Driven by Excellence.
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SLX-[redacted]
Whole Genome Characterization using Selexis SUREscan® Service

Scope of Work : 15/YO/F/001

SIGNATURE PAGE

Author:

Myriam Adam Operations Program Manager	Date

Content Review:

Alexandre Regamey Director, DNA Technology	Date

Approval:

Regine Brokamp Chief Operating Officer	Date

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Table 1: Goal and Techniques used to characterize the genome of SLX-[redacted]

Goal	Technique
Identification of transgene integration sites followed by Validation of transgene integration sites (junctions)	Next Generation Sequencing (NGS) followed by Polymerase Chain Reaction (PCR) and Sanger Sequencing
Determination of integration sites number	Fluorescence In Situ Hybridization (FISH)
Determination of the number of transgene copy integrated in the genome cell line	Taqman Quantitative Polymerase Chain Reaction (Taqman qPCR)

2 IDENTIFICATION OF TRANSGENE INTEGRATION SITES AND THEIR VALIDATION

2.1 Next Generation Sequencing

Shotgun libraries preparation and sequencing on Illumina HiSeq 2500 were performed by Eurofins Genomics (Ebersberg, D). *In silico* analysis of the raw data was performed by the Swiss Institute of Bioinformatics (SIB, Lausanne, CH), with the help of the scaffolds that have been defined for the CHO-M genome.

Five (5) integrations sites were detected by in silico analysis; only three (3) were identified with both left and right junctions. The two (2) remaining sites were identified with either a left or a right junction

2.2 PCR and Sanger sequencing

Based on the *in silico* data generated by the Swiss Institute of Bioinformatics, Selexis designed primers pairs that allow to amplify by PCR each predicted junction (one (1) primer is designed to bind to the hypothetical predicted genomic region and the second primer is designed to bind to the vector/transgene sequence at the site of integration).

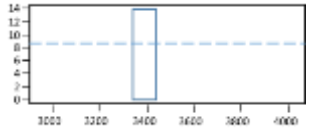
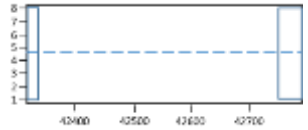
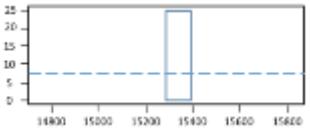
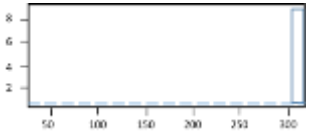
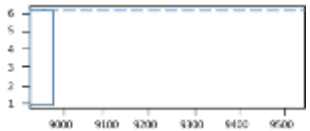
High fidelity PCR was performed using these primers pairs on [redacted] genomic DNA. Amplified DNA fragments were electrophoresed on agarose gel and purified using manufacturers' protocols prior Sanger sequencing with the primers that were used for the PCR amplification step. PCR reactions were performed following manufacturer's protocol. Sanger sequencing was outsourced to Fasteris SA (Geneva, CH).

The PCR validation of *in silico* integration sites is summarized below.

Complete data set is provided in appendix 5.4.

2.3 Summary of NGS integration sites identification for SLX-[redacted]

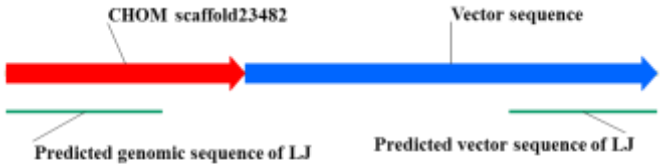
Table 2: Summary of NGS integration sites identification for SLX-[redacted]

Integration site Identification			Junction Site				Genomic Integration site	
Bioinformatics Analysis Summary	Site #	Scaffold / Contig	Left Junction		Right Junction		Deletion at the genome site of integration	Genomic sequence identification
			Identity	PCR	Identity	PCR		
	1	Scaffold 23482	[redacted]	✓	[redacted]	✓	8 bp deletion	intronic
	2	Scaffold 34597	[redacted]	✓	[redacted]	✓	No	exon
	3	Scaffold 215980	[redacted]	✓	[redacted]	✓	24 bp deletion	N/A
	4	Contig 20134276	[redacted]	✓	[redacted]	×	N/A	intronic
	5	Scaffold 46670	[redacted]	×	[redacted]	✓	N/A	exon

N/A: information not available

2.3.1 Left and right junctions of the integration site 1 (CHOM Scaffold 23482)

Left Junction at integration site 1



Left junction scaffold 23482

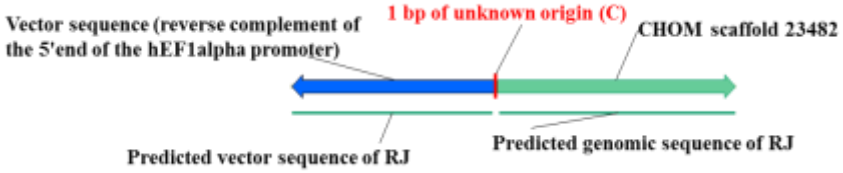
690 bp

CHO-M genomic scaffold before vector integration

Scaffold 23482 at the integration site

1096 bp

Right Junction at integration site 1

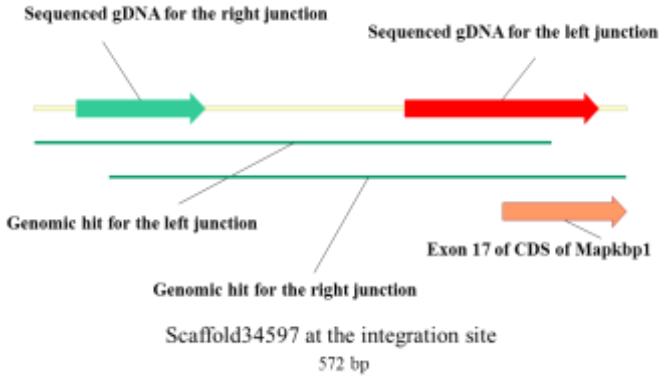


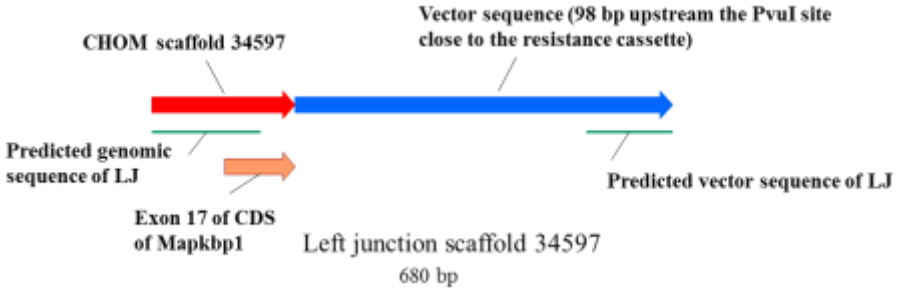
Right junction scaffold 23482

283 bp

Integration occurred between exons 7 and 8 of the PMS2 isoform X3

2.3.2 Left and right junctions of the integration site 2 (CHOM 34597)





Scaffold Left Junction at integration site 2

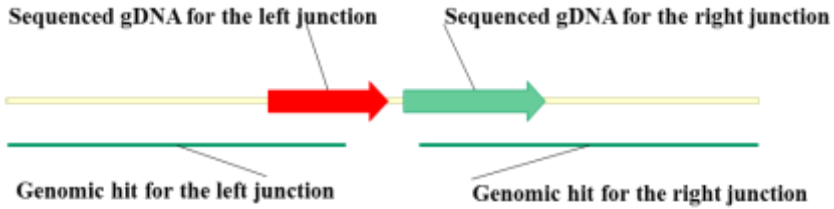
CHO-M genomic scaffold before vector integration

[redacted]

[redacted]

Right Junction at integration site 2

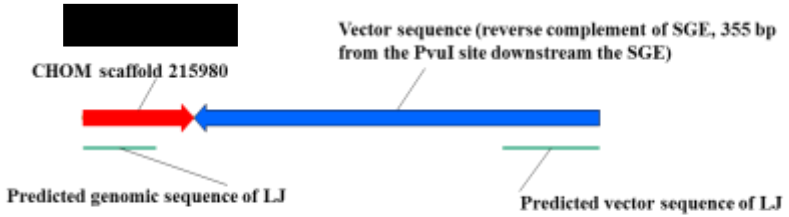
Integration occurred in exon 17 of the coding sequence of Mapkbp1



Scaffold 215980 at the integration site

1107 bp

CHO-M genomic scaffold before vector integration

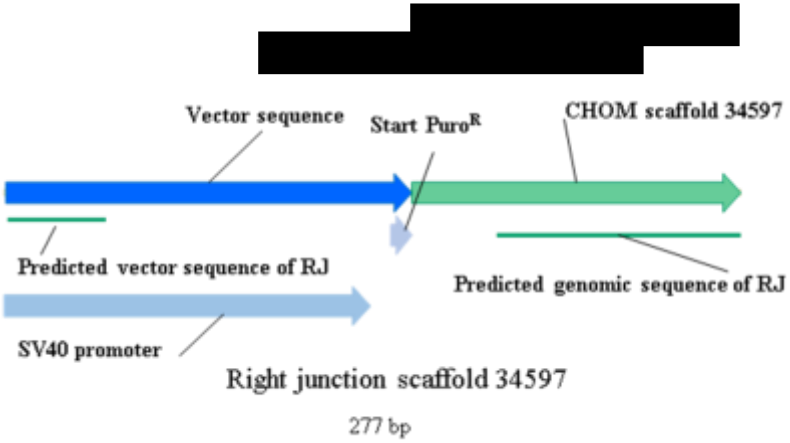


Left junction scaffold 215980

818 bp

2.3.3 Left and right junctions of the integration site 3 (CHOM Scaffold 215980)

Left Junction at integration site 3

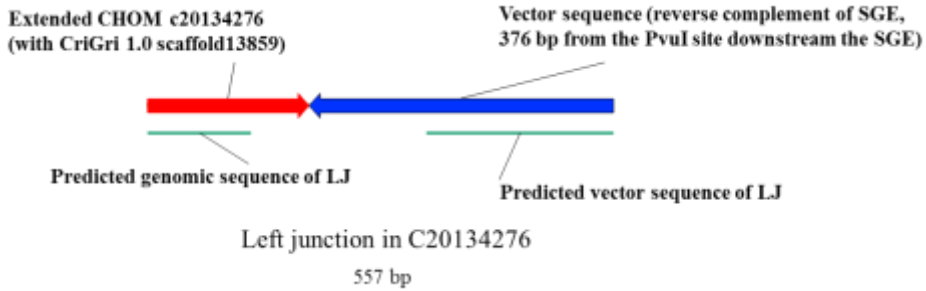


Right junction scaffold 34597

277 bp

Right Junction at integration site 3

[redacted]



Left junction in C20134276

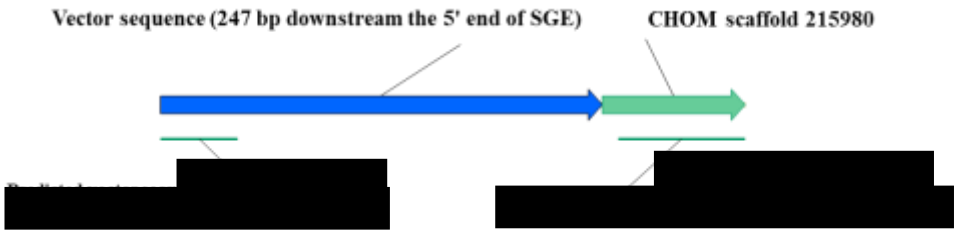
557 bp

2.3.4 Left junction of the integration site 4 (CHOM Contig 20134276)

No right junction could be predicted through *in silico* analysis; this might be due to the fact that the contig is short and/or the integration site

is on the edge of the contig.

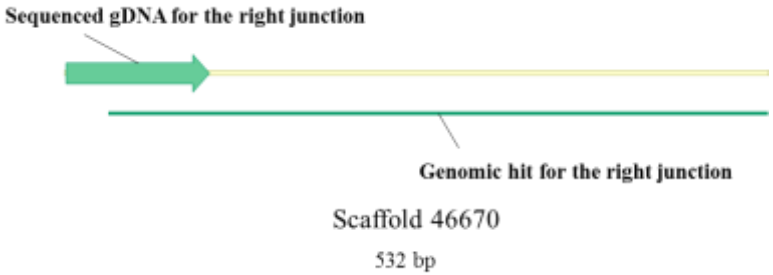
Left Junction at integration site 4



Right junction scaffold 215980

848 bp

CHO-M genomic scaffold before vector integration

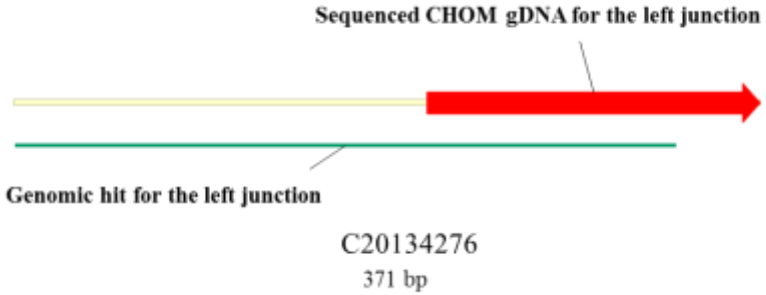


Integration occurred between exons 2 and 3 of the Hmcn2 isoform X2

2.3.5 Right junction of the integration site 5 (CHOM Scaffold 46670)

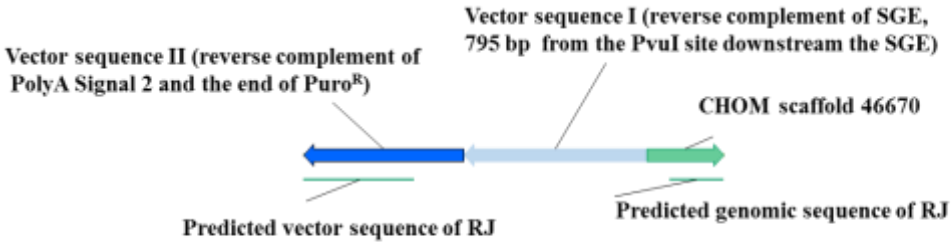
No left junction could be predicted through *in silico* analysis.

CHO-M genomic scaffold before vector integration



Right Junction at integration site 5

Integration occurred in exon 2 of some variants of Hipk2



Right junction scaffold 46670

595 bp

3 IDENTIFICATION OF TRANSGENE INSERTION SITES BY FISH

3.1 Cell line

Cells from vial # 214 from the [redacted] were prepared according to protocols adapted from Lattenmayer *et al.* (Cytotechnology. 2006; 51(3):171-82) for the generation of metaphasic and interphasic samples; corresponding samples were then analyzed by FISH.

3.2 Metaphase chromosome number determination

Twenty metaphase nuclei of [redacted] cells were examined for chromosome numbers. 90% of the examined cells contain 21 chromosomes (see table 3); these data indicate that the chromosome number is stable within this cell population.

Table 3: Distribution of chromosome numbers per cell

Chromosomes number	Cell counts
20	1
21	18
22	1

3.3 Metaphase FISH

DNA-FISH applied to metaphase cell (metaphase FISH) gives information on the number of insertions and the chromosomal position of these insertions. Furthermore, cytogenetic analysis of metaphases spreads allows the identification of chromosome rearrangements.
The metaphase chromosome spreads of twelve independent nuclei were examined. Similar results were obtained for all of them (see complete data set in appendix 5.5). Figure 1 shows two (2) chromosome spreads representative of this analysis using two (2) independently labeled probes. Imaging and analysis were performed using the ImageJ software (Open source Java-based image processing program developed at the National Institute of Health, NIH).

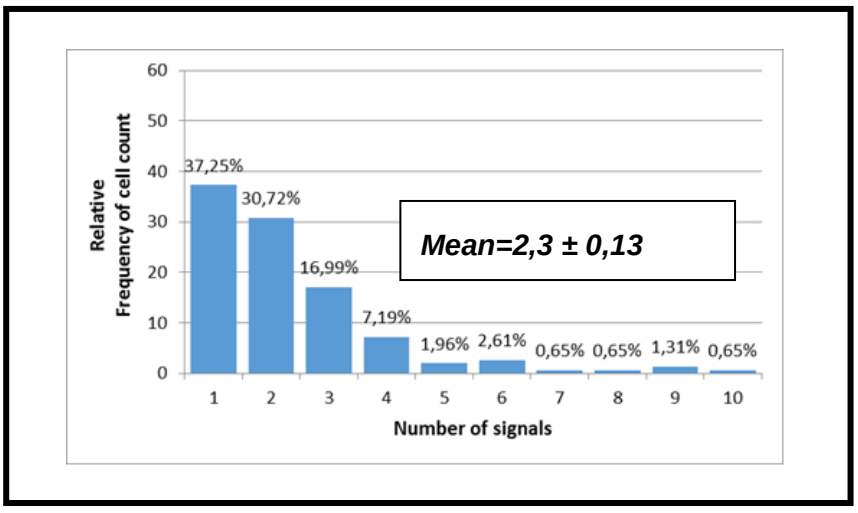
Figure 1: **Transgene mapping on [redacted] chromosomes. Two sites of integration of the transgene are detected and marked with arrows (1 and 2). Two independently prepared probes (Alexa488- or Alexa555- labeled probes) lead to similar results (A and B, respectively). Note that the chromosomes were classified following a chromosome area criterion and not banding pattern.**

3.4 Interphase FISH

Pictures of 168 nuclei were acquired using Zeiss LSM700 fluorescence confocal microscope. Images processing and analysis was performed using the ImageJ software (Open source Java-based image processing program developed at the National Institute of Health, NIH).

Figure 2: Interphase FISH
(A-B) Snapshots of DNA-FISH on interphase nuclei of 549807-MCB1 cells
(C-F) Number of signals per nucleus: C = 1 spot, D= 2 spots, E= 3 spots, F= 4 spots

Figure 3: Relative frequency histogram of the percentage of DNA-FISH signals on interphase nuclei of [redacted]



DNA FISH analyses on metaphase chromosome spreads allowed the detection of two (2) probe signals. This result is in alignment with the result of the interphase FISH analysis (average number of signals is 2,1). However, this does not exclude the possibility that there are other vector sequences integrated elsewhere in the chromosomes that were not detected due to the intrinsic limitation of the method. Indeed, regions specifically targeted by the probe used must be of the size or larger than the probe used. The signals measured for the 2 insertions sites display some differences in intensity and size suggesting some differences in the copy number per insertion site. It could be assessed that site #2 contains 2x to 3x more copies than site # 1.

4 QUANTIFICATION OF INTEGRATED COPIES OF HEAVY AND LIGHT CHAIN BY TAQMAN QPCR

Transgene copy number was analyzed by TaqMan qPCR. Specific primers sets were designed to amplify parts of the VH3 and VK2 sequence; TaqMan probes consisting of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end were designed such that they anneal within the DNA region amplified by the specific set of primers. Fluorescence detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR.

Data were analysed using the Rotor-Gene Q Series Software from Qiagen.

The calculation method used for relative quantification is the comparative CT method. *

Each qPCR value (targets and reference) is the results of four independent measures in two different PCR runs.

Based on Selexis knowledge and the genome assembly of the CHOK1 RefSeq Genome Assembly (Ref Seq Asssembly ID GCF_000223135.1), the reference gene B2M is present at one copy per haploid genome. Therefore all relative concentration values have to be multiplied by 2 in order to get the absolute copy number of the Gene of Interest.

Table 4: Taqman qPCR quantification of integrated heavy and light chain copies

Sample #	Absolute copy number VH3	Absolute copy number VK2
SLX-[redacted]	17.5±4.6	9.6±3.0
CHOM	0	0

* "Analysis of relative gene expression data using real-time quantitative PCR and the 2^[-delta delta C(T)] Method." Livak KJ & Schmittgen TD. Methods 2001 Dec;25(4): 402-408).

5 APPENDIX

5.1 Material for identification of transgene integration sites and their validation

5.1.1 Sample

☐ Fresh cell pellet Lot.

☒ Frozen cell pellet (NGS) Lot. [redacted]

30x10E6 cells [redacted]

5.1.1.1 ***Handling***

☐ Tissue grinding

☒ Freeze/thaw

5.1.1.2 ***gDNA lot***

SLX-[redacted]_ gDNA Lot. SLX-[redacted]

(out of frozen pellet Lot. [redacted],30x10E6 cells [redacted])

5.1.2 Nucleic acid extraction

PBS 1x Lot. SLX06022015-1/C

DNeasy Blood and Tissues kit (Qiagen, Hombrechtikon, CH, # 69506) Lot. 145045205

5.1.3 PCR primers for integration sites validation

215980_LJ_gDNA Forward primer (Microsynth, Balgach, CH) Lot. 2428016

215980_LJ_V Reverse primer (Microsynth, Balgach, CH) Lot. 2428017

215980_RJ_V Forward primer (Microsynth, Balgach, CH) Lot. 2428018

215980_RJ_gDNA Reverse primer (Microsynth, Balgach, CH) Lot. 2428019

23482_LJ_gDNA Forward primer (Microsynth, Balgach, CH) Lot. 2428020

23482_LJ_V Reverse primer (Microsynth, Balgach, CH) Lot. 2428021

23482_RJ_V Forward primer2 (Microsynth, Balgach, CH)	Lot. 2430312
23482_RJ_gDNA Reverse primer (Microsynth, Balgach, CH)	Lot. 2428023
23482_RJ_V Forward primer (Microsynth, Balgach, CH)	Lot. 2428022
20134276_LJ_gDNA Forward primer (Microsynth, Balgach, CH)	Lot. 2428026
20134276_LJ_V Reverse primer (Microsynth, Balgach, CH)	Lot. 2428027
34597_LJ_gDNA Forward primer (Microsynth, Balgach, CH)	Lot. 2428028
34597_LJ_V Reverse primer (Microsynth, Balgach, CH)	Lot. 2428029
34597_RJ_V Forward primer (Microsynth, Balgach, CH)	Lot. 2428030
34597_RJ_V Forward primer2 (Microsynth, Balgach, CH)	Lot. 2438178
34597_RJ_V Forward primer3 (Microsynth, Balgach, CH)	Lot. 2446089
34597_RJ_gDNA Reverse primer (Microsynth, Balgach, CH)	Lot. 2428031
34597_RJ_gDNA Reverse primer2 (Microsynth, Balgach, CH)	Lot. 2438179
34597_RJ_gDNA Reverse primer3 (Microsynth, Balgach, CH)	Lot. 2446090
46670_RJ_V Forward primer (Microsynth, Balgach, CH)	Lot. 2428032
46670_RJ_V Forward primer2 (Microsynth, Balgach, CH)	Lot. 2430313
46670_RJ_V Forward primer2b (Microsynth, Balgach, CH)	Lot. 2438180
46670_RJ_V Forward primer3 (Microsynth, Balgach, CH)	Lot. 2430314
46670_RJ_gDNA Reverse primer (Microsynth, Balgach, CH)	Lot. 2428033
46670_RJ_gDNA Reverse primer2 (Microsynth, Balgach, CH)	Lot. 2438181

5.1.4 PCR material

Pwo SuperYield DNA polymerase, DNTPack	
(Roche, Reinach, CH #04743750001)	Lot. 14111842
PCR machine Biometra,T3 Thermoblock	
(Labgene Scientific SA, Châtel-St-Denis, CH)	Lot. 1800403

5.2 Material for determination of integration sites number

5.2.1 Source of cells

☒ Cryo Tube vial (FISH) Lot. [redacted]
P:04 Vial 214

☐ Fresh cells

5.2.2 FISH material

KaryoMAX® (Colcemid) (Gibco, LuBio Science, Lucerne, CH,#15212012)
Lot. 1688618

Fluoromount G containing DAPI (BioConcept, Allschwil, CH,#0100-20)
Lot. 10615-W325

FISH Tag DNA Multicolor Kit (Molecular Probes, Lubio Science, Lucerne, CH, #F32951)
Lot. 1729887

5.2.3 Imaging

Samples were observed using either Plan-Apochromat 63X/1.4 Oil or Plan-Apochromat 100X /1.4 Oil immersion objective on a Zeiss700 laser scanning microscope (LSM700; Carl Zeiss AG).

5.3 Material for determination of the number of transgene copy integrated in the genome cell line

5.3.1 gDNA

CHOM control Lot. CHOM_131104MA01

SLX-[redacted] Lot. [redacted]
(out of frozen pellet Lot. [redacted] 30x10E6 cells Scp 11 August 2015)

5.3.2 PCR primers and probes for Taqman qPCR

5.3.2.1 *Gene of Interest (GOI)*

qPCR_[redacted] Forward primer (Microsynth, Balgach, CH) Lot. 2487990
qPCR_[redacted] Reverse primer (Microsynth, Balgach, CH) Lot. 2487991
qPCR_[redacted] Probe (Microsynth, Balgach, CH) Lot. 2487992
qPCR_[redacted] Forward primer (Microsynth, Balgach, CH) Lot. 2487993
qPCR_[redacted] Reverse primer (Microsynth, Balgach, CH) Lot. 2487994
qPCR_[redacted] Probe (Microsynth, Balgach, CH) Lot. 2487995

5.3.2.2 *Reference gene used as normaliser : beta-2-microglobulin (B2M)*

qPCR_[redacted] Forward primer2 (Eurofins Genomics, Ebersberg, D) Lot. 020865747
qPCR_[redacted] Reverse primer2 (Eurofins Genomics, Ebersberg, D) Lot. 020865748
qPCR_[redacted] Probe2 (Eurofins Genomics, Ebersberg, D) Lot. 020865749

5.3.3 qPCR material

Rotor Gene Multiplex PCR Master Mix 2x (Qiagen, Hombrechtikon, CH # 1054603)
Lot. 151044386

Strip tubes and caps 0.1mL (Qiagen, Hombrechtikon, CH, # 981103) Lot. 148018084

qPCR machine Rotor-Gene Q 5PLEX (Qiagen, Hombrechtikon, CH, # 9001570)
Lot. R1015108

5.4 Results for identification of transgene integration sites and their validation

5.4.1 Left and right junctions of the integration site 1 (CHOM Scaffold 23482)

5.4.1.1 Left junction in CHOM Scaffold 23482

PCR conditions:

The 690-bp sequenced PCR band corresponding to the left juntion of the integration site was obtained as follows: [redacted] gDNA as DNA template; PCR amplification was performed using the 23482_LJ_gDNA_Fw and 23482_LJ_V_Rv primers.

Sequenced PCR product:

The CHOM scaffold 23482 is in italics and green

The vector sequence in blue corresponds to the SGE

[redacted]

5.4.1.2 Right junction in CHOM Scaffold 23482

PCR conditions:

The 283-bp sequenced PCR band corresponding to the right juntion of the integration site was obtained as follows: SLX-[redacted] gDNA as DNA template; PCR amplification was performed using the 23482_RJ_V_Fw and 23482_RJ_gDNA_Rv primers.

Sequenced PCR product:

The CHOM scaffold 23482 is in italics and green

The underlined vector sequence in blue corresponds to the reverse complement of the 5'end of the EF1alpha promoter

One C base of unknown origin is in red and in bold.

[redacted]

5.4.1.3 *CHOM genomic sequence before vector integration in site 1*

Sequenced genomic DNA fragment for the left junction (PCR validation) is in red and underlined

One genome deletion is in orange (8 bp, ATCTTCTT) and italic

Sequenced genomic DNA fragment for the right junction (PCR validation) is in green and bold

[redacted]

5.4.2 Left and right junctions of the integration site 2 (CHOM scaffold 34597)

5.4.2.1 *Left junctions in CHOM scaffold 34597*

PCR conditions:

The 680-bp sequenced PCR band corresponding to the left junction of the integration site was obtained as follows: [redacted] gDNA as DNA template; PCR amplification was performed using the 34597_LJ_gDNA_Fw and 34597_LJ_V_Rv primers.

Sequenced PCR product:

The CHOM scaffold 34597 is in italics and green.

The exon 17 of the [redacted] is in bold.

The underlined vector sequence in blue starts 98 bp upstream the [redacted] site close to the resistance cassette

[redacted]

5.4.2.2 *Right junction CHOM scaffold 34597*

PCR conditions:

The 277-bp sequenced PCR band corresponding to the right juntion of the integration site was obtained as follows: [redacted] gDNA as DNA template; PCR amplification was performed using the 34597_RJ_V_Fw3 and 34597_RJ_gDNA_Rv3 primers.

Sequenced PCR product:

The CHOM scaffold 34597 is in italics and green.

The underlined vector sequence in blue corresponds to the [redacted] (in italics) and to the 5‘ end of the [redacted] (**start codon ATG in bold**)

[redacted]

5.4.2.3 ***CHOM genomic sequence before vector integration in site 2***

Sequenced genomic DNA fragment for the left junction (PCR validation) is underlined and red

Sequenced genomic DNA fragment for the right junction (PCR validation) is in italics and green

The exon 17 of the [redacted] is in orange in the map and in bold in the nucleotide sequence

[redacted]

5.4.3 Left and right junctions of the integration site 3 (CHOM Scaffold 215980)

5.4.3.1 *Left junction in CHOM Scaffold 215980*

PCR conditions:

The 818-bp sequenced PCR band corresponding to the left juntion of the integration site was obtained as follows: SLX-[redacted] gDNA as DNA template; PCR amplification was performed using the 215980_LJ_gDNA_Fw and 215980_LJ_V_Rv primers.

Sequenced PCR product:

The CHOM scaffold 215980 is in italics and green

The underlined vector sequence in blue corresponds to the reverse complement of SGE, 355 bp from the PvuI site downstream the SGE

[redacted]

5.4.3.2 *Right junction in CHOM Scaffold 215980*

PCR conditions:

The 848-bp sequenced PCR band corresponding to the right juntion of the integration site was obtained as follows: SLX-[redacted] gDNA as DNA template; PCR amplification was peformed using the 215980_RJ_V_Fw and 215980_RJ_gDNA_Rv primers.

Sequenced PCR product:

The CHOM scaffold 215980 is in italics and green

The underlined vector sequence in blue corresponds to the SGE, 247 bp [redacted]

[redacted]

5.4.3.3 *CHOM genomic sequence before vector integration in site 3*

Sequenced genomic DNA fragment for the left junction (PCR validation) is underlined and in red

One genome deletion is in pink and bold (24 bp, [redacted]

Sequenced genomic DNA fragment for the right junction (PCR validation) is in italics and green

[redacted]

5.4.4 Left junction of the integration site 4 (CHOM contig 20134276)

No right junction prediction for this insertion.

5.4.4.1 *Left junction in CHOM contig 20134276*

PCR conditions:

The 557-bp sequenced PCR band corresponding to the left juntion of the integration site was obtained as follows: SLX-[redacted] gDNA as DNA template; PCR amplification was peformed using the 20134276_LJ_g_F and 20134276_LJ_V_Rv primers.

Sequenced PCR product:

The CHOM contig c20134276 completed with the [redacted] *is in italics and green*

The underlined vector sequence in blue corresponds to the reverse complement of SGE, 376 bp [redacted]

[redacted]

5.4.4.2 *CHOM genomic sequence before vector integration in site 4*

Sequenced genomic DNA fragment for the left junction (PCR validation) is underlined and in red

[redacted]

5.4.5 Right junction of the integration site 5 (CHOM scaffold 46670)

No left junction prediction for this scaffold

5.4.5.1 ***Right junction in CHOM scaffold 46670***

PCR conditions:

The 595-bp sequenced PCR band corresponding to the left juntion of the integration site was obtained as follows: SLX-[redacted] gDNA as DNA template; PCR amplification was peformed using the 46670_RJ_V_Fw2 and 46670_RJ_gDNA_Rv2 primers.

Sequenced PCR product:

The CHOM scaffold 46670 is in italics and green

The underlined vector sequence in blue corresponds to the reverse complement of the [redacted]

The 259-bp vector sequence fragment in bold and in red corresponds to the reverse complement of SGE, [redacted]

[redacted]

5.4.5.2 *CHOM genomic sequence before vector integration in site 5*

Sequenced genomic DNA fragment for the right junction (PCR validation) is in italics and green

5.5 Results for determination of integration sites number

Figure 4: Karyotypes analysis. [redacted] karyotypes on [redacted] cells were analyzed and all show 2 insertions on two distinct chromosomes. Some differences are seen in the distribution of chromosomes in these karyotypes since chromosomes were classified following a chromosome area criterion and not banding pattern or morphology.