



# NextSeq™ 500 System User Guide

FOR RESEARCH USE ONLY

ILLUMINA PROPRIETARY

Part # 15046563 Rev. A  
January 2014



# Table of Contents

Table of Contents .....	vii
<b>Chapter 1 Getting Started .....</b>	<b>1</b>
Introduction .....	2
Instrument Components .....	3
Reagent Kit Overview .....	5
User-Supplied Consumables .....	8
Starting the Instrument .....	9
Instrument Software .....	11
Additional Resources .....	22
<b>Chapter 2 Performing a Run .....</b>	<b>23</b>
Introduction .....	24
NextSeq 500 Workflow .....	25
Prepare the Reagent Cartridge .....	27
Prepare the Flow Cell .....	29
Load Libraries onto the Reagent Cartridge .....	32
Set Up a Run on the Instrument .....	33
Monitor the Run .....	42
Automatic Post-Run Wash .....	44
<b>Chapter 3 Primary Analysis .....</b>	<b>45</b>
Primary Analysis Overview .....	46
Analysis Metrics .....	48
Primary Analysis Files .....	50
Flow Cell Tiles .....	52
<b>Chapter 4 Maintenance Procedures .....</b>	<b>57</b>
Maintenance Frequency .....	58
Perform an Instrument Wash .....	59
Software Updates .....	63
Shut Down the Instrument .....	65
<b>Chapter 5 Troubleshooting .....</b>	<b>67</b>

Introduction .....	68
Live Help .....	69
Resolve Pre-Run Check Errors .....	70
System Check .....	72
Index .....	75
Technical Assistance .....	79

# Getting Started

Introduction .....	2
Instrument Components .....	3
Reagent Kit Overview .....	5
User-Supplied Consumables .....	8
Starting the Instrument .....	9
Instrument Software .....	11
Additional Resources .....	22



## Introduction

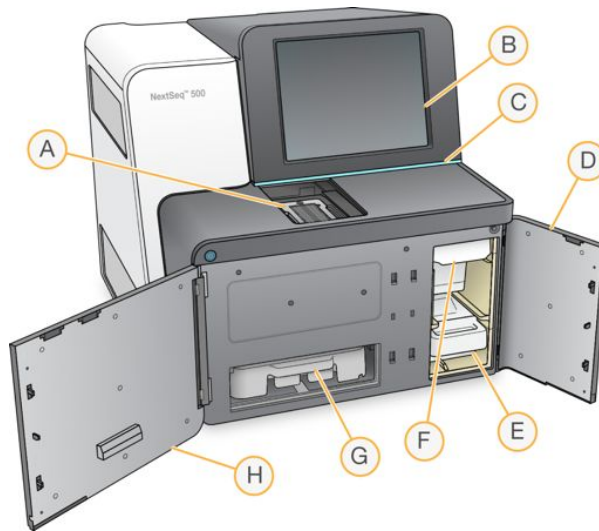
The Illumina® NextSeq™ 500 system combines the power of high-throughput sequencing with the simplicity of a desktop sequencer. The NextSeq 500 enables sequencing of exomes, whole genomes, and transcriptomes while providing the flexibility to perform lower throughput sequencing as needed.

## Features

- ▶ **High-throughput sequencing**—The NextSeq 500 performs whole-genome sequencing, exome sequencing, and transcriptome sequencing and supports TruSeq® and Nextera® libraries.
- ▶ **Flow cell types**—Flow cells are available in configurations that range from 20 Gb to 120 Gb, which provides the option of lower and higher throughput sequencing on a single instrument. Each flow cell type is kitted with a compatible pre-filled reagent cartridge.
- ▶ **Real-time analysis (RTA)**—Integrated primary analysis software performs real-time on-instrument data analysis during the sequencing run, which includes image analysis and base calling, and saves valuable downstream analysis time. The NextSeq uses a new implementation of RTA called RTA2.0, which includes important architecture and feature differences. For more information, see *Primary Analysis* on page 45.
- ▶ **BaseSpace® integration**—The sequencing workflow is integrated with BaseSpace, the Illumina genomics computing environment for data analysis, storage, and collaboration. Before beginning a sequencing run, you can enter library information and run parameters in BaseSpace. Runs that were set up in BaseSpace appear on the software interface for selection during run setup.

During the sequencing run, data can be streamed in real time to BaseSpace. BaseSpace is available on the cloud or on BaseSpace Onsite, an on-premises computing platform.

## Instrument Components



- A Flow cell compartment**—Holds the flow cell throughout the run. The flow cell compartment door automatically slides open and closes as you progress through the run setup steps.
- B Touch screen monitor**—Enables on-instrument configuration and run setup using the control software interface.
- C Status bar**—Uses three colors to indicate instrument status. Blue indicates that the instrument is processing, orange indicates the instrument needs attention, and green indicates that the instrument is ready to begin the next run.
- D Buffer compartment door**—Encloses the buffer compartment, which holds the pre-filled buffer cartridge and the spent reagents tray.
- E Spent reagents tray**—Spent reagents are pumped to the spent reagents tray for disposal after each run.
- F Buffer cartridge**—The buffer cartridge is a pre-filled consumable that provides buffers during the run and wash solution for the automatic post-run wash.
- G Reagent cartridge**—The reagent cartridge is a pre-filled consumable that provides clustering and sequencing reagents for one run.
- H Reagent compartment door**—Encloses the reagent compartment, which holds the pre-filled reagent cartridge.

## Flow Cell Compartment

The flow cell compartment houses the flow cell stage, thermal station, and fluidics connections to the flow cell. The flow cell is positioned over three pins that align the flow cell. When the flow cell door is closed, the flow cell is secured into position.

## Reagent Compartment

The reagent compartment houses the reagent cartridge. During the command to load the reagent cartridge, the software automatically raises the reagent cartridge to engage the sippers. Reagents are pumped through the sippers and fluidics lines, and then to the flow cell.

## Buffer Compartment

The buffer compartment houses the buffer cartridge and spent reagents tray. The software automatically lower sippers into the buffer cartridge to provide reagents at the appropriate time during a run and wash solution during the automatic post-run wash.

Spent reagents are delivered to the spent reagents tray throughout the process.

## Reagent Kit Overview

To perform a run on the Illumina®NextSeq™ 500, you need one single-use NextSeq 500 Kit. Each kit includes one flow cell and the clustering and sequencing reagents required for performing one run.

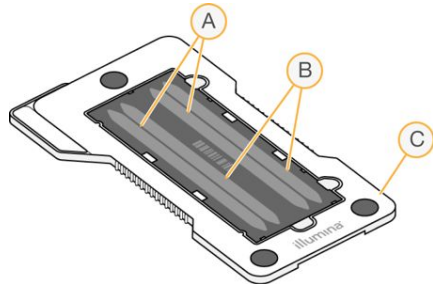
Kit components are labeled with color-coded indicators to show compatibility between flow cells and reagent cartridges. It is important to load the appropriate reagent cartridge depending on the flow cell type that you are using for the sequencing run. The buffer cartridge is universal.

The flow cell, reagent cartridge, and buffer cartridge use radio-frequency identification (RFID) for accurate consumable tracking and compatibility.

For information about kit contents and preparation instructions, see the *NextSeq 500 Kit Reference Guide* (part # 15048775).

## Flow Cell Overview

Figure 1 Flow Cell Cartridge



- A Lane pair A—Lanes 1 and 3
- B Lane pair B—Lanes 2 and 4
- C Flow cell cartridge frame

The flow cell is a glass-based substrate on which clusters are generated and the sequencing reaction is performed. The flow cell is encased in a flow cell cartridge.

The flow cell contains four lanes that are imaged in pairs. Lane pair A (lanes 1 and 3) is imaged at the same time. Lane pair B (lanes 2 and 4) is imaged when imaging of lane pair