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MiSeq® System User Guide

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October 2013

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Revision History

Part #	Revision	Date	Description of Change
15027617	L	October 2013	Added the reboot of system software as a pre-run step. Added microcentrifuge tubes to the list of user-supplied consumables. Eliminated *MiSeq Software* as a separate chapter and distributed chapter contents throughout the guide. Removed information about folders for custom recipes. Removed information about recommended cluster density ranges for MiSeq reagent kits. Removed details about MiSeq reagent kits and added an overview of reagent kit features. For detailed information, see the reagent prep documentation for the kit you are using. Added content to the trademarks notice.
15027617	K	August 2013	Corrected formatting errors.
15027617	J	August 2013	Added run descriptions for MCS v2.3 and MiSeq Reagent Kit v3. Updated the following information: • Reagent kit and version compatibility to include MiSeq Reagent Kit v3 • Custom Recipes folder description to include a v3 subfolder • Changed cluster density range for v2; added range for v3 • Output path for image files Corrected flow cell bar codes for nano flow cells (D) and micro flow cells (G). Removed information about MiSeq Reagent Kit, including contents and flow cell types. For more information, see *MiSeq Reagent Preparation Guide (part # 15044983)*.

Part #	Revision	Date	Description of Change
15027617	H	March 2013	Added section titled *MiSeq Concepts* that introduces the analysis workflow, manifest file, and sample sheet. Removed information about FASTQ file generation, manifest file formats, analysis workflow details, and sample sheet details. For information about these topics, see the *MiSeq Reporter User Guide*, part # 15028784, or the *MiSeq Sample Sheet Quick Reference Guide*, part # 15028392. Removed instructions for preparing custom primers. For more information, see *Using Custom Primers on the MiSeq*, part # 15041638.
15027617	G	January 2013	Removed instructions for denaturing and diluting DNA libraries and preparing an Illumina PhiX control. See *Preparing DNA Libraries for Sequencing on the MiSeq*, part # 15039740. Updated instrument wash instructions to add 25 ml 10% Tween 20 to 475 ml laboratory-grade water, instead of 500 ml laboratory-grade water.
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/mm^2 • Noted that the procedure for denaturing and diluting libraries, *Preparing Your Libraries*, 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 *MiSeq Reporter User Guide* to Additional Resources list.

Part #	Revision	Date	Description of Change
15027617	D	July 2012	Updated software descriptions to MCS v2.0. Added the following new information: • Added a section titled *What's New in MCS* 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 • 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 *MiSeq Sample Sheet Quick Reference Guide*, part # 15028392 and the *Illumina Experiment Manager User Guide*, part # 15031335)

Part #	Revision	Date	Description of Change
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 • 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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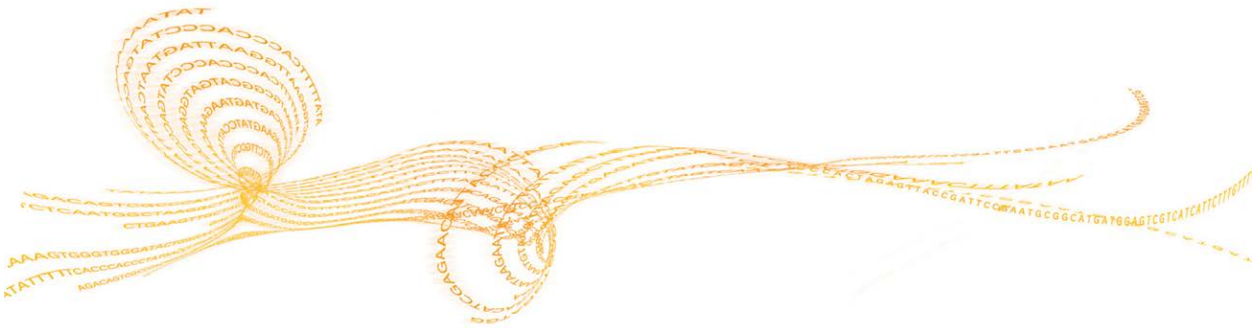
Getting Started

Chapter 1



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 8 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

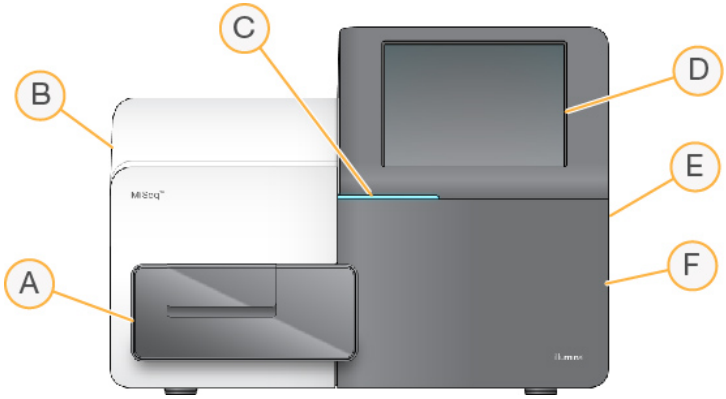
The following documentation is available for download from the Illumina website.

Resource	Description
MiSeq System Site Prep 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 Workflow Quick Reference Cards	Provides a two-page graphical representation of the workflow for the experienced user. The quick reference cards summarize sample prep, run setup, and run monitoring, as well as provide an overview of the analysis performed by MiSeq Reporter.
Illumina Experiment Manager User Guide	Provides instructions for creating sample plates and sample sheets for different workflows and library types. Illumina recommends that you create your sample sheet during the sample preparation step.
MiSeq Sample Sheet Quick Reference Guide	Provides information about adding sample sheet settings to your sample sheet.
MiSeq Reagent Prep Guide	Provides a description of kit contents and instructions for preparing the reagent cartridge before beginning your sequencing run.
Preparing DNA Libraries for Sequencing on the MiSeq	Provides instructions for denaturing and diluting prepared sample libraries before sequencing on the MiSeq, and preparing a PhiX control. This step applies to most library types.
Using Custom Primers on the MiSeq	Provides instructions for preparing and loading custom primers, and editing the samples sheet for custom primers.
MiSeq Reporter User Guide	Provides a comprehensive overview of analysis procedures, analysis workflows, and output files generated by MiSeq Reporter, as well as computing requirements, off-instrument installation instructions, and troubleshooting information.
MiSeq Reporter Online Help	Provides instructions for using the Illumina Experiment Manager software.
BaseSpace Online Help	Provides instructions for using BaseSpace and descriptions of the graphs generated for each analysis workflow.

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 support.illumina.com/sequencing/sequencing_instruments/miseq/training.ilmn.

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. Flow cell stage motors move the stage out of the enclosed optical module for flow cell loading and returns the stage 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 that the instrument is processing, orange indicates the instrument needs attention, and green indicates that the instrument is ready to begin the next run.

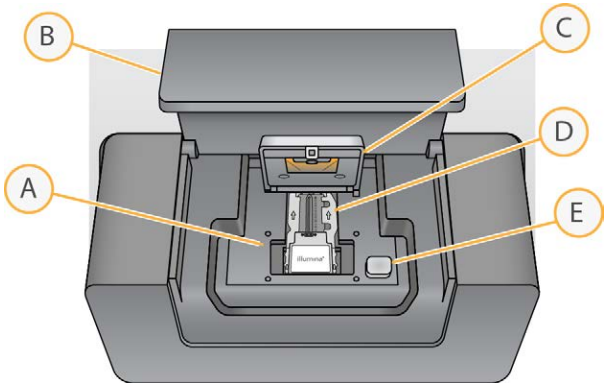
D **Touch screen monitor**—Enables on-instrument configuration and run setup using the software interface.

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. A magnetic latch secures the reagent compartment door.

The MiSeq interface guides you through the run setup steps using the touch screen monitor. Loading run components requires access to the reagent compartment and the flow cell compartment.

Flow Cell Compartment

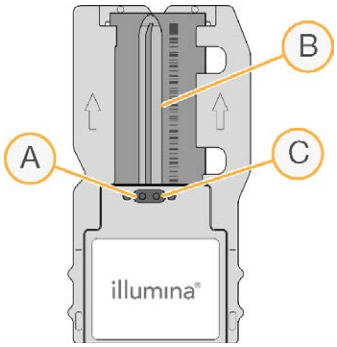


A Flow Cell Stage
B Flow Cell Compartment Door
C Flow Cell Latch
D Flow Cell
E Flow Cell Latch Release Button

The flow cell compartment houses the flow cell stage, thermal station, and fluidics connections to the flow cell. The flow cell stage holds the flow cell and the flow cell latch secures and positions the flow cell. 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

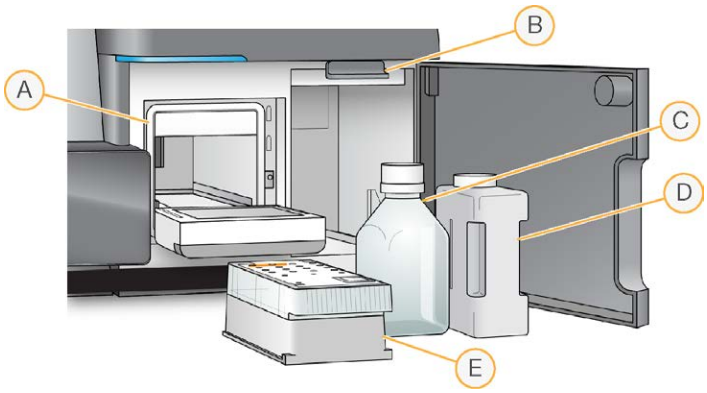
The MiSeq flow cell is a single-use glass-based substrate on which clusters are generated and the sequencing reaction is performed.

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 before setting up 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.

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.

During the run, the reagent chiller holds a single-use reagent cartridge. During the instrument wash, the reagent chiller holds the wash tray. 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. 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**.

MiSeq Reagent Kit Overview

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

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

Always use the reagent cartridge associated with your flow cell type. If the reagent cartridge is not compatible, a message appears during run setup that prompts you to load a compatible reagent cartridge.

For a description of available reagent kits, visit the Illumina website at support.illumina.com/sequencing/sequencing_kits/miseq_reagent_kit.ilmn.

For information about kit contents and requirements, see the reagent prep documentation for the kit you are using.

User-Supplied Consumables

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

Consumable	Supplier	Purpose
Stock 1.0 N NaOH, molecular biology-grade	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
Microcentrifuge tubes	General lab supplier	Denaturing and diluting sample libraries and PhiX control DNA
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:

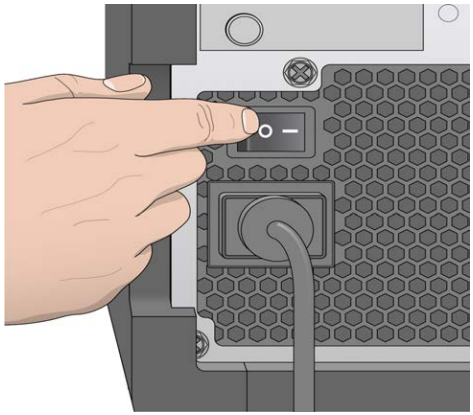
- Illumina PW1
- 18 Megohm (MΩ) water
- Milli-Q water
- Super-Q water
- Molecular biology-grade water

Starting the MiSeq

NOTE
Illumina recommends that you leave the instrument on continuously. However, if the instrument must be turned off follow the shutdown procedure described in *Shut Down the Instrument* on page 68. 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.

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 the initialization step is complete, the Welcome screen appears.

MiSeq Concepts

The following concepts and terms are common to the run setup steps on the MiSeq.

Concept	Description
Analysis Workflow	A secondary analysis procedure performed by MiSeq Reporter. The analysis workflow for each run is specified in the sample sheet.
Manifest	The file that specifies a reference genome and targeted reference regions to be used in the alignment step. For workflows that require a manifest, the manifest file is specified in the sample sheet and copied to the manifest folder designated in MCS.
Reference Genome	A FASTA format file that contains the genome sequences used during analysis. For most analysis workflows, the reference genome file is specified in the sample sheet.
Run Folder	The folder structure populated by RTA primary analysis software (MiSeqOutput folder) or the folder populated by MiSeq Reporter (MiSeqAnalysis). For more information, see *Run Folders* on page 27.
Sample Sheet	A comma-separated values file (*.csv) that contains information required to set up and analyze a sequencing run, including a list of samples and their index sequences. The sample sheet must be provided during the run setup steps on the MiSeq. After the run begins, the sample sheet is renamed to SampleSheet.csv and copied to the run folders: MiSeqTemp, MiSeqOutput, and MiSeqAnalysis.

For more information about analysis workflows and manifest file formats, see the *MiSeq Reporter User Guide (part # 15042295)*.

For more information about sample sheets, see the *MiSeq Sample Sheet Quick Reference Guide (part # 15028392)*.

MiSeq Software

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

- **MiSeq Control Software (MCS)**—Controls instrument operation. The MiSeq Control Software (MCS) interface guides you through the steps to load the flow cell and reagents before beginning the run. An overview of quality statistics appears as the run progresses.

  During the run, MCS operates the flow cell stage, dispenses reagents, controls flow cell temperatures, and captures images of clusters on the flow cell. MCS performs the run according to parameters specified in the sample sheet.
- **Real-time analysis (RTA) software**—Performs primary analysis. Real-time analysis (RTA) is an integrated primary analysis software that 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**—Performs secondary analysis. The MiSeq Reporter analysis software processes base calls generated during primary analysis, and produces information about alignment, variants, and contig assemblies for each genome requested. The analysis workflow specified in the sample sheet determines the type of analysis performed. For more information, see *MiSeq Reporter Overview* on page 25.

Optional software used off-instrument includes the Sequencing Analysis Viewer (SAV). For more information, see *Sequencing Analysis Viewer* on page 23.

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 16.
- **Perform Wash**—Provides options to start two types of instrument washes, either a maintenance wash or standby wash. See *Instrument Washes* on page 57.
- **Manage Files**—Provides controls for moving, deleting, and uploading files on the instrument computer. See *Manage Files Screen* on page 17.
- **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 19.
- **Manage Instrument**—Provides options to go to system settings, perform a systems check, update software manually, and reboot or shut down the instrument. See *Manage Instrument Screen* on page 22.
- **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 67.

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

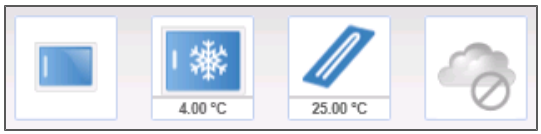
From left to right, the activity indicators represent the following activities:

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

Sensor Indicators

Four sensor indicators at the base of each interface screen represent 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 in the closed or open positions
- 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	Important information. Action is recommended.
	Warning	Warnings do not stop a run. However, some warnings 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. Select the icon to open the status window and view a 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.

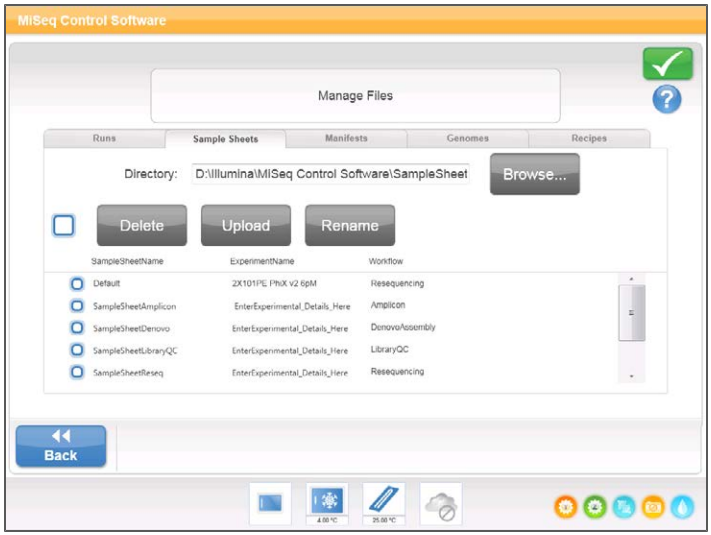
Run Setup Screens

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. For more information, see *Set Up a Run Using MCS* on page 38

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 5 Manage Files Screen



Manage Files Options

From any tab on the Manage Files screen, select **Browse** to navigate to any files accessible to the instrument.

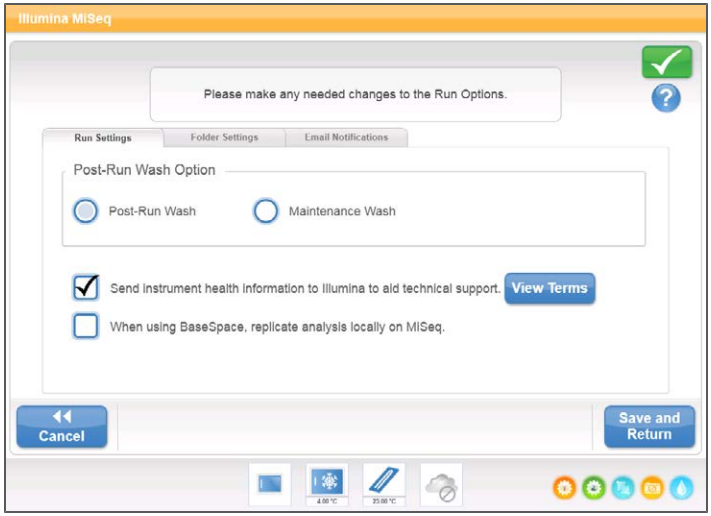
Tab	Features
Runs	Delete or Move
Sample Sheets	Delete, Upload, or Rename
Manifests	Delete or Upload
Genomes	Delete or Upload
Recipes	Delete or Upload

- **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** *copies* 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 6 Run Settings Tab on Run Options Screen



Run Settings Tab

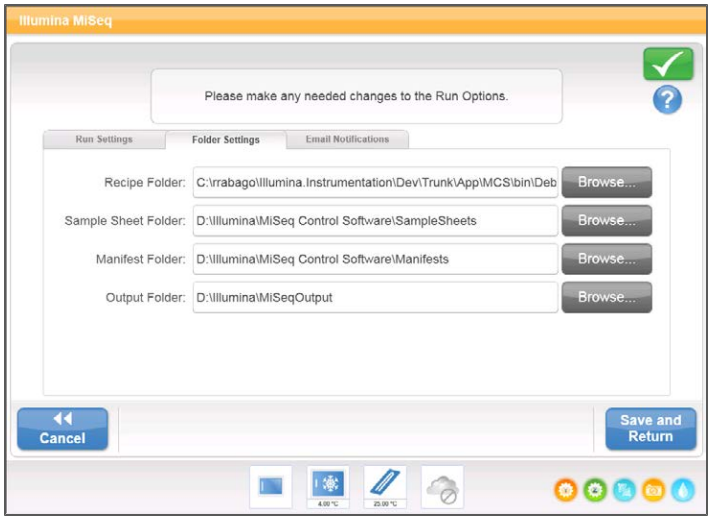
- **Post-Run Wash Option**—An instrument wash is required after each run. The software requires that a wash is performed before setting up a subsequent run. You can specify which type of wash is performed by default. A post-run wash takes about 20 minutes and a maintenance wash takes about 1 hour.
- **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 select this option when using BaseSpace, MiSeq Reporter launches automatically after the run and performs analysis locally.

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

Folder Settings Tab

You can specify the default folder locations on the Folder Settings tab. Folders can be on a local network or on the instrument computer.

Figure 7 Folder Settings Tab



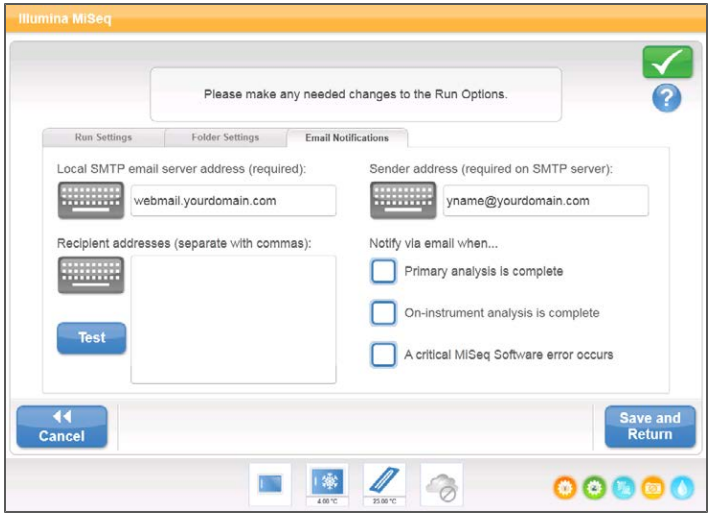
- **Recipes**—Sets the default location for recipes. Recipes are XML files that the software uses to perform the sequencing run. A recipe is created at the start of the run based on parameters in the sample sheet, and then the recipe is copied to the output folder.
- **Sample Sheets**—Sets the default location for sample sheets. Sample sheets are created before library preparation and contain parameters for the run.
- **Manifests**—Sets the default location for manifest files. Manifest files are required for some library types. See the sample prep documentation for your sample prep kit, as well as the *Sample Sheet Quick Reference Guide (part # 15028392)*.
- **MiSeqOutput**—Sets the default location for analysis output files. Illumina recommends changing the default output folder to a network location for sharing, long-term storage, and optionally using MiSeq Reporter off-line.

  For more information, see *Run Folders* on page 27.

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 8 Email Notifications Tab

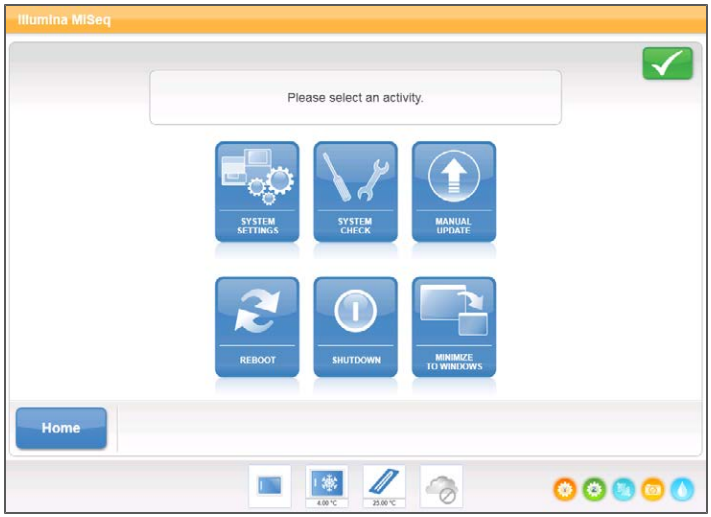


- **Local SMTP email server address**—Use the on-screen keyboard to enter the local SMTP email server address. If necessary, contact the facility administrator for this information.
- **Sender email address**—Use the on-screen keyboard to enter the sender email address. This address can be your email 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, manual software updates, rebooting or shutting down the instrument software.

Figure 9 Manage Instrument Screen



- **System Settings**—Provides the option to change IP Settings, machine name, or domain. See *System Settings Screen* on page 71.
- **System Check**—Provides troubleshooting options for checking the operational status of instrument components. See *System Check Screen* on page 73.
- **Manual Update**—Provides the option to update software on the instrument computer manually. See *Manual Update Screen* on page 67.
- **Reboot**—Use the Reboot command to reboot the system software.
- **Shut Down**—Use the Shut Down command to shut down the control software and Windows on the instrument computer. See *Shut Down the Instrument* on page 68.
- **Minimize to Windows**—Provides quick access to the instrument operating system and any folders on the instrument computer when MCS is running in kiosk mode opposed to Windows mode.

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. Install SAV onto a computer independent of the MiSeq with access to the same network connected to the instrument. After launching the software, browse to the output folder for your run.

After 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)*.

Run Duration

Run duration depends on the number of cycles that you perform. You can perform a paired-end run up to 2 x 301 sequencing cycles plus any Index Reads with MCS v2.3.

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 300-cycle run performs two 301-cycle reads (2 x 301) for a total of 602 cycles. At the end of the run, 2 x 300 cycles are analyzed.

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.

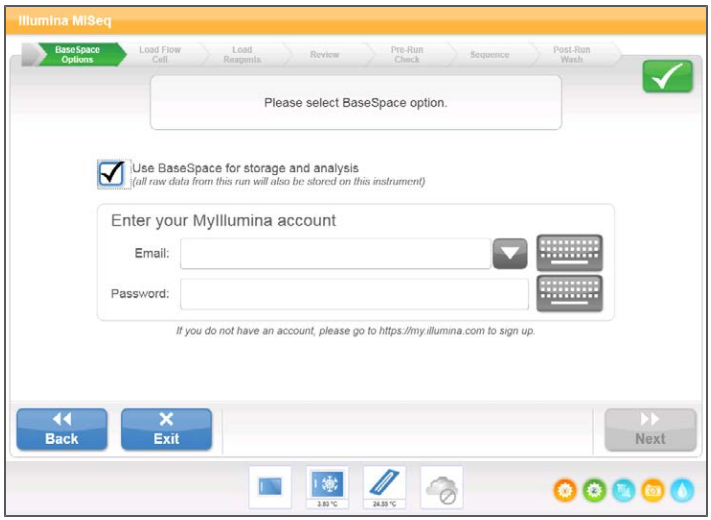
BaseSpace Overview

BaseSpace is the Illumina cloud computing 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
- Allows a new sequencing run to be started while data are being analyzed
- Provides tools for global collaboration and sharing

You can log in to BaseSpace when you set up the sequencing run. When using BaseSpace, you can set up your run so that raw data from the run is also stored locally. For more information, see *Run Options Screen* on page 19.

Figure 10 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 11 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.

Several genomes are provided with MiSeq Reporter installation. BaseSpace only supports genomes included with MiSeq Reporter.

You can connect to BaseSpace at basespace.com. Log in using your MyIllumina account login.

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 # 15042295)*.

Sequencing During Analysis

The MiSeq system computing resources are dedicated to either sequencing or 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.

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

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 MiSeqTemp folder. Contents of this folder are deleted after 7 days.
- **D:\Illumina\MiSeqOutput**—RTA copies files from the MiSeqTemp folder to the MiSeqOutput folder. As primary analysis files are generated, RTA copies files back to the MiSeqTemp folder and populates the MiSeqAnalysis folder. Focus images and thumbnail images are not copied to the MiSeqAnalysis 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 19.
- **D:\Illumina\MiSeqAnalysis**—When primary analysis is complete, MiSeq Reporter accesses the MiSeqAnalysis folder on the instrument local drive to begin secondary analysis. All files written to the MiSeqAnalysis folder are copied back to the MiSeqOutput folder. For more information, see *MiSeqOutput Folder Contents* on page 27.
  If you are using BaseSpace for analysis without replicating analysis locally, the MiSeqAnalysis 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.

MiSeqOutput Folder Contents

After primary analysis, the MiSeqOutput folder is populated with files necessary for secondary analysis by MiSeq Reporter. When secondary analysis is complete, the MiSeqOutput and MiSeqAnalysis folders are identical with one exception that the MiSeqOutput folder contains two subfolders for images files: Images and Thumbnail_Images. These subfolders are not required for secondary analysis.

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. Copies are written to 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 a read is indexed.

Folders

The folders that are copied to the output and analysis folders include the following folders generated during the sequencing run:

- **<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 files, and phasing files. MiSeq Reporter writes FASTQ files to this folder during secondary analysis. For more information, see the *MiSeq Reporter User Guide (part # 15042295)*.
- **<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.

MiSeq Reporter adds other folders, such as the Alignment folder, during secondary analysis. For more information, see the *MiSeq Reporter User Guide (part # 15042295).*

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 17. After clearing adequate disk space, select **Restart Check**.

Anti-Virus Software

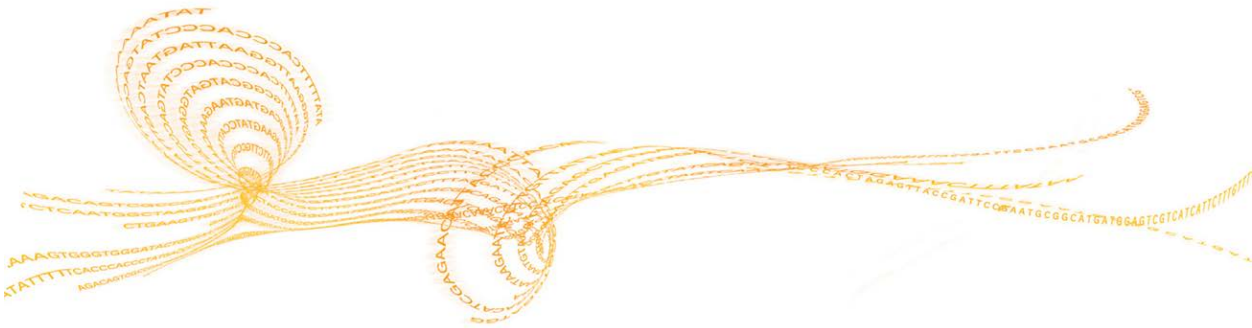
Illumina strongly recommends that you purchase and install the anti-virus software of your choice to protect the computer against viruses.

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.

Performing a Run

Chapter 2



Introduction

To perform a run on the MiSeq, follow the setup steps described in this chapter. After the run begins, 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 sequencing run is complete, perform an instrument wash.

MiSeq Workflow

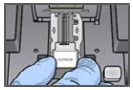
Denature and dilute libraries (does not apply to all library types). See *Preparing Libraries for Sequencing on the MiSeq (part # 15039740)*.

Prepare the pre-filled reagent cartridge for use. See *MiSeq Reagent Preparation Guide (part # 15044983)*.

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

From the software interface, select **Sequence** to start the run setup 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 MiSeq Reporter 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 25.

Load Sample Libraries

NOTE
If necessary for your library type, denature and dilute libraries, and add optional PhiX control. See *Preparing Libraries for Sequencing on the MiSeq (part # 15039740)*.

This step does not apply to all library types. Some Illumina sample preparation methods result in a ready-to-use normalized concentration of pooled libraries. Refer to the sample preparation guide for the kit used to prepare sample libraries.

NOTE
If you are using custom primers, prepare primers and set up the sample sheet as described in *Using Custom Primers on the MiSeq (part # 15041638)*.

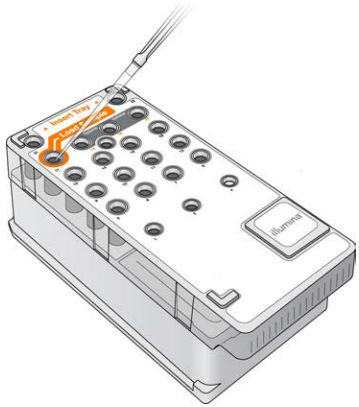
When the reagent cartridge is fully thawed and ready for use, you are ready to load prepared libraries onto the cartridge.

1 Use a clean 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 sequencing run.

2 Pipette 600 µl of prepared libraries into the **Load Samples** reservoir. Avoid touching the foil seal as you dispense the sample.

Figure 12 Load Libraries



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

Set Up a Run Using MCS

1. From the Welcome screen, select **Manage Instrument**.
2. From the Manage Instrument screen, select **Reboot** to reboot the system software.
3. [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 19.
4. From the Welcome screen, select **Sequence** to begin the run setup steps. The BaseSpace Options screen opens.
   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.

Set BaseSpace Option

To enable access to BaseSpace, 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**. Analysis is performed in BaseSpace.
   b. Clear the checkbox **Use BaseSpace for storage and analysis**. Analysis is performed on the instrument.
2. Select **Next**. The Load Flow Cell screen opens.

Clean the Flow Cell

The flow cell is immersed in storage buffer in a flow cell container.

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

- The standard flow cell container cap is clear.
- The micro flow cell container cap is green.
- The nano flow cell container cap is yellow.

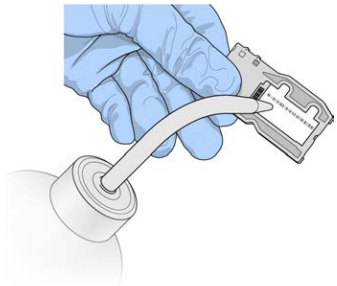
1 Put on a new pair of powder-free gloves.

2 Using plastic forceps, grip the flow cell by the base of the plastic cartridge and remove it from the flow cell container.

Figure 13 Remove Flow Cell

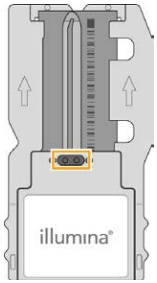
3 Lightly rinse the flow cell with laboratory-grade water, making sure that both the glass and plastic cartridge are thoroughly rinsed of excess salts. Excess salts can affect flow cell seating on the instrument. If salts dry in the imaging area, imaging can also be affected.

Figure 14 Rinse Flow Cell



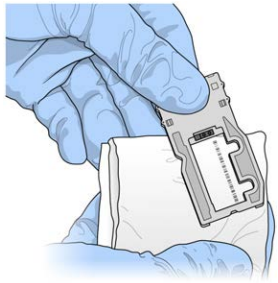
4 Using care around the black flow cell port gasket, thoroughly dry the flow cell and cartridge using a lint-free lens cleaning tissue. Gently pat dry in the area of the gasket and adjacent glass.

Figure 15 Flow Cell Ports and Gasket



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

Figure 16 Dry Flow Cell



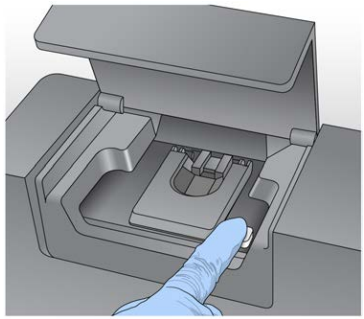
6 Dry any excess alcohol with a lint-free lens cleaning tissue. Visually inspect to make sure that the flow cell ports are free of obstructions and that the gasket is well-seated around the flow cell ports.

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

Load the Flow Cell

The Load Flow Cell screen prompts you to load the flow cell.

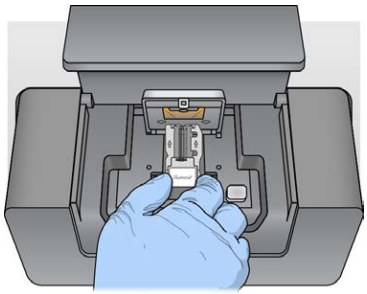
Figure 17 Open Flow Cell Latch



7 Visually inspect the flow cell stage to make sure that 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.

8 Holding the flow cell by the edges of the flow cell cartridge, place the flow cell on the flow cell stage.

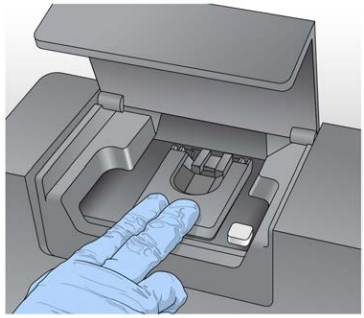
Figure 18 Place Flow Cell on Stage



9 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 that the flow cell latch is secure.

Figure 19 Close Flow Cell Latch



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

NOTE
If the RFID cannot be read, the software prompts you through the steps to obtain a temporary bypass code and proceed with setting up the run. For more information, see *Resolve RFID Read Failure* on page 79.

11 Close the flow cell compartment door.

12 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.

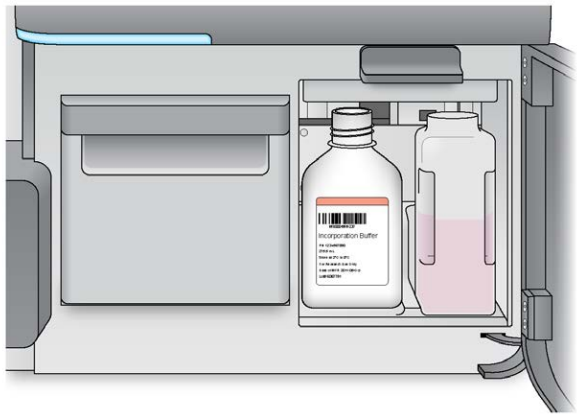
NOTE

Always 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.

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.

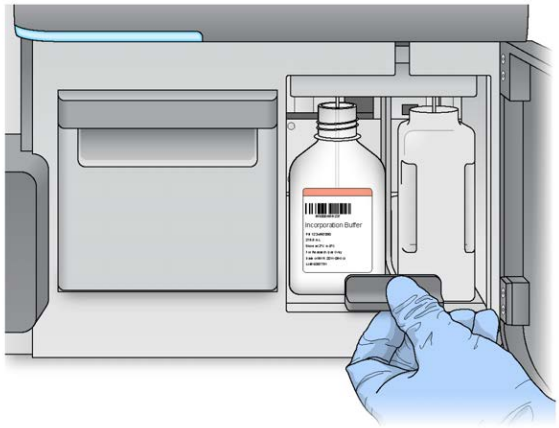
Figure 20 Load the PR2 Bottle



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 21 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 cannot be read, the software prompts you through the steps to obtain a temporary bypass code and proceed with setting up the run. For more information, see *Resolve RFID Read Failure* on page 79.

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

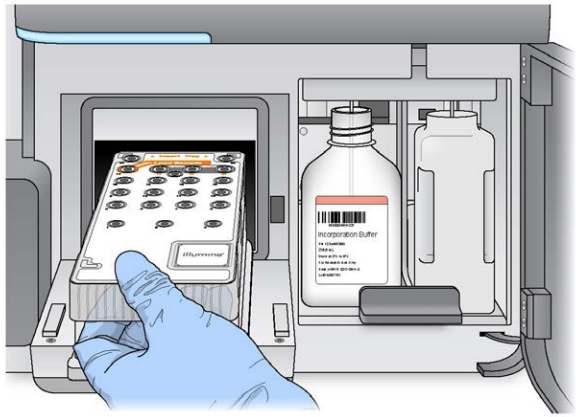
Load the Reagent Cartridge

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 22 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 cannot be read, the software prompts you through the steps to obtain a temporary bypass code and proceed with setting up the run. For more information, see *Resolve RFID Read Failure* on page 79.

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.

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.

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

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.

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.

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 *Resolve Run Setup Errors* on page 77.

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

Important Note Before Starting the Run

WARNING

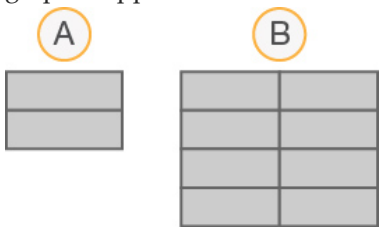
The MiSeq is sensitive to vibration. Touching the instrument after starting a run could adversely affect sequencing results.

After selecting **Start Run**, do not open the flow cell compartment or the reagent compartment doors, or touch the instrument monitor except to pause the run. For more information, see *Pause a Run* on page 75.

Monitor the Run

1 During the run, monitor run progress, intensities, and quality scores appear on the Sequencing screen. The Sequencing screen is view-only.
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.
Alternatively, if you are connected to BaseSpace, you can monitor your run using SAV in BaseSpace.

- **Run Progress**—Shows run progress in a status bar and lists the number of cycles completed.
- **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 Indicates two tiles, top surface only
B Indicates 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.
- **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. If more than one base call has a chastity value of less than 0.6 in the first 25 cycles, reads do not pass the quality filter.

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

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 you select Next, 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.
  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.
  Alternatively, if you are connected to BaseSpace, you can monitor your run using SAV in BaseSpace.

Template Generation

Real-time analysis (RTA) uses early cycles of the 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 is generated, images produced over every subsequent cycle of imaging are aligned against the template. Individual cluster intensities in all four nucleotide color channels are extracted and base calls are produced from the normalized cluster intensities.

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).

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 *Flow Cell Tile Numbering* on page 53.
*.locs files	Data\Intensities\BaseCalls\L001	Reports the cluster coordinates. Each *.locs file represents one 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.

The standard v3 flow cell is imaged in 19 tiles on the top surface and 19 tiles on the bottom surface, which results in the following tile numbering format:

- Image files named 1101 through 1119 are tiles 1–19 on the top surface.
- Image files named 2101 through 2119 are tiles 1–19 on the bottom surface.

The standard v2 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:

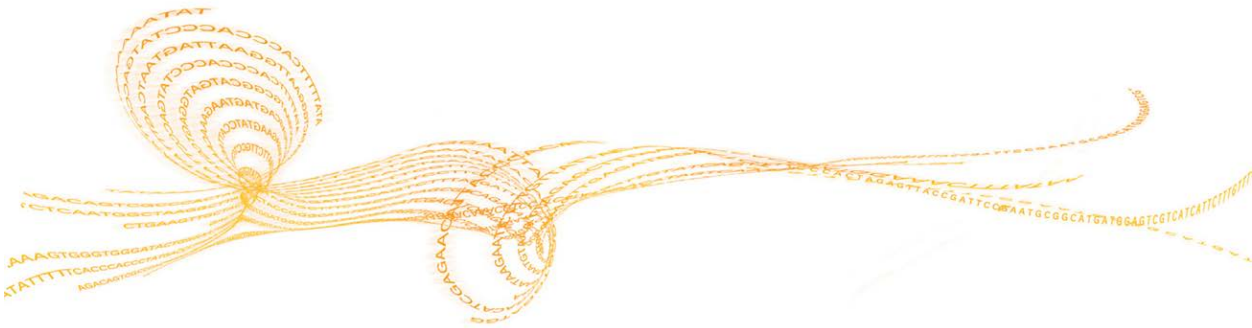
- Image files named 1101 through 1104 are tiles 1–4 on the top surface.
- Image files named 2101 through 2104 are tiles 1–4 on the bottom surface.

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\BaseCalls\L001.

Maintenance Procedures

Chapter 3



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

Perform the following maintenance procedures at the recommended intervals.

Table 1 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 2 Maintenance During Idle Mode (≥ 7 days unused)

Activity	Frequency
Standby Wash	Monthly
Instrument Shutdown	As needed

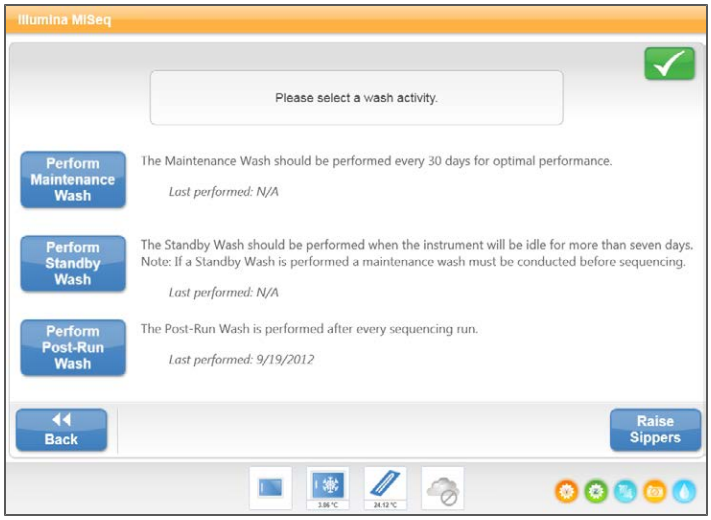
Instrument Washes

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 *Perform a Maintenance Wash* on page 61.
- **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 you expect the instrument to remain idle for up to 7 days. See *Perform a Standby Wash* on page 64. When the instrument is placed in an idle state, a maintenance wash is required before setting up 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. 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 19.

Figure 23 Perform Wash Screen



Select **Raise Sippers** to raise the reagent cartridge sippers manually so the cartridge can be removed from the instrument. Use this command if the run was interrupted unexpectedly or if an error occurred during the run. Under these conditions, the sippers do not raise automatically.

Perform 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. An instrument wash is required before you can set up a subsequent 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. These volumes result in 10% Tween 20.
   b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
   c. Invert five 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 slide the used reagent cartridge 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
A component in 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 remain in the down position, which 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.

Perform a Maintenance Wash

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

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. These volumes result in 10% Tween 20.
   b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
   c. Invert five 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 slide the used reagent cartridge or wash tray 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. These volumes result in 10% Tween 20.

b Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.

c Invert five 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 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. These volumes result in 10% Tween 20.
   b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
   c. Invert five 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 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 remain in the down position, which 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.

Perform a Standby Wash

If there are no plans to use the instrument 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. These volumes result in 10% Tween 20.
   b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
   c. Invert five 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 slide the used reagent cartridge or wash tray 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 sipers 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. These volumes result in 10% Tween 20.
b Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
c Invert five 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 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 remain in the down position, which 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.

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.

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.

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 navigate 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**.

If your instrument is connected to a network, you can update your software automatically. For more information, see *Software Updates* on page 67.

Shut Down the Instrument

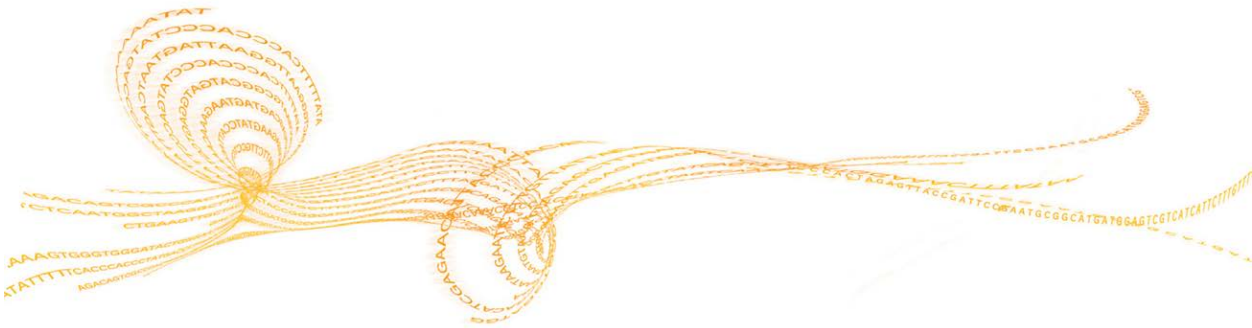
It is best to leave the instrument on at all times. However, if the instrument must be turned off, use the following procedure to shut down Windows and prepare the fluidics lines.

1. Perform a maintenance wash. For more information, see *Perform a Maintenance Wash* on page 61.
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 shuts down the software.
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

Chapter 4



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 91.

Illumina Technical Support representative typically request copies of run-specific files for troubleshooting purposes. You can find the following files at the root level of the MiSeqOutput folder:

- SampleSheet.csv
- RunParameters.xml
- RunInfo.xml
- InterOp folder

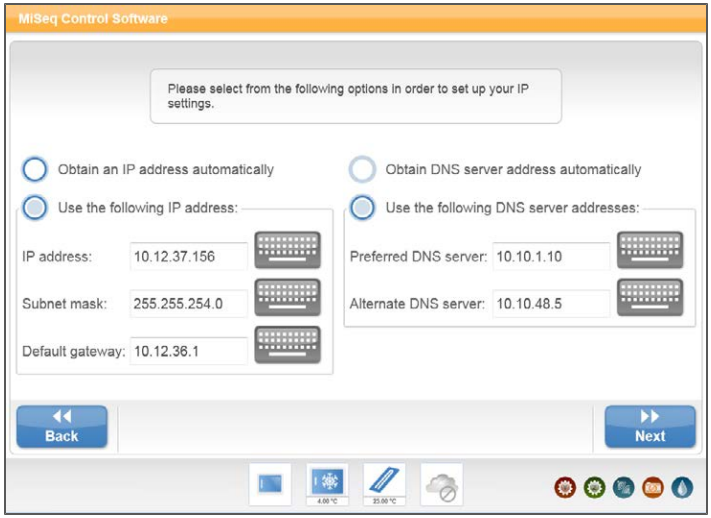
Software Settings

The MCS includes several screens that access commands to configure the system and manage the instrument.

System Settings Screen

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

Figure 24 System Settings



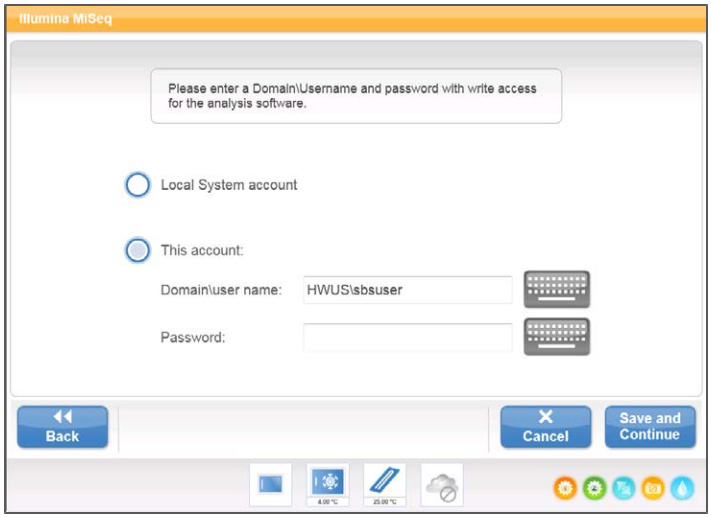
Contact the facility administrator to get information about what network settings to enter.

Changing System Credentials

Change the system user name and password 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**. Enter the domain name (Domain\MiSeq1, for example) and password. Select **Save and Continue**. The credentials for MiSeq Reporter and BaseSpace are also updated.

Figure 25 System Settings



System Check Screen

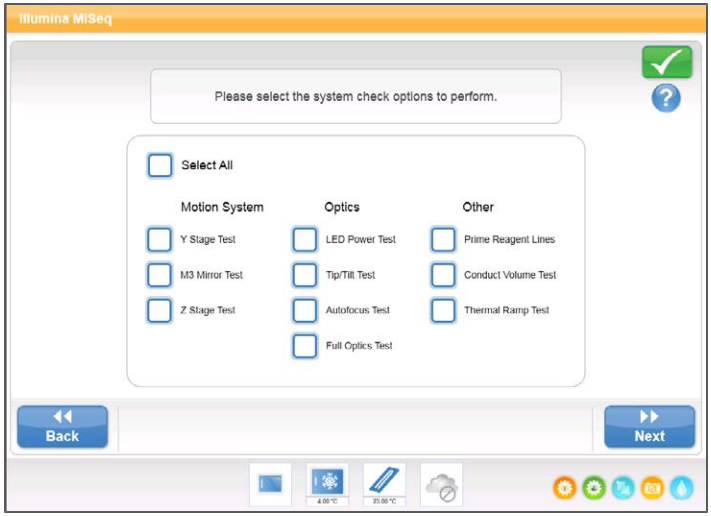
The System Check screen is typically used to connect with an Illumina Technical Support representative during a Live Help session. Use of this feature is not required during normal operation or for instrument maintenance.

Some system checks can be performed before 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. For more information, see *Perform a Volume Test* on page 82.

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 file format to a USB drive.

Figure 26 System Check Options



Live Help

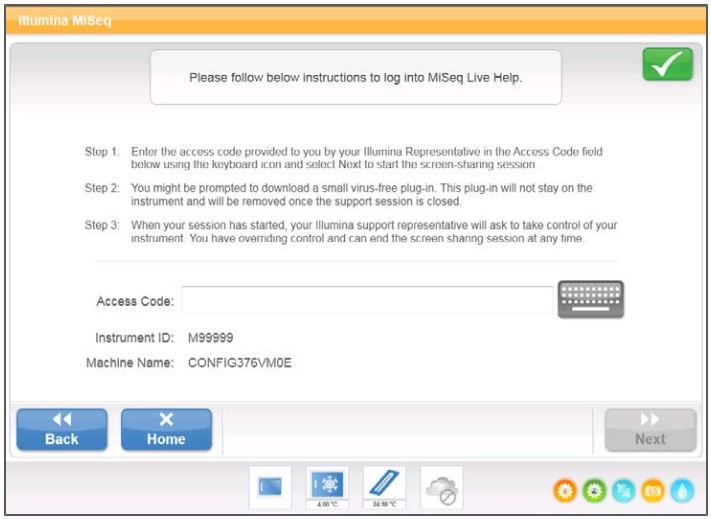
The MiSeq must be connected to a network with internet access to enable Live Help. The Live Help feature is an online assistance tool that enables a representative from Illumina Technical Support to view the MiSeq screen with your permission, and share control of the instrument. You have overriding control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

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

Figure 27 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 28 Live Help Screen



Pause or Stop 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.

Pause a Run

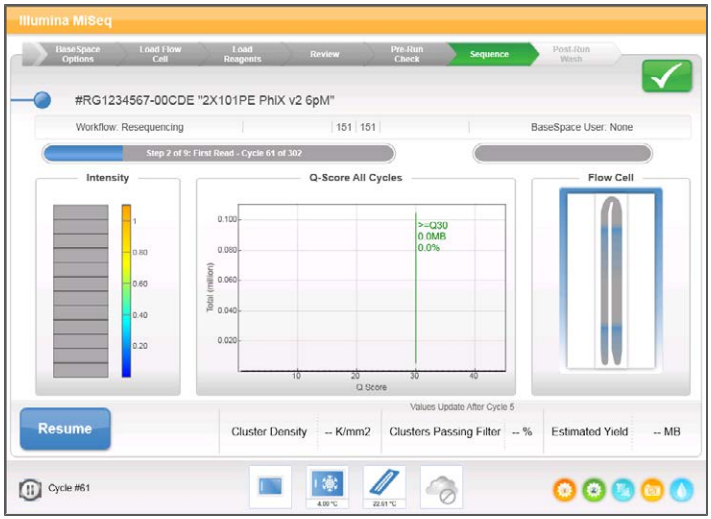
You can temporarily pause a run before it has completed. You might pause a run if you suspect that the waste bottle is full, for example. You can resume a paused run.

CAUTION
Do ***not*** pause a run during cluster generation or within the first five cycles of sequencing. It is not possible to resume a run that was paused during this time.

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. When you are ready to resume the run, select **Resume**.

Figure 29 Sequence Screen of a Paused Run

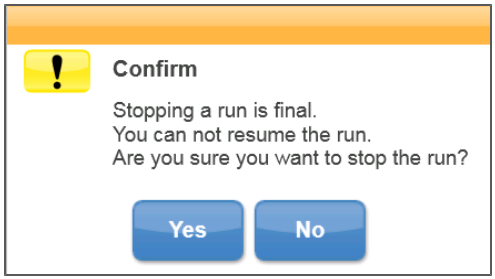


Stop a Run

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 continues for the last completed cycle.

Figure 30 Stopping a Run



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

Resolve Run Setup Errors

If any checks in the pre-run check fail, a red icon ✗ appears next to the item. A message appears on the screen that describes the error and how 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, reseat the flow cell to make sure that flow is not interrupted due to misalignment. Inspect the flow cell gasket for lint or irregularities.
✗ **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 that 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**. Secondary analysis stops for any incomplete cycles.

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**.

Resolve RFID Read Failure

If 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 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 31 MiSeq Self-Service Page

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), G (micro), or D (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. Select **Enter Reagent Kit Barcode** to enter the reagent cartridge barcode number and kit version number manually.

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

11 Select **Enter**.

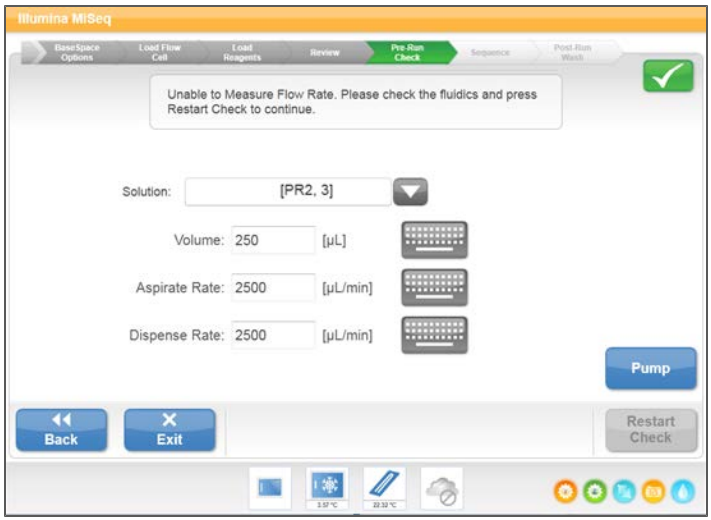
Troubleshoot Flow Rate Error

The flow rate is the speed in which fluids pass through the fluidics system (µl/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 µl/min**
- Dispense Rate: **2500 µl/min**

2 Select **Pump**.

Figure 32 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.

Perform a Volume Test

An obstruction in the fluidics lines can cause poor reagent delivery and affect sequencing results. If an obstruction in the fluidics lines is suspected, perform a volume test.

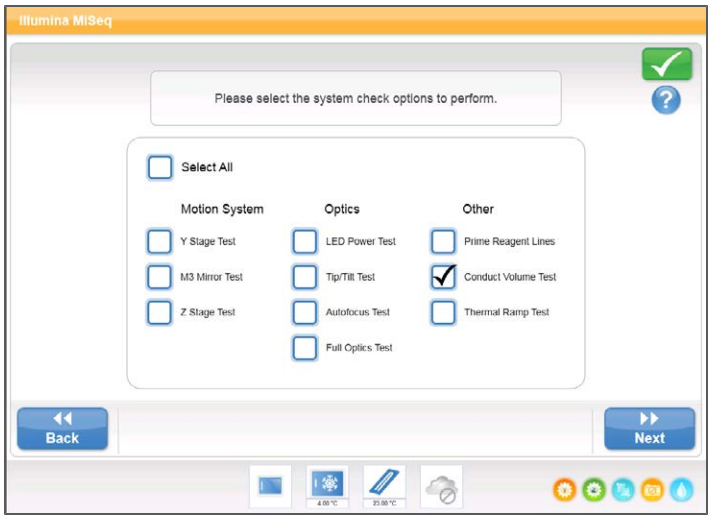
A volume test checks the health of the fluidics system by estimating the volume between two bubbles as they pass by the sensors. To perform a volume test, the wash tray and wash bottle must be loaded with laboratory-grade water and a used flow cell must be in place. Follow the onscreen prompts to perform 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 33 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:

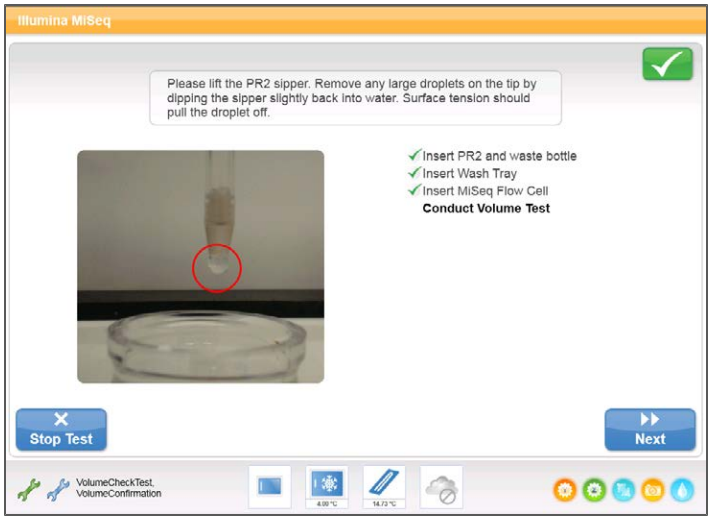
a When prompted, slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.

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.

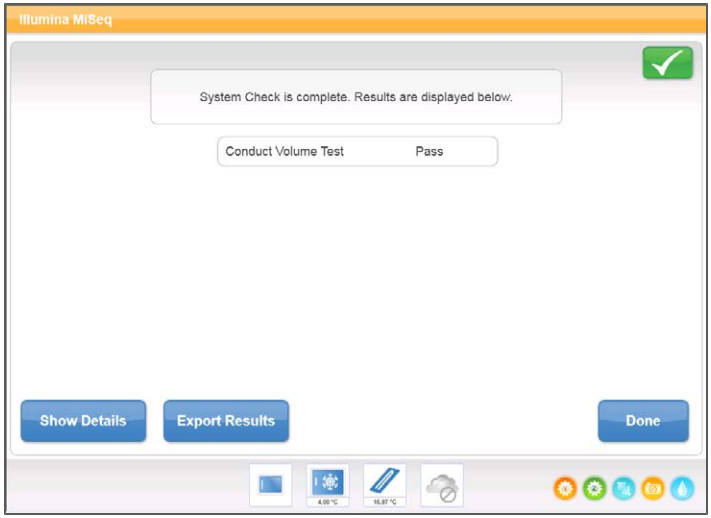
Figure 34 Remove Droplet from Sipper



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

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

Figure 35 Volume Test Results



If the test did not pass, perform a maintenance wash. See *Perform a Maintenance Wash* on page 61.

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

Measure 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 3 Illumina General Contact Information

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

Table 4 Illumina Customer Support Telephone Numbers

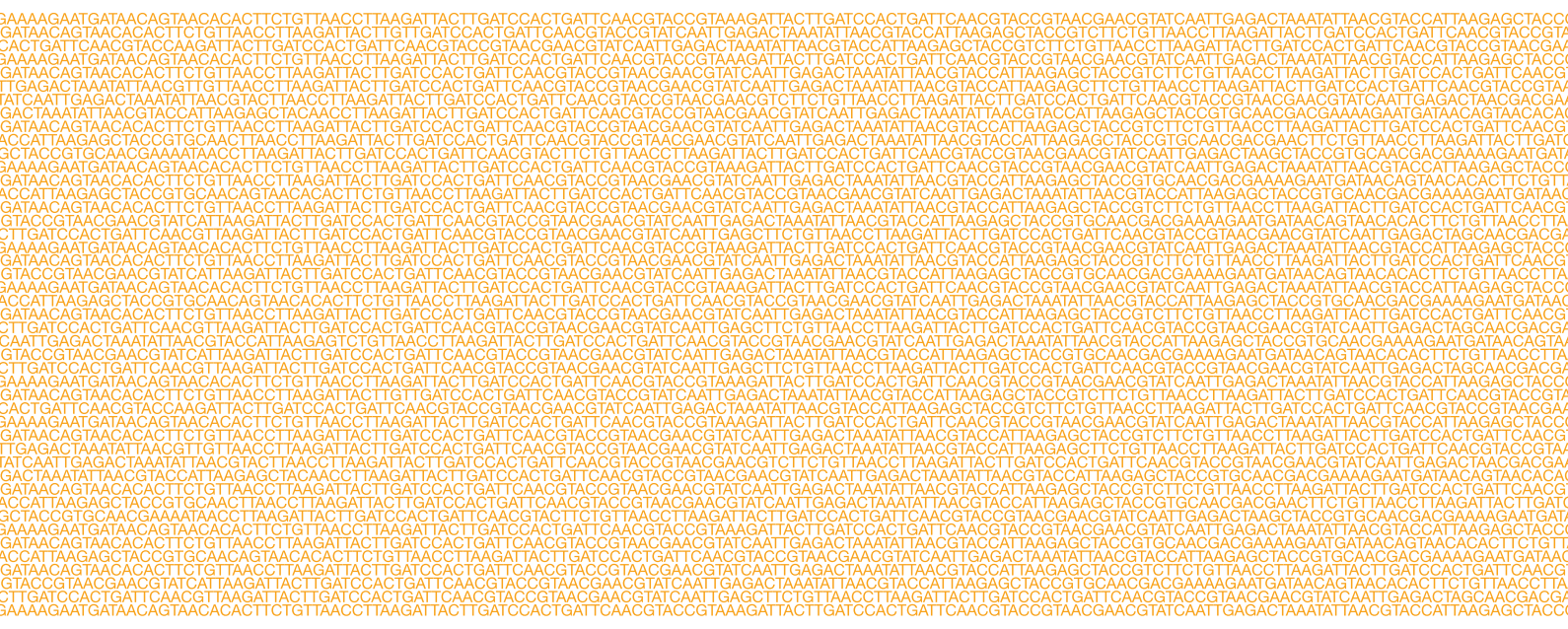
Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Austria	0800.296575	Netherlands	0800.0223859
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	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

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



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