31.87	2.40
G000490	15.23	1.08
G000491	6.37	0.38
G000492	0.70	0.28
G000493	7.63	1.14
G000494	14.30	1.06
G000495	0.73	0.06
G000497	0.23	0.06
G000498	37.90	1.42
G000499	14.63	0.70
G000500	10.47	0.32
G000501	1.37	0.31
G000567	0.10	0.00
G000568	9.25	0.21
G000570	17.30	0.85
G000571	20.20	2.26
G000572	30.60	0.42

[0610] Table 18 shows the average and standard deviation at 12.5 ng of mRNA and 3.9 nM guide concentration for % Edit, % Insertion (Ins), and % Deletion (Del) for the tested cyno specific TTR sgRNAs formulated in lipid nanoparticles with Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG. **6**.

TABLE 18

TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA (SEQ ID NO: 2) and cyno matched sgRNAs

GUIDE ID	12.5 ng mRNA, 3.9 nM sgRNA % Edit	Std Dev % Edit
G000502	80.70	0.14
G000506	60.13	0.70
G000509	74.47	7.28
G000510	61.87	2.54

Cross Screening of LNP Formulated TTR sgRNAs with Spy Cas9 mRNA in Primary Human Hepatocytes and Primary Cyno Hepatocytes

[0611] Lipid nanoparticle formulations of modified sgRNAs targeting human TTR and the cyno matched sgRNA sequences were tested on primary human hepatocytes and primary cyno hepatocytes in a dose response curve. Primary human and cyno hepatocytes were plated as described in Example 1. Both cell lines were incubated at 37° C., 5% CO_2 for 24 hours prior to treatment with LNPs. The LNPs used in the experiments detailed in Tables 20-22 were prepared using the cross-flow procedure described above but purified using PD-10 columns (GE Healthcare Life Sciences) and concentrated using Amicon centrifugal filter units (Millipore Sigma), each containing the specified sgRNA and Cas9 mRNA (SEQ ID NO:1). The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had a N:P ratio of 6.0. LNPs were incubated in hepatocyte maintenance media containing 6% cyno serum at 37° C., 5% CO_2 for 5 minutes. Post incubation the LNPs were added onto the primary human or cyno hepatocytes in an 8 point 3-fold dose response curve starting at 300 ng mRNA. The cells were lysed 72 hours post treatment for NGS analysis as described in Example 1. Percent editing was determined for crRNAs comprising each guide sequence across each cell type and the guide sequences were then rank ordered based on EC50 values and maximum editing percent. The dose response curve data for the guide sequences in both cell lines is shown in FIGS. **4** through **7**. The EC 50 values and maximum editing percent are listed below (Table 19 through 22).

[0612] Table 19 shows the EC50 and maximum editing the tested human specific TTR sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary human hepatocytes as dose response curves. The data are shown graphically in FIG. **4**.

TABLE 19

TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA and human specific sgRNAs

GUIDE ID	EC50	Max Editing
G000480	0.10	98.69
G000481	1.43	87.05
G000482	0.65	97.02

TABLE 19-continued

TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA and human specific sgRNAs

GUIDE ID	EC50	Max Editing
G000483	1.88	77.39
G000484	0.95	94.14
G000488	0.72	95.83
G000489	1.38	86.33
G000490	1.52	94.16
G000493	2.42	63.95
G000494	1.28	75.70
G000499	0.63	96.31
G000500	0.39	88.70
G000568	0.78	95.72
G000570	0.23	98.22
G000571	2.21	71.28
G000572	0.42	97.94

[0613] Table 20 shows the EC50 and maximum editing the tested human specific TTR sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG. **16**.

TABLE 20

TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA and human specific sgRNAs

GUIDE ID	EC50	Max Editing
G000480	5.28	20.32
G000481	0.93	95.07
G000482	0.89	97.47
G000483	4.40	56.52
G000484	3.47	0.22
G000488	11.56	21.63
G000489	1.79	89.21
G000490	3.09	90.76
G000493	4.97	61.15
G000494	2.77	60.84
G000499	2.00	74.94
G000500	4.42	58.04
G000567	1.76	97.06
G000568	1.87	87.93
G000570	2.00	96.73
G000571	1.55	97.03
G000572	0.79	100.31

[0614] Table 21 shows the EC50 and maximum editing the tested cyno matched TTR sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary human hepatocytes as dose response curves. The data are shown graphically in FIG. **17**.

TABLE 21

TTR editing data in primary human hepatocytes treated with LNP formulated Spy Cas9 mRNA and cyno specific sgRNAs

GUIDE ID	EC50	Max Editing
G000502	0.70	91.50
G000504	5.16	7.16
G000505	3.57	13.48
G000506	1.26	89.49

[0615] Table 22 shows the EC50 and maximum editing the tested cyno matched TTR sgRNAs formulated in lipid nanoparticles with U-depleted Spy Cas9 mRNA on primary cyno hepatocytes as dose response curves. The data are shown graphically in FIG. **18**.

TABLE 22

TTR editing data in primary cyno hepatocytes treated with LNP formulated Spy Cas9 mRNA and cyno specific sgRNAs

GUIDE ID	EC50	Max Editing
G000502	0.26	100.05
G000503	2.26	83.41
G000504	1.42	98.04
G000505	1.10	99.97
G000506	0.66	99.18

Example 5. Off-Target Analysis of TTR dgRNAs and sgRNAs Off-Target Analysis of TTR Guides

[0616] An oligo insertion based assay (See, e.g., Tsai et al., Nature Biotechnology 33, 187-197; 2015) was used to determine potential off-target genomic sites cleaved by Cas9 targeting TTR. Forty-five dgRNAs from Table 1 (and two control guides with known off-target profiles) were screened in the HEK293_Cas9 cells. The human embryonic kidney adenocarcinoma cell line HEK293 constitutively expressing Spy Cas9 ("HEK293_Cas9") was cultured in DMEM media supplemented with 10% fetal bovine serum and 500 μg/ml G418. Cells were plated at a density of 30,000 cells/well in a 96-well plate 24 hours prior to transfection. Cells were transfected with Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) per the manufacturer's protocol. Cells were transfected with a lipoplex containing individual crRNA (15 nM), trRNA (15 nM), and donor oligo with (10 nM) Lipofectamine RNAiMAX (0.3 μL/well) and OptiMem. Cells were lysed 24 hours post transfection and genomic DNA was extracting using Zymo's Quick gDNA 96 Extraction kit (catalog # D3012) following the manufacturer's recommended protocol. The gDNA was quantified using the Qubit High Sensitivity dsDNA kit (Life Technologies). Libraries were prepared per the previously described method in Tsai et al, 2015 with minor modifications. Sequencing was performed on Illumina's MiSeq and HiSeq 2500. The assay identified potential off-target sites for some of the crRNAs which are plotted in FIG. **2**.

[0617] Table 23 shows the number of off-target integration sites detected in HekCas9 cells transfected with TTR dgRNAs along with a double stranded DNA oligo donor sequence.

TABLE 23

Number of off-target integration sites detected for TTR dgRNAs via an oligo insertion based assay

GUIDE ID	# Sites
CR003335	0
CR003336	2
CR003337	10
CR003338	2
CR003339	3
CR003340	0
CR003342	0
CR003343	2
CR003344	0
CR003345	0
CR003346	0

TABLE 23-continued

Number of off-target integration sites detected for TTR dgRNAs via an oligo insertion based assay

GUIDE ID	# Sites
CR003347	1
CR003348	3
CR003351	1
CR003352	2
CR003353	2
CR003355	1
CR003356	4
CR003357	3
CR003359	6
CR003360	0
CR003363	4
CR003365	3
CR003366	1
CR003367	1
CR003368	2
CR003369	2
CR003377	0
CR003380	0
CR003382	34
CR003383	1
CR003385	3
CR003386	1
CR003387	6
CR003388	2
CR003389	2
CR003390	1
CR003391	0
CR003392	0
CR005298	0
CR005300	0
CR005301	0
CR005302	1
CR005303	1
CR005304	0

[0618] Additionally, a subset of the guides was assessed for off-target potential as modified sgRNAs in the Hek_Cas9 cells via the oligo based insertion method described above. The off-target results were plotted in FIG. **4**.

[0619] Table 24 shows the number of off-target integration sites detected in HekCas9 cells transfected with TTR sgRNAs along with a double stranded DNA oligo donor sequence.

TABLE 24

Number of off-target integration sites detected for TTR sgRNAs via an insertion detection method

GUIDE ID	# Sites
G000480	11
G000481	3
G000482	13
G000483	5
G000484	7
G000485	22
G000486	12
G000487	14
G000488	0
G000489	19
G000490	12
G000491	28
G000492	97
G000493	7
G000494	4
G000495	13
G000496	1

TABLE 24-continued

Number of off-target integration sites detected for TTR sgRNAs via an insertion detection method

GUIDE ID	# Sites
G000497	26
G000498	82
G000499	4
G000500	46
G000501	4
G000567	9
G000568	937
G000570	19
G000571	16
G000572	15

Example 6. Targeted Sequencing for Validating Potential Off-Target Sites

[0620] The HEK293_Cas9 cells used in Example 5 for detecting potential off-targets constitutively overexpress Cas9, leading to a higher number of potential off-target "hits" as compared to a transient delivery paradigm in various cell types. Further, when delivering sgRNAs (as opposed to dgRNAs), the number of potential off-target hits may be further inflated as sgRNA molecules are more stable than dgRNAs (especially when chemically modified). Accordingly, potential off-target sites identified by an oligo insertion method as used in Example 5 may be validated using targeted sequencing of the identified potential off-target sites.

[0621] In one approach, primary hepatocytes are treated with LNPs comprising Cas9 mRNA and a sgRNA of interest (e.g., a sgRNA having potential off-target sites for evaluation). The primary hepatocytes are then lysed and primers flanking the potential off-target site(s) are used to generate an amplicon for NGS analysis. Identification of indels at a certain level may validate potential off-target site, whereas the lack of indels found at the potential off-target site may indicate a false positive in the HEK293_Cas9 cell assay.

Example 7. Phenotypic Analysis

Western Blot Analysis of Secreted TTR

[0622] The hepatocellular carcinoma cell line, HepG2, was transfected as described in Example 1 with select guides from Table 1 in triplicate. Two days post-transfection, one replicate was harvested for genomic DNA and analysis by NGS sequencing for editing efficiency. Five days post-transfection, media without serum was replaced on one replicate. After 4 hrs the media was harvested for analysis of secreted TTR by WB as previously described. The data for % edit for each guide and reduction of extracellular TTR is provided in FIG. **7**.

Western Blot Analysis of Intracellular TTR

[0623] The hepatocellular carcinoma cell line, HUH7, was transfected as described in Example 1 with crRNA comprising the guides from Table 1. The transfected pools of cells were retained in tissue culture and passaged for further analysis. At seven days post-transfection, cells were harvested and whole cell extracts (WCEs) were prepared and subjected to analysis by Western Blot as previously described.

[0624] WCEs were analyzed by Western Blot for reduction of TTR protein. Full length TTR protein has a predicted molecular weight of ~16 kD. A band at this molecular weight was observed in the control lanes in the Western Blot.

[0625] Percent reduction of TTR protein was calculated using the Licor Odyssey Image Studio Ver 5.2 software. GAPDH was used as a loading control and probed simultaneously with TTR. A ratio was calculated for the densitometry values for GAPDH within each sample compared to the total region encompassing the TTR band. Percent reduction of TTR protein was determined after the ratios were normalized to control lanes. Results are shown in FIG. **8**.

Example 8. LNP Delivery to Humanized TTR Mice and Mice Having Wt (Murine) TTR

[0626] Mice humanized with respect to the TTR gene were dosed with LNP formulations 701-704 containing the guides indicated in Table 25 (5 mice per formulation). These humanized TTR mice were engineered such that a region of the endogenous murine TTR locus was deleted and replaced with an orthologous human TTR sequence so that the locus encodes a human TTR protein. For comparison, 6 mice with murine TTR were dosed with LNP700, containing a guide (G000282) targeting murine TTR. LNPs with Formulation Numbers 1-5 in Table 25 were prepared using the Nanoassemblr™ procedure as described above while LNPs with Formulation Numbers 6-16 were prepared using the cross-flow procedure described above but purified using PD-10 columns (GE Healthcare Life Sciences) and concentrated using Amicon centrifugal filter units (Millipore Sigma). As negative controls, mice of the corresponding genotype were dosed with vehicle alone (Tris-saline-sucrose buffer (TSS)). The background of the humanized TTR mice administered LNPs with Formulation Numbers 2-5 in Table 25 was 50% 12956/SvEvTac 50% C57BL/6NTac; the background of the humanized TTR mice administered LNPs having Formulation Numbers 6-16 in Table 25 as well as the mice with murine TTR (administered LNP700, Formulation Number 1) was 75% C57BL/6NTac 25% 12956/SvEvTac.

TABLE 25

LNP formulations for dosing humanized TTR mice.

Formulation Number	LNP	Guide	RNA concentration (mg/ml)	N:P Ratio	Molar Ratios (Lipid A, Cholesterol, DSPC, and PEG2k-DMG, respectively)
1	LNP700	G000282	0.53	4.5	45:44:9:2
2	LNP701	G000481	0.46	4.5	45:44:9:2
3	LNP702	G000489	0.61	4.5	45:44:9:2
4	LNP703	G000494	0.57	4.5	45:44:9:2
5	LNP704	G000499	0.59	4.5	45:44:9:2
6	LNP1148	G000481	0.73	4.5	45:44:9:2
7	LNP1152	G000499	0.45	6.0	50:38:9:3
8	LNP1153	G000482	0.53	6.0	50:38:9:3
9	LNP1155	G000571	0.70	6.0	50:38:9:3
10	LNP1156	G000572	0.58	6.0	50:38:9:3
11	LNP1157	G000480	0.84	6.0	50:38:9:3
12	LNP1159	G000488	0.79	6.0	50:38:9:3
13	LNP1160	G000493	0.71	6.0	50:38:9:3
14	LNP1161	G000500	0.66	6.0	50:38:9:3
15	LNP1162	G000567	0.69	6.0	50:38:9:3
16	LNP1163	G000570	0.66	6.0	50:38:9:3

[0627] LNPs having Formulation numbers 1-5 contained Cas9 mRNA of SEQ ID NO:2 and LNPs having Formulation Numbers 6-16 contained Cas9 mRNA of SEQ ID NO: 1, all in a 1:1 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in the molar ratios recited in Table 25, respectively. Dosing with LNPs having Formulation Numbers 1-5 was at 2 mg/kg (total RNA content) and dosing with LNPs having Formulation Numbers 6-16 was at 1 mg/kg (total RNA content). Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis. Liver editing results for Formulation Numbers 1-5 are shown in FIG. **9** and indicate editing of the human TTR sequence with each of the four guides tested at a level >35% editing (mean values) with G000494 and G000499 providing values near 60%. Liver editing results for formulation numbers 6-8, 10-13, and 15-16 are shown in FIG. **13** and Table 26, which show efficient editing of the human TTR sequence with each of the formulations tested. Greater than 38% editing was seen for all formulations, with several formulations providing editing values greater than 60%. Formulations 9 and 14 are not shown due to the design of the PCR amplicon and a resulting low number of sequencing reads.

[0628] The level of human TTR in serum was measured in the mice provided formulation numbers 6-8, 10-13, and 15-16. See FIG. **14**B. FIG. **14**A is a repeat of FIG. **13** provided for comparison purposes. Knockdown of serum human TTR was detected for each formulation tested, which correlated with the amount of editing detected in liver (See FIG. **14**A vs **14**B, Table 26).

TABLE 26

GUIDE ID	% Editing	Serum TTR(% TSS)
TSS (vehicle)	0.06	100
G481	61.28	10.52
G499	65.66	8.39
G482	70.86	4.65
G572	73.52	2.11
G480	77.34	3.48
G488	59.125	27.78
G493	38.55	49.73
G567	47.525	44.24
G570	45.5	41.73
G571	33.88	11.39
G500	44.44	34.28

[0629] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000480, G000488, G000489 and G000502. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and having a N:P ratio of 6. Dosing was at 1, 0.3, 0.1, or 0.03 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG. **26**A and serum human TTR levels in FIG. **26**B-C. A dose response for both editing and serum TTR levels was evident.

[0630] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000481, G000482, G000486 and G000499. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had an N:P ratio of 6. Dosing was at 1, 0.3, or 0.1 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG. **27**A and serum human TTR levels in FIG. **27**B-C. A dose response for both editing and serum TTR levels was evident.

[0631] In another set of experiments, humanized TTR mice were dosed with LNP formulations across a range of doses with guides G000480, G000481, G000486, G000499 and G000502. The formulations contained Cas9 mRNA (SEQ ID NO: 1) in a 1:2 ratio by weight to the guide. The LNPs contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio, respectively, and had an N:P ratio of 6. Dosing was at 1, 0.3, or 0.1 mg/kg (n=5/group). The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis and serum human TTR levels were measured as described above. Results for liver editing are shown in FIG. **28**A and serum human TTR levels in FIG. **28**B-C. A dose response for both editing and serum TTR levels was evident.

[0632] In separate experiments using wild type CD-1 mice, an LNP formulation comprising guide G000502, which is cross homologous between mouse and cyno, was tested in a dose response study. The formulation contained Cas9 mRNA (SEQ ID NO: 1) in a 1:1 ratio by weight to the guide. The LNP contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 45:44:9:2 molar ratio, respectively, and having a N:P ratio of 6. Dosing was at 1, 0.3, 0.1, 0.03, or 0.01 mg/kg (n=5/group). Liver editing results were determined using primers designed to amplify the region of interest for NGS analysis. Results for liver editing are shown in FIG. **15**A and serum mouse TTR levels in FIG. **15**B. A dose response for both editing and serum TTR levels was evident.

Example 9. LNP Delivery to Mice in Multiple Doses

[0633] Mice (females from Charles River Laboratory, aged approximately 6-7 weeks) were dosed with an LNP formulation LNP705, prepared using cross-flow and TFF procedures as described above containing G000282 ("G282") and Cas9 mRNA (SEQ ID NO: 2) in a 1:1 ratio by weight and a total RNA concentration of 0.5 mg/ml. The LNP had an N:P ratio of 4.5 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 45:44:9:2 molar ratio, respectively. Groups were dosed either once weekly up to one, two, three, or four weeks (QWx1-4) or once monthly up to two or three months (QMx2-3). Dosages were 0.5 mg/kg or 1 mg/kg (total RNA content). Control groups received a single dose on day 1 of 0.5, 1, or 2 mg/kg. Each group contained 5 mice. Serum TTR was analyzed by ELISA and at necropsy the liver, spleen and muscle were each collected for NGS editing analysis. Groups are shown in Table 27. X=sacrifice and necropsy. MPK=mg/kg.

TABLE 27

Study Groups										
Group	Duration/ Dose Regimen	Dose (MPK)	Total Dose (MPK) Given	Dose Day 1	Dose Day 8	Dose Day 15	Dose Day 22	NX Day 28	Dose Day 43	NX Day 49
1	4 Week Multi Dose/ QW × 4	0 (TSS control)	0	X	X	X	X	X		
2	2 Month	1	3	X			X		X	X
3	Multi Dose/ QM × 3	0.5	1.5	X			X		X	X
4	1 Month	1	2	X			X	X		
5	Multi Dose/ QM × 2	0.5	1	X			X	X		
6	4 Week	1	4	X	X	X	X	X		
7	Multi Dose/ QW × 4	0.5	2	X	X	X	X	X		
8	3 Week	1	3		X	X	X	X		
9	Multi Dose/ QW × 3	0.5	1.5		X	X	X	X		
10	2 Week	1	2			X	X	X		
11	Multi Dose/ QW × 2	0.5	1			X	X	X		

TABLE 27-continued

Study Groups										
Group	Duration/ Dose Regimen	Dose (MPK)	Total Dose (MPK) Given	Dose Day 1	Dose Day 8	Dose Day 15	Dose Day 22	NX Day 28	Dose Day 43	NX Day 49
12	Single	1	1				X	X		
13	Dose/	0.5	0.5				X	X		
14	QW × 1	2	2				Day 26	Day 32		

[0634] Table 28 and FIGS. **10**A-**11**B show serum TTR level results (% KD=% knockdown). Table 29 and FIGS. **12**A-C show liver editing results.

TABLE 28

Serum TTR Results.

Time Regimen	Dose	Serum TTR (μg/mL)	Serum TTR (% KD)
QWx4	TSS	1190.7	—
QMx3	0.5	245.01	79.42
QMx2	0.5	776.73	34.77
QWx4	0.5	347.43	70.82
QWx3	0.5	405.70	65.93
QWx2	0.5	432.25	63.70
QWx1	0.5	804.06	32.47
QMx3	1	91.95	92.28
QMx2	1	176.81	85.15
QWx4	1	119.52	89.96
QWx3	1	167.15	85.96
QWx2	1	130.98	89.00
QWx1	1	573.02	51.88
QWx1	2	219.07	81.60

TABLE 29

Liver Editing Results.

Time Regimen	Dose	Liver Editing (%)
QWx4	TSS	0.38
QMx3	0.5	48.18
QMx2	0.5	36.66
QWx4	0.5	56.03
QWx3	0.5	51.35
QWx2	0.5	34.77
QWx1	0.5	24.16
QMx3	1	63.40
QMx2	1	57.37
QWx4	1	62.89
QWx3	1	59.22
QWx2	1	60.12
QWx1	1	35.16
QWx1	2	60.57

[0635] The results show that it is possible to build up a cumulative dose and effect with multiple administrations over time, including at weekly or monthly intervals, to achieve increasing editing levels and % KD of TTR.

Example 10. RNA Cargo: Varying mRNA and gRNA Ratios

[0636] This study evaluated in vivo efficacy in mice of different ratios of gRNA to mRNA. CleanCap™ capped Cas9 mRNAs with the ORF of SEQ ID NO: 4, HSD 5' UTR, human albumin 3' UTR, a Kozak sequence, and a poly-A tail were made by IVT synthesis as indicated in Example 1 with N1-methylpseudouridine triphosphate in place of uridine triphosphate.

[0637] LNP formulations prepared from the mRNA described and G282 (SEQ ID NO: 124) as described in Example 1 with Lipid A, cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:3 molar ratio and with an N:P ratio of 6. The gRNA:Cas9 mRNA weight ratios of the formulations were as shown in FIGS. **19**A and **19**B.

[0638] For in vivo characterization, the LNPs were administered to mice at 0.1 mg total RNA (mg guide RNA+mg mRNA) per kg (n=5 per group). At 7-9 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured as described in Example 1. Serum TTR and liver editing results are shown in FIGS. **19**A and **19**B. Negative control mice were dosed with TSS vehicle.

[0639] In addition, the above LNPs were administered to mice at a constant mRNA dose of 0.05 mg mRNA per kg (n=5 per group), while varying the gRNA dose from 0.06 mg per kg to 0.4 mg per kg. At 7-9 days post-dose, animals were sacrificed, blood and the liver were collected, and serum TTR and liver editing were measured. Serum TTR and liver editing results are shown in FIG. **19**C and FIG. **19**D. Negative control mice were dosed with TSS vehicle.

Example 11. Off-Target Analysis of TTR sgRNAs in Primary Human Hepatocyes

[0640] Off-target analysis of sgRNAs targeting TTR was performed in primary human hepatocytes (PHH) as described in Example 5, with the following modifications. PHH were plated at a density of 33,000 cells per well on collagen-coated 96-well plates as described in Example 1. Twenty-four hours post plating, cells were washed with media and transfected using Lipofectamine RNAiMAX (ThermoFisher, Cat. 13778150) as described in Example 1. Cells were transfected with a lipoplex containing 100 ng Cas9 mRNA, immediately followed by the addition of another lipoplex containing 25 nM of the sgRNA and 12.5 nM of the donor oligo (0.3 μL/well). Cells were lysed 48 hours post-transfection and gDNA was extracted and analyzed as further described in Example 5. The data is graphically represented in FIG. **20**.

[0641] Table 30 shows the number of off-target integration sites detected in PHH, and compares to the number of sites that were detected in the HekCas9 cells used in Example 5. Fewer sites were detected in PHH for every guide tested as compared to the HekCas9 cell line, with no unique sites detected in PHH alone.

TABLE 30

Number of off-target integration sites detected for TTR sgRNAs in PHH via an oligo insertion based assay

GUIDE ID	# Sites in PHH	# Sites in HekCas9 cells (Example 5)
G000480	2	11
G000481	0	3
G000482	2	13
G000483	0	5
G000484	0	7
G000485	3	22
G000486	0	12
G000487	0	14
G000488	0	0
G000489	2	19
G000490	0	12
G000491	7	28
G000492	5	97
G000493	1	7
G000494	0	4
G000495	1	13
G000496	0	1
G000497	3	26
G000498	19	82
G000499	1	4
G000500	12	46
G000501	0	4
G000567	0	9
G000568	11	936
G000570	1	19
G000571	1	16
G000572	2	15

[0642] Following the identification of potential off-target sites in PHH via the oligo insertion assay, certain potential sites were further evaluated by targeted amplicon sequencing, e.g., as described in Example 6. In addition to the potential off-target sites identified by the oligo insertion strategy, additional potential off-target sites identified by in silico prediction were included in the analysis.

[0643] To this end, PHH were treated with LNPs comprising 100 ng of Cas9 mRNA (SEQ ID NO:1) and the gRNA of interest at 14.68 nM (in a 1:1 ratio by weight), as described in Example 4. The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. Following LNP treatment, isolated genomic DNA was analyzed by NGS (e.g., as described in Examples 1 and 6) to determine whether indels could be detected at the potential off-target site, which would be indicative of a Cas9-mediated cleavage event. Tables 31 and 32 show the potential off-target sites that were evaluated for the gRNAs G000480 and G000486, respectively.

[0644] As shown in FIGS. **21**A-B and **22**A-B and Table 33 below, indels were detected at low levels for only two of the potential off-target sites identified by the oligo insertion assay for G000480, and only one for G000486. No indels were detected at any of the in silico predicted sites for either guide. Further, indels were only detected at these sites using a near-saturating dose of LNP, as the indel rates observed at the on-target sites for G000480 and G000486 were ~97% and ~91%, respectively (See Table 33). The genomic coordinates of these sites are also reported in Tables 31 and 32, and each correspond to sequences that do not code for any protein.

[0645] A dose response assay was then performed in order to determine the highest dose of LNP in which no off-targets were detected. PHH were treated with LNPs comprising either G000480 or G000486 as described in Example 4. The doses ranged across 11 points with respect to gRNA concentration (0.001 nM, 0.002 nM, 0.007 nM, 0.02 nM, 0.06 nM, 0.19 nM, 0.57 nM, 1.72 nM, 5.17 nM, 15.51 nM, and 46.55 nM). As represented by the dashed vertical line in FIGS. **21**A-B and **22**A-B, the highest concentrations (with respect to the concentration of gRNA) at which the potential off-target sites were no longer detected for G000480 and G000486 were 0.57 nM and 15.51 nM, respectively, which resulted in on-target indel rates of 84.60% and 89.50%, respectively.

TABLE 31

Identified potential off target sites via insertion detection and in silico prediction for G000480 evaluated via targeted amplicon sequencing

GUIDE ID	Off-target (OT) Site ID	Assay Used	Chromosomal Coordinates (hg38)	Strand
G000480	INS-OT.1	Insertion Detection	chr7: 94767406-94767426	+
G000480	INS-OT.2	Insertion Detection	chr2: 192658562-192658582	+
G000480	INS-OT.3	Insertion Detection	chr7: 4834390-4834410	+
G000480	INS-OT.4	Insertion Detection	chr20: 9216118-9216138	–
G000480	INS-OT.5	Insertion Detection	chr10: 12547071-12547091	+
G000480	INS-OT.6	Insertion Detection	chr6: 168377978-168377998	–
G000480	INS-OT.7	Insertion Detection	chr12: 114144669-114144689	–
G000480	INS-OT.8	Insertion Detection	chr10: 7376755-7376775	+
G000480	INS-OT.9	Insertion Detection	chr2: 52950299-52950319	+
G000480	INS-OT.10	Insertion Detection	chr8: 56579165-56579185	–
G000480	INS-OT.11	Insertion Detection	chr1: 189992255-189992275	+
G000480	PRED-OT.1	in silico prediction	chr10:12547071-12547091	+
G000480	PRE-DOT.2	in silico prediction	chrX: 119702782-119702802	+
G000480	PRED-OT.3	in silico prediction	chr1: 116544586-116544606	+
G000480	PRED-OT.4	in silico prediction	chr6: 88282884-88282904	+
G000480	PRED-OT.6	in silico prediction	chr5: 121891868-121891888	+
G000480	PRED-OT.7	in silico prediction	chr3: 52544945-52544965	+
G000480	PRED-OT.8	in silico prediction	chr15: 36949639-36949659	+
G000480	PRED-OT.9	in silico prediction	chr5: 33866486-33866506	+
G000480	PRED-OT.10	in silico prediction	chr5: 159755754-159755774	+

TABLE 31-continued

Identified potential off target sites via insertion detection and in silico prediction for G000480 evaluated via targeted amplicon sequencing

GUIDE ID	Off-target (OT) Site ID	Assay Used	Chromosomal Coordinates (hg38)	Strand
G000480	PRED-OT.11	in silico prediction	chr5: 31349859-31349879	+
G000480	PRED-OT.12	in silico prediction	chr11: 79485652-79485672	+
G000480	PRED-OT.13	in silico prediction	chr15: 29448864-29448884	+
G000480	PRED-OT.14	in silico prediction	chr5: 171153565-171153585	+
G000480	PRED-OT.15	in silico prediction	chr9: 84855273-84855293	+
G000480	PRED-OT.16	in silico prediction	chr6: 159953060-159953080	+
G000480	PRED-OT.17	in silico prediction	chr16: 51849024-51849044	+
G000480	PRED-OT.18	in silico prediction	chr3: 24108809-24108829	+
G000480	PRED-OT.19	in silico prediction	chr18: 41118310-41118330	+
G000480	PRED-OT.20	in silico prediction	chr10: 108975241-108975261	+
G000480	PREDO-T.21	in silico prediction	chr1: 44683633-44683653	+
G000480	PRED-OT.22	in silico prediction	chr2: 196214849-196214869	+
G000480	PRED-OT.23	in silico prediction	chr9: 117353544-117353564	+
G000480	PRED-OT.24	in silico prediction	chr1: 55583322-55583342	+
G000480	PRED-OT.25	in silico prediction	chr12: 28246827-28246847	+
G000480	PRED-OT.26	in silico prediction	chr4: 54545361-54545381	+
G000480	PRED-OT.27	in silico prediction	chr13: 22364836-22364856	+
G000480	PRED-OT.28	in silico prediction	chr13: 80816049-80816069	+
G000480	PRED-OT.29	in silico prediction	chr7: 39078622-39078642	+
G000480	PRED-OT.30	in silico prediction	chr2: 59944386-59944406	+

"INS-OT.N" refers to an off-target site ID detected by oligo insertion, where N is an integer specified above; "PRED-OT.N" refers to an off-target site ID predicted via in silico methods, where N is an integer specified above.

TABLE 32

Identified potential off target sites via insertion detection and in silico prediction for G000486 evaluated via targeted amplicon sequencing

GUIDE ID	Off-target (OT) Site ID	Assay Used	Chromosomal Coordinates (hg38)	Strand
G000486	INS-OT.1	Insertion Detection	chr14: 77332157-77332177	+
G000486	INS-OT.2	Insertion Detection	chr14: 54672059-54672079	–
G000486	INS-OT.3	Insertion Detection	chr4: 108513169-108513189	–
G000486	INS-OT.4	Insertion Detection	chr5: 91397023-91397043	–
G000486	INS-OT.5	Insertion Detection	chr9:116626135-116626155	–
G000486	INS-OT.6	Insertion Detection	chr6: 73201226-73201246	+
G000486	INS-OT.7	Insertion Detection	chr16: 89368352-89368372	–
G000486	INS-OT.8	Insertion Detection	chr7: 56308371-56308391	–
G000486	INS-OT.9	Insertion Detection	chr21:43605667-43605687	+
G000486	INS-OT.10	Insertion Detection	chr5: 26758030-26758050	+
G000486	INS-OT.11	Insertion Detection	chr17: 30656428-30656448	+
G000486	INS-OT.12	Insertion Detection	chr8: 130486452-130486472	+
G000486	PRED-OT.1	in silico prediction	chr11: 44707064-44707084	+
G000486	PRED-OT.2	in silico prediction	chr5: 50775396-50775416	+
G000486	PRED-OT.3	in silico prediction	chr4: 141623949-141623969	+
G000486	PRED-OT.4	in silico prediction	chr1: 223481186-223481206	+
G000486	PRED-OT.5	in silico prediction	chr6: 39951487-39951507	+
G000486	PRED-OT.6	in silico prediction	chrY: 5456047-5456067	+
G000486	PRED-OT.8	in silico prediction	chr6: 129868719-129868739	+
G000486	PRED-OT.9	in silico prediction	chrX: 80450312-80450332	+
G000486	PRED-OT.10	in silico prediction	chr7: 27256771-27256791	+
G000486	PRED-OT.11	in silico prediction	chr3: 181416528-181416548	+
G000486	PRED-OT12	in silico prediction	chr7: 146425020-146425040	+
G000486	PRED-OT.13	in silico prediction	chr3: 16980977-16980997	+
G000486	PRED-OT.14	in silico prediction	chr7: 118161002-118161022	+
G000486	PRED-OT.15	in silico prediction	chr6: 102220539-102220559	+
G000486	PRED-OT.16	in silico prediction	chr12: 127278991-127279011	+
G000486	PRED-OT.17	in silico prediction	chr2: 67686631-67686651	+
G000486	PRED-OT.18	in silico prediction	chr1: 114467665-114467685	+
G000486	PRED-OT.19	in silico prediction	chr3: 194514436-194514456	+
G000486	PRED-OT.20	in silico prediction	chr14: 31767581-31767601	+
G000486	PRED-OT.21	in silico prediction	chr16: 28706209-28706229	+
G000486	PRED-OT.22	in silico prediction	chr8: 110526279-110526299	+
G000486	PRED-OT.23	in silico prediction	chr19: 2899814-2899834	+
G000486	PRED-OT.25	in silico prediction	chr3: 130760261-130760281	+
G000486	PRED-OT.26	in silico prediction	chr11: 2506046-2506066	+
G000486	PRED-OT.27	in silico prediction	chr2: 153918318-153918338	+

TABLE 32-continued

Identified potential off target sites via insertion detection and in silico prediction for G000486 evaluated via targeted amplicon sequencing

GUIDE ID	Off-target (OT) Site ID	Assay Used	Chromosomal Coordinates (hg38)	Strand
G000486	PRED-OT.28	in silico prediction	chr14: 40590226-40590246	+
G000486	PRED-OT.29	in silico prediction	chr18: 806650-806670	+
G000486	PRED-OT.30	in silico prediction	chr2: 117707480-117707500	+

"INS-OT.N" refers to an off-target site ID detected by oligo insertion, where N is an integer specified above; "PRED-OT.N" refers to an off-target site ID predicted via in silico methods, where N is an integer specified.

TABLE 33

Detected Off Target sites in PHH treated with LNP containing 100 ng mRNA and 31.03 nM gRNA

GUIDE ID	Off-target (OT) Site ID	Site Type	Indel Frequency (using LNP with 100 ng Cas9 mRNA and 14.68 nM gRNA)	Indel Frequency std. dev.
G000480	n/a	On-Target	97.33%	1.10%
G000480	INS-OT.2	Off-Target	1.43%	0.40%
G000480	INS-OT.4	Off-Target	0.97%	0.25%
G000486	n/a	On-Target	91.33%	1.97%
G000486	INS-OT.4	Off-Target	0.47%	0.06%

Example 12. LNP Delivery to Humanized Mouse Model of ATTR

[0646] A well-established humanized transgenic mouse model of hereditary ATTR amyloidosis that expresses the V30M pathogenic mutant form of human TTR protein was used in this Example. This mouse model recapitulates the TTR deposition phenotype in tissues observed in ATTR patients, including within the peripheral nervous system and gastrointestinal (GI) tract (See Santos et al., Neurobiol Aging. 2010 February; 31(2):280-9).

[0647] Mice (aged approximately 4-5 months) were dosed with LNP formulations prepared using the cross-flow and TFF procedures as described in Example 1. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. The LNPs contained Cas9 mRNA (SEQ ID NO: 1) and either G000481 ("G481") or a non-targeting control guide G000395 ("G395"; SEQ ID NO: 273), in a 1:1 ratio of gRNA:mRNA by weight.

[0648] Mice were injected via the lateral tail vein as described in Example 1 with a single 1 mg/kg (of total RNA content) dose of LNP with an n=10/group. At 8 weeks post treatment, the mice were euthanized for sample collection. Human TTR protein levels were measured in serum and cerebrospinal fluid (CSF) by ELISA as previously described by Butler et al., Amyloid. 2016 June; 23(2):109-18. Liver tissue was assayed for editing levels as described in Example 1. Other tissues (stomach, colon, sciatic nerve, dorsal root ganglion (DRG)) were collected and processed for semi-quantitative immunohistochemistry as previously described by Gonçalves et al., Amyloid. 2014 September; 21(3): 175-184. Statistical analysis for the immunohistochemistry data was performed using Mann Whitney test with a p-value<0.0001.

[0649] As shown in FIG. **23**A-B, robust editing (49.4%) of TTR was observed in livers of the humanized mice following the single dose of LNP comprising G481, with no editing detected in the control group. Analysis of the editing events demonstrated that 96.8% of the events were insertions, with the remainder deletions.

[0650] As shown in FIG. **24**A-B, TTR protein levels were decreased in plasma but not in CSF from the treated mice, with greater than 99% knockdown of TTR plasma levels observed ($p<0.001$).

[0651] The near complete knockdown of TTR observed in the plasma of treated animals correlated with the clearance of TTR protein amyloid deposition in the assayed tissues. As shown in FIG. **25**, control mice exhibited amyloid staining in tissues which resembles the pathophysiology observed in human subjects with ATTR. Decreasing circulating TTR by editing the HuTTR V30M locus resulted in a dramatic decrease of amyloid deposition in tissues. Approximately 85% or better reduction in TTR staining was observed across the treated tissues 8 weeks post-treatment (FIG. **25**).

Example 13. TTR mRNA Knockdown in Primary Human Hepatocytes (PHH)

[0652] In one experiment, PHH were cultured and treated with LNPs comprising Cas9 mRNA (SEQ ID NO:1) and a gRNA of interest (See FIG. **29**, Table 34), as described in Example 4. The LNPs were prepared using the cross-flow procedure described above and purified and concentrated using PD-10 columns and Amicon centrifugal filter units, respectively. The LNPs were formulated with an N:P ratio of 6.0 and contained Lipid A, Cholesterol, DSPC, and PEG2k-DMG in a 50:38:9:2 molar ratio, respectively. The LNPs comprised a gRNA:mRNA ratio of 1:2, and the cells were treated at a dose of 300 ng (with respect to the amount of mRNA cargo delivered).

[0653] Ninety-six (96) hours following LNP treatment (with biological triplicates for each condition), mRNA was purified from PHH cells using the Dynabeads mRNA DIRECT Kit (ThermoFisher Scientific) according to the manufacturer's protocol. Reverse Transcription (RT) was performed with Maxima reverse transcriptase (ThermoFisher Scientific) and a poly-dT primer. The resulting cDNA was purified with Ampure XP Beads (Agencourt). For Quantitative PCR, 2% of the purified cDNA was amplified with Taqman Fast Advanced Mastermix and 3 Taqman probe sets, TTR (Assay ID: Hs00174914_m1), GAPDH (Assay ID: Hs02786624_g1), and PPIB (Assay ID: Hs00168719_m1). The assays were run on the QuantStudio 7 Flex Real Time PCR System according to the manufacturer's instructions (Life Technologies). Relative expression of TTR mRNA was calculated by normalizing to the endogenous controls (GAPDH and PPIB) individually, and then averaged.

[0654] As shown in FIG. **29** and reproduced numerically in Table 34 below, each of the LNP formulations tested resulted in knockdown of TTR mRNA, as compared to the negative (untreated) control. The groups in FIG. **29** and Table 34 are identified by the gRNA ID used in each LNP preparation. Relative expression of TTR mRNA is plotted in FIG. **29**, whereas the percent knockdown of TTR mRNA is provided in Table 34.

TABLE 34

GUIDE ID	Avg % Knockdown	Std Dev
G000480	95.19	1.68
G000481	91.39	2.39
G000482	82.31	4.51
G000483	68.78	13.45
G000484	75.22	9.05
G000488	92.77	3.76
G000489	91.85	2.77
G000490	78.34	5.76
G000493	87.53	4.54
G000494	91.15	3.63
G000499	91.38	1.71
G000500	92.90	3.15
G000567	90.89	5.39
G000568	53.44	20.20
G000570	93.38	2.66
G000571	96.17	2.07
G000572	55.92	24.53

[0655] In a separate experiment, TTR mRNA knockdown was evaluated following treatment with LNPs comprising G000480, G000486, and G000502. The LNPs were formulated and PHH were cultured and treated with the LNPs, each as described in the experiment above in this Example with the exception that the cells were treated at a dose of 100 ng (with respect to the amount of mRNA cargo delivered).

[0656] Ninety-six (96) hours following LNP treatment (single treatment for each condition), mRNA was purified from PHH cells using the Dynabeads mRNA DIRECT Kit (ThermoFisher Scientific) according to the manufacturer's protocol. Reverse Transcription (RT) was performed with the High Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific) according to the manufacturer's instructions. For Quantitative PCR, 2% of the cDNA was amplified with Taqman Fast Advanced Mastermix and 3 Taqman probe sets, TTR (Assay ID: Hs00174914_m1), GAPDH (Assay ID: Hs02786624_g1), and PPIB (Assay ID: Hs00168719_m1). The assays were run on the QuantStudio 7 Flex Real Time PCR System according to the manufacturer's instructions (Life Technologies). Relative expression of TTR mRNA was calculated by normalizing to the endogenous controls (GAPDH and PPIB) individually, and then averaged.

[0657] As shown in FIG. **30** and reproduced numerically in Table 35 below, each of the LNP formulations tested resulted in knockdown of TTR mRNA, as compared to the negative (untreated) control. The groups in FIG. **30** and Table 35 are identified by the gRNA ID used in each LNP preparation. Relative expression of TTR mRNA is plotted in FIG. **30**, whereas the percent knockdown of TTR mRNA is provided in Table 35.

TABLE 35

GUIDE ID	Avg % Knockdown	Std Dev
G000480	95.61	0.92
G000486	97.36	0.63
G000502	90.94	2.63

Sequence Table

Description	Sequence	SEQ ID No.
Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAA GGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCAT CAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGA AGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAA CAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGA CGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAA GAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATA CCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAG CACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGAT CAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAG CGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTT CGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAG CGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCC GGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGG ACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCT GCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACA GATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGA CGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGC ACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCT GACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGA AATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGG AGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGAT GGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAG AAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGG AGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAA GGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAA	1

-continued

Sequence Table

Description	Sequence	SEQ ID No.
	GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGG	
	AGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCT	
	GCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCAC	
	AGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAA	
	GCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTT	
	CAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAA	
	GAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATT	
	CAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAA	
	GGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCT	
	GACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGAC	
	ATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAG	
	ATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGA	
	CAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC	
	AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGA	
	AGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACA	
	CATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGAC	
	AGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGA	
	AAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACA	
	GAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACT	
	GGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAA	
	CGAAAAGCTGTACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGA	
	CCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGT	
	CCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAG	
	AAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGT	
	CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC	
	ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGA	
	ACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGAT	
	CACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGA	
	CGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA	
	GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAAT	
	CAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAAC	
	AGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGA	
	CTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAAT	
	CGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTT	
	CAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGAT	
	CGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTT	
	CGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAA	
	GACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAG	
	AAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTA	
	CGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA	
	GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGG	
	AATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCT	
	GGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCC	
	GAAGTACAGCCTGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAG	
	CGCAGGAGAACTGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGT	
	CAACTTCCTGTACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGA	
	AGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGA	
	CGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGA	
	CGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCC	
	GATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCT	
	GGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAG	
	ATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCAT	
	CACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGG	
	AGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAA	
	AAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAG	
	CTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA	
	AAAAACATAAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATT	
	AATAAAAAATGGAAAGAACCTCGAG	
Cas9 transcript comprising Cas9 ORF corresponding to SEQ ID NO: 205 using codons with generally high expression in humans	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCCATGCCTAAGAAAAAGCGGAAGGTCGACGGGGA TAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGTTGGGC AGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTCCTGGG GAACACCGATAGACACAGCATCAAGAAAAATCTCATCGGAGCCCTGCTGTT TGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCGAGGCG ACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAGATCTTTTC GAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGAAGAATC TTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTTGGAAA CATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTACCATCT GCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATTGATCTA CTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATCGAAGG CGATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCAACTGGT	2

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Sequence Table

Description	Sequence	SEQ ID No.
	GCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCTAGCGGCGT	
	CGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGCCTCGA	
	AAACCTGATCGCACAGCTGCCGGGAGAGAAAAAGAACGGACTTTTCGGCAA	
	CTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAATTTTGA	
	CCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGACGACGA	
	CTTGGACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCTGTTCCT	
	TGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTGCGCGT	
	GAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAAGCGGTA	
	CGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGACAGCA	
	ACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAATGGGTA	
	CGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGTTCTATAAGTTCAT	
	CAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTCAAGCT	
	GAACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGATCCAT	
	TCCCCACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGCCAGGA	
	GGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAAAATTCT	
	GACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAATTCGCG	
	CTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGGAATTT	
	CGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAAAGCTTCATCGAACGAAT	
	GACCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAGCACAG	
	CCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGTGAAATA	
	CGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCCGGAGAACAGAAGAA	
	AGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGTCAAGCA	
	GCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTGGAAAT	
	CAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCATGATCT	
	CCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAACGAGGA	
	CATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGCGAGAT	
	GATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAGGTCAT	
	GAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCCCGCAA	
	GCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTGGATTT	
	CCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAATTGATCCACGA	
	CGACAGCCTGACCTTTAAGGAGGACATCCAAAAAGCACAAGTGTCCGGACA	
	GGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCCGGCGAT	
	TAAGAAGGGAATTCTGCAAACTGTGAAGGTGGTCGACGAGCTGGTGAAGGT	
	CATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCCGAGAAAA	
	CCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAAGCGGAT	
	CGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCACCCGGT	
	GGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTGCAAAA	
	TGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTGTCTGA	
	TTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGACTCGAT	
	CGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCAGATAA	
	TGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGGCAGCT	
	CCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACTAAAGC	
	CGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAACGGCA	
	GCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCTTGGACTC	
	CCGCATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGAAGTGAA	
	GGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGGACTTTCA	
	GTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGACGCATA	
	CCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTAAACTTGA	
	ATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAGATGAT	
	AGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTCTTTTA	
	CTCAAACATCATGAACTTTTTCAAGACTGAAATTACGCTGGCCAATGGAGA	
	AATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAAATCGT	
	GTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCTATGCC	
	GCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTTTCAAA	
	GGAATCGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGCAAGAA	
	AGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTCGCATA	
	CTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAAAGCTCAA	
	ATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTCGTTCGA	
	GAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTGAAGAA	
	GGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAAAATGG	
	TCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAATGAGCT	
	GGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCACTACGA	
	AAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTCGTGGA	
	GCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAGTTTTC	
	AAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCGGCCTA	
	CAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATTATCCA	
	CTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTACTTCGA	
	TACTACTATCGATCGCAAAAGATACACGTCCACCAAGGAAGTTCTGGACGC	
	GACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATCGATCT	
	GTCGCAGCTGGGTGGCGATTGATAGTCTAGCCATCACATTTAAAAGCATCT	

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Sequence Table

Description	Sequence	SEQ ID No.
	CAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAGCTTATTCA TCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTAAAAAACAT AAATTTCTTTAATCATTTTGCCTCTTTTCTCTGTGCTTCAATTAATAAAAA ATGGAAAGAACCTCGAG	
modified sgRNA sequence ("N" may be any natural or non-natural nucleotide)	mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	3
30/30/39 poly-A sequence	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGCGAAAAAAAAAAAAAAAAAA AAAAAAAAAAAACCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAA	3
CR003335 gRNA targeting Human TTR (Exon 1)	CUGCUCCUCCUCUGCCUUGC	5
CR003336 gRNA targeting Human TTR (Exon 1)	CCUCCUCUGCCUUGCUGGAC	6
CR003337 gRNA targeting Human TTR (Exon 1)	CCAGUCCAGCAAGGCAGAGG	7
CR003338 gRNA targeting Human TTR (Exon 1)	AUACCAGUCCAGCAAGGCAG	8
CR003339 gRNA targeting Human TTR (Exon 1)	ACACAAAUACCAGUCCAGCA	9
CR003340 gRNA targeting Human TTR (Exon 1)	UGGACUGGUAUUUGUGUCUG	10
CR003341 gRNA targeting Human TTR (Exon 1)	CUGGUAUUUGUGUCUGAGGC	11
CR003342 gRNA targeting Human TTR (Exon 2)	CUUCUCUACACCCAGGGCAC	12
CR003343 gRNA targeting Human TTR (Exon 2)	CAGAGGACACUUGGAUUCAC	13

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Sequence Table

Description	Sequence	SEQ ID No.
CR003344 gRNA targeting Human TTR (Exon 2)	UUUGACCAUCAGAGGACACU	14
CR003345 gRNA targeting Human TTR (Exon 2)	UCUAGAACUUUGACCAUCAG	15
CR003346 gRNA targeting Human TTR (Exon 2)	AAAGUUCUAGAUGCUGUCCG	16
CR003347 gRNA targeting Human TTR (Exon 2)	CAUUGAUGGCAGGACUGCCU	17
CR003348 gRNA targeting Human TTR (Exon 2)	AGGCAGUCCUGCCAUCAAUG	18
CR003349 gRNA targeting Human TTR (Exon 2)	UGCACGGCCACAUUGAUGGC	19
CR003350 gRNA targeting Human TTR (Exon 2)	CACAUGCACGGCCACAUUGA	20
CR003351 gRNA targeting Human TTR (Exon 2)	AGCCUUUCUGAACACAUGCA	21
CR003352 gRNA targeting Human TTR (Exon 2)	GAAAGGCUGCUGAUGACACC	22
CR003353 gRNA targeting Human TTR (Exon 2)	AAAGGCUGCUGAUGACACCU	23
CR003354 gRNA targeting Human TTR (Exon 2)	ACCUGGGAGCCAUUUGCCUC	24
CR003355 gRNA targeting Human TTR (Exon 2)	CCCAGAGGCAAAUGGCUCCC	25

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Sequence Table

Description	Sequence	SEQ ID No.
CR003356 gRNA targeting Human TTR (Exon 2)	GCAACUUACCCAGAGGCAAA	26
CR003357 gRNA targeting Human TTR (Exon 2)	UUCUUUGGCAACUUACCCAG	27
CR003358 gRNA targeting Human TTR (Exon 3)	AUGCAGCUCUCCAGACUCAC	28
CR003359 gRNA targeting Human TTR (Exon 3)	AGUGAGUCUGGAGAGCUGCA	29
CR003360 gRNA targeting Human TTR (Exon 3)	GUGAGUCUGGAGAGCUGCAU	30
CR003361 gRNA targeting Human TTR (Exon 3)	GCUGCAUGGGCUCACAACUG	31
CR003362 gRNA targeting Human TTR (Exon 3)	GCAUGGGCUCACAACUGAGG	32
CR003363 gRNA targeting Human TTR (Exon 3)	ACUGAGGAGGAAUUUGUAGA	33
CR003364 gRNA targeting Human TTR (Exon 3)	CUGAGGAGGAAUUUGUAGAA	34
CR003365 gRNA targeting Human TTR (Exon 3)	UGUAGAAGGGAUAUACAAAG	35
CR003366 gRNA targeting Human TTR (Exon 3)	AAAUAGACACCAAAUCUUAC	36
CR003367 gRNA targeting Human TTR (Exon 3)	AGACACCAAAUCUUACUGGA	37

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Sequence Table

Description	Sequence	SEQ ID No.
CR003368 gRNA targeting Human TTR (Exon 3)	AAGUGCCUUCCAGUAAGAUU	38
CR003369 gRNA targeting Human TTR (Exon 3)	CUCUGCAUGCUCAUGGAAUG	39
CR003370 gRNA targeting Human TTR (Exon 3)	CCUCUGCAUGCUCAUGGAAU	40
CR003371 gRNA targeting Human TTR (Exon 3)	ACCUCUGCAUGCUCAUGGAA	41
CR003372 gRNA targeting Human TTR (Exon 3)	UACUCACCUCUGCAUGCUCA	42
CR003373 gRNA targeting Human TTR (Exon 4)	GUAUUCACAGCCAACGACUC	43
CR003374 gRNA targeting Human TTR (Exon 4)	GCGGCGGGGGCCGGAGUCGU	44
CR003375 gRNA targeting Human TTR (Exon 4)	AAUGGUGUAGCGGCGGGGGC	45
CR003376 gRNA targeting Human TTR (Exon 4)	CGGCAAUGGUGUAGCGGCGG	46
CR003377 gRNA targeting Human TTR (Exon 4)	GCGGCAAUGGUGUAGCGGCG	47
CR003378 gRNA targeting Human TTR (Exon 4)	GGCGGCAAUGGUGUAGCGGC	48

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Sequence Table

Description	Sequence	SEQ ID No.
CR003379 gRNA targeting Human TTR (Exon 4)	GGGCGGCAAUGGUGUAGCGG	49
CR003380 gRNA targeting Human TTR (Exon 4)	GCAGGGCGGCAAUGGUGUAG	50
CR003381 gRNA targeting Human TTR (Exon 4)	GGGGCUCAGCAGGGCGGCAA	51
CR003382 gRNA targeting Human TTR (Exon 4)	GGAGUAGGGGCUCAGCAGGG	52
CR003383 gRNA targeting Human TTR (Exon 4)	AUAGGAGUAGGGGCUCAGCA	53
CR003384 gRNA targeting Human TTR (Exon 4)	AAUAGGAGUAGGGGCUCAGC	54
CR003385 gRNA targeting Human TTR (Exon 4)	CCCCUACUCCUAUUCCACCA	55
CR003386 gRNA targeting Human TTR (Exon 4)	CCGUGGUGGAAUAGGAGUAG	56
CR003387 gRNA targeting Human TTR (Exon 4)	GCCGUGGUGGAAUAGGAGUA	57
CR003388 gRNA targeting Human TTR (Exon 4)	GACGACAGCCGUGGUGGAAU	58
CR003389 gRNA targeting Human TTR (Exon 4)	AUUGGUGACGACAGCCGUGG	59
CR003390 gRNA targeting Human TTR (Exon 4)	GGGAUUGGUGACGACAGCCG	60

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Sequence Table

Description	Sequence	SEQ ID No.
CR003391 gRNA targeting Human TTR (Exon 4)	GGCUGUCGUCACCAAUCCCA	61
CR003392 gRNA targeting Human TTR (Exon 4)	AGUCCCUCAUUCCUUGGGAU	62
CR005298 gRNA targeting Human TTR (Exon 1)	UCCACUCAUUCUUGGCAGGA	63
CR005299 gRNA targeting Human TTR (Exon 4)	AGCCGUGGUGGAAUAGGAGU	64
CR005300 gRNA targeting Human TTR (Exon 1)	UCACAGAAACACUCACCGUA	65
CR005301 gRNA targeting Human TTR (Exon 1)	GUCACAGAAACACUCACCGU	66
CR005302 gRNA targeting Human TTR (Exon 2)	ACGUGUCUUCUCUACACCCA	67
CR005303 gRNA targeting Human TTR (Exon 2)	UGAAUCCAAGUGUCCUCUGA	68
CR005304 gRNA targeting Human TTR (Exon 2)	GGCCGUGCAUGUGUUCAGAA	69
CR005305 gRNA targeting Human TTR (Exon 3)	UAUAGGAAAACCAGUGAGUC	70
CR005306 gRNA targeting Human TTR (Exon 3)	AAAUCUUACUGGAAGGCACU	71
CR005307 gRNA targeting Human TTR (Exon 4)	UGUCUGUCUUCUCUCAUAGG	72

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Sequence Table

Description	Sequence	SEQ ID No.
CR000689 gRNA targeting Cyno TTR	ACACAAAUACCAGUCCAGCG	73
CR005364 gRNA targeting Cyno TTR	AAAGGCUGCUGAUGAGACCU	74
CR005365 gRNA targeting Cyno TTR	CAUUGACAGCAGGACUGCCU	75
CR005366 gRNA targeting Cyno TTR	AUACCAGUCCAGCGAGGCAG	76
CR005367 gRNA targeting Cyno TTR	CCAGUCCAGCGAGGCAGAGG	77
CR005368 gRNA targeting Cyno TTR	CCUCCUCUGCCUCGCUGGAC	78
CR005369 gRNA targeting Cyno TTR	AAAGUUCUAGAUGCCGUCCG	79
CR005370 gRNA targeting Cyno TTR	ACUUGUCUUCUCUAUACCCA	80
CR005371 gRNA targeting Cyno TTR	AAGUGACUUCCAGUAAGAUU	81
CR005372 gRNA targeting Cyno TTR	AAAAGGCUGCUGAUGAGACC	82
	Not Used	83
	Not Used	84
	Not Used	85
	Not Used	86
G000480 sgRNA modified sequence targeting Human TTR	mA*mA*mA*GGCUGCUGAUGACACCUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	87
G000481 sgRNA modified sequence targeting Human TTR	mU*mC*mU*AGAACUUUGACCAUCAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	88

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Sequence Table

Description	Sequence	SEQ ID No.
G000482 sgRNA modified sequence targeting Human TTR	mU*mG*mU*AGAAGGGAUAUACAAAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	89
G000483 sgRNA modified sequence targeting Human TTR	mU*mC*mC*ACUCAUUCUUGGCAGGAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	90
G000484 sgRNA modified sequence targeting Human TTR	mA*mG*mA*CACCAAAUCUUACUGGAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	91
G000485 sgRNA modified sequence targeting Human TTR	mC*mC*mU*CCUCUGCCUUGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	92
G000486 sgRNA modified sequence targeting Human TTR	mA*mC*mA*CAAAUACCAGUCCAGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	93
G000487 sgRNA modified sequence targeting Human TTR	mU*mU*mC*UUUGGCAACUUACCCAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	94
G000488 sgRNA modified sequence targeting Human TTR	mA*mA*mA*GUUCUAGAUGCUGUCCGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	95
G000489 sgRNA modified sequence targeting Human TTR	mU*mU*mU*GACCAUCAGAGGACACUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	96
G000490 sgRNA modified sequence targeting Human TTR	mA*mA*mA*UAGACACCAAAUCUUACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	97
G000491 sgRNA modified sequence targeting Human TTR	mA*mU*mA*CCAGUCCAGCAAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	98

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Sequence Table

Description	Sequence	SEQ ID No.
G000492 sgRNA modified sequence targeting Human TTR	mC*mU*mU*CUCUACACCCAGGGCACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	99
G000493 sgRNA modified sequence targeting Human TTR	mA*mA*mG*UGCCUUCCAGUAAGAUUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	100
G000494 sgRNA modified sequence targeting Human TTR	mG*mU*mG*AGUCUGGAGAGCUGCAUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	101
G000495 sgRNA modified sequence targeting Human TTR	mC*mA*mG*AGGACACUUGGAUUCACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	102
G000496 sgRNA modified sequence targeting Human TTR	mG*mG*mC*CGUGCAUGUGUUCAGAAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	103
G000497 sgRNA modified sequence targeting Human TTR	mC*mU*mG*CUCCUCCUCUGCCUUGCGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	104
G000498 sgRNA modified sequence targeting Human TTR	mA*mG*mU*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	105
G000499 sgRNA modified sequence targeting Human TTR	mU*mG*mA*AUCCAAGUGUCCUCUGAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	106
G000500 sgRNA modified sequence targeting Human TTR	mC*mC*mA*GUCCAGCAAGGCAGAGGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	107
G000501 sgRNA modified sequence targeting Human TTR	mU*mC*mA*CAGAAACACUCACCGUAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	108

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Sequence Table

Description	Sequence	SEQ ID No.
G000567 sgRNA modified sequence targeting Human TTR	mG*mA*mA*AGGCUGCUGAUGACACCGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	109
G000568 sgRNA modified sequence targeting Human TTR	mG*mG*mC*UGUCGUCACCAAUCCCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	110
G000570 sgRNA modified sequence targeting Human TTR	mC*mA*mU*UGAUGGCAGGACUGCCUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	111
G000571 sgRNA modified sequence targeting Human TTR	mG*mU*mC*ACAGAAACACUCACCGUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	112
G000572 sgRNA modified sequence targeting Human TTR	mC*mC*mC*CUACUCCUAUUCCACCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	113
G000502 sgRNA modified sequence targeting Cyno TTR	mA*mC*mA*CAAAUACCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	114
G000503 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*AGGCUGCUGAUGAGACCGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	115
G000504 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*GGCUGCUGAUGAGACCUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	116
G000505 sgRNA modified sequence targeting Cyno TTR	mC*mA*mU*UGACAGCAGGACUGCCUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	117
G000506 sgRNA modified sequence targeting Cyno TTR	mA*mU*mA*CCAGUCCAGCGAGGCAGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	118

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Sequence Table

Description	Sequence	SEQ ID No.
G000507 sgRNA modified sequence targeting Cyno TTR	mC*mC*mA*GUCCAGCGAGGCAGAGGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	119
G000508 sgRNA modified sequence targeting Cyno TTR	mC*mC*mU*CCUCUGCCUCGCUGGACGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	120
G000509 sgRNA modified sequence targeting Cyno TTR	mA*mA*mA*GUUCUAGAUGCCGUCCGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	121
G000510 sgRNA modified sequence targeting Cyno TTR	mA*mC*mU*UGUCUUCUCUAUACCCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	122
G000511 sgRNA modified sequence targeting Cyno TTR	mA*mA*mG*UGACUUCCAGUAAGAUUGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	123
G000282 sgRNA modified sequence targeting Mouse TTR	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	124
	Not used	125 to 200
DNA coding sequence of Cas9 using the thymidine analog of the minimal uridine codons listed in Table 3, with start and stop codons	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGA TGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTC CTGGGAAACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTG CTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCA AGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATC TTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAA GAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTC GGAAACATCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTAC CACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTG ATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAG CTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC GGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGA CTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTC GGAAACCTGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAAC TTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGAC GACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTG TTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTG AGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGA CAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAG TTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTC AAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGA AGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGA	201

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Sequence Table

Description	Sequence	SEQ ID No.
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	ATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAAC	
	AGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGG	
	AACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAA	
	AGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAG	
	CACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTC	
	AAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAG	
	AAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTC	
	AAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTC	
	GAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCAC	
	GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAAC	
	GAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGA	
	GAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAG	
	GTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGC	
	AGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTG	
	GACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATC	
	CACGACGACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGC	
	GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCG	
	GCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTC	
	AAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGA	
	GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAG	
	AGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACAC	
	CCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGTACCTGTACTACCTG	
	CAGAACGGAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTG	
	AGCGACTACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGAC	
	AGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
	GACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGA	
	CAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACA	
	AAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAG	
	AGACAGCTGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTG	
	GACAGCAGAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAA	
	GTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGAC	
	TTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGAC	
	GCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAG	
	CTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAG	
	ATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTC	
	TTCTACAGCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAAC	
	GGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAA	
	ATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGC	
	ATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTC	
	AGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGA	
	AAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTC	
	GCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAG	
	CTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGC	
	TTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTC	
	AAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAA	
	AACGGAAGAAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAAC	
	GAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCAC	
	TACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTC	
	GTCGAACAGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAA	
	TTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGC	
	GCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATC	
	ATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTAC	
	TTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTG	
	GACGCAACACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATC	
	GACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA	
	AAGGTCTAG	
DNA coding sequence of Cas9 using codons with generally high expression in humans	ATGGATAAGAAGTACTCAATCGGGCTGGATATCGGAACTAATTCCGTGGGT	202
	TGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTC	
	CTGGGGAACACCGATAGACACAGCATCAAGAAAAATCTCATCGGAGCCCTG	
	CTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCG	
	AGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAGATC	
	TTTTCGAACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACCGCCTGGAA	
	GAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTT	
	GGAAACATCGTCGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTAC	
	CATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATTG	
	ATCTACTTGGCCCTCGCCCATATGATCAAATTCCGCGGACACTTCCTGATC	
	GAAGGCGATCTGAACCCTGATAACTCCGACGTGGATAAGCTTTTCATTCAA	
	CTGGTGCAGACCTACAACCAACTGTTCGAAGAAAACCCAATCAATGCTAGC	
	GGCGTCGATGCCAAGGCCATCCTGTCCGCCCGGCTGTCGAAGTCGCGGCGC	

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Sequence Table

Description	Sequence	SEQ ID No.
	CTCGAAAACCTGATCGCACAGCTGCCGGGAGAGAAAAAGAACGGACTTTTC	
	GGCAACTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAAT	
	TTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGAC	
	GACGACTTGGACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCTG	
	TTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTG	
	CGCGTGAACACCGAAATAACCAAAGCGCCGCTTAGCGCCTCGATGATTAAG	
	CGGTACGACGAGCATCACCAGGATCTCACGCTGCTCAAAGCGCTCGTGAGA	
	CAGCAACTGCCTGAAAAGTACAAGGAGATCTTCTTCGACCAGTCCAAGAAT	
	GGGTACGCAGGGTACATCGATGGAGGCGCTAGCCAGGAAGAGTTCTATAAG	
	TTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTC	
	AAGCTGAACAGGGAGGATCTGCTCCGGAAACAGAGAACCTTTGACAACGGA	
	TCCATTCCCCACCAGATCCATCTGGGTGAGCTGCACGCCATCTTGCGGCGC	
	CAGGAGGACTTTTACCCATTCCTCAAGGACAACCGGGAAAAGATCGAGAAA	
	ATTCTGACGTTCCGCATCCCGTATTACGTGGGCCCACTGGCGCGCGGCAAT	
	TCGCGCTTCGCGTGGATGACTAGAAAATCAGAGGAAACCATCACTCCTTGG	
	AATTTCGAGGAAGTTGTGGATAAGGGAGCTTCGGCACAAAGCTTCATCGAA	
	CGAATGACCAACTTCGACAAGAATCTCCCAAACGAGAAGGTGCTTCCTAAG	
	CACAGCCTCCTTTACGAATACTTCACTGTCTACAACGAACTGACTAAAGTG	
	AAATACGTTACTGAAGGAATGAGGAAGCCGGCCTTTCTGTCCGGAGAACAG	
	AAGAAAGCAATTGTCGATCTGCTGTTCAAGACCAACCGCAAGGTGACCGTC	
	AAGCAGCTTAAAGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCAGTG	
	GAAATCAGCGGGGTGGAGGACAGATTCAACGCTTCGCTGGGAACCTATCAT	
	GATCTCCTGAAGATCATCAAGGACAAGGACTTCCTTGACAACGAGGAGAAC	
	GAGGACATCCTGGAAGATATCGTCCTGACCTTGACCCTTTTCGAGGATCGC	
	GAGATGATCGAGGAGAGGCTTAAGACCTACGCTCATCTCTTCGACGATAAG	
	GTCATGAAACAACTCAAGCGCCGCCGGTACACTGGTTGGGGCCGCCTCTCC	
	CGCAAGCTGATCAACGGTATTCGCGATAAACAGAGCGGTAAAACTATCCTG	
	GATTTCCTCAAATCGGATGGCTTCGCTAATCGTAACTTCATGCAATTGATC	
	CACGACGACAGCCTGACCTTTAAGGAGGACATCCAAAAAGCACAAGTGTCC	
	GGACAGGGAGACTCACTCCATGAACACATCGCGAATCTGGCCGGTTCGCCG	
	GCGATTAAGAAGGGAATTCTGCAAACTGTGAAGGTGGTCGACGAGCTGGTG	
	AAGGTCATGGGACGGCACAAACCGGAGAATATCGTGATTGAAATGGCCCGA	
	GAAAACCAGACTACCCAGAAGGGCCAGAAAAACTCCCGCGAAAGGATGAAG	
	CGGATCGAAGAAGGAATCAAGGAGCTGGGCAGCCAGATCCTGAAAGAGCAC	
	CCGGTGGAAAACACGCAGCTGCAGAACGAGAAGCTCTACCTGTACTATTTG	
	CAAAATGGACGGGACATGTACGTGGACCAAGAGCTGGACATCAATCGGTTG	
	TCTGATTACGACGTGGACCACATCGTTCCACAGTCCTTTCTGAAGGATGAC	
	TCGATCGATAACAAGGTGTTGACTCGCAGCGACAAGAACAGAGGGAAGTCA	
	GATAATGTGCCATCGGAGGAGGTCGTGAAGAAGATGAAGAATTACTGGCGG	
	CAGCTCCTGAATGCGAAGCTGATTACCCAGAGAAAGTTTGACAATCTCACT	
	AAAGCCGAGCGCGGCGGACTCTCAGAGCTGGATAAGGCTGGATTCATCAAA	
	CGGCAGCTGGTCGAGACTCGGCAGATTACCAAGCACGTGGCGCAGATCTTG	
	GACTCCCGCATGAACACTAAATACGACGAGAACGATAAGCTCATCCGGGAA	
	GTGAAGGTGATTACCCTGAAAAGCAAACTTGTGTCGGACTTTCGGAAGGAC	
	TTTCAGTTTTACAAAGTGAGAGAAATCAACAACTACCATCACGCGCATGAC	
	GCATACCTCAACGCTGTGGTCGGTACCGCCCTGATCAAAAAGTACCCTAAA	
	CTTGAATCGGAGTTTGTGTACGGAGACTACAAGGTCTACGACGTGAGGAAG	
	ATGATAGCCAAGTCCGAACAGGAAATCGGGAAAGCAACTGCGAAATACTTC	
	TTTTACTCAAACATCATGAACTTTTTCAAGACTGAAATTACGCTGGCCAAT	
	GGAGAAATCAGGAAGAGGCCACTGATCGAAACTAACGGAGAAACGGGCGAA	
	ATCGTGTGGGACAAGGGCAGGGACTTCGCAACTGTTCGCAAAGTGCTCTCT	
	ATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTT	
	TCAAAGGAATCGATCCTCCCAAAGAGAAATAGCGACAAGCTCATTGCACGC	
	AAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTC	
	GCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAAAAG	
	CTCAAATCCGTCAAAGAGCTGCTGGGGATTACCATCATGGAACGATCCTCG	
	TTCGAGAAGAACCCGATTGATTTCCTCGAGGCGAAGGGTTACAAGGAGGTG	
	AAGAAGGATCTGATCATCAAACTCCCCAAGTACTCACTGTTCGAACTGGAA	
	AATGGTCGGAAGCGCATGCTGGCTTCGGCCGGAGAACTCCAAAAAGGAAAT	
	GAGCTGGCCTTGCCTAGCAAGTACGTCAACTTCCTCTATCTTGCTTCGCAC	
	TACGAAAAACTCAAAGGGTCACCGGAAGATAACGAACAGAAGCAGCTTTTC	
	GTGGAGCAGCACAAGCATTATCTGGATGAAATCATCGAACAAATCTCCGAG	
	TTTTCAAAGCGCGTGATCCTCGCCGACGCCAACCTCGACAAAGTCCTGTCG	
	GCCTACAATAAGCATAGAGATAAGCCGATCAGAGAACAGGCCGAGAACATT	
	ATCCACTTGTTCACCCTGACTAACCTGGGAGCCCCAGCCGCCTTCAAGTAC	
	TTCGATACTACTATCGATCGCAAAAGATACACGTCCACCAAGGAAGTTCTG	
	GACGCGACCCTGATCCACCAAAGCATCACTGGACTCTACGAAACTAGGATC	
	GATCTGTCGCAGCTGGGTGGCGATGGCGGTGGATCTCCGAAAAAGAAGAGA	
	AAGGTGTAATGA	

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Sequence Table

Description	Sequence	SEQ ID No.
Amino acid sequence of Cas9 with one nuclear localization signal (1xNLS) as the C-terminal 7 amino acids	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESELVEEDKKHERHPIEGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNEKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAELSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLTKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENITHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKR KV	203
Cas9 mRNA ORF using minimal uridine codons, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG	204

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Sequence Table

Description	Sequence	SEQ ID No.
	GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGUCUAG	
Cas9 mRNA ORF using codons with generally high expression in humans, with start and stop codons	AUGGAUAAGAAGUACUCAAUCGGGCUGGAUAUCGGAACUAAUUCCGUGGGU UGGGCAGUGAUCACGGAUGAAUACAAAGUGCCGUCCAAGAAGUUCAAGGUC CUGGGGAACACCGAUAGACACAGCAUCAAGAAAAAUCUCAUCGGAGCCCUG CUGUUUGACUCCGGCGAAACCGCAGAAGCGACCCGGCUCAAACGUACCGCG AGGCGACGCUACACCCGGCGGAAGAAUCGCAUCUGCUAUCUGCAAGAGAUC UUUUCGAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACCGCCUGGAA GAAUCUUUCCUGGUGGAGGAGGACAAGAAGCAUGAACGGCAUCCUAUCUUU GGAAACAUCGUCGACGAAGUGGCGUACCACGAAAAGUACCCGACCAUCUAC CAUCUGCGGAAGAAGUUGGUUGACUCAACUGACAAGGCCGACCUCAGAUUG AUCUACUUGGCCCUCGCCCAUAUGAUCAAAUUCCGCGGACACUUCCUGAUC GAAGGCGAUCUGAACCCUGAUAACUCCGACGUGGAUAAGCUUUUCAUUCAA CUGGUGCAGACCUACAACCAACUGUUCGAAGAAAACCCAAUCAAUGCUAGC GGCGUCGAUGCCAAGGCCAUCCUGUCCGCCCGGCUGUCGAAGUCGCGGCGC CUCGAAAACCUGAUCGCACAGCUGCCGGGAGAGAAAAAGAACGGACUUUUC GGCAACUUGAUCGCUCUCUCACUGGGACUCACUCCCAAUUUCAAGUCCAAU UUUGACCUGGCCGAGGACGCGAAGCUGCAACUCUCAAAGGACACCUACGAC GACGACUUGGACAAUUUGCUGGCACAAAUUGGCGAUCAGUACGCGGAUCUG UUCCUUGCCGCUAAGAACCUUUCGGACGCAAUCUUGCUGUCCGAUAUCCUG CGCGUGAACACCGAAAUAACCAAAGCGCCGCUUAGCGCCUCGAUGAUUAAG CGGUACGACGAGCAUCACCAGGAUCUCACGCUGCUCAAAGCGCUCGUGAGA CAGCAACUGCCUGAAAAGUACAAGGAGAUCUUCUUCGACCAGUCCAAGAAU GGGUACGCAGGGUACAUCGAUGGAGGCGCUAGCCAGGAAGAGUUCUAUAAG UUCAUCAAGCCAAUCCUGGAAAAGAUGGACGGAACCGAAGAACUGCUGGUC AAGCUGAACAGGGAGGAUCUGCUCCGGAAACAGAGAACCUUUGACAACGGA UCCAUUCCCCACCAGAUCCAUCUGGGUGAGCUGCACGCCAUCUUGCGGCGC CAGGAGGACUUUUACCCAUUCCUCAAGGACAACCGGGAAAAGAUCGAGAAA AUUCUGACGUUCCGCAUCCCGUAUUACGUGGGCCCACUGGCGCGCGGCAAU UCGCGCUUCGCGUGGAUGACUAGAAAAUCAGAGGAAACCAUCACUCCUUGG AAUUUCGAGGAAGUUGUGGAUAAGGGAGCUUCGGCACAAAGCUUCAUCGAA CGAAUGACCAACUUCGACAAGAAUCUCCCAAACGAGAAGGUGCUUCCUAAG CACAGCCUCCUUUACGAAUACUUCACUGUCUACAACGAACUGACUAAAGUG	205

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Sequence Table

Description	Sequence	SEQ ID No.
	AAAUACGUUACUGAAGGAAUGAGGAAGCCGGCCUUUCUGUCCGGAGAACAG	
	AAGAAAGCAAUUGUCGAUCUGCUGUUCAAGACCAACCGCAAGGUGACCGUC	
	AAGCAGCUUAAAGAGGACUACUUCAAGAAGAUCGAGUGUUUCGACUCAGUG	
	GAAAUCAGCGGGGUGGAGGACAGAUUCAACGCUUCGCUGGGAACCUAUCAU	
	GAUCUCCUGAAGAUCAUCAAGGACAAGGACUUCCUUGACAACGAGGAGAAC	
	GAGGACAUCCUGGAAGAUAUCGUCCUGACCUUGACCCUUUUCGAGGAUCGC	
	GAGAUGAUCGAGGAGAGGCUUAAGACCUACGCUCAUCUCUUCGACGAUAAG	
	GUCAUGAAACAACUCAAGCGCCGCCGGUACACUGGUUGGGGCCGCCUCUCC	
	CGCAAGCUGAUCAACGGUAUUCGCGAUAAACAGAGCGGUAAAACUAUCCUG	
	GAUUUCCUCAAAUCGGAUGGCUUCGCUAAUCGUAACUUCAUGCAAUUGAUC	
	CACGACGACAGCCUGACCUUUAAGGAGGACAUCCAAAAAGCACAAGUGUCC	
	GGACAGGGAGACUCACUCCAUGAACACAUCGCGAAUCUGGCCGGUUCGCCG	
	GCGAUUAAGAAGGGAAUUCUGCAAACUGUGAAGGUGGUCGACGAGCUGGUG	
	AAGGUCAUGGGACGGCACAAACCGGAGAAUAUCGUGAUUGAAAUGGCCCGA	
	GAAAACCAGACUACCCAGAAGGGCCAGAAAAACUCCCGCGAAAGGAUGAAG	
	CGGAUCGAAGAAGGAAUCAAGGAGCUGGGCAGCCAGAUCCUGAAAGAGCAC	
	CCGGUGGAAAACACGCAGCUGCAGAACGAGAAGCUCUACCUGUACUAUUUG	
	CAAAAUGGACGGGACAUGUACGUGGACCAAGAGCUGGACAUCAAUCGGUUG	
	UCUGAUUACGACGUGGACCACAUCGUUCCACAGUCCUUUCUGAAGGAUGAC	
	UCGAUCGAUAACAAGGUGUUGACUCGCAGCGACAAGAACAGAGGGAAGUCA	
	GAUAAUGUGCCAUCGGAGGAGGUCGUGAAGAAGAUGAAGAAUUACUGGCGG	
	CAGCUCCUGAAUGCGAAGCUGAUUACCCAGAGAAAGUUUGACAAUCUCACU	
	AAAGCCGAGCGCGGCGGACUCUCAGAGCUGGAUAAGGCUGGAUUCAUCAAA	
	CGGCAGCUGGUCGAGACUCGGCAGAUUACCAAGCACGUGGCGCAGAUCUUG	
	GACUCCCGCAUGAACACUAAAUACGACGAGAACGAUAAGCUCAUCCGGGAA	
	GUGAAGGUGAUUACCCUGAAAAGCAAACUUGUGUCGGACUUUCGGAAGGAC	
	UUUCAGUUUUACAAAGUGAGAGAAAUCAACAACUACCAUCACGCGCAUGAC	
	GCAUACCUCAACGCUGUGGUCGGUACCGCCCUGAUCAAAAAGUACCCUAAA	
	CUUGAAUCGGAGUUUGUGUACGGAGACUACAAGGUCUACGACGUGAGGAAG	
	AUGAUAGCCAAGUCCGAACAGGAAAUCGGGAAAGCAACUGCGAAAUACUUC	
	UUUUACUCAAACAUCAUGAACUUUUUCAAGACUGAAAUUACGCUGGCCAAU	
	GGAGAAAUCAGGAAGAGGCCACUGAUCGAAACUAACGGAGAAACGGGCGAA	
	AUCGUGUGGGACAAGGGCAGGGACUUCGCAACUGUUCGCAAAGUGCUCUCU	
	AUGCCGCAAGUCAAUAUUGUGAAGAAAACCGAAGUGCAAACCGGCGGAUUU	
	UCAAAGGAAUCGAUCCUCCCAAAGAGAAAUAGCGACAAGCUCAUUGCACGC	
	AAGAAAGACUGGGACCCGAAGAAGUACGGAGGAUUCGAUUCGCCGACUGUC	
	GCAUACUCCGUCCUCGUGGUGGCCAAGGUGGAGAAGGGAAAGAGCAAAAAG	
	CUCAAAUCCGUCAAAGAGCUGCUGGGGAUUACCAUCAUGGAACGAUCCUCG	
	UUCGAGAAGAACCCGAUUGAUUUCCUCGAGGCGAAGGGUUACAAGGAGGUG	
	AAGAAGGAUCUGAUCAUCAAACUCCCCAAGUACUCACUGUUCGAACUGGAA	
	AAUGGUCGGAAGCGCAUGCUGGCUUCGGCCGGAGAACUCCAAAAAGGAAAU	
	GAGCUGGCCUUGCCUAGCAAGUACGUCAACUUCCUCUAUCUUGCUUCGCAC	
	UACGAAAAACUCAAAGGGUCACCGGAAGAUAACGAACAGAAGCAGCUUUUC	
	GUGGAGCAGCACAAGCAUUAUCUGGAUGAAAUCAUCGAACAAAUCUCCGAG	
	UUUUCAAAGCGCGUGAUCCUCGCCGACGCCAACCUCGACAAAGUCCUGUCG	
	GCCUACAAUAAGCAUAGAGAUAAGCCGAUCAGAGAACAGGCCGAGAACAUU	
	AUCCACUUGUUCACCCUGACUAACCUGGGAGCCCCAGCCGCCUUCAAGUAC	
	UUCGAUACUACUAUCGAUCGCAAAAGAUACACGUCCACCAAGGAAGUUCUG	
	GACGCGACCCUGAUCCACCAAAGCAUCACUGGACUCUACGAAACUAGGAUC	
	GAUCUGUCGCAGCUGGGUGGCGAUGGCGGUGGAUCUCCGAAAAAGAAGAGA	
	AAGGUGUAAUGA	
Cas9 nickase (D10A) amino acid sequence	MDKKYSIGLAIGINSVGWAVITDEYKVPSKKFKVLGNIDRHSIKKNLIGAL	206
	LFDSGETAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE	
	ESELVEEDKKHERHPIEGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL	
	IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS	
	GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLIPNFKSN	
	FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL	
	RVNTEITKAPLSASMIKRYDEHHQDLILLKALVRQQLPEKYKEIFFDQSKN	
	GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG	
	SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN	
	SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMINFDKNLPNEKVLPK	
	HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVIV	
	KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN	
	EDILEDIVLILTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS	
	RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLIFKEDIQKAQVS	
	GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR	
	ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL	
	QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLIRSDKNRGKS	
	DNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLIKAERGGLSELDKAGFIK	
	RQLVETRQIIKHVAQILDSRMNIKYDENDKLIREVKVITLKSKLVSDFRKD	

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Sequence Table

Description	Sequence	SEQ ID No.
	FQFYKVREINNYHHAHDAYLNAVVGIALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNSDKLIAR KKDWDPKKYGGFDSPIVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKR KV	
Cas9 nickase (D10A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC	207

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Sequence Table

Description	Sequence	SEQ ID No.
	UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC GACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA AAGGUCUAG	
dCas9 (D10A H840A) amino acid sequence	MDKKYSIGLAIGINSVGWAVITDEYKVPSKKFKVLGNIDRHSIKKNLIGAL LFDSGETAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESELVEEDKKHERHPIEGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLIPNEKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLILLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMINFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVIV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLILTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLIFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLIRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLIKAERGGLSELDKAGFIK RQLVETRQIIKHVAQILDSRMNIKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGIALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNSDKLIAR KKDWDPKKYGGFDSPIVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLINLGAPAAFKY FDTTIDRKRYISTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSPKKKR KV	208
dCas9 (D10A H840A) mRNA ORF	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA	209

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Sequence Table

Description	Sequence	SEQ ID No.
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	UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC	
	AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA	
	AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA	
	CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG	
	AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC	
	AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG	
	AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA	
	AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG	
	CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC	
	AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG	
	AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC	
	AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC	
	GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC	
	GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC	
	GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA	
	GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG	
	GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC	
	AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG	
	GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC	
	CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC	
	GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG	
	GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC	
	AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA	
	GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG	
	AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC	
	CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG	
	CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG	
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	AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
	GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA	
	CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA	
	AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG	
	AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG	
	GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA	
	GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC	
	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	
	GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG	
	CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG	
	AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC	
	UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC	
	GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA	
	AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC	
	AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC	
	AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA	
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	CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC	
	UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC	
	AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA	
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	GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC	
	UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC	
	GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA	
	UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC	
	GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC	
	AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC	
	UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG	
	GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC	
	GACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA	
	AAGGUCUAG	
Cas9 mRNA coding sequence using minimal uridine codons (no start or	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC	210

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Sequence Table

Description	Sequence	SEQ ID No.
stop codons; suit able for inclusion in fusion protein coding sequence)	CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC	
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	GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA	
	GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG	
	GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA	
	AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC	
	GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC	
	GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC	
	CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA	
	GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA	
	UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG	
	CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA	
	UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC	
	AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG	
	CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC	
	AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG	
	GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC	
	CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC	
	AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC	
	UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA	
	AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC	
	AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG	
	UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG	
	AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG	
	CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA	
	AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC	
	CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA	
	GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA	
	AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC	
	AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA	
	AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC	
	UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC	
	GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA	
	CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA	
	AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG	
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	GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC	
	AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC	
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	AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC	
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	GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG	
	AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC	
	GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA	
	CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC	
	GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC	
	GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC	
	AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA	
	UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC	
	CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC	
	GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC	

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Sequence Table

Description	Sequence	SEQ ID No.
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Cas9 nickase coding sequence using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACCACACUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA	211

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Sequence Table

Description	Sequence	SEQ ID No.
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dCas9 coding sequence using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC GACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC	212

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Sequence Table

Description	Sequence	SEQ ID No.
	AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAG GUC	
Amino acid sequence of Cas9 (without NLS)	MDKKYSIGLDIGINSVGWAVITDEYKVPSKKFKVLGNIDRHSIKKNLIGAL LFDSGETAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESELVEEDKKHERHPIEGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLIPNEKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLILLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMINFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVIV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLILTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLIFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLIRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLIKAERGGLSELDKAGFIK RQLVETRQIIKHVAQILDSRMNIKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGIALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNSDKLIAR KKDWDPKKYGGFDSPIVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLINLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	213
Cas9 mRNA ORF encoding SEQ ID NO: 213 using minimal uridine codons, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC	214

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Sequence Table

Description	Sequence	SEQ ID No.
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	AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG	
	AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA	
	CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC	
	GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG	
	UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC	
	AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA	
	AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA	
	CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG	
	AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC	
	AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG	
	AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA	
	AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG	
	CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC	
	AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG	
	AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC	
	AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC	
	GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC	
	GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC	
	GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA	
	GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG	
	GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC	
	AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG	
	GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC	
	CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC	
	GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG	
	GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC	
	AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA	
	GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG	
	AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC	
	CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG	
	CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG	
	AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC	
	AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
	GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA	
	CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA	
	AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG	
	AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG	
	GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA	
	GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC	
	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	
	GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG	
	CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG	
	AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC	
	UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC	
	GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA	
	AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC	
	AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC	
	AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA	
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	GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG	
	CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC	
	UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC	
	AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA	
	AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC	
	GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC	
	UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC	
	GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA	
	UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC	
	GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC	
	AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC	
	UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG	
	GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC	
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Cas9 coding sequence encoding SEQ ID NO: 213	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGG GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA	215

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Sequence Table

Description	Sequence	SEQ ID No.
using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC	
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	AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC	
	CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC	
	UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA	
	GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG	
	GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA	
	GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG	
	GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA	
	AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC	
	GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC	
	GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC	
	CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA	
	GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA	
	UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG	
	CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA	
	UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC	
	AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG	
	CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC	
	AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG	
	GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC	
	CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC	
	AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC	
	UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA	
	AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC	
	AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG	
	UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG	
	AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG	
	CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA	
	AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC	
	CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA	
	GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA	
	AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC	
	AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA	
	AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC	
	UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC	
	GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA	
	CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA	
	AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG	
	GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA	
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	AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC	
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	GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG	
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	UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA	
	GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC	
	GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG	
	CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC	
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	AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA	
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	AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC	
	GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG	
	AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC	
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	CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC	
	GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC	
	GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC	

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Sequence Table

Description	Sequence	SEQ ID No.
	AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGAC	
Amino acid sequence of Cas9 nickase (without NLS)	MDKKYSIGLAIGINSVGWAVITDEYKVPSKKFKVLGNIDRHSIKKNLIGAL LFDSGETAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESELVEEDKKHERHPIEGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLIPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLILLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMINFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVIV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLILTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLIFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLIRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLIKAERGGLSELDKAGFIK RQLVETRQIIKHVAQILDSRMNIKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGIALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNSDKLIAR KKDWDPKKYGGFDSPIVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLINLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	216
Cas9 nickase mRNA ORF encoding SEQ ID NO: 216 using minimal uridine codons as listed in Table 3, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC	217

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Sequence Table

Description	Sequence	SEQ ID No.
	GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA	
	GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG	
	GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC	
	AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG	
	GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC	
	CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC	
	GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG	
	GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC	
	AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA	
	GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG	
	AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC	
	CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG	
	CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG	
	AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC	
	AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
	GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA	
	CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA	
	AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG	
	AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG	
	GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA	
	GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC	
	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	
	GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG	
	CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG	
	AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC	
	UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC	
	GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA	
	AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC	
	AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC	
	AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA	
	AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC	
	GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG	
	CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC	
	UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC	
	AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA	
	AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC	
	GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC	
	UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC	
	GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA	
	UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC	
	GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC	
	AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC	
	UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG	
	GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC	
	GACCUGAGCCAGCUGGGAGGAGACUAG	
Cas9 nickase coding sequence encoding SEQ ID NO: 216 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG	218
	GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG	
	GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG	
	UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA	
	AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC	
	AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA	
	AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA	
	AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC	
	CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC	
	UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA	
	GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG	
	GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA	
	GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG	
	GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA	
	AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC	
	GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC	
	GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC	
	CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA	
	GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA	
	UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG	
	CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA	
	UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC	
	AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG	
	CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC	
	AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG	

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Sequence Table

Description	Sequence	SEQ ID No.
	GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC	
	CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC	
	AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC	
	UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA	
	AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC	
	AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG	
	UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG	
	AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG	
	CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA	
	AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC	
	CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA	
	GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA	
	AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC	
	AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA	
	AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC	
	UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC	
	GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA	
	CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA	
	AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG	
	GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA	
	AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA	
	AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG	
	GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG	
	AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC	
	GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC	
	AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC	
	AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG	
	CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG	
	GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA	
	CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC	
	AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC	
	AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC	
	CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA	
	UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG	
	GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG	
	AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC	
	UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA	
	GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC	
	GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG	
	CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC	
	AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG	
	AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA	
	UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG	
	AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC	
	GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG	
	AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC	
	GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA	
	CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC	
	GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC	
	GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC	
	AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA	
	UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC	
	CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC	
	GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC	
	GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC	
	CUGAGCCAGCUGGGAGGAGAC	
Amino acid sequence of dCas9 (without NLS)	MDKKYSIGLAIGINSVGWAVITDEYKVPSKKFKVLGNIDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESELVEEDKKHERHPIEGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLIPNEKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLILLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMINFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVIV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLILTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLIFKEDIQKAQVS	219

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Sequence Table

Description	Sequence	SEQ ID No.
	GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLIRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLIKAERGGLSELDKAGFIK RQLVETRQIIKHVAQILDSRMNIKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGIALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNSDKLIAR KKDWDPKKYGGFDSPIVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	
dCas9 mRNA ORF encoding SEQ ID NO: 219 using minimal uridine codons as listed in Table 3, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG AGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGAC AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC	220

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Sequence Table

Description	Sequence	SEQ ID No.
	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	
	GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG	
	CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG	
	AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC	
	UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC	
	GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA	
	AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC	
	AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC	
	AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA	
	AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC	
	GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG	
	CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC	
	UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC	
	AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA	
	AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC	
	GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC	
	UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC	
	GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA	
	UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC	
	GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC	
	AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC	
	UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG	
	GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC	
	GACCUGAGCCAGCUGGGAGGAGACUAG	
dCas9 coding sequence encoding SEQ ID NO: 219 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG	221
	GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG	
	GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG	
	UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA	
	AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC	
	AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA	
	AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA	
	AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC	
	CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC	
	UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA	
	GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG	
	GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA	
	GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG	
	GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA	
	AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC	
	GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC	
	GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC	
	CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA	
	GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA	
	UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG	
	CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA	
	UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC	
	AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG	
	CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC	
	AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG	
	GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC	
	CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC	
	AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC	
	UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA	
	AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC	
	AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG	
	UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG	
	AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG	
	CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA	
	AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC	
	CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA	
	GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA	
	AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC	
	AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA	
	AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC	
	UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC	
	GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA	
	CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA	
	AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG	
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	AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA	

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Sequence Table

Description	Sequence	SEQ ID No.
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Amino acid sequence of Cas9 with two nuclear localization signals (2xNLS) as the C-terminal amino acids	MDKKYSIGLDIGINSVGWAVITDEYKVPSKKFKVLGNIDRHSIKKNLIGAL LFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLIPNFKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLILLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMINFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKINRKVIV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLILTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLIFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLIRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLIKAERGGLSELDKAGFIK RQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIAR KKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKY FDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGSPKKKR KVDGSPKKKRKVDSG	222
Cas9 mRNA ORF encoding SEQ ID NO: 222 using minimal uridine	AUGGACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA	223

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Sequence Table

Description	Sequence	SEQ ID No.
codons, with start and stop codons	GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC	
	GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC	
	CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG	
	AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC	
	GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG	
	CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC	
	GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA	
	CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC	
	GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC	
	UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC	
	GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG	
	UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG	
	AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG	
	AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA	
	CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC	
	GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG	
	UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC	
	AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA	
	AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA	
	CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG	
	AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC	
	AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG	
	AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA	
	AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG	
	CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC	
	AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG	
	AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC	
	AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC	
	GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC	
	GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC	
	GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA	
	GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG	
	GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC	
	AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG	
	GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC	
	CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC	
	GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG	
	GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC	
	AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA	
	GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG	
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	CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG	
	CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG	
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	AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
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	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	
	GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG	
	CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG	
	AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC	
	UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC	
	GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA	
	AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC	
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	CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC	
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	GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC	
	UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC	
	GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA	
	UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC	
	GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC	

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Sequence Table

Description	Sequence	SEQ ID No.
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	UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG	
	GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC	
	GACCUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGA	
	AAGGUCCCGAAGAAGAAGAGAAAGGUC	
	GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAG	
	AAGAGAAAGGUCGACAGCGGAUAG	
Cas9 coding sequence SEQ encoding ID NO: 222 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGACAUCGGAACAAACAGCGUCGGAUGG	224
	GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG	
	GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG	
	UUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA	
	AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC	
	AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA	
	AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA	
	AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC	
	CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC	
	UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA	
	GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG	
	GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA	
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	GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA	
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	GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC	
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	UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC	
	AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG	
	CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC	
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	UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG	
	AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG	
	CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA	
	AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC	
	CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA	
	GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA	
	AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC	
	AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA	
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	GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC	
	AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC	
	AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG	
	CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG	
	GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA	
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	AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC	
	CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA	
	UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG	
	GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG	
	AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC	
	UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA	
	GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC	

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Sequence Table

Description	Sequence	SEQ ID No.
	GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC CUGAGCCAGCUGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAG GUCCCGAAGAAGAAGAGAAAGGUC GGAAGCGGAAGCCCGAAGAAGAAGAGAAAGGUCGACGGAAGCCCGAAGAAG AAGAGAAAGGUCGACAGCGGA	
Amino acid sequence of Cas9 nickase with two nuclear localization signals as the C-terminal amino acids	MDKKYSIGLAIGINSVGWAVITDEYKVPSKKFKVLGNIDRHSIKKNLIGAL LFDSGETAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESELVEEDKKHERHPIEGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLIPNEKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLILLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMINFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVIV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLILTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLIFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLIRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLIKAERGGLSELDKAGFIK RQLVETRQIIKHVAQILDSRMNIKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGIALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNSDKLIAR KKDWDPKKYGGFDSPIVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLINLGAPAAFKY FDTTIDRKRYISTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGSPKKKR KVDGSPKKKRKVDSG	225
Cas9 nickase mRNA ORF encoding SEQ ID NO: 25 using minimal uridine codons as listed in Table 3, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA	226

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Sequence Table

Description	Sequence	SEQ ID No.
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	GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG	
	UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC	
	AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA	
	AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA	
	CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG	
	AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC	
	AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG	
	AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA	
	AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG	
	CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC	
	AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG	
	AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC	
	AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC	
	GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC	
	GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC	
	GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA	
	GAAAUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAG	
	GUCAUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGC	
	AGAAAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUG	
	GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC	
	CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC	
	GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG	
	GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC	
	AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA	
	GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG	
	AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC	
	CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG	
	CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG	
	AGCGACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGAC	
	AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
	GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA	
	CAGCUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACA	
	AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG	
	AGACAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUG	
	GACAGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAA	
	GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC	
	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	
	GCAUACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAG	
	CUGGAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAG	
	AUGAUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUC	
	UUCUACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAAC	
	GGAGAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAA	
	AUCGUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGC	
	AUGCCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUC	
	AGCAAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGA	
	AAGAAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUC	
	GCAUACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAG	
	CUGAAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGC	
	UUCGAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUC	
	AAGAAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAA	
	AACGGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAAC	
	GAACUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCAC	
	UACGAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUC	
	GUCGAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAA	
	UUCAGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGC	
	GCAUACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUC	
	AUCCACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUAC	
	UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG	
	GACGCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUC	
	GACCUGAGCCAGCUGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAAGAGA	
	AAGGUCGACGGAAGCCCGAAGAAGAAGAGAAAGGUCGACAGCGGAUAG	
Cas9 nickase coding sequence encoding SEQ ID NO: 25 using minimal uridine	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG	227
	GCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUCCUG	
	GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG	
	UUCGACAGGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCAAGA	
	AGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUCUUC	
	AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA	
	AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA	
	AACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUACCAC	

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Sequence Table

Description	Sequence	SEQ ID No.
codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	CUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUGAUC	
	UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA	
	GGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAGCUG	
	GUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGCGGA	
	GUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGACUG	
	GAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUCGGA	
	AACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAACUUC	
	GACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGACGAC	
	GACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUGUUC	
	CUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUGAGA	
	GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA	
	UACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGACAG	
	CAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAACGGA	
	UACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAGUUC	
	AUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUCAAG	
	CUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGAAGC	
	AUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGACAG	
	GAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAGAUC	
	CUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAACAGC	
	AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC	
	UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA	
	AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC	
	AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG	
	UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG	
	AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG	
	CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA	
	AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC	
	CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA	
	GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA	
	AUGAUCGAAGAAAGACUGAAGACAUACGCACACCUGUUCGACGACAAGGUC	
	AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA	
	AAGCUGAUCAACGGAAUCAGAGACAAGCAGAGCGGAAAGACAAUCCUGGAC	
	UUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUCCAC	
	GACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGCGGA	
	CAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCGGCA	
	AUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUCAAG	
	GUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGAGAA	
	AACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAGAGA	
	AUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACACCCG	
	GUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUGCAG	
	AACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUGAGC	
	GACUACGACGUCGACCACAUCGUCCCGCAGAGCUUCCUGAAGGACGACAGC	
	AUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGCGAC	
	AACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGACAG	
	CUGCUGAACGCAAAGCUGAUCACACAGAGAAAGUUCGACAACCUGACAAAG	
	GCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAGAGA	
	CAGCUGGUCGAAACAAGACAGAUCACAAAGCACGUCGCACAGAUCCUGGAC	
	AGCAGAAUGAACACAAAGUACGACGAAAACGACAAGCUGAUCAGAGAAGUC	
	AAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGACUUC	
	CAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGACGCA	
	UACCUGAACGCAGUCGUCGGAACAGCACUGAUCAAGAAGUACCCGAAGCUG	
	GAAAGCGAAUUCGUCUACGGAGACUACAAGGUCUACGACGUCAGAAAGAUG	
	AUCGCAAAGAGCGAACAGGAAAUCGGAAAGGCAACAGCAAAGUACUUCUUC	
	UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA	
	GAAAUCAGAAAGAGACCGCUGAUCGAAACAAACGGAGAAACAGGAGAAAUC	
	GUCUGGGACAAGGGAAGAGACUUCGCAACAGUCAGAAAGGUCCUGAGCAUG	
	CCGCAGGUCAACAUCGUCAAGAAGACAGAAGUCCAGACAGGAGGAUUCAGC	
	AAGGAAAGCAUCCUGCCGAAGAGAAACAGCGACAAGCUGAUCGCAAGAAAG	
	AAGGACUGGGACCCGAAGAAGUACGGAGGAUUCGACAGCCCGACAGUCGCA	
	UACAGCGUCCUGGUCGUCGCAAAGGUCGAAAAGGGAAAGAGCAAGAAGCUG	
	AAGAGCGUCAAGGAACUGCUGGGAAUCACAAUCAUGGAAAGAAGCAGCUUC	
	GAAAAGAACCCGAUCGACUUCCUGGAAGCAAAGGGAUACAAGGAAGUCAAG	
	AAGGACCUGAUCAUCAAGCUGCCGAAGUACAGCCUGUUCGAACUGGAAAAC	
	GGAAGAAAGAGAAUGCUGGCAAGCGCAGGAGAACUGCAGAAGGGAAACGAA	
	CUGGCACUGCCGAGCAAGUACGUCAACUUCCUGUACCUGGCAAGCCACUAC	
	GAAAAGCUGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCUGUUCGUC	
	GAACAGCACAAGCACUACCUGGACGAAAUCAUCGAACAGAUCAGCGAAUUC	
	AGCAAGAGAGUCAUCCUGGCAGACGCAAACCUGGACAAGGUCCUGAGCGCA	
	UACAACAAGCACAGAGACAAGCCGAUCAGAGAACAGGCAGAAAACAUCAUC	
	CACCUGUUCACACUGACAAACCUGGGAGCACCGGCAGCAUUCAAGUACUUC	
	GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC	

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Sequence Table

Description	Sequence	SEQ ID No.
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Amino acid sequence of dCas9 with two nuclear localization signals as the C-terminal amino acids	MDKKYSIGLAIGINSVGWAVITDEYKVPSKKFKVLGNIDRHSIKKNLIGAL LFDSGETAEATRLKRTARRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLE ESELVEEDKKHERHPIEGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRL IYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINAS GVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLIPNEKSN FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL RVNTEITKAPLSASMIKRYDEHHQDLILLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMINFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVIV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN EDILEDIVLILTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLS RKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLIFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAR ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLIRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKEDNLIKAERGGLSELDKAGFIK RQLVETRQIIKHVAQILDSRMNIKYDENDKLIREVKVITLKSKLVSDFRKD FQFYKVREINNYHHAHDAYLNAVVGIALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGESKESILPKRNSDKLIAR KKDWDPKKYGGFDSPIVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGN ELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISE FSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLINLGAPAAFKY FDTTIDRKRYISTKEVLDATLIHQSITGLYETRIDLSQLGGDGSGSPKKKR KVDGSPKKKRKVDSG	228
dCas9 mRNA ORF encoding SEQ ID NO: 228 using minimal uridine codons, with start and stop codons	AUGGACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGA UGGGCAGUCAUCACAGACGAAUACAAGGUCCCGAGCAAGAAGUUCAAGGUC CUGGGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUG CUGUUCGACAGCGGAGAAACAGCAGAAGCAACAAGACUGAAGAGAACAGCA AGAAGAAGAUACACAAGAAGAAAGAACAGAAUCUGCUACCUGCAGGAAAUC UUCAGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAA GAAAGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUC GGAAACAUCGUCGACGAAGUCGCAUACCACGAAAAGUACCCGACAAUCUAC CACCUGAGAAAGAAGCUGGUCGACAGCACAGACAAGGCAGACCUGAGACUG AUCUACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUC GAAGGAGACCUGAACCCGGACAACAGCGACGUCGACAAGCUGUUCAUCCAG CUGGUCCAGACAUACAACCAGCUGUUCGAAGAAAACCCGAUCAACGCAAGC GGAGUCGACGCAAAGGCAAUCCUGAGCGCAAGACUGAGCAAGAGCAGAAGA CUGGAAAACCUGAUCGCACAGCUGCCGGGAGAAAAGAAGAACGGACUGUUC GGAAACCUGAUCGCACUGAGCCUGGGACUGACACCGAACUUCAAGAGCAAC UUCGACCUGGCAGAAGACGCAAAGCUGCAGCUGAGCAAGGACACAUACGAC GACGACCUGGACAACCUGCUGGCACAGAUCGGAGACCAGUACGCAGACCUG UUCCUGGCAGCAAAGAACCUGAGCGACGCAAUCCUGCUGAGCGACAUCCUG AGAGUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAG AGAUACGACGAACACCACCAGGACCUGACACUGCUGAAGGCACUGGUCAGA CAGCAGCUGCCGGAAAAGUACAAGGAAAUCUUCUUCGACCAGAGCAAGAAC GGAUACGCAGGAUACAUCGACGGAGGAGCAAGCCAGGAAGAAUUCUACAAG UUCAUCAAGCCGAUCCUGGAAAAGAUGGACGGAACAGAAGAACUGCUGGUC AAGCUGAACAGAGAAGACCUGCUGAGAAAGCAGAGAACAUUCGACAACGGA AGCAUCCCGCACCAGAUCCACCUGGGAGAACUGCACGCAAUCCUGAGAAGA CAGGAAGACUUCUACCCGUUCCUGAAGGACAACAGAGAAAAGAUCGAAAAG AUCCUGACAUUCAGAAUCCCGUACUACGUCGGACCGCUGGCAAGAGGAAAC AGCAGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGG AACUUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAA AGAAUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAG CACAGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUC AAGUACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAG AAGAAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUC AAGCAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUC GAAAUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCAC GACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAAC GAAGACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGA	229

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Sequence Table

Description	Sequence	SEQ ID No.
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	GACUUCCUGAAGAGCGACGGAUUCGCAAACAGAAACUUCAUGCAGCUGAUC	
	CACGACGACAGCCUGACAUUCAAGGAAGACAUCCAGAAGGCACAGGUCAGC	
	GGACAGGGAGACAGCCUGCACGAACACAUCGCAAACCUGGCAGGAAGCCCG	
	GCAAUCAAGAAGGGAAUCCUGCAGACAGUCAAGGUCGUCGACGAACUGGUC	
	AAGGUCAUGGGAAGACACAAGCCGGAAAACAUCGUCAUCGAAAUGGCAAGA	
	GAAAACCAGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAAUGAAG	
	AGAAUCGAAGAAGGAAUCAAGGAACUGGGAAGCCAGAUCCUGAAGGAACAC	
	CCGGUCGAAAACACACAGCUGCAGAACGAAAAGCUGUACCUGUACUACCUG	
	CAGAACGGAAGAGACAUGUACGUCGACCAGGAACUGGACAUCAACAGACUG	
	AGCGACUACGACGUCGACGCAAUCGUCCCGCAGAGCUUCCUGAAGGACGAC	
	AGCAUCGACAACAAGGUCCUGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
	GACAACGUCCCGAGCGAAGAAGUCGUCAAGAAGAUGAAGAACUACUGGAGA	
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	AAGGCAGAGAGAGGAGGACUGAGCGAACUGGACAAGGCAGGAUUCAUCAAG	
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	GUCAAGGUCAUCACACUGAAGAGCAAGCUGGUCAGCGACUUCAGAAAGGAC	
	UUCCAGUUCUACAAGGUCAGAGAAAUCAACAACUACCACCACGCACACGAC	
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	UUCGACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUG	
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	AAGAGAAAGGUCGACAGCGGAUAG	
dCas9 coding sequence encoding SEQ ID NO: 228 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GACAAGAAGUACAGCAUCGGACUGGCAAUCGGAACAAACAGCGUCGGAUGG	230
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	GGAAACACAGACAGACACAGCAUCAAGAAGAACCUGAUCGGAGCACUGCUG	
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	AGCAACGAAAUGGCAAAGGUCGACGACAGCUUCUUCCACAGACUGGAAGAA	
	AGCUUCCUGGUCGAAGAAGACAAGAAGCACGAAAGACACCCGAUCUUCGGA	
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	UACCUGGCACUGGCACACAUGAUCAAGUUCAGAGGACACUUCCUGAUCGAA	
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	GUCAACACAGAAAUCACAAAGGCACCGCUGAGCGCAAGCAUGAUCAAGAGA	
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Sequence Table

Description	Sequence	SEQ ID No.
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	AGAUUCGCAUGGAUGACAAGAAAGAGCGAAGAAACAAUCACACCGUGGAAC	
	UUCGAAGAAGUCGUCGACAAGGGAGCAAGCGCACAGAGCUUCAUCGAAAGA	
	AUGACAAACUUCGACAAGAACCUGCCGAACGAAAAGGUCCUGCCGAAGCAC	
	AGCCUGCUGUACGAAUACUUCACAGUCUACAACGAACUGACAAAGGUCAAG	
	UACGUCACAGAAGGAAUGAGAAAGCCGGCAUUCCUGAGCGGAGAACAGAAG	
	AAGGCAAUCGUCGACCUGCUGUUCAAGACAAACAGAAAGGUCACAGUCAAG	
	CAGCUGAAGGAAGACUACUUCAAGAAGAUCGAAUGCUUCGACAGCGUCGAA	
	AUCAGCGGAGUCGAAGACAGAUUCAACGCAAGCCUGGGAACAUACCACGAC	
	CUGCUGAAGAUCAUCAAGGACAAGGACUUCCUGGACAACGAAGAAAACGAA	
	GACAUCCUGGAAGACAUCGUCCUGACACUGACACUGUUCGAAGACAGAGAA	
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	AUGAAGCAGCUGAAGAGAAGAAGAUACACAGGAUGGGGAAGACUGAGCAGA	
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	UACAGCAACAUCAUGAACUUCUUCAAGACAGAAAUCACACUGGCAAACGGA	
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	GACACAACAAUCGACAGAAAGAGAUACACAAGCACAAAGGAAGUCCUGGAC	
	GCAACACUGAUCCACCAGAGCAUCACAGGACUGUACGAAACAAGAAUCGAC	
	CUGAGCCAGCUGGGAGGAGACGGAAGCGGAAGCCCGAAGAAGAAGAGAAAG	
	GUCGACGGAAGCCCGAAGAAGAAGAGAAAGGUCGACAGCGGA	
T7 Promoter	TAATACGACTCACTATA	231
Human beta-globin 5' UTR	ACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACC	232
Human beta-globin 3' UTR	GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAG TCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTC TGCCTAATAAAAAACATTTATTTTCATTGC	233
Human alpha-globin 5' UTR	CATAAACCCTGGCGCGCTCGCGGCCCGGCACTCTTCTGGTCCCCACAGACT CAGAGAGAACCCACC	234

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Sequence Table

Description	Sequence	SEQ ID No.
Human alpha-globin 3' UTR	GCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCCAGCCC CTCCTCCCCTTCCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAG TGGGCGGC	235
Xenopus laevis beta-globin 5' UTR	AAGCTCAGAATAAACGCTCAACTTTGGCC	236
Xenopus laevis beta-globin 3' UTR	ACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAATACCAACT TACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTCGTATCTGCTCC TAATAAAAAGAAAGTTTCTTCACATTCT	237
Bovine Growth Hormone 5' UTR	CAGGGTCCTGTGGACAGCTCACCAGCT	238
Bovine Growth Hormone 3' UTR	TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCA	239
Mus musculus hemoglobin alpha, adult chain 1 (Hba-a1), 3' UTR	GCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTG CACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAG	240
HSD17B4 5' UTR	TCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTTGCA GGCCTTATTC	241
G282 single guide RNA targeting the mouse TTR gene	mU*mU*mA*CAGCCACGUCUACAGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	242
	Not used	243
Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 204, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCCATGGACAAGAAGTACAGCATCGGACTGGACAT CGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCC GAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAA GAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAAC AAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAAT CTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAG CTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCA CGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGA AAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGA CAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTT CAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGT CGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGA AAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAG ACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGA AAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGAC ACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCT GAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGG AGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAAT CCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCT GAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACT GCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTT CTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAG CCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGG AACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCA GAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACT GCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAA	244

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Sequence Table

Description	Sequence	SEQ ID No.
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	CGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAA	
	CGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTA	
	CAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGC	
	ATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTTCAAGAC	
	AAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGAT	
	CGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGC	
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	CCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACT	
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	AAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAAAAGCAT	
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	AAATGGAAAGAACCTCGAG	
Alternative Cas9 ORF with 19.36% U content	ATGGATAAGAAGTACTCGATCGGGCTGGATATCGGAACTAATTCCGTGGGT	245
	TGGGCAGTGATCACGGATGAATACAAAGTGCCGTCCAAGAAGTTCAAGGTC	
	CTGGGGAACACCGATAGACACAGCATCAAGAAGAATCTCATCGGAGCCCTG	
	CTGTTTGACTCCGGCGAAACCGCAGAAGCGACCCGGCTCAAACGTACCGCG	
	AGGCGACGCTACACCCGGCGGAAGAATCGCATCTGCTATCTGCAAGAAATC	
	TTTTCGAACGAAATGGCAAAGGTGGACGACAGCTTCTTCCACCGCCTGGAA	
	GAATCTTTCCTGGTGGAGGAGGACAAGAAGCATGAACGGCATCCTATCTTT	
	GGAAACATCGTGGACGAAGTGGCGTACCACGAAAAGTACCCGACCATCTAC	
	CATCTGCGGAAGAAGTTGGTTGACTCAACTGACAAGGCCGACCTCAGATTG	
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Sequence Table

Description	Sequence	SEQ ID No.
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	CTCGAAAACCTGATCGCACAGCTGCCGGGAGAGAAGAAGAACGGACTTTTC	
	GGCAACTTGATCGCTCTCTCACTGGGACTCACTCCCAATTTCAAGTCCAAT	
	TTTGACCTGGCCGAGGACGCGAAGCTGCAACTCTCAAAGGACACCTACGAC	
	GACGACTTGGACAATTTGCTGGCACAAATTGGCGATCAGTACGCGGATCTG	
	TTCCTTGCCGCTAAGAACCTTTCGGACGCAATCTTGCTGTCCGATATCCTG	
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	TTCATCAAGCCAATCCTGGAAAAGATGGACGGAACCGAAGAACTGCTGGTC	
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	ATGCCGCAAGTCAATATTGTGAAGAAAACCGAAGTGCAAACCGGCGGATTT	
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	AAGAAAGACTGGGACCCGAAGAAGTACGGAGGATTCGATTCGCCGACTGTC	
	GCATACTCCGTCCTCGTGGTGGCCAAGGTGGAGAAGGGAAAGAGCAAGAAG	
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	ATCCACTTGTTCACCCTGACTAACCTGGGAGCTCCAGCCGCCTTCAAGTAC	
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Sequence Table

Description	Sequence	SEQ ID No.
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Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 245, Kozak sequence, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCTGCCACCATGGATAAGAAGTACTCGATCGGGCT GGATATCGGAACTAATTCCGTGGGTTGGGCAGTGATCACGGATGAATACAA AGTGCCGTCCAAGAAGTTCAAGGTCCTGGGGAACACCGATAGACACAGCAT CAAGAAGAATCTCATCGGAGCCCTGCTGTTTGACTCCGGCGAAACCGCAGA AGCGACCCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAA TCGCATCTGCTATCTGCAAGAAATCTTTTCGAACGAAATGGCAAAGGTGGA CGACAGCTTCTTCCACCGCCTGGAAGAATCTTTCCTGGTGGAGGAGGACAA GAAGCATGAACGGCATCCTATCTTTGGAAACATCGTGGACGAAGTGGCGTA CCACGAAAAGTACCCGACCATCTACCATCTGCGGAAGAAGTTGGTTGACTC AACTGACAAGGCCGACCTCAGATTGATCTACTTGGCCCTCGCCCATATGAT CAAATTCCGCGGACACTTCCTGATCGAAGGCGATCTGAACCCTGATAACTC CGACGTGGATAAGCTGTTCATTCAACTGGTGCAGACCTACAACCAACTGTT CGAAGAAAACCCAATCAATGCCAGCGGCGTCGATGCCAAGGCCATCCTGTC CGCCCGGCTGTCGAAGTCGCGGCGCCTCGAAAACCTGATCGCACAGCTGCC GGGAGAGAAGAAGAACGGACTTTTCGGCAACTTGATCGCTCTCTCACTGGG ACTCACTCCCAATTTCAAGTCCAATTTTGACCTGGCCGAGGACGCGAAGCT GCAACTCTCAAAGGACACCTACGACGACGACTTGGACAATTTGCTGGCACA AATTGGCGATCAGTACGCGGATCTGTTCCTTGCCGCTAAGAACCTTTCGGA CGCAATCTTGCTGTCCGATATCCTGCGCGTGAACACCGAAATAACCAAAGC GCCGCTTAGCGCCTCGATGATTAAGCGGTACGACGAGCATCACCAGGATCT CACGCTGCTCAAAGCGCTCGTGAGACAGCAACTGCCTGAAAAGTACAAGGA GATTTTCTTCGACCAGTCCAAGAATGGGTACGCAGGGTACATCGATGGAGG CGCCAGCCAGGAAGAGTTCTATAAGTTCATCAAGCCAATCCTGGAAAAGAT GGACGGAACCGAAGAACTGCTGGTCAAGCTGAACAGGGAGGATCTGCTCCG CAAACAGAGAACCTTTGACAACGGAAGCATTCCACACCAGATCCATCTGGG TGAGCTGCACGCCATCTTGCGGCGCCAGGAGGACTTTTACCCATTCCTCAA GGACAACCGGGAAAAGATCGAGAAAATTCTGACGTTCCGCATCCCGTATTA CGTGGGCCCACTGGCGCGCGGCAATTCGCGCTTCGCGTGGATGACTAGAAA ATCAGAGGAAACCATCACTCCTTGGAATTTCGAGGAAGTTGTGGATAAGGG AGCTTCGGCACAATCCTTCATCGAACGAATGACCAACTTCGACAAGAATCT CCCAAACGAGAAGGTGCTTCCTAAGCACAGCCTCCTTTACGAATACTTCAC TGTCTACAACGAACTGACTAAAGTGAAATACGTTACTGAAGGAATGAGGAA GCCGGCCTTTCTGAGCGGAGAACAGAAGAAAGCGATTGTCGATCTGCTGTT CAAGACCAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAA GAAGATCGAGTGTTTCGACTCAGTGGAAATCAGCGGAGTGGAGGACAGATT CAACGCTTCGCTGGGAACCTATCATGATCTCCTGAAGATCATCAAGGACAA GGACTTCCTTGACAACGAGGAGAACGAGGACATCCTGGAAGATATCGTCCT GACCTTGACCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGAC CTACGCTCATCTCTTCGACGATAAGGTCATGAAACAACTCAAGCGCCGCCG GTACACTGGTTGGGGCCGCCTCTCCCGCAAGCTGATCAACGGTATTCGCGA TAAACAGAGCGGTAAAACTATCCTGGATTTCCTCAAATCGGATGGCTTCGC TAATCGTAACTTCATGCAGTTGATCCACGACGACAGCCTGACCTTTAAGGA GGACATCCAGAAAGCACAAGTGAGCGGACAGGGAGACTCACTCCATGAACA CATCGCGAATCTGGCCGGTTCGCCGGCGATTAAGAAGGGAATCCTGCAAAC TGTGAAGGTGGTGGACGAGCTGGTGAAGGTCATGGGACGGCACAAACCGGA GAATATCGTGATTGAAATGGCCCGAGAAAACCAGACTACCCAGAAGGGCCA GAAGAACTCCCGCGAAAGGATGAAGCGGATCGAAGAAGGAATCAAGGAGCT GGGCAGCCAGATCCTGAAAGAGCACCCGGTGGAAAACACGCAGCTGCAGAA CGAGAAGCTCTACCTGTACTATTTGCAAAATGGACGGGACATGTACGTGGA CCAAGAGCTGGACATCAATCGGTTGTCTGATTACGACGTGGACCACATCGT TCCACAGTCCTTTCTGAAGGATGACTCCATCGATAACAAGGTGTTGACTCG CAGCGACAAGAACAGAGGGAAGTCAGATAATGTGCCATCGGAGGAGGTCGT GAAGAAGATGAAGAATTACTGGCGGCAGCTCCTGAATGCGAAGCTGATTAC CCAGAGAAAGTTTGACAATCTCACTAAAGCCGAGCGCGGCGGACTCTCAGA GCTGGATAAGGCTGGATTCATCAAACGGCAGCTGGTCGAGACTCGGCAGAT TACCAAGCACGTGGCGCAGATCCTGGACTCCCGCATGAACACTAAATACGA CGAGAACGATAAGCTCATCCGGGAAGTGAAGGTGATTACCCTGAAAAGCAA ACTTGTGTCGGACTTTCGGAAGGACTTTCAGTTTTACAAAGTGAGAGAAAT CAACAACTACCATCACGCGCATGACGCATACCTCAACGCTGTGGTCGGCAC CGCCCTGATCAAGAAGTACCCTAAACTTGAATCGGAGTTTGTGTACGGAGA CTACAAGGTCTACGACGTGAGGAAGATGATAGCCAAGTCCGAACAGGAAAT CGGGAAAGCAACTGCGAAATACTTCTTTTACTCAAACATCATGAACTTCTT CAAGACTGAAATTACGCTGGCCAATGGAGAAATCAGGAAGAGGCCACTGAT CGAAACTAACGGAGAAACGGGCGAAATCGTGTGGGACAAGGGCAGGGACTT CGCAACTGTTCGCAAAGTGCTCTCTATGCCGCAAGTCAATATTGTGAAGAA AACCGAAGTGCAAACCGGCGGATTTTCAAAGGAATCGATCCTCCCAAAGAG AAATAGCGACAAGCTCATTGCACGCAAGAAAGACTGGGACCCGAAGAAGTA	246

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Sequence Table

Description	Sequence	SEQ ID No.
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	GGAGGCGAAGGGTTACAAGGAGGTGAAGAAGGATCTGATCATCAAACTGCC	
	CAAGTACTCACTGTTCGAACTGGAAAATGGTCGGAAGCGCATGCTGGCTTC	
	GGCCGGAGAACTCCAGAAAGGAAATGAGCTGGCCTTGCCTAGCAAGTACGT	
	CAACTTCCTCTATCTTGCTTCGCACTACGAGAAACTCAAAGGGTCACCGGA	
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	CGCCAACCTCGACAAAGTCCTGTCGGCCTACAATAAGCATAGAGATAAGCC	
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	GGGAGCTCCAGCCGCCTTCAAGTACTTCGATACTACTATCGACCGCAAAAG	
	ATACACGTCCACCAAGGAAGTTCTGGACGCGACCCTGATCCACCAAAGCAT	
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	TGGCCCCAAGAAGAAACGGAAGGTGTGATAGCTAGCCATCACATTTAAAAG	
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	AAAAAATGGAAAGAACCTCGAG	
Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 245, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT	247
	GCAGGCCTTATTCGGATCTATGGATAAGAAGTACTCGATCGGGCTGGATAT	
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	CCGGCTCAAACGTACCGCGAGGCGACGCTACACCCGGCGGAAGAATCGCAT	
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	CAACCGCAAGGTGACCGTCAAGCAGCTTAAAGAGGACTACTTCAAGAAGAT	
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	CCTTGACAACGAGGAGAACGAGGACATCCTGGAAGATATCGTCCTGACCTT	
	GACCCTTTTCGAGGATCGCGAGATGATCGAGGAGAGGCTTAAGACCTACGC	
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Sequence Table

Description	Sequence	SEQ ID No.
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Cas9 transcript comprising Kozak sequence with Cas9 ORF using codons with generally high expression in humans	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT	249
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Sequence Table

Description	Sequence	SEQ ID No.
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Cas9 ORF with splice junctions removed; 12.75% U content	ATGGACAAGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGA	250
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	ATCTACCTGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATC	
	GAAGGAGACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAG	
	CTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGC	
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Sequence Table

Description	Sequence	SEQ ID No.
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	GACGACCTGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTG	
	TTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTG	
	AGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAG	
	AGATACGACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGA	
	CAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAAC	
	GGATACGCAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAG	
	TTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTC	
	AAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGA	
	AGCATCCCGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGA	
	CAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAG	
	ATCCTGACATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAAC	
	AGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGG	
	AACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAA	
	AGAATGACAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAG	
	CACAGCCTGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTC	
	AAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAG	
	AAGAAGGCAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTC	
	AAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTC	
	GAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCAC	
	GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAAC	
	GAAGACATCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGA	
	GAAATGATCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAG	
	GTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGC	
	AGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTG	
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	GGACAGGGAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCG	
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	AGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGC	
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	GCATACCTGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAG	
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	GGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAA	
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	ATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTC	
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	TTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGC	
	GCATACAACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATC	
	ATCCACCTGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTAC	
	TTCGACACAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTG	
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	AAGGTCTAG	

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Sequence Table

Description	Sequence	SEQ ID No.
Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 250, Kozak sequence, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT GCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACT GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAA GGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCAT CAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGA AGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAA CAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGA CGACAGCTTCTTCCACcggCTGGAAGAAAGCTTCCTGGTCGAAGAAGACAA GAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATA CCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAG CACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGAT CAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAG CGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTT CGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAG CGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCC GGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGG ACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCT GCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACA GATCGGAGACCAGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGA CGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGC ACCGCTGAGCGCAAGCATGATCAAGAGATACGACGAACACCACCAGGACCT GACACTGCTGAAGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGA AATCTTCTTCGACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGG AGCAAGCCAGGAAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGAT GGACGGAACAGAAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAG AAAGCAGAGAACATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGG AGAACTGCACGCAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAA GGACAACAGAGAAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTA CGTCGGACCGCTGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAA GAGCGAAGAAACAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGG AGCAAGCGCACAGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCT GCCGAACGAAAAGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCAC AGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAA GCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTT CAAGACAAACAGAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAA GAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATT CAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAA GGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCT GACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGAC ATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAG ATACACAGGATGGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGA CAAGCAGAGCGGAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGC AAACAGAAACTTCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGA AGACATCCAGAAGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACA CATCGCAAACCTGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGAC AGTCAAGGTCGTCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGA AAACATCGTCATCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACA GAAGAACAGCAGAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACT GGGAAGCCAGATCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAA CGAAAAGCTGTACCTGTACTACCTGCAaAACGGAAGAGACATGTACGTCGA CCAGGAACTGGACATCAACAGACTGAGCGACTACGACGTCGACCACATCGT CCCGCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAG AAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGT CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGA ACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGAT CACAAAGCACGTCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGA CGAAAACGACAAGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAA GCTGGTCAGCGACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAAT CAACAACTACCACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAAC AGCACTGATCAAGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGA CTACAAGGTCTACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAAT CGGAAAGGCAACAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTT CAAGACAGAAATCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGAT CGAAACAAACGGAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTT CGCAACAGTCAGAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAA GACAGAAGTCCAGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAG AAACAGCGACAAGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTA CGGAGGATTCGACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAA GGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGG AATCACAATCATGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCT GGAAGCAAAGGGATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCC	251

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Sequence Table

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	AGACAACGAACAGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGA	
	CGAAATCATCGAACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGA	
	CGCAAACCTGGACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCC	
	GATCAGAGAACAGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCT	
	GGGAGCACCGGCAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAG	
	ATACACAAGCACAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCAT	
	CACAGGACTGTACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGG	
	AGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCTAGCTAGCCATCACATTTAA	
	AAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAATGAAGATCAATAG	
	CTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGCCAACACCCTGTCTA	
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Cas9 ORF with minimal uridine codons frequently used in humans in general; 12.75% U content	ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACAGCGTGGGC	252
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	AGAAGAAGATACACCAGAAGAAAGAACAGAATCTGCTACCTGCAGGAGATC	
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	GAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGAGACACCCCATCTTC	
	GGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTAC	
	CACCTGAGAAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTGAGACTG	
	ATCTACCTGGCCCTGGCCCACATGATCAAGTTCAGAGGCCACTTCCTGATC	
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	CTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGC	
	GGCGTGGACGCCAAGGCCATCCTGAGCGCCAGACTGAGCAAGAGCAGAAGA	
	CTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAACGGCCTGTTC	
	GGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAAC	
	TTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGAC	
	GACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTG	
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	GACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAAC	
	GAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACAGA	
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	GACTTCCTGAAGAGCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATC	
	CACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGC	
	GGCCAGGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCC	
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	AAGGTGATGGGCAGACACAAGCCCGAGAACATCGTGATCGAGATGGCCAGA	
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	GACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACTGGAGA	
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Sequence Table

Description	Sequence	SEQ ID No.
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	CTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGAGAAAG	
	ATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTC	
	TTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCCTGGCCAAC	
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	GTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAG	
	TTCAGCAAGAGAGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGC	
	GCCTACAACAAGCACAGAGACAAGCCCATCAGAGAGCAGGCCGAGAACATC	
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	TTCGACACCACCATCGACAGAAAGAGATACACCAGCACCAAGGAGGTGCTG	
	GACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACCAGAATC	
	GACCTGAGCCAGCTGGGCGGCGACGGCGGCGGCAGCCCCAAGAAGAAGAGA	
	AAGGTGTGA	
Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 252, Kozak sequence, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT	253
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	GGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGACAGACACAGCAT	
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	GAGCGAGGAGACCATCACCCCCTGGAACTTCGAGGAGGTGGTGGACAAGGG	
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	GCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGGACCTGCTGTT	
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	CAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAA	
	GGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCT	
	GACCCTGACCCTGTTCGAGGACAGAGAGATGATCGAGGAGAGACTGAAGAC	
	CTACGCCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGAGAAGAAG	
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Sequence Table

Description	Sequence	SEQ ID No.
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	GAAGAACAGCAGAGAGAGAATGAAGAGAATCGAGGAGGGCATCAAGGAGCT	
	GGGCAGCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAA	
	CGAGAAGCTGTACCTGTACTACCTGCAGAACGGCAGAGACATGTACGTGGA	
	CCAGGAGCTGGACATCAACAGACTGAGCGACTACGACGTGGACCACATCGT	
	GCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCAG	
	AAGCGACAAGAACAGAGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGT	
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Cas9 ORF with minimal uridine codons infrequently used in humans in general; 12.75% U content	ATGGACAAAAAATACAGCATAGGGCTAGACATAGGGACGAACAGCGTAGGG	254
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Sequence Table

Description	Sequence	SEQ ID No.
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Cas9 transcript with 5' UTR of HSD, ORF corresponding to SEQ ID NO: 254, Kozak sequence, and 3' UTR of ALB	GGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT	255
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Sequence Table

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	CGCGATACTACTAAGCGACATACTACGAGTAAACACGGAAATAACGAAAGC	
	GCCGCTAAGCGCGAGCATGATAAAACGATACGACGAACACCACCAAGACCT	
	AACGCTACTAAAAGCGCTAGTACGACAACAACTACCGGAAAAATACAAAGA	
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Sequence Table

Description	Sequence	SEQ ID No.
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Sequence Table

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Cas9 transcript with 5' UTR from CMV, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3' UTR of ALB	GGGCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGA	257
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	ACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACC	
	TGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACC	
	TGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACC	
	TGGACAACCTGCTGGCACAGATCGGAGACCAGTACGCAGACCTGTTCCTGG	
	CAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCA	
	ACACAGAAATCACAAAGGCACCGCTGAGCGCAAGCATGATCAAGAGATACG	
	ACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGC	
	TGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACG	
	CAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCA	
	AGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGA	
	ACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCC	
	CGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAG	
	ACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGA	
	CATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGAT	
	TCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCG	
	AAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGA	
	CAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCC	
	TGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACG	
	TCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGG	
	CAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGC	
	TGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCA	
	GCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGC	
	TGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACA	
	TCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGA	
	TCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGA	
	AGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGC	
	TGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCC	
	TGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACG	
	ACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGG	
	GAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCA	
	AGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCA	
	TGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACC	
	AGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCG	
	AAGAAGGAATCAAGGAACTGGGAAGCCAGATCCTGAAGGAACACCCGGTCG	
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Sequence Table

Description	Sequence	SEQ ID No.
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	ACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACG	
	TCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGC	
	TGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAG	
	AGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGC	
	TGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCA	
	GAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGG	
	TCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGT	
	TCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACC	
	TGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAA	
	GCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCG	
	CAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACA	
	GCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAA	
	TCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCT	
	GGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC	
	AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGG	
	AAAGCATCCTGCCGAAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGG	
	ACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACA	
	GCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGA	
	GCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA	
	AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGG	
	ACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAA	
	GAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGG	
	CACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAA	
	AGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAAC	
	AGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCA	
	AGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA	
	ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACC	
	TGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACA	
	CAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAA	
	CACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGA	
	GCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCT	
	AGCTAGCCATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAA	
	AGAAAATGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGT	
	AAAGCCAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTC	
	TTTTCTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCTCGAG	
Cas9 transcript with 5' UTR from HBB, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3' UTR of HBB	GGGACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACA	258
	CCGGATCTGCCACCATGGACAAGAAGTACAGCATCGGACTGGACATCGGAA	
	CAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAAGGTCCCGAGCA	
	AGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCATCAAGAAGAACC	
	TGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGAAGCAACAAGAC	
	TGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAACAGAATCTGCT	
	ACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGACGACAGCTTCT	
	TCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAAGAAGCACGAAA	
	GACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATACCACGAAAAGT	
	ACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAGCACAGACAAGG	
	CAGACCTGAGACTGATCTACCTGGCACTGGCACACATGATCAAGTTCAGAG	
	GACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAGCGACGTCGACA	
	AGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTTCGAAGAAAACC	
	CGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAGCGCAAGACTGA	
	GCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCCGGGAGAAAAGA	
	AGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGGACTGACACCGA	
	ACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCTGCAGCTGAGCA	
	AGGACACATACGACGACGACCTGGACAACCTGCTGGCACAGATCGGAGACC	
	AGTACGCAGACCTGTTCCTGGCAGCAAAGAACCTGAGCGACGCAATCCTGC	
	TGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGCACCGCTGAGCG	
	CAAGCATGATCAAGAGATACGACGAACACCACCAGGACCTGACACTGCTGA	
	AGGCACTGGTCAGACAGCAGCTGCCGGAAAAGTACAAGGAAATCTTCTTCG	
	ACCAGAGCAAGAACGGATACGCAGGATACATCGACGGAGGAGCAAGCCAGG	
	AAGAATTCTACAAGTTCATCAAGCCGATCCTGGAAAAGATGGACGGAACAG	
	AAGAACTGCTGGTCAAGCTGAACAGAGAAGACCTGCTGAGAAAGCAGAGAA	
	CATTCGACAACGGAAGCATCCCGCACCAGATCCACCTGGGAGAACTGCACG	
	CAATCCTGAGAAGACAGGAAGACTTCTACCCGTTCCTGAAGGACAACAGAG	
	AAAAGATCGAAAAGATCCTGACATTCAGAATCCCGTACTACGTCGGACCGC	
	TGGCAAGAGGAAACAGCAGATTCGCATGGATGACAAGAAAGAGCGAAGAAA	
	CAATCACACCGTGGAACTTCGAAGAAGTCGTCGACAAGGGAGCAAGCGCAC	
	AGAGCTTCATCGAAAGAATGACAAACTTCGACAAGAACCTGCCGAACGAAA	
	AGGTCCTGCCGAAGCACAGCCTGCTGTACGAATACTTCACAGTCTACAACG	
	AACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAAGCCGGCATTCC	

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Sequence Table

Description	Sequence	SEQ ID No.
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	GAAAGGTCACAGTCAAGCAGCTGAAGGAAGACTACTTCAAGAAGATCGAAT	
	GCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATTCAACGCAAGCC	
	TGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGG	
	ACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCTGACACTGACAC	
	TGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGACATACGCACACC	
	TGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAGATACACAGGAT	
	GGGGAAGACTGAGCAGAAAGCTGATCAACGGAATCAGAGACAAGCAGAGCG	
	GAAAGACAATCCTGGACTTCCTGAAGAGCGACGGATTCGCAAACAGAAACT	
	TCATGCAGCTGATCCACGACGACAGCCTGACATTCAAGGAAGACATCCAGA	
	AGGCACAGGTCAGCGGACAGGGAGACAGCCTGCACGAACACATCGCAAACC	
	TGGCAGGAAGCCCGGCAATCAAGAAGGGAATCCTGCAGACAGTCAAGGTCG	
	TCGACGAACTGGTCAAGGTCATGGGAAGACACAAGCCGGAAAACATCGTCA	
	TCGAAATGGCAAGAGAAAACCAGACAACACAGAAGGGACAGAAGAACAGCA	
	GAGAAAGAATGAAGAGAATCGAAGAAGGAATCAAGGAACTGGGAAGCCAGA	
	TCCTGAAGGAACACCCGGTCGAAAACACACAGCTGCAGAACGAAAAGCTGT	
	ACCTGTACTACCTGCAGAACGGAAGAGACATGTACGTCGACCAGGAACTGG	
	ACATCAACAGACTGAGCGACTACGACGTCGACCACATCGTCCCGCAGAGCT	
	TCCTGAAGGACGACAGCATCGACAACAAGGTCCTGACAAGAAGCGACAAGA	
	ACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGTCAAGAAGATGA	
	AGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCACACAGAGAAAGT	
	TCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGAACTGGACAAGG	
	CAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGATCACAAAGCACG	
	TCGCACAGATCCTGGACAGCAGAATGAACACAAAGTACGACGAAAACGACA	
	AGCTGATCAGAGAAGTCAAGGTCATCACACTGAAGAGCAAGCTGGTCAGCG	
	ACTTCAGAAAGGACTTCCAGTTCTACAAGGTCAGAGAAATCAACAACTACC	
	ACCACGCACACGACGCATACCTGAACGCAGTCGTCGGAACAGCACTGATCA	
	AGAAGTACCCGAAGCTGGAAAGCGAATTCGTCTACGGAGACTACAAGGTCT	
	ACGACGTCAGAAAGATGATCGCAAAGAGCGAACAGGAAATCGGAAAGGCAA	
	CAGCAAAGTACTTCTTCTACAGCAACATCATGAACTTCTTCAAGACAGAAA	
	TCACACTGGCAAACGGAGAAATCAGAAAGAGACCGCTGATCGAAACAAACG	
	GAGAAACAGGAGAAATCGTCTGGGACAAGGGAAGAGACTTCGCAACAGTCA	
	GAAAGGTCCTGAGCATGCCGCAGGTCAACATCGTCAAGAAGACAGAAGTCC	
	AGACAGGAGGATTCAGCAAGGAAAGCATCCTGCCGAAGAGAAACAGCGACA	
	AGCTGATCGCAAGAAAGAAGGACTGGGACCCGAAGAAGTACGGAGGATTCG	
	ACAGCCCGACAGTCGCATACAGCGTCCTGGTCGTCGCAAAGGTCGAAAAGG	
	GAAAGAGCAAGAAGCTGAAGAGCGTCAAGGAACTGCTGGGAATCACAATCA	
	TGGAAAGAAGCAGCTTCGAAAAGAACCCGATCGACTTCCTGGAAGCAAAGG	
	GATACAAGGAAGTCAAGAAGGACCTGATCATCAAGCTGCCGAAGTACAGCC	
	TGTTCGAACTGGAAAACGGAAGAAAGAGAATGCTGGCAAGCGCAGGAGAAC	
	TGCAGAAGGGAAACGAACTGGCACTGCCGAGCAAGTACGTCAACTTCCTGT	
	ACCTGGCAAGCCACTACGAAAAGCTGAAGGGAAGCCCGGAAGACAACGAAC	
	AGAAGCAGCTGTTCGTCGAACAGCACAAGCACTACCTGGACGAAATCATCG	
	AACAGATCAGCGAATTCAGCAAGAGAGTCATCCTGGCAGACGCAAACCTGG	
	ACAAGGTCCTGAGCGCATACAACAAGCACAGAGACAAGCCGATCAGAGAAC	
	AGGCAGAAAACATCATCCACCTGTTCACACTGACAAACCTGGGAGCACCGG	
	CAGCATTCAAGTACTTCGACACAACAATCGACAGAAAGAGATACACAAGCA	
	CAAAGGAAGTCCTGGACGCAACACTGATCCACCAGAGCATCACAGGACTGT	
	ACGAAACAAGAATCGACCTGAGCCAGCTGGGAGGAGACGGAGGAGGAAGCC	
	CGAAGAAGAAGAGAAAGGTCTAGCTAGCGCTCGCTTTCTTGCTGTCCAATT	
	TCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATA	
	TTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTT	
	TCATTGCCTCGAG	
Cas9 transcript with 5' UTR from XBG, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3' UTR of XBG	GGGAAGCTCAGAATAAACGCTCAACTTTGGCCGGATCTGCCACCATGGACA	259
	AGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAG	
	TCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAA	
	ACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCG	
	ACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAA	
	GATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCA	
	ACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCT	
	TCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACA	
	TCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGA	
	GAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACC	
	TGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAG	
	ACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCC	
	AGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG	
	ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAA	
	ACCTGATCGCACAGCTGCCGGGAGAAAAGAAGAACGGACTGTTCGGAAACC	
	TGATCGCACTGAGCCTGGGACTGACACCGAACTTCAAGAGCAACTTCGACC	
	TGGCAGAAGACGCAAAGCTGCAGCTGAGCAAGGACACATACGACGACGACC	

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Sequence Table

Description	Sequence	SEQ ID No.
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	ACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGC	
	TGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACG	
	CAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCA	
	AGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGA	
	ACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCC	
	CGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAG	
	ACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGA	
	CATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGAT	
	TCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCG	
	AAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGA	
	CAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCC	
	TGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACG	
	TCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGG	
	CAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGC	
	TGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCA	
	GCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGC	
	TGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACA	
	TCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGA	
	TCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGA	
	AGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGC	
	TGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCC	
	TGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACG	
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	GAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCA	
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	TGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACC	
	AGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCG	
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	GAAGAGACATGTACGTCGACCAGGAACTGGACATCAACAGACTGAGCGACT	
	ACGACGTCGACCACATCGTCCCGCAGAGCTTCCTGAAGGACGACAGCATCG	
	ACAACAAGGTCCTGACAAGAAGCGACAAGAACAGAGGAAAGAGCGACAACG	
	TCCCGAGCGAAGAAGTCGTCAAGAAGATGAAGAACTACTGGAGACAGCTGC	
	TGAACGCAAAGCTGATCACACAGAGAAAGTTCGACAACCTGACAAAGGCAG	
	AGAGAGGAGGACTGAGCGAACTGGACAAGGCAGGATTCATCAAGAGACAGC	
	TGGTCGAAACAAGACAGATCACAAAGCACGTCGCACAGATCCTGGACAGCA	
	GAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGG	
	TCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGT	
	TCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACC	
	TGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAA	
	GCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCG	
	CAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACA	
	GCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAA	
	TCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCT	
	GGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC	
	AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGG	
	AAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGG	
	ACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACA	
	GCGTCCTGGTCGTCGCAAAGGTCGAAAAGGGAAAGAGCAAGAAGCTGAAGA	
	GCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA	
	AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGG	
	ACCTGATCATCAAGCTGCCGAAGTACAGCCTGTTCGAACTGGAAAACGGAA	
	GAAAGAGAATGCTGGCAAGCGCAGGAGAACTGCAGAAGGGAAACGAACTGG	
	CACTGCCGAGCAAGTACGTCAACTTCCTGTACCTGGCAAGCCACTACGAAA	
	AGCTGAAGGGAAGCCCGGAAGACAACGAACAGAAGCAGCTGTTCGTCGAAC	
	AGCACAAGCACTACCTGGACGAAATCATCGAACAGATCAGCGAATTCAGCA	
	AGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA	
	ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACC	
	TGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACA	
	CAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAA	
	CACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGA	
	GCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCT	
	AGCTAGCACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAAT	
	ACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTCGTAT	
	CTGCTCCTAATAAAAAGAAAGTTTCTTCACATTCTCTCGAG	
Cas9 transcript	AGGAAGCTCAGAATAAACGCTCAACTTTGGCCGGATCTGCCACCATGGACA AGAAGTACAGCATCGGACTGGACATCGGAACAAACAGCGTCGGATGGGCAG	260

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Sequence Table

Description	Sequence	SEQ ID No.
with AGG as first three nucleotides for use with CleanCap ™, 5' UTR from XBG, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3' UTR of XBG	TCATCACAGACGAATACAAGGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAA	
	ACACAGACAGACACAGCATCAAGAAGAACCTGATCGGAGCACTGCTGTTCG	
	ACAGCGGAGAAACAGCAGAAGCAACAAGACTGAAGAGAACAGCAAGAAGAA	
	GATACACAAGAAGAAAGAACAGAATCTGCTACCTGCAGGAAATCTTCAGCA	
	ACGAAATGGCAAAGGTCGACGACAGCTTCTTCCACAGACTGGAAGAAAGCT	
	TCCTGGTCGAAGAAGACAAGAAGCACGAAAGACACCCGATCTTCGGAAACA	
	TCGTCGACGAAGTCGCATACCACGAAAAGTACCCGACAATCTACCACCTGA	
	GAAAGAAGCTGGTCGACAGCACAGACAAGGCAGACCTGAGACTGATCTACC	
	TGGCACTGGCACACATGATCAAGTTCAGAGGACACTTCCTGATCGAAGGAG	
	ACCTGAACCCGGACAACAGCGACGTCGACAAGCTGTTCATCCAGCTGGTCC	
	AGACATACAACCAGCTGTTCGAAGAAAACCCGATCAACGCAAGCGGAGTCG	
	ACGCAAAGGCAATCCTGAGCGCAAGACTGAGCAAGAGCAGAAGACTGGAAA	
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	CAGCAAAGAACCTGAGCGACGCAATCCTGCTGAGCGACATCCTGAGAGTCA	
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	ACGAACACCACCAGGACCTGACACTGCTGAAGGCACTGGTCAGACAGCAGC	
	TGCCGGAAAAGTACAAGGAAATCTTCTTCGACCAGAGCAAGAACGGATACG	
	CAGGATACATCGACGGAGGAGCAAGCCAGGAAGAATTCTACAAGTTCATCA	
	AGCCGATCCTGGAAAAGATGGACGGAACAGAAGAACTGCTGGTCAAGCTGA	
	ACAGAGAAGACCTGCTGAGAAAGCAGAGAACATTCGACAACGGAAGCATCC	
	CGCACCAGATCCACCTGGGAGAACTGCACGCAATCCTGAGAAGACAGGAAG	
	ACTTCTACCCGTTCCTGAAGGACAACAGAGAAAAGATCGAAAAGATCCTGA	
	CATTCAGAATCCCGTACTACGTCGGACCGCTGGCAAGAGGAAACAGCAGAT	
	TCGCATGGATGACAAGAAAGAGCGAAGAAACAATCACACCGTGGAACTTCG	
	AAGAAGTCGTCGACAAGGGAGCAAGCGCACAGAGCTTCATCGAAAGAATGA	
	CAAACTTCGACAAGAACCTGCCGAACGAAAAGGTCCTGCCGAAGCACAGCC	
	TGCTGTACGAATACTTCACAGTCTACAACGAACTGACAAAGGTCAAGTACG	
	TCACAGAAGGAATGAGAAAGCCGGCATTCCTGAGCGGAGAACAGAAGAAGG	
	CAATCGTCGACCTGCTGTTCAAGACAAACAGAAAGGTCACAGTCAAGCAGC	
	TGAAGGAAGACTACTTCAAGAAGATCGAATGCTTCGACAGCGTCGAAATCA	
	GCGGAGTCGAAGACAGATTCAACGCAAGCCTGGGAACATACCACGACCTGC	
	TGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAAGAAAACGAAGACA	
	TCCTGGAAGACATCGTCCTGACACTGACACTGTTCGAAGACAGAGAAATGA	
	TCGAAGAAAGACTGAAGACATACGCACACCTGTTCGACGACAAGGTCATGA	
	AGCAGCTGAAGAGAAGAAGATACACAGGATGGGGAAGACTGAGCAGAAAGC	
	TGATCAACGGAATCAGAGACAAGCAGAGCGGAAAGACAATCCTGGACTTCC	
	TGAAGAGCGACGGATTCGCAAACAGAAACTTCATGCAGCTGATCCACGACG	
	ACAGCCTGACATTCAAGGAAGACATCCAGAAGGCACAGGTCAGCGGACAGG	
	GAGACAGCCTGCACGAACACATCGCAAACCTGGCAGGAAGCCCGGCAATCA	
	AGAAGGGAATCCTGCAGACAGTCAAGGTCGTCGACGAACTGGTCAAGGTCA	
	TGGGAAGACACAAGCCGGAAAACATCGTCATCGAAATGGCAAGAGAAAACC	
	AGACAACACAGAAGGGACAGAAGAACAGCAGAGAAAGAATGAAGAGAATCG	
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	GAATGAACACAAAGTACGACGAAAACGACAAGCTGATCAGAGAAGTCAAGG	
	TCATCACACTGAAGAGCAAGCTGGTCAGCGACTTCAGAAAGGACTTCCAGT	
	TCTACAAGGTCAGAGAAATCAACAACTACCACCACGCACACGACGCATACC	
	TGAACGCAGTCGTCGGAACAGCACTGATCAAGAAGTACCCGAAGCTGGAAA	
	GCGAATTCGTCTACGGAGACTACAAGGTCTACGACGTCAGAAAGATGATCG	
	CAAAGAGCGAACAGGAAATCGGAAAGGCAACAGCAAAGTACTTCTTCTACA	
	GCAACATCATGAACTTCTTCAAGACAGAAATCACACTGGCAAACGGAGAAA	
	TCAGAAAGAGACCGCTGATCGAAACAAACGGAGAAACAGGAGAAATCGTCT	
	GGGACAAGGGAAGAGACTTCGCAACAGTCAGAAAGGTCCTGAGCATGCCGC	
	AGGTCAACATCGTCAAGAAGACAGAAGTCCAGACAGGAGGATTCAGCAAGG	
	AAAGCATCCTGCCGAAGAGAAACAGCGACAAGCTGATCGCAAGAAAGAAGG	
	ACTGGGACCCGAAGAAGTACGGAGGATTCGACAGCCCGACAGTCGCATACA	
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	GCGTCAAGGAACTGCTGGGAATCACAATCATGGAAAGAAGCAGCTTCGAAA	
	AGAACCCGATCGACTTCCTGGAAGCAAAGGGATACAAGGAAGTCAAGAAGG	
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Sequence Table

Description	Sequence	SEQ ID No.
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	AGAGAGTCATCCTGGCAGACGCAAACCTGGACAAGGTCCTGAGCGCATACA	
	ACAAGCACAGAGACAAGCCGATCAGAGAACAGGCAGAAAACATCATCCACC	
	TGTTCACACTGACAAACCTGGGAGCACCGGCAGCATTCAAGTACTTCGACA	
	CAACAATCGACAGAAAGAGATACACAAGCACAAAGGAAGTCCTGGACGCAA	
	CACTGATCCACCAGAGCATCACAGGACTGTACGAAACAAGAATCGACCTGA	
	GCCAGCTGGGAGGAGACGGAGGAGGAAGCCCGAAGAAGAAGAGAAAGGTCT	
	AGCTAGCACCAGCCTCAAGAACACCCGAATGGAGTCTCTAAGCTACATAAT	
	ACCAACTTACACTTTACAAAATGTTGTCCCCCAAAATGTAGCCATTCGTAT	
	CTGCTCCTAATAAAAAGAAAGTTTCTTCACATTCTCTCGAG	
Cas9 transcript with AGG as first three nucleotides for use with CleanCap ™, 5' UTR from HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3' UTR of ALB	AGGTCCCGCAGTCGGCGTCCAGCGGCTCTGCTTGTTCGTGTGTGTGTCGTT	261
	GCAGGCCTTATTCGGATCCGCCACCATGGACAAGAAGTACAGCATCGGACT	
	GGACATCGGAACAAACAGCGTCGGATGGGCAGTCATCACAGACGAATACAA	
	GGTCCCGAGCAAGAAGTTCAAGGTCCTGGGAAACACAGACAGACACAGCAT	
	CAAGAAGAACCTGATCGGAGCACTGCTGTTCGACAGCGGAGAAACAGCAGA	
	AGCAACAAGACTGAAGAGAACAGCAAGAAGAAGATACACAAGAAGAAAGAA	
	CAGAATCTGCTACCTGCAGGAAATCTTCAGCAACGAAATGGCAAAGGTCGA	
	CGACAGCTTCTTCCACAGACTGGAAGAAAGCTTCCTGGTCGAAGAAGACAA	
	GAAGCACGAAAGACACCCGATCTTCGGAAACATCGTCGACGAAGTCGCATA	
	CCACGAAAAGTACCCGACAATCTACCACCTGAGAAAGAAGCTGGTCGACAG	
	CACAGACAAGGCAGACCTGAGACTGATCTACCTGGCACTGGCACACATGAT	
	CAAGTTCAGAGGACACTTCCTGATCGAAGGAGACCTGAACCCGGACAACAG	
	CGACGTCGACAAGCTGTTCATCCAGCTGGTCCAGACATACAACCAGCTGTT	
	CGAAGAAAACCCGATCAACGCAAGCGGAGTCGACGCAAAGGCAATCCTGAG	
	CGCAAGACTGAGCAAGAGCAGAAGACTGGAAAACCTGATCGCACAGCTGCC	
	GGGAGAAAAGAAGAACGGACTGTTCGGAAACCTGATCGCACTGAGCCTGGG	
	ACTGACACCGAACTTCAAGAGCAACTTCGACCTGGCAGAAGACGCAAAGCT	
	GCAGCTGAGCAAGGACACATACGACGACGACCTGGACAACCTGCTGGCACA	
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	CGCAATCCTGCTGAGCGACATCCTGAGAGTCAACACAGAAATCACAAAGGC	
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	AGTCTACAACGAACTGACAAAGGTCAAGTACGTCACAGAAGGAATGAGAAA	
	GCCGGCATTCCTGAGCGGAGAACAGAAGAAGGCAATCGTCGACCTGCTGTT	
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	GAAGATCGAATGCTTCGACAGCGTCGAAATCAGCGGAGTCGAAGACAGATT	
	CAACGCAAGCCTGGGAACATACCACGACCTGCTGAAGATCATCAAGGACAA	
	GGACTTCCTGGACAACGAAGAAAACGAAGACATCCTGGAAGACATCGTCCT	
	GACACTGACACTGTTCGAAGACAGAGAAATGATCGAAGAAAGACTGAAGAC	
	ATACGCACACCTGTTCGACGACAAGGTCATGAAGCAGCTGAAGAGAAGAAG	
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	AAGCGACAAGAACAGAGGAAAGAGCGACAACGTCCCGAGCGAAGAAGTCGT	
	CAAGAAGATGAAGAACTACTGGAGACAGCTGCTGAACGCAAAGCTGATCAC	
	ACAGAGAAAGTTCGACAACCTGACAAAGGCAGAGAGAGGAGGACTGAGCGA	
	ACTGGACAAGGCAGGATTCATCAAGAGACAGCTGGTCGAAACAAGACAGAT	
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Sequence Table

Description	Sequence	SEQ ID No.
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30/30/39 poly-A sequence	Not used	262
poly-A 100 sequence	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	263
G209 single guide RNA targeting the mouse TTR gene	AAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACC	264
ORF encoding *Neisseria meningitidis* Cas9 using minimal uridine codons, with start and stop codons	ATGGCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATC GGAATCGCATCGGTCGGATGGGCAATGGTCGAAATCGACGAAGAAGAAAAC CCGATCAGACTGATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTC CCGAAGACAGGAGACTCGCTGGCAATGGCAAGAAGACTGGCAAGATCGGTC AGAAGACTGACAAGAAGAAGAGCACACAGACTGCTGAGAACAAGAAGACTG CTGAAGAGAGAAGGAGTCCTGCAGGCAGCAAACTTCGACGAAAACGGACTG ATCAAGTCGCTGCCGAACACACCGTGGCAGCTGAGAGCAGCAGCACTGGAC AGAAAGCTGACACCGCTGGAATGGTCGGCAGTCCTGCTGCACCTGATCAAG CACAGAGGATACCTGTCGCAGAGAAAGAACGAAGGAGAAACAGCAGACAAG GAACTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAG ACAGGAGACTTCAGAACACCGGCAGAACTGGCACTGAACAAGTTCGAAAAG GAATCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACACATTCTCG AGAAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAAGGAA TTCGGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAACACTG CTGATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTG GGACACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATAC ACAGCAGAAAGATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTG GAACAGGGATCGGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATG GACGAACCGTACAGAAAGTCGAAGCTGACATACGCACAGGCAAGAAAGCTG CTGGGACTGGAAGACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGAC AACGCAGAAGCATCGACACTGATGGAAATGAAGGCATACCACGCAATCTCG AGAGCACTGGAAAAGGAAGGACTGAAGGACAAGAAGTCGCCGCTGAACCTG TCGCCGGAACTGCAGGACGAAATCGGAACAGCATTCTCGCTGTTCAAGACA GACGAAGACATCACAGGAAGACTGAAGGACAGAATCCAGCCGGAAATCCTG GAAGCACTGCTGAAGCACATCTCGTTCGACAAGTTCGTCCAGATCTCGCTG AAGGCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATACGAC GAAGCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAACACAGAA GAAAAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTC	265

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Sequence Table

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	AAGGACAGAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTC	
	GTCGGAGAACCGAAGTCGAAGGACATCCTGAAGCTGAGACTGTACGAACAG	
	CAGCACGGAAAGTGCCTGTACTCGGGAAAGGAAATCAACCTGGGAAGACTG	
	AACGAAAAGGGATACGTCGAAATCGACCACGCACTGCCGTTCTCGAGAACA	
	TGGGACGACTCGTTCAACAACAAGGTCCTGGTCCTGGGATCGGAAAACCAG	
	AACAAGGGAAACCAGACACCGTACGAATACTTCAACGGAAAGGACAACTCG	
	AGAGAATGGCAGGAATTCAAGGCAAGAGTCGAAACATCGAGATTCCCGAGA	
	TCGAAGAAGCAGAGAATCCTGCTGCAGAAGTTCGACGAAGACGGATTCAAG	
	GAAAGAAACCTGAACGACACAAGATACGTCAACAGATTCCTGTGCCAGTTC	
	GTCGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAAGAGAGTCTTCGCA	
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	AAGAGACTGGACGAAGGAGTCTCGGTCCTGAGAGTCCCGCTGACACAGCTG	
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	CAGGTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGACAGGAGTCTGGGTC	
	AGAAACCACAACGGAATCGCAGACAACGCAACAATGGTCAGAGTAGACGTC	
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	GACCACAAGATCGGAAAGAACGGAATCCTGGAAGGAATCGGAGTCAAGACA	
	GCACTGTCGTTCCAGAAGTACCAGATCGACGAACTGGGAAAGGAAATCAGA	
	CCGTGCAGACTGAAGAAGAGACCGCCGGTCAGATCCGGAAAGAGAACAGCA	
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ORF encoding *Neisseria meningitidis* Cas9 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)	GCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGGA	266
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	AAGCTGACACCGCTGGAATGGTCGGCAGTCCTGCTGCACCTGATCAAGCAC	
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	CTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAGACA	
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	TCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACACATTCTCGAGA	
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	GGACTGGAAGACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGACAAC	
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	CCGGAACTGCAGGACGAAATCGGAACAGCATTCTCGCTGTTCAAGACAGAC	
	GAAGACATCACAGGAAGACTGAAGGACAGAATCCAGCCGGAAATCCTGGAA	
	GCACTGCTGAAGCACATCTCGTTCGACAAGTTCGTCCAGATCTCGCTGAAG	
	GCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATACGACGAA	
	GCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAACACAGAAGAA	
	AAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTCGTC	
	CTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGAAGA	
	TACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGAAAG	
	TCGTTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGAAAG	
	GACAGAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGTC	
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Sequence Table

Description	Sequence	SEQ ID No.
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Transcript comprising SEQ ID NO: 265 (encoding *Neisseria meningitidis* Cas9)	GGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTGGATCCGCCACCAT GGCAGCATTCAAGCCGAACTCGATCAACTACATCCTGGGACTGGACATCGG AATCGCATCGGTCGGATGGGCAATGGTCGAAATCGACGAAGAAGAAAACCC GATCAGACTGATCGACCTGGGAGTCAGAGTCTTCGAAAGAGCAGAAGTCCC GAAGACAGGAGACTCGCTGGCAATGGCAAGAAGACTGGCAAGATCGGTCAG AAGACTGACAAGAAGAAGAGCACACAGACTGCTGAGAACAAGAAGACTGCT GAAGAGAGAAGGAGTCCTGCAGGCAGCAAACTTCGACGAAAACGGACTGAT CAAGTCGCTGCCGAACACACCGTGGCAGCTGAGAGCAGCAGCACTGGACAG AAAGCTGACACCGCTGGAATGGTCGGCAGTCCTGCTGCACCTGATCAAGCA CAGAGGATACCTGTCGCAGAGAAAGAACGAAGGAGAAACAGCAGACAAGGA ACTGGGAGCACTGCTGAAGGGAGTCGCAGGAAACGCACACGCACTGCAGAC AGGAGACTTCAGAACACCGGCAGAACTGGCACTGAACAAGTTCGAAAAGGA ATCGGGACACATCAGAAACCAGAGATCGGACTACTCGCACACATTCTCGAG AAAGGACCTGCAGGCAGAACTGATCCTGCTGTTCGAAAAGCAGAAGGAATT CGGAAACCCGCACGTCTCGGGAGGACTGAAGGAAGGAATCGAAACACTGCT GATGACACAGAGACCGGCACTGTCGGGAGACGCAGTCCAGAAGATGCTGGG ACACTGCACATTCGAACCGGCAGAACCGAAGGCAGCAAAGAACACATACAC AGCAGAAAGATTCATCTGGCTGACAAAGCTGAACAACCTGAGAATCCTGGA ACAGGGATCGGAAAGACCGCTGACAGACACAGAAAGAGCAACACTGATGGA CGAACCGTACAGAAAGTCGAAGCTGACATACGCACAGGCAAGAAAGCTGCT GGGACTGGAAGACACAGCATTCTTCAAGGGACTGAGATACGGAAAGGACAA CGCAGAAGCATCGACACTGATGGAAATGAAGGCATACCACGCAATCTCGAG AGCACTGGAAAAGGAAGGACTGAAGGACAAGAAGTCGCCGCTGAACCTGTC GCCGGAACTGCAGGACGAAATCGGAACAGCATTCTCGCTGTTCAAGACAGA CGAAGACATCACAGGAAGACTGAAGGACAGAATCCAGCCGGAAATCCTGGA AGCACTGCTGAAGCACATCTCGTTCGACAAGTTCGTCCAGATCTCGCTGAA GGCACTGAGAAGAATCGTCCCGCTGATGGAACAGGGAAAGAGATACGACGA AGCATGCGCAGAAATCTACGGAGACCACTACGGAAAGAAGAACACAGAAGA AAAGATCTACCTGCCGCCGATCCCGGCAGACGAAATCAGAAACCCGGTCGT CCTGAGAGCACTGTCGCAGGCAAGAAAGGTCATCAACGGAGTCGTCAGAAG ATACGGATCGCCGGCAAGAATCCACATCGAAACAGCAAGAGAAGTCGGAAA GTCGTTCAAGGACAGAAAGGAAATCGAAAAGAGACAGGAAGAAAACAGAAA GGACAGAGAAAAGGCAGCAGCAAAGTTCAGAGAATACTTCCCGAACTTCGT CGGAGAACCGAAGTCGAAGGACATCCTGAAGCTGAGACTGTACGAACAGCA GCACGGAAAGTGCCTGTACTCGGGAAAGGAAATCAACCTGGGAAGACTGAA CGAAAAGGGATACGTCGAAATCGACCACGCACTGCCGTTCTCGAGAACATG GGACGACTCGTTCAACAACAAGGTCCTGGTCCTGGGATCGGAAAACCAGAA CAAGGGAAACCAGACACCGTACGAATACTTCAACGGAAAGGACAACTCGAG	267

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Sequence Table

Description	Sequence	SEQ ID No.
	AGAATGGCAGGAATTCAAGGCAAGAGTCGAAACATCGAGATTCCCGAGATC GAAGAAGCAGAGAATCCTGCTGCAGAAGTTCGACGAAGACGGATTCAAGGA AAGAAACCTGAACGACACAAGATACGTCAACAGATTCCTGTGCCAGTTCGT CGCAGACAGAATGAGACTGACAGGAAAGGGAAAGAAGAGAGTCTTCGCATC GAACGGACAGATCACAAACCTGCTGAGAGGATTCTGGGGACTGAGAAAGGT CAGAGCAGAAAACGACAGACACCACGCACTGGACGCAGTCGTCGTCGCATG CTCGACAGTCGCAATGCAGCAGAAGATCACAAGATTCGTCAGATACAAGGA AATGAACGCATTCGACGGAAAGACAATCGACAAGGAAACAGGAGAAGTCCT GCACCAGAAGACACACTTCCCGCAGCCGTGGGAATTCTTCGCACAGGAAGT CATGATCAGAGTCTTCGGAAAGCCGGACGGAAAGCCGGAATTCGAAGAAGC AGACACACTGGAAAAGCTGAGAACACTGCTGGCAGAAAAGCTGTCGTCGAG ACCGGAAGCAGTCCACGAATACGTCACACCGCTGTTCGTCTCGAGAGCACC GAACAGAAAGATGTCGGGACAGGGACACATGGAAACAGTCAAGTCGGCAAA GAGACTGGACGAAGGAGTCTCGGTCCTGAGAGTCCCGCTGACACAGCTGAA GCTGAAGGACCTGGAAAAGATGGTCAACAGAGAAAGAGAACCGAAGCTGTA CGAAGCACTGAAGGCAAGACTGGAAGCACACAAGGACGACCCGGCAAAGGC ATTCGCAGAACCGTTCTACAAGTACGACAAGGCAGGAAACAGAACACAGCA GGTCAAGGCAGTCAGAGTCGAACAGGTCCAGAAGACAGGAGTCTGGGTCAG AAACCACAACGGAATCGCAGACAACGCAACAATGGTCAGAGTAGACGTCTT CGAAAAGGGAGACAAGTACTACCTGGTCCCGATCTACTCGTGGCAGGTCGC AAAGGGAATCCTGCCGGACAGAGCAGTCGTCCAGGGAAAGGACGAAGAAGA CTGGCAGCTGATCGACGACTCGTTCAACTTCAAGTTCTCGCTGCACCCGAA CGACCTGGTCGAAGTCATCACAAAGAAGGCAAGAATGTTCGGATACTTCGC ATCGTGCCACAGAGGAACAGGAAACATCAACATCAGAATCCACGACCTGGA CCACAAGATCGGAAAGAACGGAATCCTGGAAGGAATCGGAGTCAAGACAGC ACTGTCGTTCCAGAAGTACCAGATCGACGAACTGGGAAAGGAAATCAGACC GTGCAGACTGAAGAAGAGACCGCCGGTCAGATCCGGAAAGAGAACAGCAGA CGGATCGGAATTCGAATCGCCGAAGAAGAAGAGAAAGGTCGAATGATAGCT AGCTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGC CTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGA CCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTG CATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGC AGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATG CGGTGGGCTCTATGG	
Amino acid sequence of *Neisseria meningitidis* Cas9	MAAFKPNSINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRVFERAEV PKTGDSLAMARRLARSVRRLIRRRAHRLLRIRRLLKREGVLQAANFDENGL IKSLPNTPWQLRAAALDRKLIPLEWSAVLLHLIKHRGYLSQRKNEGETADK ELGALLKGVAGNAHALQTGDFRIPAELALNKFEKESGHIRNQRSDYSHIFS RKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDAVQKML GHCIFEPAEPKAAKNTYTAERFIWLIKLNNLRILEQGSERPLIDTERAILM DEPYRKSKLIYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKAYHAIS RALEKEGLKDKKSPLNLSPELQDEIGTAFSLEKTDEDITGRLKDRIQPEIL EALLKHISFDKFVQISLKALRRIVPLMEQGKRYDEACAEIYGDHYGKKNTE EKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPARIHIETARAVG KSFKDRKEIEKRQEENRKDREKAAAKFREYFPNEVGEPKSKDILKLRLYEQ QHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSENNKVLVLGSENQ NKGNQTPYEYENGKDNSREWQEFKARVETSRFPRSKKQRILLQKFDEDGEK ERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVFASNGQITNLLRGFWGLRK VRAENDRHHALDAVVVACSTVAMQQKITREVRYKEMNAFDGKTIDKETGEV LHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEADTLEKLRTLLAEKLSS RPEAVHEYVTPLEVSRAPNRKMSGQGHMETVKSAKRLDEGVSVLRVPLTQL KLKDLEKMVNRREREPKLYEALKARLEAHKDDPAKAFAEPFYKYDKAGNRTQ QVKAVRVEQVQKTGVWVRNHNGIADNATMVRVDVFEKGDKYYLVPIYSWQV AKGILPDRAVVQGKDEEDWQLIDDSFNEKESLHPNDLVEVITKKARMFGYF ASCHRGTGNINIRIHDLDHKIGKNGILEGIGVKTALSFQKYQIDELGKEIR PCRLKKRPPVRSGKRTADGSEFESPKKKRKVE	268
G390 single guide RNA targeting the rat TTR gene	mG*mC*mC*GAGUCUGGAGAGCUGCAGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	269
trRNA	AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUUUUU	270
	Not Used	271
G534 single guide RNA	mA*mC*mG*CAAAUAUCAGUCCAGCGGUUUUAGAmGmCmUmAmGmAmAmAm UmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmA	272

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Sequence Table

Description	Sequence	SEQ ID No.
targeting the rat TTR gene	mAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	
G000395 5' truncated inactive sgRNA modified sequence	mG*mC*mA*AUGGUGUAGCGGGUUUUAGAmGmCmUmAmGmAmAmAmUmAmG mCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmG GmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU	273
SV40 NLS	PKKKRKV	274
Alternate SV40 NLS	PKKKRRV	275
Nucleoplasmin NLS	KRPAATKKAGQAKKKK	276
Exemplary Kozak sequence	gccRccAUGG	277
Exemplary Kozak sequence	gccgccRccAUGG	278

* = PS linkage; 'm' = 2T-O-Me nucleotide

[0658] The following sequence table provides a listing of sequences disclosed herein. It is understood that if a DNA sequence (comprising Ts) is referenced with respect to an RNA, then Ts should be replaced with Us (which may be modified or unmodified depending on the context), and vice versa.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 278

<210> SEQ ID NO 1
<211> LENGTH: 4411
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3 UTR of ALB

<400> SEQUENCE: 1

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attcggatcc gccaccatgg acaagaagta cagcatcgga ctggacatcg gaacaaacag 120

cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

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gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag aaaagaagaa 780

cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg acgacgacct 900

ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg cagcaaagaa 960

cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc tgacactgct 1080

gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat tctacaagtt 1200

catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc accagatcca 1320

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caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg tcggaccgct 1440

ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa caatcacacc 1500

gtggaacttc gaagaagtcg tcgacaaggg agcaagcgca cagagcttca tcgaaagaat 1560

gacaaacttc gacaagaacc tgccgaacga aaaggtcctg ccgaagcaca gcctgctgta 1620

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aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc tgttcaagac 1740

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actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga agagaagaag 2040

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cggaaagaca atcctggact tcctgaagag cgacggattc gcaaacagaa acttcatgca 2160

gctgatccac gacgacagcc tgacattcaa ggaagacatc cagaaggcac aggtcagcgg 2220

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attcatcaag agacagctgg tcgaaacaag acagatcaca aagcacgtcg cacagatcct 2880

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cagcccgaca gtcgcataca gcgtcctggt cgtcgcaaag gtcgaaaagg gaaagagcaa 3540

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gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

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<210> SEQ ID NO 2
<211> LENGTH: 4403
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript comprising Cas9 ORF corresponding to SEQ ID NO: 205 using codons with generally high expression in humans

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gctggatatc ggaactaatt ccgtgggttg ggcagtgatc acggatgaat acaaagtgcc 180

gtccaagaag ttcaaggtcc tggggaacac cgatagacac agcatcaaga aaaatctcat 240

cggagccctg ctgtttgact ccggcgaaac cgcagaagcg acccggctca aacgtaccgc 300

gaggcgacgc tacacccggc ggaagaatcg catctgctat ctgcaagaga tcttttcgaa 360

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ccacgaaaag tacccgacca tctaccatct gcggaagaag ttggttgact caactgacaa 540

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taaaaaatgg aaagaacctc gag 4403

<210> SEQ ID NO 3
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: modified sgRNA sequence
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<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
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<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: n is a, c, g, or u
<220> FEATURE:

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<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
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<223> OTHER INFORMATION: 2'-O-Me nucleotide
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<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 3

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 4
<211> LENGTH: 105
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: 30/30/39 poly-A sequence

<400> SEQUENCE: 4

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aaaccgaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa 105

<210> SEQ ID NO 5
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003335 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 5

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<210> SEQ ID NO 6
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003336 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 6

ccuccucugc cuugcuggac 20

<210> SEQ ID NO 7
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003337 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 7

ccaguccagc aaggcagagg 20

<210> SEQ ID NO 8
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003338 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 8

auaccagucc agcaaggcag 20

<210> SEQ ID NO 9
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003339 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 9

acacaaauac caguccagca 20

<210> SEQ ID NO 10
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003340 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 10

uggacuggua uuugugucug 20

<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003341 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 11

cugguauuug ugucugaggc 20

<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003342 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 12

cuucucuaca cccagggcac 20

<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003343 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 13

cagaggacac uuggauucac 20

<210> SEQ ID NO 14
<211> LENGTH: 20

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003344 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 14

uuugaccauc agaggacacu 20

<210> SEQ ID NO 15
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003345 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 15

ucuagaacuu ugaccaucag 20

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003346 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 16

aaaguucuag augcuguccg 20

<210> SEQ ID NO 17
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003347 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 17

cauugauggc aggacugccu 20

<210> SEQ ID NO 18
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003348 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 18

aggcaguccu gccaucaaug 20

<210> SEQ ID NO 19
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003349 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 19

ugcacggcca cauugauggc 20

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<210> SEQ ID NO 20
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003350 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 20

cacaugcacg gccacauuga 20

<210> SEQ ID NO 21
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003351 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 21

agccuuucug aacacaugca 20

<210> SEQ ID NO 22
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003352 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 22

gaaaggcugc ugaugacacc 20

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003353 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 23

aaaggcugcu gaugacaccu 20

<210> SEQ ID NO 24
<211> LENGTH: 20
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003354 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 24

accugggagc cauuugccuc 20

<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003355 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 25

cccagaggca aauggcuccc 20

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<210> SEQ ID NO 26
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003356 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 26

gcaacuuacc cagaggcaaa 20

<210> SEQ ID NO 27
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003357 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 27

uucuuuggca acuuacccag 20

<210> SEQ ID NO 28
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003358 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 28

augcagcucu ccagacucac 20

<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003359 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 29

agugagucug gagagcugca 20

<210> SEQ ID NO 30
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003360 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 30

gugagucugg agagcugcau 20

<210> SEQ ID NO 31
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003361 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 31

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gcugcauggg cucacaacug 20

<210> SEQ ID NO 32
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003362 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 32

gcaugggcuc acaacugagg 20

<210> SEQ ID NO 33
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003363 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 33

acugaggagg aauuuguaga 20

<210> SEQ ID NO 34
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003364 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 34

cugaggagga auuuguagaa 20

<210> SEQ ID NO 35
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003365 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 35

uguagaaggg auauacaaag 20

<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003366 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 36

aaauagacac caaaucuuac 20

<210> SEQ ID NO 37
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003367 gRNA targeting Human TTR (Exon 3)

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<400> SEQUENCE: 37

agacaccaaa ucuuacugga 20

<210> SEQ ID NO 38
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003368 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 38

aagugccuuc caguaagauu 20

<210> SEQ ID NO 39
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003369 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 39

cucugcaugc ucauggaaug 20

<210> SEQ ID NO 40
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003370 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 40

ccucugcaug cucauggaau 20

<210> SEQ ID NO 41
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003371 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 41

accucugcau gcucauggaa 20

<210> SEQ ID NO 42
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003372 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 42

uacucaccuc ugcaugcuca 20

<210> SEQ ID NO 43
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic: CR003373 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 43

guauucacag ccaacgacuc 20

<210> SEQ ID NO 44
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003374 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 44

gcggcggggg ccggagucgu 20

<210> SEQ ID NO 45
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003375 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 45

aaugguguag cggcgggggc 20

<210> SEQ ID NO 46
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003376 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 46

cggcaauggu guagcggcgg 20

<210> SEQ ID NO 47
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003377 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 47

gcggcaaugg uguagcggcg 20

<210> SEQ ID NO 48
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003378 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 48

ggcggcaaug guguagcggc 20

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003379 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 49

gggcggcaau gguguagcgg 20

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003380 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 50

gcagggcggc aaugguguag 20

<210> SEQ ID NO 51
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003381 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 51

ggggcucagc agggcggcaa 20

<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003382 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 52

ggaguagggg cucagcaggg 20

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003383 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 53

auaggaguag gggcucagca 20

<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003384 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 54

aauaggagua ggggcucagc 20

<210> SEQ ID NO 55

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<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003385 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 55

ccccuacucc uauuccacca 20

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003386 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 56

ccguggugga auaggaguag 20

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003387 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 57

gccguggugg aauaggagua 20

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003388 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 58

gacgacagcc gugguggaau 20

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003389 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 59

auuggugacg acagccgugg 20

<210> SEQ ID NO 60
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003390 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 60

gggauuggug acgacagccg 20

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<210> SEQ ID NO 61
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003391 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 61

ggcugucguc accaauccca 20

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR003392 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 62

agucccucau uccuugggau 20

<210> SEQ ID NO 63
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic: CR005298 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 63

uccacucauu cuuggcagga 20

<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005299 gRNA targeting Human TTR (Exon 4)

<400> SEQUENCE: 64

agccguggug gaauaggagu 20

<210> SEQ ID NO 65
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005300 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 65

ucacagaaac acucaccgua 20

<210> SEQ ID NO 66
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005301 gRNA targeting Human TTR (Exon 1)

<400> SEQUENCE: 66

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gucacagaaa cacucaccgu 20

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005302 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 67

acgugucuuc ucuacaccca 20

<210> SEQ ID NO 68
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005303 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 68

ugaauccaag uguccucuga 20

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005304 gRNA targeting Human TTR (Exon 2)

<400> SEQUENCE: 69

ggccgugcau guguucagaa 20

<210> SEQ ID NO 70
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005305 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 70

uauaggaaaa ccagugaguc 20

<210> SEQ ID NO 71
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005306 gRNA targeting Human TTR (Exon 3)

<400> SEQUENCE: 71

aaaucuuacu ggaaggcacu 20

<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005307 gRNA targeting Human TTR (Exon 4)

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<400> SEQUENCE: 72

ugucugucuu cucucauagg 20

<210> SEQ ID NO 73
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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic: CR000689 gRNA targeting Cyno TTR

<400> SEQUENCE: 73

acacaaauac caguccagcg 20

<210> SEQ ID NO 74
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005364 gRNA targeting Cyno TTR

<400> SEQUENCE: 74

aaaggcugcu gaugagaccu 20

<210> SEQ ID NO 75
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005365 gRNA targeting Cyno TTR

<400> SEQUENCE: 75

cauugacagc aggacugccu 20

<210> SEQ ID NO 76
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005366 gRNA targeting Cyno TTR

<400> SEQUENCE: 76

auaccagucc agcgaggcag 20

<210> SEQ ID NO 77
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005367 gRNA targeting Cyno TTR

<400> SEQUENCE: 77

ccaguccagc gaggcagagg 20

<210> SEQ ID NO 78
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005368 gRNA targeting Cyno TTR

<400> SEQUENCE: 78

ccuccucugc cucgcuggac 20

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<210> SEQ ID NO 79
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005369 gRNA targeting Cyno TTR

<400> SEQUENCE: 79

aaaguucuag augccguccg 20

<210> SEQ ID NO 80
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005370 gRNA targeting Cyno TTR

<400> SEQUENCE: 80

acuugucuuc ucuauaccca 20

<210> SEQ ID NO 81
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005371 gRNA targeting Cyno TTR

<400> SEQUENCE: 81

aagugacuuc caguaagauu 20

<210> SEQ ID NO 82
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: CR005372 gRNA targeting Cyno TTR

<400> SEQUENCE: 82

aaaaggcugc ugaugagacc 20

<210> SEQ ID NO 83

<400> SEQUENCE: 83

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<210> SEQ ID NO 84

<400> SEQUENCE: 84

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<210> SEQ ID NO 85

<400> SEQUENCE: 85

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<210> SEQ ID NO 86

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<210> SEQ ID NO 87
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000480 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 87

aaaggcugcu gaugacaccu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

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<210> SEQ ID NO 88
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000481 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
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<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 88

ucuagaacuu ugaccaucag guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 89
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000482 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:

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<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 89

uguagaaggg auauacaaag guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 90
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000483 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 90

uccacucauu cuuggcagga guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 91
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000484 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
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<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 91

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agacaccaaa ucuuacugga guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 92
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000485 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 92

ccuccucugc cuugcuggac guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 93
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000486 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
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<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 93

acacaaauac caguccagca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 94
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000487 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base

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<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
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<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
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<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 94

uucuuuggca acuuacccag guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 95
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000488 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 95

aaaguucuag augcuguccg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 96
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000489 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

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<400> SEQUENCE: 96

uuugaccauc agaggacacu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 97
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000490 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 97

aaauagacac caaaucuuac guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 98
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000491 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 98

auaccagucc agcaaggcag guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 99
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000492 sgRNA modified sequence

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targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 99

cuucucuaca cccagggcac guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 100
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000493 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 100

aagugccuuc caguaagauu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 101
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000494 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:

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<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 101

gugagucugg agagcugcau guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 102
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000495 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 102

cagaggacac uuggauucac guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 103
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000496 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 103

ggccgugcau guguucagaa guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 104
<211> LENGTH: 100
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000497 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 104

cugcuccucc ucugccuugc guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 105
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000498 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 105

agugagucug gagagcugca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 106
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000499 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base

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<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 106

ugaauccaag uguccucuga guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 107
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000500 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
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<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 107

ccaguccagc aaggcagagg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 108
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000501 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
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<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 108

ucacagaaac acucaccgua guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

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<210> SEQ ID NO 109
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic: G000567 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 109

gaaaggcugc ugaugacacc guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 110
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000568 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
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<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 110

ggcugucguc accaauccca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 111
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000570 sgRNA modified sequence
targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)

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<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 111

cauugauggc aggacugccu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 112
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000571 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 112

gucacagaaa cacucaccgu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 113
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000572 sgRNA modified sequence targeting Human TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 113

ccccuacucc uauuccacca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

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cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 114
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000502 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 114

acacaaauac caguccagcg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 115
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000503 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 115

aaaaggcugc ugaugagacc guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 116
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000504 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

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<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 116

aaaggcugcu gaugagaccu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 117
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000505 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 117

cauugacagc aggacugccu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 118
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000506 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 118

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auaccagucc agcgaggcag guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 119
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000507 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 119

ccaguccagc gaggcagagg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 120
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000508 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 120

ccuccucugc cucgcuggac guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 121
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000509 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:

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<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 121

aaaguucuag augccguccg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 122
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000510 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 122

acuugucuuc ucuauaccca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 123
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000511 sgRNA modified sequence targeting Cyno TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)

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<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 123

aagugacuuc caguaagauu guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 124
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000282 sgRNA modified sequence targeting Mouse TTR
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 124

uuacagccac gucuacagca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 125

<400> SEQUENCE: 125

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<210> SEQ ID NO 126

<400> SEQUENCE: 126

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<210> SEQ ID NO 199

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<210> SEQ ID NO 200

<400> SEQUENCE: 200

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<210> SEQ ID NO 201
<211> LENGTH: 4140
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: DNA coding sequence of Cas9 using the thymidine analog of the minimal uridine codons listed in Table 3, with start and stop codons

<400> SEQUENCE: 201

atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg atgggcagtc 60

atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa cacagacaga 120

cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccacaga 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

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gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct gagcgcaagc 960

atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc actggtcaga 1020

cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa cttcgacaag 1500

aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata cttcacagtc 1560

tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac aggatgggga 1980

agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

gacttcctga agagcgacgg attcgcaaac agaaacttca tgcagctgat ccacgacgac 2100

agcctgacat tcaaggaaga catccagaag gcacaggtca gcggacaggg agacagcctg 2160

cacgaacaca tcgcaaacct ggcaggaagc ccggcaatca agaagggaat cctgcagaca 2220

gtcaaggtcg tcgacgaact ggtcaaggtc atgggaagac acaagccgga aaacatcgtc 2280

atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca gaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

ctggtcgaaa caagacagat cacaaagcac gtcgcacaga tcctggacag cagaatgaac 2820

acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

taccaccacg cacacgacgc atacctgaac gcagtcgtcg gaacagcact gatcaagaag 3000

tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

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atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

gacctgagcc agctgggagg agacggagga ggaagcccga agaagaagag aaaggtctag 4140

<210> SEQ ID NO 202
<211> LENGTH: 4143
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: DNA coding sequence of Cas9 using codons with generally high expression in humans

<400> SEQUENCE: 202

atggataaga agtactcaat cgggctggat atcggaacta attccgtggg ttgggcagtg 60

atcacggatg aatacaaagt gccgtccaag aagttcaagg tcctggggaa caccgataga 120

cacagcatca agaaaaatct catcggagcc ctgctgtttg actccggcga aaccgcagaa 180

gcgacccggc tcaaacgtac cgcgaggcga cgctacaccc ggcggaagaa tcgcatctgc 240

tatctgcaag agatcttttc gaacgaaatg gcaaaggtcg acgacagctt cttccaccgc 300

ctggaagaat ctttcctggt ggaggaggac aagaagcatg aacggcatcc tatctttgga 360

aacatcgtcg acgaagtggc gtaccacgaa aagtacccga ccatctacca tctgcggaag 420

aagttggttg actcaactga caaggccgac ctcagattga tctacttggc cctcgcccat 480

atgatcaaat tccgcggaca cttcctgatc gaaggcgatc tgaaccctga taactccgac 540

gtggataagc ttttcattca actggtgcag acctacaacc aactgttcga agaaaaccca 600

atcaatgcta gcggcgtcga tgccaaggcc atcctgtccg cccggctgtc gaagtcgcgg 660

cgcctcgaaa acctgatcgc acagctgccg ggagagaaaa agaacggact tttcggcaac 720

ttgatcgctc tctcactggg actcactccc aatttcaagt ccaattttga cctggccgag 780

gacgcgaagc tgcaactctc aaaggacacc tacgacgacg acttggacaa tttgctggca 840

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caaattggcg atcagtacgc ggatctgttc cttgccgcta agaacctttc ggacgcaatc 900

ttgctgtccg atatcctgcg cgtgaacacc gaaataacca aagcgccgct tagcgcctcg 960

atgattaagc ggtacgacga gcatcaccag gatctcacgc tgctcaaagc gctcgtgaga 1020

cagcaactgc ctgaaaagta caaggagatc ttcttcgacc agtccaagaa tgggtacgca 1080

gggtacatcg atggaggcgc tagccaggaa gagttctata agttcatcaa gccaatcctg 1140

gaaaagatgg acggaaccga agaactgctg gtcaagctga acagggagga tctgctccgg 1200

aaacagagaa cctttgacaa cggatccatt ccccaccaga tccatctggg tgagctgcac 1260

gccatcttgc ggcgccagga ggacttttac ccattcctca aggacaaccg ggaaaagatc 1320

gagaaaattc tgacgttccg catcccgtat tacgtgggcc cactggcgcg cggcaattcg 1380

cgcttcgcgt ggatgactag aaaatcagag gaaaccatca ctccttggaa tttcgaggaa 1440

gttgtggata agggagcttc ggcacaaagc ttcatcgaac gaatgaccaa cttcgacaag 1500

aatctcccaa acgagaaggt gcttcctaag cacagcctcc tttacgaata cttcactgtc 1560

tacaacgaac tgactaaagt gaaatacgtt actgaaggaa tgaggaagcc ggcctttctg 1620

tccggagaac agaagaaagc aattgtcgat ctgctgttca agaccaaccg caaggtgacc 1680

gtcaagcagc ttaaagagga ctacttcaag aagatcgagt gtttcgactc agtggaaatc 1740

agcggggtgg aggacagatt caacgcttcg ctgggaacct atcatgatct cctgaagatc 1800

atcaaggaca aggacttcct tgacaacgag gagaacgagg acatcctgga agatatcgtc 1860

ctgaccttga ccctttttcga ggatcgcgag atgatcgagg agaggcttaa gacctacgct 1920

catctcttcg acgataaggt catgaaacaa ctcaagcgcc gccggtacac tggttggggc 1980

cgcctctccc gcaagctgat caacggtatt cgcgataaac agagcggtaa aactatcctg 2040

gatttcctca aatcggatgg cttcgctaat cgtaacttca tgcaattgat ccacgacgac 2100

agcctgacct ttaaggagga catccaaaaa gcacaagtgt ccggacaggg agactcactc 2160

catgaacaca tcgcgaatct ggccggttcg ccggcgatta agaagggaat tctgcaaact 2220

gtgaaggtgg tcgacgagct ggtgaaggtc atgggacggc acaaaccgga gaatatcgtg 2280

attgaaatgg cccgagaaaa ccagactacc cagaagggcc agaaaaactc ccgcgaaagg 2340

atgaagcgga tcgaagaagg aatcaaggag ctgggcagcc agatcctgaa agagcacccg 2400

gtggaaaaca cgcagctgca gaacgagaag ctctacctgt actatttgca aaatggacgg 2460

gacatgtacg tggaccaaga gctggacatc aatcggttgt ctgattacga cgtggaccac 2520

atcgttccac agtcctttct gaaggatgac tcgatcgata acaaggtgtt gactcgcagc 2580

gacaagaaca gagggaagtc agataatgtg ccatcggagg aggtcgtgaa gaagatgaag 2640

aattactggc ggcagctcct gaatgcgaag ctgattaccc agagaaagtt tgacaatctc 2700

actaaagccg agcgcggcgg actctcagag ctggataagg ctggattcat caaacggcag 2760

ctggtcgaga ctcggcagat taccaagcac gtggcgcaga tcttggactc ccgcatgaac 2820

actaaatacg acgagaacga taagctcatc cgggaagtga aggtgattac cctgaaaagc 2880

aaacttgtgt cggactttcg gaaggacttt cagttttaca aagtgagaga aatcaacaac 2940

taccatcacg cgcatgacgc atacctcaac gctgtggtcg gtaccgccct gatcaaaaag 3000

taccctaaac ttgaatcgga gtttgtgtac ggagactaca aggtctacga cgtgaggaag 3060

atgatagcca agtccgaaca ggaaatcggg aaagcaactg cgaaatactt cttttactca 3120

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aacatcatga acttttcaa gactgaaatt acgctggcca atggagaaat caggaagagg   3180

ccactgatcg aaactaacgg agaaacgggc gaaatcgtgt gggacaaggg cagggacttc   3240

gcaactgttc gcaaagtgct ctctatgccg caagtcaata ttgtgaagaa aaccgaagtg   3300

caaaccggcg gattttcaaa ggaatcgatc ctcccaaaga gaaatagcga caagctcatt   3360

gcacgcaaga aagactggga cccgaagaag tacggaggat tcgattcgcc gactgtcgca   3420

tactccgtcc tcgtggtggc caaggtggag aagggaaaga gcaaaaagct caaatccgtc   3480

aaagagctgc tggggattac catcatggaa cgatcctcgt tcgagaagaa cccgattgat   3540

ttcctcgagg cgaagggtta caaggaggtg aagaaggatc tgatcatcaa actccccaag   3600

tactcactgt tcgaactgga aaatggtcgg aagcgcatgc tggcttcggc cggagaactc   3660

caaaaaggaa atgagctggc cttgcctagc aagtacgtca acttcctcta tcttgcttcg   3720

cactacgaaa aactcaaagg gtcaccggaa gataacgaac agaagcagct tttcgtggag   3780

cagcacaagc attatctgga tgaaatcatc gaacaaatct ccgagttttc aaagcgcgtg   3840

atcctcgccg acgccaacct cgacaaagtc ctgtcggcct acaataagca tagagataag   3900

ccgatcagag aacaggccga gaacattatc cacttgttca ccctgactaa cctgggagcc   3960

ccagccgcct tcaagtactt cgatactact atcgatcgca aaagatacac gtccaccaag   4020

gaagttctgg acgcgaccct gatccaccaa agcatcactg gactctacga aactaggatc   4080

gatctgtcgc agctgggtgg cgatggcggt ggatctccga aaaagaagag aaaggtgtaa   4140

tga                                                                4143
```

<210> SEQ ID NO 203
<211> LENGTH: 1379
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Amino acid sequence of Cas9 with one nuclear localization signal (1xNLS) as the C-terminal 7 amino acids

<400> SEQUENCE: 203

```
Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
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145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560
```

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```
Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
               565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960
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```
Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
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    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Gly  Gly Ser Pro Lys Lys  Lys Arg Lys Val
    1370                1375
```

<210> SEQ ID NO 204
<211> LENGTH: 4140
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 mRNA ORF using minimal uridine codons, with start and stop codons

<400> SEQUENCE: 204

```
auggacaaga aguacagcau cggacuggac aucggaacaa acagcgucgg augggcaguc     60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga    120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa    180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc    240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga    300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga    360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag    420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac    480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac    540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg    600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga    660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac    720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa    780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca    840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc    900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc    960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga   1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca   1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug   1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga   1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac   1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc   1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc   1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa   1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag   1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc   1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug   1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca   1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc   1740
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agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

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gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggagga ggaagcccga agaagaagag aaaggucuag 4140

<210> SEQ ID NO 205
<211> LENGTH: 4143
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 mRNA ORF using codons with generally high expression in humans, with start and stop codons

<400> SEQUENCE: 205

auggauaaga aguacucaau cgggcuggau aucggaacua auuccguggg uugggcagug 60

aucacggaug aauacaaagu gccguccaag aaguucaagg uccuggggaa caccgauaga 120

cacagcauca agaaaaaucu caucggagcc cugcuguuug acuccggcga aaccgcagaa 180

gcgacccggc ucaaacguac cgcgaggcga cgcuacaccc ggcggaagaa ucgcaucugc 240

uaucugcaag agaucuuuuc gaacgaaaug gcaaaggucg acgacagcuu cuuccaccgc 300

cuggaagaau cuuuccuggu ggaggaggac aagaagcaug aacggcaucc uaucuuugga 360

aacaucgucg acgaaguggc guaccacgaa aaguacccga ccaucuacca ucugcggaag 420

aaguugguug acucaacuga caaggccgac cucagauuga ucuacuuggc ccucgcccau 480

augaucaaau uccgcggaca cuuccugauc gaaggcgauc ugaacccuga uaacuccgac 540

guggauaagc uuuucauuca acuggugcag accuacaacc aacuguucga agaaaaccca 600

aucaaugcua gcggcgucga ugccaaggcc auccuguccg cccggcuguc gaagucgcgg 660

cgccucgaaa accugaucgc acagcugccg ggagagaaaa agaacggacu uuucggcaac 720

uugaucgcuc ucucacuggg acucacuccc aauuucaagu ccaauuuuga ccuggccgag 780

gacgcgaagc ugcaacucuc aaaggacacc uacgacgacg acuuggacaa uuugcuggca 840

caaauuggcg aucaguacgc ggaucuguuc cuugccgcua agaaccuuuc ggacgcaauc 900

uugcuguccg auauccugcg cgugaacacc gaaauaacca aagcgccgcu uagcgccucg 960

augauuaagc gguacgacga gcaucaccag gaucucacgc ugcucaaagc gcucgugaga 1020

cagcaacugc cugaaaagua caaggagauc uucuucgacc aguccaagaa uggguacgca 1080

ggguacaucg auggaggcgc uagccaggaa gaguucuaua aguucaucaa gccaauccug 1140

gaaaagaugg acggaaccga agaacugcug gucaagcuga acagggagga ucugcuccgg 1200

aaacagagaa ccuuugacaa cggauccauu ccccaccaga uccaucuggg ugagcugcac 1260

gccaucuugc ggcgccagga ggacuuuuac ccauuccuca aggacaaccg ggaaaagauc 1320

gagaaaauuc ugacguuccg caucccguau uacgugggcc cacuggcgcg cggcaauucg 1380

cgcuucgcgu ggaugacuag aaaaucagag gaaaccauca cuccuuggaa uuucgaggaa 1440

guuguggaua agggagcuuc ggcacaaagc uucaucgaac gaaugaccaa cuucgacaag 1500

aaucucccaa acgagaaggu gcuuccuaag cacagccucc uuuacgaaua cuucacuguc 1560

uacaacgaac ugacuaaagu gaaauacguu acugaaggaa ugaggaagcc ggccuuucug 1620

uccggagaac agaagaaagc aauugucgau cugcuguuca agaccaaccg caaggugacc 1680

gucaagcagc uuaaagagga cuacuucaag aagaucgagu guuucgacuc aguggaaauc 1740

agcggggugg aggacagauu caacgcuucg cugggaaccu aucaugaucu ccugaagauc 1800

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aucaaggaca aggacuuccu ugacaacgag gagaacgagg acauccugga agauaucguc 1860

cugaccuuga cccuuuucga ggaucgcgag augaucgagg agaggcuuaa gaccuacgcu 1920

caucucuucg acgauaaggu caugaaacaa cucaagcgcc gccgguacac ugguuggggc 1980

cgccucuccc gcaagcugau caacgguauu cgcgauaaac agagcgguaa aacuauccug 2040

gauuuccuca aaucggaugg cuucgcuaau cguaacuuca ugcaauugau ccacgacgac 2100

agccugaccu uuaaggagga cauccaaaaa gcacaagugu ccggacaggg agacucacuc 2160

caugaacaca ucgcgaaucu ggccgguucg ccggcgauua agaagggaau ucugcaaacu 2220

gugaaggugg ucgacgagcu ggugaagguc augggacggc acaaaccgga gaauaucgug 2280

auugaaaugg cccgagaaaa ccagacuacc cagaagggcc agaaaaacuc ccgcgaaagg 2340

augaagcgga ucgaagaagg aaucaaggag cugggcagcc agauccugaa agagcacccg 2400

guggaaaaca cgcagcugca gaacgagaag cucuaccugu acuauuugca aaauggacgg 2460

gacauguacg uggaccaaga gcuggacauc aaucgguugu cugauuacga cguggaccac 2520

aucguuccac aguccuuucu gaaggaugac ucgaucgaua acaagguguu gacucgcagc 2580

gacaagaaca gagggaaguc agauaaugug ccaucggagg aggucgugaa gaagaugaag 2640

aauuacuggc ggcagcuccu gaaugcgaag cugauuaccc agagaaaguu ugacaaucuc 2700

acuaaagccg agcgcggcgg acucucagag cuggauaagg cuggauucau caaacggcag 2760

cuggucgaga cucggcagau uaccaagcac guggcgcaga ucuuggacuc ccgcaugaac 2820

acuaaauacg acgagaacga uaagcucauc cgggaaguga aggugauuac ccugaaaagc 2880

aaacuugugu cggacuuucg gaaggacuuu caguuuuaca aagugagaga aaucaacaac 2940

uaccaucacg cgcaugacgc auaccucaac gcuguggucg guaccgcccu gaucaaaaag 3000

uacccuaaac uugaaucgga guuuguguac ggagacuaca aggucuacga cgugaggaag 3060

augauagcca aguccgaaca ggaaaucggg aaagcaacug cgaaauacuu cuuuuacuca 3120

aacaucauga acuuuuucaa gacugaaauu acgcuggcca auggagaaau caggaagagg 3180

ccacugaucg aaacuaacgg agaaacgggc gaaaucgugu gggacaaggg cagggacuuc 3240

gcaacuguuc gcaaagugcu cucuaugccg caagucaaua uugugaagaa aaccgaagug 3300

caaaccggcg gauuuucaaa ggaaucgauc cucccaaaga gaaauagcga caagcucauu 3360

gcacgcaaga aagacuggga cccgaagaag uacggaggau ucgauucgcc gacugucgca 3420

uacuccgucc ucgugguggc caagguggag aagggaaaga gcaaaaagcu caaauccguc 3480

aaagagcugc uggggauuac caucauggaa cgauccucgu ucgagaagaa cccgauugau 3540

uuccucgagg cgaaggguua caaggaggug aagaaggauc ugaucaucaa acucccccaag 3600

uacucacugu ucgaacugga aaauggucgg aagcgcaugc uggcuucggc cggagaacuc 3660

caaaaaggaa augagcuggc cuugccuagc aaguacguca acuuccucua ucuugcuucg 3720

cacuacgaaa aacucaaagg gucaccggaa gauaacgaac agaagcagcu uuucguggag 3780

cagcacaagc auuaucugga ugaaaucauc gaacaaaucu ccgaguuuuc aaagcgcgug 3840

auccucgccg acgccaaccu cgacaaaguc cugucggccu acaauaagca uagagauaag 3900

ccgaucagag aacaggccga gaacauuauc cacuuguuca cccugacuaa ccugggagcc 3960

ccagccgccu ucaaguacuu cgauacuacu aucgaucgca aaagauacac guccaccaag 4020

gaaguucugg acgcgacccu gauccaccaa agcaucacug gacucuacga aacuaggauc 4080

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gaucugucgc agcugggugg cgauggcggu ggaucuccga aaaagaagag aaagguguaa 4140

uga 4143

<210> SEQ ID NO 206
<211> LENGTH: 1379
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 nickase (D10A) amino acid sequence

<400> SEQUENCE: 206

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys

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               325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735
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Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125
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Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Gly  Gly Ser Pro Lys Lys  Lys Arg Lys Val
    1370                1375
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<210> SEQ ID NO 207
<211> LENGTH: 4140
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 nickase (D10A) mRNA ORF

<400> SEQUENCE: 207

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auggacaaga aguacagcau cggacuggca aucggaacaa acagcgucgg augggcaguc     60

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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa    180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc    240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga    300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga    360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag    420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac    480
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augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

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aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

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cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggagga ggaagcccga agaagaagag aaaggucuag 4140

<210> SEQ ID NO 208
<211> LENGTH: 1379
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: dCas9 (D10A H840A) amino acid sequence

<400> SEQUENCE: 208

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

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```
Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
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            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910
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Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290
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Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                 1300                 1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                 1315                 1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                 1330                 1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                 1345                 1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                 1360                 1365

Gly Gly  Gly Ser Pro Lys Lys  Lys Arg Lys Val
    1370                 1375
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<210> SEQ ID NO 209
<211> LENGTH: 4140
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: dCas9 (D10A H840A) mRNA ORF

<400> SEQUENCE: 209

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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa    180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc    240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga    300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga    360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag    420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac    480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac    540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg    600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga    660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac    720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa    780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca    840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc    900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc    960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga   1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca   1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug   1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga   1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac   1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc   1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc   1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa   1440
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gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

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aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgacgca 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

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aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

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cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggagga ggaagcccga agaagaagag aaaggucuag 4140

<210> SEQ ID NO 210
<211> LENGTH: 4134
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 mRNA coding sequence using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> SEQUENCE: 210

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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

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gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

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ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

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gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

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cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa gguc 4134

<210> SEQ ID NO 211
<211> LENGTH: 4134
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 nickase bare coding sequence

<400> SEQUENCE: 211

gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

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ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

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aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa gguc 4134

<210> SEQ ID NO 212
<211> LENGTH: 4134
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: dCas9 bare coding sequence

<400> SEQUENCE: 212

gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

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ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgacgcaauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

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guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa gguc 4134

<210> SEQ ID NO 213
<211> LENGTH: 1368
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Amino acid sequence of Cas9 (without NLS)

<400> SEQUENCE: 213

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys

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               325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735
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Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125
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Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365
```

<210> SEQ ID NO 214
<211> LENGTH: 4107
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 mRNA ORF encoding SEQ ID NO: 213 using minimal uridine codons, with start and stop codons

<400> SEQUENCE: 214

```
auggacaaga aguacagcau cggacuggac aucggaacaa acagcgucgg augggcaguc     60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga    120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa    180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc    240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga    300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga    360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag    420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac    480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac    540
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gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

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acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaaggUc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacuag 4107

<210> SEQ ID NO 215
<211> LENGTH: 4101
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 coding sequence encoding SEQ ID NO: 213 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> SEQUENCE: 215

gacaagaagu acagcaucgg acuggacauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggUccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

-continued

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

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cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga c 4101

<210> SEQ ID NO 216
<211> LENGTH: 1368
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Amino acid sequence of Cas9 nickase (without NLS)

<400> SEQUENCE: 216

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr

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        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525
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```
Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925
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Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
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    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365
```

<210> SEQ ID NO 217
<211> LENGTH: 4107
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 nickase mRNA ORF encoding SEQ ID NO: 216 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> SEQUENCE: 217

```
auggacaaga aguacagcau cggacuggca aucggaacaa acagcgucgg augggcaguc     60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga    120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa    180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc    240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga    300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga    360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag    420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac    480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac    540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg    600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga    660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac    720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa    780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca    840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc    900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc    960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga   1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca   1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug   1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga   1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac   1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc   1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc   1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa   1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag   1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc   1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug   1620
```

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agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

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ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacuag 4107

<210> SEQ ID NO 218
<211> LENGTH: 4101
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 nickase coding sequence encoding SEQ ID NO: 216 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> SEQUENCE: 218

gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

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ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

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aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga c 4101

<210> SEQ ID NO 219
<211> LENGTH: 1368
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Amino acid sequence of dCas9 (without NLS)

<400> SEQUENCE: 219

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

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Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
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705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110
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Arg Asn Ser Asp Lys Leu Ile Ala Arg Lys Lys Asp Trp Asp Pro
1115 1120 1125

Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val
1130 1135 1140

Leu Val Val Ala Lys Val Glu Lys Gly Lys Ser Lys Lys Leu Lys
1145 1150 1155

Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
1160 1165 1170

Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala Lys Gly Tyr Lys
1175 1180 1185

Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
1190 1195 1200

Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly
1205 1210 1215

Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val
1220 1225 1230

Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245

Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys
1250 1255 1260

His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys
1265 1270 1275

Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala
1280 1285 1290

Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
1295 1300 1305

Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala
1310 1315 1320

Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser
1325 1330 1335

Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr
1340 1345 1350

Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp
1355 1360 1365

<210> SEQ ID NO 220
<211> LENGTH: 4107
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: dCas9 mRNA ORF encoding SEQ ID NO: 219 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> SEQUENCE: 220

auggacaaga aguacagcau cggacuggca aucggaacaa acagcgucgg augggcaguc 60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga 120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga 300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360

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aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgacgca 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

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aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacuag 4107

<210> SEQ ID NO 221
<211> LENGTH: 4113
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: dCas9 coding sequence encoding SEQ ID NO: 219 using minimal uridine codons as listed in Table 3 (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> SEQUENCE: 221

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acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

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cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgacgcaauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

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aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaggagga agc 4113

<210> SEQ ID NO 222
<211> LENGTH: 1392
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Amino acid sequence of Cas9 with two nuclear localization signals (2xNLS) as the C-terminal amino acids

<400> SEQUENCE: 222

Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

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```
Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
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               485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895
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Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
           900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
       915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
   930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
               965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
           980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
       995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
   1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
   1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
   1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
   1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
   1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
   1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
   1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
   1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
   1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
   1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
   1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
   1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
   1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
   1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
   1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
   1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
   1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
   1265                1270                1275
```

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```
Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                 1285                 1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                 1300                 1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                 1315                 1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                 1330                 1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                 1345                 1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                 1360                 1365

Gly Ser  Gly Ser Pro Lys Lys  Lys Arg Lys Val Asp  Gly Ser Pro
    1370                 1375                 1380

Lys Lys  Lys Arg Lys Val Asp  Ser Gly
    1385                 1390
```

<210> SEQ ID NO 223
<211> LENGTH: 4233
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 mRNA ORF encoding SEQ ID NO: 222 using minimal uridine codons, with start and stop codons

<400> SEQUENCE: 223

```
auggacaaga aguacagcau cggacuggac aucggaacaa acagcgucgg augggcaguc     60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga    120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa    180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc    240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga    300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga    360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag    420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac    480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac    540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg    600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga    660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac    720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa    780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca    840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc    900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc    960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga   1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca   1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug   1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga   1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac   1260
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gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

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uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggagga ggaagcccga agaagaagag aaaggucccg 4140

aagaagaaga gaaaggucgg aagcggaagc ccgaagaaga agagaaaggu cgacggaagc 4200

ccgaagaaga agagaaaggu cgacagcgga uag 4233

<210> SEQ ID NO 224
<211> LENGTH: 4227
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 coding sequence encoding SEQ ID NO: 222 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> SEQUENCE: 224

gacaagaagu acagcaucgg acuggacauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

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cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

aaggucgucg acgaacuggu caaggucaug ggaagacaca agccggaaaa caucgucauc 2280

gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

auguacgucg accaggaacu ggacaucaac agacugagcg acuacgacgu cgaccacauc 2520

gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

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gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaggagga agcccgaaga agaagagaaa ggucccgaag 4140

aagaagagaa aggucggaag cggaagcccg aagaagaaga gaaaggucga cggaagcccg 4200

aagaagaaga gaaaggucga cagcgga 4227

<210> SEQ ID NO 225
<211> LENGTH: 1392
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Amino acid sequence of Cas9 nickase with two nuclear localization signals as the C-terminal amino acids

<400> SEQUENCE: 225

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala

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        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605
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Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005
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Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Ser  Gly Ser Pro Lys Lys  Lys Arg Lys Val Asp  Gly Ser Pro
    1370                1375                1380

Lys Lys  Lys Arg Lys Val Asp  Ser Gly
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1385 1390

<210> SEQ ID NO 226
<211> LENGTH: 4179
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 nickase mRNA ORF encoding SEQ ID NO: 25 using minimal uridine codons as listed in Table 3, with start and stop codons

<400> SEQUENCE: 226

auggacaaga aguacagcau cggacuggca aucggaacaa acagcgucgg augggcaguc 60

aucacagacg aauacaaggu cccgagcaag aaguucaagg uccugggaaa cacagacaga 120

cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa 180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc 240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga 300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga 360

aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

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ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

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agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

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cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

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agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

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aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgaccac 2520

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gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

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aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggaagc ggaagcccga agaagaagag aaaggucgac 4140

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ggaagcccga agaagaagag aaaggucgac agcggauag 4179

<210> SEQ ID NO 227
<211> LENGTH: 4173
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 nickase coding sequence encoding SEQ ID NO: 25 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> SEQUENCE: 227

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agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

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aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

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gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

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ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

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cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

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gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

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gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

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aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

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cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

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agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

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uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

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agcccgaaga agaagagaaa ggucgacagc gga 4173

<210> SEQ ID NO 228
<211> LENGTH: 1392
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Amino acid sequence of dCas9 with two nuclear localization signals as the C-terminal amino acids

<400> SEQUENCE: 228

Met Asp Lys Lys Tyr Ser Ile Gly Leu Ala Ile Gly Thr Asn Ser Val
1 5 10 15

Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305 310 315 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325 330 335

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Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380

Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735
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Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp Ala Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
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    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365

Gly Ser  Gly Ser Pro Lys Lys  Lys Arg Lys Val Asp  Gly Ser Pro
    1370                1375                1380

Lys Lys  Lys Arg Lys Val Asp  Ser Gly
    1385                1390


<210> SEQ ID NO 229
<211> LENGTH: 4179
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: dCas9 mRNA ORF encoding SEQ ID NO:
      228 using minimal uridine codons, with start and stop codons

<400> SEQUENCE: 229

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cacagcauca agaagaaccu gaucggagca cugcuguucg acagcggaga aacagcagaa    180

gcaacaagac ugaagagaac agcaagaaga agauacacaa gaagaaagaa cagaaucugc    240

uaccugcagg aaaucuucag caacgaaaug gcaaaggucg acgacagcuu cuuccacaga    300

cuggaagaaa gcuuccuggu cgaagaagac aagaagcacg aaagacaccc gaucuucgga    360
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aacaucgucg acgaagucgc auaccacgaa aaguacccga caaucuacca ccugagaaag 420

aagcuggucg acagcacaga caaggcagac cugagacuga ucuaccuggc acuggcacac 480

augaucaagu ucagaggaca cuuccugauc gaaggagacc ugaacccgga caacagcgac 540

gucgacaagc uguucaucca gcugguccag acauacaacc agcuguucga agaaaacccg 600

aucaacgcaa gcggagucga cgcaaaggca auccugagcg caagacugag caagagcaga 660

agacuggaaa accugaucgc acagcugccg ggagaaaaga agaacggacu guucggaaac 720

cugaucgcac ugagccuggg acugacaccg aacuucaaga gcaacuucga ccuggcagaa 780

gacgcaaagc ugcagcugag caaggacaca uacgacgacg accuggacaa ccugcuggca 840

cagaucggag accaguacgc agaccuguuc cuggcagcaa agaaccugag cgacgcaauc 900

cugcugagcg acauccugag agucaacaca gaaaucacaa aggcaccgcu gagcgcaagc 960

augaucaaga gauacgacga acaccaccag gaccugacac ugcugaaggc acuggucaga 1020

cagcagcugc cggaaaagua caaggaaauc uucuucgacc agagcaagaa cggauacgca 1080

ggauacaucg acggaggagc aagccaggaa gaauucuaca aguucaucaa gccgauccug 1140

gaaaagaugg acggaacaga agaacugcug gucaagcuga acagagaaga ccugcugaga 1200

aagcagagaa cauucgacaa cggaagcauc ccgcaccaga uccaccuggg agaacugcac 1260

gcaauccuga gaagacagga agacuucuac ccguuccuga aggacaacag agaaaagauc 1320

gaaaagaucc ugacauucag aaucccguac uacgucggac cgcuggcaag aggaaacagc 1380

agauucgcau ggaugacaag aaagagcgaa gaaacaauca caccguggaa cuucgaagaa 1440

gucgucgaca agggagcaag cgcacagagc uucaucgaaa gaaugacaaa cuucgacaag 1500

aaccugccga acgaaaaggu ccugccgaag cacagccugc uguacgaaua cuucacaguc 1560

uacaacgaac ugacaaaggu caaguacguc acagaaggaa ugagaaagcc ggcauuccug 1620

agcggagaac agaagaaggc aaucgucgac cugcuguuca agacaaacag aaaggucaca 1680

gucaagcagc ugaaggaaga cuacuucaag aagaucgaau gcuucgacag cgucgaaauc 1740

agcggagucg aagacagauu caacgcaagc cugggaacau accacgaccu gcugaagauc 1800

aucaaggaca aggacuuccu ggacaacgaa gaaaacgaag acauccugga agacaucguc 1860

cugacacuga cacuguucga agacagagaa augaucgaag aaagacugaa gacauacgca 1920

caccuguucg acgacaaggu caugaagcag cugaagagaa gaagauacac aggaugggga 1980

agacugagca gaaagcugau caacggaauc agagacaagc agagcggaaa gacaauccug 2040

gacuuccuga agagcgacgg auucgcaaac agaaacuuca ugcagcugau ccacgacgac 2100

agccugacau ucaaggaaga cauccagaag gcacagguca gcggacaggg agacagccug 2160

cacgaacaca ucgcaaaccu ggcaggaagc ccggcaauca agaagggaau ccugcagaca 2220

gucaaggucg ucgacgaacu ggucaagguc augggaagac acaagccgga aaacaucguc 2280

aucgaaaugg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

augaagagaa ucgaagaagg aaucaaggaa cugggaagcc agauccugaa ggaacacccg 2400

gucgaaaaca cacagcugca gaacgaaaag cuguaccugu acuaccugca gaacggaaga 2460

gacauguacg ucgaccagga acuggacauc aacagacuga gcgacuacga cgucgacgca 2520

aucgucccgc agagcuuccu gaaggacgac agcaucgaca acaagguccu gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacguc ccgagcgaag aagucgucaa gaagaugaag 2640

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aacuacugga gacagcugcu gaacgcaaag cugaucacac agagaaaguu cgacaaccug 2700

acaaaggcag agagaggagg acugagcgaa cuggacaagg caggauucau caagagacag 2760

cuggucgaaa caagacagau cacaaagcac gucgcacaga uccuggacag cagaaugaac 2820

acaaaguacg acgaaaacga caagcugauc agagaaguca aggucaucac acugaagagc 2880

aagcugguca gcgacuucag aaaggacuuc caguucuaca aggucagaga aaucaacaac 2940

uaccaccacg cacacgacgc auaccugaac gcagucgucg gaacagcacu gaucaagaag 3000

uacccgaagc uggaaagcga auucgucuac ggagacuaca aggucuacga cgucagaaag 3060

augaucgcaa agagcgaaca ggaaaucgga aaggcaacag caaaguacuu cuucuacagc 3120

aacaucauga acuucuucaa gacagaaauc acacuggcaa acggagaaau cagaaagaga 3180

ccgcugaucg aaacaaacgg agaaacagga gaaaucgucu gggacaaggg aagagacuuc 3240

gcaacaguca gaaagguccu gagcaugccg caggucaaca ucgucaagaa gacagaaguc 3300

cagacaggag gauucagcaa ggaaagcauc cugccgaaga gaaacagcga caagcugauc 3360

gcaagaaaga aggacuggga cccgaagaag uacggaggau ucgacagccc gacagucgca 3420

uacagcgucc uggucgucgc aaaggucgaa aagggaaaga gcaagaagcu gaagagcguc 3480

aaggaacugc ugggaaucac aaucauggaa agaagcagcu ucgaaaagaa cccgaucgac 3540

uuccuggaag caaagggaua caaggaaguc aagaaggacc ugaucaucaa gcugccgaag 3600

uacagccugu ucgaacugga aaacggaaga aagagaaugc uggcaagcgc aggagaacug 3660

cagaagggaa acgaacuggc acugccgagc aaguacguca acuuccugua ccuggcaagc 3720

cacuacgaaa agcugaaggg aagcccggaa gacaacgaac agaagcagcu guucgucgaa 3780

cagcacaagc acuaccugga cgaaaucauc gaacagauca gcgaauucag caagagaguc 3840

auccuggcag acgcaaaccu ggacaagguc cugagcgcau acaacaagca cagagacaag 3900

ccgaucagag aacaggcaga aaacaucauc caccuguuca cacugacaaa ccugggagca 3960

ccggcagcau ucaaguacuu cgacacaaca aucgacagaa agagauacac aagcacaaag 4020

gaaguccugg acgcaacacu gauccaccag agcaucacag gacuguacga aacaagaauc 4080

gaccugagcc agcugggagg agacggaagc ggaagcccga agaagaagag aaaggucgac 4140

ggaagcccga agaagaagag aaaggucgac agcggauag 4179

<210> SEQ ID NO 230
<211> LENGTH: 4173
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: dCas9 coding sequence encoding SEQ ID NO: 228 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> SEQUENCE: 230

gacaagaagu acagcaucgg acuggcaauc ggaacaaaca gcgucggaug ggcagucauc 60

acagacgaau acaaggcccc gagcaagaag uucaaggucc ugggaaacac agacagacac 120

agcaucaaga agaaccugau cggagcacug cuguucgaca gcggagaaac agcagaagca 180

acaagacuga agagaacagc aagaagaaga uacacaagaa gaaagaacag aaucugcuac 240

cugcaggaaa ucuucagcaa cgaaauggca aaggucgacg acagcuucuu ccacagacug 300

gaagaaagcu uccuggucga agaagacaag aagcacgaaa gacacccgau cuucggaaac 360

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aucgucgacg aagucgcaua ccacgaaaag uacccgacaa ucuaccaccu gagaaagaag 420

cuggucgaca gcacagacaa ggcagaccug agacugaucu accuggcacu ggcacacaug 480

aucaaguuca gaggacacuu ccugaucgaa ggagaccuga acccggacaa cagcgacguc 540

gacaagcugu ucauccagcu gguccagaca uacaaccagc uguucgaaga aaacccgauc 600

aacgcaagcg gagucgacgc aaaggcaauc cugagcgcaa gacugagcaa gagcagaaga 660

cuggaaaacc ugaucgcaca gcugccggga gaaaagaaga acggacuguu cggaaaccug 720

aucgcacuga gccugggacu gacaccgaac uucaagagca acuucgaccu ggcagaagac 780

gcaaagcugc agcugagcaa ggacacauac gacgacgacc uggacaaccu gcuggcacag 840

aucggagacc aguacgcaga ccuguuccug gcagcaaaga accugagcga cgcaauccug 900

cugagcgaca uccugagagu caacacagaa aucacaaagg caccgcugag cgcaagcaug 960

aucaagagau acgacgaaca ccaccaggac cugacacugc ugaaggcacu ggucagacag 1020

cagcugccgg aaaaguacaa ggaaaucuuc uucgaccaga gcaagaacgg auacgcagga 1080

uacaucgacg gaggagcaag ccaggaagaa uucuacaagu ucaucaagcc gauccuggaa 1140

aagauggacg gaacagaaga acugcugguc aagcugaaca gagaagaccu gcugagaaag 1200

cagagaacau ucgacaacgg aagcaucccg caccagaucc accugggaga acugcacgca 1260

auccugagaa gacaggaaga cuucuacccg uuccugaagg acaacagaga aaagaucgaa 1320

aagauccuga cauucagaau cccguacuac gucggaccgc uggcaagagg aaacagcaga 1380

uucgcaugga ugacaagaaa gagcgaagaa acaaucacac cguggaacuu cgaagaaguc 1440

gucgacaagg gagcaagcgc acagagcuuc aucgaaagaa ugacaaacuu cgacaagaac 1500

cugccgaacg aaaagguccu gccgaagcac agccugcugu acgaauacuu cacagucuac 1560

aacgaacuga caaaggucaa guacgucaca gaaggaauga gaaagccggc auuccugagc 1620

ggagaacaga agaaggcaau cgucgaccug cuguucaaga caaacagaaa ggucacaguc 1680

aagcagcuga aggaagacua cuucaagaag aucgaaugcu ucgacagcgu cgaaaucagc 1740

ggagucgaag acagauucaa cgcaagccug ggaacauacc acgaccugcu gaagaucauc 1800

aaggacaagg acuuccugga caacgaagaa aacgaagaca uccuggaaga caucguccug 1860

acacugacac uguucgaaga cagagaaaug aucgaagaaa gacugaagac auacgcacac 1920

cuguucgacg acaaggucau gaagcagcug aagagaagaa gauacacagg auggggaaga 1980

cugagcagaa agcugaucaa cggaaucaga gacaagcaga gcggaaagac aauccuggac 2040

uuccugaaga gcgacggauu cgcaaacaga aacuucaugc agcugaucca cgacgacagc 2100

cugacauuca aggaagacau ccagaaggca caggucagcg gacagggaga cagccugcac 2160

gaacacaucg caaaccuggc aggaagcccg gcaaucaaga agggaauccu gcagacaguc 2220

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gaaauggcaa gagaaaacca gacaacacag aagggacaga agaacagcag agaaagaaug 2340

aagagaaucg aagaaggaau caaggaacug ggaagccaga uccugaagga acacccgguc 2400

gaaaacacac agcugcagaa cgaaaagcug uaccuguacu accugcagaa cggaagagac 2460

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gucccgcaga gcuuccugaa ggacgacagc aucgacaaca agguccugac aagaagcgac 2580

aagaacagag gaaagagcga caacgucccg agcgaagaag ucgucaagaa gaugaagaac 2640

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uacuggagac agcugcugaa cgcaaagcug aucacacaga gaaaguucga caaccugaca 2700

aaggcagaga gaggaggacu gagcgaacug gacaaggcag gauucaucaa gagacagcug 2760

gucgaaacaa gacagaucac aaagcacguc gcacagaucc uggacagcag aaugaacaca 2820

aaguacgacg aaaacgacaa gcugaucaga gaagucaagg ucaucacacu gaagagcaag 2880

cuggucagcg acuucagaaa ggacuuccag uucuacaagg ucagagaaau caacaacuac 2940

caccacgcac acgacgcaua ccugaacgca gucgucggaa cagcacugau caagaaguac 3000

ccgaagcugg aaagcgaauu cgucuacgga gacuacaagg ucuacgacgu cagaaagaug 3060

aucgcaaaga gcgaacagga aaucggaaag gcaacagcaa aguacuucuu cuacagcaac 3120

aucaugaacu ucuucaagac agaaaucaca cuggcaaacg gagaaaucag aaagagaccg 3180

cugaucgaaa caaacggaga aacaggagaa aucgucuggg acaagggaag agacuucgca 3240

acagucagaa agguccugag caugccgcag gucaacaucg ucaagaagac agaaguccag 3300

acaggaggau ucagcaagga aagcauccug ccgaagagaa acagcgacaa gcugaucgca 3360

agaaagaagg acugggaccc gaagaaguac ggaggauucg acagcccgac agucgcauac 3420

agcguccugg ucgucgcaaa ggucgaaaag ggaaagagca agaagcugaa gagcgucaag 3480

gaacugcugg gaaucacaau cauggaaaga agcagcuucg aaaagaaccc gaucgacuuc 3540

cuggaagcaa agggauacaa ggaagucaag aaggaccuga ucaucaagcu gccgaaguac 3600

agccuguucg aacuggaaaa cggaagaaag agaaugcugg caagcgcagg agaacugcag 3660

aagggaaacg aacuggcacu gccgagcaag uacgucaacu uccuguaccu ggcaagccac 3720

uacgaaaagc ugaagggaag cccggaagac aacgaacaga agcagcuguu cgucgaacag 3780

cacaagcacu accuggacga aaucaucgaa cagaucagcg aauucagcaa gagagucauc 3840

cuggcagacg caaaccugga caagguccug agcgcauaca acaagcacag agacaagccg 3900

aucagagaac aggcagaaaa caucauccac cuguucacac ugacaaaccu gggagcaccg 3960

gcagcauuca aguacuucga cacaacaauc gacagaaaga gauacacaag cacaaaggaa 4020

guccuggacg caacacugau ccaccagagc aucacaggac uguacgaaac aagaaucgac 4080

cugagccagc ugggaggaga cggaagcgga agcccgaaga agaagagaaa ggucgacgga 4140

agcccgaaga agaagagaaa ggucgacagc gga 4173

<210> SEQ ID NO 231
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: T7 Promoter

<400> SEQUENCE: 231

taatacgact cactata 17

<210> SEQ ID NO 232
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(50)
<223> OTHER INFORMATION: Human beta-globin 5 UTR

<400> SEQUENCE: 232

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acatttgctt ctgacacaac tgtgttcact agcaacctca aacagacacc 50

<210> SEQ ID NO 233
<211> LENGTH: 132
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(132)
<223> OTHER INFORMATION: Human beta-globin 3 UTR

<400> SEQUENCE: 233

gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac 60

taaactgggg gatattatga agggccttga gcatctggat tctgcctaat aaaaaacatt 120

tattttcatt gc 132

<210> SEQ ID NO 234
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(66)
<223> OTHER INFORMATION: Human alpha-globin 5 UTR

<400> SEQUENCE: 234

cataaaccct ggcgcgctcg cggcccggca ctcttctggt ccccacagac tcagagagaa 60

cccacc 66

<210> SEQ ID NO 235
<211> LENGTH: 110
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(110)
<223> OTHER INFORMATION: Human alpha-globin 3 UTR

<400> SEQUENCE: 235

gctggagcct cggtggccat gcttcttgcc ccttgggcct ccccccagcc cctcctcccc 60

ttcctgcacc cgtacccccg tggtctttga ataaagtctg agtgggcggc 110

<210> SEQ ID NO 236
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Xenopus laevis
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(29)
<223> OTHER INFORMATION: Xenopus laevis beta-globin 5 UTR

<400> SEQUENCE: 236

aagctcagaa taaacgctca actttggcc 29

<210> SEQ ID NO 237
<211> LENGTH: 130
<212> TYPE: DNA
<213> ORGANISM: Xenopus laevis
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(130)
<223> OTHER INFORMATION: Xenopus laevis beta-globin 3 UTR

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<400> SEQUENCE: 237

accagcctca agaacacccg aatggagtct ctaagctaca taataccaac ttacacttta 60

caaaatgttg tcccccaaaa tgtagccatt cgtatctgct cctaataaaa agaaagtttc 120

ttcacattct 130

<210> SEQ ID NO 238
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Bos taurus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(27)
<223> OTHER INFORMATION: Bovine Growth Hormone 5 UTR

<400> SEQUENCE: 238

cagggtcctg tggacagctc accagct 27

<210> SEQ ID NO 239
<211> LENGTH: 102
<212> TYPE: DNA
<213> ORGANISM: Bos taurus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(102)
<223> OTHER INFORMATION: Bovine Growth Hormone 3 UTR

<400> SEQUENCE: 239

ttgccagcca tctgttgttt gcccctcccc cgtgccttcc ttgaccctgg aaggtgccac 60

tcccactgtc ctttcctaat aaaatgagga attgcatcg ca 102

<210> SEQ ID NO 240
<211> LENGTH: 93
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(93)
<223> OTHER INFORMATION: Mus musculus hemoglobin alpha, adult chain 1
(Hba-a1), 3UTR

<400> SEQUENCE: 240

gctgccttct gcggggcttg ccttctggcc atgcccttct tctctccctt gcacctgtac 60

ctcttggtct ttgaataaag cctgagtagg aag 93

<210> SEQ ID NO 241
<211> LENGTH: 61
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: HSD17B4 5 UTR

<400> SEQUENCE: 241

tcccgcagtc ggcgtccagc ggctctgctt gttcgtgtgt gtgtcgttgc aggccttatt 60

c 61

<210> SEQ ID NO 242
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G282 single guide RNA targeting the

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mouse TTR gene
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 242

uuacagccac gucuacagca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 243

<400> SEQUENCE: 243

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<210> SEQ ID NO 244
<211> LENGTH: 4405
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 204, and 3 UTR of ALB

<400> SEQUENCE: 244

gggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc atggacaaga agtacagcat cggactggac atcggaacaa acagcgtcgg 120

atgggcagtc atcacagacg aatacaaggt cccgagcaag aagttcaagg tcctgggaaa 180

cacagacaga cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga 240

aacagcagaa gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa 300

cagaatctgc tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt 360

cttccacaga ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc 420

gatcttcgga aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca 480

cctgagaaag aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc 540

actggcacac atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga 600

caacagcgac gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga 660

agaaaacccg atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag 720

caagagcaga agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact 780

gttcggaaac ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga 840

cctggcagaa gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa 900

cctgctggca cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag 960

cgacgcaatc ctgctgagcg acatcctgag agtcaacaca gaaatcacaa aggcaccgct 1020

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gagcgcaagc atgatcaaga gatacgacga acaccaccag gacctgacac tgctgaaggc 1080

actggtcaga cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa 1140

cggatacgca ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa 1200

gccgatcctg gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga 1260

cctgctgaga aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg 1320

agaactgcac gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag 1380

agaaaagatc gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag 1440

aggaaacagc agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa 1500

cttcgaagaa gtcgtcgaca agggagcaag cgcacagagc ttcatcgaaa gaatgacaaa 1560

cttcgacaag aacctgccga acgaaaaggt cctgccgaag cacagcctgc tgtacgaata 1620

cttcacagtc tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc 1680

ggcattcctg agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag 1740

aaaggtcaca gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag 1800

cgtcgaaatc agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct 1860

gctgaagatc atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga 1920

agacatcgtc ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa 1980

gacatacgca cacctgttcg acgacaaggt catgaagcag ctgaagagaa gaagatacac 2040

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gacaatcctg gacttcctga agagcgacgg attcgcaaac agaaacttca tgcagctgat 2160

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aaacatcgtc atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag 2400

cagagaaaga atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa 2460

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caagagacag ctggtcgaaa caagacagat cacaaagcac gtcgcacaga tcctggacag 2880

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cagaaagaga ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg 3300

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cctgggagca ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac 4080

aagcacaaag gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga 4140

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gaaaatgaag atcaatagct tattcatctc ttttttcttt tcgttggtgt aaagccaaca 4320

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aataaaaaat ggaaagaacc tcgag 4405

<210> SEQ ID NO 245
<211> LENGTH: 4188
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Alternative Cas9 ORF with 19.36% U content

<400> SEQUENCE: 245

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atcacggatg aatacaaagt gccgtccaag aagttcaagg tcctggggaa caccgataga 120

cacagcatca agaagaatct catcggagcc ctgctgtttg actccggcga aaccgcagaa 180

gcgacccggc tcaaacgtac cgcgaggcga cgctacaccc ggcggaagaa tcgcatctgc 240

tatctgcaag aaatcttttc gaacgaaatg gcaaaggtgg acgacagctt cttccaccgc 300

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aacatcgtgg acgaagtggc gtaccacgaa aagtacccga ccatctacca tctgcggaag 420

aagttggttg actcaactga caaggccgac ctcagattga tctacttggc cctcgcccat 480

atgatcaaat tccgcggaca cttcctgatc gaaggcgatc tgaaccctga taactccgac 540

gtggataagc tgttcattca actggtgcag acctacaacc aactgttcga agaaaaccca 600

atcaatgcca gcggcgtcga tgccaaggcc atcctgtccg cccggctgtc gaagtcgcgg 660

cgcctcgaaa acctgatcgc acagctgccg ggagagaaga agaacggact tttcggcaac 720

ttgatcgctc tctcactggg actcactccc aatttcaagt ccaattttga cctggccgag 780

gacgcgaagc tgcaactctc aaaggacacc tacgacgacg acttggacaa tttgctggca 840

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caaattggcg atcagtacgc ggatctgttc cttgccgcta agaacctttc ggacgcaatc 900

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atgattaagc ggtacgacga gcatcaccag gatctcacgc tgctcaaagc gctcgtgaga 1020

cagcaactgc ctgaaaagta caaggagatt ttcttcgacc agtccaagaa tgggtacgca 1080

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gagaaaattc tgacgttccg catcccgtat tacgtgggcc cactggcgcg cggcaattcg 1380

cgcttcgcgt ggatgactag aaaatcagag gaaaccatca ctccttggaa tttcgaggaa 1440

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catctcttcg acgataaggt catgaaacaa ctcaagcgcc gccggtacac tggttggggc 1980

cgcctctccc gcaagctgat caacggtatt cgcgataaac agagcggtaa aactatcctg 2040

gatttcctca aatcggatgg cttcgctaat cgtaacttca tgcagttgat ccacgacgac 2100

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attgaaatgg cccgagaaaa ccagactacc cagaagggcc agaagaactc ccgcgaaagg 2340

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ccactgatcg aaactaacgg agaaacgggc gaaatcgtgt gggacaaggg cagggacttc 3240

gcaactgttc gcaaagtgct ctctatgccg caagtcaata ttgtgaagaa aaccgaagtg 3300

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ccagccgcct tcaagtactt cgatactact atcgaccgca aaagatacac gtccaccaag 4020

gaagttctgg acgcgaccct gatccaccaa agcatcactg gactctacga aactaggatc 4080

gatctgtcgc agctgggtgg cgatggtggc ggtggatcct acccatacga cgtgcctgac 4140

tacgcctccg gaggtggtgg ccccaagaag aaacggaagg tgtgatag 4188

<210> SEQ ID NO 246
<211> LENGTH: 4459
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 245, Kozak sequence, and 3 UTR of ALB

<400> SEQUENCE: 246

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cgtgggttgg gcagtgatca cggatgaata caaagtgccg tccaagaagt tcaaggtcct 180

ggggaacacc gatagacaca gcatcaagaa gaatctcatc ggagccctgc tgtttgactc 240

cggcgaaacc gcagaagcga cccggctcaa acgtaccgcg aggcgacgct acacccggcg 300

gaagaatcgc atctgctatc tgcaagaaat cttttcgaac gaaatggcaa aggtggacga 360

cagcttcttc caccgcctgg aagaatcttt cctggtggag gaggacaaga agcatgaacg 420

gcatcctatc tttggaaaca tcgtggacga agtggcgtac cacgaaaagt acccgaccat 480

ctaccatctg cggaagaagt tggttgactc aactgacaag gccgacctca gattgatcta 540

cttggccctc gcccatatga tcaaattccg cggacacttc ctgatcgaag gcgatctgaa 600

ccctgataac tccgacgtgg ataagctgtt cattcaactg gtgcagacct acaaccaact 660

gttcgaagaa aacccaatca atgccagcgg cgtcgatgcc aaggccatcc tgtccgcccg 720

gctgtcgaag tcgcggcgcc tcgaaaacct gatcgcacag ctgccgggag agaagaagaa 780

cggacttttc ggcaacttga tcgctctctc actgggactc actcccaatt tcaagtccaa 840

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ggacaatttg ctggcacaaa ttggcgatca gtacgcggat ctgttccttg ccgctaagaa 960

cctttcggac gcaatcttgc tgtccgatat cctgcgcgtg aacaccgaaa taaccaaagc 1020

gccgcttagc gcctcgatga ttaagcggta cgacgagcat caccaggatc tcacgctgct 1080

caaagcgctc gtgagacagc aactgcctga aaagtacaag gagattttct tcgaccagtc 1140

caagaatggg tacgcagggt acatcgatgg aggcgccagc caggaagagt tctataagtt 1200

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tctgggtgag ctgcacgcca tcttgcggcg ccaggaggac ttttacccat tcctcaagga 1380

caaccgggaa aagatcgaga aaattctgac gttccgcatc ccgtattacg tgggcccact 1440

ggcgcgcggc aattcgcgct tcgcgtggat gactagaaaa tcagaggaaa ccatcactcc 1500

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gttgatccac gacgacagcc tgacctttaa ggaggacatc cagaaagcac aagtgagcgg 2220

acagggagac tcactccatg aacacatcgc gaatctggcc ggttcgccgg cgattaagaa 2280

gggaatcctg caaactgtga aggtggtgga cgagctggtg aaggtcatgg gacggcacaa 2340

accggagaat atcgtgattg aaatggcccg agaaaaccag actacccaga agggccagaa 2400

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cctgaaagag cacccggtgg aaaacacgca gctgcagaac gagaagctct acctgtacta 2520

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cgtgaagaag atgaagaatt actggcggca gctcctgaat gcgaagctga ttacccagag 2760

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gagagaaatc aacaactacc atcacgcgca tgacgcatac ctcaacgctg tggtcggcac 3060

cgccctgatc aagaagtacc ctaaacttga atcggagttt gtgtacggag actacaaggt 3120

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atagctagcc atcacattta aaagcatctc agcctaccat gagaataaga gaaagaaaat 4320

gaagatcaat agcttattca tctttttttc ttttttcgttg gtgtaaagcc aacaccctgt 4380

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aaatggaaag aacctcgag 4459

<210> SEQ ID NO 247
<211> LENGTH: 4453
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 245, and 3 UTR of ALB

<400> SEQUENCE: 247

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attcggatct atggataaga agtactcgat cgggctggat atcggaacta attccgtggg 120

ttgggcagtg atcacggatg aatacaaagt gccgtccaag aagttcaagg tcctggggaa 180

caccgataga cacagcatca agaagaatct catcggagcc ctgctgtttg actccggcga 240

aaccgcagaa gcgacccggc tcaaacgtac cgcgaggcga cgctacaccc ggcggaagaa 300

tcgcatctgc tatctgcaag aaatcttttc gaacgaaatg gcaaaggtgg acgacagctt 360

cttccaccgc ctggaagaat ctttcctggt ggaggaggac aagaagcatg aacggcatcc 420

tatctttgga aacatcgtgg acgaagtggc gtaccacgaa aagtacccga ccatctacca 480

tctgcggaag aagttggttg actcaactga caaggccgac ctcagattga tctacttggc 540

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cctcgcccat atgatcaaat tccgcggaca cttcctgatc gaaggcgatc tgaaccctga 600

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agaaaaccca atcaatgcca gcggcgtcga tgccaaggcc atcctgtccg cccggctgtc 720

gaagtcgcgg cgcctcgaaa acctgatcgc acagctgccg ggagagaaga agaacggact 780

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cttcgacaag aatctcccaa acgagaaggt gcttcctaag cacagcctcc tttacgaata 1620

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ggcctttctg agcggagaac agaagaaagc gattgtcgat ctgctgttca agaccaaccg 1740

caaggtgacc gtcaagcagc ttaaagagga ctacttcaag aagatcgagt gtttcgactc 1800

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gacctacgct catctcttcg acgataaggt catgaaacaa ctcaagcgcc gccggtacac 2040

tggttggggc cgcctctccc gcaagctgat caacggtatt cgcgataaac agagcggtaa 2100

aactatcctg gatttcctca aatcggatgg cttcgctaat cgtaacttca tgcagttgat 2160

ccacgacgac agcctgacct ttaaggagga catccagaaa gcacaagtga gcggacaggg 2220

agactcactc catgaacaca tcgcgaatct ggccggttcg ccggcgatta agaagggaat 2280

cctgcaaact gtgaaggtgg tggacgagct ggtgaaggtc atgggacggc acaaaccgga 2340

gaatatcgtg attgaaatgg cccgagaaaa ccagactacc cagaagggcc agaagaactc 2400

ccgcgaaagg atgaagcgga tcgaagaagg aatcaaggag ctgggcagcc agatcctgaa 2460

agagcacccg gtggaaaaca cgcagctgca gaacgagaag ctctacctgt actatttgca 2520

aaatggacgg gacatgtacg tggaccaaga gctggacatc aatcggttgt ctgattacga 2580

cgtggaccac atcgttccac agtcctttct gaaggatgac tccatcgata acaaggtgtt 2640

gactcgcagc gacaagaaca gagggaagtc agataatgtg ccatcggagg aggtcgtgaa 2700

gaagatgaag aattactggc ggcagctcct gaatgcgaag ctgattaccc agagaaagtt 2760

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ccgcatgaac actaaatacg acgagaacga taagctcatc cgggaagtga aggtgattac 2940

cctgaaaagc aaacttgtgt cggactttcg gaaggacttt cagttttaca aagtgagaga 3000

aatcaacaac taccatcacg cgcatgacgc atacctcaac gctgtggtcg gcaccgccct 3060

gatcaagaag taccctaaac ttgaatcgga gtttgtgtac ggagactaca aggtctacga 3120

cgtgaggaag atgatagcca agtccgaaca ggaaatcggg aaagcaactg cgaaatactt 3180

cttttactca aacatcatga acttcttcaa gactgaaatt acgctggcca atggagaaat 3240

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cagggacttc gcaactgttc gcaaagtgct ctctatgccg caagtcaata ttgtgaagaa 3360

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caagctcatt gcacgcaaga aagactggga cccgaagaag tacggaggat tcgattcgcc 3480

gactgtcgca tactccgtcc tcgtggtggc caaggtggag aagggaaaga gcaagaagct 3540

caaatccgtc aaagagctgc tggggattac catcatggaa cgatcctcgt tcgagaagaa 3600

cccgattgat ttcctggagg cgaagggtta caaggaggtg aagaaggatc tgatcatcaa 3660

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cctgggagct ccagccgcct tcaagtactt cgatactact atcgaccgca aaagatacac 4080

gtccaccaag gaagttctgg acgcgaccct gatccaccaa agcatcactg gactctacga 4140

aactaggatc gatctgtcgc agctgggtgg cgatggtggc ggtggatcct acccatacga 4200

cgtgcctgac tacgcctccg gaggtggtgg ccccaagaag aaacggaagg tgtgatagct 4260

agccatcaca tttaaaagca tctcagccta ccatgagaat aagagaaaga aaatgaagat 4320

caatagctta ttcatctctt tttctttttc gttggtgtaa agccaacacc ctgtctaaaa 4380

aacataaatt tctttaatca ttttgcctct tttctctgtg cttcaattaa taaaaaatgg 4440

aaagaacctc gag 4453

<210> SEQ ID NO 248

<400> SEQUENCE: 248

000

<210> SEQ ID NO 249
<211> LENGTH: 4409
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript comprising Kozak sequence with Cas9 ORF using codons with generally high expression in humans

<400> SEQUENCE: 249

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attcggatcc gccaccatgc ctaagaaaaa gcggaaggtc gacggggata agaagtactc 120

aatcgggctg gatatcggaa ctaattccgt gggttgggca gtgatcacgg atgaatacaa 180

agtgccgtcc aagaagttca aggtcctggg gaacaccgat agacacagca tcaagaaaaa 240

tctcatcgga gccctgctgt ttgactccgg cgaaaccgca gaagcgaccc ggctcaaacg 300

taccgcgagg cgacgctaca cccggcggaa gaatcgcatc tgctatctgc aagagatctt 360

ttcgaacgaa atggcaaagg tcgacgacag cttcttccac cgcctggaag aatctttcct 420

ggtggaggag gacaagaagc atgaacggca tcctatcttt ggaaacatcg tcgacgaagt 480

ggcgtaccac gaaaagtacc cgaccatcta ccatctgcgg aagaagttgg ttgactcaac 540

tgacaaggcc gacctcagat tgatctactt ggccctcgcc catatgatca aattccgcgg 600

acacttcctg atcgaaggcg atctgaaccc tgataactcc gacgtggata agcttttcat 660

tcaactggtg cagacctaca accaactgtt cgaagaaaac ccaatcaatg ctagcggcgt 720

cgatgccaag gccatcctgt ccgcccggct gtcgaagtcg cggcgcctcg aaaacctgat 780

cgcacagctg ccgggagaga aaaagaacgg acttttcggc aacttgatcg ctctctcact 840

gggactcact cccaatttca agtccaattt tgacctggcc gaggacgcga agctgcaact 900

ctcaaaggac acctacgacg acgacttgga caatttgctg gcacaaattg gcgatcagta 960

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cgaagaactg ctggtcaagc tgaacaggga ggatctgctc cggaaacaga gaacctttga 1320

caacggatcc attccccacc agatccatct gggtgagctg cacgccatct tgcggcgcca 1380

ggaggacttt tacccattcc tcaaggacaa ccgggaaaag atcgagaaaa ttctgacgtt 1440

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ttcggcacaa agcttcatcg aacgaatgac caacttcgac aagaatctcc caaacgagaa 1620

ggtgcttcct aagcacagcc tcctttacga atacttcact gtctacaacg aactgactaa 1680

agtgaaatac gttactgaag gaatgaggaa gccggccttt ctgtccggag aacagaagaa 1740

agcaattgtc gatctgctgt tcaagaccaa ccgcaaggtg accgtcaagc agcttaaaga 1800

ggactacttc aagaagatcg agtgtttcga ctcagtggaa atcagcgggg tggaggacag 1860

attcaacgct tcgctgggaa cctatcatga tctcctgaag atcatcaagg acaaggactt 1920

ccttgacaac gaggagaacg aggacatcct ggaagatatc gtcctgacct tgacccttt 1980

cgaggatcgc gagatgatcg aggagaggct taagacctac gctcatctct tcgacgataa 2040

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gatcaacggt attcgcgata aacagagcgg taaaactatc ctggatttcc tcaaatcgga 2160

tggcttcgct aatcgtaact tcatgcaatt gatccacgac gacagcctga cctttaagga 2220

ggacatccaa aaagcacaag tgtccggaca gggagactca ctccatgaac acatcgcgaa 2280

tctggccggt tcgccggcga ttaagaaggg aattctgcaa actgtgaagg tggtcgacga 2340

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gctggtgaag gtcatgggac ggcacaaacc ggagaatatc gtgattgaaa tggcccgaga 2400

aaaccagact acccagaagg gccagaaaaa ctcccgcgaa aggatgaagc ggatcgaaga 2460

aggaatcaag gagctgggca gccagatcct gaaagagcac ccggtggaaa acacgcagct 2520

gcagaacgag aagctctacc tgtactattt gcaaaatgga cgggacatgt acgtggacca 2580

agagctggac atcaatcggt tgtctgatta cgacgtggac cacatcgttc cacagtcctt 2640

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cggactctca gagctggata aggctggatt catcaaacgg cagctggtcg agactcggca 2880

gattaccaag cacgtggcgc agatcttgga ctcccgcatg aacactaaat acgacgagaa 2940

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cgcatacctc aacgctgtgg tcggtaccgc cctgatcaaa aagtacccta aacttgaatc 3120

ggagtttgtg tacggagact acaaggtcta cgacgtgagg aagatgatag ccaagtccga 3180

acaggaaatc gggaaagcaa ctgcgaaata cttcttttac tcaaacatca tgaacttttt 3240

caagactgaa attacgctgg ccaatggaga aatcaggaag aggccactga tcgaaactaa 3300

cggagaaacg ggcgaaatcg tgtgggacaa gggcagggac ttcgcaactg ttcgcaaagt 3360

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ggccaaggtg gagaagggaa agagcaaaaa gctcaaatcc gtcaaagagc tgctggggat 3600

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ggaaaatggt cggaagcgca tgctggcttc ggccggagaa ctccaaaaag gaaatgagct 3780

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cgagaacatt atccacttgt tcaccctgac taacctggga gccccagccg ccttcaagta 4080

cttcgatact actatcgatc gcaaaagata cacgtccacc aaggaagttc tggacgcgac 4140

cctgatccac caaagcatca ctggactcta cgaaactagg atcgatctgt cgcagctggg 4200

tggcgattga tagtctagcc atcacattta aaagcatctc agcctaccat gagaataaga 4260

gaaagaaaat gaagatcaat agcttattca tctcttttttc tttttcgttg gtgtaaagcc 4320

aacaccctgt ctaaaaaaca taaatttctt taatcatttt gcctcttttc tctgtgcttc 4380

aattaataaa aaatggaaag aacctcgag 4409

<210> SEQ ID NO 250
<211> LENGTH: 4140
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 ORF with splice junctions removed; 12.75% U content

<400> SEQUENCE: 250

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cacagcatca agaagaacct gatcggagca ctgctgttcg acagcggaga aacagcagaa 180

gcaacaagac tgaagagaac agcaagaaga agatacacaa gaagaaagaa cagaatctgc 240

tacctgcagg aaatcttcag caacgaaatg gcaaaggtcg acgacagctt cttccaccgg 300

ctggaagaaa gcttcctggt cgaagaagac aagaagcacg aaagacaccc gatcttcgga 360

aacatcgtcg acgaagtcgc ataccacgaa aagtacccga caatctacca cctgagaaag 420

aagctggtcg acagcacaga caaggcagac ctgagactga tctacctggc actggcacac 480

atgatcaagt tcagaggaca cttcctgatc gaaggagacc tgaacccgga caacagcgac 540

gtcgacaagc tgttcatcca gctggtccag acatacaacc agctgttcga agaaaacccg 600

atcaacgcaa gcggagtcga cgcaaaggca atcctgagcg caagactgag caagagcaga 660

agactggaaa acctgatcgc acagctgccg ggagaaaaga agaacggact gttcggaaac 720

ctgatcgcac tgagcctggg actgacaccg aacttcaaga gcaacttcga cctggcagaa 780

gacgcaaagc tgcagctgag caaggacaca tacgacgacg acctggacaa cctgctggca 840

cagatcggag accagtacgc agacctgttc ctggcagcaa agaacctgag cgacgcaatc 900

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cagcagctgc cggaaaagta caaggaaatc ttcttcgacc agagcaagaa cggatacgca 1080

ggatacatcg acggaggagc aagccaggaa gaattctaca agttcatcaa gccgatcctg 1140

gaaaagatgg acggaacaga agaactgctg gtcaagctga acagagaaga cctgctgaga 1200

aagcagagaa cattcgacaa cggaagcatc ccgcaccaga tccacctggg agaactgcac 1260

gcaatcctga gaagacagga agacttctac ccgttcctga aggacaacag agaaaagatc 1320

gaaaagatcc tgacattcag aatcccgtac tacgtcggac cgctggcaag aggaaacagc 1380

agattcgcat ggatgacaag aaagagcgaa gaaacaatca caccgtggaa cttcgaagaa 1440

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tacaacgaac tgacaaaggt caagtacgtc acagaaggaa tgagaaagcc ggcattcctg 1620

agcggagaac agaagaaggc aatcgtcgac ctgctgttca agacaaacag aaaggtcaca 1680

gtcaagcagc tgaaggaaga ctacttcaag aagatcgaat gcttcgacag cgtcgaaatc 1740

agcggagtcg aagacagatt caacgcaagc ctgggaacat accacgacct gctgaagatc 1800

atcaaggaca aggacttcct ggacaacgaa gaaaacgaag acatcctgga agacatcgtc 1860

ctgacactga cactgttcga agacagagaa atgatcgaag aaagactgaa gacatacgca 1920

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agactgagca gaaagctgat caacggaatc agagacaagc agagcggaaa gacaatcctg 2040

gacttcctga agagcgacgg attcgcaaac agaaacttca tgcagctgat ccacgacgac 2100

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atcgaaatgg caagagaaaa ccagacaaca cagaagggac agaagaacag cagagaaaga 2340

atgaagagaa tcgaagaagg aatcaaggaa ctgggaagcc agatcctgaa ggaacacccg 2400

gtcgaaaaca cacagctgca gaacgaaaag ctgtacctgt actacctgca aaacggaaga 2460

gacatgtacg tcgaccagga actggacatc aacagactga gcgactacga cgtcgaccac 2520

atcgtcccgc agagcttcct gaaggacgac agcatcgaca acaaggtcct gacaagaagc 2580

gacaagaaca gaggaaagag cgacaacgtc ccgagcgaag aagtcgtcaa gaagatgaag 2640

aactactgga gacagctgct gaacgcaaag ctgatcacac agagaaagtt cgacaacctg 2700

acaaaggcag agagaggagg actgagcgaa ctggacaagg caggattcat caagagacag 2760

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acaaagtacg acgaaaacga caagctgatc agagaagtca aggtcatcac actgaagagc 2880

aagctggtca gcgacttcag aaaggacttc cagttctaca aggtcagaga aatcaacaac 2940

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tacccgaagc tggaaagcga attcgtctac ggagactaca aggtctacga cgtcagaaag 3060

atgatcgcaa agagcgaaca ggaaatcgga aaggcaacag caaagtactt cttctacagc 3120

aacatcatga acttcttcaa gacagaaatc acactggcaa acggagaaat cagaaagaga 3180

ccgctgatcg aaacaaacgg agaaacagga gaaatcgtct gggacaaggg aagagacttc 3240

gcaacagtca gaaaggtcct gagcatgccg caggtcaaca tcgtcaagaa gacagaagtc 3300

cagacaggag gattcagcaa ggaaagcatc ctgccgaaga gaaacagcga caagctgatc 3360

gcaagaaaga aggactggga cccgaagaag tacggaggat tcgacagccc gacagtcgca 3420

tacagcgtcc tggtcgtcgc aaaggtcgaa aagggaaaga gcaagaagct gaagagcgtc 3480

aaggaactgc tgggaatcac aatcatggaa agaagcagct tcgaaaagaa cccgatcgac 3540

ttcctggaag caaagggata caaggaagtc aagaaggacc tgatcatcaa gctgccgaag 3600

tacagcctgt tcgaactgga aaacggaaga aagagaatgc tggcaagcgc aggagaactg 3660

cagaagggaa acgaactggc actgccgagc aagtacgtca acttcctgta cctggcaagc 3720

cactacgaaa agctgaaggg aagcccggaa gacaacgaac agaagcagct gttcgtcgaa 3780

cagcacaagc actacctgga cgaaatcatc gaacagatca gcgaattcag caagagagtc 3840

atcctggcag acgcaaacct ggacaaggtc ctgagcgcat acaacaagca cagagacaag 3900

ccgatcagag aacaggcaga aaacatcatc cacctgttca cactgacaaa cctgggagca 3960

ccggcagcat tcaagtactt cgacacaaca atcgacagaa agagatacac aagcacaaag 4020

gaagtcctgg acgcaacact gatccaccag agcatcacag gactgtacga aacaagaatc 4080

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<210> SEQ ID NO 251
<211> LENGTH: 4411
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR of HSD,
ORF corresponding to SEQ ID NO: 250, Kozak sequence, and 3 UTR of

-continued

ALB

<400> SEQUENCE: 251

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cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

cagcttcttc caccggctgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag aaaagaagaa 780

cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg acgacgacct 900

ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg cagcaaagaa 960

cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc tgacactgct 1080

gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat tctacaagtt 1200

catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc accagatcca 1320

cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt tcctgaagga 1380

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cgaatacttc acagtctaca acgaactgac aaaggtcaag tacgtcacag aaggaatgag 1680

aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc tgttcaagac 1740

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cgacagcgtc gaaatcagcg gagtcgaaga cagattcaac gcaagcctgg gaacatacca 1860

cgacctgctg aagatcatca aggacaagga cttcctggac aacgaagaaa acgaagacat 1920

cctggaagac atcgtcctga cactgacact gttcgaagac agagaaatga tcgaagaaag 1980

actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga agagaagaag 2040

atacacagga tggggaagac tgagcagaaa gctgatcaac ggaatcagag acaagcagag 2100

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<210> SEQ ID NO 252
<211> LENGTH: 4140
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 ORF with minimal uridine codons frequently used in humans in general; 12.75% U content

<400> SEQUENCE: 252

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cacagcatca agaagaacct gatcggcgcc ctgctgttcg acagcggcga gaccgccgag 180

gccaccagac tgaagagaac cgccagaaga agatacacca gaagaaagaa cagaatctgc 240

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<210> SEQ ID NO 253
<211> LENGTH: 4411

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 252, Kozak sequence, and 3 UTR of ALB

<400> SEQUENCE: 253

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cgtgggctgg gccgtgatca ccgacgagta caaggtgccc agcaagaagt tcaaggtgct 180

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<210> SEQ ID NO 254
<211> LENGTH: 4140
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 ORF with minimal uridine codons infrequently used in humans in general; 12.75% U content

<400> SEQUENCE: 254

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cacagcataa aaaaaaacct aataggggcg ctactattcg acagcgggga aacggcggaa 180

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gtaaaagtag tagacgaact agtaaaagta atggggcgac acaaaccgga aaacatagta 2280

atagaaatgg cgcgagaaaa ccaaacgacg caaaaagggc aaaaaaacag ccgagaacga 2340

atgaaacgaa tagaagaagg gataaaagaa ctagggagcc aaatactaaa agaacacccg 2400

gtagaaaaca cgcaactaca aaacgaaaaa ctatacctat actacctaca aaacgggcga 2460

gacatgtacg tagaccaaga actagacata aaccgactaa gcgactacga cgtagaccac 2520

atagtaccgc aaagcttcct aaaagacgac agcatagaca acaaagtact aacgcgaagc 2580

gacaaaaacc gagggaaaag cgacaacgta ccgagcgaag aagtagtaaa aaaaatgaaa 2640

aactactggc gacaactact aaacgcgaaa ctaataacgc aacgaaaatt cgacaaccta 2700

acgaaagcgg aacgaggggg gctaagcgaa ctagacaaag cggggttcat aaaacgacaa 2760

ctagtagaaa cgcgacaaat aacgaaacac gtagcgcaaa tactagacag ccgaatgaac 2820

acgaaatacg acgaaaacga caaactaata cgagaagtaa aagtaataac gctaaaaagc 2880

aaactagtaa gcgacttccg aaaagacttc caattctaca aagtacgaga aataaacaac 2940

taccaccacg cgcacgacgc gtacctaaac gcggtagtag ggacggcgct aataaaaaaa 3000

tacccgaaac tagaaagcga attcgtatac ggggactaca aagtatacga cgtacgaaaa 3060

atgatagcga aaagcgaaca agaaatagggg aaagcgacgg cgaaatactt cttctacagc 3120

aacataatga acttcttcaa aacggaaata acgctagcga acggggaaat acgaaaacga 3180

ccgctaatag aaacgaacgg ggaaacgggg gaaatagtat gggacaaagg gcgagacttc 3240

gcgacggtac gaaaagtact aagcatgccg caagtaaaca tagtaaaaaa aacggaagta 3300

caaacggggg ggttcagcaa agaaagcata ctaccgaaac gaaacagcga caaactaata 3360

gcgcgaaaaa aagactggga cccgaaaaaa tacgggggggt tcgacagccc gacggtagcg 3420

tacagcgtac tagtagtagc gaaagtagaa aaagggaaaa gcaaaaaact aaaaagcgta 3480

aaagaactac tagggataac gataatggaa cgaagcagct tcgaaaaaaa cccgatagac 3540

ttcctagaag cgaaagggta caaagaagta aaaaaagacc taataataaa actaccgaaa 3600

tacagcctat tcgaactaga aaacgggcga aaacgaatgc tagcgagcgc gggggaacta 3660

caaaaaggga acgaactagc gctaccgagc aaatacgtaa acttcctata cctagcgagc 3720

cactacgaaa aactaaaagg gagcccggaa gacaacgaac aaaaacaact attcgtagaa 3780

caacacaaac actacctaga cgaaataata gaacaaataa gcgaattcag caaacgagta 3840

atactagcgg acgcgaacct agacaaagta ctaagcgcgt acaacaaaca ccgagacaaa 3900

ccgatacgag aacaagcgga aaacataata cacctattca cgctaacgaa cctaggggcg 3960

ccggcggcgt tcaaatactt cgacacgacg atagaccgaa aacgatacac gagcacgaaa 4020

gaagtactag acgcgacgct aatacaccaa agcataacgg ggctatacga aacgcgaata 4080

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gacctaagcc aactaggggg ggacgggggg gggagcccga aaaaaaaacg aaaagtatga 4140

<210> SEQ ID NO 255
<211> LENGTH: 4411
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR of HSD, ORF corresponding to SEQ ID NO: 254, Kozak sequence, and 3 UTR of ALB

<400> SEQUENCE: 255

gggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc gccaccatgg acaaaaaata cagcataggg ctagacatag ggacgaacag 120

cgtagggtgg gcggtaataa cggacgaata caaagtaccg agcaaaaaat tcaaagtact 180

agggaacacg gaccgacaca gcataaaaaa aaacctaata ggggcgctac tattcgacag 240

cggggaaacg gcggaagcga cgcgactaaa acgaacggcg cgacgacgat acacgcgacg 300

aaaaaaccga atatgctacc tacaagaaat attcagcaac gaaatggcga aagtagacga 360

cagcttcttc caccgactag aagaaagctt cctagtagaa gaagacaaaa aacacgaacg 420

acacccgata ttcgggaaca tagtagacga agtagcgtac cacgaaaaat acccgacgat 480

ataccaccta cgaaaaaaac tagtagacag cacggacaaa gcggacctac gactaatata 540

cctagcgcta gcgcacatga taaaattccg agggcacttc ctaatagaag gggacctaaa 600

cccggacaac agcgacgtag acaaactatt catacaacta gtacaaacgt acaaccaact 660

attcgaagaa aacccgataa acgcgagcgg ggtagacgcg aaagcgatac taagcgcgcg 720

actaagcaaa agccgacgac tagaaaacct aatagcgcaa ctaccggggg aaaaaaaaaa 780

cgggctattc gggaacctaa tagcgctaag cctagggcta acgccgaact tcaaaagcaa 840

cttcgaccta gcggaagacg cgaaactaca actaagcaaa gacacgtacg acgacgacct 900

agacaaccta ctagcgcaaa taggggacca atacgcggac ctattcctag cggcgaaaaa 960

cctaagcgac gcgatactac taagcgacat actacgagta aacacggaaa taacgaaagc 1020

gccgctaagc gcgagcatga taaaacgata cgacgaacac caccaagacc taacgctact 1080

aaaagcgcta gtacgacaac aactaccgga aaaatacaaa gaaatattct tcgaccaaag 1140

caaaaacggg tacgcggggt acatagacgg gggggcgagc caagaagaat tctacaaatt 1200

cataaaaccg atactagaaa aaatggacgg gacggaagaa ctactagtaa aactaaaccg 1260

agaagaccta ctacgaaaac aacgaacgtt cgacaacggg agcataccgc accaaataca 1320

cctaggggaa ctacacgcga tactacgacg acaagaagac ttctacccgt tcctaaaaga 1380

caaccgagaa aaaatagaaa aaatactaac gttccgaata ccgtactacg tagggccgct 1440

agcgcgaggg aacagccgat tcgcgtggat gacgcgaaaa agcgaagaaa cgataacgcc 1500

gtggaacttc gaagaagtag tagacaaagg ggcgagcgcg caaagcttca tagaacgaat 1560

gacgaacttc gacaaaaacc taccgaacga aaaagtacta ccgaaacaca gcctactata 1620

cgaatacttc acggtataca acgaactaac gaaagtaaaa tacgtaacgg aagggatgcg 1680

aaaaccggcg ttcctaagcg gggaacaaaa aaaagcgata gtagacctac tattcaaaac 1740

gaaccgaaaa gtaacggtaa aacaactaaa agaagactac ttcaaaaaaa tagaatgctt 1800

cgacagcgta gaaataagcg gggtagaaga ccgattcaac gcgagcctag ggacgtacca 1860

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cgacctacta aaaataataa aagacaaaga cttcctagac aacgaagaaa acgaagacat 1920

actagaagac atagtactaa cgctaacgct attcgaagac cgagaaatga tagaagaacg 1980

actaaaaacg tacgcgcacc tattcgacga caaagtaatg aaacaactaa aacgacgacg 2040

atacacgggg tgggggcgac taagccgaaa actaataaac gggatacgag acaaacaaag 2100

cgggaaaacg atactagact tcctaaaaag cgacgggttc gcgaaccgaa acttcatgca 2160

actaatacac gacgacagcc taacgttcaa agaagacata caaaaagcgc aagtaagcgg 2220

gcaaggggac agcctacacg aacacatagc gaacctagcg gggagcccgg cgataaaaaa 2280

agggatacta caaacggtaa aagtagtaga cgaactagta aaagtaatgg ggcgacacaa 2340

accggaaaac atagtaatag aaatggcgcg agaaaaccaa acgacgcaaa aagggcaaaa 2400

aaacagccga gaacgaatga aacgaataga agaagggata aaagaactag ggagccaaat 2460

actaaaagaa cacccggtag aaaacacgca actacaaaac gaaaaactat acctatacta 2520

cctacaaaac gggcgagaca tgtacgtaga ccaagaacta gacataaacc gactaagcga 2580

ctacgacgta gaccacatag taccgcaaag cttcctaaaa gacgacagca tagacaacaa 2640

agtactaacg cgaagcgaca aaaaccgagg gaaaagcgac aacgtaccga gcgaagaagt 2700

agtaaaaaaa atgaaaaact actggcgaca actactaaac gcgaaactaa taacgcaacg 2760

aaaattcgac aacctaacga aagcggaacg agggggggcta agcgaactag acaaagcggg 2820

gttcataaaa cgacaactag tagaaacgcg acaaataacg aaacacgtag cgcaaatact 2880

agacagccga atgaacacga aatacgacga aaacgacaaa ctaatacgag aagtaaaagt 2940

aataacgcta aaaagcaaac tagtaagcga cttccgaaaa gacttccaat tctacaaagt 3000

acgagaaata aacaactacc accacgcgca cgacgcgtac ctaaacgcgg tagtagggac 3060

ggcgctaata aaaaaatacc cgaaactaga aagcgaattc gtatacgggg actacaaagt 3120

atacgacgta cgaaaaatga tagcgaaaag cgaacaagaa atagggaaag cgacggcgaa 3180

atacttcttc tacagcaaca taatgaactt cttcaaaacg gaaataacgc tagcgaacgg 3240

ggaaatacga aaacgaccgc taatagaaac gaacggggaa acgggggaaa tagtatggga 3300

caaagggcga gacttcgcga cggtacgaaa agtactaagc atgccgcaag taaacatagt 3360

aaaaaaaacg gaagtacaaa cgggggggtt cagcaaagaa agcatactac cgaaacgaaa 3420

cagcgacaaa ctaatagcgc gaaaaaaaga ctgggacccg aaaaaatacg gggggttcga 3480

cagcccgacg gtagcgtaca gcgtactagt agtagcgaaa gtagaaaaag ggaaaagcaa 3540

aaaactaaaa agcgtaaaag aactactagg gataacgata atggaacgaa gcagcttcga 3600

aaaaaacccg atagacttcc tagaagcgaa agggtacaaa gaagtaaaaa aagacctaat 3660

aataaaacta ccgaaataca gcctattcga actagaaaac gggcgaaaac gaatgctagc 3720

gagcgcgggg gaactacaaa aagggaacga actagcgcta ccgagcaaat acgtaaactt 3780

cctataccta gcgagccact acgaaaaact aaaagggagc ccggaagaca acgaacaaaa 3840

acaactattc gtagaacaac acaaacacta cctagacgaa ataatagaac aaataagcga 3900

attcagcaaa cgagtaatac tagcggacgc gaacctagac aaagtactaa gcgcgtacaa 3960

caaacaccga gacaaaccga tacgagaaca agcggaaaac ataatacacc tattcacgct 4020

aacgaaccta ggggcgccgg cggcgttcaa atacttcgac acgacgatag accgaaaacg 4080

atacacgagc acgaaagaag tactagacgc gacgctaata caccaaagca taacggggct 4140

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atacgaaacg cgaatagacc taagccaact agggggggac gggggggga gcccgaaaaa 4200

aaaacgaaaa gtatgactag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt tctctgtgct 4380

tcaattaata aaaaatggaa agaacctcga g 4411

<210> SEQ ID NO 256
<211> LENGTH: 4411
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with AGG as first three nucleotides for use with CleanCapTM, 5 UTR of HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3 UTR of ALB

<400> SEQUENCE: 256

aggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc gccaccatgg acaagaagta cagcatcgga ctggacatcg gaacaaacag 120

cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag aaaagaagaa 780

cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg acgacgacct 900

ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg cagcaaagaa 960

cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc tgacactgct 1080

gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat tctacaagtt 1200

catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc accagatcca 1320

cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt tcctgaagga 1380

caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg tcggaccgct 1440

ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa caatcacacc 1500

gtggaacttc gaagaagtcg tcgacaaggg agcaagcgca cagagcttca tcgaaagaat 1560

gacaaacttc gacaagaacc tgccgaacga aaaggtcctg ccgaagcaca gcctgctgta 1620

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cgaatacttc acagtctaca acgaactgac aaaggtcaag tacgtcacag aaggaatgag 1680

aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc tgttcaagac 1740

aaacagaaag gtcacagtca agcagctgaa ggaagactac ttcaagaaga tcgaatgctt 1800

cgacagcgtc gaaatcagcg gagtcgaaga cagattcaac gcaagcctgg gaacatacca 1860

cgacctgctg aagatcatca aggacaagga cttcctggac aacgaagaaa acgaagacat 1920

cctggaagac atcgtcctga cactgacact gttcgaagac agagaaatga tcgaagaaag 1980

actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga agagaagaag 2040

atacacagga tggggaagac tgagcagaaa gctgatcaac ggaatcagag acaagcagag 2100

cggaaagaca atcctggact tcctgaagag cgacggattc gcaaacagaa acttcatgca 2160

gctgatccac gacgacagcc tgacattcaa ggaagacatc cagaaggcac aggtcagcgg 2220

acagggagac agcctgcacg aacacatcgc aaacctggca ggaagcccgg caatcaagaa 2280

gggaatcctg cagacagtca aggtcgtcga cgaactggtc aaggtcatgg gaagacacaa 2340

gccggaaaac atcgtcatcg aaatggcaag agaaaaccag acaacacaga agggacagaa 2400

gaacagcaga gaaagaatga agagaatcga agaaggaatc aaggaactgg gaagccagat 2460

cctgaaggaa cacccggtcg aaaacacaca gctgcagaac gaaaagctgt acctgtacta 2520

cctgcagaac ggaagagaca tgtacgtcga ccaggaactg gacatcaaca gactgagcga 2580

ctacgacgtc gaccacatcg tcccgcagag cttcctgaag gacgacagca tcgacaacaa 2640

ggtcctgaca agaagcgaca agaacagagg aaagagcgac aacgtcccga gcgaagaagt 2700

cgtcaagaag atgaagaact actggagaca gctgctgaac gcaaagctga tcacacagag 2760

aaagttcgac aacctgacaa aggcagagag aggaggactg agcgaactgg acaaggcagg 2820

attcatcaag agacagctgg tcgaaacaag acagatcaca aagcacgtcg cacagatcct 2880

ggacagcaga atgaacacaa agtacgacga aaacgacaag ctgatcagag aagtcaaggt 2940

catcacactg aagagcaagc tggtcagcga cttcagaaag gacttccagt tctacaaggt 3000

cagagaaatc aacaactacc accacgcaca cgacgcatac ctgaacgcag tcgtcggaac 3060

agcactgatc aagaagtacc cgaagctgga aagcgaattc gtctacggag actacaaggt 3120

ctacgacgtc agaaagatga tcgcaaagag cgaacaggaa atcggaaagg caacagcaaa 3180

gtacttcttc tacagcaaca tcatgaactt cttcaagaca gaaatcacac tggcaaacgg 3240

agaaatcaga aagagaccgc tgatcgaaac aaacggagaa acaggagaaa tcgtctggga 3300

caagggaaga gacttcgcaa cagtcagaaa ggtcctgagc atgccgcagg tcaacatcgt 3360

caagaagaca gaagtccaga caggaggatt cagcaaggaa agcatcctgc cgaagagaaa 3420

cagcgacaag ctgatcgcaa gaaagaagga ctgggacccg aagaagtacg gaggattcga 3480

cagcccgaca gtcgcataca gcgtcctggt cgtcgcaaag gtcgaaaagg gaaagagcaa 3540

gaagctgaag agcgtcaagg aactgctggg aatcacaatc atggaaagaa gcagcttcga 3600

aaagaacccg atcgacttcc tggaagcaaa gggatacaag gaagtcaaga aggacctgat 3660

catcaagctg ccgaagtaca gcctgttcga actggaaaac ggaagaaaga gaatgctggc 3720

aagcgcagga gaactgcaga agggaaacga actggcactg ccgagcaagt acgtcaactt 3780

cctgtacctg gcaagccact acgaaaagct gaagggaagc ccggaagaca acgaacagaa 3840

gcagctgttc gtcgaacagc acaagcacta cctggacgaa atcatcgaac agatcagcga 3900

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attcagcaag agagtcatcc tggcagacgc aaacctggac aaggtcctga gcgcatacaa 3960

caagcacaga gacaagccga tcagagaaca ggcagaaaac atcatccacc tgttcacact 4020

gacaaacctg ggagcaccgg cagcattcaa gtacttcgac acaacaatcg acagaaagag 4080

atacacaagc acaaaggaag tcctggacgc aacactgatc caccagagca tcacaggact 4140

gtacgaaaca agaatcgacc tgagccagct gggaggagac ggaggaggaa gcccgaagaa 4200

gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt tctctgtgct 4380

tcaattaata aaaaatggaa agaacctcga g 4411

<210> SEQ ID NO 257
<211> LENGTH: 4481
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR from CMV, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3 UTR of ALB

<400> SEQUENCE: 257

gggcagatcg cctggagacg ccatccacgc tgttttgacc tccatagaag acaccgggac 60

cgatccagcc tccgcggccg ggaacggtgc attggaacgc ggattccccg tgccaagagt 120

gactcaccgt ccttgacacg gccaccatgg acaagaagta cagcatcgga ctggacatcg 180

gaacaaacag cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt 240

tcaaggtcct gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc 300

tgttcgacag cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat 360

acacaagaag aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa 420

aggtcgacga cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga 480

agcacgaaag acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt 540

acccgacaat ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga 600

gactgatcta cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag 660

gagacctgaa cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat 720

acaaccagct gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc 780

tgagcgcaag actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag 840

aaaagaagaa cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact 900

tcaagagcaa cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg 960

acgacgacct ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg 1020

cagcaaagaa cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa 1080

tcacaaaggc accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc 1140

tgacactgct gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct 1200

tcgaccagag caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat 1260

tctacaagtt catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca 1320

agctgaacag agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc 1380

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accagatcca cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt 1440

tcctgaagga caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg 1500

tcggaccgct ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa 1560

caatcacacc gtggaacttc gaagaagtcg tcgacaaggg agcaagcgca cagagcttca 1620

tcgaaagaat gacaaacttc gacaagaacc tgccgaacga aaaggtcctg ccgaagcaca 1680

gcctgctgta cgaatacttc acagtctaca acgaactgac aaaggtcaag tacgtcacag 1740

aaggaatgag aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc 1800

tgttcaagac aaacagaaag gtcacagtca agcagctgaa ggaagactac ttcaagaaga 1860

tcgaatgctt cgacagcgtc gaaatcagcg gagtcgaaga cagattcaac gcaagcctgg 1920

gaacatacca cgacctgctg aagatcatca aggacaagga cttcctggac aacgaagaaa 1980

acgaagacat cctggaagac atcgtcctga cactgacact gttcgaagac agagaaatga 2040

tcgaagaaag actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga 2100

agagaagaag atacacagga tggggaagac tgagcagaaa gctgatcaac ggaatcagag 2160

acaagcagag cggaaagaca atcctggact tcctgaagag cgacggattc gcaaacagaa 2220

acttcatgca gctgatccac gacgacagcc tgacattcaa ggaagacatc cagaaggcac 2280

aggtcagcgg acagggagac agcctgcacg aacacatcgc aaacctggca ggaagcccgg 2340

caatcaagaa gggaatcctg cagacagtca aggtcgtcga cgaactggtc aaggtcatgg 2400

gaagacacaa gccggaaaac atcgtcatcg aaatggcaag agaaaaccag acaacacaga 2460

agggacagaa gaacagcaga gaaagaatga agagaatcga agaaggaatc aaggaactgg 2520

gaagccagat cctgaaggaa cacccggtcg aaaacacaca gctgcagaac gaaaagctgt 2580

acctgtacta cctgcagaac ggaagagaca tgtacgtcga ccaggaactg gacatcaaca 2640

gactgagcga ctacgacgtc gaccacatcg tcccgcagag cttcctgaag gacgacagca 2700

tcgacaacaa ggtcctgaca agaagcgaca agaacagagg aaagagcgac aacgtcccga 2760

gcgaagaagt cgtcaagaag atgaagaact actggagaca gctgctgaac gcaaagctga 2820

tcacacagag aaagttcgac aacctgacaa aggcagagag aggaggactg agcgaactgg 2880

acaaggcagg attcatcaag agacagctgg tcgaaacaag acagatcaca aagcacgtcg 2940

cacagatcct ggacagcaga atgaacacaa agtacgacga aaacgacaag ctgatcagag 3000

aagtcaaggt catcacactg aagagcaagc tggtcagcga cttcagaaag gacttccagt 3060

tctacaaggt cagagaaatc aacaactacc accacgcaca cgacgcatac ctgaacgcag 3120

tcgtcggaac agcactgatc aagaagtacc cgaagctgga aagcgaattc gtctacggag 3180

actacaaggt ctacgacgtc agaaagatga tcgcaaagag cgaacaggaa atcggaaagg 3240

caacagcaaa gtacttcttc tacagcaaca tcatgaactt cttcaagaca gaaatcacac 3300

tggcaaacgg agaaatcaga aagagaccgc tgatcgaaac aaacggagaa acaggagaaa 3360

tcgtctggga caagggaaga gacttcgcaa cagtcagaaa ggtcctgagc atgccgcagg 3420

tcaacatcgt caagaagaca gaagtccaga caggaggatt cagcaaggaa agcatcctgc 3480

cgaagagaaa cagcgacaag ctgatcgcaa gaaagaagga ctgggacccg aagaagtacg 3540

gaggattcga cagcccgaca gtcgcataca gcgtcctggt cgtcgcaaag gtcgaaaagg 3600

gaaagagcaa gaagctgaag agcgtcaagg aactgctggg aatcacaatc atggaaagaa 3660

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gcagcttcga aaagaacccg atcgacttcc tggaagcaaa gggatacaag gaagtcaaga 3720

aggacctgat catcaagctg ccgaagtaca gcctgttcga actggaaaac ggaagaaaga 3780

gaatgctggc aagcgcagga gaactgcaga agggaaacga actggcactg ccgagcaagt 3840

acgtcaactt cctgtacctg gcaagccact acgaaaagct gaagggaagc ccggaagaca 3900

acgaacagaa gcagctgttc gtcgaacagc acaagcacta cctggacgaa atcatcgaac 3960

agatcagcga attcagcaag agagtcatcc tggcagacgc aaacctggac aaggtcctga 4020

gcgcatacaa caagcacaga gacaagccga tcagagaaca ggcagaaaac atcatccacc 4080

tgttcacact gacaaacctg ggagcaccgg cagcattcaa gtacttcgac acaacaatcg 4140

acagaaagag atacacaagc acaaaggaag tcctggacgc aacactgatc caccagagca 4200

tcacaggact gtacgaaaca agaatcgacc tgagccagct gggaggagac ggaggaggaa 4260

gcccgaagaa gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc 4320

atgagaataa gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt 4380

tggtgtaaag ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt 4440

tctctgtgct tcaattaata aaaaatggaa agaacctcga g 4481

<210> SEQ ID NO 258
<211> LENGTH: 4348
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR from HBB, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3 UTR of HBB

<400> SEQUENCE: 258

gggacatttg cttctgacac aactgtgttc actagcaacc tcaaacagac accggatctg 60

ccaccatgga caagaagtac agcatcggac tggacatcgg aacaaacagc gtcggatggg 120

cagtcatcac agacgaatac aaggtcccga gcaagaagtt caaggtcctg ggaaacacag 180

acagacacag catcaagaag aacctgatcg gagcactgct gttcgacagc ggagaaacag 240

cagaagcaac aagactgaag agaacagcaa gaagaagata cacaagaaga aagaacagaa 300

tctgctacct gcaggaaatc ttcagcaacg aaatggcaaa ggtcgacgac agcttcttcc 360

acagactgga agaaagcttc ctggtcgaag aagacaagaa gcacgaaaga cacccgatct 420

tcggaaacat cgtcgacgaa gtcgcatacc acgaaaagta cccgacaatc taccacctga 480

gaaagaagct ggtcgacagc acagacaagg cagacctgag actgatctac ctggcactgg 540

cacacatgat caagttcaga ggacacttcc tgatcgaagg agacctgaac ccggacaaca 600

gcgacgtcga caagctgttc atccagctgg tccagacata caaccagctg ttcgaagaaa 660

acccgatcaa cgcaagcgga gtcgacgcaa aggcaatcct gagcgcaaga ctgagcaaga 720

gcagaagact ggaaaacctg atcgcacagc tgccgggaga aaagaagaac ggactgttcg 780

gaaacctgat cgcactgagc ctgggactga caccgaactt caagagcaac ttcgacctgg 840

cagaagacgc aaagctgcag ctgagcaagg acacatacga cgacgacctg gacaacctgc 900

tggcacagat cggagaccag tacgcagacc tgttcctggc agcaaagaac ctgagcgacg 960

caatcctgct gagcgacatc ctgagagtca acacagaaat cacaaaggca ccgctgagcg 1020

caagcatgat caagagatac gacgaacacc accaggacct gacactgctg aaggcactgg 1080

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tcagacagca gctgccggaa aagtacaagg aaatcttctt cgaccagagc aagaacggat 1140

acgcaggata catcgacgga ggagcaagcc aggaagaatt ctacaagttc atcaagccga 1200

tcctggaaaa gatggacgga acagaagaac tgctggtcaa gctgaacaga gaagacctgc 1260

tgagaaagca gagaacattc gacaacggaa gcatcccgca ccagatccac ctgggagaac 1320

tgcacgcaat cctgagaaga caggaagact tctacccgtt cctgaaggac aacagagaaa 1380

agatcgaaaa gatcctgaca ttcagaatcc cgtactacgt cggaccgctg gcaagaggaa 1440

acagcagatt cgcatggatg acaagaaaga gcgaagaaac aatcacaccg tggaacttcg 1500

aagaagtcgt cgacaaggga gcaagcgcac agagcttcat cgaaagaatg acaaacttcg 1560

acaagaacct gccgaacgaa aaggtcctgc cgaagcacag cctgctgtac gaatacttca 1620

cagtctacaa cgaactgaca aaggtcaagt acgtcacaga aggaatgaga aagccggcat 1680

tcctgagcgg agaacagaag aaggcaatcg tcgacctgct gttcaagaca aacagaaagg 1740

tcacagtcaa gcagctgaag gaagactact tcaagaagat cgaatgcttc gacagcgtcg 1800

aaatcagcgg agtcgaagac agattcaacg caagcctggg aacataccac gacctgctga 1860

agatcatcaa ggacaaggac ttcctggaca acgaagaaaa cgaagacatc ctggaagaca 1920

tcgtcctgac actgacactg ttcgaagaca gagaaatgat cgaagaaaga ctgaagacat 1980

acgcacacct gttcgacgac aaggtcatga agcagctgaa gagaagaaga tacacaggat 2040

ggggaagact gagcagaaag ctgatcaacg gaatcagaga caagcagagc ggaaagacaa 2100

tcctggactt cctgaagagc gacggattcg caaacagaaa cttcatgcag ctgatccacg 2160

acgacagcct gacattcaag gaagacatcc agaaggcaca ggtcagcgga cagggagaca 2220

gcctgcacga acacatcgca aacctggcag gaagcccggc aatcaagaag ggaatcctgc 2280

agacagtcaa ggtcgtcgac gaactggtca aggtcatggg aagacacaag ccggaaaaca 2340

tcgtcatcga aatggcaaga gaaaaccaga caacacagaa gggacagaag aacagcagag 2400

aaagaatgaa gagaatcgaa gaaggaatca aggaactggg aagccagatc ctgaaggaac 2460

acccggtcga aaacacacag ctgcagaacg aaaagctgta cctgtactac ctgcagaacg 2520

gaagagacat gtacgtcgac caggaactgg acatcaacag actgagcgac tacgacgtcg 2580

accacatcgt cccgcagagc ttcctgaagg acgacagcat cgacaacaag gtcctgacaa 2640

gaagcgacaa gaacagagga aagagcgaca acgtcccgag cgaagaagtc gtcaagaaga 2700

tgaagaacta ctggagacag ctgctgaacg caaagctgat cacacagaga aagttcgaca 2760

acctgacaaa ggcagagaga ggaggactga gcgaactgga caaggcagga ttcatcaaga 2820

gacagctggt cgaaacaaga cagatcacaa agcacgtcgc acagatcctg gacagcagaa 2880

tgaacacaaa gtacgacgaa aacgacaagc tgatcagaga agtcaaggtc atcacactga 2940

agagcaagct ggtcagcgac ttcagaaagg acttccagtt ctacaaggtc agagaaatca 3000

acaactacca ccacgcacac gacgcatacc tgaacgcagt cgtcggaaca gcactgatca 3060

agaagtaccc gaagctggaa agcgaattcg tctacggaga ctacaaggtc tacgacgtca 3120

gaaagatgat cgcaaagagc gaacaggaaa tcggaaaggc aacagcaaag tacttcttct 3180

acagcaacat catgaacttc ttcaagacag aaatcacact ggcaaacgga gaaatcagaa 3240

agagaccgct gatcgaaaca aacggagaaa caggagaaat cgtctgggac aagggaagag 3300

acttcgcaac agtcagaaag gtcctgagca tgccgcaggt caacatcgtc aagaagacag 3360

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aagtccagac aggaggattc agcaaggaaa gcatcctgcc gaagagaaac agcgacaagc 3420

tgatcgcaag aaagaaggac tgggacccga agaagtacgg aggattcgac agcccgacag 3480

tcgcatacag cgtcctggtc gtcgcaaagg tcgaaaaggg aaagagcaag aagctgaaga 3540

gcgtcaagga actgctggga atcacaatca tggaaagaag cagcttcgaa aagaacccga 3600

tcgacttcct ggaagcaaag ggatacaagg aagtcaagaa ggacctgatc atcaagctgc 3660

cgaagtacag cctgttcgaa ctggaaaacg gaagaaagag aatgctggca agcgcaggag 3720

aactgcagaa gggaaacgaa ctggcactgc cgagcaagta cgtcaacttc ctgtacctgg 3780

caagccacta cgaaaagctg aagggaagcc cggaagacaa cgaacagaag cagctgttcg 3840

tcgaacagca caagcactac ctggacgaaa tcatcgaaca gatcagcgaa ttcagcaaga 3900

gagtcatcct ggcagacgca aacctggaca aggtcctgag cgcatacaac aagcacagag 3960

acaagccgat cagagaacag gcagaaaaca tcatccacct gttcacactg acaaacctgg 4020

gagcaccggc agcattcaag tacttcgaca caacaatcga cagaaagaga tacacaagca 4080

caaaggaagt cctggacgca acactgatcc accagagcat cacaggactg tacgaaacaa 4140

gaatcgacct gagccagctg ggaggagacg gaggaggaag cccgaagaag aagagaaagg 4200

tctagctagc gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa 4260

gtccaactac taaactgggg gatattatga agggccttga gcatctggat tctgcctaat 4320

aaaaaacatt tattttcatt gcctcgag 4348

<210> SEQ ID NO 259
<211> LENGTH: 4325
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with 5 UTR from XBG, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3 UTR of XBG

<400> SEQUENCE: 259

gggaagctca gaataaacgc tcaactttgg ccggatctgc caccatggac aagaagtaca 60

gcatcggact ggacatcgga acaaacagcg tcggatgggc agtcatcaca gacgaataca 120

aggtcccgag caagaagttc aaggtcctgg gaaacacaga cagacacagc atcaagaaga 180

acctgatcgg agcactgctg ttcgacagcg gagaaacagc agaagcaaca agactgaaga 240

gaacagcaag aagaagatac acaagaagaa agaacagaat ctgctacctg caggaaatct 300

tcagcaacga aatggcaaag gtcgacgaca gcttcttcca cagactggaa gaaagcttcc 360

tggtcgaaga agacaagaag cacgaaagac acccgatctt cggaaacatc gtcgacgaag 420

tcgcatacca cgaaaagtac ccgacaatct accacctgag aaagaagctg gtcgacagca 480

cagacaaggc agacctgaga ctgatctacc tggcactggc acacatgatc aagttcagag 540

gacacttcct gatcgaagga gacctgaacc cggacaacag cgacgtcgac aagctgttca 600

tccagctggt ccagacatac aaccagctgt tcgaagaaaa cccgatcaac gcaagcggag 660

tcgacgcaaa ggcaatcctg agcgcaagac tgagcaagag cagaagactg gaaaacctga 720

tcgcacagct gccgggagaa aagaagaacg gactgttcgg aaacctgatc gcactgagcc 780

tgggactgac accgaacttc aagagcaact tcgacctggc agaagacgca aagctgcagc 840

tgagcaagga cacatacgac gacgacctgg acaacctgct ggcacagatc ggagaccagt 900

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acgcagacct gttcctggca gcaaagaacc tgagcgacgc aatcctgctg agcgacatcc 960

tgagagtcaa cacagaaatc acaaaggcac cgctgagcgc aagcatgatc aagagatacg 1020

acgaacacca ccaggacctg acactgctga aggcactggt cagacagcag ctgccggaaa 1080

agtacaagga aatcttcttc gaccagagca agaacggata cgcaggatac atcgacggag 1140

gagcaagcca ggaagaattc tacaagttca tcaagccgat cctggaaaag atggacggaa 1200

cagaagaact gctggtcaag ctgaacagag aagacctgct gagaaagcag agaacattcg 1260

acaacggaag catcccgcac cagatccacc tgggagaact gcacgcaatc ctgagaagac 1320

aggaagactt ctacccgttc ctgaaggaca acagagaaaa gatcgaaaag atcctgacat 1380

tcagaatccc gtactacgtc ggaccgctgg caagaggaaa cagcagattc gcatggatga 1440

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caagcgcaca gagcttcatc gaaagaatga caaacttcga caagaacctg ccgaacgaaa 1560

aggtcctgcc gaagcacagc ctgctgtacg aatacttcac agtctacaac gaactgacaa 1620

aggtcaagta cgtcacagaa ggaatgagaa agccggcatt cctgagcgga gaacagaaga 1680

aggcaatcgt cgacctgctg ttcaagacaa acagaaaggt cacagtcaag cagctgaagg 1740

aagactactt caagaagatc gaatgcttcg acagcgtcga aatcagcgga gtcgaagaca 1800

gattcaacgc aagcctggga acataccacg acctgctgaa gatcatcaag gacaaggact 1860

tcctggacaa cgaagaaaac gaagacatcc tggaagacat cgtcctgaca ctgacactgt 1920

tcgaagacag agaaatgatc gaagaaagac tgaagacata cgcacacctg ttcgacgaca 1980

aggtcatgaa gcagctgaag agaagaagat acacaggatg gggaagactg agcagaaagc 2040

tgatcaacgg aatcagagac aagcagagcg gaaagacaat cctggacttc ctgaagagcg 2100

acggattcgc aaacagaaac ttcatgcagc tgatccacga cgacagcctg acattcaagg 2160

aagacatcca gaaggcacag gtcagcggac agggagacag cctgcacgaa cacatcgcaa 2220

acctggcagg aagcccggca atcaagaagg gaatcctgca gacagtcaag gtcgtcgacg 2280

aactggtcaa ggtcatggga agacacaagc cggaaaacat cgtcatcgaa atggcaagag 2340

aaaaccagac aacacagaag ggacagaaga acagcagaga aagaatgaag agaatcgaag 2400

aaggaatcaa ggaactggga agccagatcc tgaaggaaca cccggtcgaa aacacacagc 2460

tgcagaacga aaagctgtac ctgtactacc tgcagaacgg aagagacatg tacgtcgacc 2520

aggaactgga catcaacaga ctgagcgact acgacgtcga ccacatcgtc ccgcagagct 2580

tcctgaagga cgacagcatc gacaacaagg tcctgacaag aagcgacaag aacagaggaa 2640

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tgctgaacgc aaagctgatc acacagagaa agttcgacaa cctgacaaag gcagagagag 2760

gaggactgag cgaactggac aaggcaggat tcatcaagag acagctggtc gaaacaagac 2820

agatcacaaa gcacgtcgca cagatcctgg acagcagaat gaacacaaag tacgacgaaa 2880

acgacaagct gatcagagaa gtcaaggtca tcacactgaa gagcaagctg gtcagcgact 2940

tcagaaagga cttccagttc tacaaggtca gagaaatcaa caactaccac cacgcacacg 3000

acgcatacct gaacgcagtc gtcggaacag cactgatcaa gaagtacccg aagctggaaa 3060

gcgaattcgt ctacggagac tacaaggtct acgacgtcag aaagatgatc gcaaagagcg 3120

aacaggaaat cggaaaggca acagcaaagt acttcttcta cagcaacatc atgaacttct 3180

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tcaagacaga aatcacactg gcaaacggag aaatcagaaa gagaccgctg atcgaaacaa 3240

acggagaaac aggagaaatc gtctgggaca agggaagaga cttcgcaaca gtcagaaagg 3300

tcctgagcat gccgcaggtc aacatcgtca agaagacaga agtccagaca ggaggattca 3360

gcaaggaaag catcctgccg aagagaaaca gcgacaagct gatcgcaaga aagaaggact 3420

gggacccgaa gaagtacgga ggattcgaca gcccgacagt cgcatacagc gtcctggtcg 3480

tcgcaaaggt cgaaaaggga aagagcaaga agctgaagag cgtcaaggaa ctgctgggaa 3540

tcacaatcat ggaaagaagc agcttcgaaa agaacccgat cgacttcctg gaagcaaagg 3600

gatacaagga agtcaagaag gacctgatca tcaagctgcc gaagtacagc ctgttcgaac 3660

tggaaaacgg aagaaagaga atgctggcaa gcgcaggaga actgcagaag ggaaacgaac 3720

tggcactgcc gagcaagtac gtcaacttcc tgtacctggc aagccactac gaaaagctga 3780

agggaagccc ggaagacaac gaacagaagc agctgttcgt cgaacagcac aagcactacc 3840

tggacgaaat catcgaacag atcagcgaat tcagcaagag agtcatcctg gcagacgcaa 3900

acctggacaa ggtcctgagc gcatacaaca agcacagaga caagccgatc agagaacagg 3960

cagaaaacat catccacctg ttcacactga caaacctggg agcaccggca gcattcaagt 4020

acttcgacac aacaatcgac agaaagagat acacaagcac aaaggaagtc ctggacgcaa 4080

cactgatcca ccagagcatc acaggactgt acgaaacaag aatcgacctg agccagctgg 4140

gaggagacgg aggaggaagc ccgaagaaga agagaaaggt ctagctagca ccagcctcaa 4200

gaacacccga atggagtctc taagctacat aataccaact tacactttac aaaatgttgt 4260

cccccaaaat gtagccattc gtatctgctc ctaataaaaa gaaagtttct tcacattctc 4320

tcgag 4325

<210> SEQ ID NO 260
<211> LENGTH: 4325
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with AGG as first three nucleotides for use with CleanCapTM, 5 UTR from XBG, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3 UTR of XBG

<400> SEQUENCE: 260

aggaagctca gaataaacgc tcaactttgg ccggatctgc caccatggac aagaagtaca 60

gcatcggact ggacatcgga acaaacagcg tcggatgggc agtcatcaca gacgaataca 120

aggtcccgag caagaagttc aaggtcctgg gaaacacaga cagacacagc atcaagaaga 180

acctgatcgg agcactgctg ttcgacagcg gagaaacagc agaagcaaca agactgaaga 240

gaacagcaag aagaagatac acaagaagaa agaacagaat ctgctacctg caggaaatct 300

tcagcaacga aatggcaaag gtcgacgaca gcttcttcca cagactggaa gaaagcttcc 360

tggtcgaaga agacaagaag cacgaaagac acccgatctt cggaaacatc gtcgacgaag 420

tcgcatacca cgaaaagtac ccgacaatct accacctgag aaagaagctg gtcgacagca 480

cagacaaggc agacctgaga ctgatctacc tggcactggc acacatgatc aagttcagag 540

gacacttcct gatcgaagga gacctgaacc cggacaacag cgacgtcgac aagctgttca 600

tccagctggt ccagacatac aaccagctgt tcgaagaaaa cccgatcaac gcaagcggag 660

tcgacgcaaa ggcaatcctg agcgcaagac tgagcaagag cagaagactg gaaaacctga 720

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tcgcacagct gccgggagaa aagaagaacg gactgttcgg aaacctgatc gcactgagcc 780

tgggactgac accgaacttc aagagcaact tcgacctggc agaagacgca aagctgcagc 840

tgagcaagga cacatacgac gacgacctgg acaacctgct ggcacagatc ggagaccagt 900

acgcagacct gttcctggca gcaaagaacc tgagcgacgc aatcctgctg agcgacatcc 960

tgagagtcaa cacagaaatc acaaaggcac cgctgagcgc aagcatgatc aagagatacg 1020

acgaacacca ccaggacctg acactgctga aggcactggt cagacagcag ctgccggaaa 1080

agtacaagga aatcttcttc gaccagagca agaacggata cgcaggatac atcgacggag 1140

gagcaagcca ggaagaattc tacaagttca tcaagccgat cctggaaaag atggacggaa 1200

cagaagaact gctggtcaag ctgaacagag aagacctgct gagaaagcag agaacattcg 1260

acaacggaag catcccgcac cagatccacc tgggagaact gcacgcaatc ctgagaagac 1320

aggaagactt ctacccgttc ctgaaggaca acagagaaaa gatcgaaaag atcctgacat 1380

tcagaatccc gtactacgtc ggaccgctgg caagaggaaa cagcagattc gcatggatga 1440

caagaaagag cgaagaaaca atcacaccgt ggaacttcga agaagtcgtc gacaagggag 1500

caagcgcaca gagcttcatc gaaagaatga caaacttcga caagaacctg ccgaacgaaa 1560

aggtcctgcc gaagcacagc ctgctgtacg aatacttcac agtctacaac gaactgacaa 1620

aggtcaagta cgtcacagaa ggaatgagaa agccggcatt cctgagcgga gaacagaaga 1680

aggcaatcgt cgacctgctg ttcaagacaa acagaaaggt cacagtcaag cagctgaagg 1740

aagactactt caagaagatc gaatgcttcg acagcgtcga aatcagcgga gtcgaagaca 1800

gattcaacgc aagcctggga acataccacg acctgctgaa gatcatcaag gacaaggact 1860

tcctggacaa cgaagaaaac gaagacatcc tggaagacat cgtcctgaca ctgacactgt 1920

tcgaagacag agaaatgatc gaagaaagac tgaagacata cgcacacctg ttcgacgaca 1980

aggtcatgaa gcagctgaag agaagaagat acacaggatg gggaagactg agcagaaagc 2040

tgatcaacgg aatcagagac aagcagagcg gaaagacaat cctggacttc ctgaagagcg 2100

acggattcgc aaacagaaac ttcatgcagc tgatccacga cgacagcctg acattcaagg 2160

aagacatcca gaaggcacag gtcagcggac agggagacag cctgcacgaa cacatcgcaa 2220

acctggcagg aagcccggca atcaagaagg gaatcctgca gacagtcaag gtcgtcgacg 2280

aactggtcaa ggtcatggga agacacaagc cggaaaacat cgtcatcgaa atggcaagag 2340

aaaaccagac aacacagaag ggacagaaga acagcagaga aagaatgaag agaatcgaag 2400

aaggaatcaa ggaactggga agccagatcc tgaaggaaca cccggtcgaa aacacacagc 2460

tgcagaacga aaagctgtac ctgtactacc tgcagaacgg aagagacatg tacgtcgacc 2520

aggaactgga catcaacaga ctgagcgact acgacgtcga ccacatcgtc ccgcagagct 2580

tcctgaagga cgacagcatc gacaacaagg tcctgacaag aagcgacaag aacagaggaa 2640

agagcgacaa cgtcccgagc gaagaagtcg tcaagaagat gaagaactac tggagacagc 2700

tgctgaacgc aaagctgatc acacagagaa agttcgacaa cctgacaaag gcagagagag 2760

gaggactgag cgaactggac aaggcaggat tcatcaagag acagctggtc gaaacaagac 2820

agatcacaaa gcacgtcgca cagatcctgg acagcagaat gaacacaaag tacgacgaaa 2880

acgacaagct gatcagagaa gtcaaggtca tcacactgaa gagcaagctg gtcagcgact 2940

tcagaaagga cttccagttc tacaaggtca gagaaatcaa caactaccac cacgcacacg 3000

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acgcatacct gaacgcagtc gtcggaacag cactgatcaa gaagtacccg aagctggaaa 3060

gcgaattcgt ctacggagac tacaaggtct acgacgtcag aaagatgatc gcaaagagcg 3120

aacaggaaat cggaaaggca acagcaaagt acttcttcta cagcaacatc atgaacttct 3180

tcaagacaga aatcacactg gcaaacggag aaatcagaaa gagaccgctg atcgaaacaa 3240

acggagaaac aggagaaatc gtctgggaca agggaagaga cttcgcaaca gtcagaaagg 3300

tcctgagcat gccgcaggtc aacatcgtca agaagacaga agtccagaca ggaggattca 3360

gcaaggaaag catcctgccg aagagaaaca gcgacaagct gatcgcaaga aagaaggact 3420

gggacccgaa gaagtacgga ggattcgaca gcccgacagt cgcatacagc gtcctggtcg 3480

tcgcaaaggt cgaaaaggga aagagcaaga agctgaagag cgtcaaggaa ctgctgggaa 3540

tcacaatcat ggaaagaagc agcttcgaaa agaacccgat cgacttcctg gaagcaaagg 3600

gatacaagga agtcaagaag gacctgatca tcaagctgcc gaagtacagc ctgttcgaac 3660

tggaaaacgg aagaaagaga atgctggcaa gcgcaggaga actgcagaag ggaaacgaac 3720

tggcactgcc gagcaagtac gtcaacttcc tgtacctggc aagccactac gaaaagctga 3780

agggaagccc ggaagacaac gaacagaagc agctgttcgt cgaacagcac aagcactacc 3840

tggacgaaat catcgaacag atcagcgaat tcagcaagag agtcatcctg gcagacgcaa 3900

acctggacaa ggtcctgagc gcatacaaca agcacagaga caagccgatc agagaacagg 3960

cagaaaacat catccacctg ttcacactga caaacctggg agcaccggca gcattcaagt 4020

acttcgacac aacaatcgac agaaagagat acacaagcac aaaggaagtc ctggacgcaa 4080

cactgatcca ccagagcatc acaggactgt acgaaacaag aatcgacctg agccagctgg 4140

gaggagacgg aggaggaagc ccgaagaaga agagaaaggt ctagctagca ccagcctcaa 4200

gaacacccga atggagtctc taagctacat aataccaact tacactttac aaaatgttgt 4260

cccccaaaat gtagccattc gtatctgctc ctaataaaaa gaaagtttct tcacattctc 4320

tcgag 4325

<210> SEQ ID NO 261
<211> LENGTH: 4411
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Cas9 transcript with AGG as first three nucleotides for use with CleanCapTM, 5 UTR from HSD, ORF corresponding to SEQ ID NO: 204, Kozak sequence, and 3 UTR of ALB

<400> SEQUENCE: 261

aggtcccgca gtcggcgtcc agcggctctg cttgttcgtg tgtgtgtcgt tgcaggcctt 60

attcggatcc gccaccatgg acaagaagta cagcatcgga ctggacatcg gaacaaacag 120

cgtcggatgg gcagtcatca cagacgaata caaggtcccg agcaagaagt tcaaggtcct 180

gggaaacaca gacagacaca gcatcaagaa gaacctgatc ggagcactgc tgttcgacag 240

cggagaaaca gcagaagcaa caagactgaa gagaacagca agaagaagat acacaagaag 300

aaagaacaga atctgctacc tgcaggaaat cttcagcaac gaaatggcaa aggtcgacga 360

cagcttcttc cacagactgg aagaaagctt cctggtcgaa gaagacaaga agcacgaaag 420

acacccgatc ttcggaaaca tcgtcgacga agtcgcatac cacgaaaagt acccgacaat 480

ctaccacctg agaaagaagc tggtcgacag cacagacaag gcagacctga gactgatcta 540

-continued

cctggcactg gcacacatga tcaagttcag aggacacttc ctgatcgaag gagacctgaa 600

cccggacaac agcgacgtcg acaagctgtt catccagctg gtccagacat acaaccagct 660

gttcgaagaa aacccgatca acgcaagcgg agtcgacgca aaggcaatcc tgagcgcaag 720

actgagcaag agcagaagac tggaaaacct gatcgcacag ctgccgggag aaaagaagaa 780

cggactgttc ggaaacctga tcgcactgag cctgggactg acaccgaact tcaagagcaa 840

cttcgacctg gcagaagacg caaagctgca gctgagcaag gacacatacg acgacgacct 900

ggacaacctg ctggcacaga tcggagacca gtacgcagac ctgttcctgg cagcaaagaa 960

cctgagcgac gcaatcctgc tgagcgacat cctgagagtc aacacagaaa tcacaaaggc 1020

accgctgagc gcaagcatga tcaagagata cgacgaacac caccaggacc tgacactgct 1080

gaaggcactg gtcagacagc agctgccgga aaagtacaag gaaatcttct tcgaccagag 1140

caagaacgga tacgcaggat acatcgacgg aggagcaagc caggaagaat tctacaagtt 1200

catcaagccg atcctggaaa agatggacgg aacagaagaa ctgctggtca agctgaacag 1260

agaagacctg ctgagaaagc agagaacatt cgacaacgga agcatcccgc accagatcca 1320

cctgggagaa ctgcacgcaa tcctgagaag acaggaagac ttctacccgt tcctgaagga 1380

caacagagaa aagatcgaaa agatcctgac attcagaatc ccgtactacg tcggaccgct 1440

ggcaagagga aacagcagat tcgcatggat gacaagaaag agcgaagaaa caatcacacc 1500

gtggaacttc gaagaagtcg tcgacaaggg agcaagcgca cagagcttca tcgaaagaat 1560

gacaaacttc gacaagaacc tgccgaacga aaaggtcctg ccgaagcaca gcctgctgta 1620

cgaatacttc acagtctaca acgaactgac aaaggtcaag tacgtcacag aaggaatgag 1680

aaagccggca ttcctgagcg gagaacagaa gaaggcaatc gtcgacctgc tgttcaagac 1740

aaacagaaag gtcacagtca agcagctgaa ggaagactac ttcaagaaga tcgaatgctt 1800

cgacagcgtc gaaatcagcg gagtcgaaga cagattcaac gcaagcctgg gaacatacca 1860

cgacctgctg aagatcatca aggacaagga cttcctggac aacgaagaaa acgaagacat 1920

cctggaagac atcgtcctga cactgacact gttcgaagac agagaaatga tcgaagaaag 1980

actgaagaca tacgcacacc tgttcgacga caaggtcatg aagcagctga agagaagaag 2040

atacacagga tggggaagac tgagcagaaa gctgatcaac ggaatcagag acaagcagag 2100

cggaaagaca atcctggact tcctgaagag cgacggattc gcaaacagaa acttcatgca 2160

gctgatccac gacgacagcc tgacattcaa ggaagacatc cagaaggcac aggtcagcgg 2220

acagggagac agcctgcacg aacacatcgc aaacctggca ggaagcccgg caatcaagaa 2280

gggaatcctg cagacagtca aggtcgtcga cgaactggtc aaggtcatgg gaagacacaa 2340

gccggaaaac atcgtcatcg aaatggcaag agaaaaccag acaacacaga agggacagaa 2400

gaacagcaga gaaagaatga agagaatcga agaaggaatc aaggaactgg gaagccagat 2460

cctgaaggaa cacccggtcg aaaacacaca gctgcagaac gaaaagctgt acctgtacta 2520

cctgcagaac ggaagagaca tgtacgtcga ccaggaactg gacatcaaca gactgagcga 2580

ctacgacgtc gaccacatcg tcccgcagag cttcctgaag gacgacagca tcgacaacaa 2640

ggtcctgaca agaagcgaca agaacagagg aaagagcgac aacgtcccga gcgaagaagt 2700

cgtcaagaag atgaagaact actggagaca gctgctgaac gcaaagctga tcacacagag 2760

aaagttcgac aacctgacaa aggcagagag aggaggactg agcgaactgg acaaggcagg 2820

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attcatcaag agacagctgg tcgaaacaag acagatcaca aagcacgtcg cacagatcct 2880

ggacagcaga atgaacacaa agtacgacga aaacgacaag ctgatcagag aagtcaaggt 2940

catcacactg aagagcaagc tggtcagcga cttcagaaag gacttccagt tctacaaggt 3000

cagagaaatc aacaactacc accacgcaca cgacgcatac ctgaacgcag tcgtcggaac 3060

agcactgatc aagaagtacc cgaagctgga aagcgaattc gtctacggag actacaaggt 3120

ctacgacgtc agaaagatga tcgcaaagag cgaacaggaa atcggaaagg caacagcaaa 3180

gtacttcttc tacagcaaca tcatgaactt cttcaagaca gaaatcacac tggcaaacgg 3240

agaaatcaga aagagaccgc tgatcgaaac aaacggagaa acaggagaaa tcgtctggga 3300

caagggaaga gacttcgcaa cagtcagaaa ggtcctgagc atgccgcagg tcaacatcgt 3360

caagaagaca gaagtccaga caggaggatt cagcaaggaa agcatcctgc cgaagagaaa 3420

cagcgacaag ctgatcgcaa gaaagaagga ctgggacccg aagaagtacg gaggattcga 3480

cagcccgaca gtcgcataca gcgtcctggt cgtcgcaaag gtcgaaaagg gaaagagcaa 3540

gaagctgaag agcgtcaagg aactgctggg aatcacaatc atggaaagaa gcagcttcga 3600

aaagaacccg atcgacttcc tggaagcaaa gggatacaag gaagtcaaga aggacctgat 3660

catcaagctg ccgaagtaca gcctgttcga actggaaaac ggaagaaaga gaatgctggc 3720

aagcgcagga gaactgcaga agggaaacga actggcactg ccgagcaagt acgtcaactt 3780

cctgtacctg gcaagccact acgaaaagct gaagggaagc ccggaagaca acgaacagaa 3840

gcagctgttc gtcgaacagc acaagcacta cctggacgaa atcatcgaac agatcagcga 3900

attcagcaag agagtcatcc tggcagacgc aaacctggac aaggtcctga gcgcatacaa 3960

caagcacaga gacaagccga tcagagaaca ggcagaaaac atcatccacc tgttcacact 4020

gacaaacctg ggagcaccgg cagcattcaa gtacttcgac acaacaatcg acagaaagag 4080

atacacaagc acaaaggaag tcctggacgc aacactgatc caccagagca tcacaggact 4140

gtacgaaaca agaatcgacc tgagccagct gggaggagac ggaggaggaa gcccgaagaa 4200

gaagagaaag gtctagctag ccatcacatt taaaagcatc tcagcctacc atgagaataa 4260

gagaaagaaa atgaagatca atagcttatt catctctttt tctttttcgt tggtgtaaag 4320

ccaacaccct gtctaaaaaa cataaatttc tttaatcatt ttgcctcttt tctctgtgct 4380

tcaattaata aaaaatggaa agaacctcga g 4411

<210> SEQ ID NO 262

<400> SEQUENCE: 262

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<210> SEQ ID NO 263
<211> LENGTH: 93
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: poly-A 100 sequence

<400> SEQUENCE: 263

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 60

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa 93

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<210> SEQ ID NO 264
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G209 single guide RNA targeting the mouse TTR gene

<400> SEQUENCE: 264

aaataagaga gaaaagaaga gtaagaagaa atataagagc cacc 44

<210> SEQ ID NO 265
<211> LENGTH: 3312
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: ORF encoding Neisseria meningitidis Cas9 using minimal uridine codons, with start and stop codons

<400> SEQUENCE: 265

atggcagcat tcaagccgaa ctcgatcaac tacatcctgg gactggacat cggaatcgca 60

tcggtcggat gggcaatggt cgaaatcgac gaagaagaaa acccgatcag actgatcgac 120

ctgggagtca gagtcttcga aagagcagaa gtcccgaaga caggagactc gctggcaatg 180

gcaagaagac tggcaagatc ggtcagaaga ctgacaagaa gaagagcaca cagactgctg 240

agaacaagaa gactgctgaa gagagaagga gtcctgcagg cagcaaactt cgacgaaaac 300

ggactgatca agtcgctgcc gaacacaccg tggcagctga gagcagcagc actggacaga 360

aagctgacac cgctggaatg gtcggcagtc ctgctgcacc tgatcaagca cagaggatac 420

ctgtcgcaga gaaagaacga aggagaaaca gcagacaagg aactgggagc actgctgaag 480

ggagtcgcag gaaacgcaca cgcactgcag acaggagact tcagaacacc ggcagaactg 540

gcactgaaca agttcgaaaa ggaatcggga cacatcagaa accagagatc ggactactcg 600

cacacattct cgagaaagga cctgcaggca gaactgatcc tgctgttcga aaagcagaag 660

gaattcggaa acccgcacgt ctcgggagga ctgaaggaag gaatcgaaac actgctgatg 720

acacagagac cggcactgtc gggagacgca gtccagaaga tgctgggaca ctgcacattc 780

gaaccggcag aaccgaaggc agcaaagaac acatacacag cagaaagatt catctggctg 840

acaaagctga acaacctgag aatcctggaa cagggatcgg aaagaccgct gacagacaca 900

gaaagagcaa cactgatgga cgaaccgtac agaaagtcga agctgacata cgcacaggca 960

agaaagctgc tgggactgga agacacagca ttcttcaagg gactgagata cggaaaggac 1020

aacgcagaag catcgacact gatggaaatg aaggcatacc acgcaatctc gagagcactg 1080

gaaaaggaag gactgaagga caagaagtcg ccgctgaacc tgtcgccgga actgcaggac 1140

gaaatcggaa cagcattctc gctgttcaag acagacgaag acatcacagg aagactgaag 1200

gacagaatcc agccggaaat cctggaagca ctgctgaagc acatctcgtt cgacaagttc 1260

gtccagatct cgctgaaggc actgagaaga atcgtcccgc tgatggaaca gggaaagaga 1320

tacgacgaag catgcgcaga aatctacgga gaccactacg gaaagaagaa cacagaagaa 1380

aagatctacc tgccgccgat cccggcagac gaaatcagaa acccggtcgt cctgagagca 1440

ctgtcgcagg caagaaaggt catcaacgga gtcgtcagaa gatacggatc gccggcaaga 1500

atccacatcg aaacagcaag agaagtcgga aagtcgttca aggacagaaa ggaaatcgaa 1560

aagagacagg aagaaaacag aaaggacaga gaaaaggcag cagcaaagtt cagagaatac 1620

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ttcccgaact tcgtcggaga accgaagtcg aaggacatcc tgaagctgag actgtacgaa 1680

cagcagcacg gaaagtgcct gtactcggga aaggaaatca acctgggaag actgaacgaa 1740

aagggatacg tcgaaatcga ccacgcactg ccgttctcga gaacatggga cgactcgttc 1800

aacaacaagg tcctggtcct gggatcggaa aaccagaaca agggaaacca gacaccgtac 1860

gaatacttca acggaaagga caactcgaga gaatggcagg aattcaaggc aagagtcgaa 1920

acatcgagat tcccgagatc gaagaagcag agaatcctgc tgcagaagtt cgacgaagac 1980

ggattcaagg aaagaaacct gaacgacaca agatacgtca acagattcct gtgccagttc 2040

gtcgcagaca gaatgagact gacaggaaag ggaaagaaga gagtcttcgc atcgaacgga 2100

cagatcacaa acctgctgag aggattctgg ggactgagaa aggtcagagc agaaaacgac 2160

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atcacaagat tcgtcagata caaggaaatg aacgcattcg acggaaagac aatcgacaag 2280

gaaacaggag aagtcctgca ccagaagaca cacttcccgc agccgtggga attcttcgca 2340

caggaagtca tgatcagagt cttcggaaag ccggacggaa agccggaatt cgaagaagca 2400

gacacactgg aaaagctgag aacactgctg gcagaaaagc tgtcgtcgag accggaagca 2460

gtccacgaat acgtcacacc gctgttcgtc tcgagagcac cgaacagaaa gatgtcggga 2520

cagggacaca tggaaacagt caagtcggca aagagactgg acgaaggagt ctcggtcctg 2580

agagtcccgc tgacacagct gaagctgaag gacctggaaa agatggtcaa cagagaaaga 2640

gaaccgaagc tgtacgaagc actgaaggca agactggaag cacacaagga cgacccggca 2700

aaggcattcg cagaaccgtt ctacaagtac gacaaggcag gaaacagaac acagcaggtc 2760

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atcgcagaca acgcaacaat ggtcagagta gacgtcttcg aaaagggaga caagtactac 2880

ctggtcccga tctactcgtg gcaggtcgca aagggaatcc tgccggacag agcagtcgtc 2940

cagggaaagg acgaagaaga ctggcagctg atcgacgact cgttcaactt caagttctcg 3000

ctgcacccga acgacctggt cgaagtcatc acaaagaagg caagaatgtt cggatacttc 3060

gcatcgtgcc acagaggaac aggaaacatc aacatcagaa tccacgacct ggaccacaag 3120

atcggaaaga acggaatcct ggaaggaatc ggagtcaaga cagcactgtc gttccagaag 3180

taccagatcg acgaactggg aaaggaaatc agaccgtgca gactgaagaa gagaccgccg 3240

gtcagatccg gaaagagaac agcagacgga tcggaattcg aatcgccgaa gaagaagaga 3300

aaggtcgaat ga 3312

<210> SEQ ID NO 266
<211> LENGTH: 3306
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: ORF encoding Neisseria meningitidis Cas9 using minimal uridine codons (no start or stop codons; suitable for inclusion in fusion protein coding sequence)

<400> SEQUENCE: 266

gcagcattca agccgaactc gatcaactac atcctgggac tggacatcgg aatcgcatcg 60

gtcggatggg caatggtcga aatcgacgaa gaagaaaacc cgatcagact gatcgacctg 120

ggagtcagag tcttcgaaag agcagaagtc ccgaagacag gagactcgct ggcaatggca 180

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agaagactgg caagatcggt cagaagactg acaagaagaa gagcacacag actgctgaga 240

acaagaagac tgctgaagag agaaggagtc ctgcaggcag caaacttcga cgaaaacgga 300

ctgatcaagt cgctgccgaa cacaccgtgg cagctgagag cagcagcact ggacagaaag 360

ctgacaccgc tggaatggtc ggcagtcctg ctgcacctga tcaagcacag aggatacctg 420

tcgcagagaa agaacgaagg agaaacagca gacaaggaac tgggagcact gctgaaggga 480

gtcgcaggaa acgcacacgc actgcagaca ggagacttca gaacaccggc agaactggca 540

ctgaacaagt tcgaaaagga atcgggacac atcagaaacc agagatcgga ctactcgcac 600

acattctcga gaaaggacct gcaggcagaa ctgatcctgc tgttcgaaaa gcagaaggaa 660

ttcggaaacc cgcacgtctc gggaggactg aaggaaggaa tcgaaacact gctgatgaca 720

cagagaccgg cactgtcggg agacgcagtc cagaagatgc tgggacactg cacattcgaa 780

ccggcagaac cgaaggcagc aaagaacaca tacacagcag aaagattcat ctggctgaca 840

aagctgaaca acctgagaat cctggaacag ggatcggaaa gaccgctgac agacacagaa 900

agagcaacac tgatggacga accgtacaga aagtcgaagc tgacatacgc acaggcaaga 960

aagctgctgg gactggaaga cacagcattc ttcaagggac tgagatacgg aaaggacaac 1020

gcagaagcat cgacactgat ggaaatgaag gcataccacg caatctcgag agcactggaa 1080

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tcgcaggcaa gaaaggtcat caacggagtc gtcagaagat acggatcgcc ggcaagaatc 1500

cacatcgaaa cagcaagaga agtcggaaag tcgttcaagg acagaaagga aatcgaaaag 1560

agacaggaag aaaacagaaa ggacagagaa aaggcagcag caaagttcag agaatacttc 1620

ccgaacttcg tcggagaacc gaagtcgaag gacatcctga agctgagact gtacgaacag 1680

cagcacggaa agtgcctgta ctcgggaaag gaaatcaacc tgggaagact gaacgaaaag 1740

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aacaaggtcc tggtcctggg atcggaaaac cagaacaagg gaaaccagac accgtacgaa 1860

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ttcaaggaaa gaaacctgaa cgacacaaga tacgtcaaca gattcctgtg ccagttcgtc 2040

gcagacagaa tgagactgac aggaaaggga aagaagagag tcttcgcatc gaacggacag 2100

atcacaaacc tgctgagagg attctgggga ctgagaaagg tcagagcaga aaacgacaga 2160

caccacgcac tggacgcagt cgtcgtcgca tgctcgacag tcgcaatgca gcagaagatc 2220

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cacgaatacg tcacaccgct gttcgtctcg agagcaccga acagaaagat gtcgggacag 2520

ggacacatgg aaacagtcaa gtcggcaaag agactggacg aaggagtctc ggtcctgaga 2580

gtcccgctga cacagctgaa gctgaaggac ctggaaaaga tggtcaacag agaaagagaa 2640

ccgaagctgt acgaagcact gaaggcaaga ctggaagcac acaaggacga cccggcaaag 2700

gcattcgcag aaccgttcta caagtacgac aaggcaggaa acagaacaca gcaggtcaag 2760

gcagtcagag tcgaacaggt ccagaagaca ggagtctggg tcagaaacca caacggaatc 2820

gcagacaacg caacaatggt cagagtagac gtcttcgaaa agggagacaa gtactacctg 2880

gtcccgatct actcgtggca ggtcgcaaag ggaatcctgc cggacagagc agtcgtccag 2940

ggaaaggacg aagaagactg gcagctgatc gacgactcgt tcaacttcaa gttctcgctg 3000

cacccgaacg acctggtcga agtcatcaca aagaaggcaa gaatgttcgg atacttcgca 3060

tcgtgccaca gaggaacagg aaacatcaac atcagaatcc acgacctgga ccacaagatc 3120

ggaaagaacg gaatcctgga aggaatcgga gtcaagacag cactgtcgtt ccagaagtac 3180

cagatcgacg aactgggaaa ggaaatcaga ccgtgcagac tgaagaagag accgccggtc 3240

agatccggaa agagaacagc agacggatcg gaattcgaat cgccgaagaa gaagagaaag 3300

gtcgaa 3306

<210> SEQ ID NO 267
<211> LENGTH: 3636
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Transcript comprising SEQ ID NO: 265 (encoding Neisseria meningitidis Cas9)

<400> SEQUENCE: 267

gggagaccca agctggctag cgtttaaact taagcttgga tccgccacca tggcagcatt 60

caagccgaac tcgatcaact acatcctggg actggacatc ggaatcgcat cggtcggatg 120

ggcaatggtc gaaatcgacg aagaagaaaa cccgatcaga ctgatcgacc tgggagtcag 180

agtcttcgaa agagcagaag tcccgaagac aggagactcg ctggcaatgg caagaagact 240

ggcaagatcg gtcagaagac tgacaagaag aagagcacac agactgctga gaacaagaag 300

actgctgaag agagaaggag tcctgcaggc agcaaacttc gacgaaaacg gactgatcaa 360

gtcgctgccg aacacaccgt ggcagctgag agcagcagca ctggacagaa agctgacacc 420

gctggaatgg tcggcagtcc tgctgcacct gatcaagcac agaggatacc tgtcgcagag 480

aaagaacgaa ggagaaacag cagacaagga actgggagca ctgctgaagg gagtcgcagg 540

aaacgcacac gcactgcaga caggagactt cagaacaccg gcagaactgg cactgaacaa 600

gttcgaaaag gaatcgggac acatcagaaa ccagagatcg gactactcgc acacattctc 660

gagaaaggac ctgcaggcag aactgatcct gctgttcgaa aagcagaagg aattcggaaa 720

cccgcacgtc tcgggaggac tgaaggaagg aatcgaaaca ctgctgatga cacagagacc 780

ggcactgtcg ggagacgcag tccagaagat gctgggacac tgcacattcg aaccggcaga 840

accgaaggca gcaaagaaca catacacagc agaaagattc atctggctga caaagctgaa 900

caacctgaga atcctggaac agggatcgga aagaccgctg acagacacag aaagagcaac 960

actgatggac gaaccgtaca gaaagtcgaa gctgacatac gcacaggcaa gaaagctgct 1020

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gggactggaa gacacagcat tcttcaaggg actgagatac ggaaaggaca acgcagaagc 1080

atcgacactg atggaaatga aggcatacca cgcaatctcg agagcactgg aaaaggaagg 1140

actgaaggac aagaagtcgc cgctgaacct gtcgccggaa ctgcaggacg aaatcggaac 1200

agcattctcg ctgttcaaga cagacgaaga catcacagga agactgaagg acagaatcca 1260

gccggaaatc ctggaagcac tgctgaagca catctcgttc gacaagttcg tccagatctc 1320

gctgaaggca ctgagaagaa tcgtcccgct gatggaacag ggaaagagat acgacgaagc 1380

atgcgcagaa atctacggag accactacgg aaagaagaac acagaagaaa agatctacct 1440

gccgccgatc ccggcagacg aaatcagaaa cccggtcgtc ctgagagcac tgtcgcaggc 1500

aagaaaggtc atcaacggag tcgtcagaag atacggatcg ccggcaagaa tccacatcga 1560

aacagcaaga gaagtcggaa agtcgttcaa ggacagaaag gaaatcgaaa agagacagga 1620

agaaaacaga aaggacagag aaaaggcagc agcaaagttc agagaatact tcccgaactt 1680

cgtcggagaa ccgaagtcga aggacatcct gaagctgaga ctgtacgaac agcagcacgg 1740

aaagtgcctg tactcgggaa aggaaatcaa cctgggaaga ctgaacgaaa agggatacgt 1800

cgaaatcgac cacgcactgc cgttctcgag aacatgggac gactcgttca acaacaaggt 1860

cctggtcctg ggatcggaaa accagaacaa gggaaaccag acaccgtacg aatacttcaa 1920

cggaaaggac aactcgagag aatggcagga attcaaggca agagtcgaaa catcgagatt 1980

cccgagatcg aagaagcaga gaatcctgct gcagaagttc gacgaagacg gattcaagga 2040

aagaaacctg aacgacacaa gatacgtcaa cagattcctg tgccagttcg tcgcagacag 2100

aatgagactg acaggaaagg gaaagaagag agtcttcgca tcgaacggac agatcacaaa 2160

cctgctgaga ggattctggg gactgagaaa ggtcagagca gaaaacgaca gacaccacgc 2220

actggacgca gtcgtcgtcg catgctcgac agtcgcaatg cagcagaaga tcacaagatt 2280

cgtcagatac aaggaaatga acgcattcga cggaaagaca atcgacaagg aaacaggaga 2340

agtcctgcac cagaagacac acttcccgca gccgtgggaa ttcttcgcac aggaagtcat 2400

gatcagagtc ttcggaaagc cggacggaaa gccggaattc gaagaagcag acacactgga 2460

aaagctgaga acactgctgg cagaaaagct gtcgtcgaga ccggaagcag tccacgaata 2520

cgtcacaccg ctgttcgtct cgagagcacc gaacagaaag atgtcgggac agggacacat 2580

ggaaacagtc aagtcggcaa agagactgga cgaaggagtc tcggtcctga gagtcccgct 2640

gacacagctg aagctgaagg acctggaaaa gatggtcaac agagaaagag aaccgaagct 2700

gtacgaagca ctgaaggcaa gactggaagc acacaaggac gacccggcaa aggcattcgc 2760

agaaccgttc tacaagtacg acaaggcagg aaacagaaca cagcaggtca aggcagtcag 2820

agtcgaacag gtccagaaga caggagtctg ggtcagaaac cacaacggaa tcgcagacaa 2880

cgcaacaatg gtcagagtag acgtcttcga aaagggagac aagtactacc tggtcccgat 2940

ctactcgtgg caggtcgcaa agggaatcct gccggacaga gcagtcgtcc agggaaagga 3000

cgaagaagac tggcagctga tcgacgactc gttcaacttc aagttctcgc tgcacccgaa 3060

cgacctggtc gaagtcatca caaagaaggc aagaatgttc ggatacttcg catcgtgcca 3120

cagaggaaca ggaaacatca acatcagaat ccacgacctg gaccacaaga tcggaaagaa 3180

cggaatcctg gaaggaatcg gagtcaagac agcactgtcg ttccagaagt accagatcga 3240

cgaactggga aaggaaatca gaccgtgcag actgaagaag agaccgccgg tcagatccgg 3300

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aaagagaaca gcagacggat cggaattcga atcgccgaag aagaagagaa aggtcgaatg 3360

atagctagct cgagtctaga gggcccgttt aaacccgctg atcagcctcg actgtgcctt 3420

ctagttgcca gccatctgtt gtttgcccct cccccgtgcc ttccttgacc ctggaaggtg 3480

ccactcccac tgtcctttcc taataaaatg aggaaattgc atcgcattgt ctgagtaggt 3540

gtcattctat tctgggggggt ggggtggggc aggacagcaa gggggaggat tgggaagaca 3600

atagcaggca tgctggggat gcggtgggct ctatgg 3636

<210> SEQ ID NO 268
<211> LENGTH: 1103
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Amino acid sequence of Neisseria meningitidis Cas9

<400> SEQUENCE: 268

Met Ala Ala Phe Lys Pro Asn Ser Ile Asn Tyr Ile Leu Gly Leu Asp
1 5 10 15

Ile Gly Ile Ala Ser Val Gly Trp Ala Met Val Glu Ile Asp Glu Glu
20 25 30

Glu Asn Pro Ile Arg Leu Ile Asp Leu Gly Val Arg Val Phe Glu Arg
35 40 45

Ala Glu Val Pro Lys Thr Gly Asp Ser Leu Ala Met Ala Arg Arg Leu
50 55 60

Ala Arg Ser Val Arg Arg Leu Thr Arg Arg Arg Ala His Arg Leu Leu
65 70 75 80

Arg Thr Arg Arg Leu Leu Lys Arg Glu Gly Val Leu Gln Ala Ala Asn
85 90 95

Phe Asp Glu Asn Gly Leu Ile Lys Ser Leu Pro Asn Thr Pro Trp Gln
100 105 110

Leu Arg Ala Ala Ala Leu Asp Arg Lys Leu Thr Pro Leu Glu Trp Ser
115 120 125

Ala Val Leu Leu His Leu Ile Lys His Arg Gly Tyr Leu Ser Gln Arg
130 135 140

Lys Asn Glu Gly Glu Thr Ala Asp Lys Glu Leu Gly Ala Leu Leu Lys
145 150 155 160

Gly Val Ala Gly Asn Ala His Ala Leu Gln Thr Gly Asp Phe Arg Thr
165 170 175

Pro Ala Glu Leu Ala Leu Asn Lys Phe Glu Lys Glu Ser Gly His Ile
180 185 190

Arg Asn Gln Arg Ser Asp Tyr Ser His Thr Phe Ser Arg Lys Asp Leu
195 200 205

Gln Ala Glu Leu Ile Leu Leu Phe Glu Lys Gln Lys Glu Phe Gly Asn
210 215 220

Pro His Val Ser Gly Gly Leu Lys Glu Gly Ile Glu Thr Leu Leu Met
225 230 235 240

Thr Gln Arg Pro Ala Leu Ser Gly Asp Ala Val Gln Lys Met Leu Gly
245 250 255

His Cys Thr Phe Glu Pro Ala Glu Pro Lys Ala Ala Lys Asn Thr Tyr
260 265 270

Thr Ala Glu Arg Phe Ile Trp Leu Thr Lys Leu Asn Asn Leu Arg Ile
275 280 285

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Leu Glu Gln Gly Ser Glu Arg Pro Leu Thr Asp Thr Glu Arg Ala Thr
    290                 295                 300

Leu Met Asp Glu Pro Tyr Arg Lys Ser Lys Leu Thr Tyr Ala Gln Ala
305                 310                 315                 320

Arg Lys Leu Leu Gly Leu Glu Asp Thr Ala Phe Phe Lys Gly Leu Arg
                325                 330                 335

Tyr Gly Lys Asp Asn Ala Glu Ala Ser Thr Leu Met Glu Met Lys Ala
            340                 345                 350

Tyr His Ala Ile Ser Arg Ala Leu Glu Lys Glu Gly Leu Lys Asp Lys
        355                 360                 365

Lys Ser Pro Leu Asn Leu Ser Pro Glu Leu Gln Asp Glu Ile Gly Thr
    370                 375                 380

Ala Phe Ser Leu Phe Lys Thr Asp Glu Asp Ile Thr Gly Arg Leu Lys
385                 390                 395                 400

Asp Arg Ile Gln Pro Glu Ile Leu Glu Ala Leu Leu Lys His Ile Ser
                405                 410                 415

Phe Asp Lys Phe Val Gln Ile Ser Leu Lys Ala Leu Arg Arg Ile Val
            420                 425                 430

Pro Leu Met Glu Gln Gly Lys Arg Tyr Asp Glu Ala Cys Ala Glu Ile
        435                 440                 445

Tyr Gly Asp His Tyr Gly Lys Lys Asn Thr Glu Glu Lys Ile Tyr Leu
    450                 455                 460

Pro Pro Ile Pro Ala Asp Glu Ile Arg Asn Pro Val Val Leu Arg Ala
465                 470                 475                 480

Leu Ser Gln Ala Arg Lys Val Ile Asn Gly Val Val Arg Arg Tyr Gly
                485                 490                 495

Ser Pro Ala Arg Ile His Ile Glu Thr Ala Arg Glu Val Gly Lys Ser
            500                 505                 510

Phe Lys Asp Arg Lys Glu Ile Glu Lys Arg Gln Glu Glu Asn Arg Lys
        515                 520                 525

Asp Arg Glu Lys Ala Ala Ala Lys Phe Arg Glu Tyr Phe Pro Asn Phe
    530                 535                 540

Val Gly Glu Pro Lys Ser Lys Asp Ile Leu Lys Leu Arg Leu Tyr Glu
545                 550                 555                 560

Gln Gln His Gly Lys Cys Leu Tyr Ser Gly Lys Glu Ile Asn Leu Gly
                565                 570                 575

Arg Leu Asn Glu Lys Gly Tyr Val Glu Ile Asp His Ala Leu Pro Phe
            580                 585                 590

Ser Arg Thr Trp Asp Asp Ser Phe Asn Asn Lys Val Leu Val Leu Gly
        595                 600                 605

Ser Glu Asn Gln Asn Lys Gly Asn Gln Thr Pro Tyr Glu Tyr Phe Asn
    610                 615                 620

Gly Lys Asp Asn Ser Arg Glu Trp Gln Glu Phe Lys Ala Arg Val Glu
625                 630                 635                 640

Thr Ser Arg Phe Pro Arg Ser Lys Lys Gln Arg Ile Leu Leu Gln Lys
                645                 650                 655

Phe Asp Glu Asp Gly Phe Lys Glu Arg Asn Leu Asn Asp Thr Arg Tyr
            660                 665                 670

Val Asn Arg Phe Leu Cys Gln Phe Val Ala Asp Arg Met Arg Leu Thr
        675                 680                 685
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Gly Lys Gly Lys Lys Arg Val Phe Ala Ser Asn Gly Gln Ile Thr Asn
    690                 695                 700

Leu Leu Arg Gly Phe Trp Gly Leu Arg Lys Val Arg Ala Glu Asn Asp
705                 710                 715                 720

Arg His His Ala Leu Asp Ala Val Val Val Ala Cys Ser Thr Val Ala
                725                 730                 735

Met Gln Gln Lys Ile Thr Arg Phe Val Arg Tyr Lys Glu Met Asn Ala
            740                 745                 750

Phe Asp Gly Lys Thr Ile Asp Lys Glu Thr Gly Glu Val Leu His Gln
        755                 760                 765

Lys Thr His Phe Pro Gln Pro Trp Glu Phe Phe Ala Gln Glu Val Met
    770                 775                 780

Ile Arg Val Phe Gly Lys Pro Asp Gly Lys Pro Glu Phe Glu Glu Ala
785                 790                 795                 800

Asp Thr Leu Glu Lys Leu Arg Thr Leu Leu Ala Glu Lys Leu Ser Ser
                805                 810                 815

Arg Pro Glu Ala Val His Glu Tyr Val Thr Pro Leu Phe Val Ser Arg
            820                 825                 830

Ala Pro Asn Arg Lys Met Ser Gly Gln Gly His Met Glu Thr Val Lys
        835                 840                 845

Ser Ala Lys Arg Leu Asp Glu Gly Val Ser Val Leu Arg Val Pro Leu
    850                 855                 860

Thr Gln Leu Lys Leu Lys Asp Leu Glu Lys Met Val Asn Arg Glu Arg
865                 870                 875                 880

Glu Pro Lys Leu Tyr Glu Ala Leu Lys Ala Arg Leu Glu Ala His Lys
                885                 890                 895

Asp Asp Pro Ala Lys Ala Phe Ala Glu Pro Phe Tyr Lys Tyr Asp Lys
            900                 905                 910

Ala Gly Asn Arg Thr Gln Gln Val Lys Ala Val Arg Val Glu Gln Val
        915                 920                 925

Gln Lys Thr Gly Val Trp Val Arg Asn His Asn Gly Ile Ala Asp Asn
    930                 935                 940

Ala Thr Met Val Arg Val Asp Val Phe Glu Lys Gly Asp Lys Tyr Tyr
945                 950                 955                 960

Leu Val Pro Ile Tyr Ser Trp Gln Val Ala Lys Gly Ile Leu Pro Asp
                965                 970                 975

Arg Ala Val Val Gln Gly Lys Asp Glu Glu Asp Trp Gln Leu Ile Asp
            980                 985                 990

Asp Ser Phe Asn Phe Lys Phe Ser  Leu His Pro Asn Asp  Leu Val Glu
        995                 1000                1005

Val Ile  Thr Lys Lys Ala Arg  Met Phe Gly Tyr Phe  Ala Ser Cys
    1010                1015                1020

His Arg  Gly Thr Gly Asn Ile  Asn Ile Arg Ile His  Asp Leu Asp
    1025                1030                1035

His Lys  Ile Gly Lys Asn Gly  Ile Leu Glu Gly Ile  Gly Val Lys
    1040                1045                1050

Thr Ala  Leu Ser Phe Gln Lys  Tyr Gln Ile Asp Glu  Leu Gly Lys
    1055                1060                1065

Glu Ile  Arg Pro Cys Arg Leu  Lys Lys Arg Pro Pro  Val Arg Ser
    1070                1075                1080

Gly Lys  Arg Thr Ala Asp Gly  Ser Glu Phe Glu Ser  Pro Lys Lys
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1085 1090 1095

Lys Arg Lys Val Glu
1100

<210> SEQ ID NO 269
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Synthetic: G390 single guide RNA targeting the rat TTR gene
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
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<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 269

gccgagucug gagagcugca guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 270
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: trRNA

<400> SEQUENCE: 270

aacagcauag caaguuaaaa uaaggcuagu ccguuaucaa cuugaaaaag uggcaccgag 60

ucggugcuuu uuuu 74

<210> SEQ ID NO 271

<400> SEQUENCE: 271

000

<210> SEQ ID NO 272
<211> LENGTH: 100
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G534 single guide RNA targeting the rat TTR gene
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (29)..(40)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (69)..(96)

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<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (97)..(100)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 272

acgcaaauau caguccagcg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu 100

<210> SEQ ID NO 273
<211> LENGTH: 95
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: G000395 5 truncated inactive sgRNA modified sequence
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(3)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (24)..(35)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (64)..(91)
<223> OTHER INFORMATION: 2'-O-Me nucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (92)..(95)
<223> OTHER INFORMATION: PS linkage, 2'-O-Me nucleotide

<400> SEQUENCE: 273

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ucaacuugaa aaaguggcac cgagucggug cuuuu 95

<210> SEQ ID NO 274
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: SV40 NLS

<400> SEQUENCE: 274

Pro Lys Lys Lys Arg Lys Val
1 5

<210> SEQ ID NO 275
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Alternate SV40 NLS

<400> SEQUENCE: 275

Pro Lys Lys Lys Arg Arg Val
1 5

<210> SEQ ID NO 276
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Nucleoplasmin NLS

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<400> SEQUENCE: 276

Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys
1 5 10 15

<210> SEQ ID NO 277
<211> LENGTH: 10
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Exemplary Kozak sequence

<400> SEQUENCE: 277

gccrccaugg 10

<210> SEQ ID NO 278
<211> LENGTH: 13
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: Exemplary Kozak sequence

<400> SEQUENCE: 278

gccgccrcca ugg 13

What is claimed is:

1. A method of inducing a double-stranded break (DSB) within the TTR gene, comprising delivering a composition to a cell, wherein the composition comprises

a. a guide RNA comprising a guide sequence selected from SEQ ID NOs: 5-82;

b. a guide RNA comprising at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide RNA comprising a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

2. A method of modifying the TTR gene comprising delivering a composition to a cell, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

3. A method of treating amyloidosis associated with TTR (ATTR), comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82, thereby treating ATTR.

4. A method of reducing TTR serum concentration, comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82, thereby reducing TTR serum concentration.

5. A method for reducing or preventing the accumulation of amyloids or amyloid fibrils comprising TTR in a subject, comprising administering a composition to a subject in need thereof, wherein the composition comprises (i) an RNA-guided DNA binding agent or a nucleic acid encoding an RNA-guided DNA binding agent and (ii) a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82, thereby reducing accumulation of amyloids or amyloid fibrils.

6. A composition comprising a guide RNA comprising:

a. a guide sequence selected from SEQ ID NOs: 5-82;

b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or

c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

7. A composition comprising a vector encoding a guide RNA, wherein the guide RNA comprises:

a. a guide sequence selected from SEQ ID NOs: 5-82;
b. at least 17, 18, 19, or 20 contiguous nucleotides of a sequence selected from SEQ ID NOs: 5-82; or
c. a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID NOs: 5-82.

8. The composition of claim **6** or **7**, for use in inducing a double-stranded break (DSB) within the TTR gene in a cell or subject.

9. The composition of claim **6** or **7**, for use in modifying the TTR gene in a cell or subject.

10. The composition of claim **6** or **7**, for use in treating amyloidosis associated with TTR (ATTR) in a subject.

11. The composition of claim **6** or **7**, for use in reducing TTR serum concentration in a subject.

12. The composition of claim **6** or **7**, for use in reducing or preventing the accumulation of amyloids or amyloid fibrils in a subject.

13. The method of any one of claims **1-5** or the composition for use of any one of claims **8-12**, wherein the composition reduces serum TTR levels.

14. The method or composition for use of claim **13**, wherein the serum TTR levels are reduced by at least 50% as compared to serum TTR levels before administration of the composition.

15. The method or composition for use of claim **13**, wherein the serum TTR levels are reduced by 50-60%, 60-70%, 70-80%, 80-90%, 90-95%, 95-98%, 98-99%, or 99-100% as compared to serum TTR levels before administration of the composition.

16. The method or composition for use of any one of claim **1-5** or **8-15**, wherein the composition results in editing of the TTR gene.

17. The method or composition for use of claim **16**, wherein the editing is calculated as a percentage of the population that is edited (percent editing).

18. The method or composition for use of claim **17**, wherein the percent editing is between 30 and 99% of the population.

19. The method or composition for use of claim **17**, wherein the percent editing is between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the population.

20. The method of any one of claims **1-5** or the composition for use of any one of claims **8-19**, wherein the composition reduces amyloid deposition in at least one tissue.

21. The method or composition for use of claim **20**, wherein the at least one tissue comprises one or more of stomach, colon, sciatic nerve, or dorsal root ganglion.

22. The method or composition for use of claim **20** or **21**, wherein amyloid deposition is measured 8 weeks after administration of the composition.

23. The method or composition for use of any one of claims **20-22**, wherein amyloid deposition is compared to a negative control or a level measured before administration of the composition.

24. The method or composition for use of any one of claims **20-23**, wherein amyloid deposition is measured in a biopsy sample and/or by immunostaining.

25. The method or composition for use of any one of claims **20-24**, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen in a negative control.

26. The method or composition for use of any one of claims **20-25**, wherein amyloid deposition is reduced by between 30 and 35%, 35 and 40%, 40 and 45%, 45 and 50%, 50 and 55%, 55 and 60%, 60 and 65%, 65 and 70%, 70 and 75%, 75 and 80%, 80 and 85%, 85 and 90%, 90 and 95%, or 95 and 99% of the amyloid deposition seen before administration of the composition.

27. The method or composition for use of any one of claim **1-5** or **8-26**, wherein the composition is administered or delivered at least two times.

28. The method or composition for use of claim **27**, wherein the composition is administered or delivered at least three times.

29. The method or composition for use of claim **27**, wherein the composition is administered or delivered at least four times.

30. The method or composition for use of claim **27**, wherein the composition is administered or delivered up to five, six, seven, eight, nine, or ten times.

31. The method or composition for use of any one of claims **27-30**, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 days.

32. The method or composition for use of any one of claims **27-30**, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 weeks.

33. The method or composition for use of any one of claims **27-30**, wherein the administration or delivery occurs at an interval of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 months.

34. The method or composition of any one of the preceding claims, wherein the guide sequence is selected from SEQ ID NOs: 5-82.

35. The method or composition of any one of the preceding claims, wherein the guide RNA is at least partially complementary to a target sequence present in the human TTR gene.

36. The method or composition of claim **35**, wherein the target sequence is in exon 1, 2, 3, or 4 of the human TTR gene.

37. The method or composition of claim **35**, wherein the target sequence is in exon 1 of the human TTR gene.

38. The method or composition of claim **35**, wherein the target sequence is in exon 2 of the human TTR gene.

39. The method or composition of claim **35**, wherein the target sequence is in exon 3 of the human TTR gene.

40. The method or composition of claim **35**, wherein the target sequence is in exon 4 of the human TTR gene.

41. The method or composition of any one of claims **1-40**, wherein the guide sequence is complementary to a target sequence in the positive strand of TTR.

42. The method or composition of any one of claims **1-40**, wherein the guide sequence is complementary to a target sequence in the negative strand of TTR.

43. The method or composition of any one of claims **1-40**, wherein the first guide sequence is complementary to a first target sequence in the positive strand of the TTR gene, and wherein the composition further comprises a second guide

sequence that is complementary to a second target sequence in the negative strand of the TTR gene.

44. The method or composition of any one of the preceding claims, wherein the guide RNA comprises a crRNA that comprises the guide sequence and further comprises a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end.

45. The method or composition of any one of the preceding claims, wherein the guide RNA is a dual guide (dgRNA).

46. The method or composition of claim **45**, wherein the dual guide RNA comprises a crRNA comprising a nucleotide sequence of SEQ ID NO: 126, wherein the nucleotides of SEQ ID NO: 126 follow the guide sequence at its 3' end, and a trRNA.

47. The method or composition of any one of claims **1-43**, wherein the guide RNA is a single guide (sgRNA).

48. The method or composition of claim **47**, wherein the sgRNA comprises a guide sequence that has the pattern of SEQ ID NO: 3.

49. The method or composition of claim **47**, wherein the sgRNA comprises the sequence of SEQ ID NO: 3.

50. The method or composition of claim **48** or **49**, wherein each N in SEQ ID NO: 3 is any natural or non-natural nucleotide, wherein the N's form the guide sequence, and the guide sequence targets Cas9 to the TTR gene.

51. The method or composition of any one of claims **47-50**, wherein the sgRNA comprises any one of the guide sequences of SEQ ID NOs: 5-82 and the nucleotides of SEQ ID NO: 126.

52. The method or composition of any one of claims **47-51**, wherein the sgRNA comprises a guide sequence that is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, or 90% identical to a sequence selected from SEQ ID Nos: 87-124.

53. The method or composition of claim **47**, wherein the sgRNA comprises a sequence selected from SEQ ID Nos: 87-124.

54. The method or composition of any one of the preceding claims, wherein the guide RNA comprises at least one modification.

55. The method or composition of claim **54**, wherein the at least one modification includes a 2'-O-methyl (2'-O-Me) modified nucleotide.

56. The method or composition of claim **54** or **55**, wherein the at least one modification includes a phosphorothioate (PS) bond between nucleotides.

57. The method or composition of any one of claims **54-56**, wherein the at least one modification includes a 2'-fluoro (2'-F) modified nucleotide.

58. The method or composition of any one of claims **54-57**, wherein the at least one modification includes a modification at one or more of the first five nucleotides at the 5' end.

59. The method or composition of any one of claims **54-58**, wherein the at least one modification includes a modification at one or more of the last five nucleotides at the 3' end.

60. The method or composition of any one of claims **54-59**, wherein the at least one modification includes PS bonds between the first four nucleotides.

61. The method or composition of any one of claims **54-60**, wherein the at least one modification includes PS bonds between the last four nucleotides.

62. The method or composition of any one of claims **54-61**, wherein the at least one modification includes 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

63. The method or composition of any one of claims **54-62**, wherein the at least one modification includes 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

64. The method or composition of any one of claims **54-63**, wherein the guide RNA comprises the modified nucleotides of SEQ ID NO: 3.

65. The method or composition of any one of claims **1-64**, wherein the composition further comprises a pharmaceutically acceptable excipient.

66. The method or composition of any one of claims **1-65**, wherein the guide RNA is associated with a lipid nanoparticle (LNP).

67. The method or composition of claim **66**, wherein the LNP comprises a CCD lipid.

68. The method or composition of claim **67**, wherein the CCD lipid is Lipid A or Lipid B.

69. The method or composition of claim **66-68**, wherein the LNP comprises a neutral lipid.

70. The method or composition of claim **69**, wherein the neutral lipid is DSPC

71. The method or composition of any one of claims **66-70**, wherein the LNP comprises a helper lipid.

72. The method or composition of claim **71**, wherein the helper lipid is cholesterol.

73. The method or composition of any one of claims **66-72**, wherein the LNP comprises a stealth lipid.

74. The method or composition of claim **73**, wherein the stealth lipid is PEG2k-DMG.

75. The method or composition of any one of the preceding claims, wherein the composition further comprises an RNA-guided DNA binding agent.

76. The method or composition of any one of the preceding claims, wherein the composition further comprises an mRNA that encodes an RNA-guided DNA binding agent.

77. The method or composition of claim **75** or **76**, wherein the RNA-guided DNA binding agent is a Cas cleavase.

78. The method or composition of claim **77**, wherein the RNA-guided DNA binding agent is Cas9.

79. The method or composition of any one of claims **75-78**, wherein the RNA-guided DNA binding agent is modified.

80. The method or composition of any one of claims **75-79**, wherein the RNA-guided DNA binding agent is a nickase.

81. The method or composition of claim **79** or **80**, wherein the modified RNA-guided DNA binding agent comprises a nuclear localization signal (NLS).

82. The method or composition of any one of claims **75-81**, wherein the RNA-guided DNA binding agent is a Cas from a Type-II CRISPR/Cas system.

83. The method or composition of any one of the preceding claims, wherein the composition is a pharmaceutical formulation and further comprises a pharmaceutically acceptable carrier.

84. The method or composition for use of any one of claim **1-5** or **8-83**, wherein the composition reduces or prevents amyloids or amyloid fibrils comprising TTR.

85. The method or composition for use of claim **84**, wherein the amyloids or amyloid fibrils are in the nerves, heart, or gastrointestinal track.

86. The method or composition for use of any one of claim **1-5** or **8-83**, wherein non-homologous ending joining (NHEJ) leads to a mutation during repair of a DSB in the TTR gene.

87. The method or composition for use of claim **86**, wherein NHEJ leads to a deletion or insertion of a nucleotide (s) during repair of a DSB in the TTR gene.

88. The method or composition for use of claim **87**, wherein the deletion or insertion of a nucleotide(s) induces a frame shift or nonsense mutation in the TTR gene.

89. The method or composition for use of claim **87**, wherein a frame shift or nonsense mutation is induced in the TTR gene of at least 50% of liver cells.

90. The method or composition for use of claim **89**, wherein a frame shift or nonsense mutation is induced in the TTR gene of 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100% of liver cells.

91. The method or composition for use of any one of claims **87-90**, wherein a deletion or insertion of a nucleotide (s) occurs in the TTR gene at least 50-fold or more than in off-target sites.

92. The method or composition for use of claim **91**, wherein the deletion or insertion of a nucleotide(s) occurs in the TTR gene 50-fold to 150-fold, 150-fold to 500-fold, 500-fold to 1500-fold, 1500-fold to 5000-fold, 5000-fold to 15000-fold, 15000-fold to 30000-fold, or 30000-fold to 60000-fold more than in off-target sites.

93. The method or composition for use of any one of claims **87-92**, wherein the deletion or insertion of a nucleotide(s) occurs at less than or equal to 3, 2, 1, or 0 off-target site(s) in primary human hepatocytes, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

94. The method or composition for use of claim **93**, wherein the deletion or insertion of a nucleotide(s) occurs at a number of off-target sites in primary human hepatocytes that is less than the number of off-target sites at which a deletion or insertion of a nucleotide(s) occurs in Cas9-overexpressing cells, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

95. The method or composition for use of claim **94**, wherein the Cas9-overexpressing cells are HEK293 cells stably expressing Cas9.

96. The method or composition for use of any one of claims **93-95**, wherein the number of off-target sites in primary human hepatocytes is determined by analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA and the guide RNA, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

97. The method or composition for use of any one of claims **93-95**, wherein the number of off-target sites in primary human hepatocytes is determined by an oligonucleotide insertion assay comprising analyzing genomic DNA from primary human hepatocytes transfected in vitro with Cas9 mRNA, the guide RNA, and a donor oligonucleotide, optionally wherein the off-target site(s) does (do) not occur in a protein coding region in the genome of the primary human hepatocytes.

98. The method or composition of any one of claim **1-43** or **47-97**, wherein the sequence of the guide RNA is:

a) SEQ ID NO: 92 or 104;
b) SEQ ID NO: 87, 89, 96, or 113;
c) SEQ ID NO: 100, 102, 106, 111, or 112; or
d) SEQ ID NO: 88, 90, 91, 93, 94, 95, 97, 101, 103, 108, or 109,

optionally wherein the guide RNA does not produce indels at off-target site(s) that occur in a protein coding region in the genome of primary human hepatocytes.

99. The method or composition for use of any one of claim **1-5** or **8-98**, wherein administering the composition reduces levels of TTR in the subject.

100. The method or composition for use of claim **99**, wherein the levels of TTR are reduced by at least 50%.

101. The method or composition for use of claim **100**, wherein the levels of TTR are reduced by 50%-60%, 60%-70%, 70% or 80%, 80%-90%, 90-95%, 95%-99%, or 99%-100%.

102. The method or composition for use of claim **100** or **101**, wherein the levels of TTR are measured in serum, plasma, blood, cerebral spinal fluid, or sputum.

103. The method or composition for use of claim **100** or **101**, wherein the levels of TTR are measured in liver, choroid plexus, and/or retina.

104. The method or composition for use of any one of claims **99-103**, wherein the levels of TTR are measured via enzyme-linked immunosorbent assay (ELISA).

105. The method or composition for use of any one of claim **1-5** or **8-104**, wherein the subject has ATTR.

106. The method or composition for use of any one of claim **1-5** or **8-105**, wherein the subject is human.

107. The method or composition for use of claim **105** or **106**, wherein the subject has ATTRwt.

108. The method or composition for use of claim **105** or **106**, wherein the subject has hereditary ATTR.

109. The method or composition for use of any one of claim **1-5**, **8-106**, or **108**, wherein the subject has a family history of ATTR.

110. The method or composition for use of any one of claim **1-5**, **8-106**, or **108-109**, wherein the subject has familial amyloid polyneuropathy.

111. The method or composition for use of any one of claim **1-5** or **8-110**, wherein the subject has only or predominantly nerve symptoms of ATTR.

112. The method or composition for use of any one of claim **1-5** or **8-110**, wherein the subject has familial amyloid cardiomyopathy.

113. The method or composition for use of any one of claim **1-5**, **8-109**, or **112**, wherein the subject has only or predominantly cardiac symptoms of ATTR.

114. The method or composition for use of any one of claim **1-5** or **8-113**, wherein the subject expresses TTR having a V30 mutation.

115. The method or composition for use of claim **114**, wherein the V30 mutation is V30A, V30G, V30L, or V30M.

116. The method or composition for use of claim any one of claim **1-5** or **8-113**, wherein the subject expresses TTR having a T60 mutation.

117. The method or composition for use of claim **116**, wherein the T60 mutation is T60A.

118. The method or composition for use of claim any one of claim **1-5** or **8-113**, wherein the subject expresses TTR having a V122 mutation.

119. The method or composition for use of claim **118**, wherein the V122 mutation is V122A, V122I, or V122(–).

120. The method or composition for use of any one of claim **1-5** or **8-119**, wherein the subject expresses wild-type TTR.

121. The method or composition for use of any one of claim **1-5**, **8-107**, or **120**, wherein the subject does not express TTR having a V30, T60, or V122 mutation.

122. The method or composition for use of any one of claim **1-5**, **8-107**, or **120-121**, wherein the subject does not express TTR having a pathological mutation.

123. The method or composition for use of claim **121**, wherein the subject is homozygous for wild-type TTR.

124. The method or composition for use of any one of claim **1-5** or **8-123**, wherein after administration the subject has an improvement, stabilization, or slowing of change in symptoms of sensorimotor neuropathy.

125. The method or composition for use of claim **124**, wherein the improvement, stabilization, or slowing of change in sensory neuropathy is measured using electromyogram, nerve conduction tests, or patient-reported outcomes.

126. The method or composition for use of any one of claim **1-5** or **8-125**, wherein the subject has an improvement, stabilization, or slowing of change in symptoms of congestive heart failure.

127. The method or composition for use of claim **126**, wherein the improvement, stabilization, or slowing of change in congestive heart failure is measured using cardiac biomarker tests, lung function tests, chest x-rays, or electrocardiography.

128. The method or composition for use of any one of claim **1-5** or **8-127**, wherein the composition or pharmaceutical formulation is administered via a viral vector.

129. The method or composition for use of any one of claim **1-5** or **8-127**, wherein the composition or pharmaceutical formulation is administered via lipid nanoparticles.

130. The method or composition for use of any one of claim **1-5** or **8-129**, wherein the subject is tested for specific mutations in the TTR gene before administering the composition or formulation.

131. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 5.

132. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 6.

133. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 7.

134. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 8.

135. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 9.

136. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 10.

137. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 11.

138. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 12.

139. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 13.

140. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 14.

141. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 15.

142. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 16.

143. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 17.

144. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 18.

145. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 19.

146. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 20.

147. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 21.

148. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 22.

149. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 23.

150. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 24.

151. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 25.

152. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 26.

153. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 27.

154. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 28.

155. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 29.

156. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 30.

157. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 31.

158. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 32.

159. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 33.

160. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 34.

161. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 35.

162. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 36.

163. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 37.

164. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 38.

165. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 39.

166. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 40.

167. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 41.

168. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 42.

169. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 43.

170. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 44.

171. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 45.

172. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 46.

173. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 47.

174. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 48.

175. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 49.

176. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 50.

177. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 51.

178. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 52.

179. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 53.

180. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 54.

181. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 55.

182. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 56.

183. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 57.

184. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 58.

185. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 59.

186. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 60.

187. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 61.

188. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 62.

189. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 63.

190. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 64.

191. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 65.

192. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 66.

193. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 67.

194. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 68.

195. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 69.

196. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 70.

197. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 71.

198. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 72.

199. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 73.

200. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 74.

201. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 75.

202. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 76.

203. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 77.

204. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 78.

205. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 79.

206. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 80.

207. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 81.

208. The method or composition of any one of claims **1-130**, wherein the sequence selected from SEQ ID NOs: 5-82 is SEQ ID NO: 82.

209. Use of a composition or formulation of any of claims **6-208** for the preparation of a medicament for treating a human subject having ATTR.

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