



MiSeq[®] System User Guide

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Revision History

Part #	Revision	Date	Description of Change
15027617	F	November 2012	Added the following new information: • Added kit descriptions for new MiSeq reagent kits: MiSeq Reagent Nano Kit and MiSeq Reagent Micro Kit • Added overview of flow cell types • Added description of Enrichment analysis workflow Updated the following information: • New in MCS v2.1, updated Perform Wash screen to add a post-run wash option and command to raise sippers • Updated version compatibility table to include nano and micro kit dependencies • Updated version compatibility information to include new reagent kits
15027617	E	October 2012	Updated the following information: • Corrected PhiX control preparation instructions and expected cluster density of prepared PhiX control to 1000–1200 K/mm² • Noted that the procedure for denaturing and diluting libraries, <i>Preparing Your Libraries</i>, does not apply to Nextera XT libraries as well as TruSeq Amplicon libraries • Changed upgrade name from MiSeq Expansion Pack to MiSeq hardware upgrade • Add the <i>MiSeq Reporter User Guide</i> to Additional Resources list.
15027617	D	July 2012	Updated software descriptions to MCS v2.0. Added the following new information: • Added a section titled <i>What's New in MCS</i> to describe new software features, interface changes, and workflow changes • Added catalog number and description of the MiSeq Reagent Kit v2, 500 Cycles • Added Version Compatibility and Requirements section

Part #	Revision	Date	Description of Change
			• Added description of MiSeq Expansion Pack, which is required for 14-tile dual-surface flow cell imaging • Added description of dual-surface flow cell tile numbering • Added the PCR Amplicon analysis workflow for Nextera XT libraries • Added the use of 10% Tween 20 in wash procedures and expected wash volumes • Added the reagent cartridge version to the RFID read failure procedure Updated the following information: • Changed reagent acronyms for IMF, CMF, and AMX to v2 reagent names IMS, CMS, and AMS, respectively • Changed the PhiX concentration from 8 pM to 12.5 pM • Changed the maximum recommended NaOH concentration to 1 mM in final solution • Noted that a maintenance wash is required to remove the instrument from standby mode and begin the setup steps for a subsequent run • Removed Sample Sheet Parameters section and sample sheet setup step in the workflow; Illumina recommends creating the sample sheet prior to sample preparation (See the <i>MiSeq Sample Sheet Quick Reference Guide</i>, Part # 15028392 and the <i>Illumina Experiment Manager User Guide</i>, Part # 15031335)
15027617	C	April 2012	Updated software descriptions to MCS v1.2 Added the following new procedures and sections: BaseSpace overview, Using Custom Primers, Generating FASTQ files, Troubleshooting Flow Rate Error, Performing a Volume Test, Performing a Maintenance Wash, and Idling the Instrument, which includes a standby wash. Updated the following information: • Updated name of Amplicon workflow to Custom Amplicon; updated name of DenovoAssembly workflow to Assembly; added GenerateFASTQ workflow • Added descriptions of run folders and files; updated run folder naming; added output file size • Listed genome folder as required for amplicon sequencing in Sample Sheet Parameters • Added instructions for diluting NaOH to denature libraries

Part #	Revision	Date	Description of Change
			• Updated Resolving RFID Read Failure to include MiSeq Self-Service instructions • Listed files and folders used for troubleshooting run performance
15027617	B	December 2011	Updated software descriptions to MCS v1.1 Added information about anti-virus protection Updated the following information: • Instructions to resolve RFID failure • Preparing libraries—Changed to 0.2 N NaOH • Run folder naming convention • Required disk space and storage capacity • Run setup steps—Added more information to Setting Up the Sample Sheet • Run setup steps—Added note to dispose of remaining PR2 • Analysis duration—Added when analysis exceeds two hours • Analysis Input Requirements—Listed manifest files as required for TruSeq Custom Amplicon libraries • Corrected HT1 tube size in MiSeq Reagent Kit Contents • Changed iCom references to MyIllumina
15027617	A	September 2011	Initial release

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Getting Started

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Introduction

The Illumina MiSeq® System combines proven sequencing by synthesis (SBS) technology with a revolutionary workflow that enables you to go from DNA to analyzed data in as little as eight hours. The MiSeq integrates cluster generation, sequencing, and data analysis on a single instrument.

Features

- ▶ **Walkaway Automation**—After setting up your run, which includes loading the pre-filled reagent cartridge, buffer bottle, and flow cell, no additional hands-on time is required.
- ▶ **Pre-filled Reagent Cartridge**—A specially designed single-use pre-filled reagent cartridge provides reagents for cluster generation and sequencing, including paired-end sequencing reagents and indexing reagents. Integrated radio-frequency identification (RFID) tracking enables accurate consumable tracking.
- ▶ **Interface Controls**—The MiSeq Control Software (MCS) interface provides controls to configure the instrument, set up and monitor runs, and perform maintenance procedures.
- ▶ **Convenient Flow Cell Loading**—A clamping mechanism auto-positions the flow cell as it is loaded onto the instrument. Integrated radio-frequency identification (RFID) tracking enables accurate consumable tracking.
- ▶ **Innovative Fluidics Architecture**—The MiSeq fluidics system enables unmatched efficiency in chemistry cycle time during sequencing.
- ▶ **Real Time Analysis (RTA)**—Integrated primary analysis software performs real time on-instrument data analysis during the sequencing run, which includes image analysis and base calling, and saves valuable downstream analysis time.
- ▶ **MiSeq Reporter**—Integrated secondary analysis software processes data from primary analysis to perform alignment and provide information about each sample analyzed.

Additional Resources

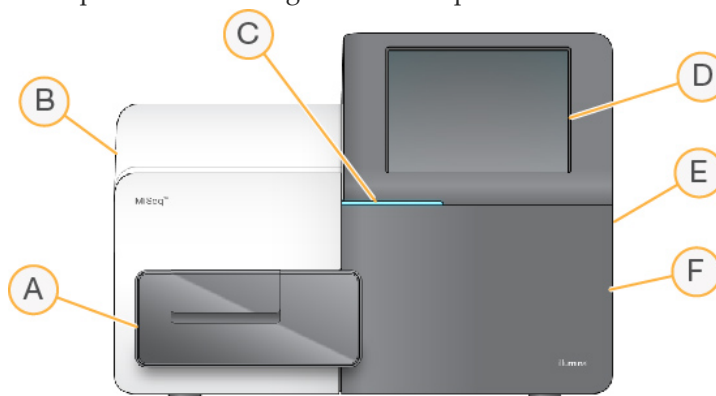
The following documentation is available for download from the MiSeq support page on the Illumina website. A MyIllumina login is required.

- ▶ ***MiSeq System Site Preparation Guide***—Provides specifications for laboratory space, electrical requirements, and environmental considerations.
- ▶ ***MiSeq System Safety and Compliance Guide***—Provides information about instrument labeling, compliance certifications, and safety considerations.
- ▶ ***MiSeq Reagent Preparation Guide***—Provides a description of kit contents, and instructions for preparing the reagent cartridge before beginning your sequencing run.
- ▶ ***MiSeq Sample Sheet Quick Reference Guide***—Provides information about sample sheet requirements. This guide provides a quick reference to sample sheet information whether you are creating it manually or using the Illumina Experiment Manager.
- ▶ ***Illumina Experiment Manager User Guide***—Provides instructions for creating sample plates and sample sheets for different workflows and library types.
- ▶ ***MiSeq Reporter User Guide***—Provides a comprehensive overview of analysis procedures, analysis workflows, and output files generated by MiSeq Reporter interface, as well as computing requirements, off-instrument installation instructions, and troubleshooting information.
- ▶ ***MiSeq Reporter Online Help***—Provides instructions for using the MiSeq Reporter interface. Go to http://support.illumina.com/help/miseq_reporter/default.htm.
- ▶ ***BaseSpace Online Help***—Provides instructions for using BaseSpace and descriptions of the graphs generated for each analysis workflow. Go to <http://support.illumina.com/help/BaseSpaceHelp/BaseSpaceHelp.htm>.

Visit the MiSeq support page on the Illumina website for access to documentation, software downloads, and frequently asked questions. To view a comprehensive list of MiSeq training courses, go to www.illumina.com/training/miseq.

Components

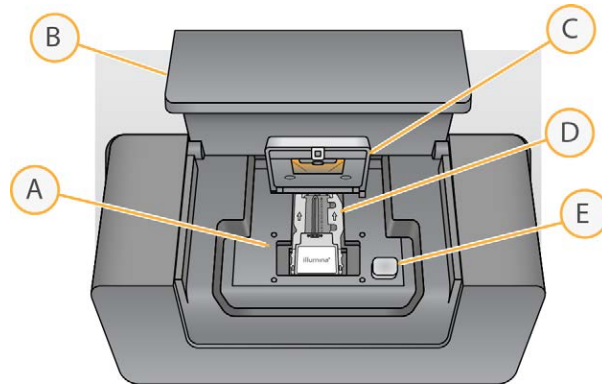
The MiSeq has the following exterior components:



- A Flow Cell Compartment**—Contains the flow cell stage that houses the flow cell throughout the run. The flow cell stage is controlled by motors that move the stage out of the enclosed optical module for flow cell loading, and returns it when the run begins.
- B Enclosed Optics Module**—Contains optical components that enable imaging of the flow cell.
- C Status Bar**—Uses three colors to indicate instrument status. Blue indicates the instrument is processing, orange indicates the instrument needs attention, and green indicates that the instrument is ready to begin the next run.
- D Touch Screen Monitor**—Enables on-instrument configuration and run setup using the software interface.
- E External USB Ports**—Facilitates the transfer of files and data to the instrument computer from the touch screen monitor.
- F Reagent Compartment**—Holds reagents at proper temperatures, wash solutions, and the waste bottle. The reagent compartment door is secured by a magnetic latch.

The MiSeq interface guides you through the run setup steps using the touch screen monitor. To load run components, you need to access the reagent compartment and the flow cell compartment.

Flow Cell Compartment

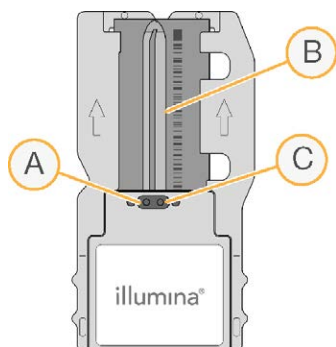


- A** Flow Cell Stage
- B** Flow Cell Compartment Door
- C** Flow Cell Latch
- D** Flow Cell
- E** Flow Cell Latch Release Button

The flow cell compartment houses the flow cell stage, thermal station, and fluidics connections to the flow cell. The flow cell stage moves in and out of the optics module and holds the flow cell, which is secured by the flow cell latch. When the flow cell latch closes, two pins near the latch hinge auto-position the flow cell.

The thermal station, located beneath the flow cell stage, controls changes in flow cell temperature required for cluster generation and sequencing.

Flow Cell



- A** Outlet Port
- B** Imaging Area
- C** Inlet Port

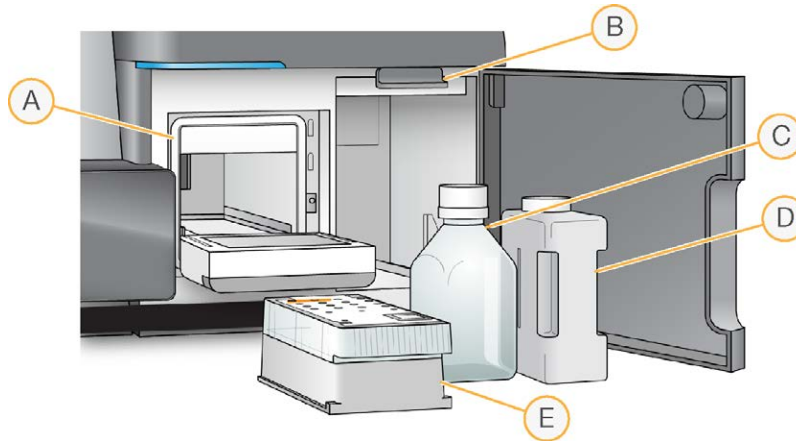
The MiSeq flow cell is a single-use glass-based substrate on which clusters are generated and the sequencing reaction is performed.

Reagents enter the flow cell through the inlet port, pass through the single-lane imaging area, and then exit the flow cell through the outlet port. Waste exiting the flow cell is delivered to the waste bottle.

Samples are loaded onto the reagent cartridge prior to beginning the run, and then automatically transferred to the flow cell after the run begins.

During the sequencing run, the single lane is imaged in small imaging areas called tiles. All MiSeq flow cells have a single lane, but the number of tiles differ depending on the type of flow cell you are using. For more information, see *Flow Cell Types* on page 13.

Reagent Compartment



- A Reagent Chiller
- B Sipper Handle (shown in raised position)
- C PR2 Bottle
- D Waste Bottle
- E Reagent Cartridge

The reagent compartment contains the reagent chiller, and positions for the wash buffer (PR2) bottle and the waste bottle.

The reagent chiller holds a single-use reagent cartridge during the run, or a wash tray during the instrument wash. The software automatically lowers sippers into each reservoir of the reagent cartridge at the appropriate time during a run depending on the process being performed.

To the right of the reagent chiller are two form-fitted slots, one for the PR2 bottle and one for the waste bottle. The sipper handle locks the bottles in place and lowers the appropriate sipper into each bottle.

During a run, reagents are pumped through the sippers and fluidics lines, and then to the flow cell. Reagent waste is delivered to the waste bottle throughout the process.

Reagent Cartridge

The MiSeq reagent cartridge is a single-use consumable consisting of foil-sealed reservoirs pre-filled with clustering and sequencing reagents sufficient for sequencing one flow cell. Each reservoir on the cartridge is numbered.

Sample libraries are loaded onto the cartridge in position 17, which is labeled **Load Samples**.

Positions 18, 19, and 20 are empty and can be used for optional custom sequencing primers. For more information, see *Using Custom Primers* on page 75.

MiSeq Upgrade

The MiSeq upgrade includes hardware and software enhancements that enable dual-surface imaging of the flow cell when running MCS v2.0. Additionally, the standard flow cell is imaged 14 tiles instead of 12 tiles, for a total of 28 tiles: 14 tiles on the top surface and 14 tiles on the bottom surface.

- ▶ **Hardware**—Hardware components installed by an Illumina representative. If your instrument requires this upgrade, you will receive a notification from Illumina.
- ▶ **Software**—MiSeq Control Software (MCS) v2.0.

Dual-surface imaging generates significantly more data from your run. For more information, visit the MiSeq System specifications page on the Illumina website (www.illumina.com/systems/miseq/performance_specifications.ilmn).



NOTE

The number of possible tiles on the flow cell differs between flow cell types. For more information, see *Flow Cell Types* on page 13.

MiSeq Reagent Kits

To perform a run on the MiSeq, you need one single-use MiSeq Reagent Kit, which is available in three kit types. Each type of MiSeq reagent kit includes a kit-specific flow cell type and reagents required for performing one run.

Kit Type	Flow Cell Type	Available Kit Sizes
MiSeq Reagent Kit v2	Includes a standard (14 tile) flow cell	500 cycles 300 cycles 50 cycles
MiSeq Reagent Micro Kit v2	Includes a micro (4 tile) flow cell	300 cycles
MiSeq Reagent Nano Kit v2	Includes a nano (2 tile) flow cell	500 cycles 300 cycles

The flow cell, PR2 bottle, and reagent cartridge provided in the kit use radio-frequency identification (RFID) for accurate consumable tracking and compatibility.

You must use the reagent cartridge associated with your flow cell type. If the reagent cartridge is not compatible, a message appears on the screen during the run setup steps. To proceed, you must load a compatible reagent cartridge.

Reagent Kit Sizes

Reagent kit size is measured by the number of cycles that can be performed with one kit. Possible sizes are 500 cycles, 300 cycles, and 50 cycles.

Kit Size	Number of Cycles
500-Cycle Kit	Provides reagents for up to 525 cycles of sequencing, which is sufficient for up to a 251-cycle paired-end run plus two eight-cycle index reads.
300-Cycle Kit	Provides reagents for up to 325 cycles of sequencing, which is sufficient for up to a 151-cycle paired-end run plus two eight-cycle index reads.
50-Cycle Kit	Provides reagents for up to 75 cycles of sequencing, which is sufficient for up to a 26-cycle paired-end run plus two eight-cycle index reads.

MiSeq Reagent Kit v2

Consumable	Catalog #
MiSeq Reagent Kit v2, 500 Cycles	Catalog # MS-102-2003
MiSeq Reagent Kit v2, 300 Cycles	Catalog # MS-102-2002
TG MiSeq Reagent Kit v2, 300 Cycles	Catalog # TG-142-1003
MiSeq Reagent Kit v2, 50 Cycles	Catalog # MS-102-2001

MiSeq Reagent Micro Kit v2

Consumable	Catalog #
MiSeq Reagent Micro Kit v2, 300 Cycles	Catalog # MS-103-1002
TG MiSeq Reagent Micro Kit v2, 300 Cycles	Catalog # TG-142-1002

MiSeq Reagent Nano Kit v2

Consumable	Catalog #
MiSeq Reagent Nano Kit v2, 500 Cycles	Catalog # MS-103-1003
MiSeq Reagent Nano Kit v2, 300 Cycles	Catalog # MS-103-1001
TG MiSeq Reagent Nano Kit v2, 300 Cycles	Catalog # TG-142-1001

Kit Contents and Storage Requirements

Box 1: Store at -15° to -25°C

Quantity	Component	Storage	Description
1	Reagent Cartridge	-15° to -25°C	Single-use pre-filled cartridge
1	HT1	-15° to -25°C	5 ml tube, Hybridization Buffer

Box 2: Store at 2° to 8°C

Quantity	Component	Storage	Description
1	PR2 Bottle	2° to 8°C	500 ml bottle, Incorporation Buffer
1	MiSeq Flow Cell	2° to 8°C	Single-use PE flow cell

Reagent Cartridge Contents

Table 1 Reagent Cartridge Contents

Position	Reagent Name	Description
1	IMS	Incorporation Mix
2	SRE	Scan Mix
4	CMS	Cleavage Mix
5	AMS1	Amplification Mix, Read 1
6	AMS2	Amplification Mix, Read 2
7	LPM	Linearization Premix
8	LDR	Formamide
9	LMX1	Linearization Mix
10	LMX2	Read 2 Linearization Mix
11	RMF	Resynthesis Mix
12	HP10	Read 1 Primer Mix
13	HP12	Index Primer Mix
14	HP11	Read 2 Primer Mix
15	PW1	Laboratory-grade water
16	PW1	Laboratory-grade water
17	Empty	Load Samples (Reserved for sample libraries)

Position	Reagent Name	Description
18	Empty	Optional use for custom Read 1 primer
19	Empty	Optional use for custom Index Read primer
20	Empty	Optional use for custom Read 2 primer
21	PW1	Laboratory-grade water
22	Empty	Empty

Flow Cell Types

The flow cell is immersed in storage buffer in a flow cell container. The flow cell container cap color indicates the flow cell type:

- ▶ **Clear cap**—Cap color for a standard (14 tile) flow cell.
- ▶ **Green cap**—Cap color for a micro (4 tile) flow cell.
- ▶ **Yellow cap**—Cap color for a nano (2 tile) flow cell.

Flow cell types differ in output and number of tiles imaged. Dual-surface imaging, which is possible on the standard and micro flow cells, doubles the number of tiles imaged.

Flow cell output depends on the number of reads possible from each flow cell. Reads are measured as the number of clusters passing filter.

Flow Cell Output

Flow Cell	# of Reads	Kit Size (Cycles)	Output (Max.)	2 x 75 Output	2 x 150 Output	2 x 250 Output
Standard Flow Cell	15 M	50, 300, 500	7.5 Gb	2.25 Gb	4.5 Gb	7.5 Gb
Micro Flow Cell	4 M	300	1.2 Gb	600 Mb	1.2 Gb	--
Nano Flow Cell	1 M	300, 500	500 Mb	150 Mb	300 Mb	500 Mb

Flow Cell Tiles

Flow Cell	Tiles	Imaging Surface	Total Tiles Imaged
Standard Flow Cell	14 tiles	Top and bottom	28 tiles total
Micro Flow Cell	4 tiles	Top and bottom	8 tiles total
Nano Flow Cell	2 tiles	Top only	2 tiles total

Version Compatibility and Requirements

The following table lists version compatibility of MiSeq reagents, MiSeq Control Software (MCS), and supported read lengths.

MiSeq Reagent Kit Version	MCS Version	Read Length
MiSeq Reagent Kit v2	MCS v2.0 or later	Up to 2 x 251 cycles
MiSeq Reagent Micro Kit v2	MCS v2.1 or later	Up to 2 x 151 cycles
MiSeq Reagent Nano Kit v2	MCS v2.0 or later	Up to 2 x 251 cycles

The following table lists requirements specific to workflow enhancements and components. For more information, see *What's New in MCS* on page 21.

Workflow Component	Requirement
2 x 251 cycle runs	• MCS v2.0 or later • MiSeq Reagent Kit v2, 500 Cycles
Imaging of 14 tiles on a standard flow cell	• MCS v2.0 or later • Upgraded hardware components • MiSeq Reagent Kit v2
Dual-surface flow cell imaging	• MCS v2.0 or later • Upgraded hardware components • MiSeq Reagent Kit v2
Sequencing on a micro flow cell (four-tile dual-surface imaging)	• MCS v2.1 or later • Upgraded hardware components • MiSeq Micro Reagent Kit v2 Note: You can use the micro flow cell with MCS v2.1 on an instrument <i>without</i> upgraded hardware. However, this configuration results in four-tile single-surface imaging.
Sequencing on a nano flow cell (two-tile single-surface imaging)	• MCS v2.0 or later • MiSeq Nano Reagent Kit v2

For information about upgraded hardware components, see *MiSeq Upgrade* on page 8. Additional information is available on the MiSeq support page on the Illumina website. Go to http://support.illumina.com/sequencing/sequencing_instruments/miseq.ilmn.

User-Supplied Consumables

Make sure the following user-supplied consumables are available before beginning a run.

Consumable	Supplier	Purpose
1 N NaOH	General lab supplier	Denaturing sample libraries Denaturing sample libraries and PhiX control DNA
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 15648-981* General lab supplier	Cleaning the flow cell holder
Disposable gloves, powder-free	General lab supplier	General use
Lab tissue, low-lint	VWR, catalog # 21905-026*	Cleaning the flow cell stage
Lens paper, 4 x 6 in.	VWR, catalog # 52846-001*	Cleaning the flow cell
Tween 20	Sigma-Aldrich, catalog # P7949	Washing the instrument
Tweezers, square-tip plastic (optional)	McMaster-Carr, catalog # 7003A22*	Removing flow cell from flow cell shipping container
Water, laboratory-grade	General lab supplier	Washing the instrument

* or equivalent

Guidelines for Laboratory-Grade Water

Always use laboratory-grade water to perform instrument procedures. Never use tap water or deionized water. Any of the following are acceptable examples:

- ▶ Illumina PW1
- ▶ 18 Megohm (MΩ) water
- ▶ Milli-Q water
- ▶ Super-Q water
- ▶ Molecular biology-grade water

Anti-Virus Software

Illumina strongly recommends that you purchase and install the anti-virus software of your choice to protect the computer against viruses.

To avoid interfering with MiSeq operation or losing data, configure the anti-virus software updates as follows:

- ▶ Set for manual scans, not automatic scans.
- ▶ Perform scans only when the instrument is not in use.
- ▶ Set updates to download but not install without user authorization.
- ▶ Do not automatically reboot the computer upon update.
- ▶ Exclude the data drive and application directory from any real-time file system protection.

Starting the MiSeq

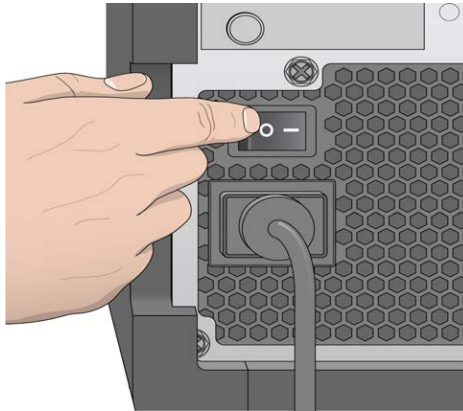


NOTE

It is best to leave the instrument on at all times. However, if the instrument must be turned off follow the shut down procedure described in *Shutting Down the Instrument* on page 103, and then wait a **minimum** of 60 seconds before turning the power switch back to the ON position.

- 1 If the MiSeq is not already on, reach around the right side of the instrument to locate the power switch on the back panel. It is in the lower corner directly above the power cord.

Figure 1 Power Switch Location



- 2 Turn the power switch to the **ON** position. The integrated instrument computer starts. It might take several seconds before the touch screen shows activity.
- 3 Log in to the operating system using the default user name and password:
 - User name: sbsuser
 - Password: sbs123

Wait until the operating system has finished loading. When the system is ready, the MiSeq Control Software (MCS) launches and initializes the system automatically. After initialization is complete, the Welcome screen appears.

MiSeq Software

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Introduction

Three software applications are pre-installed on the instrument computer:

- ▶ **MiSeq Control Software (MCS)**—Controls instrument operation.
- ▶ **Real Time Analysis (RTA)**—Performs primary analysis.
- ▶ **MiSeq Reporter**—Performs secondary analysis.

Optional software used off-instrument includes the Sequencing Analysis Viewer (SAV) and the Illumina Experiment Manager. For more information, see *Sequencing Analysis Viewer* on page 49 and *Illumina Experiment Manager* on page 52.

MiSeq Control Software (MCS)

The MiSeq Control Software (MCS) interface guides you through the steps to load the flow cell and reagents prior to starting the run, and then provides an overview of quality statistics that you can monitor as the run progresses.

During the run, MCS operates the flow cell stage in the enclosed optics module, gives commands to dispense reagents, changes temperatures of the flow cell, and captures images of clusters on the flow cell for image analysis. MCS performs the run according to parameters specified in the sample sheet.

Real Time Analysis (RTA)

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. Images are temporarily stored in the run folder for processing by RTA, and then automatically deleted when RTA analysis is complete.

MiSeq Reporter

MiSeq Reporter is the secondary analysis software on the MiSeq that processes base calls generated during primary analysis, and produces information about alignment, variants, and contig assemblies for each genome requested and each sample based on the analysis workflow specified in the sample sheet. For more information, see *MiSeq Reporter Overview* on page 65.

What's New in MCS

- ▶ **Micro flow cell**—MCS v2.1 enables sequencing on the four-tile micro flow cell.
- ▶ **Enables 2 x 251 cycle runs**—MCS v2.0 together with reagents provided in the MiSeq Reagent Kit v2 (500 Cycles) enable runs up to 2 x 251 cycles. This version of the software contains the RFID recognition components for v2 kit components and requisite recipes to perform the run. For more information, see *Version Compatibility and Requirements* on page 14.



NOTE

Longer read lengths can require optimization of library insert sizes to reduce incidences of sequencing beyond the sample DNA into the library adapter. For information about settings for adapter trimming, see the *MiSeq Sample Sheet Quick Reference Guide*, Part # 15028392.

- ▶ **Enables flow cell imaging in 14 tiles**—MCS v2.0 images a standard flow cell lane in 14 tiles instead of 12 tiles as in previous versions of the software. Additional hardware components and reagents provided in the MiSeq Reagent Kit v2 are required for imaging 14 tiles on a standard flow cell. For more information, see *MiSeq Upgrade* on page 8.



NOTE

Micro flow cells provided in the MiSeq Reagent Micro Kit v2 are imaged in four tiles. Nano flow cells provided in the MiSeq Reagent Nano Kit v2 are imaged in two tiles. For more information, see *Flow Cell Types* on page 13.

- ▶ **Enables dual-surface flow cell imaging in conjunction with hardware changes**—MCS v2.0 enables imaging of the flow cell on the top surface and the bottom surface. Additional hardware components are required for dual-surface imaging. For more information, see *MiSeq Upgrade* on page 8.

To download the latest version of MCS, go to the MiSeq support page on www.illumina.com, and click the Downloads tab. A MyIllumina login is required.

MCS Interface Changes

MCS v2.1

- ▶ **Perform Wash**—MCS v2.1 introduces the option to initiate a post-run wash from the Perform Wash screen, as well as to raise the reagent cartridge sippers. For more

information, see *Perform Wash Screen* on page 28.

MCS v2.0

- ▶ **Perform Wash**—The Perform Wash command on the Welcome screen (previously labeled Wash Options) provides the option to perform a maintenance wash or a standby wash. For more information, see *Perform Wash Screen* on page 28.
- ▶ **Run Options**—The Run Options command on the Welcome screen (previously labeled Setup Options) opens to three tabbed categories: Run Settings, Folder Settings, and Email Notifications. For more information, see *Run Options Screen* on page 32.
- ▶ **Email Notifications**—The Email Notifications tab contains options for specifying email addresses of intended notification recipients and events that trigger an email notification. A network connection is required. For more information, see *Email Notifications Tab* on page 34.
- ▶ **Minimize to Windows**—The Minimize to Windows command on the Manage Instrument screen allows you to minimize the MCS interface to access files and folders stored on the instrument computer. For more information, see *Manage Instrument Screen* on page 36.

MCS Workflow Changes

- ▶ **Kit version required for RFID bypass code**—MCS v2.0 requires that you enter the version number of the kit, Version 1 or Version 2, in addition to the barcode number of the reagent cartridge to activate a temporary RFID bypass code in the event of a reagent cartridge RFID read failure.
- ▶ **Location of custom recipe folder**—MCS v2.0 requires that you save custom recipes in one of two subfolders of D:\Illumina\MiSeq Control Software\CustomRecipes named **V1** or **V2**:
 - Custom recipes created for use with the original MiSeq Reagent Kit (v1) must be stored in D:\Illumina\MiSeq Control Software\CustomRecipes\V1.
 - Custom recipes created for use with the MiSeq Reagent Kit v2 must be stored in D:\Illumina\MiSeq Control Software\CustomRecipes\V2.
- ▶ **Setting for percentage of tiles to scan**—MCS v2.0 allows you to specify a percentage of tiles to be scanned using **PercentTilesToScan**. This is a setting in the Settings section of the sample sheet. If set to the default value of 1, 100% of the tiles

are scanned. Any value represents the percentage of tiles to be scanned. For more information, see *MiSeq Sample Sheet Quick Reference Guide*, Part # 15028392.

Welcome Screen

The MCS interface opens to the Welcome screen when the software launches.

Figure 2 Welcome Screen



- ▶ **Sequence**—This option opens a series of run setup screens that guide you through the setup steps for your run. See *Run Setup Screens* on page 40.
- ▶ **Perform Wash**—Provides options to start two types of instrument washes, either a maintenance wash or standby wash. See *Perform Wash Screen* on page 28.
- ▶ **Manage Files**—Provides controls for moving, deleting, and uploading files on the instrument computer. See *Manage Files Screen* on page 30.
- ▶ **Run Options**—Provides options for the post-run wash, changing default locations of data folders, and specifying email notification preferences. See *Run Options Screen* on page 32.
- ▶ **Manage Instrument**—Provides options to go to system settings, to perform a systems check, to manually update software, and to reboot or shut down the instrument. See *Manage Instrument Screen* on page 36.
- ▶ **Updates Available**—This option appears on the Welcome screen only if a software update is available. Your MiSeq must be connected to a network with internet access to enable this option. See *Software Updates* on page 26.

Activity Indicators

A series of icons are located in the lower-right corner of each interface screen. Each icon is an activity indicator that shows which activity the instrument is performing.

Figure 3 Activity Indicators



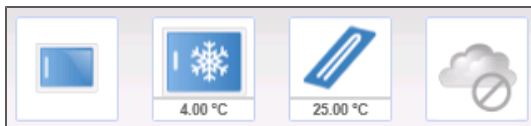
From left to right, the activity indicators represent the following activities:

- Moving the Y-stage
- Moving the Z-stage
- Activating electronics functionality
- Using the camera
- Pumping through the fluidics system

Sensor Indicators

Four sensor indicators are located at the base of each interface screen, each representing the status of an instrument component.

Figure 4 Sensor Indicators



From left to right, the sensor indicators represent the following components:

- Flow cell compartment door, closed or open
- Temperature of the reagent chiller in °C
- Temperature of the flow cell in °C
- Status of BaseSpace® connection (not connected shown)

Status Icons

In the top-right corner of the Welcome screen is a status icon that signals any change in conditions during run setup or during the run.

Status Icon	Status Name	Description
	Status OK	No change. System is normal.
	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 to the associated image 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 any item listed to see a detailed description of the condition and instructions to resolve the condition, if applicable.
- ▶ 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 hide.

Software Updates

If your system is connected to a network with internet access, you can automatically update the instrument software from the Welcome screen.

When software updates are available, the Update Available button appears on the Welcome screen. Otherwise, this button is not visible.

Figure 5 Update Available Button on Welcome Screen



To start a software update, select **Update Available**. A dialog box opens to confirm the command, at which time a reboot of the instrument is required. Installation of the update begins automatically upon reboot.

If your instrument is not connected to a network with internet access, you can update the software manually. For more information, see *Manual Update Screen* on page 39.

Perform Wash Screen

You can initiate three types of washes from the Perform Wash screen: maintenance wash, standby wash, and post-run wash.

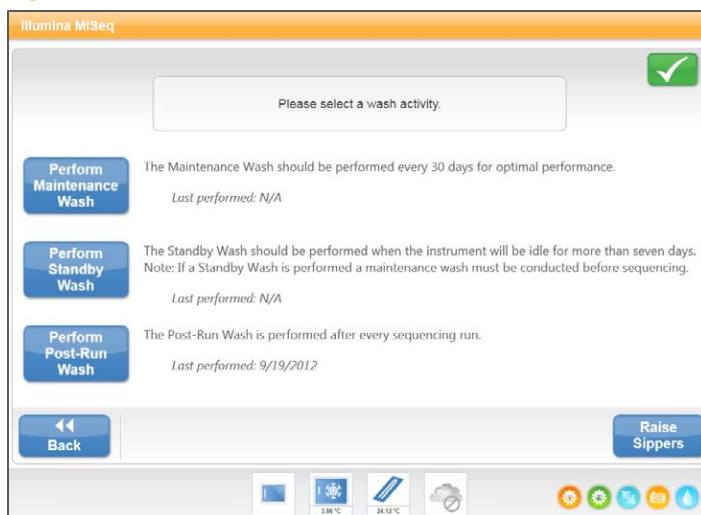
- ▶ **Maintenance Wash**—The maintenance wash consists of three consecutive wash cycles that thoroughly flush the system. Perform a maintenance wash at least every 30 days. See *Procedure* on page 97.
- ▶ **Standby Wash**—The standby wash properly prepares the fluidics lines for sitting idle and consists of two consecutive wash cycles. Perform a standby wash if the instrument will be idle for up to seven days. See *Performing a Standby Wash* on page 100.

After the instrument has been placed in an idle state, you must perform a maintenance wash before beginning a new sequencing run.

- ▶ **Post-Run Wash**—The post-run wash is the standard instrument wash performed between sequencing runs and consists of a single wash cycle. If you need to perform a post-run wash at a time other than directly following a run, use the command on the Perform Wash screen to initiate the wash.

You can configure your instrument to perform a maintenance wash between runs. For more information, see *Run Options Screen* on page 32.

Figure 6 Perform Wash Screen

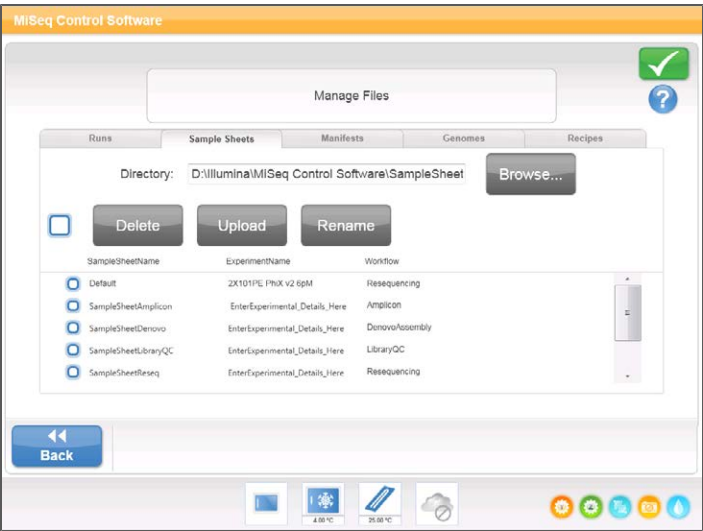


Introduced in MCS v2.1, select **Raise Sippers** to raise the reagent cartridge sippers. You might need to use this command if the run was interrupted unexpectedly or if an error occurred during the run, and you need to remove the reagent cartridge from the instrument. Following standard run procedures, the sippers are raised by the software at the end of the sequencing run and prior to beginning a post-run wash.

Manage Files Screen

Use the Manage Files feature to move, upload, or delete files on the instrument computer. The screen is divided into five tabs: Runs, Sample Sheets, Manifests, Genomes, and Recipes.

Figure 7 Manage Files Screen



Manage Files Options

From any tab on the Manage Files screen, select **Browse** to view files located in folders other than those currently selected.

Tab	Features
Runs	Delete or Move
Sample Sheets	Delete, Upload, or Rename

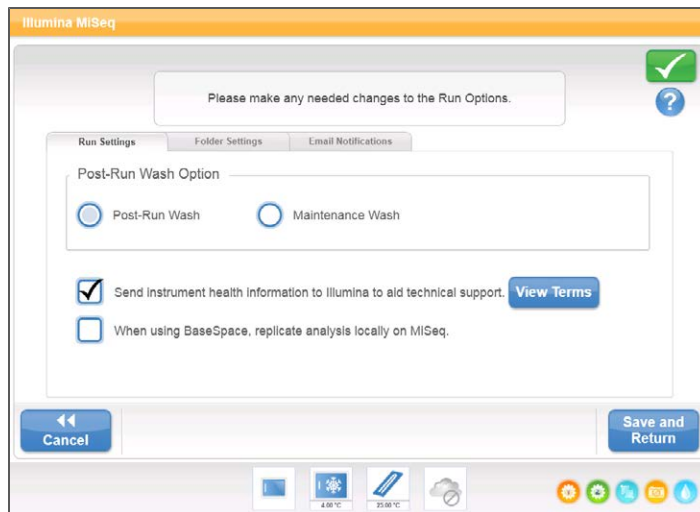
Tab	Features
Manifests	Delete or Upload
Genomes	Delete or Upload
Recipes	Delete or Upload

- ▶ **Delete**—Select the checkbox next to the file or folder listed, and then select **Delete**. The Delete feature is available on all tabs.
- ▶ **Move**—Available only for run folders. Select the checkbox next to the folder name, select **Move**, and then browse to an appropriate location. **Move** will *copy* the run folder to the new location and then *delete* the folder from the old location.
- ▶ **Select All Files**—Select the checkbox to the left of the Delete button, and then select an action, Delete or Move. The action is applied to all files or folders.
- ▶ **Upload Files**—Available for sample sheets, manifests, genomes, and recipes. If the MiSeq is not connected to a network, use this feature to upload files to the instrument computer from a USB drive. Select **Upload** and browse to the location on a USB drive where the file resides. The file is uploaded to the folder indicated in the Directory field.
- ▶ **Rename**—Available only for sample sheets. Select the checkbox next to the sample sheet file, and then select **Rename**. Use the on-screen keyboard to rename the sample sheet.

Run Options Screen

The Run Options screen has three tabs for specifying the default settings for a run: Run Settings, Folder Settings, and Email Notifications.

Figure 8 Run Settings Tab on Run Options Screen



Run Settings Tab

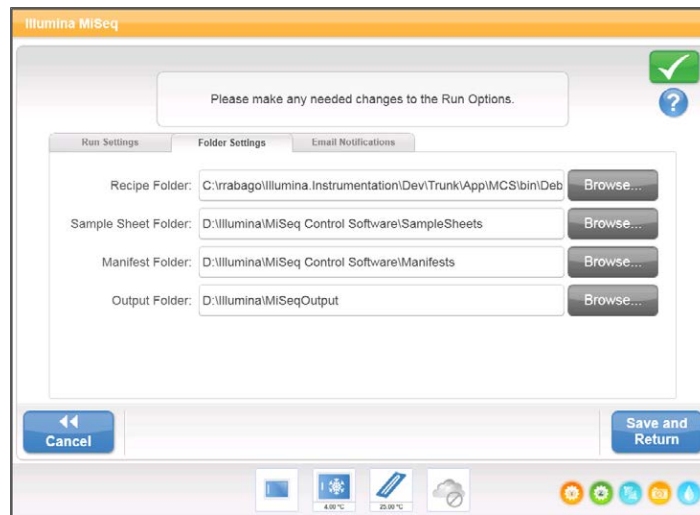
- ▶ **Post-Run Wash Option**—An instrument wash is required after each run. You must perform a wash before the software will proceed to the run setup steps for a subsequent run. You can specify which type of wash is performed by default. A post-run wash takes about 20 minutes and a maintenance wash takes about 1 hour.
- ▶ **Send Instrument Health**—Illumina recommends selecting this option to help Illumina Technical Support in troubleshooting possible problems. The only files sent to Illumina are log files (interop files and log files). The instrument must be connected to a network with internet access to use this feature.
- ▶ **Replicate Analysis Locally**—This setting provides the option to perform analysis both locally on the instrument and in BaseSpace:
 - If you are using BaseSpace and select this option, MiSeq Reporter will launch automatically after the run and perform analysis locally.

- If you are using BaseSpace and do not select this option, MiSeq Reporter will not launch automatically after the run and analysis will only be performed in BaseSpace.

Folder Settings Tab

You can specify the default folder locations on the Folder Settings tab. For ease of access, the folders can be located on a local network or on the instrument computer.

Figure 9 Folder Settings Tab



- **Recipes**—Sets the default location for recipes. Recipes are XML files that the software uses to perform the sequencing run. A recipe is created at the start of the run based on parameters in the sample sheet. After a run-specific recipe is created, it is copied to the output folder.



NOTE

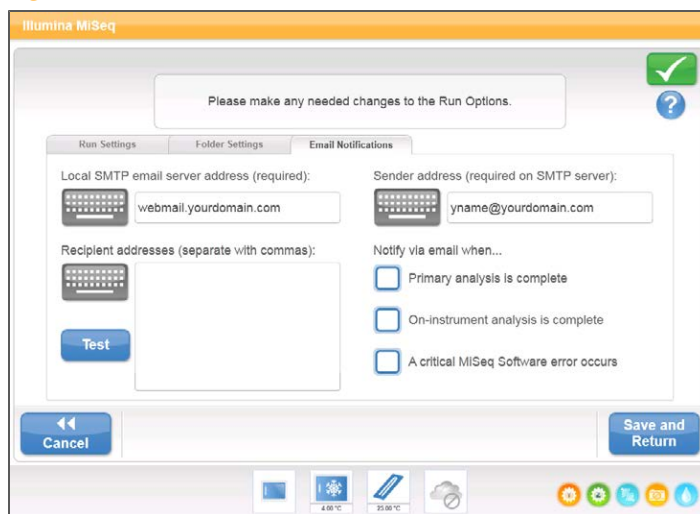
By default, custom recipes are stored in D:\Illumina\MiSeq Control Software\CustomRecipes. MiSeq Control Software (MCS) requires two subfolders: V1 and V2. Any custom recipes created for use with the original MiSeq Reagent Kit (v1) must be placed in the V1 subfolder, and recipes created for use with the MiSeq Reagent Kit v2 must be placed in the V2 subfolder. You can browse to the custom recipes folder and create subfolders from the Manage Files screen. For more information, see *Manage Files Screen* on page 30.

- ▶ **Sample Sheets**—Sets the default location for sample sheets. Sample sheets are files that you create prior to starting a run and that contain the parameters for your run. For more information, see *Sample Sheets* on page 52.
- ▶ **Manifests**—Sets the default location for manifest files. Manifests are files used only for sequencing TruSeq® Custom Amplicon and Nextera® XT libraries. They contain the amplicon sequences to which clusters are aligned during analysis. For more information, see *Manifests* on page 53.
- ▶ **MiSeqOutput**—Sets the default location for analysis output files. Illumina recommends changing the default output folder to a network location for the purpose of sharing, long-term storage, and optionally using MiSeq Reporter off-line. For more information, see *Run Folders* on page 56.

Email Notifications Tab

MiSeq can be configured to send an email notification when primary analysis is complete, when on-instrument secondary analysis is complete, or if a critical MiSeq software error occurs.

Figure 10 Email Notifications Tab



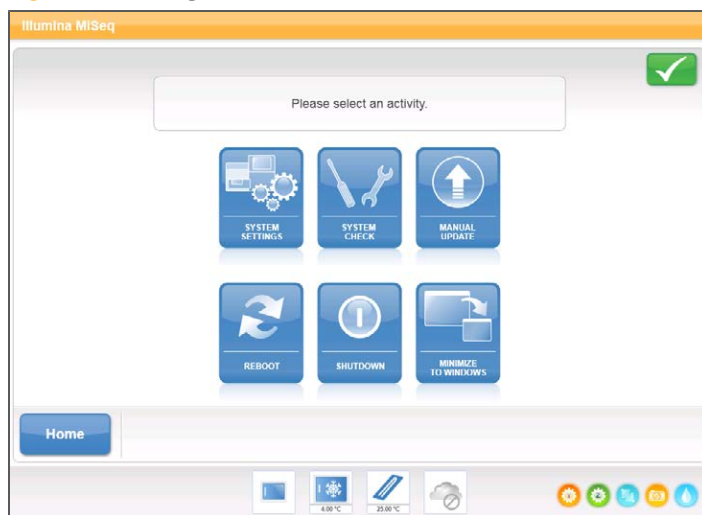
- ▶ **Local SMTP email server address**—Use the on-screen keyboard to enter the local SMTP email server address. If necessary, contact the facility administrator for this information.

- ▶ **Sender email address**—Use the on-screen keyboard to enter the sender email address. This can be your email address or a different address specified for sending email notifications. The sender email address must have the same domain name as the email server address.
- ▶ **Email addresses**—Use the on-screen keyboard to enter the email addresses of each recipient to receive notifications. Separate each email address with a comma. Select **Test** to send a test email to notification recipients.
- ▶ **Notify via email when**—Select the checkbox for each of the run events that trigger a notification.

Manage Instrument Screen

The Manage Instrument screen contains controls for system settings, troubleshooting, manually updating software, and safely rebooting or shutting down the instrument software.

Figure 11 Manage Instrument Screen



- ▶ **System Settings**—Provides the option to change IP Settings, machine name, or domain. See *System Settings Screen* on page 37.
- ▶ **System Check**—Provides troubleshooting options for checking the operational status of instrument components. See *System Check Screen* on page 38.
- ▶ **Manual Update**—Provides the option to manually update software on the instrument computer. See *Manual Update Screen* on page 39.
- ▶ **Reboot**—Use the Reboot command to safely reboot the system software if needed for troubleshooting. There is no requirement to periodically reboot the software.
- ▶ **Shut Down**—Use the Shut Down command to safely shut down the control software and Windows on the instrument computer. See *Shutting Down the Instrument* on page 103.

- **Minimize to Windows**—Provides quick access to the instrument operating system and any folders located on the instrument computer when MCS is running in kiosk mode opposed to Windows mode.

System Settings Screen

System Settings are normally configured when the instrument is initially installed and started for the first time. If any settings need to be changed due to a network or facility change, use the System Settings feature.

Figure 12 System Settings



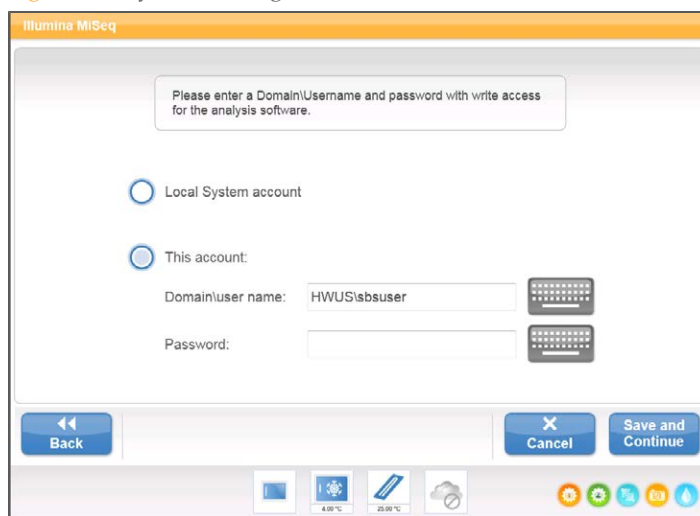
Contact the facility administrator to get information about what network settings to enter.

Changing System Credentials

To change the system user name and password, make the change on the Systems Settings screen. Select **System Settings** on the Manage Instrument screen, and then select **Save and Continue** to progress to the third screen in the series of screens.

Select **This account**, and then enter the domain name (Domain\MiSeq1, for example) and password. Select **Save and Continue**. At this time the credentials for MiSeq Reporter and BaseSpace are also updated.

Figure 13 System Settings



System Check Screen

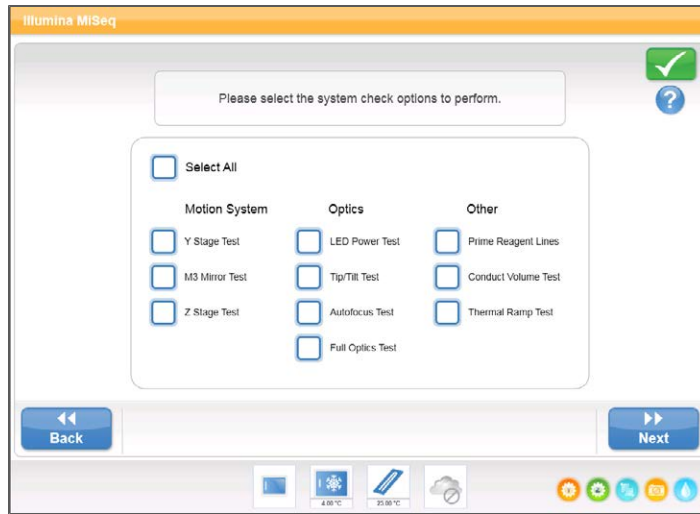
The System Check is typically used by an Illumina Technical Support representative during a Live Help session. Use of this feature is not required during normal operation or for instrument maintenance.

Some system checks can be performed prior to contacting Illumina Technical Support, such as the Volume Test. A volume test checks the health of the fluidics system by estimating the flow volume as bubbles pass by the sensors. This test is followed by a line wash. For more information, see *Performing a Volume Test* on page 112.

Upon completion of a system check, the test results appear on the screen:

- ▶ Select **Show Details** to see a summary of the results on the software interface.
- ▶ Select **Export Results** to export the results in a *.csv format to a USB drive.

Figure 14 System Check Options



Manual Update Screen

Use the Manual Update feature to update instrument control software and analysis software from the MiSeq interface by browsing to the location of the installable software file.

Select **Browse** to browse to the location where the installable file for the new software version is located. When the path to the installable software file appears on the screen, select **Update**.

Alternatively, you can update your software automatically if your instrument is connected to a network. For more information, see *Software Updates* on page 26.

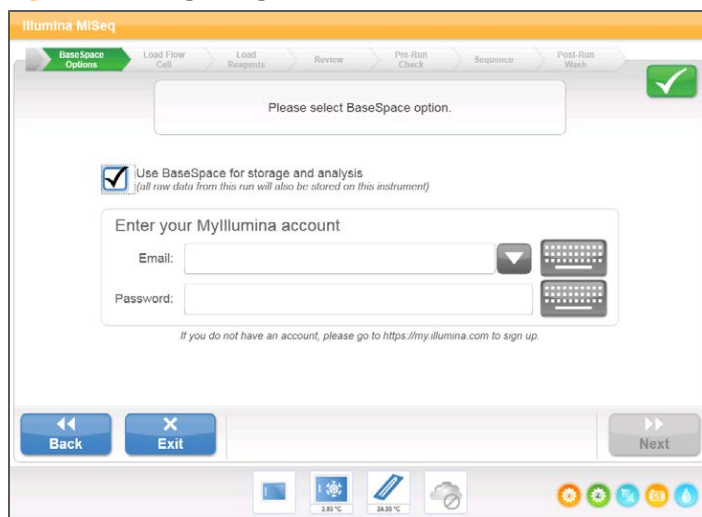
Run Setup Screens

When you select **Sequence** on the Welcome screen, a series of run setup screens open in the following order: BaseSpace Option, Load Flow Cell, Load Reagents, Review, and Pre-Run Check.

BaseSpace Options Screen

The first screen in the run setup steps provides the option to use BaseSpace for storage and analysis. To enable access, you need a network connection and a MyIllumina account. For more information, see *BaseSpace Overview* on page 64.

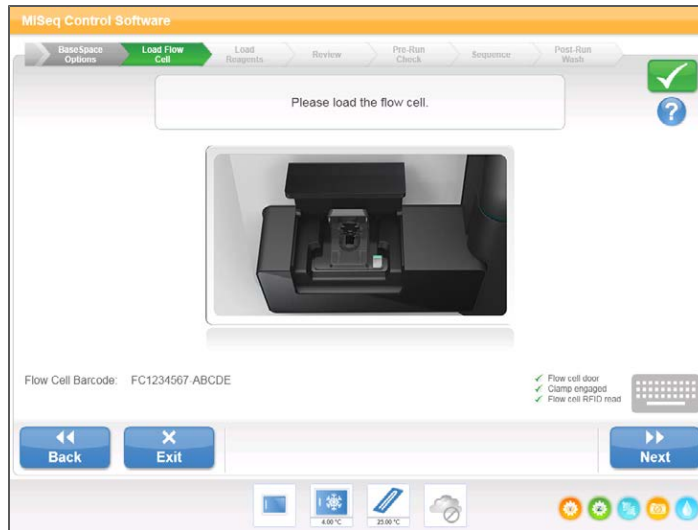
Figure 15 BaseSpace Options Screen



Load Flow Cell Screen

The Load Flow Cell screen prompts you to load the flow cell. After the flow cell is in place, close the flow cell latch and flow cell compartment door. Both the latch and compartment door must be closed before beginning the run.

Figure 16 Load Flow Cell Screen



When the flow cell is loaded, the software reads and records the RFID. A confirmation that the RFID was successfully read appears in the lower-right corner of the screen.

In the event that the RFID cannot be read, you can obtain a temporary bypass code from Illumina that enables you to proceed with the run setup steps. For more information, see *Resolving RFID Read Failure* on page 109.

Select the help icon in the upper-right corner of the screen. The help file contains a video that illustrates the steps for loading the flow cell.

Load Reagents Screen

The Load Reagents screen has two steps: first, load the PR2 bottle and empty waste bottle, and then load the reagent cartridge. When the PR2 bottle and reagent cartridge are loaded, the software reads and records the RFID. A confirmation that the RFID was successfully read appears in the lower-right corner of the screen.

Figure 17 Load PR2 Bottle and Waste Bottle

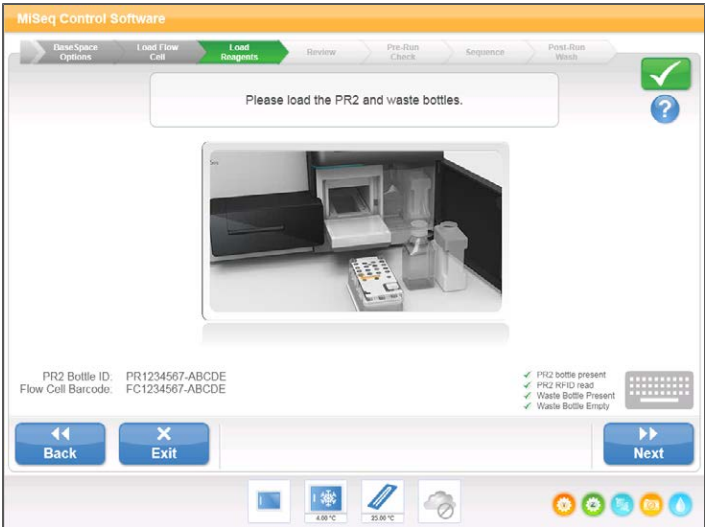
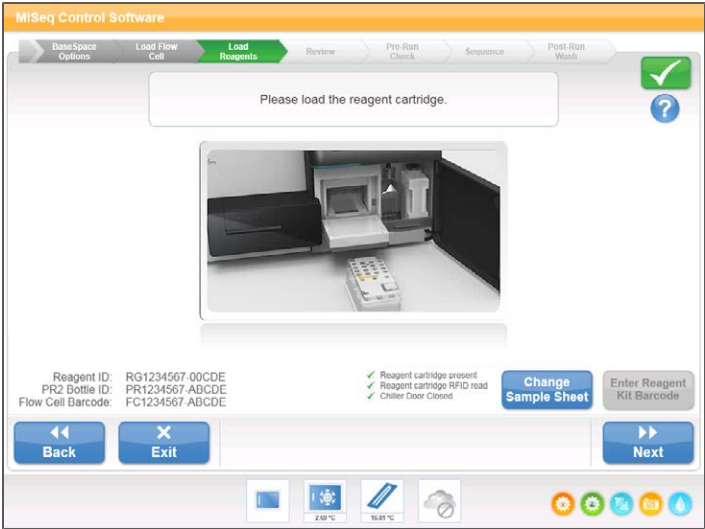


Figure 18 Load Reagent Cartridge



In the event that the RFID cannot be read, you can obtain a temporary bypass code from Illumina that enables you to proceed with the run setup steps. For more information, see *Resolving RFID Read Failure* on page 109.

You must use the reagent cartridge associated with the type of flow cell that you loaded. If the reagent cartridge is not compatible, a message appears on the screen. Select **Back** to load the appropriate reagent cartridge or **Exit** to return to the Welcome screen.

Select the help icon in the upper-right corner of the screen. The help file contains a video that illustrates the steps for loading the reagent cartridge and for loading the PR2 bottle.

Change Sample Sheet

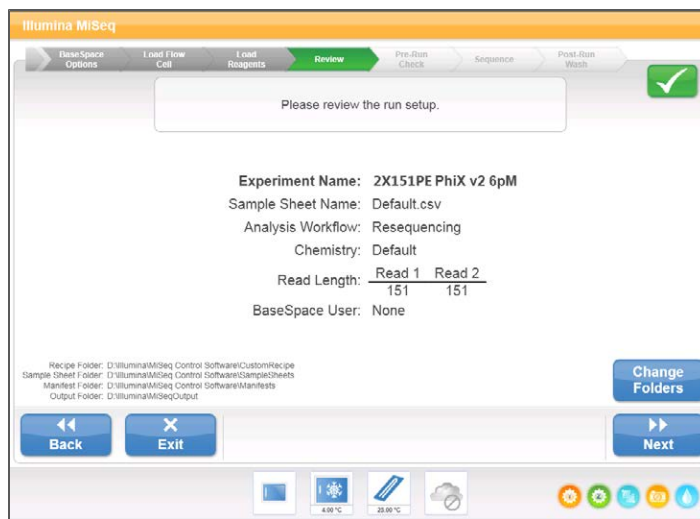
Every run must have a sample sheet. By default, the software looks for a sample sheet file with a name matching the barcode number of the reagent cartridge loaded on the instrument. If a sample sheet is not found, a message appears that prompts you to browse to the location of the correct sample sheet for your run. For more information, see *Sample Sheets* on page 52.

To prevent the software from searching unsuccessfully, use the **Change Sample Sheet** option on the Load Reagents screen to direct the software to the appropriate sample sheet.

Review Screen

The Review screen confirms that the software has located the sample sheet for the run, and lists the sample sheet name and parameters provided in the sample sheet.

Figure 19 Review Screen



Change Folders

In the lower-left corner of the Review screen, the current folder locations for recipes, sample sheets, manifests, and output folders are listed. To change folder locations, select **Change Folders** and browse to a preferred location. Using this option from the Review screen changes folder locations for the current run only.

Sample Sheet Not Found

If a sample sheet was not specified from the Load Reagents screen and the software cannot locate a sample sheet named with the barcode number of the reagent cartridge loaded for the run, a screen appears for browsing to the sample sheet associated with the run. After browsing to the sample sheet, select **Restart Check**.

Figure 20 Sample Sheet Not Found

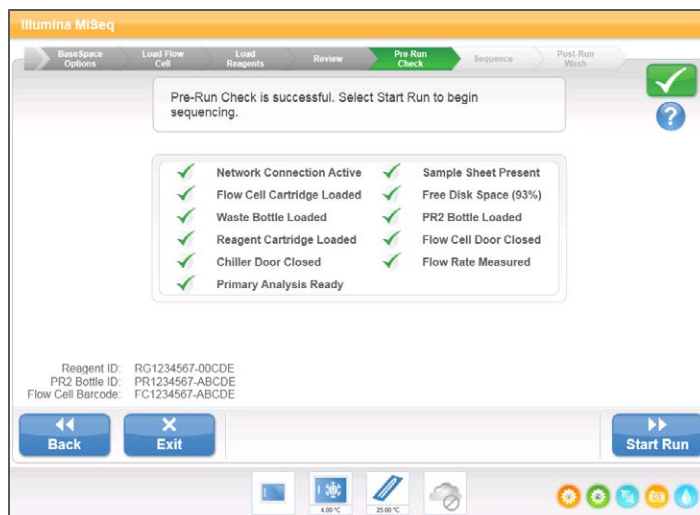


Pre-Run Check Screen

The software automatically performs a pre-run check of required run components and conditions before allowing the run to begin. If any errors occur during the pre-run check, a message appears on the screen that describes the error and the action to correct it.

For more information, see *Resolving Run Setup Errors* on page 107.

Figure 21 Pre-Run Check Screen

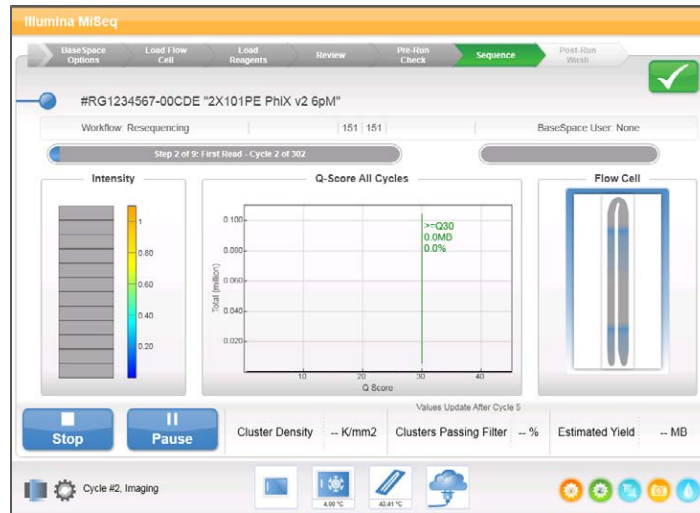


When the pre-run check is successful, the **Start Run** button becomes active.

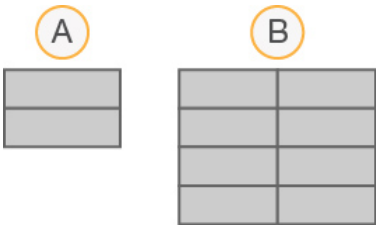
Sequencing Screen

The Sequencing screen opens when the run begins. This screen provides a visual representation of the run in progress, including intensities and quality scores (Q-scores). For MiSeq run specifications, visit the MiSeq System specifications page on the Illumina website (www.illumina.com/systems/miseq/performance_specifications.ilmn).

Figure 22 Sequencing Screen



- ▶ **Run Progress**—Shows run progress in a status bar and lists the number of cycles completed.
- ▶ **BaseSpace User**—Shows the BaseSpace user name and shows the progress as files are uploaded to BaseSpace.
- ▶ **Intensity**—Shows the value of cluster intensities of the 90th percentile for each tile. The graphic in the Intensity area represents the number of tiles and number of surfaces being imaged:
 - If the flow cell is imaged on the top surface only, a single-column graphic appears.
 - If the flow cell is image on the top surface and bottom surface, a two-column graphic appears.



- A** Two tiles, top surface only
- B** Four tiles, top and bottom surface

► **Q-Score All Cycles**—Shows the average percentage of bases greater than Q30, which is a quality score (Q-score) measurement. A Q-score is a prediction of the probability of a wrong base call. Q-scores are calculated after cycle 25.

Q-Score	Probability of Wrong Base Call
Q40	1 in 10,000
Q30	1 in 1,000
Q20	1 in 100
Q10	1 in 10

- **Cluster Density (K/mm²)**—Shows the number of clusters per square millimeter for the run. Optimally, the cluster density should be 800K/mm². However, a range of 50K–1300K/mm² is acceptable.
- **Clusters Passing Filter (%)**—Shows the percentage of clusters passing filter based on the Illumina chastity filter, which measures quality. This data appears only after cycle 25.



NOTE
The chastity of a base call is the ratio of the intensity of the greatest signal divided by the sum of the two greatest signals. Reads do not pass the quality filter if more than one base call with chastity of less than 0.6 in the first 25 cycles.

- **Estimated Yield (Mb)**—Shows the projected number of bases called for the run, measured in megabases. This data appears only after cycle 25.

Template Generation

Real Time Analysis (RTA) uses the first four cycles of the sequencing run for template generation. Template generation is the process by which cluster positions over the entire

flow cell surface are defined according to X and Y coordinate position.

After the template of cluster positions has been generated, the images produced over every subsequent cycle of imaging are aligned against the template so that individual cluster intensities in all four nucleotide color channels can be extracted, and base calls produced from the normalized cluster intensities.

Sequencing Analysis Viewer

You can monitor your run in greater detail without interfering with the run using the Illumina Sequencing Analysis Viewer (SAV). Your MiSeq must be networked to view primary analysis results with SAV.

SAV allows you to review metrics during a run as metrics are generated, and later after a run has completed. You *must* install SAV onto a computer independent of the MiSeq with access to the same network connected to the instrument. After launching the software, you can browse to the output folder for your run.

After cycle four following template generation, SAV provides metrics generated by RTA and organizes the metrics into plots, graphs, and tables.



NOTE

SAV is universal to Illumina sequencers, most of which use an eight-lane flow cell. Some views include drop-down lists showing lanes 1–8. The MiSeq flow cell is a single-lane flow cell, so your data appears when you select **All** or **Lane 1**.

For more information, see the *Sequencing Analysis Viewer User Guide*, Part # 15020619.

Analysis Workflows

The analysis workflow is a procedure performed by MiSeq Reporter. One analysis workflow must be specified in the sample sheet for each sequencing run. When the run is complete, MiSeq Reporter performs secondary analysis according to that workflow.

Analysis Workflow	Applications	Output
Assembly	Assembles small genomes from reads without the use of a genomic reference. If a genomic reference is specified, a dot-plot is generated with respect to the reference of genome position vs assembled contigs.	Contigs in FASTA format.
Custom Amplicon	Sequences TruSeq Custom amplicon from probes targeting particular genome positions (up to approx. 384 loci from up to approx. 96 samples). Aligns reads against a manifest file specified in the sample sheet. Two manifests can be specified: one for the control and one for the sample.	Aligned reads in BAM format. Variant calls in VCF format.
Enrichment	Analyzes DNA that has been enriched for particular target sequences using a pulldown assay. Aligns reads against the whole genome reference, and performs variant analysis for the regions of interest specified in the manifest file. Reporting accumulates coverage and other statistics for each amplicon.	Aligned reads in BAM format. Variant calls in VCF format.
Generate FASTQ	Generates intermediate analysis files in FASTQ format and then exits the workflow. Enables the use of third-party tools to analyze sequencing data.	Sequence files in FASTQ format.

Analysis Workflow	Applications	Output
Library QC	Aligns reads against reference genomes specified in the sample sheet, and then generates a sample report in LibraryQC.html.	Per-sample summary statistics.
Metagenomics	Classifies bacteria from a metagenomic sample by amplifying specific regions in 16S ribosomal RNA. No genomic reference is required for the metagenomics workflow. Reads are classified using a database of 16S rRNA data. For paired-end runs, each cluster is classified using base calls from both reads.	Read classifications by taxonomic group from kingdom through genus.
PCR Amplicon	Sequences any number of PCR amplicons that have been fragmented using Nextera tagmentation. Aligns reads against the reference genomes specified in the sample sheet. Performs variant analysis for the regions of interest specified in the manifest file.	Aligned reads in BAM format. Variant calls in VCF format.
Resequencing	Sequences a small genome, such as <i>E. coli</i> . Aligns reads against the reference genomes specified in the sample sheet and performs variant analysis.	Aligned reads in BAM format. Variant calls in VCF format.
Small RNA	Sequences miRNA. Aligns reads against databases for contaminants, mature miRNA, small RNA, and a genomic reference, in that order.	Reports on the relative abundance of each record.

Sample Sheets

The sample sheet is a comma separated values (*.csv) file that stores information needed to set up, perform, and analyze a sequencing run.

Illumina recommends that you create your sample sheet prior to preparing your sample libraries. You can create your sample sheet using the Illumina Experiment Manager or create it manually using a text editor, such as Excel or Notepad.

Before starting the run, make sure that the sample sheet is accessible to the instrument by copying the sample sheet to a network location accessible from the instrument computer, or copy the sample sheet from a USB flash drive to the instrument computer using the Manage Files feature in MiSeq Control Software (MCS). For more information, see *Manage Files Screen* on page 30.

When the run begins, the software copies the sample sheet from the designated sample sheet folder to the root of the output folder, and then copies it to the root of the analysis folder. At the end of the run, the sample sheet is used for secondary analysis by the MiSeq Reporter software.

Illumina Experiment Manager

The Illumina Experiment Manager is a wizard-based application that guides you through the steps to create your sample sheet.

Using the Illumina Experiment Manager not only reduces syntax errors, but also prompts you to provide information for sections that apply to your sample type and analysis workflow. It provides a feature for recording parameters for your sample plate, such as sample ID, project name, dual indices, and barcode information. Using the Illumina Experiment Manager, you can import sample plate parameters to your sample sheet.

The Illumina Experiment Manager can be run on any Windows platform. You can download a copy from the Illumina website at www.illumina.com. Go to the MiSeq support page and click **Downloads**. A MyIllumina login is required.

For more information, see the *Illumina Experiment Manager User Guide*, Part # 15031335.

Manifests

A manifest is a file that specifies the alignments to a reference and the targeted reference regions used in the workflow.

A manifest file is *required* for the following analysis workflows:

- ▶ **Custom Amplicon**—The manifest file for the Custom Amplicon workflow is provided by Illumina with your custom assay (CAT) and uses a *.txt file format. You can obtain a manifest file for your TruSeq Custom Amplicon experiment from the Illumina website. Log in to MyIllumina, and click **Custom Products**. From the options for TruSeq Custom Amplicon, click **Product Files**.
- ▶ **PCR Amplicon**—The manifest file for the PCR Amplicon workflow is generated using the Illumina Experiment Manager and uses a *.AmpliconManifest file format. The genome reference must be specified in the manifest file. For more information see the *Illumina Experiment Manager User Guide*, Part # 15031335.
- ▶ **Enrichment**—Like the PCR Amplicon workflow, the manifest file for the Enrichment workflow is generated using the Illumina Experiment Manager and uses a *.AmpliconManifest file format. For more information see the *Illumina Experiment Manager User Guide*, Part # 15031335.

Load your manifest file onto the instrument using the Manage Files feature to the specified location. For more information see *Manage Files Screen* on page 30.

Generating FASTQ Files

For each analysis workflow, MiSeq Reporter generates intermediate analysis files in the FASTQ format. MiSeq Reporter places the FASTQ files in the BaseCalls folder of the analysis folder (Data\Intensities\BaseCalls).

Normally, you do not need to access FASTQ files. However, if you plan to perform secondary analysis with a third-party analysis tool, you can set up your sample sheet so that MiSeq Reporter only generates the FASTQ files and then exits the workflow before proceeding to alignment.

To instruct MiSeq Reporter to only generate FASTQ files, specify **GenerateFASTQ** as an analysis workflow. If you use this workflow, you do not need to specify a path to a genome folder. This workflow is an option in Illumina Experiment Manager v1.2, or later.

Figure 23 Example of Sample Sheet Set to OnlyGenerateFASTQ

[Header]			
Investigator Name	John Smith		
Project Name	Alpha		
Experiment Name	Custom analysis		
Date	5/4/2012		
Workflow	GenerateFASTQ		
[Reads]			
	51		
	51		
[Settings]			
[Data]			
Sample_ID	Sample_Name	Index	GenomeFolder
10002	Alpha	TAAGCT	PhiX\Illumina\RTA\Sequence\WholeGenomeFASTA
10003	Beta	CGGATG	PhiX\Illumina\RTA\Sequence\WholeGenomeFASTA
10004	Gamma	ACCTGA	PhiX\Illumina\RTA\Sequence\WholeGenomeFASTA
10005	Delta	GTTCAC	PhiX\Illumina\RTA\Sequence\WholeGenomeFASTA

FASTQ File Naming

FASTQ files are named with the sample name and the sample number, which is a numeric assignment based on the order that the sample is listed in the sample sheet. For example:

Data\Intensities\BaseCalls\samplename_S1_L001_R1_001.fastq.gz

- **samplename**—The sample name provided in the sample sheet. If a sample name is not provided, the file name includes the sample ID, which is a required field in the sample sheet and must be unique.
- **S1**—The sample number based on the order that samples are listed in the sample sheet starting with 1. In this example, S1 indicates that this sample is the first sample listed in the sample sheet.



NOTE

Reads that cannot be assigned to any sample are written to a FASTQ file for sample number 0, and excluded from downstream analysis.

- **L001**—The lane number. This segment is always L001 with the single-lane flow cell.
- **R1**—The read. In this example, R1 means Read 1. For a paired-end run, there will be at least one file with R2 in the file name for Read 2.
- **001**—The last segment is always 001.

FASTQ files are compressed in the GNU zip format, as indicated by the *.gz. FASTQ files can be uncompressed using tools such as gzip (command-line) or 7-zip (GUI).

Demultiplexed FASTQ Files

MiSeq Reporter generates FASTQ files that have been demultiplexed (separated by sample). Each file contains sequences for a particular sample and read.

Run Folders

Each run on the MiSeq generates three run folders, each with a specific purpose:

- ▶ **D:\Illumina\MiSeqTemp**—When the run begins, a temporary run folder is written to the local drive of the instrument computer and used as a working area for MCS and RTA. There is no need to access the Temp folder. Contents of this folder are deleted after seven days.
- ▶ **D:\Illumina\MiSeqOutput**—RTA copies files from the Temp folder to the Output folder. As primary analysis files are generated, RTA copies files back to the Temp folder and populates the Analysis folder. Focus images and thumbnail images are not copied to the Analysis folder.

You can change the location of the output folder in the Output Folder field on the Run Options screen. For more information, see *Run Options Screen* on page 32.

- ▶ **D:\Illumina\MiSeqAnalysis**—When primary analysis is complete, MiSeq Reporter accesses the Analysis folder on the instrument local drive to begin secondary analysis. All files written to the Analysis folder are copied to the Output folder. For more information, see *Output and Analysis Folder Contents* on page 56.

If you are using BaseSpace for analysis without replicating analysis locally, the Analysis folder on the instrument local drive is empty.

Root Folder Naming

The root run folder name identifies the date of the run, the instrument number, and the flow cell used for the run. For any one run, each run folder has the same root folder name.

By default, the folder name uses the following format:

YYMMDD_<InstrumentNumber>_<Run Number>_A<FlowCellBarcode>

The run number increments by one each time a run is performed on a given instrument.

Output and Analysis Folder Contents

When secondary analysis by MiSeq Reporter is complete, the output and analysis folders are identical with one exception that the Output folder contains two subfolders for images files: Images and Thumbnail_Images.

Files

The files that are copied to the output and analysis folders include the following:

- ▶ **SampleSheet.csv**—Provides parameters for the run and subsequent analysis. At the start of the run, the sample sheet is copied to the root folder and renamed SampleSheet.csv. Additional copies are located in Data\Intensities and Data\Intensities\BaseCalls.
- ▶ **runParameters.xml**—Contains a summary of run parameters and information about run components, such as the RFID of the flow cell and reagents associated with the run.
- ▶ **RunInfo.xml**—Contains high-level run information, such as the number of reads and cycles in the sequencing run, and whether or not a read is indexed.

Folders

The folders that are copied to the output and analysis folders include the following:

- ▶ **<Run folder name>\Config**—Contains configuration files for the run.
- ▶ **<Run folder name>\Data**—Contains subfolders Intensities, BaseCalls, and Alignment. Data generated from MiSeq Reporter are located in the Alignment subfolder.
- ▶ **<Run folder name>\Data\RTA Logs**—Contains log files that describe each step performed by RTA for each Read.
- ▶ **<Run folder name>\Data\Intensities\BaseCalls**—Contains subfolders with base call (*.bcl) files, matrix and phasing files, and FASTQ files. FASTQ files can be used for downstream analysis with third-party tools.
- ▶ **<Run folder name>\Data\Intensities\BaseCalls\Alignment**—Contains alignment (*.bam) and variant call (*.vcf) files. This folder is not created if you set up your run with the GenerateFASTQ workflow.
- ▶ **<Run folder name>\Recipe**—Contains the recipe used for the run.
- ▶ **<Run folder name>\Logs**—Contains log files that describe every step performed by the instrument for each cycle.
- ▶ **<Run folder name>\Interop**—Contains binary files used by Sequencing Analysis Viewer (SAV) to summarize various primary analysis metrics such as cluster density, intensities, quality scores, and overall run quality.

All other files and folders created in the temporary run folder are not copied to the output and analysis folders. They contain temporary files that are not required for analysis or troubleshooting.

Primary Analysis Results

The primary analysis output from a sequencing run is a set of quality-scored base call files (*.bcl), which are generated from the raw image files.

The following table describes the folders and files generated by Real Time Analysis (RTA) during primary analysis. Many of these files are used for secondary analysis by the MiSeq Reporter software.

Key File	Subfolder	Description
RTAComplete.txt	Root folder	A marker file generated when base call analysis is complete. The presence of this file triggers the start of secondary analysis.
SampleSheet.csv	Root folder	This file is read and copied to the run folder before the run, and later used for secondary analysis.
RunInfo.xml	Root folder	Identifies the boundaries of the reads (including index reads) and the quality table selected for run.
*.bcl files	Data\Intensities\BaseCalls\L001\CX.X	Each *.bcl file contains RTA base calling and base quality scoring results for one cycle, one tile.
*.stats files	Data\Intensities\BaseCalls\L001\CX.X	*.stats files contain RTA base calling statistics for a given cycle/tile.
*.filter files	Data\Intensities\BaseCalls	*.filter files contain filter results per tile.
*.txt	Data\RTALogs	Log files from primary analysis.
*.cif files	Data\Intensities\L001\CX.X	Each binary *.cif file contains RTA image analysis results for one cycle, one tile. For more information, see <i>Flow Cell Tile Numbering</i> on page 59.
*.locs files	Data\Intensities\BaseCalls\L001	Reports the cluster coordinates. There is one *.locs file for each tile..
*.jpg files	Thumbnail_Images\L001\CX.X	Thumbnail images generated for each cycle and base, and can be used to troubleshoot a run. These files are not required for secondary analysis and are not copied to the Analysis folder.

Flow Cell Tile Numbering

When the tiles are imaged during the sequencing run, one output file is generated for each tile and named with the tile number in a four digit format.

With MCS v2.0 and upgraded hardware components, the standard flow cell is imaged in 14 tiles on the top surface and 14 tiles on the bottom surface, which results in the following tile numbering format:

- ▶ Image files named 1101 through 1114 are tiles 1–14 on the top surface.
- ▶ Image files named 2101 through 2114 are tiles 1–14 on the bottom surface.

The same tile numbering format is used with micro flow cells:

- ▶ Image files named 1101 through 1104 are tiles 1–4 on the top surface.
- ▶ Image files named 2101 through 2104 are tiles 1–4 on the bottom surface.

For nano flow cells, image files are named for tiles one and two on the top surface, 1101 and 1102.

The output files for each tile are located in the run folder in `Data\Intensities\L001`.

Run Duration

Run duration depends on the number of cycles you perform. You can perform a paired-end run up to 2×251 sequencing cycles plus any Index Reads with MCS v2.0.

Additionally, run duration depends on the version of MiSeq reagents you are using and any performance enhancing upgrades installed on your instrument.

For expected durations and other specifications, visit the MiSeq System specifications page on the Illumina website (www.illumina.com/systems/miseq/performance_specifications.ilmn).

Number of Cycles in a Read

The number of cycles performed in a read is one more cycle than the number of cycles analyzed. The one extra cycle is required for phasing and prephasing calculations.

For example, a paired-end 150-cycle run performs two 151-cycle reads (2×151) for a total of 302 cycles. At the end of the run, 2×150 cycles are analyzed.

In this guide, read lengths are listed as the number of cycles performed including the final cycle.

Required Disk Space

The integrated instrument computer has approximately 550 GB of storage capacity. Before starting a run, the software checks available disk space. If there is not enough disk space for the run, a software prompt appears. The message indicates how much disk space is required for the run and how much disk space must be cleared before the run can proceed.

If prompted to make disk space available, go to the Welcome screen and select **Manage Files**. From the Manage Files screen, select the **Runs** tab. Move or delete older run folders as appropriate. For more information, see *Manage Files Screen* on page 30. After clearing adequate disk space, select **Restart Check**.

Pausing or Stopping a Run

The MiSeq is designed to complete a run from beginning to end without user intervention. However, it is possible to pause a run or stop a run from the Sequencing screen.

Pausing a Run

You can temporarily pause a run before it has completed using the Pause button on the Sequencing screen. You might pause a run if you suspect that the waste bottle is full or to check the volume in the PR2 bottle. You can resume a paused run.



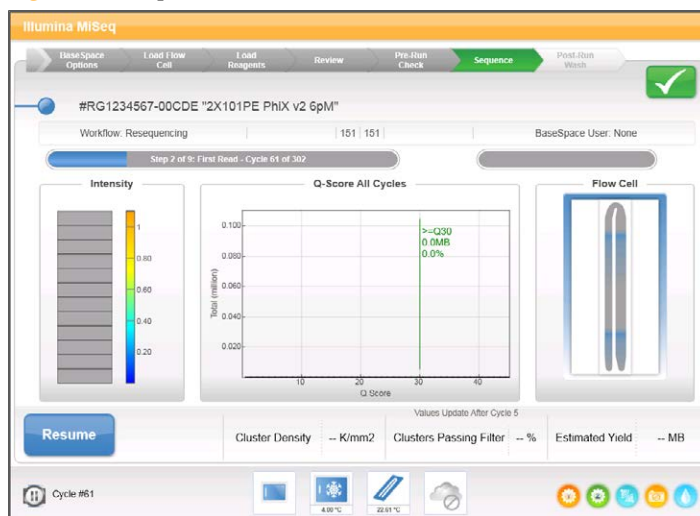
CAUTION

Do **not** pause a run during cluster generation or within the first five cycles of sequencing. Runs paused prior to cycle six cannot be resumed.

When you select Pause, the current command is completed, after which the run is paused and the flow cell is placed in a safe state.

To pause a run from the Sequencing screen, select **Pause**. The button changes to Resume. At this point you can empty the waste bottle, for example. When you are ready to resume the run, select **Resume**.

Figure 24 Sequence Screen of a Paused Run

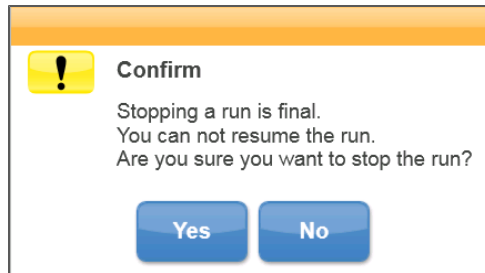


Stopping a Run

You can stop a run during sequencing before the run has completed using the **Stop** button on the Sequencing screen. You might stop a run if the run was set up incorrectly, if the data quality is bad, or if you experience a hardware error.

When a run is stopped, the current command is not completed and the flow cell stage moves to the forward position. Primary analysis will continue for the last completed cycle.

Figure 25 Stopping a Run



Stopping a run is final. A stopped run cannot be resumed. The only option is to proceed to an instrument wash.

Secondary Analysis Options

MiSeq sequencing data can be analyzed on the instrument computer using MiSeq Reporter or on the cloud using BaseSpace. Both BaseSpace and MiSeq Reporter produce information about alignment, variants, and contig assemblies for each genome requested and for each sample of a multi-sample run.

BaseSpace Overview

BaseSpace is Illumina's analysis environment. Using BaseSpace to store and analyze your run data provides the following benefits:

- ▶ Eliminates the need for onsite storage and computing
- ▶ Enables web-based data management and analysis
- ▶ Provides tools for global collaboration and sharing

When setting up runs on the MiSeq, you have the option to log in to BaseSpace. When logged in to BaseSpace, raw data from the run is also stored on the instrument, or in the location of the output folder that you specified in Run Options. For more information, see *Run Options Screen* on page 32.

Figure 26 BaseSpace Option Screen

When you begin your sequencing run, the BaseSpace icon changes to indicate that the MiSeq is connected to BaseSpace and data files are being transferred to your secure location. Data files are encrypted in transit, decrypted during analysis, and encrypted again when stored.

Figure 27 Connected to BaseSpace Icon



BaseSpace automatically disconnects from the MiSeq at the end of the run or as soon as all primary analysis files have finished uploading. If the internet connection is interrupted, analysis files will continue uploading after the connection is restored from the point when the interruption occurred.

As soon as the last base call file is uploaded to BaseSpace, secondary analysis of your data begins. The same analysis workflows are supported on BaseSpace as with on-instrument analysis using MiSeq Reporter.

You can connect to BaseSpace at <https://basespace.illumina.com>. Log in using your MyIllumina account login. The first time you visit, you will be asked to accept the BaseSpace agreement. After that, you are ready to view your analysis results.

For more information about using BaseSpace, see the BaseSpace online help on the Illumina website: www.illumina.com/help/BaseSpaceHelp/BaseSpaceHelp.htm.

MiSeq Reporter Overview

MiSeq Reporter is a Windows Service application that processes base calls generated by primary analysis. MiSeq Reporter begins secondary analysis immediately after the completion of primary analysis of the sequencing run.

MiSeq Reporter runs on the instrument computer. However, the software interface must be viewed through a web browser on another computer that is connected to the same network as the MiSeq Reporter.

When secondary analysis is complete, a file named `CompletedJobInfo.xml` is written to the run folder. For more information, see the *MiSeq Reporter User Guide*, Part # 15028784.

Sequencing During Analysis

If a new sequencing run is started on the MiSeq before secondary analysis of an earlier run is complete, secondary analysis is stopped automatically. MiSeq computing resources are dedicated to either sequencing or analysis and the system is designed in such a way that the sequencing run setup command overrides the analysis command.

You can restart secondary analysis using the Requeue feature on the MiSeq Reporter interface after the new sequencing run is complete. At that point, secondary analysis starts from the beginning.

The MiSeq must be connected to a network with internet access to enable Live Help. The Live Help feature is an online assistance tool that enables a representative from Illumina Technical Support to view the MiSeq screen with your permission, and share control of the instrument. You have overriding control and can end the screen-sharing session at any time.

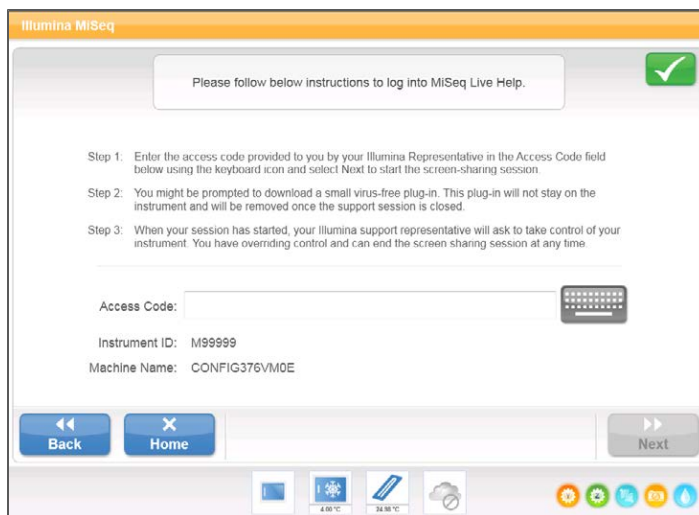
Access Live Help from the help icon on the Welcome screen.

Figure 28 Help Menu



To enable a connection, obtain a unique access code from Illumina Technical Support, enter that code on the Live Help screen, and then select **Next**.

Figure 29 Live Help Screen



Performing a Run

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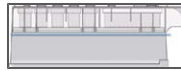
Introduction

To perform a run on the MiSeq, follow the setup steps described in this chapter. After the run begins, the MiSeq is completely automated and no other user intervention is required.

The sequencing run can be monitored from the Sequencing screen or monitored remotely using the Sequencing Analysis Viewer (SAV), an optional software application that you can download from the Illumina website.

After the run is complete, always perform an instrument wash.

MiSeq Workflow



Prepare the pre-filled reagent cartridge for use.



Dilute and denature your libraries (except for TruSeq Custom Amplicon libraries)

Load the library mix onto the reagent cartridge in the designated reservoir.



From the software interface, select **Sequence** to start the run set up steps.

[Optional] Connect to BaseSpace.



Wash and thoroughly dry the flow cell.

Load the flow cell.



Load the PR2 bottle and make sure that the waste bottle is empty.

Load the reagent cartridge.



Review run parameters and pre-run check results.

Select **Start Run**.



Monitor your run from the MCS interface or from another computer using Sequencing Analysis Viewer (SAV).



Perform a post-run wash using laboratory-grade water mixed with Tween 20.

Cluster Generation

During cluster generation, single DNA molecules are bound to the surface of the flow cell, and then bridge-amplified to form clusters.

Sequencing

Following cluster generation, clusters are imaged using LED and filter combinations specific to each of the four fluorescently-labeled dideoxynucleotides. After imaging of one tile of the flow cell is complete, the flow cell is moved into place to expose the next tile. The process is repeated for each cycle of sequencing. Following image analysis, the software performs primary analysis, which includes base calling, filtering, and quality scoring.

Analysis

When the run is complete, the Illumina Experiment Manager analysis software launches automatically to perform secondary analysis, which includes alignment and variant calling. You can monitor secondary analysis using an internet connection from another computer. For more information, see *MiSeq Reporter Overview* on page 65.

Preparing Reagents

The MiSeq Reagent Kit contains a tube of HT1, the reagent cartridge, and a bottle of PR2. For best results, carefully follow the reagent preparation instructions.

Component	Storage	Preparation Requirements
HT1	-15° to -25°C	Thaw for use in diluting denatured libraries, if applicable.
Reagent Cartridge	-15° to -25°C	Thoroughly thaw and inspect prior to use.
PR2 Bottle	2° to 8°C	Use directly from 2° to 8°C storage.

Prepare HT1

The tube of HT1 (Hybridization Buffer) is used to dilute denatured libraries prior to loading them onto the reagent cartridge for sequencing. This step does not apply to TruSeq Amplicon libraries (either Custom Amplicon or Cancer Panel) or Nextera XT libraries. For all other libraries, you need to thaw HT1.

- 1 Remove the tube of HT1 (Hybridization Buffer) from -15° to -25°C storage and set aside at room temperature to thaw.
- 2 When thawed, store at 2° to 8°C until you are ready to dilute denatured libraries. For more information, see *Preparing Your Libraries* on page 76.

Thaw Reagent Cartridge

The following instructions describe how to thaw the reagent cartridge using a room temperature water bath. This method requires approximately 1 hour.

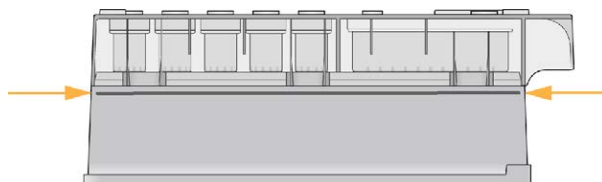


NOTE

Alternatively, you can thaw reagents overnight in 2° to 8°C storage. Reagents are stable up to one week when stored at this temperature.

- 3 Remove the reagent cartridge from -15° to -25°C storage.
- 4 Place the reagent cartridge in a water bath containing enough room temperature deionized water to submerge the base of the reagent cartridge. Do not allow the water to exceed the maximum water line printed on the reagent cartridge.

Figure 30 Maximum Water Line



- 5 Allow the reagent cartridge to thaw in the room temperature water bath for approximately 1 hour or until completely thawed.
- 6 Remove the cartridge from the water bath and gently tap it on the bench to dislodge water from the base of the cartridge. Dry the base of the cartridge.

Inspect the Reagent Cartridge

- 1 Invert the reagent cartridge ten times to mix the thawed reagents, and then visually inspect that all positions are thawed.
- 2 Visually inspect the reagent in position 1 to make sure that it is fully mixed and free of precipitates.
- 3 Gently tap the cartridge on the bench to reduce air bubbles in the reagents.



NOTE

The MiSeq sipper tubes go to the bottom of each reservoir to aspirate the reagents, so it is important that the reservoirs are free of air bubbles.

- 4 Place the reagent cartridge on ice or set aside at 2° to 8°C until ready to set up the run. For best results, proceed directly to loading the sample and setting up the run.



WARNING

This set of reagents 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 applicable local governmental safety standards.

For more information, see the MSDS for this kit, at www.illumina.com/msds.

Using Custom Primers

It is possible to use custom sequencing primers for a run on the MiSeq by loading custom primers into the three empty reservoirs on the MiSeq reagent cartridge. The empty reservoirs are labeled 18, 19, and 20, and each one is associated with a specific read in a sequencing run.

Read	Reservoir
Custom Primer for Read 1	18
Custom Primer for the Index Read	19
Custom Primer for Read 2	20

Optionally, you can use a combination of custom primers and Illumina primers provided in the reagent cartridge. For example, you can use the Illumina primer for Read 1, a custom primer for the Index Read, and the Illumina primer for Read 2.

Using custom primers on the MiSeq requires a change to the sample sheet. For more information, see the *MiSeq Sample Sheet Quick Reference Guide*, Part # 15028392.

Preparing Custom Primers

- 1 Prepare 600 µl of custom primer at 0.5 µM. Use HT1 (Hybridization Buffer) to dilute the custom primer.
- 2 With a clean pipette tip, pierce the foil seal covering the port associated with the primer you are loading.
- 3 Load 600 µl of custom primer in the appropriate port on the reagent cartridge. Take care to avoid touching the foil seal as you dispense the primer.
- 4 Inspect the bottom of the cartridge to make sure that bubbles are not present.

Preparing Your Libraries



NOTE

This Step Does Not Apply to All Library Types—If you are sequencing TruSeq Amplicon libraries (either Custom Amplicon or Cancer Panel) or Nextera XT libraries, ***do not*** perform this step. TruSeq Amplicon and Nextera XT protocols result in a ready-to-use normalized concentration of pooled libraries.

Illumina-Supplied Consumables

- ▶ HT1 (Hybridization Buffer), pre-chilled
(Shipped frozen in the MiSeq reagent cartridge box)

User-Supplied Consumables

- ▶ Stock 1.0 N NaOH (diluted to 0.2 N NaOH)
- ▶ Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20
- ▶ PhiX (optional)

Prepare a Fresh Dilution of NaOH



CAUTION

Using freshly diluted NaOH is essential in order to completely denature samples for cluster generation on the MiSeq.

To denature your samples, prepare 1 ml of 0.2 N NaOH. Preparing a volume of 1 ml prevents small pipetting errors from affecting the final NaOH concentration.

- 1 Combine the following volumes in a microcentrifuge tube:
 - Laboratory-grade water (800 μ l)
 - Stock 1.0 N NaOH (200 μ l)
- 2 Invert the tube several times to mix.

Denature DNA



NOTE

If your application requires higher than a 20 pM final concentration of your library, make sure that your concentration of NaOH is equal to 0.2 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 will inhibit library hybridization to the flow cell and decrease cluster density.

Use the following instructions to denature the DNA to a concentration of 20 pM.

- 1 Combine the following volumes of sample DNA and 0.2 N NaOH in a microcentrifuge tube:
 - 2 nM sample DNA (10 µl)
 - 0.2 N NaOH (10 µl)



NOTE

0.2 N NaOH is required to denature and dilute PhiX. If you plan to prepare a PhiX control within the next **12 hours**, set aside remaining 0.2 N NaOH. For more information, see *Denature and Dilute PhiX Control* on page 78.

Otherwise, discard the remaining dilution of 0.2 N NaOH.

- 2 Vortex briefly to mix the sample solution, and then centrifuge the sample solution to 280 xg for 1 minute.
- 3 Incubate for 5 minutes at room temperature to denature the DNA into single strands.
- 4 Add the following volume of pre-chilled HT1 to the tube containing denatured DNA to result in a 20 pM denatured library:
 - Denatured DNA (20 µl)
 - Pre-chilled HT1 (980 µl)
- 5 Place the denatured DNA on ice until you are ready to proceed to final dilution.

Dilute Denatured DNA

Use the following instructions to further dilute the 20 pM DNA to give 1000 µl of the desired input concentration.

- 1 Dilute the denatured DNA to the desired concentration using the following example:

Final Concentration	6 pM	8 pM	10 pM	12 pM	15 pM
20 pM denatured DNA	300 µl	400 µl	500 µl	600 µl	750 µl
Pre-chilled HT1	700 µl	600 µl	500 µl	400 µl	250 µl

- 2 Invert several times to mix the DNA solution.
- 3 Pulse centrifuge the DNA solution.
- 4 Place the denatured and diluted DNA on ice until you are ready to load your samples onto the MiSeq reagent cartridge.

Denature and Dilute PhiX Control

PhiX is used as an internal control for all libraries except TruSeq Amplicon libraries (either Custom Amplicon or Cancer Panel) and Nextera XT libraries.

Use the following instructions to denature and dilute the 10 nM PhiX library to 12.5 pM. This should result in a cluster density of 1000–1200 K/mm².

- 1 Combine the following volumes to dilute the PhiX library to 4 nM:
 - 10 nM PhiX library (2 µl)
 - 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20 (3 µl)
- 2 If not already prepared for denaturing your samples within the last **12 hours**, prepare a fresh dilution of 0.2 N NaOH. For more information, see *Prepare a Fresh Dilution of NaOH* on page 76.
- 3 Combine the following volumes of 4 nM PhiX library and 0.2 N NaOH in a microcentrifuge tube to result in a 2 nM PhiX library:
 - 4 nM PhiX library (5 µl)
 - 0.2 N NaOH (5 µl)
- 4 Vortex briefly to mix the 2 nM PhiX library solution.
- 5 Centrifuge the template solution to 280 xg for 1 minute.
- 6 Incubate for 5 minutes at room temperature to denature the PhiX library into single strands.
- 7 Add the following volume of pre-chilled HT1 to the tube containing denatured PhiX library to result in a 20 pM PhiX library.
 - Denatured PhiX library (10 µl)
 - Pre-chilled HT1 (990 µl)



NOTE

You can store the denatured 20 pM PhiX library up to three weeks at -15° to -25°C. After 3 weeks, cluster numbers tend to decrease.

- 8 Dilute the denatured 20 pM PhiX library to 12.5 pM as follows:
- 20 pM denatured PhiX library (375 µl)

• Pre-chilled HT1 (225 µl)
- 9 Discard the remaining dilution of 0.2 N NaOH.

Combine Sample Library and PhiX Control

For most libraries, Illumina recommends a low-concentration PhiX control spike-in at 1%. However, for metagenomics or low diversity libraries, increase the PhiX control spike-in to 25%. For more information, see *About Low Diversity Libraries* on page 79.

- 1 Combine the following volumes of denatured PhiX control library and your denatured sample library.

	For Metagenomics or Low Diversity Libraries (25%)	For All Other Libraries (1%)
PhiX control library	250 µl	10 µl
Denatured sample library	750 µl	990 µl

- 2 Set the combined sample library and PhiX control aside on ice until you are ready to load it onto the MiSeq reagent cartridge.

About Low Diversity Libraries

Low diversity libraries are libraries where a significant number of the reads have the same sequence. This shifts the base composition because the reads are no longer random. Low diversity can occur with some expression studies with > 25% one type of transcript, low plexity amplicon pools, adapter dimer, or bisulfite sequencing, for example. A higher concentration spike-in helps balance the overall lack of sequence diversity.

To optimize the performance of your MiSeq and to obtain higher quality data, Illumina recommends sequencing samples with high diversity and avoiding monotemplate stretches during sequencing. Low diversity can occur with the following libraries, for example:

- ▶ Libraries derived from RNA with more than 25% of the reads from a single transcript

▶ Pools of one or a few amplicons

- ▶ Libraries with initial cycle indexing

When sequencing PCR amplicons, 16S rRNA libraries, or other samples where diversity is low, one approach is to concatamerize the amplicons and fragment to create sequencing diversity. Other options include indexing your libraries and sequencing them with other diverse libraries, or pooling together different amplicons, ensuring that the index read has diversity. For recommendations, see low plexity pooling guidelines in the *Nextera DNA Sample Preparation Guide*, Part # 15027987.



NOTE

In order to focus, the MiSeq needs to detect signal in the C or T channel in the first cycle.

Loading Sample Libraries onto Cartridge

After you have prepared the MiSeq reagent cartridge for use, you are ready to load your samples. You can load a single library or a pool of indexed libraries.

- 1 Use a separate, clean, and empty 1 ml pipette tip to pierce the foil seal over the reservoir labeled **Load Samples**.

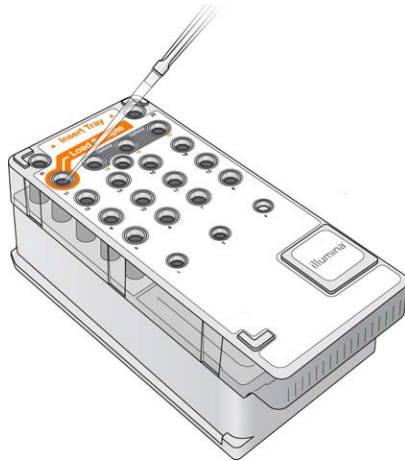


NOTE

Do not pierce any other reagent positions. Other reagent positions are pierced automatically during the run.

- 2 Pipette 600 μ l of your sample libraries into the **Load Samples** reservoir. Take care to avoid touching the foil seal as you dispense your sample.

Figure 31 Load Libraries



- 3 Proceed directly to the run setup steps using the MiSeq Control Software (MCS) interface.

Setting Up a Run Using MCS

- 1 [Optional] From the Run Options screen, check the folder locations for recipes, sample sheets, manifests, and the MiSeqOutput folder. For more information, see *Run Options Screen* on page 32.
- 2 From the Welcome screen, select **Sequence** to begin the run set up steps. The BaseSpace Options screen opens.

Set BaseSpace Option

The first screen in the run setup steps provides the option to use BaseSpace for storage and analysis. To enable access, you need a network connection and a MyIllumina account.

- 1 From the BaseSpace Options screen, do one of the following:
 - a Select the checkbox **Use BaseSpace for storage and analysis** and log in to MyIllumina using the on-screen keyboard:
 - Enter the email address associated with your MyIllumina account.
 - Enter your MyIllumina password.
 - b Clear the checkbox **Use BaseSpace for storage and analysis**.
- 2 Select **Next**. The Load Flow Cell screen opens.

Cleaning the Flow Cell

The flow cell is immersed in storage buffer in a flow cell container. Before loading the flow cell on the MiSeq, rinse the flow cell and thoroughly dry it, and then clean the flow cell with an alcohol wipe, as described here.

The cap color of the flow cell container indicates the flow cell type:

- ▶ The standard (14 tile) flow cell container cap is clear.
- ▶ The micro (4 tile) flow cell container cap is green.
- ▶ The nano (2 tile) flow cell container cap is yellow.

- 1 Put on a new pair of powder-free gloves.
- 2 Using plastic forceps, grip the flow cell by the base of the plastic cartridge and remove it from the flow cell container.

Figure 32 Remove Flow Cell



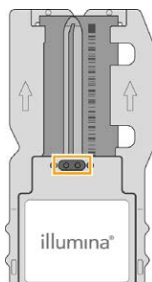
- 3 Lightly rinse the flow cell assembly with laboratory-grade water, making sure that both the glass and plastic cartridge are thoroughly rinsed of excess salts. Excess salts can affect flow cell seating on the instrument. If salts dry in the imaging area, imaging can also be affected.

Figure 33 Rinse Flow Cell



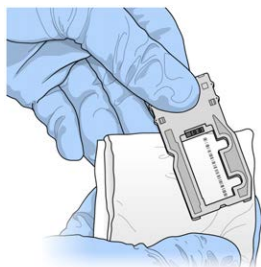
- 4 Using care around the black flow cell port gasket, thoroughly dry the flow cell and cartridge using a lint-free lens cleaning tissue. Gently pat dry in the area of the gasket and adjacent glass.

Figure 34 Flow Cell Ports and Gasket



- 5 Using an alcohol wipe, clean the flow cell glass, making sure that the glass is free of streaks and fingerprints, and that the flow cell is completely free of lint or tissue fibers. Avoid using the alcohol wipe on the flow cell port gasket.

Figure 35 Dry Flow Cell



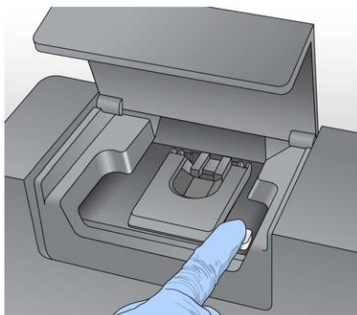
- 6 Dry any excess alcohol with a lint-free lens cleaning tissue, and visually inspect to make sure that the flow cell ports are free of obstructions and that the gasket is well-seated around the flow cell ports.

If the gasket appear to be dislodged, gently press it back into place until it sits securely around the flow cell ports.

Loading the Flow Cell

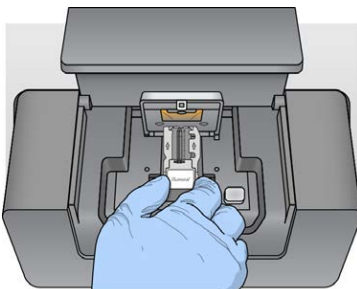
- 1 Raise the flow cell compartment door, and then press the release button to the right of the flow cell latch. The flow cell latch opens.

Figure 36 Open Flow Cell Latch



- 2 Visually inspect the flow cell stage to make sure it is free of lint. If lint or other debris is present, clean the flow cell stage using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol. Carefully wipe the surface of the flow cell stage until it is clean and dry.
- 3 Hold the flow cell by the edges of the flow cell cartridge near the Illumina label. Make sure the label is facing upward and place the flow cell on the flow cell stage.

Figure 37 Place Flow Cell on Stage

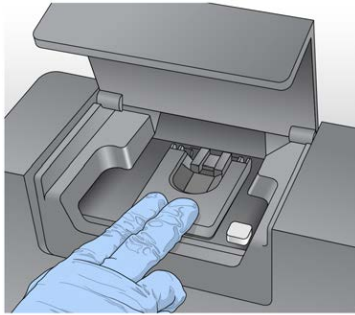


- 4 Gently press down on the flow cell latch to close it over the flow cell.

**NOTE**

As the flow cell latch is closed, two alignment pins near the hinge of the flow cell latch properly align and position the flow cell. An audible click indicates the flow cell latch is secure.

Figure 38 Close Flow Cell Latch



- 5 Check the lower-left corner of the screen to confirm that the flow cell RFID was successfully read.

**NOTE**

If the RFID is not read by the system, the software prompts you through the steps to obtain a temporary bypass code and proceed with setting up the run. For more information, see *Resolving RFID Read Failure* on page 109.

- 6 Close the flow cell compartment door.
- 7 Select **Next** on the Load Flow Cell screen. The Load Reagents screen opens.

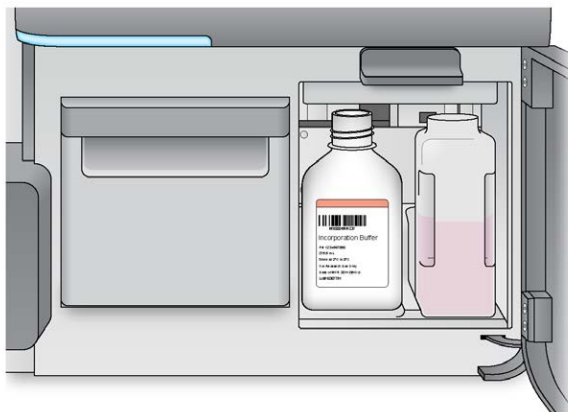
Loading Reagents

There are two steps to loading reagents. First, load the PR2 bottle and make sure that the waste bottle is empty, and then load the reagent cartridge.

Load PR2 and Check the Waste Bottle

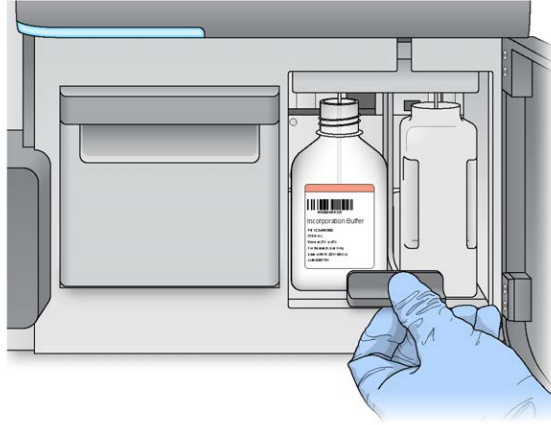
- 1 Remove the bottle of PR2 from 2° to 8°C storage. Gently invert the bottle to mix the PR2 bottle, and then remove the lid.
- 2 Open the reagent compartment door.
- 3 Raise the sipper handle until it locks into place.
- 4 Place the PR2 bottle in the indentation to the right of the reagent chiller.

Figure 39 Load the PR2 Bottle



- 5 Make sure that the waste bottle is empty. If it is not empty, empty the contents into the appropriate waste container.
- 6 Slowly lower the sipper handle. Make sure that the sippers lower into the PR2 and waste bottles.

Figure 40 Lower Sipper Handle



- 7 Check the lower-left corner of the screen to confirm that the RFID of the PR2 bottle was read successfully.



NOTE

If the RFID is not read by the system, the software prompts you through the steps to obtain a temporary bypass code and proceed with setting up the run. For more information, see *Resolving RFID Read Failure* on page 109.

- 8 Select **Next** on the Load Reagents screen.

Load the Reagent Cartridge

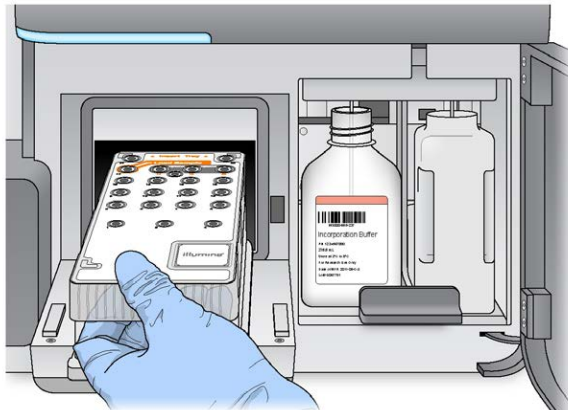


NOTE

Do not leave the reagent chiller door open for extended periods of time.

- 1 Open the reagent chiller door.
- 2 Hold the reagent cartridge on the end with the Illumina label, and slide the reagent cartridge into the reagent chiller until the cartridge stops.

Figure 41 Load Reagent Cartridge



- 3 Close the reagent chiller door.
- 4 Check the lower-left corner of the screen to confirm that the RFID of the reagent cartridge was read successfully.



NOTE

If the RFID is not read by the system, the software prompts you through the steps to obtain a temporary bypass code and proceed with setting up the run. For more information, see *Resolving RFID Read Failure* on page 109.

If the reagent cartridge is not compatible with the flow cell, a message appears. Select **Back** to load a compatible cartridge, or select **Exit** to return to the Welcome screen.

- 5 Close the reagent compartment door.
- 6 Select **Next** on the Load Reagents screen. The Review screen opens.

Starting the Run

After loading the flow cell and reagents, review the run parameters and perform a pre-run check before starting the run.

Review Run Parameters

- 1 Review Experiment Name, Analysis Workflow, and Read Length. These parameters are specified in the sample sheet.
- 2 Review the folder locations in the lower-left corner.
If any changes are needed, select **Change Folders**. When the changes are complete, select **Save**, and then select **Next**.
- 3 Select **Next**. The Pre-Run Check screen opens.

Review Pre-Run Check

The system performs a check of all run components, disk space, and network connections before starting the run.

If any items do not pass the pre-run check, a message appears on the screen with instructions on how to correct the error. For more information, see *Resolving Run Setup Errors* on page 107.

When all items successfully pass the pre-run check, select **Start Run**.

Important Note Before Starting the Run



WARNING

The MiSeq is sensitive to vibration. Touching the instrument after starting a run could adversely impact sequencing results.

After selecting **Start Run**, do not open the flow cell compartment or the reagent compartment door, or touch the instrument monitor except to pause the run. For more information, see *Pausing a Run* on page 62.

Monitoring the Run

- 1 During the run, monitor run progress, intensities, and quality scores on the Sequencing screen. The Sequencing screen is view-only. For more information, see *Sequencing Screen* on page 47.
To monitor the run in greater detail, use the Sequencing Analysis Viewer (SAV) installed on another computer independent of the instrument computer. A network connection is required. For more information, see *Sequencing Analysis Viewer* on page 49 and the *Sequencing Analysis Viewer User Guide*, Part # 15020619.
- 2 When the run is complete, the Next button appears. Review the results on the Sequencing screen before proceeding.

NOTE
The Sequencing screen remains viewable until Next is selected. After Next is selected, it is not possible to return to the Sequencing screen.
- 3 Select **Next** to exit the Sequencing screen and proceed to a post-run wash.

Run Metrics

Run metrics appear on the Sequencing screen at different points in a run. During cluster generation steps, no metrics appear. After sequencing begins, the following metrics appear at the indicated cycles:

Cycle	Metric
Cycle 1–4	Intensity
Cycle 4–25	Intensity and Cluster Density
Cycle 25 through run completion	Intensity, Cluster Density, % PF, Yield, and Q-scores

For MiSeq run specifications, visit the MiSeq System specifications page on the Illumina website (www.illumina.com/systems/miseq/performance_specifications.ilmn).

Maintenance Procedures

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Introduction

Always perform an instrument wash following the completion of a sequencing run.

Regular instrument washes ensure continued performance in the following ways:

- ▶ Flushes any remaining reagents from the fluidics lines and sippers
- ▶ Prevents salt accumulation and crystallization in the fluidics lines and sippers
- ▶ Prevents cross-contamination from the previous run

Maintenance Frequency

Illumina recommends that you perform the following maintenance procedures at the intervals shown below.

Table 2 Maintenance During Normal Operation

Activity	Frequency
Post-Run Wash	After every run
Maintenance Wash	Monthly
Standby Wash	To prepare for idle mode (if unused for ≥ 7 days)
Instrument Shutdown	As needed

Table 3 Maintenance During Idle Mode (≥7 days unused)

Activity	Frequency
Standby Wash	Monthly
Instrument Shutdown	As needed

Performing a Post-Run Wash

Always perform an instrument wash after completing a sequencing run. Follow the software prompts to load the wash components and perform the wash. The post-run wash takes approximately 20 minutes.

Illumina recommends that you perform the wash directly following the completion of a run. If the wash is not performed at this time, you must perform a wash prior to the next run.



NOTE

Leave the used flow cell on the instrument. A flow cell must be loaded on the instrument to perform an instrument wash.

User-Supplied Consumables

- ▶ Tween 20 (Sigma-Aldrich, catalog # P7949)
- ▶ Laboratory-grade water

Procedure

- 1 Prepare fresh wash solution with Tween 20 and laboratory-grade water, as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. This results in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 500 ml laboratory-grade water. This results in a 0.5% Tween 20 wash solution.
 - c Invert several times to mix.
- 2 Prepare the wash components with fresh wash solution, as follows:
 - a Add 6 ml wash solution to each reservoir of the wash tray.
 - b Add 350 ml wash solution to the 500 ml wash bottle.
- 3 When the run is complete, select **Start Wash**. The software automatically raises the sippers in the reagent chiller.
- 4 Open the reagent compartment door and reagent chiller door, and remove the used reagent cartridge from the chiller. The reagent cartridge should slide easily from the chiller.

- 5 Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
- 6 Raise the sipper handle in front of the PR2 bottle and waste bottle until it locks into place.
- 7 Remove the PR2 bottle and replace it with the wash bottle.



NOTE

Discard the PR2 bottle after each run. Do not reuse any remaining PR2.

- 8 Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.



WARNING

This set of reagents 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 applicable local governmental safety standards.

For more information, see the MSDS for this kit, at www.illumina.com/msds.

- 9 Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
- 10 Close the reagent compartment door.
- 11 Select **Next**. The post-run wash begins.

When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.



NOTE

The sippers will remain in the down position. This is normal. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Performing a Maintenance Wash

Perform a maintenance wash every 30 days to ensure optimal performance. The maintenance performs a series of three wash steps using a wash solution of laboratory-grade water mixed with Tween 20. The maintenance wash takes approximately 90 minutes.

User-Supplied Consumables

- ▶ Tween 20 (Sigma-Aldrich, catalog # P7949)
- ▶ Laboratory-grade water

Procedure

- 1 Make sure that a used flow cell is loaded on the instrument.
- 2 From the Welcome screen, select **Perform Wash**.
- 3 From the Perform Wash screen, select **Maintenance Wash**. The software automatically raises the sippers in the reagent chiller.

Perform First Wash

- 1 Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. This results in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 500 ml laboratory-grade water. This results in a 0.5% Tween 20 wash solution.
 - c Invert several times to mix.
- 2 Prepare the wash components with fresh wash solution as follows:
 - a Add 6 ml wash solution to each reservoir of the wash tray.
 - b Add 350 ml wash solution to the 500 ml wash bottle.
- 3 Load the wash tray and wash bottle onto the instrument:
 - a Open the reagent compartment door and reagent chiller door, and remove the used reagent cartridge or wash tray from the previous run. The reagent cartridge should slide easily from the chiller.

- b Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
- c Raise the sipper handle in front of the PR2 bottle and waste bottle until it locks into place, and replace the PR2 bottle with the wash bottle.



NOTE

Discard the PR2 bottle after each run. Do not reuse any remaining PR2.

- d Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
- e Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
- f Close the reagent compartment door.

- 4 Select **Next**. The first wash begins.

Perform Second Wash



NOTE

Always use fresh wash solution for each wash step. Reusing wash solution from the previous wash can return waste to the fluidics lines.

- 1 Prepare fresh wash solution with Tween 20 and laboratory-grade water, as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. This results in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 500 ml laboratory-grade water. This results in a 0.5% Tween 20 wash solution.
 - c Invert several times to mix.
- 2 When the first wash is complete, remove the wash tray and wash bottle, and discard the remaining wash solution.
- 3 Refill the wash components with fresh wash solution, as follows:
 - a Add 6 ml wash solution to each reservoir of the wash tray.
 - b Add 350 ml wash solution to the 500 ml wash bottle.
- 4 Load the freshly-filled wash tray and wash bottle, as follows:
 - a Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.

- b Load the wash bottle and slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - c Close the reagent compartment door.
- 5 Select **Next**. The second wash begins.

Perform Final Wash

- 1 Prepare fresh wash solution with Tween 20 and laboratory-grade water, as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. This results in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 500 ml laboratory-grade water. This results in a 0.5% Tween 20 wash solution.
 - c Invert several times to mix.
- 2 When the second wash is complete, remove the wash tray and wash bottle, and discard the remaining wash solution.
- 3 Refill the wash components with fresh wash solution, as follows:
 - a Add 6 ml wash solution to each reservoir of the wash tray.
 - b Add 350 ml wash solution to the 500 ml wash bottle.
- 4 Load the freshly-filled wash tray and wash bottle, as follows:
 - a Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - b Load the wash bottle and slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - c Close the reagent compartment door.
- 5 Select **Next**. The final wash begins.

After the Wash

When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.



NOTE

The sippers will remain in the down position. This is normal. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Performing a Standby Wash

If the instrument will not be used within the next seven days, prepare the instrument to sit idle by performing a standby wash. A standby wash performs two consecutive washes that flush each position of any remaining reagents or salt accumulation. Each wash takes approximately 60 minutes. Allow approximately 2 hours to complete the standby wash.

When the standby wash is complete, the instrument is in standby mode and a message appears on the Welcome screen stating the status of the instrument. When the instrument is in standby mode, a maintenance wash must be performed before a sequencing run can be initiated.



NOTE

Illumina recommends repeating the standby wash *every 30 days* that the instrument remains idle.

User-Supplied Consumables

- ▶ Tween 20 (Sigma-Aldrich, catalog # P7949)
- ▶ Laboratory-grade water

Procedure

- 1 Make sure that a used flow cell is loaded on the instrument.
- 2 From the Welcome screen, select **Perform Wash**.
- 3 From the Wash Options screen, select **Standby Wash**. The software automatically raises the sippers in the reagent chiller.

Perform First Wash

- 1 Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. This results in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 500 ml laboratory-grade water. This results in a 0.5% Tween 20 wash solution.
 - c Invert several times to mix.

- 2 Prepare the wash components with fresh wash solution as follows:
 - a Add 6 ml wash solution to each reservoir of the wash tray.
 - b Add 350 ml wash solution to the 500 ml wash bottle.
 - 3 Load the wash tray and wash bottle onto the instrument:
 - a Open the reagent compartment door and reagent chiller door, and remove the used reagent cartridge or wash tray from the previous run. The reagent cartridge should slide easily from the chiller.
 - b Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - c Raise the sipper handle in front of the PR2 bottle and waste bottle until it locks into place, and replace the PR2 bottle with the wash bottle.
-  **NOTE**
Discard the PR2 bottle after each run. Do not reuse any remaining PR2.
- d Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
 - e Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - f Close the reagent compartment door.
- 4 Select **Next**. The first wash begins.

Perform Second Wash



NOTE

Always use fresh wash solution for each wash step. Reusing wash solution from the previous wash can return waste to the fluidics lines.

- 1 Prepare fresh wash solution with Tween 20 and laboratory-grade water, as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. This results in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 500 ml laboratory-grade water. This results in a 0.5% Tween 20 wash solution.
 - c Invert several times to mix.
- 2 When the first wash is complete, remove the wash tray and wash bottle, and discard the remaining wash solution.
- 3 Refill the wash components with fresh wash solution, as follows:

- a Add 6 ml wash solution to each reservoir of the wash tray.
- b Add 350 ml wash solution to the 500 ml wash bottle.
- 4 Load the freshly-filled wash tray and wash bottle, as follows:
 - a Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - b Load the wash bottle and slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - c Close the reagent compartment door.
- 5 Select **Next**. The second wash begins.

After the Wash

When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.



NOTE

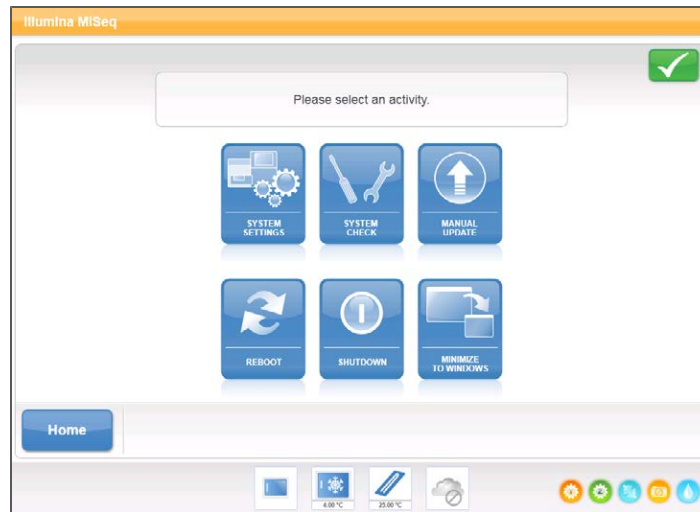
The sippers will remain in the down position. This is normal. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Shutting Down the Instrument

It is best to leave the instrument on at all times. However, if the instrument must be turned off, use the following procedure to safely shut down Windows and prepare the fluidics lines.

- 1 Perform a maintenance wash to thoroughly wash the system. For more information, see *Procedure* on page 97.
- 2 Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
- 3 Close the reagent compartment door.
- 4 From the Manage Instrument screen, select **Shut Down**. This command safely shuts down the software.

Figure 42 Manage Instrument Screen



- 5 Toggle the power switch to the OFF position.



NOTE

Any time that you turn off the instrument, wait a *minimum* of 60 seconds before turning the power switch back to the ON position.

Troubleshooting

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Introduction

This section describes common troubleshooting steps to take before contacting Illumina Technical Support. For most errors, an on-screen message appears with instructions for correcting the error.

For technical questions, visit the MiSeq support pages on the Illumina website for access to frequently asked questions, or log in to your MyIllumina account for access to support bulletins.

For problems with run quality or performance, contact Illumina Technical Support. For more information, see *Technical Assistance* on page 121.

The Illumina Technical Support representative might ask for copies of run-specific files for troubleshooting purposes. The following files are located at the root level of the output run folder:

- ▶ SampleSheet.csv
- ▶ RunParameters.xml
- ▶ RunInfo.xml
- ▶ Interop folder

Resolving Run Setup Errors

If any checks in the pre-run check fail, a red icon  appears next to the item to indicate the item is not ready, and a message appears on the screen that describes the error and the action needed to correct it.

Error	Action
 Flow Rate Measured	The flow rate check screen opens. Use the drop-down list or on-screen keyboard to enter the following: • Solution: PR2 • Volume: 250 • Aspirate Rate: 2500 • Dispense Rate: 2500 Select Pump. If the error persists, set the volume to pump 500 µl PR2 and repeat the process. When fluids have been pumped, select Restart Check. When the pre-run check is successful, the Start Run button becomes active. If the flow check fails again, reseal the flow cell to make sure that flow is not interrupted due to misalignment. Inspect the flow cell gasket for lint or irregularities that might prevent flow.
 Free Disk Space	If disk space is low, a message appears indicating how much disk space is required. Use the Manage Files feature to clear the required space from the instrument computer.
 Network Connection Active	Make sure the network cable is plugged into the instrument. If the network connection is not restored, select Reboot on the Manage Instrument screen to reboot the software. If the connection is still not restored, select Shut Down on the Manage Instrument screen, and then turn off the instrument using the power switch. Wait at least 60 seconds, and then turn on the instrument and start the software.
 Primary Analysis Ready	Primary analysis from the previous run is not complete. The default time to allow primary analysis to complete is 1 hour, and a countdown appears on the screen. The options are to wait 1 hour or select Terminate Analysis. Any incomplete cycles will not be available for secondary analysis.

Error	Action
 Sample Sheet Present	If you did not name your sample sheet with the reagent cartridge ID for your run, the instrument cannot locate the appropriate sample sheet automatically. Browse to the sample sheet for your run. If you named your sample sheet with the reagent cartridge ID for your run, make sure that the sample sheet is located in the default sample sheet folder. Check the default folder location in Run Options on the Welcome screen. Make sure that the sample sheet file extension is *.csv. If the sample sheet is missing, create one and copy it to the sample sheet locations specified in Run Options. When you have located a sample sheet, select Restart Check.

Resolving RFID Read Failure

In the event that the system cannot read the RFID of a consumable, you can obtain a temporary bypass code from the Illumina website. A temporary bypass code expires in seven days.

- 1 Always select **Retry** before proceeding. If the RFID failed a second time, select **Get Code**.
- 2 From a computer with internet access, go to <https://my.illumina.com> and log in to your MyIllumina account.
- 3 From the MyIllumina page, click **Account**. In the Resources column, click **MiSeq Self-Service**.
- 4 On the MiSeq Self-Service page, enter the **MiSeq serial number**.
- 5 From the Type of Override Code drop-down list, select **RFID Override**.

Figure 43 MiSeq Self-Service Page

MyIllumina / Account / MiSeq Self Service

Self Service for MiSeq

MiSeq Serial Number

Note: The MiSeq serial number can be found under the title "Instrument Name" in the About menu.

Description of the Issue

Type of Override Code

Please select...

GET CODE

- 6 To generate the code, select **Get Code**.
- 7 Return to the MCS interface and select **Enter Code**.
- 8 Enter the temporary bypass code using the on-screen keyboard, and then select **Next**.
- 9 Enter the barcode number of the flow cell, PR2 bottle, or reagent cartridge.

Consumable	Barcode Number Location
Flow Cell	Above the barcode on the flow cell container label. Flow cell barcode numbers begin with an A (standard), D (micro), or G (nano). Example: A0E61
PR2 Bottle	Below the barcode on the PR2 bottle label. Example: MS0011881-PR2
Reagent Cartridge	Below the barcode on the reagent cartridge label. Example: MS0010744-300

- 10 If you are entering a bypass code for the reagent cartridge, enter the version number of the kit: Version 1 or Version 2. Select **Enter Reagent Kit Barcode** to manually enter the reagent cartridge barcode number and kit version number.



CAUTION
Entering the incorrect reagent kit version can negatively impact sequencing data.

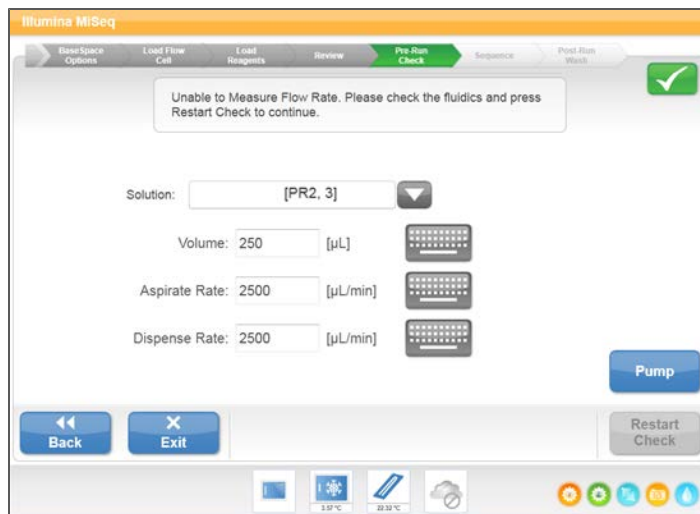
- 11 Select **Enter**.

Troubleshooting Flow Rate Error

The flow rate is the speed in which fluids pass through the fluidics system ($\mu\text{L}/\text{min}$). It is measured before each run during the pre-run check. If the system is unable to measure the flow rate, you are prompted to pump a volume of reagent (PR2) through the system before checking the flow rate again.

- 1 Use the drop-down list or on-screen keyboard to enter the following information:
 - Solution: **PR2**
 - Volume: **250 μL**
 - Aspirate Rate: **2500 $\mu\text{L}/\text{min}$**
 - Dispense Rate: **2500 $\mu\text{L}/\text{min}$**
- 2 Select **Pump**.

Figure 44 Measure Flow Rate



- 3 When the pump step is complete, select **Restart Check**.
- 4 If the error persists, set the volume to pump 500 μL PR2 and repeat the process one more time. Contact Illumina Technical Support if the second attempt does not resolve the error.

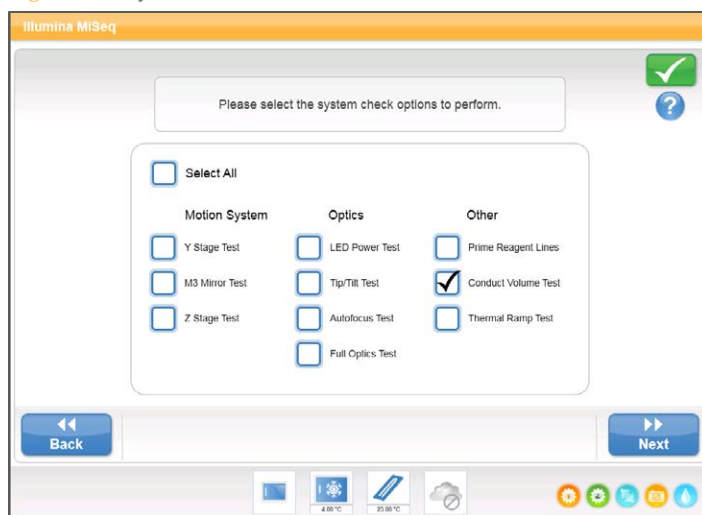
Performing a Volume Test

An obstruction in the fluidics lines might be the cause of poor reagent delivery or sequencing results. If an obstruction in the fluidics lines is suspected, perform a volume test.

A volume test checks the health of the fluidics system by estimating the volume between two bubbles as they pass by the sensors. To perform a volume test, the wash tray and wash bottle must be loaded with laboratory-grade water and a used flow cell must be in place. Follow the onscreen prompts to perform the test.

- 1 Make sure that a used flow cell is loaded on the instrument.
- 2 From the Manage Instrument screen, select **System Check**.
- 3 Select **Conduct Volume Test**, and then select **Next**.

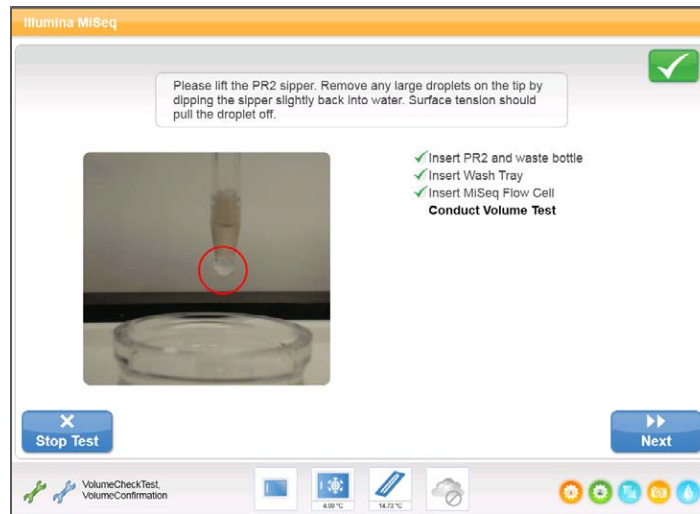
Figure 45 System Check Screen



- 4 Fill each reservoir of the wash tray with 6 ml laboratory-grade water.
- 5 Fill the 500 ml wash bottle with 350 ml laboratory-grade water.
- 6 Load the wash tray and wash bottle onto the instrument.

- a Open the reagent compartment door and reagent chiller door, and slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - b Raise the sipper handle until it locks into place, and load the wash bottle.
 - c Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
 - d Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
- 7 Following the on-screen prompts, remove any droplets from the wash bottle sipper, as follows:
- a When prompted, slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.
 - b When prompted, slowly lower the sipper handle far enough into the water to allow the surface tension to remove the droplet.
 - c When prompted, slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.
 - d When prompted, slowly lower the sipper handle completely, making sure that the sippers lower into the wash bottle and waste bottle.

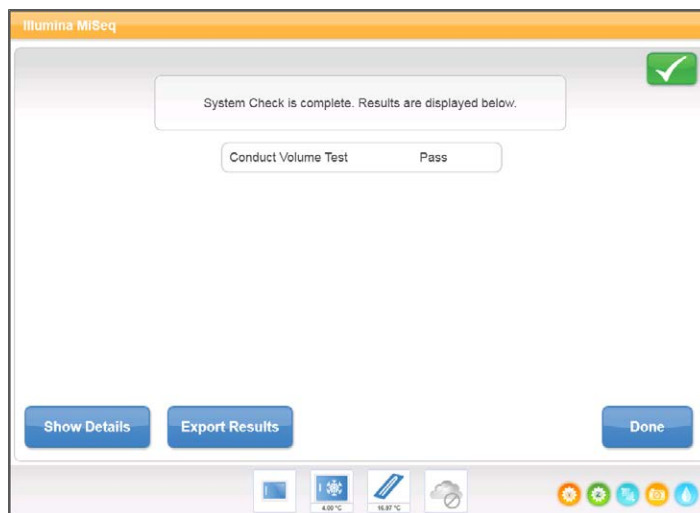
Figure 46 Remove Droplet from Sipper



- 8 Select **Next**. The volume test begins.

When the volume test is complete, the results appear on the screen.

Figure 47 Volume Test Results



If the test did not pass, perform a maintenance wash. See *Procedure* on page 97.

- 9 When the maintenance wash is complete, repeat the volume test.

Measuring Expected Wash Volumes

Measuring expected wash volumes confirms that wash fluidics are performing efficiently.

- 1 Before beginning a wash, empty the waste bottle.
- 2 When the wash is complete, measure the wash volume in the waste bottle.

Wash Type	Expected Wash Volume
Post Run Wash	17.25 ml
Standby Wash	46 ml
Maintenance Wash	17.25 ml

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 4 Illumina General Contact Information

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

Table 5 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

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at www.illumina.com/msds.

Product Documentation

Additional product documentation in PDF is available for download from the Illumina website. Go to www.illumina.com/support and select a product. A MyIllumina login is required. To register for a MyIllumina account, please visit my.illumina.com/Account/Register.

