



# HiSeq<sup>®</sup> 2500 System User Guide

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# Revision History

Part #	Rev.	Date	Description of Change
15035786	B	November 2013	Restructured content. Replaced the following reagents: <ul style="list-style-type: none"><li>• RMR for RMX</li></ul>
15035786	A	October 2012	Initial release.





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## Introduction

The Illumina HiSeq® System combines innovative engineering with proven SBS technology to set new standards in output, simplicity, and cost-effectiveness.

The HiSeq includes the following features:

- ▶ **Dual Surface Imaging**—The HiSeq uses a four camera epifluorescence system with cutting-edge scanning technology to enable dual surface imaging.
- ▶ **High-Capacity Reagent Chiller**—The reagent compartment is a high-capacity chiller that holds enough reagents for the entire sequencing run.
- ▶ **Integrated Fluidics for Paired-End Runs**—Integrated paired-end fluidics provide reagents from the reagent compartment to the flow cell for Read 2 resynthesis and for indexed sequencing.
- ▶ **Interface Control Options**—The instrument software interface provides options for setting up a run and operating the instrument using the touch screen monitor or the integrated keyboard.
- ▶ **Real-Time Analysis**—The instrument software performs real-time data analysis on the instrument computer, which allows you to monitor important quality metrics during the run and save valuable downstream analysis time. Downstream analysis of sequencing data can be performed with Illumina and third-party software applications, on IlluminaCompute computing and storage appliances, in the BaseSpace genomics cloud solution, or on a custom infrastructure.
- ▶ **BaseSpace Connectivity**—The HiSeq features an option to send instrument health and sequencing data to the BaseSpace genomics cloud solution in real time to streamline both instrument quality control and analysis. Real-time monitoring of runs from anywhere, including Illumina tech support, enables fast troubleshooting. BaseSpace facilitates collaboration by enabling you to share results instantly with anyone anywhere in the world. Free alignment and variant calling and BaseSpace app store provide many workflows that tailor analysis for diverse biological applications.
- ▶ **Dual Flow Cells**—The HiSeq System is equipped with a dual flow cell system. You can sequence a single flow cell or two flow cells with different read lengths simultaneously and start and stop experiments independently.

## Audience and Purpose

This guide contains information about the HiSeq 2500. It provides an overview of instrument components and software features along with instructions for performing a

sequencing run.

This guide is for laboratory personnel and other individuals responsible for the following:

- ▶ Operating the instrument
- ▶ Performing sequencing runs
- ▶ Performing instrument and component maintenance
- ▶ Training personnel on the instrument

## Delivery and Installation

An Illumina-authorized service provider delivers, uncrates, and places the HiSeq 2500 on the lab bench. The space and bench must be ready in advance of delivery. For more information, see Lab Bench Guidelines in the *HiSeq Site Prep Guide*, part # 15006407.



### CAUTION

Allow only Illumina-authorized personnel to uncrate, install, or move the HiSeq 2500. Mishandling of the instrument can affect the alignment or damage instrument components.

An Illumina representative installs and aligns the instrument. If you plan to connect the instrument to a data management system or remote network location, have the path for data storage selected before the date of installation. Having the path already selected allows your Illumina representative to test the data transfer process during installation. For more information, see Network Considerations in the *HiSeq Site Prep Guide*, part # 15006407.



### CAUTION

After your Illumina representative has installed and aligned the HiSeq 2500, **do not** relocate the instrument. Moving the instrument improperly can impact the optical alignment and compromise data integrity. If you need to relocate the HiSeq 2500, contact your Illumina representative.

## Crate Dimensions and Contents

The HiSeq 2500 and its components are shipped in three crates. Use the following dimensions to determine the minimum door width required to accommodate the shipping crates.

- ▶ Crate #1 contains the HiSeq 2500 instrument.
- ▶ Crate #2 contains one uninterruptible power supply (Japan, North America, or International version).
- ▶ Crate #3 contains the following items:
  - Workstation PC, monitor, and monitor arm
  - Waste bottle and reagent racks
  - Instrument panels
  - Power cords—One domestic power cord or three international power cords (UK, EU, and China)

Table 1 Crated Dimensions

Measurement	Crate #1: Instrument	Crate #2: UPS	Crate #3: Workstation, Covers, and Power Cords
Width	164 cm (64.5 in.)	77.5 cm (30.5 in.)	104 cm (41 in.)
Height	121 cm (47.5 in.)	51 cm (20 in.)	99.1 cm (39 in.)
Depth	104 cm (41 in.)	61 cm (24 in.)	81.3 cm (32 in.)
Weight	312 kg (688 lbs.)	80.3 kg (177 lbs.)	125 kg (275 lbs.)

## HiSeq 2500 Configuration and Components

The HiSeq is shipped with a dedicated workstation that is customized to the latest system requirements. The computer workstation is a dedicated subsystem of the instrument and is not intended for use nor supported as a general-purpose workstation.

The HiSeq system comprises the HiSeq instrument. It includes a monitor, a dedicated workstation that is customized to the latest system requirements, a barcode scanner, a keyboard, a mouse, and a universal power supply.

Figure 1 HiSeq 2500 Main Compartments



- A Optics Module—Contains optical components that enable dual surface imaging of the flow cell, imaging A, C, G, and T at the same time using epifluorescence. The excitation laser beam passes through the objective and the fluorescence is collected through the same objective.
- B Flow Cell Compartment and Sample Loading Station—Contains the vacuum-controlled flow cell stage. The flow cell stage holds the flow cell in place during sequencing runs as well as the sippers that enable the introduction of the sample for the rapid run mode of operation.
- C Fluidics Compartment—Contains fluidics pumps that deliver reagents to the flow cell, and then to the waste container.
- D Status Bar—Uses three colors to indicate instrument status. Blue indicates that the instrument is running, orange indicates the instrument needs attention, and green indicates that the instrument is ready to begin the next run.



- E** Reagent Compartment—Contains racks that hold reagents for sequencing runs, or wash solution for instrument washes.



**NOTE**

Tubes with screw caps cannot be used in the Sample Loading Station.

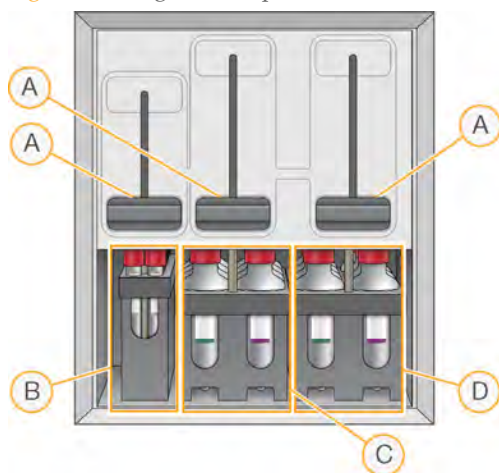
To set up a sequencing run on the HiSeq 2500, access the reagent compartment and the flow cell compartment.

## Reagent Compartment

The reagent compartment is a high-capacity reagent chiller that holds three reagent racks: two for SBS reagents and one for cluster and paired-end reagents. Sipper handles lower the sippers into the reagent bottles.

- ▶ **SBS Reagent Racks**—SBS reagent racks hold 250 ml conical bottles. The reagent rack for flow cell A is located in the center position, and the rack for flow cell B is located in the far right position. Each reagent rack has numbered positions that correspond to connections on the fluidics pump.
- ▶ **Cluster, Paired-End, and Index Reagent Rack**—The cluster and paired-end reagent rack is located to the left of racks A and B. It has two rows of numbered positions that hold 15 ml conical tubes containing cluster and paired-end reagents and indexing reagents. The left row is for flow cell A, and the right row is for flow cell B.
- ▶ **Reagent Chiller**—The reagent chiller is designed to maintain reagent temperature at 4°C. The reagent chiller is not intended to cool down reagents. Reagents must be cooled before they are loaded onto the instrument.

Figure 2 Reagent Compartment

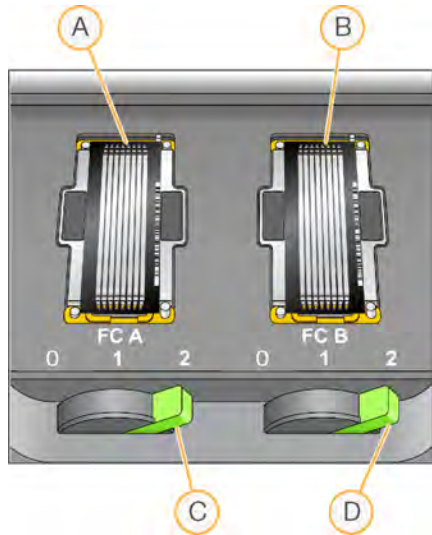


- A Sipper Handles
- B Reagent Rack for Clustering, Paired-End, or Indexing Reagents
- C Reagent Rack for SBS Reagents, Flow Cell A
- D Reagent Rack for SBS Reagents, Flow Cell B

## Flow Cell Compartment

The flow cell compartment houses the flow cell stage, the thermal stations, the vacuum system, and the fluidics connections to each flow cell.

Figure 3 Flow Cell Stage With High Output Flow Cells



- A Flow Cell A
- B Flow Cell B
- C Flow Cell Lever A
- D Flow Cell Lever B

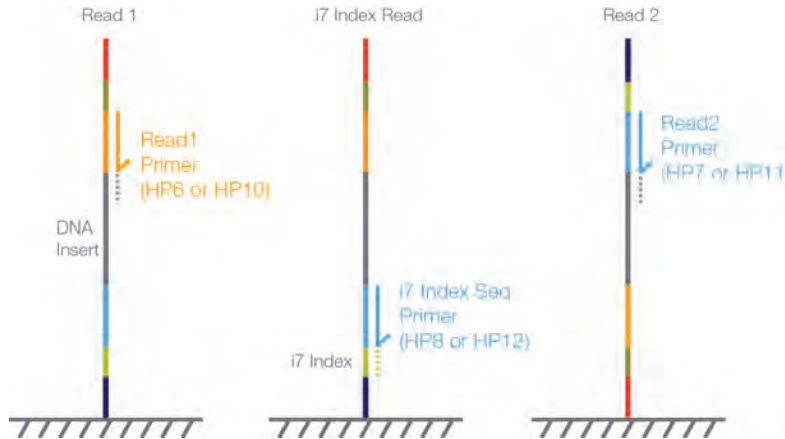
The flow cells are seated on the flow cell stage, which moves in and out of the optics module. For the dual flow cell system in the HiSeq 2500, flow cell A is on the left, and flow cell B is on the right. The flow cell is positioned on the flow cell holder with the inlet and outlet ports facing down, and held in place by a vacuum. The illuminated flow cell lever in front of each flow cell holder controls the vacuum. The flow cell lever turns green when the vacuum seal is secure.

You cannot open the flow cell compartment if the flow cell stage is not in forward-most position. The HiSeq Control Software controls the flow cell stage. The software positions the flow cell stage in the forward-most position when it prompts you to load or remove a flow cell.

# Single-Indexed Sequencing Overview

Single-indexed sequencing includes one Index Read following Read 1. The details of the workflow depend on whether you are running high output or rapid run mode.

Figure 4 Single-Indexed Sequencing



## Read 1: High Output

Read 1 follows the standard Read 1 sequencing protocol using reagents provided in the TruSeq SBS Kit. The Read 1 sequencing primer is annealed to the template strand during the cluster generation process on the cBot.

- ▶ When sequencing dual-indexed Nextera libraries, use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP10**. HP10 is used on the cBot in place of HP6. For more information, see the *cBot User Guide*, part # 15006165.
- ▶ When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP6 or HP10.

## Read 1: Rapid Run

Read 1 preparation is performed entirely on the HiSeq instrument, including sample loading, cluster generation, and sequencing primer annealing. The Read 1 sequencing primer HP6, included in TruSeq Rapid Cluster Generation Kits, is compatible with Nextera, TruSeq LT/v2, dual-indexed TruSeq HT libraries, TruSeq Custom Amplicon, and TruSeq Amplicon-Cancer Panel.

## Index Read Preparation: High Output

The Read 1 product is removed and the Index 1 (i7) sequencing primer is annealed to the same template strand, producing the Index 1 (i7) Read.

- ▶ When sequencing dual-indexed, use the appropriate sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP12**, which you use in place of HP8.
- ▶ When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP8 or HP12.

## Index Read Preparation: Rapid Run

The Read 1 product is removed and the Index 1 (i7) sequencing primer HP12 is annealed to the same template strand producing the Index 1 (i7) Read. HP12 is included in the TruSeq Rapid Cluster Generation Kits and is compatible with dual-indexed Nextera, TruSeq LT/v2, dual-indexed TruSeq HT libraries, TruSeq Custom Amplicon, and TruSeq Amplicon-Cancer Panel.

## Index 1 (i7) Read

Following Index Read preparation, the Index 1 (i7) Read is performed.

- ▶ When sequencing single-indexed libraries, the Index Read consists of seven cycles of sequencing.
- ▶ When sequencing dual-indexed libraries (Nextera or TruSeq HT), the Index Read consists of eight cycles of sequencing.

## Read 2 Resynthesis

The Index Read product is removed and the original template strand is used to regenerate the complementary strand. Then, the original template strand is removed to allow hybridization of the Read 2 sequencing primer.

- ▶ When sequencing dual-indexed Nextera libraries, use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP11**, which you use in place of HP7.
- ▶ When sequencing single-indexed TruSeq LT/v2 libraries or dual-indexed TruSeq HT libraries, you can use either HP7 or HP11.
- ▶ For rapid run mode, the Read 2 sequencing primer HP7 is contained within the TruSeq Rapid Cluster Generation Kit. HP7 is compatible with dual-indexed Nextera, TruSeq LT/v2 and dual-indexed TruSeq HT libraries.

## Read 2

Read 2 follows the standard paired-end sequencing protocol using reagents provided in the TruSeq SBS Kit.

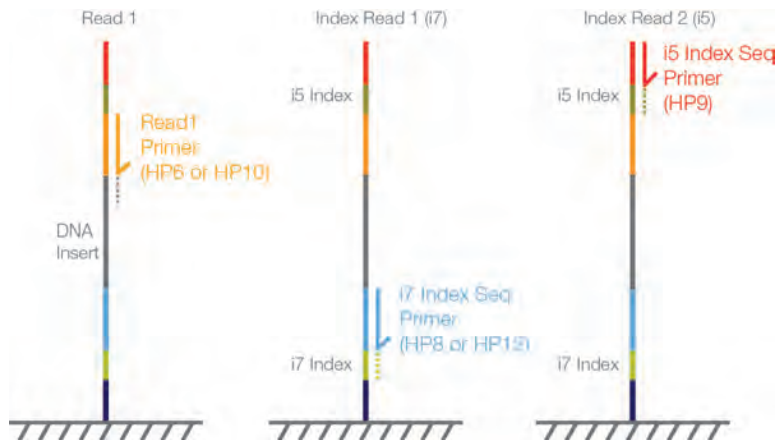
# Dual-Indexed Sequencing Overview



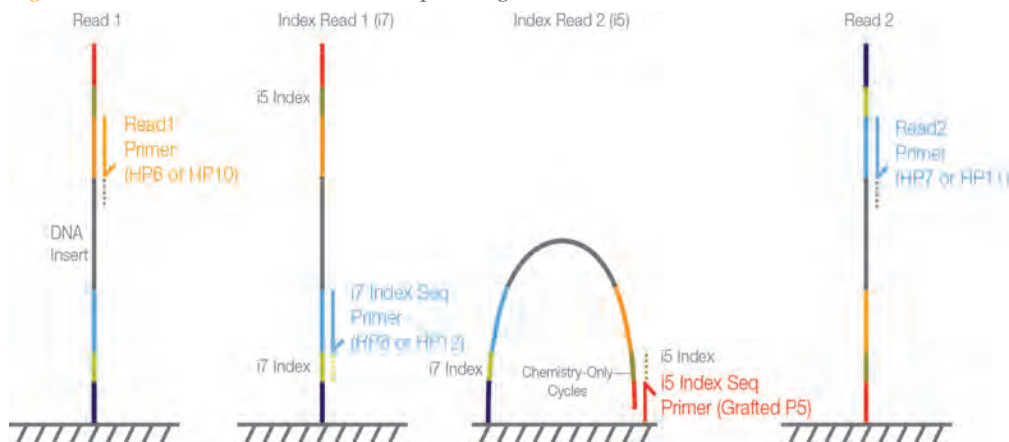
**NOTE**  
Performing dual-indexed sequencing runs requires HiSeq Control Software (HCS) v1.5, or later.

Dual-indexed sequencing includes two Index Reads following Read 1.

**Figure 5** Dual-Indexed Single-Read Sequencing



**Figure 6** Dual-Indexed Paired-End Sequencing



## Read 1: High Output

Read 1 follows the standard Read 1 sequencing protocol using reagents provided in the TruSeq SBS Kit. The Read 1 sequencing primer is annealed to the template strand during the cluster generation process on the cBot.

- ▶ When sequencing dual-indexed Nextera libraries, use the appropriate sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP10**. This is used on the cBot in place of HP6. For more information, see the *cBot User Guide*, part # 15006165.
- ▶ When sequencing dual-indexed TruSeq HT libraries, you can use either HP6 or HP10.

## Read 1: Rapid Run

Read 1 preparation is performed entirely on the HiSeq instrument, including sample loading, cluster generation, and sequencing primer annealing. The Read 1 sequencing primer HP6, included in TruSeq Rapid Cluster Generation kits, is compatible with Nextera, TruSeq LT/v2, dual-indexed TruSeq HT libraries, TruSeq Custom Amplicon, and TruSeq Amplicon-Cancer Panel.

## Index Read Preparation: High Output

The Read 1 product is removed and the Index 1 (i7) sequencing primer is annealed to the same template strand, producing the Index 1 (i7) Read.

- ▶ When sequencing dual-indexed Nextera libraries, use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP12**, which you use in place of HP8.
- ▶ When sequencing dual-indexed TruSeq HT libraries, you can use either HP8 or HP12.

## Index Read Preparation: Rapid Run

The Read 1 product is removed and the Index 1 (i7) sequencing primer HP12 is annealed to the same template strand producing the Index 1 (i7) Read. HP12 is included in the TruSeq Rapid Cluster Generation Kits and is compatible with dual-indexed Nextera, TruSeq LT/v2, dual-indexed TruSeq HT libraries, TruSeq Custom Amplicon, and TruSeq Amplicon-Cancer Panel.



## Index 1 (i7) Read

Following Index Read preparation, the Index 1 (i7) Read is performed. When sequencing dual-indexed libraries (Nextera or TruSeq HT), the Index Read consists of eight cycles of sequencing.

## Index 2 (i5) Read

The process for Index 2 (i5) Read is different for runs performed on single-read flow cells than for runs performed on paired-end flow cells:

- ▶ **Single-Read Flow Cells**—The Index 1 (i7) Read product is removed and the Index 2 (i5) Sequencing Primer Mix, **HP9**, is annealed to the same template strand. HP9 is provided in the TruSeq Dual Index Sequencing Primer Box and required for dual-indexed runs on single-read flow cells regardless of the library type (Nextera or TruSeq HT). The run proceeds through eight cycles of sequencing.
- ▶ **Paired-End Flow Cells**—The Index 1 (i7) Read product is removed. The Resynthesis Mix, **RMR**, deprotects the grafted P5 primer on the surface of the flow cell and the template anneals to the grafted P5 primer. The run proceeds through an additional seven chemistry-only cycles (no imaging occurs), followed by eight cycles of sequencing.
- ▶ For rapid run mode, index 2 (i5) sequencing primer HP9 is required for single read flow cells only. HP9 is included in the TruSeq Rapid SR Cluster Generation Kit.

## Read 2 Resynthesis

The Index Read product is removed and the original template strand is used to regenerate the complementary strand. Then, the original template strand is removed to allow hybridization of the Read 2 sequencing primer.

- ▶ When sequencing dual-indexed Nextera libraries, use the sequencing primer provided in the TruSeq Dual Index Sequencing Primer Box, **HP11**, which you use in place of HP7.
- ▶ When sequencing dual-indexed TruSeq HT libraries, you can use HP7 or HP11.
- ▶ For rapid run mode, HP7 is provided in the TruSeq Rapid PE Cluster Generation Kit and compatible with all libraries.

## Read 2

Read 2 follows the standard paired-end sequencing protocol using reagents provided in the TruSeq SBS Kit.

## Dual-Indexed Libraries

Dual-indexed libraries are libraries prepared with adapters containing two indices:

- ▶ Index 1 (i7), an eight-base index adjacent to the P7 strand
- ▶ Index 2 (i5), an eight-base index adjacent to the P5 strand

During sample preparation, up to 12 unique Index 1 sequences are arrayed across the columns of a 96-well plate. And, up to eight unique Index 2 sequences are arrayed down the rows. This creates up to 96 uniquely dual-indexed adapters.

## Sample Sheet Overview

The sample sheet is a user-generated file in \*.csv format that stores information about a sequencing run. The sample sheet is copied into the run folder and later used for analysis.

Using a sample sheet is optional. If you choose to use a sample sheet, create one *before* you start the run.



### NOTE

To view indexing results in Sequencing Analysis Viewer (SAV), you must load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to report indexing information. See the *Sequencing Analysis Viewer User Guide*, part # 15020619.

You can create your sample sheet in various software applications such as Excel or Notepad, or use the Illumina Experiment Manager.

Use the following guidelines when creating your sample sheet:

- ▶ All index sequences are specified in the Index field. If you are doing dual-indexed reads, you must separate all indices by a hyphen: ACCAGTAA–GGACATGA.
- ▶ If a sample does not contain an index sequence, leave the Index field blank.
- ▶ For a sample sheet to be valid, every cycle in an Index Read for every lane *must* have at least one base in the red channel (A,C) and one base in the green channel (G,T). This ensures proper image processing. Correct secondary analysis cannot be performed with an invalid sample sheet.

## Available Disk Space

During the run at the end of imaging each lane, the software checks available disk space on the local D: and E: drives. The software does not check the network location during the run. If disk space drops below the safe threshold, the software pauses the run and places the flow cell in a safe state.



### NOTE

Illumina recommends that you specify a network location for the output run folder. The output run folder is specified on the Storage screen. For more information, see *Storage Screen* on page 82.

If the disk space becomes low, make disk space available to continue the run. When sufficient disk space becomes available, the run automatically resumes.

The HiSeq 2500 instrument computer has a storage capacity of 2.7 TB per flow cell. This is enough space to hold a 209-cycle run of a high output flow cell if images are not saved to disk. Data from flow cell A is stored on the D: drive, while data from flow cell B is stored on the E: drive.

## Sequencing Consumables

Illumina-supplied consumables are required for sequencing on the HiSeq 2500. The kits that you need depend on the type of run you perform and the type of libraries you are sequencing.

- ▶ For any type of run, you need at least one SBS kit. If you are performing a rapid run, use a rapid run SBS kit.
- ▶ For indexed runs, you need one SR or PE cluster kit. If you are performing a rapid run, use a rapid run cluster kit.
- ▶ For paired-end runs, indexed or non-indexed, you need one PE cluster kit. If you are performing a rapid run, use a rapid run cluster kit.
- ▶ Some library types require sequencing primers that are provided in the TruSeq Dual Index Sequencing Primer Box. For more information, see the reagent preparation documentation associated with the cluster kit that you are using for your run.

## User-Supplied Consumables

### Consumables

Consumable	Supplier	Purpose
1 N NaOH	General lab supplier	Instrument maintenance wash.
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 15648-981  General lab supplier	Cleaning the flow cell and flow cell stage.
Centrifuge tubes, 250 ml	Corning, catalog # 430776	Instrument maintenance wash and water wash.
Conical tubes, 15 ml	Corning, catalog # 430052	Collecting and measuring waste volumes.
Disposable gloves, powder-free	General lab supplier	General use.
Lab tissue, low-lint	VWR, catalog # 21905-026	Cleaning the flow cell holder.
Lens paper, 4 x 6 in.	VWR, catalog # 52846-001	Cleaning the flow cell.
Water, laboratory-grade, 18 M Ohm	Millipore	SBS reagent rack, position 2. Water wash.

### Microtubes

The following microtubes are acceptable for use with the sample loading station for rapid runs.

Consumable	Supplier	Purpose
Microcentrifuge Tube, 1.5 mL	VWR, catalog #20170-038	Sample(template) loading and washing the template fluidic line.
Microcentrifuge Tube, 1.7 mL, capless	VWR, catalog #20170-575	Sample(template) loading and washing the template fluidic line.
Microcentrifuge Tube, 1.5 mL	VWR, catalog #20170-650	Sample(template) loading and washing the template fluidic line.
Microcentrifuge Tube, 1.5 mL	VWR, catalog #89000-028	Sample(template) loading and washing the template fluidic line.
Microcentrifuge Tube, 1.5 mL	Axygen, catalog #MCT-150-C	Sample(template) loading and washing the template fluidic line.
Microcentrifuge Tube, 1.7 mL	Axygen, catalog #MCT-175-C	Sample(template) loading and washing the template fluidic line.
Microcentrifuge Tube, 1.7 mL	Sorenson BioScience, catalog #16070	Sample(template) loading and washing the template fluidic line.

## Start the HiSeq 2500

Follow best practices when you turn on, turn off, and operate the computer:

- ▶ Do not power up the computer while the instrument is on.
- ▶ Allow the instrument to be off for a full minute before turning it back on.
- ▶ Never turn off the instrument while the instrument software is running.
- ▶ Connect the instrument USB cable, monitor USB, and keyboard USB to the back of the server; connect the barcode scanner and mouse to the front of the server.
- ▶ Do not use port manager to delete ports.

Use the following instructions to start the HiSeq 2500 and instrument computer.

- 1 Start the instrument control computer.
- 2 Log on to the operating system using the default user name and password. Wait until it has loaded.
  - User name: sbsuser
  - Password: sbs123


If the default values do not work, consult your IT personnel for the user name and password for your site.

- 3 Switch the main power switch to the ON position. If you are facing the front of the instrument, the power switch is on the left side.
- 4 Wait for the instrument drive called “DoNotEject” to initialize. A window opens when the drive is initialized. Close the window.



### NOTE

Never eject the “DoNotEject” flash drive located inside the instrument chassis, or modify the files on it. This drive contains hardware configuration files and initializes whenever the instrument is turned on.

- 5 To ensure adequate disk space, archive the data on the instrument computer from previous runs to a network location.
- 6 Open HCS using the shortcut icon on the computer desktop. HCS takes a few minutes to initialize. When the software is initialized, the Welcome screen opens and the initialization icon  appears on the bottom left of the screen.

## HiSeq Software

Three software applications are installed on the instrument computer:

- ▶ **HiSeq Control Software**—The HiSeq Control Software (HCS) interface guides you through the steps to specify run parameters, and load the flow cell and reagents before starting the run. During the run, HCS provides a summary of quality statistics throughout the run.
- ▶ **Real-Time Analysis**—The integrated primary analysis software, Real-Time Analysis (RTA), performs base calling and assigns a quality score to each base for each cycle. For more information, see *Real-Time Analysis Introduction* on page 124.
- ▶ **Sequencing Analysis Viewer**—The Sequencing Analysis Viewer (SAV) provides detailed quality statistics and updated metrics as the run progresses. For more information, see *Sequencing Analysis Viewer* on page 125.

### HiSeq Control Software (HCS) Interface

The HCS interface opens to run configuration screen.

From here, you can select the Rapid Run or High Output run configuration. When you change the mode, perform a gasket change and maintenance wash. HCS guides you through the process. Be sure to allow sufficient time for this.

After you have selected a mode and completed any required wash steps, the Welcome screen opens for the mode you have selected. Both runs must be in the same mode. The software returns to the Welcome screen of the given mode when a run is complete.

The Welcome screen is split into two panels, one for flow cell A and one for flow cell B. You can set up a run for flow cell A and flow cell B in parallel using the software interface.



Figure 7 HCS Interface, Welcome Screen for Rapid Run Mode



- A Welcome Screen Menu Button
- B Interface Panel for Flow Cell A
- C Interface Panel for Flow Cell B
- D Activity Indicators

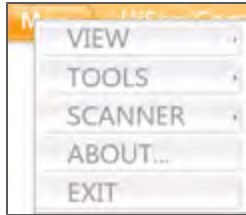
## Welcome Screen Menu

The Welcome screen menu button, located in the upper-left corner of the Welcome screen, provides the following options:

- ▶ **View**—Provides options to view the interface in full screen or in a window, or to minimize the interface.
- ▶ **Tools**—Provides access to the Options window and Show Log file:
  - **Options**—From the Options window, you can define the run Id template, default folder locations, and the LIMS server, user name, and password. See *Menu Options Window* on page 24.
  - **Show Log File**—The Show Log file is a text file used for troubleshooting.
- ▶ **Scanner**—Activates the command to initialize the software manually.

- ▶ **About**—Provides information about instrument hardware, software versions, and technical support contact information.
- ▶ **Exit**—Closes the HCS interface.

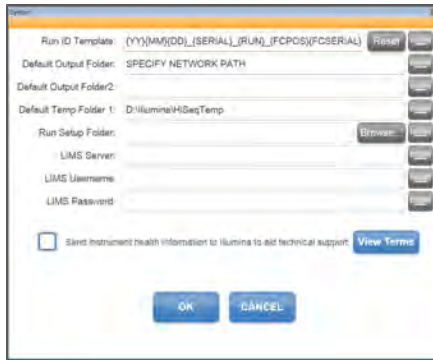
Figure 8 Menu Button



## Menu Options Window

From the Options window, you can define the run ID template, default folder locations, and the LIMS server, user name, and password.

Figure 9 Menu Options Window



**Run ID Template**—The name used to name a run folder

**Default Output Folder**—The default location for flow cell A runs to be saved. This can be changed on a per run basis

**Default Output Folder2**—The default location for flow cell B runs to be saved. This can be changed on a per run basis

**Default Temp Folder1**—The location to which temporary files can be written during a run

**Run Setup Folder**—Where the HiSeq picks up LIMS sample forms

**LIMS Server**—The server name for interactions with supported Illumina LIMS

**LIMS Username**—The LIMS user name HiSeq uses when authenticating to Illumina LIMS

**LIMS Password**—The LIMS password HiSeq uses when authenticating to Illumina LIMS

**Send instrument health information to Illumina to aid technical support**—Gives permission to the instrument to send information to BaseSpace for each run. All information remains confidential. Illumina recommends that you keep this feature enabled and never shares your information. Run sample data is sent only if you specifically indicate on the Storage screen that you want to send run sample data. See *Storage Screen* on page 82 for more information.

## Start Screen Options

The options on the Welcome screen are as follows:

- ▶ Sequence
- ▶ Wash
- ▶ Check
- ▶ Mode

**Sequence**—Select **Sequence** to begin the steps to set up a sequencing run. From the Sequence button, you can start a new run or resume an existing run.

Figure 10 Sequence Command Options



- **New Run**—The software guides you through setting run parameters, loading and priming reagents, loading the flow cell, performing fluidics checks, and starting the run.
- **Resume Run**—The software guides you through selecting the existing run folder and setting parameters for resuming the run.

**Wash**—Select **Wash** to initiate an instrument wash. From the Wash button, you can start a Water Wash or a Maintenance Wash.

Figure 11 Wash Command Options



- **Water Wash**—The water wash flushes water through the system. A water wash is recommended before you start a new run if the instrument has been idle for one day or more. For more information, see *Perform a Water Wash* on page 117.
- **Maintenance Wash**—The maintenance wash flushes water, then NaOH, and then water through the system. This wash is recommended after each sequencing run in high output mode and every 10 days in rapid run mode. *Performing an Instrument Maintenance Wash* on page 112



#### NOTE

Regardless of which mode you are running on the HiSeq 2500/1500, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.

**Check**—Select **Check** to open the fluidics check screen and confirm proper flow during instrument installation or fluidics troubleshooting.

**Mode Select**—Select **Mode Select** to return to the run configuration screen.

## Activity Indicators

The Welcome screen contains a series of icons in the lower-right corner of the screen that indicate which activity the instrument is performing.

**Figure 12** Activity Indicators. From left to right, they represent the X, Y, and Z motors, electronics functionality, the camera, the fluidics system, and processing functions.








**Figure 13** Activity Indicators. From left to right, they represent flow cell A temperature, reagent chiller temperature, BaseSpace cloud status, and flow cell B temperature.



## Status Icons

Status icons are located in the upper-right corner of each screen, and notify you of any change in conditions during run setup or during the run.

Status Icon	Status Name	Description
	Status OK	No change. System is normal.
	Information	Information only. No action is required
	Attention	Information that might require attention.
	Warning	Warnings do not stop a run, but might require action before proceeding.
	Error	Errors usually stop a run and generally require action before proceeding with the run.

When a change in condition occurs, the icon changes and blinks to alert you. If this happens, select the icon to open the status window, which contains a general description of the condition. Select **Acknowledge** to accept the message and **Close** to close the dialog box. You can filter the types of messages that appear in the status window by selecting the icons along the top margin of the window. Selecting an icon toggles the condition to show or to hide.



#### NOTE

For more information about known issues related to errors and warnings, see the release notes for the version of instrument control software you are using.

## Switch Sequencing Modes

When you switch from high output mode to rapid run mode and rapid run to high output mode, a maintenance wash and gasket change are required before you start a new run. If the instrument has been left idle for 10 days or more before switching between modes, a maintenance wash is required before you set up a run. This is the case regardless of the mode that you select. HCS guides you through the process.

Regardless of which mode you are running, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.

To change from the current mode, use the mode select option on the HCS software main page. You can change modes only when there are no active runs on the system. The mode select function is disabled while a sequencing run is in progress. All flow cells need to complete all mode change procedures in order for the system to enter the new mode properly.

Because a wash was run at the end of the high output run, when you switch to rapid run from high output, only a water wash is required. You can use clean, Milli-Q grade (18 M $\Omega$ ) water in place of PW1 for system washes.

The expected fluidics output volumes are the specified volumes +/- 10%.

### Switch to High Output Mode

After a rapid run is finished, perform the required post-run water wash (or maintenance wash if after 10 days). The software automatically guides you through this.

When switching modes, both flow cells must fully complete the mode switch washes. If the mode wash is not carried out for one or both of the flow cells, the software does not let you switch back to the previous mode without completing the full mode wash.

If you are going to leave one position idle after a mode switch, the mode switch wash can be carried out when switching to the current mode or just before switching back to the previous mode.

If one of the positions is canceled during the mode switch wash, complete the mode wash before switching back to the previous mode.

When you want to switch to high output mode when a maintenance wash is required after a rapid run is complete, cancel the rapid run maintenance wash and switch immediately to high output mode. This mitigates additional washing. However, complete the necessary gasket change, rapid run maintenance wash, and high output maintenance wash after you choose to switch to high output mode.

**CAUTION**

A gasket change and a rapid run maintenance wash followed by a high output maintenance wash (to condition the gasket) are required when you change the mode to high output.

To switch to high output mode, navigate to the Home Screen and select **MODE SELECT**. This takes you to the Run Configuration screen. Select **HIGH OUTPUT**. A warning message appears to remind you of the following:

- ▶ Change gaskets.
- ▶ Carry out a full rapid run maintenance wash using a rapid run flow cell before the software switches to high output mode.

Once in high output mode, perform a full high output maintenance wash.

**Table 2** Maintenance Wash Volumes (volumes are +/- 10%)

Flow Cell Type	Run Mode	
	Rapid (2 lane)	High Output (8-lane)
Flow Cell Gasket	10-port	8-port or 10-port
Reagents	PW1 > 1 N NaOH > PW1	PW1 > 1 N NaOH > PW1
Expected Per Flow Cell Volumes (ml)	$19 + 9.5 + 19 = 47.5$	$72 + 36 + 72 = 180$
Time (minutes)	$13 + 6 + 13 = 32$	$60 + 30 + 60 = 150$
Total Mode Change Time	~ 3 hours	

After selecting **OK**, the software automatically prompts you to change the gaskets before the rapid run maintenance wash only. Place waste lines 4 and 5 into a 50 ml tube to before starting the wash.

**NOTE**

If you cancel this rapid run maintenance wash, the software remains in rapid run mode. If you want to remain in rapid run mode, a water wash is required before starting a new rapid run. If you still want to switch to high output mode, the required rapid run maintenance wash and high output maintenance wash must be completed.

Once in high output mode, perform a full high output maintenance wash to condition the gaskets. The software automatically guides you through this. During the fluidics check, make sure that all 8 syringes are at least 75% full of water before you start the wash. Use

the manual pump function when setting up the flow cell to pump water through the flow cell manually. Continue to use this manual function until all 8 syringes are at least 75% full of water before you start the wash. A small amount of air is not a problem as the air disperses during the maintenance wash. Gaskets do not need to be changed before the high output maintenance wash. Select whether you want to wash the PE positions. Place waste lines 1–8 into an appropriate container to measure the volumes used.



**NOTE**

If you cancel the wash after the rapid run maintenance wash is complete or at any point after the software is in high output mode (displayed at top of screen), the system remains in high output mode. A high output maintenance wash must be completed before starting a run in high output mode.

After all washes are complete, you can start a new high output run.

## Switch to Rapid Run Mode

After a high output run is completed, change the gaskets and perform the required post-run maintenance wash. The software automatically guides you through this. A high output maintenance wash takes approximately 2 hours and 30 minutes. Make sure that the volume is 180 ml +/- 10% per lane. Perform this wash before starting the switching process.

To switch to rapid run mode, navigate to the Welcome Screen, and select **MODE SELECT**. This takes you to the Mode Select screen. Select **RAPID RUN**.



**NOTE**

If you cancel this wash, the software does not let you switch modes until the wash is completed.

A warning message appears telling you that a maintenance wash must be performed before a new run can be started in rapid run mode.

**Table 3** Maintenance Wash Volumes (volumes are +/- 10%)

	Run Mode
Flow Cell Type	Rapid (2 lane)
Flow Cell Gasket	10-port
Reagents	PW1 > 1 N NaOH > PW1



	Run Mode
Expected Per Flow Cell Volumes (ml)	$19 + 9.5 + 19 = 47.5$
Time (minutes)	$13 + 6 + 13 = 32$
Total Mode Change Time	~ 35 minutes

The software automatically guides you through a rapid run maintenance wash using a rapid run flow cell. Place waste lines 4 and 5 into a 50 ml tube. The wash takes ~30 minutes.



**NOTE**

You cannot start a new rapid run in rapid run mode until the required water wash is completed.

After the wash is finished, the software automatically brings up the welcome screen. Select **SEQUENCE** and then **NEW RUN** to start a rapid run.



# Perform a High Output Sequencing Run

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## Introduction

To perform a high output sequencing run on the HiSeq 2500, you first prepare reagents for your run and then set up the run using the HCS interface. Run setup steps include: entering run parameters, loading and priming reagents, loading the high output flow cell, and performing a fluidics check.

- ▶ Reagents for Read 1 and the Index Reads are loaded during run setup before starting the run.
- ▶ Reagents for Read 2 resynthesis are loaded after the completion of Read 1 and the Index Reads.
- ▶ Illumina recommends that you prepare and load fresh ICB for Read 2.

### Types of High Output Runs

You can perform six types of high output runs on the HiSeq 2500:

Run Type	Read 1 Cycles	Index 1 (i7) Read Cycles	Index 2 (i5) Read Cycles	Read 2 Cycles	Total Cycles
Single-Read, Non-Indexed	≤ 101	--	--	--	≤ 101
Single-Read, Single-Indexed	≤ 101	7 * 8 **	--	--	≤ 108 * ≤ 109 **
Single-Read, Dual-Indexed	≤ 101	8	8	--	≤ 117
Paired-End, Non-Indexed	≤ 101	--	--	≤ 101	≤ 202
Paired-End, Single-Indexed	≤ 101	7 * 8 **	--	≤ 101	≤ 209 * ≤ 210 **
Paired-End, Dual-Indexed	≤ 101	8	7 + 8 ***	≤ 101	≤ 225

\* Number of cycles for single-indexed libraries

\*\* Number of cycles for dual-indexed libraries

\*\*\* Index 2 Read of a paired-end dual-indexed run includes seven additional chemistry-only cycles

For more information, see *Single-Indexed Sequencing Overview* on page 10 and *Dual-Indexed Sequencing Overview* on page 13.

## Stagger Runs

The HiSeq 2000 and 2500 enable you to start and stop experiments independently on sides A and B and run staggered runs continuously. Before you begin the process of loading a new flow cell while an adjacent flow cell is running, pause the current run in progress.

- 1 Make sure that the run on the adjacent flow cell is performing a chemistry step, and then select **Pause**. The Pause menu opens.

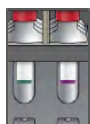


### NOTE

It is safest to pause the current flow cell run while in a chemistry and not an imaging step.

- 2 Select **Normal Pause**.
- 3 Wait for the software to complete the current chemistry step. The system is automatically placed in a safe state.
- 4 After the adjacent flow cell run has been paused successfully, set-up the new flow cell on the other lane of the system.
- 5 After you load the new flow cell, close the compartment door.
- 6 Select **Start** to start the new sequencing run.
- 7 Select **Resume** on the adjacent flow cell to continue the sequencing run on that side. The software automatically controls chemistry and imaging processes on both flow cells through the end of specified sequencing experiments. After each run ends, normal wash procedures are required.

## High Output Sequencing Workflow (8-Lane Flow Cell)



Prepare SBS reagents. This includes thawing and inverting all reagents. For details about SBS reagents, see the SBS reagent prep guides for the reagents that you use for your experiment. To find the guides, go to the Illumina website: [support.illumina.com/sequencing/kits.ilmn](http://support.illumina.com/sequencing/kits.ilmn).

### For indexed runs:

Prepare indexing reagents. Make sure that you prepare the appropriate sequencing primers for your library and type of run. For details about indexing reagents, see the indexing reagent prep guides for the reagents that you are using for your experiment. To find the guides, go to the Illumina website: [support.illumina.com/sequencing/kits.ilmn](http://support.illumina.com/sequencing/kits.ilmn).

Using HCS, enter parameters for your run.

When prompted, load SBS reagents for Read 1 and Read 2, except ICB (recommended).

### For indexed runs:

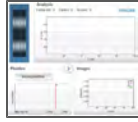
Load indexing reagents. For dual-indexed runs, load reagents for both Index Reads.

With a used flow cell on the instrument, confirm proper flow as prompted by the HCS user interface.

Prime SBS reagents and measure priming waste.

Note: For rapid run mode, you do not need to load the used flow cell as priming is completed within recipe after the run has started.

Load a flow cell that was previously clustered on the cBot. Confirm proper flow as prompted by the HCS user interface.



Start your sequencing run. After cycle 1, inspect the First Base Report (optional setting), and then continue Read 1.

**For indexed runs:**

Following Read 1, the run proceeds to the Index Read (single-indexed runs) or two Index Reads (dual-indexed runs).



**For paired-end runs:**

Prepare paired-end reagents and Read 2 reagents. For details about reagents, see the reagent prep guides for the kits that you are using for your run. To find the guides, go to the Illumina website: [support.illumina.com/sequencing/kits.ilmn](http://support.illumina.com/sequencing/kits.ilmn).

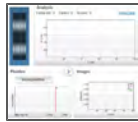
Prepare fresh ICB for Read 2 (recommended).



**For paired-end runs:**

Load paired-end reagents onto the paired-end rack.

Load fresh ICB in position 1 (recommended).



**For paired-end runs:**

Continue the run. The software automatically primes paired-end reagents and performs Read 2 resynthesis and Read 2.



When the run is complete, unload and weigh reagents.

Perform an instrument maintenance wash.

Visit the HiSeq specifications page on the Illumina website for information about run duration and other performance specifications at [www.illumina.com/systems/hiseq\\_2500\\_1500/performance\\_specifications.ilmn](http://www.illumina.com/systems/hiseq_2500_1500/performance_specifications.ilmn).

## Prepare SBS Reagents

The first step in setting up a run is to prepare SBS reagents. Reagent preparation requires overnight thawing at 2° to 8°C, or about 90 minutes in a room temperature deionized water bath. When thawed, reagents are ready for use. For high output mode, Incorporation Mix requires preparation before use.

For more information, see the reagent preparation instructions associated with the reagents that you are using for your run. Go to [support.illumina.com/sequencing/kits.ilmn](http://support.illumina.com/sequencing/kits.ilmn) to find any documentation that you may need.



### NOTE

TruSeq SBS v3 reagents enable an alternative workflow of loading all SBS reagents at the start of a paired-end sequencing run for both Read 1 and Read 2. Although this alternative SBS workflow provides acceptable sequencing performance in Read 2, Illumina recommends that you prepare and load fresh ICB at the beginning of Read 2 for optimal performance.



## Prepare Indexing Reagents


Indexed sequencing requires indexing reagents based on the type of libraries you are sequencing and the number of Index Reads that you perform.

Indexing reagents take less than 30 minutes to prepare.

For more information, see the reagent preparation instructions associated with the reagents that you are using for your run. Go to [support.illumina.com/sequencing/kits.ilmn](http://support.illumina.com/sequencing/kits.ilmn) to find any documentation that you may need.

## Enter Run Parameters

When you select **Sequence | New Run** from the Welcome screen, the software opens the run setup screens that guide you through the run setup steps, starting with the screens on the Run Configuration tab.

Each run configuration screen contains drop-down lists, checkboxes, or text fields. Use the hand-held barcode scanner to scan the flow cell or reagent kit ID, or enter the ID using the touch screen keyboard. The keyboard icon is located to the right of the text fields. 

When you have finished entering the required information on each screen, the Next button becomes active. Select **Next** to move to the next screen or select **Back** to return to the previous screen. At any time during the run setup steps, you can select **Cancel** to abort the run setup and return to the Welcome screen.

Run configuration screens are different for rapid run mode than for high output mode.

- ▶ High output includes Storage, Flowcell-Set-up, Advanced, Recipe, Reagents, and Review.
- ▶ Rapid run includes Pre Run Fluidics Check, Storage, Flowcell-Set-up, Advanced, Recipe, Sample Sheet, Reagents, and Review.

Prepare SBS reagents and indexing reagents before setting up the run. For instructions, see the reagent prep documentation for the kits that you are using.

- ▶ From the HCS Welcome screen, select **Sequence | New Run**. The Scan screen opens.

## Gasket Change Screen

When you change the run mode from the Welcome screen (rapid run to high output or high output to rapid run), the software opens the Gasket Change screens that guides you through a maintenance wash.

- 1 **Load Gasket** — You are prompted to load a new gasket.
- 2 **Load Wash Reagents** — You are prompted to load wash reagents.
- 3 **Load Wash Flow Cell** — You are prompted to load a non-sequencing flow cell and enter a wash flow cell ID.
- 4 **Fluidics Check** — You are prompted to select a solution. Use default values for Volume, Aspiration Rate, and Dispense Rate.
- 5 **Wash** — The instrument performs a wash.

- 6 **Load Solvent** — You are prompted to load NaOH in all positions for washing.
- 7 **Solvent Wash** — The instrument performs a solvent wash.
- 8 **Load Wash Reagents** — You are prompted to load wash reagents.
- 9 **Wash** — The instrument performs a wash.

## Maintenance Wash Screen

If necessary, HCS prompts you to run a maintenance wash before a run. In all cases, a maintenance wash is recommended after every high output run and every 10 days in rapid run mode.

- 1 **Load Wash Reagents** — You are prompted to load wash reagents.
- 2 **Load Wash Flow Cell** — You are prompted to load a non-sequencing flow cell and enter a wash flow cell ID.
- 3 **Fluidics Check** — You are prompted to select a solution. Use default values for Volume, Aspiration Rate, and Dispense Rate.
- 4 **Wash** — The instrument performs a wash.
- 5 **Load Solvent** — You are prompted to load NaOH in all positions for washing.
- 6 **Solvent Wash** — The instrument performs a solvent wash.
- 7 **Load Wash Reagents** — You are prompted to load wash reagents.
- 8 **Wash** — The instrument performs a wash.

## Storage Screen

The Storage screen enables you to define where the system outputs and stores your run data.

- 1 Select **Use BaseSpace for storage and analysis** if you want to save your data to a BaseSpace account. If you select this option, The system prompts you to enter a My Illumina account email and password. Zip BCL files is selected by default. Illumina recommends that you also save files locally. To save files locally, select **Save to an output folder** and enter a path. The **Run Monitoring Only** option sends only system health information to BaseSpace.



## NOTE

For every run, your HiSeq 2500 attempts to send at least general run metrics to BaseSpace. Metrics are sent anonymously, associated with the HiSeq 2500 from which they came, not to any particular user. Metrics include interoperation files (that SAV uses) and global files such as RunInfo.xml and RunParameters.xml. No data files are sent unless you specify for the system to do so.

Permissions for this feature are enabled by default, and Illumina recommends that you leave them enabled. To disable permissions, select **Menu | Tools | Options**, and then deselect **Send system health information to Illumina to aid technical support**. You can also view terms in the Options dialog box.

If permissions for this feature are not enabled, HCS prompts you to accept Terms and Conditions. If you do not accept the terms and conditions, you cannot use BaseSpace. If you are not using BaseSpace, go back to the previous screen and make sure that the BaseSpace option is de-select (otherwise the Next button is not enabled).

- 2 In **Save to an output folder**, browse to and select the path of your run folder.
- 3 Select **Zip BCL files** to reduce the footprint of disc space needed for the run. If you are using BaseSpace, this option is selected by default.



## NOTE

If you are using BaseSpace or an updated version of CASAVA for data analysis, zip the BCL files.

- 4 Select **Bin QScores** to reduce the footprint of disc space needed for the run. This is done by grouping Q-scores together over a wider range of values. For technical details regarding how data quality score resolution can be reduced without sacrificing score accuracy or standard analysis and variant calling performance, find the white paper on [www.Illumina.com](http://www.Illumina.com) titled, *Reducing Whole-Genome Data Storage Footprint*.
- 5 Select a setting from the **Save Auxiliary Files** drop-down list:
  - Select **Save All Thumbnails** to save tile level and full thumbnails. Thumbnail images are a combination of eight images from each swath, combined in one thumbnail image. A swath is a column of tiles, and there are three swaths per lane per surface.
  - Select **Save Tile Thumbnails** to save tile level thumbnails only. Tile thumbnails show just the tile rather than the whole swath.
- 6 Select **Next**.

## Flow Cell Setup Screen

The Flow Cell Setup screen is the run configuration screen with which you record information about your run, starting with the flow cell ID.



### NOTE

It is critical that you scan the flow cell barcode or accurately enter the flow cell ID when you set up your run. The software uses the flow cell ID to determine flow cell type and reagent compatibility.

- 1 Scan the flow cell barcode or enter the flow cell ID (barcode number) of the flow cell to be sequenced.
- 2 Confirm the flow cell type, which is automatically selected based on the flow cell ID. The software uses this information to confirm flow cell compatibility with instrument software and run components.
- 3 Enter the experiment name. This name appears at the top of each interface screen to help identify the sequencing run in progress.
- 4 Enter a user name.
- 5 Select the lane containing the control, if applicable. Otherwise, select **None**.
- 6 Select **Next**.

## Advanced Screen

The Advanced screen includes settings for first base confirmation and indicating alignment lanes.

- 1 Select the **Confirm First Base** checkbox to generate a First Base Report confirmation. Selecting this option requires that you confirm the results before the run can continue. If you do not select the checkbox, the First Base Report is generated as usual and the run continues without showing the First Base Report confirmation dialog box. You can access the First Base Report from the run folder at any time during the run.
- 2 Select the control lane on your flow cell containing the control library from the drop-down list. Otherwise, select **None**.
- 3 Select or deselect **Align to PhiX** for each lane of the flow cell. This enables real-time reporting of error rates for PhiX control lanes or PhiX spike-in lanes. By default, all lanes are selected for alignment by Real-Time Analysis (RTA).

Clear the checkbox for any lanes that do not have PhiX added to the sample so that RTA does not align and calculate error rates for that lane, resulting in arbitrarily high error rates. For more information, see *Using a PhiX Control for HiSeq Sequencing Runs*, which is available on the HiSeq support page on the Illumina website at [www.illumina.com](http://www.illumina.com).

- 4 Select **Keep Intensity Files** for later reanalysis or custom processing (optional). Select the checkbox to save cluster intensity files (\*.cif) for later reanalysis, troubleshooting, or custom processing. Saving intensity files is not required for on-instrument analysis. Enabling this option significantly increases the size of the data output folder. By default, this option is not selected.
- 5 Select **Next**.

## Recipe Screen

From the information that you enter on the Recipe screen, the software creates a recipe specific to the run. The recipe is automatically copied to the run folder and saved in the Recipe subfolder. The saved recipe includes only the number of cycles for each read and index; it does not save the options for the control lane, PhiX alignment, images, or cifs.

- 1 Select an indexing option:
  - **No Index**—Performs a non-indexed single-read or paired-end run.
  - **Single Index**—Performs a single-read or paired-end run with one Index Read (Index 1 (i7) Read).
  - **Dual Index**—Performs a single-read or paired-end run with two Index Reads (Index 1 (i7) Read and Index 2 (i5) Read).
  - **Custom**—Performs a single-read or paired-end run with a custom number of cycles for the Index Reads.

If you select Dual Index or Custom, select a **Flow Cell Format: Single Read or Paired End**.

A dual-indexed single-read run can be performed on either a single-read flow cell or a paired-end flow cell with recipe adjustments. Your selection in HCS, paired-end or single-read flow cell, determines the recipe. Always use the appropriate dual-index sequencing primers box according to your flow cell type.



### NOTE

A dual-indexed paired-end run must be performed on a paired-end flow cell.

- 2 Enter the number of cycles you plan to sequence for Read 1 and Read 2, if applicable.

For the **Custom** indexing option, enter the number of cycles you plan to sequence for the Index Reads.

For paired-end runs, enter the **same** number of cycles for Read 2 as you entered for Read 1. Or, if you selected the **Custom** indexing option, enter the number of cycles for each Index Read. (Read lengths do not need to be identical.)



**NOTE**

It is possible to perform a dual-indexed single-read run using a paired-end flow cell containing paired-end libraries. To do so, set up a *dual-indexed paired-end run* and enter 0 (zero) for the number of cycles in Read 2. This stops the run after the Index 2 (i5) Read.

- 3 Confirm the chemistry selections for your run:
  - a **SBS**—This setting is determined by the flow cell ID.



**NOTE**

The software defaults to the compatible kit versions based on the flow cell ID. For example, the ID for HiSeq Flow Cell v3 defaults to the TruSeq SBS Kit v3.

- b **Index**—This setting is determined by the indexing option selected. Specify the kit name to make sure that the correct number of indexing cycles are applied to your run.
    - **For non-indexed or single-indexed libraries**—Select **Multiplexing Primer Box**. This sets up your run to perform a seven-cycle Index Read.
    - **For dual-indexed libraries**—Select **TruSeq Dual Index Sequencing Primer Box**. This sets up your run to perform an eight-cycle Index Read.



**NOTE**

Select TruSeq Dual Index Sequencing Primer Box setting

- 4 Select the **Use Existing Recipe** checkbox only if you want to use a custom recipe. Otherwise, allow the software to create your recipe based on information you provide on the run setup screens.
 

ILLUMINA recommends that you use the recipe wizard to build your recipe based on the information you enter on the Recipe screen.

## Sample Sheet Screen

In the **Sample Sheet** field, enter the path of a valid sample sheet for your run. When the run begins, the software copies the sample sheet to the root level of the run folder. Unless you are using BaseSpace, a sample sheet is optional. For indexed runs, the sample sheet is required if you want indices to be reported in SAV; if you are running BaseSpace, a sample

sheet is required. For more information about sample sheets, see *Sample Sheet Overview* on page 17.



#### NOTE

To view indexing results in Sequencing Analysis Viewer (SAV), you must load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to properly report indexing information. For more information, see the *Sequencing Analysis Viewer User Guide*, part # 15020619.

- 1 Enter the path or browse to a valid sample sheet for your run. For indexed runs, the sample sheet is required if you want indices to be reported in SAV; a sample sheet is also required if you are using BaseSpace.
- 2 Select **Next**.

## Reagents Screen

The Reagents screen records information about the reagents used for your run.

- 1 Scan or enter the Reagent Kit ID. You can enter the kit ID from either box 1 or box 2.
- 2 For indexed runs, scan or enter the Indexing Reagent Kit ID.
- 3 For paired-end runs, scan or enter the Reagent Kit ID.
- 4 Select the SBS reagent kit you are using for your run:
  - Select **200 Cycles** for a 200 cycle kit
  - Select **50 Cycles** for a 50 cycle kit.
  - Select **Used Kit** for a partial kit, and then enter the number of SBS cycles remaining, or the number of cycles that reagents are expected to last.



#### NOTE

If you are combining four 50-cycle SBS kits for a dual-indexed run, select **Used Kit** instead of **50 Cycles** to set the cycle counter correctly.

In high output mode, the counter defaults to 225 cycles, which you can change according to the number of cycles in your run.

In rapid run mode, the counter defaults to 325 cycles, which you can change according to the number of cycles in your run.

- 5 The **Cycles Remaining** field is populated automatically depending on the SBS kit you are using. If you are using a partial kit, enter the number of cycles reagents are expected to last. The software counts down the number of cycles entered. When the cycles are low, the software prompts you to load fresh reagents.



**NOTE**

If you are starting a new run using less than a full reagent kit, make sure that you are present when new reagents are needed.

- ▶ Select **Prime SBS Reagents** to prime reagents before you start a run. Always prime reagents before loading a new flow cell.
- 6 Select **Next**.

## Review Screen

Review your run information. If it is correct, select **Next**.

Select **Back** if you need to change any entries.

## Load and Prime Reagents

The next step is to load sequencing reagents. The software interface guides you through the process.

## Pre-Run Setup Screens

After you have entered the required information in the run configuration screens, the pre-run setup screens open. Pre-run setup screens include: Load Reagents, Load Prime Flow Cell, Prime Fluidics Check, and Prime. The Load Prime Flow Cell, Prime Fluidics Check, and Prime screens are available in only high output mode and rapid run mode when you have performed template hybridization on the cBot.

### Load SBS Reagents

The Load Reagents screen prompts you to load SBS reagents. If you specified a number of cycles for indexing, the software prompts you to load indexing reagents on the paired-end rack.

Make sure that SBS reagents are thoroughly thawed and ready to load onto the instrument. After Read 1 of a paired-end run in high output mode, the software pauses so that you can load paired-end reagents. Paired-end reagents must be loaded before Read 2 resynthesis.



#### NOTE

To prepare for the post-run rinse at the end of a sequencing run, it is important that you load 25 ml of PW1 or laboratory-grade water in position 2 when you load SBS reagents at the beginning of your run.

The post-run rinse does *not* replace the post-run instrument wash.

### Illumina-Supplied Consumables

- ▶ Eight funnel caps (Provided in the TruSeq Cluster Kit)

### User-Supplied Consumables

- ▶ One 250 ml bottle (Corning, catalog # 430776)

### Procedure

- 1 Record the weight of each reagent on the lab tracking form.



#### NOTE

Weighing reagents before and after a sequencing run confirms proper reagent delivery.

- 2 Open the reagent compartment door.
- 3 Raise the sippers for the sequencing reagent rack using the following motion:

- a Pull the sipper handle towards you.
  - b Push the sipper handle up while pulling it towards you.
  - c Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.
- 4 Slide the reagent rack out of the reagent compartment.
  - 5 Place each reagent bottle onto the rack in the associated numbered position. Make sure that the conical end of the bottle rests in the indentation on the base of the rack.

**Table 4** SBS Reagent Positions

Position	Reagent	Description
1	ICB	Incorporation Mix
2	PW1 (25 ml)	Wash Buffer
3	SRE	Scan Mix Reagent
4	SBS Buffer 1 (SB1)	High Salt Buffer
5	SBS Buffer 2 (SB2)	Incorporation Wash Buffer
6	SBS Buffer 2 (SB2)	Incorporation Wash Buffer
7	CMR	Cleavage Mix Reagent
8	SBS Buffer 3 (SB3)	Cleavage Buffer

- 6 Load the template into the sample loading station (if applicable).
- 7 Add 25 ml of PW1 or laboratory-grade water to the bottle in position 2.



**NOTE**

HCS v2.0 and later includes an automatic post-run rinse following the completion of the sequencing run. To prepare for the post-run rinse, it is important that you load 25 ml of PW1 or laboratory-grade water in position 2 when you load SBS reagents at the beginning of your run.

The post-run rinse does *not* replace the post-run instrument wash.

- 8 Remove the cap from each reagent bottle and replace it with a funnel cap.



**CAUTION**

After handling the bottle of CMR, discard your gloves and replace them with a new pair.

- 9 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.
- 10 Lower the sippers into the sequencing reagent bottles as follows:

- a Pull the sipper handle towards you.
  - b Lower the sipper handle while pulling it towards you.
  - c Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.
  - d Release the sipper handle into the slot on the bottom end of the groove. Make sure that the sipper handle rests securely in the slot.
- 11 Select the checkbox labeled **PW1 (25 mL) loaded in Position 2**.
  - 12 Do one of the following:
    - If you are performing an indexed run, select **Next**. The software prompts you to load indexing reagents.
    - If you are performing a non-indexed run, close the reagent compartment door and select **Next**. The software proceeds to priming reagents.

## Load Indexing Reagents

If you are performing an indexed run, load indexing reagents before starting the run.

- 1 Before beginning, record the weight of each reagent on the lab tracking form.
- 2 Raise the sippers for the paired-end reagent rack using the following motion:
  - a Pull the handle towards you.
  - b Push the handle up while pulling it towards you.
  - c Release the handle into the slot on the top end of the groove. Make sure that the handle rests securely in the slot.
- 3 Slide the reagent rack out of the reagent compartment using the rack handle.
- 4 Place each reagent tube onto the rack in the associated numbered position for the type of run that you are performing.
- 5 Place tubes filled with deionized water into rack positions that are not used.
- 6 Remove the caps from each reagent tube.
- 7 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.
- 8 Lower the sippers into the tubes on the paired-end reagent rack as follows:
  - a Pull the handle towards you.
  - b Lower the handle while pulling it towards you.

- c Visually inspect the sippers to make sure that they do not bend as they lower into the tubes.
  - d Release the handle into the slot on the bottom end of the groove. Make sure that the handle rests securely in the slot.
- 9 Close the reagent compartment door.
  - 10 Select **Next**.

## Prime Reagents



### NOTE

Prime reagents only if you have done template hybridization on the cBot for a rapid run flow cell.

Perform the following steps to prime reagents:

- ▶ *Clean the Flow Cell Holder* on page 94
- ▶ *Load the Used Flow Cell* on page 54
- ▶ *Confirm Proper Flow* on page 57
- ▶ *Position Tubing and Prime Reagents* on page 57

## User-Supplied Consumables

- ▶ Lens cleaning tissue
- ▶ 70% ethanol or alcohol wipes
- ▶ Low-lint lab tissue
- ▶ Used flow cell

## Clean the Flow Cell Holder

- 1 Open the flow cell compartment door.

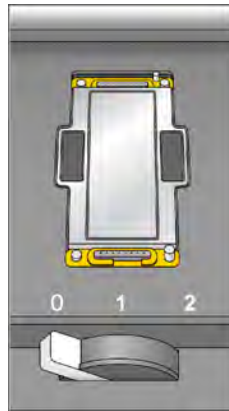


### CAUTION

Do not place fluids on the flow cell compartment door or on the flow cell stage when the door is open. Spills in this area can damage the instrument.

- 2 Make sure that the flow cell lever is in the OFF position.

Figure 14 Flow Cell Lever in Position 0



- 3 Put on a new pair of powder-free latex gloves.
- 4 If the flow cell from a previous run is present, remove it and set aside. It can be used to confirm proper flow before loading the clustered flow cell.
- 5 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.

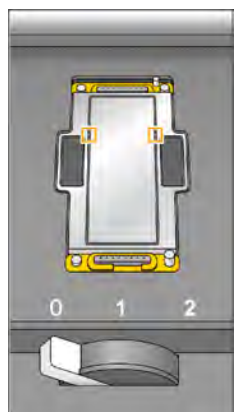


**CAUTION**

Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

- 6 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

Figure 15 Vacuum Hole Locations



### Load the Used Flow Cell

The Load Prime Flow Cell screen is available in high output mode and rapid run mode when you have performed template hybridization on the cBot.

The Load Prime Flow Cell screen prompts you to load a *used* flow cell for the priming step. After loading a used flow cell, confirm that the vacuum is engaged.



#### NOTE

Illumina recommends that you use the flow cell from a previous run for priming reagents on a subsequent run or for a post-run maintenance wash.

- 1 Rinse the used flow cell with laboratory-grade water. Dry it with lens cleaning tissue or lint-free tissue.
- 2 Clean the flow cell using alcohol wipes and lens cleaning tissue.



#### NOTE

Do not remove or replace the manifold gaskets at this time.

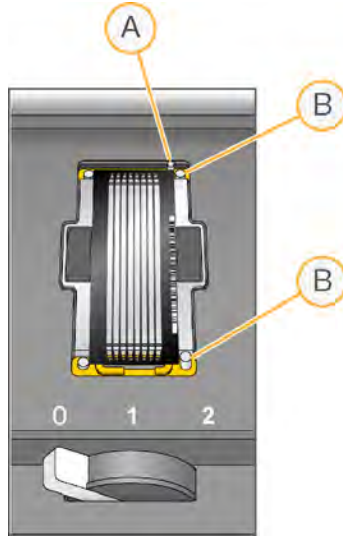
- 3 Place the used flow cell on the flow cell holder with the inlet and outlet ports facing *down* and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.
- 4 Gently slide the flow cell towards the top and right guide pins until it stops.



**NOTE**

Remove your hand from the flow cell before engaging the vacuum switch. This prevents any possible alignment drift over time.

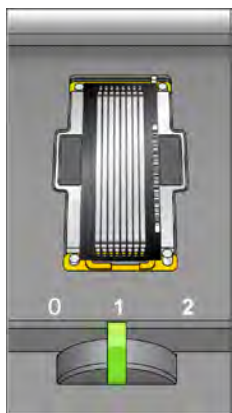
**Figure 16** Flow Cell Positioned Against Top and Right Guide Pins.



- A Top Guide Pin
- B Right Guide Pins

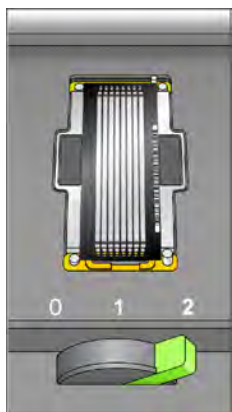
- 5 Slowly move the flow cell lever to position 1. This engages the vacuum and secures the flow cell into position. When the flow cell lever is green, the vacuum is engaged.

Figure 17 Flow Cell Lever in Position 1



- 6 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2 (far-right). When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 18 Flow Cell Lever in Position 2



- 7 Make sure that the **Vacuum Engaged** checkbox is selected on the load prime flow cell screen, and then select **Next**.

## Confirm Proper Flow

After the used flow cell is loaded, check for proper flow. Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

- 1 Select solution 2 (laboratory-grade water) from the drop-down list.



### CAUTION

You can use water to confirm proper flow on a used flow cell only. Never use water to confirm proper flow on a clustered flow cell.

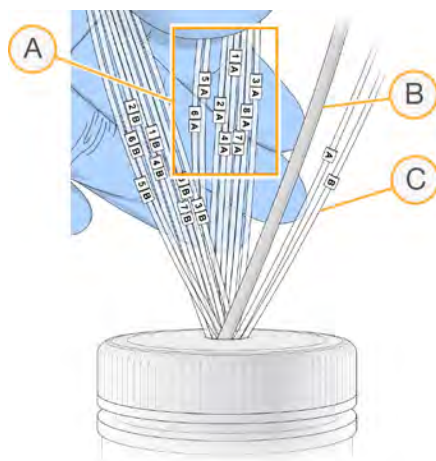
- 2 Confirm the following default values:
  - Volume: **250**
  - Aspirate Rate: **250**
  - Dispense Rate: **2000**
- 3 Select **Pump**.
- 4 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

If you see excessive bubbles, check the gaskets for obstructions, reduce the aspirate rate to 100, and pump another 250  $\mu$ l of water to the flow cell. If problems persist, remove the flow cell, repeat the cleaning steps, and reload the flow cell.

## Position Tubing and Prime Reagents

To position tubing, loosen and remove the eight lines of waste tubing for the appropriate flow cell from the waste container. Make sure that you do not include the eight lines for the opposite flow cell or the lines for the condensation pump.

**Figure 19** Waste Lines for a HiSeq 2500



- A Flow Cell Waste Lines for Reagent Positions 1–8 on high output flow cell
- B Condensation Pump Tubing (Do not remove)
- C Paired-End Priming Pump Tubing (Do not remove)

- 1 Place each waste tubing into an empty 15 ml tube, one line per tube. Priming waste is collected and measured after the priming step.
- 2 Select **Next**. The priming screen opens and the priming step starts automatically. You can monitor the progress of the priming step from the priming screen.

## Prime Fluidics Check and Prime Reagents

Prime Fluidics Check is available in high output mode and rapid run mode when you have performed template hybridization on the cBot.

The Prime Fluidics Check screen prompts you to confirm proper flow before priming reagents. Select **Pump** to start the fluidics check.

You can pause or cancel the fluidics check from this screen:

- ▶ Select **Pause** to pause the fluidics check temporarily. The Pause button changes to the Resume button.
- ▶ Select **Resume** to continue.
- ▶ Select **Stop** to cancel the fluidics check.

After the fluidics check, the Prime screen opens.

The Prime screen is available in high output mode and rapid run mode when you have performed template hybridization on the cBot.

From the Prime screen, you can monitor the priming step in progress. Select **Start Prime** to begin, or select **Skip Prime** if you have already primed reagents.

You can pause or cancel the priming step from this screen:

- ▶ Select **Pause** to temporarily pause the priming step. The Pause button changes to the Resume button.
- ▶ Select **Resume** to continue.
- ▶ Select **Stop** to stop priming.
- ▶ Select **Cancel** to abort the run setup and return to the Welcome screen.

When the priming step is complete, the initiate run screens open automatically.

- 1 When the priming step is complete, measure the collected priming waste and confirm that the volume is 2 ml from each reagent position primed for a total of **14 ml**. Record the results on the lab tracking form.



#### NOTE

The color of sequencing reagent waste might be brown in appearance. You also might see a slight discoloration on the funnel caps or in any spills. The change in waste color is not toxic and does not affect run performance.

- 2 Return the waste tubing to the waste container before proceeding.
- 3 Select **Next**. You are ready to load the clustered flow cell.

## Load a Flow Cell

The next step is to remove the used flow cell and load the flow cell that you want to sequence.

Perform the following steps to load the flow cell:

- ▶ *Remove the Used Flow Cell* on page 60
- ▶ *Clean the Flow Cell Holder* on page 100
- ▶ *Clean the Flow Cell* on page 101
- ▶ *Load the Flow Cell* on page 63
- ▶ *Confirm Proper Flow* on page 66

### User-Supplied Consumables

- ▶ Lens cleaning tissue
- ▶ 70% ethanol or alcohol wipes
- ▶ Low-lint lab tissue



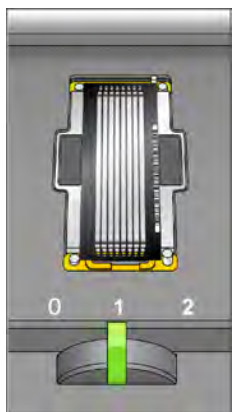
#### NOTE

Regardless of which mode you are running on the HiSeq 2500/1500, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.

## Remove the Used Flow Cell

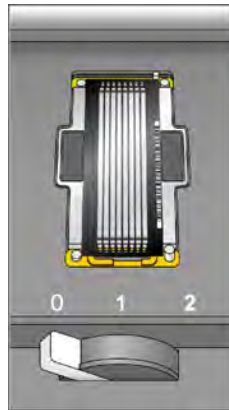
- 1 Open the flow cell compartment door.

Figure 20 Flow Cell Lever in Position 1



- 2 Slowly move the flow cell lever to position 1 to disengage the manifolds.
- 3 Slowly move the flow cell lever to position 0 to disengage the vacuum seal and release the flow cell.

Figure 21 Flow Cell Lever in Position 0



- 4 Lift the used flow cell from the flow cell holder.

## Clean the Flow Cell Holder

- 1 Put on a new pair of powder-free latex gloves.
- 2 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.



### CAUTION

Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

Figure 22 Inspect Vacuum Holes



- 3 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

## Clean the Flow Cell

- 1 Rinse the flow cell with laboratory-grade water and dry it with a lens cleaning tissue.
- 2 Fold an alcohol wipe to approximately the size of the flow cell.
- 3 Hold the edges of the clustered flow cell with two fingers. Make sure that the inlet and outlet ports are facing *up*.
- 4 Wipe off each side of the flow cell with a single sweeping motion. Repeat, refolding the alcohol wipe with each pass, until the flow cell is clean.
- 5 Dry the flow cell using a dry lens cleaning tissue.



### CAUTION

If you clean the flow cell while it is lying on the benchtop, you could easily apply too much pressure and break the flow cell. Illumina recommends cleaning the flow cell while holding the edges between your fingers.

- 6 Protect the flow cell from dust until you are ready to load it onto the instrument.



## Load the Flow Cell



### NOTE

Do not replace the manifold gaskets at this time. Leave the gaskets that were installed before the instrument maintenance wash in place when loading the flow cell. You will replace manifold gaskets after the current sequencing run is completed, before the instrument maintenance wash.

Regardless of which mode you are running on the HiSeq 2500/1500, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.

Do not perform more than one sequencing run on the same set of manifold gaskets.

- 1 Place the flow cell on the flow cell holder with the inlet and outlet ports facing *down* and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.

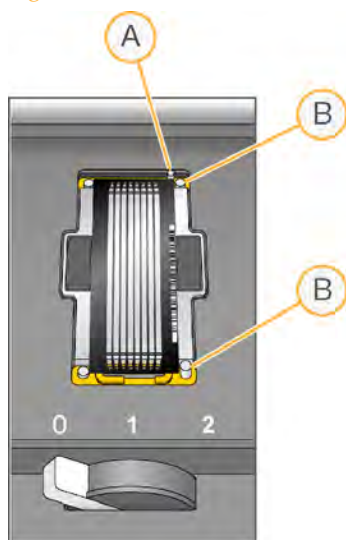


### NOTE

The flow cell has a mechanically keyed corner, which provides a visual orientation for loading the flow cell. Install the flow cell so that the keyed corner is on the output end of the flow cell facing towards the instrument, and on the left side of the flow cell by lane 1.

- 2 Gently slide the flow cell towards the top and right guide pins until it stops.

Figure 23 Flow Cell Positioned Against Top and Right Guide Pins



- A Top Guide Pin
- B Right Guide Pins

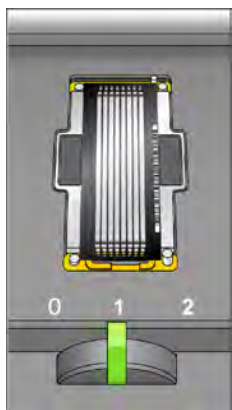


NOTE

Remove your hand from the flow cell before engaging the vacuum switch. This prevents possible alignment drift over time.

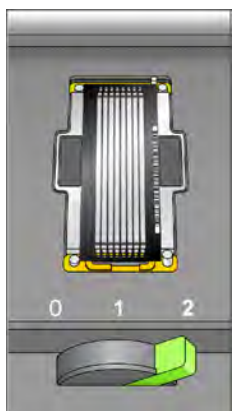
- 3 Slowly move the flow cell lever to position 1. This engages the vacuum and secures the flow cell into position. When the flow cell lever is green, the vacuum is engaged.

Figure 24 Flow Cell Lever in Position 1



- 4 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2. When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 25 Flow Cell Lever in Position 2



- 5 Make sure that the **Vacuum Engaged** checkbox is selected on the load sequencing flow cell screen.

## Confirm Proper Flow

After loading the flow cell, check for proper flow using the fluidics check screen. Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

- 1 Select solution 5 (SB2) from the drop-down list.
- 2 Enter the following default values:
  - Volume: **250**
  - Aspirate Rate: **250**
  - Dispense Rate: **2000**
- 3 Select **Pump**.
- 4 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.  
If you see excessive bubbles, check the manifold gaskets for obstructions, and repeat the process.
  - a Select solution 6 (SB2) to avoid depleting SB2 from position 5.
  - b Reduce the aspirate rate to 100, and pump another 250  $\mu\text{l}$  of SB2 to the flow cell.
- 5 After you have confirmed proper flow, select **Next** to proceed. Make sure that the flow cell lever is green, and then close the flow cell compartment door.
- 6 Confirm that the **Vacuum Engaged** and **Door Closed** checkboxes are selected, and then select **Next**.
- 7 Select **Start** to start the sequencing run.

## Close Door Screen

The Close Door screen prompts you to close the flow cell compartment door. The system confirms that the vacuum is engaged and that the flow cell compartment door is closed. The checkboxes are selected automatically when the system is ready.

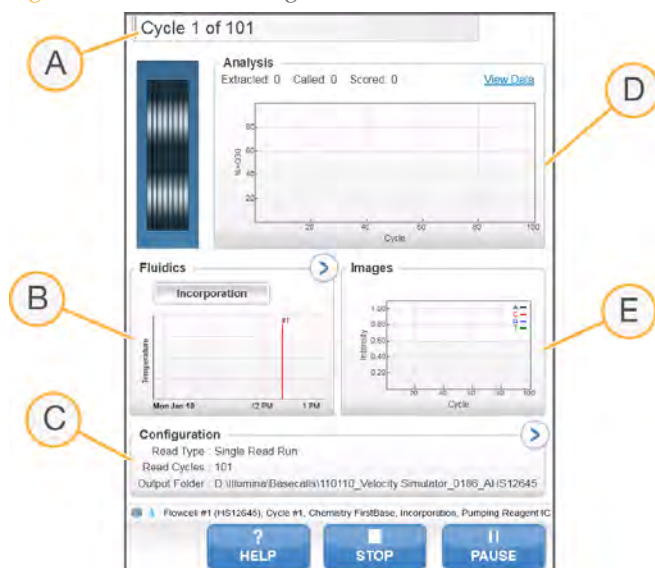
## Monitor the Run

Use the run overview screen to monitor on-instrument analysis, fluidics, and imaging.

- ▶ **Progress Bar**—Use the progress bar to monitor how many cycles have been completed.
- ▶ **Flow Cell Image**—Use the flow cell image to monitor which lanes have been imaged for that cycle.
- ▶ **Analysis Graph**—Use the analysis graph to monitor quality scores by cycle.
- ▶ **Images Graph**—Use the images graph to monitor intensities by cycle.
- ▶ **Fluidics Graph**—Use the arrow button to expand the fluidics section and monitor chemistry steps in the protocol.

In rapid run mode, HCS automatically prompts you to perform a water wash when sequencing is finished. See *Perform a Water Wash* on page 117.

Figure 26 Run Monitoring Screen Overview



- A Run Progress
- B Fluidics and Temperature
- C Run Configuration
- D Analysis
- E Images

**NOTE**

You might notice an intermittent clicking sound coming from the right side of the instrument during operation. This is normal. The clicking sound indicates that the refrigeration condensation pump is working.

## First Base Report

The First Base Report confirmation dialog box opens automatically after cycle one is complete, if you selected **Confirm First Base** on the scan screen during run setup. The run pauses at this step.

If you did not select the checkbox, the run continues after first base incorporation without prompting you to review the First Base Report.

- 1 Review the First Base Report from the First Base Report confirmation dialog box or by opening `First_Base_Report.htm` from the root level of the run folder.
- 2 If the results are satisfactory, select **Continue** to continue your run. If results are not satisfactory, abort the run.

## Sequencing Analysis Viewer

You can monitor your run using the Sequencing Analysis Viewer, which launches automatically after image analysis begins. For more information, see *Sequencing Analysis Viewer* on page 125.

For a detailed description of available metrics and installation instructions, see the *Sequencing Analysis Viewer User Guide*, part # 15020619.

## Prepare Reagents for Read 2

Before the completion of Read 1 and any Index Reads, prepare reagents for Read 2 resynthesis and fresh ICB for Read 2. Reagents take less than 30 minutes to prepare.

For more information, see the reagent preparation documentation associated with the kits that you are using for your run. Go to [support.illumina.com/sequencing/kits.ilmn](http://support.illumina.com/sequencing/kits.ilmn) to find any documentation that you may need.

## Load Reagents for Read 2

Prepare paired-end reagents and ICB for Read 2 before setting up the run. For instructions, see the reagent prep documentation for the kits that you are using.

After completion of Read 1 and any Index Reads, the software prompts you to load paired-end reagents for Read 2 resynthesis and freshly prepared ICB for Read 2.

### Load Paired-End Reagents

- 1 Record the weight of each reagent on the lab tracking form.
- 2 Make sure that the paired-end rack is not in use on the opposite flow cell for Read 2 resynthesis, Index 1 (i7) Read preparation, or Index 2 (i5) Read preparation.
- 3 Raise the sippers for the paired-end reagent rack using the following motion:
  - a Pull the handle towards you.
  - b Push the handle up while pulling it towards you.
  - c Release the handle into the slot on the top end of the groove. Make sure that the handle rests securely in the slot.
- 4 Slide the reagent rack out of the reagent compartment using the rack handle.
- 5 Place each of the reagent tubes onto the rack in the associated numbered positions depending on the type of run you are performing.

**Table 5** Paired-End Reagent Positions

Position	Reagent	Description
11	LMX2	Linearization Mix 2
12	BMX	Blocking Mix
13	AMX2	Amplification Mix 2
14	APM2	AMX2 Premix
15	AT2	100% Formamide
16	HP7 or HP11	Read 2 Sequencing Primer
18	HP3	Denaturation Solution
19	HT2	Wash Buffer



**NOTE**

You do not need to load RMR for dual-indexed paired-end runs. RMR is used during the Index 2 (i5) Read and is not used again during Read 2 resynthesis.

- 6 Remove the caps from each reagent tube.
- 7 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.
- 8 Lower the sippers into the paired-end reagent tubes as follows:
  - a Pull the handle towards you.
  - b Lower the handle while pulling it towards you.
  - c Visually inspect the sippers to make sure that they do not bend as they lower into the tubes.
  - d Release the handle into the slot on the bottom end of the groove. Make sure that the handle rests securely in the slot.

## Load ICB for Read 2

- 1 Record the weight of the reagent on the lab tracking form.
- 2 Raise the sippers for the sequencing reagent rack using the following motion:
  - a Pull the sipper handle towards you.
  - b Push the sipper handle up while pulling it towards you.
  - c Release the sipper handle into the slot on top end of the groove. Make sure that the sipper handle rests securely in the slot.
- 3 Slide the reagent rack out of the reagent compartment.
- 4 Remove the existing ICB reagent bottle from position 1 of the reagent rack and remove the funnel cap from the bottle.
- 5 Place the funnel cap on the new bottle of ICB and load the bottle in position 1, making sure that the conical end of the bottle rests in the indentation on the base of the rack.
- 6 Slide the reagent rack into the reagent compartment, aligning the rack with the raised guide on the floor of the compartment.
- 7 Lower the sippers into the sequencing reagent bottles as follows:
  - a Pull the sipper handle towards you.
  - b Lower the sipper handle while pulling it towards you.

- c Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.
  - d Release the sipper handle into the slot on the bottom end of the groove. Make sure that the sipper handle rests securely in the slot.
- 8 Close the reagent compartment door, and then select **Next** to resume the run.

# Perform a Rapid Sequencing Run

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## Introduction

To perform a rapid sequencing run on the HiSeq 2500, you first prepare reagents for your run and then set up the run using the HCS interface. Run setup steps include: entering run parameters, loading reagents, loading the rapid run flow cell, and performing a fluidics check. If you have preformed template hybridization on the cBot, you also need to prime reagents.

When loading the same low-diversity sample into both rapid flow cell lanes, you do not have a balanced control lane for matrix and phasing estimations. This can result in poor quality data. Illumina recommends spiking in PhiX at 30% to 50% to increase base diversity. Alternatively, perform template hybridization on the cBot with a balanced sample in one lane and set this as the control lane on the HiSeq.

For rapid run mode, all SBS, clustering, PE and indexing reagents are loaded at the start of the run.

### Types of Rapid Runs

You can perform six types of rapid runs on the HiSeq 2500:

Run Type	Read 1 Cycles	Index 1 (i7) Read Cycles	Index 2 (i5) Read Cycles	Read 2 Cycles	Total Cycles
Single-Read, Non-Indexed	≤ 151	--	--	--	≤ 151
Single-Read, Single-Indexed	≤ 151	7 * 8 **	--	--	≤ 158 * ≤ 159 **
Single-Read, Dual-Indexed	≤ 151	8	8	--	≤ 167
Paired-End, Non-Indexed	≤ 151	--	--	≤ 151	≤ 302
Paired-End, Single-Indexed	≤ 151	7 * 8 **	--	≤ 151	≤ 309 * ≤ 310 **
Paired-End, Dual-Indexed	≤ 151	8	7 + 8 ***	≤ 151	≤ 325

\* Number of cycles for single-indexed libraries

\*\* Number of cycles for dual-indexed libraries

\*\*\* Index 2 Read of a paired-end dual-indexed run includes seven additional chemistry-only cycles

**NOTE**

If you are running more than 100 cycles per read, combine multiple kits.

For more information, see *Single-Indexed Sequencing Overview* on page 10 and *Dual-Indexed Sequencing Overview* on page 13.

## Stagger Runs

The HiSeq 2000 and 2500 enable you to start and stop experiments independently on sides A and B and run staggered runs continuously. Before you begin the process of loading a new flow cell while an adjacent flow cell is running, pause the current run in progress.

- 1 Make sure that the run on the adjacent flow cell is performing a chemistry step, and then select **Pause**. The Pause menu opens.

**NOTE**

It is safest to pause the current flow cell run while in a chemistry and not an imaging step.

- 2 Select **Normal Pause**.
- 3 Wait for the software to complete the current chemistry step. The system is automatically placed in a safe state.
- 4 After the adjacent flow cell run has been paused successfully, set-up the new flow cell on the other lane of the system.
- 5 After you load the new flow cell, close the compartment door.
- 6 Select **Start** to start the new sequencing run.
- 7 Select **Resume** on the adjacent flow cell to continue the sequencing run on that side. The software automatically controls chemistry and imaging processes on both flow cells through the end of specified sequencing experiments. After each run ends, normal wash procedures are required.

## Rapid Sequencing Workflow (2-Lane Flow Cell)



Prepare reagents and library sample. This includes thawing and inverting all reagents and diluting your sample to the relevant concentration. For details, see the reagent prep guides for the reagents that you will be using for your experiment. To find the guides, go to the Illumina website: [support.illumina.com/sequencing/kits.ilmn](http://support.illumina.com/sequencing/kits.ilmn).

The workflow varies depending upon whether you perform template hybridization on the cBot as opposed to on the HiSeq. Workflow differences are noted in the following sections:

- *Introduction* on page 74
- *Prime Reagents* on page 93
- *Prepare SBS and Cluster Reagents* on page 78
- *Denature Library for On-Instrument Clustering* on page 78
- *Load SBS and Clustering Reagents and Template* on page 88
- *Load a Flow Cell* on page 99
- *Confirm Proper Flow* on page 105



Using HCS, perform a pre-run fluidics check and enter parameters for your run.

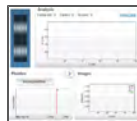


If you are performing on-board cluster generation, load clustering, SBS, PE turn, indexing reagents, and the library sample at start of run. If you have performed template hybridization on the cBot, load the reagents at the start of the run, and the library sample are not needed.



If you are performing a template hybridization on the cBot, prime clustering reagents on the HiSeq through a used flow cell.

If you are performing on-board cluster generation, prime all clustering and sequencing reagents through the new flow cell after the run has started.



Start your sequencing run. After cycle 1, inspect the First Base Report (optional setting), and then continue Read 1.

The sequencing run continues through the PE turn and Read 2 with no intervention necessary.



When the run is complete, unload and weigh reagents. You are prompted to carry out a post run water wash. After the post run water wash, the instrument is ready to use. If a maintenance wash is required, the software prompts you to perform one. This occurs every 10 days or when switching modes.

Visit the HiSeq specifications page on the Illumina website for information about run duration and other performance specifications at [www.illumina.com/systems/hiseq\\_2500\\_1500/performance\\_specifications.ilmn](http://www.illumina.com/systems/hiseq_2500_1500/performance_specifications.ilmn).

## Prepare SBS and Cluster Reagents



### NOTE

Regardless of whether you are performing on-board cluster generation or cBot template hybridization, the same clustering and SBS reagents are prepared as described in this section. If you are performing template hybridization on the cBot, you can skip the section titled, Denature Library for On-Instrument Clustering.

When running in rapid mode, the HiSeq draws all sequencing primers from a single tube; so, only a single tube of sequencing primer can be used in each primer hybridization step on the HiSeq in rapid run mode. Therefore the same sequencing primer must be used for both lanes of a rapid run flow cell.

The first step in setting up a run is to prepare SBS reagents. Reagent preparation requires overnight thawing at 2° to 8°C, or about 90 minutes in a room temperature deionized water bath. When thawed, reagents are ready for use. For rapid run mode, the Incorporation Mix does not require preparation apart from other reagents.

For instructions, see the reagent prep documentation for the kits that you are using. Go to [support.illumina.com/sequencing/kits.ilmn](http://support.illumina.com/sequencing/kits.ilmn) to find any documentation that you may need.

## Denature Library for On-Instrument Clustering



### NOTE

If you are performing the template hybridization on the cBot, you can skip this section.

This step is only for performing the template hybridization step on the HiSeq 2500.

Perform the following steps to denature the template DNA with 0.1 N NaOH to a final DNA concentration of 20 pM.



### NOTE

If your application requires higher than a 20 pM final concentration of your library, make sure that your concentration of NaOH is not higher than 0.05 N in the denaturation solution and not more than 0.001 N (1 mM) in the final solution after diluting with HT1.

Higher concentrations of NaOH in the library inhibit library hybridization and decrease cluster density.

## Illumina Supplied Consumables

- ▶ HT1



## User Supplied Consumables

- ▶ 1N NaOH
- ▶ Microcentrifuge tube. See *Load SBS and Clustering Reagents and Template* on page 88.

## Procedure

- 1 Combine the following volumes of template DNA and 0.1 N NaOH in a microcentrifuge tube:
  - 2 nM template DNA (10  $\mu$ l)
  - 0.1 N NaOH (10  $\mu$ l)
- 2 Vortex briefly to mix the template solution.
- 3 Centrifuge the template solution to 280 xg for one minute.
- 4 Incubate for five minutes at room temperature to denature the template into single strands.
- 5 Transfer denatured template (Temp) to a tube containing 980  $\mu$ l pre-chilled HT1 (Hybridization buffer).

## Diluting Denatured DNA

Perform the following steps to dilute the denatured DNA with pre-chilled HT1.

- 1 Dilute the denatured DNA in a 1.5 ml or 1.7 ml microcentrifuge tube, to the desired concentration in a total volume of 420  $\mu$ l, according to the following examples:

Required Concentration	20 pM template ( $\mu$ l)	HT1 ( $\mu$ l)
2 pM	42	378
6 pM	126	294
10 pM	210	210
12 pM	252	168
16 pM	336	84
20 pM	420	0

- 2 Invert several times to mix, and then pulse centrifuge the template solution.
- 3 Place the denatured template DNA on ice until you are ready to load it onto the instrument. See *Load SBS and Clustering Reagents and Template* on page 88 for template loading details.

## Check Pre-Run Fluidics and Run Parameters

You must prepare SBS reagents and indexing reagents before setting up the run. For instructions, see the reagent prep documentation for the kits that you are using.

The HiSeq Control Software guides you through the run setup steps. Make sure that you are in rapid run mode.



### CAUTION

Place waste lines 1, 2, 3, 6, 7, and 8 for the appropriate position in a 1 liter container filled with deionized water. If these lines are not placed in deionized water, damage to the reagent pumps can occur.

From the HCS Welcome screen, select **Sequence | New Run**. The Pre Run Fluidics Check screen opens. Illumina recommends that you perform the fluidics check to ensure proper functioning of your instrument before starting a run.

## Volume Check Screen

For rapid run mode only, the Volume Check screen enables you to perform a pre-run fluidics check for waste volume.

If you select **Yes** to perform the fluidics check at the start of the run, HCS prompts you to load water in all positions, including the sample loading station, and load a used flow cell. HCS prompts you pump 250  $\mu$ l water from position 5 at 1500 ul/min. Make sure that there is no air in the syringes. Repeat the flow check until you can confirm proper flow. After you have confirmed the flow, begin the fluidics volume check and measure the volume collected from each lane. This takes about 12 minutes. When the check is complete, the software enables you to advance to the next screen.

If you select **No** to skip the fluidics volume check, the software advances directly to the next screen.

- 1 Load laboratory-grade water onto all 8 SBS positions, 10 Clustering (i.e., PE) positions, and the sample position for the relevant flow cell. Make sure that a used rapid run flow cell is loaded on the instrument, and carry out a flow volume check.



### NOTE

Before you perform a fluidics volume check, make sure waste outlet lines 1, 2, 3, 6, 7, and 8 are in a bottle of clean water. Place lines 4 and 5 into empty 50 ml tubes. The expected delivery volume per lane per flow cell is 9.5 ml.

## Storage Screen

The Storage screen enables you to define where the system outputs and stores your run data.

- 1 Select **Use BaseSpace for storage and analysis** if you want to save your data to a BaseSpace account. If you select this option, The system prompts you to enter a My Illumina account email and password. Zip BCL files is selected by default. Illumina recommends that you also save files locally. To save files locally, select **Save to an output folder** and enter a path. The **Run Monitoring Only** option sends only system health information to BaseSpace.



### NOTE

For every run, your HiSeq 2500 attempts to send at least general run metrics to BaseSpace. Metrics are sent anonymously, associated with the HiSeq 2500 from which they came, not to any particular user. Metrics include interoperation files (that SAV uses) and global files such as RunInfo.xml and RunParameters.xml. No data files are sent unless you specify for the system to do so.

Permissions for this feature are enabled by default, and Illumina recommends that you leave them enabled. To disable permissions, select **Menu | Tools | Options**, and then deselect **Send system health information to Illumina to aid technical support**. You can also view terms in the Options dialog box.

If permissions for this feature are not enabled, HCS prompts you to accept Terms and Conditions. If you do not accept the terms and conditions, you cannot use BaseSpace. If you are not using BaseSpace, go back to the previous screen and make sure that the BaseSpace option is de-select (otherwise the Next button is not enabled).

- 2 In **Save to an output folder**, browse to and select the path of your run folder.
- 3 Select **Zip BCL files** to reduce the footprint of disc space needed for the run. If you are using BaseSpace, this option is selected by default.



### NOTE

If you are using BaseSpace or an updated version of CASAVA for data analysis, zip the BCL files.

- 4 Select **Bin QScores** to reduce the footprint of disc space needed for the run. This is done by grouping Q-scores together over a wider range of values. For technical details regarding how data quality score resolution can be reduced without sacrificing score accuracy or standard analysis and variant calling performance, find the white paper on [www.Illumina.com](http://www.Illumina.com) titled, *Reducing Whole-Genome Data Storage Footprint*.

- 5 Select a setting from the **Save Auxiliary Files** drop-down list:
  - Select **Save All Thumbnails** to save tile level and full thumbnails. Thumbnail images are a combination of eight images from each swath, combined in one thumbnail image. A swath is a column of tiles, and there are three swaths per lane per surface.
  - Select **Save Tile Thumbnails** to save tile level thumbnails only. Tile thumbnails show just the tile rather than the whole swath.
- 6 Select **Next**.

## Flow Cell Setup Screen

The Flow Cell Setup screen is the run configuration screen with which you record information about your run, starting with the flow cell ID.



### NOTE

It is critical that you scan the flow cell barcode or accurately enter the flow cell ID when you set up your run. The software uses the flow cell ID to determine flow cell type and reagent compatibility.

- 1 Scan the flow cell barcode or enter the flow cell ID (barcode number) of the flow cell to be sequenced.
- 2 Confirm the flow cell type, which is automatically selected based on the flow cell ID.
- 3 Enter the experiment name and user name.
- 4 Select the lane containing the control, if applicable. Otherwise, select **None**.
- 5 Select **Next**.

## Advanced Screen

The Advanced screen includes settings for first base confirmation and indicating alignment lanes.

- 1 Select the **Confirm First Base** checkbox to generate a First Base Report confirmation. Selecting this option requires that you confirm the results before the run can continue. If you do not select the checkbox, the First Base Report is generated as usual and the run continues without showing the First Base Report confirmation dialog box. You can access the First Base Report from the run folder at any time during the run.

- 2 Select the control lane on your flow cell containing the control library from the drop-down list. Otherwise, select **None**.
- 3 Select or deselect **Align to PhiX** for each lane of the flow cell.  
This enables real-time reporting of error rates for PhiX control lanes or PhiX spike-in lanes. By default, all lanes are selected for alignment by Real-Time Analysis (RTA). Clear the checkbox for any lanes that do not have PhiX added to the sample so that RTA does not align and calculate error rates for that lane, resulting in arbitrarily high error rates. For more information, see *Using a PhiX Control for HiSeq Sequencing Runs*, which is available on the HiSeq support page on the Illumina website at [www.illumina.com](http://www.illumina.com).
- 4 Select **Keep Intensity Files** for later reanalysis or custom processing (optional).  
Select the checkbox to save cluster intensity files (\*.cif) for later reanalysis, troubleshooting, or custom processing. Saving intensity files is not required for on-instrument analysis. Enabling this option significantly increases the size of the data output folder. By default, this option is not selected.
- 5 Select **Next**.

## Recipe Screen

- 1 Select one of the following indexing options:
  - **No Index**—Performs a non-indexed single-read or paired-end run.
  - **Single Index**—Performs a single-read or paired-end run with one Index Read (Index 1 (i7) Read).
  - **Dual Index**—Performs a single-read or paired-end run with two Index Reads (Index 1 (i7) Read and Index 2 (i5) Read).
  - **Custom**—Performs a single-read or paired-end run with a custom number of cycles for the Index Reads.
- 2 Select a flow cell format, either **Single Read** or **Paired End**.
- 3 Enter the number of cycles you plan to sequence for Read 1 and Read 2, if applicable. If you selected the **Custom** indexing option, enter the number of cycles you plan to sequence for the Index Reads.

**NOTE**

If you choose to run a custom recipe, the number of cycles is determined by that recipe. The number of cycles option in the software is grayed out when the recipe is loaded. You can enter up to 325 cycles.

**NOTE**

It is possible to perform a dual-indexed single-read run using a paired-end flow cell containing paired-end libraries. To do so, set up a *dual-indexed paired-end run* and enter zero for the number of cycles in Read 2. This stops the run after the Index 2 (i5) Read.

- 4 Select the **Use Existing Recipe** checkbox only if you want to use a custom recipe. Otherwise, allow the software to create your recipe based on information you provide on the run setup screens.

## Sample Sheet Screen

In the **Sample Sheet** field, enter the path of a valid sample sheet for your run. When the run begins, the software copies the sample sheet to the root level of the run folder. Unless you are using BaseSpace, a sample sheet is optional. For indexed runs, the sample sheet is required if you want indices to be reported in SAV. If you are running BaseSpace, a sample sheet is required. For more information about sample sheets, see *Sample Sheet Overview* on page 17.

**NOTE**

To view indexing results in Sequencing Analysis Viewer (SAV) or to use BaseSpace, load a sample sheet at the start of your run. SAV v1.8 requires a sample sheet to properly report indexing information. For more information, see the *Sequencing Analysis Viewer User Guide*, part # 15020619.

- 1 Enter the path or browse to a valid sample sheet for your run. For indexed runs, the sample sheet is required if you want indices to be reported in SAV. A sample sheet is also required if you are using BaseSpace.
- 2 If you want to perform on-board cluster generation on the HiSeq, select **On-Board Cluster Generation**. If you have already performed template hybridization on the cBot, select **Template Hybridization on cBot**.
- 3 Select **Next**.

## Reagents Screen

The Reagents screen records information about the reagents used for your run.

- 1 Scan or enter the Reagent Kit ID. You can enter the kit ID from either box 1 or box 2.
- 2 For indexed runs, scan or enter the Indexing Reagent Kit ID.
- 3 Select the SBS reagent kit you are using for your run.
  - Select **200 Cycles** for a 200 cycle kit.
  - Select **50 Cycles** for a 50 cycle kit.



#### NOTE

If you are combining four 50-cycle SBS kits for a dual-indexed run, select **Used Kit** instead of 50 Cycles to correctly set the cycle counter.

- 4 For paired-end runs, scan or enter the PE or SR Reagent Kit ID.
- 5 The Cycles Remaining field is populated automatically depending on the SBS kit you are using. If you are using a partial kit, enter the number of cycles reagents are expected to last. The software counts down the number of cycles entered. When the cycles are low, the software prompts you to load fresh reagents.



#### NOTE

If you are starting a new run using less than a full reagent kit, make sure that you are present when new reagents are needed.

- 6 If you selected Template Hybridization on cBot in the Sample Sheet window, select **Prime SBS Reagents** to prime reagents before you start a run. If you are performing on-board cluster generation, do not prime SBS Reagents.
- 7 Select **Next**.

## Review Screen

Review your run information. If it is correct, select **Next**.  
Select **Back** if you need to change any entries.



## Load and Prime Reagents

The next step is to load sequencing reagents. The software interface guides you through the process.

## Pre-Run Setup Screens

After you have entered the required information in the run configuration screens, the pre-run setup screens open. Pre-run setup screens include: Load Reagents, Load Prime Flow Cell, Prime Fluidics Check, and Prime. The Load Prime Flow Cell, Prime Fluidics Check, and Prime screens are available in only high output mode and rapid run mode when you have performed template hybridization on the cBot.

### Load SBS and Clustering Reagents and Template

Make sure that SBS reagents are thoroughly thawed and ready to load onto the instrument.

#### Illumina-Supplied Consumables

- ▶ Eight funnel caps (Provided in the SBS Kit)

#### User-Supplied Consumables

- ▶ One 250 ml bottle (Corning, catalog # 430776)
- ▶ One flip-top Eppendorf tube per flow cell (1.5 ml or 1.7 ml). The following Eppendorf tubes are acceptable for use with the HiSeq 2500. Do not use tubes with screw caps.

Consumable	Supplier	Purpose
Microcentrifuge Tube, 1.5 mL	VWR, catalog #20170-038	Sample(template) loading and washing the template fluidics line.
Microcentrifuge Tube, 1.7 mL, capless	VWR, catalog #20170-575	
Microcentrifuge Tube, 1.5 mL	VWR, catalog #20170-650	
Microcentrifuge Tube, 1.5 mL	VWR, catalog #89000-028	
Microcentrifuge Tube, 1.5 mL	Axygen, catalog #MCT-150-C	
Microcentrifuge Tube, 1.7 mL	Axygen, catalog #MCT-175-C	
Microcentrifuge Tube, 1.7 mL	Sorenson BioScience, catalog #16070	

## Procedure

- 1 Record the weight of each reagent on the lab tracking form to confirm proper reagent delivery.
- 2 Open the reagent compartment door.
- 3 Raise the sippers for the sequencing reagent rack using the following motion:
  - a Pull the sipper handle towards you.
  - b Push the sipper handle up while pulling it towards you.
  - c Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.
- 4 Slide the reagent rack out of the reagent compartment.
- 5 Place each reagent bottle onto the rack in the associated numbered position as shown on the Reagents screen, making sure that the conical end of the bottle rests in the indentation on the base of the rack.



### NOTE

Load laboratory-grade water into positions 2, 4, 8, 10, 11, and 12 for single read flow cell. Load laboratory-grade water into positions 2, 4, 8, and 19 for a paired-end flow cell. This prevents the lines from drying out.

**Table 6** Single Read Flow Cell Positions

Position	Reagent	Description
1	IMM	Incorporation Master Mix
2	PW1 (25 ml)	Wash Buffer
3	SRM	Scan Reagent Master Mix
4	PW1 (25 ml)	Wash Buffer
5	USB	Universal Sequencing Buffer
6	USB	Universal Sequencing Buffer
7	CRM	Cleavage Reagent Master Mix
8	PW1 (25 ml)	Wash Buffer
10	PW1 (10 ml)	Wash Buffer

Position	Reagent	Description
11	PW1 (10 ml)	Wash Buffer
12	PW1 (10 ml)	Wash Buffer
13	AMS	Fast Amplification Mix
14	FPM	Fast Premix
15	FDR	Fast Denaturation Reagent
16	HP9	i5 Index Primer
17	HP12	i7 Index Primer
18	HP6	Read 1 Primer
19	FLS	Fast Linearization Solution
Sample Loading Station Template	Template	420 $\mu$ l in 1.5 ml or 1.7 ml microcentrifuge tube at required concentration

**Table 7** Paired End Flow Cell Positions

Position	Reagent	Description
1	IMM	Incorporation Master Mix
2	PW1 (25 ml)	Wash Buffer
3	SRM	Scan Reagent Master Mix
4	PW1 (25 ml)	Wash Buffer
5	USB	Universal Sequencing Buffer
6	USB	Universal Sequencing Buffer
7	CRM	Cleavage Reagent Master Mix
8	PW1 (25 ml)	Wash Buffer
10	FRM	Fast Resynthesis Mix
11	FLM2	Fast Linearization Mix 2 (Read 2)
12	FLM1	Fast Linearization Mix 1 (Read 1)
13	AMS	Fast Amplification Mix
14	FPM	Fast Premix
15	FDR	Fast Denaturation Reagent
16	HP7	Read 2 Primer
17	HP12	i7 Index Primer
18	HP6	Read 1 Primer

Position	Reagent	Description
19	PW1 (10 ml)	Wash Buffer
Sample Loading Station Template	Template	420 $\mu$ l in 1.5 ml or 1.7 ml microcentrifuge tube at required concentration

## Loading the Template



### NOTE

If you used the alternative, cBot template hybridization recipe, you do not need to load the template. However, place two empty conical tubes into the Template Loading Station to deal with back flow.

- 1 The software prompts you to load the template at the same time as the other reagents. Load the template into the sample loading station. When loading the same low-diversity sample into both rapid run flow cell lanes, you do not have a balanced control lane for matrix and phasing estimations. This can result in poor quality data. Illumina recommends spiking in PhiX at 30% - 50% to increase base diversity. Alternatively, perform template hybridization on the cBot with a balanced sample in one lane, and set it as the control lane on the HiSeq.

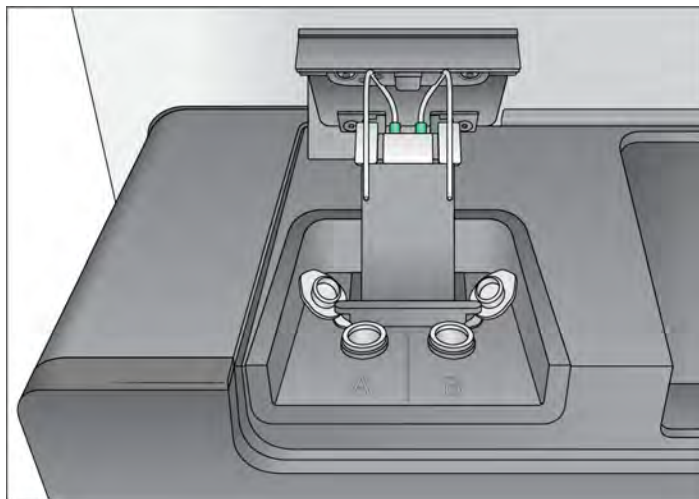


### CAUTION

It is important to add no more than the required volume of template. When the sequencing run is finished successfully, the system performs a flush-back wash adding an additional 500  $\mu$ l of water back into the template tube.

- a Make sure that the flow cell compartment door is open.
- b Lift the door of the sample loading station on the left side of the flow cell stage. As you lift the door, the sippers, which are attached to the door, also lift up.
- c Remove the Eppendorf tube containing water from the appropriate positions (A and B) and replace it with the Eppendorf tubes containing exactly 420  $\mu$ l of diluted library template per Eppendorf tube. Holding the lids away from you, push them under the bar behind the tubes (to hold them out of the way of the sippers).

Figure 27 Sample Loading Station With the Door Open



NOTE

The liquid remaining in the tube following the run is highly diluted. Discard it immediately and not keep it for further use.

- d Slowly close the sample loading station door; make sure that the sippers are properly aligned so that they enter the Eppendorf tubes when the lid is closed. (Make sure that the sample loading station door is closed before closing the flow cell compartment door.)

### Loading the SBS and Clustering Reagents



NOTE

Be sure to load all clustering reagents regardless of run type.

- 1 Add 25 ml of PW1 or laboratory-grade water to the bottles in positions 2, 4, and 8.



NOTE

HCS v2.0 and later includes an automatic post-run rinse following the completion of the sequencing run.

The post-run rinse does *not* replace the post-run instrument wash.

- 2 Add 10 ml of PW1 or laboratory-grade water to 15 ml conical tubes in positions 10, 11, and 12 for a single read flow cell or position 19 for a paired-end flow cell.

- 3 Remove the cap from each reagent bottle and replace it with a funnel cap. Replace the cap on the bottle of CRM last, and then replace your gloves.

**CAUTION**

After handling the bottle of CRM, discard your gloves and replace them with a new pair.

- 4 Slide the reagent racks into the reagent compartment, aligning the racks with the raised guide on the floor of the compartment.
- 5 Lower the sippers into the sequencing reagent bottles as follows:
  - a Pull the sipper handle towards you.
  - b Lower the sipper handle while pulling it towards you.
  - c Visually inspect the sippers to make sure that they do not bend as they lower into the funnel caps.
  - d Release the sipper handle into the slot on the bottom end of the groove. Make sure that the sipper handle rests securely in the slot.
- 6 Select the checkbox labeled **PW1 (25 ml) loaded in Position 2**.
- 7 Select **Next**.

## Prime Reagents

**NOTE**

Prime reagents only if you have done template hybridization on the cBot for a rapid run flow cell.

Perform the following steps to prime reagents:

- ▶ *Clean the Flow Cell Holder* on page 94
- ▶ *Load the Used Flow Cell* on page 54
- ▶ *Confirm Proper Flow* on page 57
- ▶ *Position Tubing and Prime Reagents* on page 57

### User-Supplied Consumables

- ▶ Lens cleaning tissue
- ▶ 70% ethanol or alcohol wipes
- ▶ Low-lint lab tissue
- ▶ Used flow cell

## Clean the Flow Cell Holder

- 1 Open the flow cell compartment door.



### CAUTION

Do not place fluids on the flow cell compartment door or on the flow cell stage when the door is open. Spills in this area can damage the instrument.

- 2 Make sure that the flow cell lever is in the OFF position.

Figure 28 Flow Cell Lever in Position 0



- 3 Put on a new pair of powder-free latex gloves.
- 4 If the flow cell from a previous run is present, remove it and set aside. It can be used to confirm proper flow before loading the clustered flow cell.
- 5 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.



### CAUTION

Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

- 6 Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.



Figure 29 Vacuum Hole Locations



### Load the Used Flow Cell

- 1 Rinse the used flow cell with laboratory-grade water. Dry it with lens cleaning tissue or lint-free tissue.
- 2 Clean the flow cell using alcohol wipes and lens cleaning tissue.



#### NOTE

Do not remove or replace the manifold gaskets at this time.

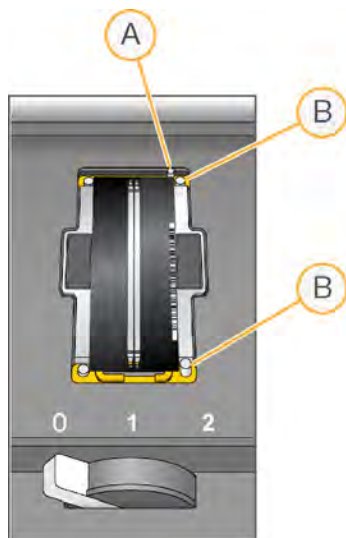
- 3 Place the used flow cell on the flow cell holder with the inlet and outlet ports facing *down* and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.
- 4 Gently slide the flow cell towards the top and right guide pins until it stops.



#### NOTE

Remove your hand from the flow cell before engaging the vacuum switch. This prevents any possible alignment drift over time.

Figure 30 Flow Cell Positioned Against Top and Right Guide Pins.



- A Top Guide Pin
- B Right Guide Pins

- 5 Slowly move the flow cell lever to position 1. This engages the vacuum and secures the flow cell into position. When the flow cell lever is green, the vacuum is engaged.

Figure 31 Flow Cell Lever in Position 1



- Wait for about 5 seconds, and then slowly move the flow cell lever to position 2 (far-right). When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 32 Flow Cell Lever in Position 2



- Make sure that the **Vacuum Engaged** checkbox is selected on the load prime flow cell screen, and then select **Next**.

### Confirm Proper Flow

After the used flow cell is loaded, check for proper flow. Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.

- Select solution 2 (laboratory-grade water) from the drop-down list.



#### CAUTION

You can use water to confirm proper flow on a used flow cell only. Never use water to confirm proper flow on a clustered flow cell.

- Confirm the following default values:
  - Volume: **250**
  - Aspirate Rate: **1500**
  - Dispense Rate: **2000**
- Select **Pump**.
- Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

If you see excessive bubbles, check the gaskets for obstructions, reduce the aspirate rate to 1000, and pump another 250  $\mu$ l of water to the flow cell. If problems persist, remove the flow cell, repeat the cleaning steps, and reload the flow cell.

## Position Tubing and Prime Reagents

- 1 Loosen and remove waste lines 4 and 5 for the appropriate flow cell from the waste container. Make sure that you do not include the eight lines for the opposite flow cell or the lines for the condensation pump or the paired-end priming pumps.
- 2 Place lines 4 and 5 into two 15 ml tubes. Place the remaining lines (1, 2, 3, 6, 7 and 8) into a container with laboratory-grade water. Priming waste is collected and measured after the priming step.
- 3 Select **Next**. The priming screen opens and the priming step starts automatically. You can monitor the progress of the priming step from the priming screen.
- 4 When the priming step is complete, measure the collected priming waste and confirm that the volume is 2.5 ml  $\pm$  10%, 500  $\mu$ l per reagent, per lane. Record the results on the lab tracking form.



### NOTE

The color of sequencing reagent waste might be brown in appearance. You also might see a slight discoloration on the funnel caps or in any spills. The change in waste color is not toxic and does not affect run performance.

- 5 Return the waste tubing to the waste container before proceeding. Leave waste lines 1, 2, 3, 6, 7 and 8 in container with laboratory-grade water
- 6 Select **Next**. You are ready to load the hybridized flow cell.

## Load a Flow Cell



### NOTE

If you have performed template hybridization on the cBot, you will load a hybridized flow cell. If you are performing template hybridization on the HiSeq, load a new flow cell.

The next step is to remove the used flow cell and load the flow cell that you want to sequence.

If you want to have individually addressable lanes on the 2-lane rapid run flow cell, use the cBot to carry out template hybridization and first extension. The rest of the clustering process is carried out on the HiSeq.

Perform the following steps to load the flow cell:

- ▶ *Remove the Used Flow Cell* on page 60
- ▶ *Clean the Flow Cell Holder* on page 100
- ▶ *Clean the Flow Cell* on page 101
- ▶ *Load the Flow Cell* on page 63
- ▶ *Confirm Proper Flow* on page 66

### User-Supplied Consumables

- ▶ Lens cleaning tissue
- ▶ 70% ethanol or alcohol wipes
- ▶ Low-lint lab tissue



### NOTE

Regardless of which mode you are running on the HiSeq 2500/1500, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.

## Remove the Used Flow Cell

- 1 Open the flow cell compartment door.

Figure 33 Flow Cell Lever in Position 1



- 2 Slowly move the flow cell lever to position 1 to disengage the manifolds.
- 3 Slowly move the flow cell lever to position 0 to disengage the vacuum seal and release the flow cell.

Figure 34 Flow Cell Lever in Position 0



- 4 Lift the used flow cell from the flow cell holder.

### Clean the Flow Cell Holder

- 1 Put on a new pair of powder-free latex gloves.

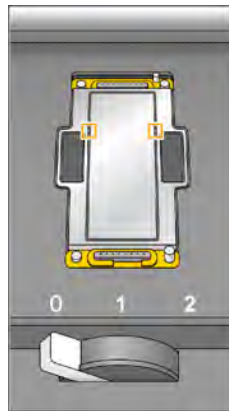
- Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.



**CAUTION**

Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

**Figure 35** Inspect Vacuum Holes



- Visually inspect the flow cell holder to make sure that it is free of lint and the vacuum holes are free of obstructions.

## Clean the Flow Cell

- Rinse the flow cell with laboratory-grade water and dry it with a lens cleaning tissue.
- Fold an alcohol wipe to approximately the size of the flow cell.
- Hold the edges of the clustered flow cell with two fingers. Make sure that the inlet and outlet ports are facing *up*.
- Wipe off each side of the flow cell with a single sweeping motion. Repeat, refolding the alcohol wipe with each pass, until the flow cell is clean.
- Dry the flow cell using a dry lens cleaning tissue.



**CAUTION**

If you clean the flow cell while it is lying on the benchtop, you could easily apply too much pressure and break the flow cell. Illumina recommends cleaning the flow cell while holding the edges between your fingers.

- 6 Protect the flow cell from dust until you are ready to load it onto the instrument.

## Load the Flow Cell



### NOTE

Do not replace the manifold gaskets at this time. Leave the gaskets that were installed before the instrument maintenance wash in place when loading the flow cell. You will replace manifold gaskets after the current sequencing run is completed, before the instrument maintenance wash.

Regardless of which mode you are running, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.

The manifold must be changed for rapid run mode every 10 days, before the instrument maintenance wash.

- 1 Place the flow cell on the flow cell holder with the inlet and outlet ports facing *down* and the barcode on the right. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.



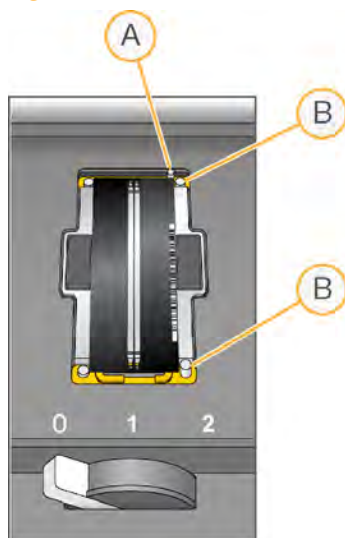
### NOTE

The flow cell has a mechanically keyed corner, which provides a visual orientation for loading the flow cell. Install the flow cell so that the keyed corner is on the output end of the flow cell facing towards the instrument, and on the left side of the flow cell by lane 1.

- 2 Gently slide the flow cell towards the top and right guide pins until it stops.



Figure 36 Flow Cell Positioned Against Top and Right Guide Pins



- A Top Guide Pin
- B Right Guide Pins



NOTE

Remove your hand from the flow cell before engaging the vacuum switch. This prevents possible alignment drift over time.

- 3 Slowly move the flow cell lever to position 1. This engages the vacuum and secures the flow cell into position. When the flow cell lever is green, the vacuum is engaged.

Figure 37 Flow Cell Lever in Position 1



- 4 Wait for about 5 seconds, and then slowly move the flow cell lever to position 2. When the flow cell lever is solid green, the manifolds are in position and the flow cell is ready for use.

Figure 38 Flow Cell Lever in Position 2



- 5 Make sure that the **Vacuum Engaged** checkbox is selected on the load sequencing flow cell screen.

## Confirm Proper Flow



### NOTE

If you are performing template hybridization in the cBot, load a used flow for reagent priming and the fluidics check. You must also check fluidics through the hybridized flow cell.

After loading the flow cell, check for proper flow using the fluidics check screen. Checking for proper flow confirms that the flow cell and gaskets are properly installed and the manifold is engaged.



### NOTE

Regardless of which mode you are running on the HiSeq 2500/1500, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.

- 1 Select solution 5 (USB) from the drop-down list.
- 2 Make sure that the following default values are entered:
  - Volume: **250**
  - Aspirate Rate: **1500**
  - Dispense Rate: **2000**
- 3 Make sure that waste outlet lines 1, 2, 3, 6, 7, and 8 are in a bottle of clean water and that lines 4 and 5 are in the waste container.
- 4 Select **Pump**.
- 5 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

If you see excessive bubbles, check the manifold gaskets for obstructions, and repeat the process.

  - a Select solution 6 (USB) to avoid depleting USB from position 5.
  - b Reduce the aspirate rate to 1000, and pump another 250  $\mu$ l of USB to the flow cell.
- 6 After you have confirmed proper flow, select **Next** to proceed. Make sure that the flow cell lever is green, and then close the flow cell compartment door.
- 7 Confirm that the **Vacuum Engaged** and **Door Closed** checkboxes are selected, and then select **Next**.
- 8 Select **Start** to start the sequencing run.

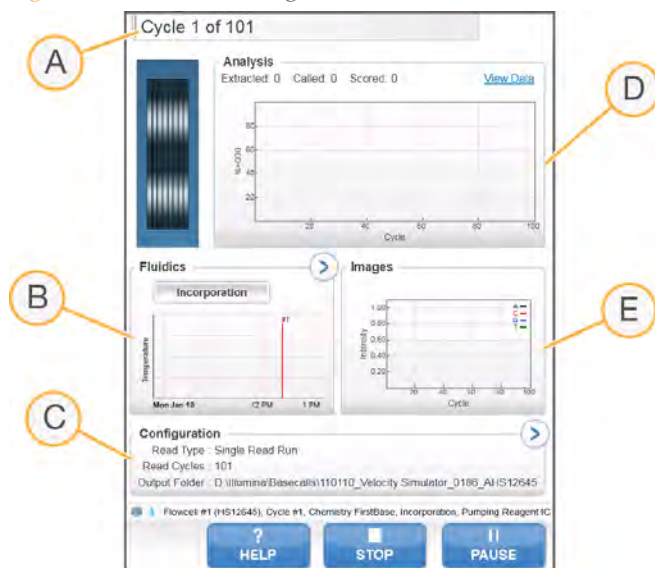
## Monitor the Run

Use the run overview screen to monitor on-instrument analysis, fluidics, and imaging.

- ▶ **Progress Bar**—Use the progress bar to monitor how many cycles have been completed.
- ▶ **Flow Cell Image**—Use the flow cell image to monitor which lanes have been imaged for that cycle.
- ▶ **Analysis Graph**—Use the analysis graph to monitor quality scores by cycle.
- ▶ **Images Graph**—Use the images graph to monitor intensities by cycle.
- ▶ **Fluidics Graph**—Use the arrow button to expand the fluidics section and monitor chemistry steps in the protocol.

In rapid run mode, HCS automatically prompts you to perform a water wash when sequencing is finished. See *Perform a Water Wash* on page 117.

Figure 39 Run Monitoring Screen Overview



- A Run Progress
- B Fluidics and Temperature
- C Run Configuration
- D Analysis
- E Images

**NOTE**

You might notice an intermittent clicking sound coming from the right side of the instrument during operation. This is normal. The clicking sound indicates that the refrigeration condensation pump is working.

## First Base Report

The First Base Report confirmation dialog box opens automatically after cycle one is complete, if you selected **Confirm First Base** on the scan screen during run setup. The run pauses at this step.

If you did not select the checkbox, the run continues after first base incorporation without prompting you to review the First Base Report.

- 1 Review the First Base Report from the First Base Report confirmation dialog box or by opening `First_Base_Report.htm` from the root level of the run folder.
- 2 If the results are satisfactory, select **Continue** to continue your run. If results are not satisfactory, abort the run.

## Sequencing Analysis Viewer

You can monitor your run using the Sequencing Analysis Viewer, which launches automatically after image analysis begins. For more information, see *Sequencing Analysis Viewer* on page 125.

For a detailed description of available metrics and installation instructions, see the *Sequencing Analysis Viewer User Guide*, part # 15020619.



# Post-Run Procedures

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## Introduction

This section and its subsections pertain to both rapid run and high output mode runs. Post-run procedures include the removal and weighing of reagents, and an instrument wash.

Regular instrument washes ensure continued performance in the following ways:

- ▶ Flushes any remaining reagents and sample from the fluidics lines and sippers
- ▶ Prevents salt accumulation and crystallization in the fluidics lines and sippers
- ▶ Prevents cross-contamination from the previous run, including cross-contamination from rapid to high output runs and high output to rapid runs.



### NOTE

A water wash is performed at the end of each run to wash the system and check fluidics. HCS does not allow you to start a new run unless a water wash or required maintenance wash has been performed within the last 24 hours. Every 10 days, HCS prompts you to perform a maintenance wash without regard to when you last performed a maintenance wash.



## Unload and Weigh Reagents

- 1 Open the reagent compartment door.
- 2 Raise the sippers for the reagent rack and clustering rack you are loading using the following motion:
  - a Pull the sipper handle towards you.
  - b Push the sipper handle up while pulling it towards you.
  - c Release the sipper handle into the slot on the top end of the groove. Make sure that the sipper handle rests securely in the slot.
- 3 Slide the reagent rack out of the reagent compartment using the rack handle.
- 4 Remove each bottle from the reagent rack and record the weight on the lab tracking form.
- 5 (For rapid run mode) Remove tubes from sample loading station.



### NOTE

The color of sequencing reagent waste might be brown in appearance. You also might see a slight discoloration on the funnel caps or in any spills. The change in waste color is not toxic and does not affect run performance.



### WARNING

**A component in the clustering kit contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.**

**Dispose of containers and any unused contents in accordance with the governmental safety standards for your region.**

**For more information, see the MSDS for this kit, at [www.illumina.com/msds](http://www.illumina.com/msds).**

## Performing an Instrument Maintenance Wash

To ensure optimal instrument performance, perform an instrument maintenance wash every 10 days, after each sequencing run, and prior to switching between Rapid Run mode and High Output mode. Change the gasket at the time of the maintenance wash. Regardless of which mode you are running, you will always have a 10 port gasket in the front manifold and an 8 port gasket in the back manifold. The maintenance wash consists of three wash steps:

- ▶ First water wash—Flushes the system with laboratory-grade water.
- ▶ NaOH wash—Washes the system with NaOH.
- ▶ Final water wash—Flushes the system with laboratory-grade water to remove NaOH.

Positions	Delivered Volume
Eight SBS positions	20 minutes
Eight SBS positions and ten clusters	60 minutes
(For rapid run mode) One sample position, eight SBS reagents, 10 cluster reagents. SBS and cluster positions are washed every time as they are used for every run.	10 minutes

### User-Supplied Consumables

- ▶ Ethanol wipes
- ▶ 24 bottles, 250 ml (Corning, catalog # 430776), eight bottles per wash step
- ▶ 30 tubes, 15 ml (Corning, catalog # 430052), 10 tubes per wash step
- ▶ Laboratory-grade water
- ▶ 1 N NaOH
- ▶ 3 eppendorfs per flow cell for sample loading station wash steps

### First Water Wash

- 1 From the HCS Welcome screen select **Wash | Maintenance**.
- 2 (For high output mode) Select **Yes** to wash PE reagent positions. Otherwise, select **No**. Select **Next** to proceed.
- 3 Load the instrument with laboratory-grade water as follows:

- a Fill eight 250 ml bottles with more laboratory-grade water than there were reagents present at the beginning of the run to make sure that the sippers are rinsed.
  - b (For rapid run mode) Fill Eppendorf tubes with 1 ml laboratory-grade water
  - c If you are washing clusters, load ten 15 ml tubes with 10 ml laboratory-grade water.
  - d Load the bottles and tubes onto the instrument in the appropriate reagent rack.
- 4 Remove the flow cell from the flow cell stage, and set it aside. Reload this flow cell before starting the instrument wash.



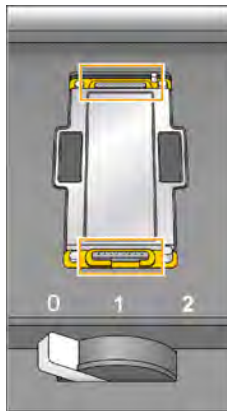
## NOTE

Do not change gaskets during the NaOH and second wash steps. Gaskets are to remain installed until the sequencing run is complete.

*Always* install new manifold gaskets before performing an instrument maintenance wash. Regardless of which mode you are running, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.

- 5 Make sure that you have protective gloves on, and remove the front and back gaskets. Do so by applying light pressure with your finger to one side of the gasket. The other side pops up. Use tweezers to grasp and remove each gasket.

Figure 40 Remove Used Manifold Gaskets



- 6 Position two new gaskets in the slots on the front end and back end of the flow cell holder. Regardless of which mode you are running, always have a 10-port gasket in the front manifold and an 8-port gasket in the back manifold.
- 7 Load the flow cell that you removed to install the new gaskets. Select **Next**.

- 8 Perform a fluidics check:
  - a Select solution 2 from the drop-down list.
  - b Enter the following default values:
    - Volume: **250**
    - (For high output mode) Aspirate Rate: **250**
    - (For rapid run mode) Aspirate Rate: **1500**
    - Dispense Rate: **2000**
  - c Select **Pump**.
  - d Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
- 9 Loosen and remove the eight lines of waste tubing for the appropriate flow cell from the waste container. Make sure that you do not include the eight lines for the opposite flow cell, or the lines from the condensation pump or the cluster priming pumps.
- 10 (For high output mode) Bundle the eight lines of waste tubing with parafilm, making sure to keep all of the ends even. Place the bundled tube ends into a 250 ml bottle. (For rapid run mode) Place the ends of tubes 4 and 5 into an empty container. Place the end of all of the other tubes into a bottle of clean water to prevent air from being introduced into the syringe pumps.
- 11 Select **Next**.
- 12 When the wash is complete, measure the delivered volume.  
The following tables list the wash volumes from the eight lines of waste tubing. They do not include the priming pump wash volume.

Positions	Delivered Volume
Eight SBS positions	32 ml
Eight SBS positions and ten clusters	72 ml
(For rapid run mode) One sample position, eight SBS reagents, 10 cluster reagents. SBS and cluster positions are washed every time as they are used for every run.	9.5 ml per lane

## NaOH Wash



### CAUTION

For safety purposes, wear protective eyewear when working with large volumes of NaOH.

- 1 Load the instrument with 1 N NaOH as follows:
  - a Load eight 250 ml bottles with 5 ml of NaOH.
  - b If you are washing cluster positions, load ten 15 ml tubes with 5 ml NaOH.
  - c (For rapid run mode) Fill Eppendorf tubes with 1 ml 1N NaOH
- 2 Load the bottles and tubes onto the instrument in the appropriate reagent rack.
- 3 Select **Next** to start the NaOH wash.
- 4 When the NaOH wash is complete, measure the delivered volume.

Positions	Delivered Volume
Eight SBS positions	16 ml
Eight SBS positions and 10 cluster positions	36 ml
(For rapid run mode) One sample position, eight SBS reagents, 10 cluster positions. SBS and cluster positions are washed every time as they are used for every run.	4.75 ml per lane

## Final Water Wash

- 1 Load the instrument with laboratory-grade water as follows:
  - a Load eight 250 ml bottles with at least 20 ml of laboratory-grade water.
  - b If you are washing cluster positions, load ten 15 ml tubes with at least 10 ml laboratory-grade water.
  - c Fill Eppendorf tubes with 1 ml laboratory-grade water.



### CAUTION

Do not reuse the same water or wash bottles that you used for the first wash step. The water from the first wash step might be contaminated with reagents that were present on the sippers.

- 2 Load the bottles and tubes onto the instrument in the appropriate reagent rack.
- 3 Select **Next** to start the final water wash.
- 4 When the final water wash is complete, measure the delivered volume.

Positions	Delivered Volume
Eight SBS positions	32 ml
Eight SBS positions and 10 cluster positions	72 ml
(For rapid run mode) One sample position, eight SBS reagents, 10 cluster positions. SBS and cluster positions are washed every time as they are used for every run.	9.5 ml per lane

- 5 Unwrap the waste lines and return them to the waste container.

## Perform a Water Wash

Perform a water wash if the instrument has been idle for one day or more before you begin a new sequencing run.

For rapid run mode, you do not need to navigate to the home screen before starting the post-run wash as HCS automatically prompts you to do so. Volume for the rapid run post run wash is greater than for the standard water wash.



### NOTE

HCS prompts you to perform a water wash after each rapid run.

HCS does not allow you to start a new run if the instrument has been idle for more than 24 hours.

Before the start of every run, be sure to carry out the recommended fluidics check as you are prompted by the software, to ensure that fluidics are working properly.

### Illumina-Supplied Consumables

- ▶ Laboratory-grade water

### User-Supplied Consumables

- ▶ Eight 250 ml bottles (Corning, catalog # 430776)
- ▶ Ten 15 ml conical tubes (Corning, catalog # 430052)
- ▶ One Eppendorf tube flip-top tube for each flow cell

### Procedure

- 1 From the HCS Welcome screen select **Wash | Water**.
- 2 (For high output mode) Select **Yes** to wash cluster positions. Otherwise, select **No**. Select **Next** to proceed.
- 3 Load the instrument with laboratory-grade water as follows:
  - a Fill eight 250 ml bottles with 20 ml laboratory-grade water.
  - b If you are washing paired-end positions, load ten 15 ml tubes with 10 ml laboratory-grade water.
  - c If you are washing cluster positions, load ten 15 ml tubes with 10 ml laboratory-grade water.
  - d (For rapid run mode) Fill the Eppendorf tube with 1 ml laboratory-grade water.



NOTE

Manifold gaskets do not require replacement before a water wash.

- 4 Make sure that a used flow cell is loaded. Load a used flow cell, if necessary. Select **Next**.



NOTE

Illumina recommends a fluidics check if you loaded a used flow cell for the wash.

- 5 If you loaded a used flow cell in the previous step, perform a fluidics check:
  - a Select solution 2 from the drop-down list.
  - b Enter the following default values:
    - Volume: **250**
    - (For high output mode) Aspirate Rate: **250**
    - (For rapid run mode) Aspirate Rate: **1500**
    - Dispense Rate: **2000**
  - c Select **Pump**.
  - d Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.
- 6 Loosen and remove the eight lines of waste tubing for the flow cell from the waste container. Make sure that you do not include the lines for the condensation pump or the cluster priming pump.
- 7 Bundle all of the lines of waste tubing with parafilm, making sure to keep all of the ends even.
  - ▶ **High Output:** Place the bundled tube ends into a 250 ml bottle.
  - ▶ **Rapid Run:** Place the ends of tubes 4 and 5 into an empty container. Place the end of all of the other tubes into a bottle of clean water to prevent air from being introduced into the syringe pumps.
- 8 Select **Next** to start the water wash.

Positions	Approximate Run Time
Eight SBS positions	20 minutes
Eight SBS positions and 10 paired-end positions	60 minutes
(For rapid run mode) Eight SBS positions, 10 cluster positions, and 1 sample loading position for each flow cell	10 minutes per SBS cluster



Positions	Approximate Run Time
Eight SBS positions	26 minutes
Eight SBS positions and 10 paired-end positions	52 minutes

- 9 When the wash is complete, measure the delivered volume. Record the delivery volume on the lab tracking form.

Positions	Delivered Volume
Eight SBS positions	32 ml
Eight SBS positions and 10 cluster positions	72 ml
(For rapid run mode) Eight SBS reagents, 10 cluster positions, and 1 sample loading position. SBS and cluster positions are washed every time as they are used for every run.	9.5 ml per lane

- 10 Return the waste tubing to the waste bottle.

## Idle the Instrument

If the instrument is not going to be used within the next 10 days, prepare it to sit idle. If it is not going to be used for more than 10 days, see *Shut Down the Instrument* on page 121.

- 1 Perform a complete maintenance wash to flush the system fully. For more information, see *Performing an Instrument Maintenance Wash* on page 112.  
You can prepare both flow cell positions or only one flow cell position to sit idle.
- 2 Leave the flow cell on the flow cell stage with the flow cell lever in position 2. This leaves the manifolds in the raised position.
- 3 (For high output mode) Load 10 ml of laboratory-grade water in each reagent position in the reagent racks. Then lower the sippers into the water.  
(For rapid run mode) Load 10 ml of laboratory-grade water in each reagent position and 1 ml of laboratory-grade water in the sample loading station position in the reagent racks. Then, lower the sippers into the water.
- 4 Leave the instrument on.
- 5 When you are ready to use the instrument for another run, perform a water wash. For more information, see *Perform a Water Wash* on page 117.

## Shut Down the Instrument

Turn off the instrument only if it is not going to be used for more than 10 days. If the instrument is going to be used within the next 10 days, see *Idle the Instrument* on page 120. If you turn off the instrument, you do not need to turn off the PC.

Use the following procedure to prepare fluidics safely and shut down the system.

- 1 Perform a complete maintenance wash to fully flush the system. For more information, see *Performing an Instrument Maintenance Wash* on page 112.
- 2 Remove the flow cell(s) from the flow cell stage.
- 3 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is clean.



### CAUTION

Do not allow alcohol to drip into the vacuum holes or around the manifolds. Use a low-lint lab tissue to dry the stage, if necessary.

- 4 (For high output mode) Load 10 ml of laboratory-grade water in each reagent position in the reagent racks. Then lower the sippers into the water.  
(For rapid run mode) Load 10 ml of laboratory-grade water in each reagent position and 1 ml laboratory-grade water in each sample loading station position in the reagent racks. Then, lower the sippers into the water.
- 5 Turn off the instrument.
- 6 When you are ready to restart the instrument, load water in all reagent positions, turn on the instrument, and perform a water wash. For more information, see *Perform a Water Wash* on page 117.



# Real-Time Analysis

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## Real-Time Analysis Introduction

The integrated primary analysis software, Real-Time Analysis (RTA), performs image analysis and base calling, and assigns a quality score to each base for each cycle. Analysis is performed during the chemistry and imaging cycles of a sequencing run. This saves downstream analysis time and allows you to quickly decide whether your run is progressing as expected.

Real-Time Analysis (RTA) runs automatically on the instrument computer, and is configured through the HCS interface at the beginning of a run.



### NOTE

If for any reason RTA stops functioning, the software automatically resumes analysis during the next cycle at the appropriate point on the flow cell. Do not restart the application manually.

## Data Transfer

The output from a sequencing run is a set of quality-scored base call (\*.bcl) files, which are generated from the raw image files and contain the base calls per cycle. By default, images are deleted from the instrument computer after image analysis. The raw image data are not needed for downstream analysis.

If for any reason image analysis falls too far behind, the software stops sequencing and places the flow cell in a safe state. Sequencing resumes when image analysis issues are resolved.

### **Basecalling\_Netcopy\_complete**

Data transfer is complete when the Basecalling\_Netcopy\_complete files are present in the root level of the run folder. There should be one generic file named Basecalling\_Netcopy\_complete.txt, plus one file for each read.

## Monitor Runtime Statistics

Real-Time Analysis automatically generates quality metrics when image analysis begins. Not all metrics are available at the early cycles because some processes need multiple cycles to generate data. The following summary describes points in the run when metrics are available:

- ▶ **Image Analysis**—Begins after cycle 4. The software first needs to generate a template with cluster locations. At cycle 4, the software catches up with the image analysis.



### NOTE

Because the software catches up with the image analysis during cycle 4, cycle 4 takes significantly longer to finish. The software waits for the analysis to end before proceeding with the next cycle. This behavior is normal.

- ▶ **Phasing Estimates and Base Calls**—The software generates phasing/prephasing estimates and base calls after cycle 12.
- ▶ **Quality Scores**—The software generates quality scoring after cycle 25. The software determines which of the reads pass the quality filter, applying the Chastity formula over the first 25 bases. At least 24 of the first 25 cycles must have a Chastity value  $>0.6$ . Reads may pass the quality filter if only one base in the first 25 has a lower Chastity value. Quality scores are reported only for reads passing the quality filter. See RTA Theory of Operations for more details.
- ▶ **Error Rates**—If a PhiX control was included, RTA generates error rates after cycle 25.
- ▶ **In-Line Controls**—If you prepared your samples with the TruSeq sample prep kits, RTA will perform in-line control alignment at cycle 52 of each read or the end of the read if the read length is fewer than 52 cycles.
- ▶ **Index Count**—After the last index read is completed, RTA will report the index count per lane if a sample sheet was provided.

## Sequencing Analysis Viewer

The Sequencing Analysis Viewer (SAV) is an application that allows you to view important sequencing metrics generated by Real-Time Analysis (RTA) in plots, graphs, and tables. SAV automatically opens to the analysis page after image analysis begins.

Statistics are updated as soon as new analysis information is available. Select **Refresh** at any time during the run to view updated metrics as the run progresses.

SAV can be viewed from a remote location by installing SAV on another computer with access to the network location of the output run folder. You can download a copy of SAV from the Illumina website. A MyIllumina login is required.

For a detailed description of available metrics and installation instructions, see the *Sequencing Analysis Viewer User Guide*, part # 15020619.

## Analysis Page

The analysis page reports the progress of the analysis, and provides access to the most important runtime statistics.

Statistics are updated as soon as new analysis information is available. The runtime statistics can be viewed from any computer with access to the experiment run folder. Navigate to the <RunFolder>\Data folder and open the Status file. The Status file is transferred to the Data subfolder in the analysis folder at the end of each cycle that is processed.



### NOTE

Your browser might warn you about active content. If so, click the warning in the information bar and allow blocked content.

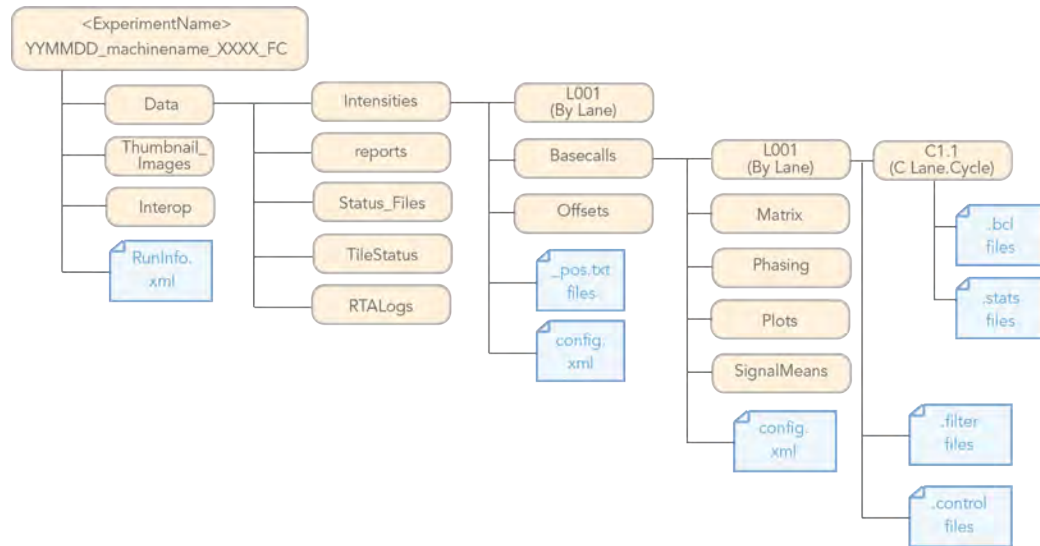


## Real-Time Analysis Output Data

RTA output results are saved to the network location that you specified during the run setup steps.

All analysis results are saved in an analysis output folder that is given the same name as the run folder on the instrument computer. The following figure illustrates the organization of the network analysis folder, including the location of the most important output files (blue boxes).

Figure 41 Real-Time Analysis Folder



## First Base Report

The First Base Report confirmation dialog box opens automatically after cycle one is complete, if you selected **Confirm First Base** on the scan screen during run setup. The run pauses at this step.

If you did not select the checkbox, the run continues after first base incorporation without prompting you to review the First Base Report.

- 1 Review the First Base Report from the First Base Report confirmation dialog box or by opening `First_Base_Report.htm` from the root level of the run folder.

- If the results are satisfactory, select **Continue** to continue your run. If results are not satisfactory, abort the run.

## Run Folders

Each sequencing run generates a run folder that contains data files and log files.

### Run Folder Name and Path

During run setup, the software prompts you to enter the path for the run folder. By default, the folder is named in the following format:

YYMMDD\_<Workstation Name>\_<Run Number>\_<Flow Cell ID>

Example: 110114\_SN106\_0716\_A90095ACXX

The run number increments by one each time you perform a run on a given instrument. The flow cell ID entered during the run setup steps appends to the end of the run folder name.

The run folder is written to the output path specified on the Scan screen during run setup. The temporary run folder for flow cell A is written to the D: drive and the temporary run folder for flow cell B is written to the E: drive.

### Contents of Run Folders

Subfolder	Key Files/Folders	Description
[Root level]	First_Base_Report.htm	File with basic metrics for the first cycle.
	IALog.txt	Log file for image analysis events.
	Sample Sheet (optional)	If you plan to use CASAVA, copy the sample sheet to the root level of the run folder. For more information, see the <i>CASAVA User Guide</i> , part # 15011196.
	RunInfo.xml	Identifies high-level run information.
	runParameters.xml	Provides the setup parameters for the run.
Config		Contains a copy of the software configuration parameters used for the run.

Subfolder	Key Files/Folders	Description
Data	Status.htm	Contains run and analysis-specific metrics generated during the run.
	Intensities folder	Contains image analysis results. This folder is saved on the network folder under <Run Folder>\Data, and is given the same name as the run folder on the instrument computer.
	CopyLog.txt	Log file for copy events.
	Log.txt	Log file for image analysis and base calling.
Data\ Intensities	BaseCalls folder	Contains RTA base calling results.
	config.xml	Contains run and image analysis-specific information.
	RTAConfiguration.xml	Configuration file for RTA in *.xml format.
Logs	Recipe_<date>_ <time>.log	Contains the run log information. Whenever the log file becomes too large (approximately 5 MB), HCS starts a new log file.
Recipe	*.xml	When you start a run, a copy of the recipe is moved to the Recipe folder. If you stop the run and resume it later, this recipe restarts where the run was stopped.
Thumbnail_Images	thumbnail images (* .jpg files)	Contains thumbnails of nine locations from each tile, which can be used to troubleshoot a run.

## Analysis Results

Table 8 Contents of Network Analysis Folder

Key File	Subfolder	Description
*.bcl files	AnalysisFolder\Data\ Intensities\Basecalls\ L00X\CX.X	Each *.bcl file contains RTA base calling and base quality scoring results for one cycle, one tile. These files are the input into CASAVA v1.8.2 or the BCL Converter.
*.cif files	AnalysisFolder\Data\ Intensities\L00X\CX.X	Each binary *.cif file contains RTA image analysis results for one cycle, one tile. Use these files in the OLB analysis software if you want to perform BUSTARD base calling. For a description, see the <i>Off-line Basecaller Software User Guide</i> .

Key File	Subfolder	Description
*.clocs/*.locs files	AnalysisFolder\Data\Intensities\L00	These files contain the x, y position for every cluster. The *.clocs file is a compressed version of the *.locs file.
*.filter files	AnalysisFolder\Data\Intensities\Basecalls	*.filter files contain filter results per tile. These files are used by CASAVA v1.8.2, or later.
*.jpg files	AnalysisFolder\Thumbnail_Images\L00X\CX.X	HCS can generate thumbnail images of nine locations from each tile. The thumbnails are generated for each cycle and base, and can be used to troubleshoot a run.
*.stats files	AnalysisFolder\Data\Intensities\Basecalls\L00X\CX.X	*.stats files contain RTA base calling statistics for a given cycle/tile. These files are used by CASAVA v1.8.2 or later.
config.xml	AnalysisFolder\Data\Intensities\ AnalysisFolder\Data\Intensities\Basecalls\	Contains meta-information about the run, image analysis, and base calling.
CopyLog.txt	AnalysisFolder\Data	All copy events during analysis are captured in the CopyLog file.
ErrorLog.txt	AnalysisFolder\Data	Any error during analysis is captured in the ErrorLog file. If there are no errors, no ErrorLog.txt file is generated.
RunInfo.xml	AnalysisFolder\	Identifies the boundaries of the reads (including indexing reads), the flow cell ID (if available), and the quality table selected for run.
SampleSheet.csv	AnalysisFolder\ AnalysisFolder\Data\Intensities\Basecalls\	Contains sample information, including indexing information. RTA copies the Sample Sheet file in the root folder of the run to the Basecalls folder.

## Tile Numbering

The tile name is a four-digit number encoding the position on the flow cell.

- ▶ The first digit encodes surface:
  - 1 is for top
  - 2 is for bottom
- ▶ The second digit encodes the swath:

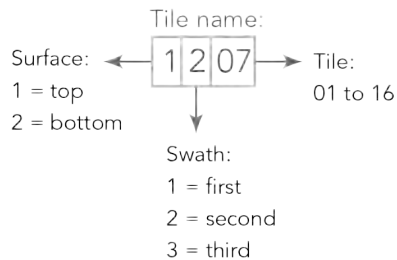
- 1 is for the first swath
- 2 is for the second swath
- 3 is for the third swath (if applicable)

**NOTE**

A swath is a column of tiles within a lane of the flow cell.

- ▶ The last two digits encode the tile, 01 through 16 (with HCS v2.0). Tile numbering starts with 01 at the output end of the flow cell through 16 at the input end.

**Figure 42** Tile Numbering



This example indicates a tile from the top surface of the flow cell, the second swath, and the seventh tile.

## Main Sequence Output Files

The following output file types contain primary analysis data generated by Real-Time Analysis:

### Base Call Files

A base call (\*.bcl) file contains base calls and quality scores for one cycle and one tile. They can be used for later analysis off-instrument.

**NOTE**

If you are using CASAVA v1.7 or earlier, the \*.bcl files need to be converted to the \_qseq.txt format in OLB or the BCL Converter to enable downstream analysis. For more information, see the *BCL Converter User Guide* or the *Off-line Basecaller Software User Guide*.

This step is not necessary with CASAVA v1.8.2, or later.

### Stats Files

The Stats file is a binary file containing base calling statistics for clusters passing filter. These files are used by the BCL Converter and CASAVA v1.8.

### Filter Files

The filter (\*.filter) files are binary files containing filter results. These files are used by the BCL Converter and CASAVA v1.8.

### Control Files

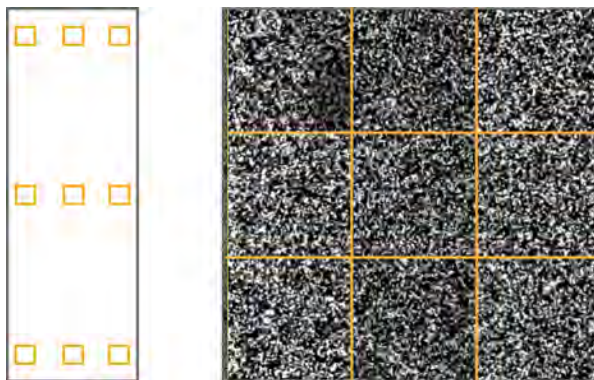
The control (\*.control) files are binary files containing control results. These files are used by the BCL Converter and CASAVA v1.8.

## Thumbnail Images

You can configure HCS to generate thumbnail images in \*.jpg format. Thumbnail images are generated for each cycle and base.

HCS collects images from nine sections of a tile. These nine images are combined in one thumbnail image and can be used to troubleshoot a run. Thumbnail images are not suitable for image analysis.

Figure 43 Thumbnail Image



# Troubleshooting

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## Introduction

This section describes what you can do if you encounter a problem during a sequencing run and how to perform a fluidics check from the Welcome screen.



## Possible Run Setup Problems

Problem	Possible Cause	Action
The software did not initialize.	The software was unable to initialize internal hardware devices.	<p>Close the error message and then relaunch the instrument software.</p> <p>If the problem persists, restart the instrument computer. If you are going to restart the computer, first shut down the instrument to make sure the DoNotEject drive is recognized correctly.</p> <p>If the problem persists after restarting the instrument computer, shut down the instrument, wait a minimum of 60 seconds, and then restart the instrument.</p>
Flow cell lever is orange.	<p>The flow cell did not seat properly.</p> <p>The vacuum did not seal.</p> <p>Manifolds did not raise.</p>	<p>Remove the flow cell and repeat the cleaning steps.</p> <p>Make sure that the gaskets are present and well-seated.</p> <p>Reload the flow cell.</p>
Poor fluid delivery.	Potential bubbles in the system.	<p>Reposition the flow cell and confirm that the holes are facing <i>down</i>.</p> <p>Look for white precipitate around the gaskets. If precipitate is present, replace the gaskets. Always replace gaskets before an instrument maintenance wash.</p> <p>Confirm that the sipper assemblies are fully lowered and each sipper is in contact with the reagents.</p>

## Perform a Fluidics Check

The Check button on the Welcome screen allows you to perform a fluidics check when you are not setting up a run. Use this option during instrument installation and fluidics troubleshooting.

- 1 Load a used flow cell onto the instrument.
- 2 Load eight 250 ml bottles with wash solution or laboratory-grade water, and load the bottles onto the corresponding reagent rack. Load the rack onto the instrument.
- 3 Select **Check** on the Welcome screen.
- 4 Select solution 5 (SB2) from the drop-down list. If you are performing a fluidics check with a used flow cell installed, you can select solution 2, which is water.
- 5 Enter the following default values:
  - Volume: **250**
  - (For high output mode) Aspirate Rate: **250**
  - (For rapid run mode) Aspirate Rate: **1500**
  - Dispense Rate: **2000**
- 6 Select **Pump**. If you need to pause the fluidics check, select **Pause**.
- 7 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifolds.

If you see excessive bubbles, check the manifold gaskets for obstructions, reduce the aspirate rate to 100, and pump another 250  $\mu$ l of water to the flow cell.

## Repeat Read 2 Primer Hybridization

It is possible to repeat Read 2 primer hybridization on the instrument; however, additional reagents are required. Contact Illumina Technical Support for further evaluation on whether a Read 2 rehybridization is appropriate.

For more information, visit the HiSeq support page on the Illumina website, and click **Troubleshooting**. A MyIllumina login is required.

## If BaseSpace is Unavailable

If BaseSpace is not available, open Windows Services and make sure Illumina BaseSpace Broker is started; if it is not started, restart it. If services are running and you are still unable to access BaseSpace, contact Illumina technical support.

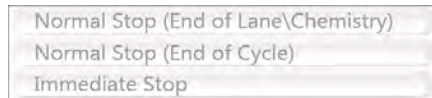
## Stop and Resume a Run

You can stop a run from the Run Overview screen. You might stop a run if the run was set up incorrectly, if the data quality is bad, or if there is a hardware error. If you plan to resume your run, make sure that you select the appropriate normal stop options with one of the RTA options that allow the run resume.

Stop Option	RTA Option	Able to Resume?
Normal Stop (End of Lane\Chemistry)	Keep As Is	Yes. The run resumes at the next chemistry or imaging command.
	Complete For Run	No. The run cannot be resumed.
	Complete For Read	Yes. The run resumes at the beginning of the next read.
Normal Stop (End of Cycle)	Keep As Is	Yes. The run resumes at the next cycle.
	Complete For Run	No. The run cannot be resumed.
	Complete For Read	Yes. The run resumes at the beginning of the next read.
Immediate Stop	No option	No.

- 1 From the Run Overview screen, select **Stop**. The stop menu opens.

Figure 44 Stop the Run



- 2 Select one of the following stop options:
  - **Normal Stop (End of Lane\Chemistry)**—Stops the run only after the current chemistry or imaging command is complete, and then places the flow cell in a safe state.
  - **Normal Stop (End of Cycle)**—Stops the run after the current cycle is complete, and then places the flow cell in a safe state.
  - **Immediate Stop**—Stops the run without completing the current operation and does *not* place the flow cell in safe state. You cannot resume a run that was stopped using the immediate stop option.
- 3 Select one of the following RTA options:
  - **Keep As Is**—The run is stopped without any modifications to RTA. You can resume the run where you left off.

- **Complete For Run**—RTA is stopped. The run info, run parameters, and recipe files are updated to reflect total cycles as the last cycle completed. Then RTA restarts to complete analysis for the run up to the point the run was stopped. You cannot resume the run.
  - **Complete For Read**—RTA is stopped. The run info, run parameters, and recipe files are updated to trim the length of the current read to the last cycle completed. Subsequent reads are not affected. Then RTA restarts to complete analysis for the current read. You can resume the run at the beginning of the next read.
- 4 After the run is stopped, select **Return to Start** on the Run Overview screen. The Welcome screen opens.

## Resume a Stopped Run

You can resume a stopped run if the run was stopped safely using a normal stop option with one of the RTA options that allow the run to resume.

- 1 From the Welcome screen, select **Sequence**, and then select **Resume Run**. The Resume screen opens.
- 2 Select the run folder for the run you want to resume from the drop-down list.



**NOTE**

The software resumes the run at the point where the run was stopped, and defaults to the correct setup on the Resume screen.

- 3 Confirm the settings on the Resume screen or select the appropriate point in the run to resume the run.
  - **Resume At** lists the read or point in the run to resume.
  - **Start At Cycle** lists the cycle to resume.

Figure 45 Points to Resume the Run





**CAUTION**

Illumina does not recommend resuming a run at the point of paired-end turnaround, other than for Read 2 primer rehybridization. For more information, see *Repeat Read 2 Primer Hybridization* on page 137.

- 4 Confirm the settings on the Resume screen or select the imaging and chemistry commands to resume. For more information, see *Example Settings for Resuming a Run* on page 141.
- 5 Select **Next** to proceed. The software guides you through the remaining run setup steps.

### Example Settings for Resuming a Run

If you stopped a run after imaging lane 1 at cycle 23 of a 101-cycle read, the software automatically sets up the resume run settings for Read #1 at cycle 23. The system displays the following settings on the Resume screen:

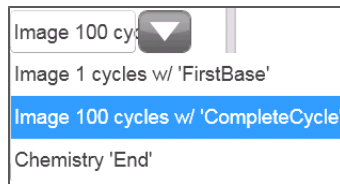
- ▶ **Resume At:** Read #1
- ▶ **Start At Cycle:** 23

Figure 46 Example of Resume at Cycle 23



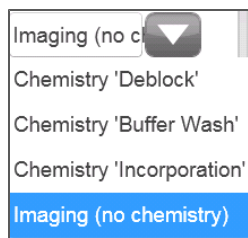
For a 101-cycle read, there is one cycle of **FirstBase** followed by 100 cycles of **CompleteCycle**. At cycle 23, you are within 100 cycles of CompleteCycle. **Image 100 cycles w/ 'CompleteCycle'** is selected automatically.

Figure 47 Examples of Imaging Commands



Because the run in this example was stopped during an imaging step, **Imaging (no chemistry)** is selected automatically.

Figure 48 Examples of Chemistry Commands



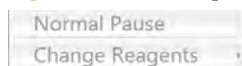


## Pause a Run

You can pause a run from the Run Overview screen. You might pause a run to check run components, such as reagent volumes, before proceeding with the run. Under normal procedures, you do not need to use this feature. The software pauses the run automatically before Read 2 in high output mode to allow you to load reagents.

- 1 From the Run Overview screen, select **Pause**. The pause menu opens.

Figure 49 Pause Options



- 2 Select **Normal Pause**.
- 3 Select **Yes** to confirm the pause command. The software completes the current chemistry or imaging command and places the flow cell in a safe state.
- 4 Select **Resume** when you are ready to resume the run.

## Change Reagents During a Run

If you started your run with a partial volume of reagents, use the Change Reagents feature to pause your run and replenish reagents. There is no need to pause a run to load fresh reagent before Read 2; the software pauses the run automatically at that point.

- 1 From the Run Overview screen, select **Pause**. The pause menu opens.
- 2 Select **Change Reagents**.
- 3 Select **Yes** to confirm the pause command. The software completes the current chemistry or imaging command and places the flow cell in a safe state.
- 4 On the Reagent screen, enter the following reagent parameters:
  - The reagent kit ID for new reagents you are loading.
  - The number of cycles the reagents are expected to last.



### NOTE

The priming checkbox is disabled on the Change Reagents screen. Priming is not required.

- 5 Select **Next** to proceed to loading reagents.



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## Technical Assistance

For technical assistance, contact Illumina Technical Support.

**Table 9** Illumina General Contact Information

Illumina Website	www.illumina.com
Email	techsupport@illumina.com

**Table 10** Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

### Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at [www.illumina.com/msds](http://www.illumina.com/msds).

### Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to [www.illumina.com/support](http://www.illumina.com/support), select a product, then click **Documentation & Literature**.