

MiSeq

System Guide



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Introduction

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

Features

- ▶ **Walk away 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.
- ▶ **Prefilled reagent cartridge**—A specially designed single-use prefilled 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 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.
- ▶ **Integrated secondary analysis software**— MiSeq Reporter software processes data from analysis by RTA to perform alignment and provides information about each sample analyzed.

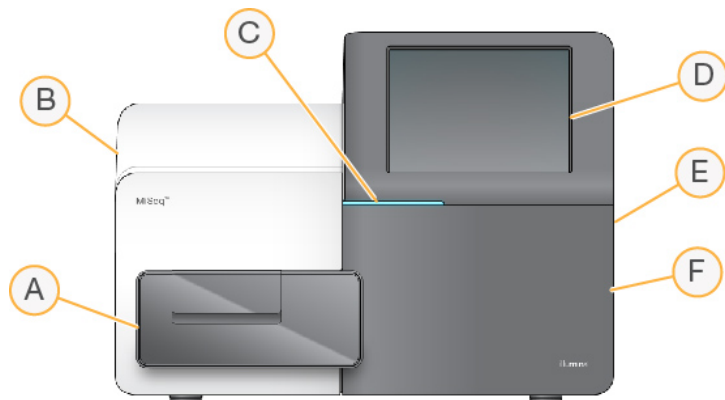
Additional Resources

The [MiSeq system support pages](#) on the Illumina website provide additional resources. These resources include software, training, compatible products, and the following documentation. Always check support pages for the latest versions.

Resource	Description
<i>MiSeq System Site Prep Guide (document # 15027615)</i>	Provides specifications for laboratory space, electrical requirements, and environmental considerations.
<i>MiSeq System Safety and Compliance Guide (document # 15027616)</i>	Provides information about instrument labeling, compliance certifications, and safety considerations.
<i>Illumina Experiment Manager User Guide (document # 15031335)</i>	Provides instructions for creating sample plates and sample sheets for different workflows and library types.
<i>BlueFuse Workflow Manager User Guide (document # 1000000028842)</i>	Provides instructions for creating sample plates and sample sheets for use with the VeriSeq PGS workflow.
<i>MiSeq Sample Sheet Quick Reference Guide (document # 15028392)</i>	Provides information about adding sample sheet settings to your sample sheet.
<i>MiSeq System Denature and Dilute Libraries Guide (document # 15039740)</i>	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.
<i>MiSeq Custom Primers Guide (document # 15041638)</i>	Provides instructions for preparing and loading custom primers, and editing the samples sheet for custom primers.
<i>MiSeq Reporter User Guide (document # 15042295)</i>	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.
<i>MiSeq Reporter Online Help</i>	Provides instructions for using the MiSeq Reporter software.
<i>BlueFuse Multi Software Guide (document # 15053620)</i>	Provides a comprehensive overview of analysis procedures, analysis workflows, and files generated by BlueFuse Multi, as well as computing requirements, and troubleshooting information. Use this guide with the VeriSeq PGS workflow.
<i>BaseSpace User Guide (document # 15044182)</i>	Provides instructions for using BaseSpace and descriptions of the graphs generated for each analysis workflow.
<i>BaseSpace Onsite System Guide (document # 15049148)</i>	Provides instructions for using the BaseSpace Onsite System.

Components

The MiSeq comprises a touch screen monitor, a status bar, a power button with adjacent USB ports, and three compartments.



- 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 compartment**—Contains optical components that enable imaging of the flow cell.
- C **Status bar**—Indicates flow cell status as ready to sequence (green), processing (blue), or needs attention (orange).
- D **Touch screen monitor**—Displays the control software interface for system configuration and run setup.
- E **External USB ports**—Facilitates the transfer of files and data to the instrument computer from the touch screen monitor.
- F **Reagent compartment**—Contains reagents at proper temperatures, wash solutions, and a bottle for used reagents. A magnetic latch secures the reagent compartment door.

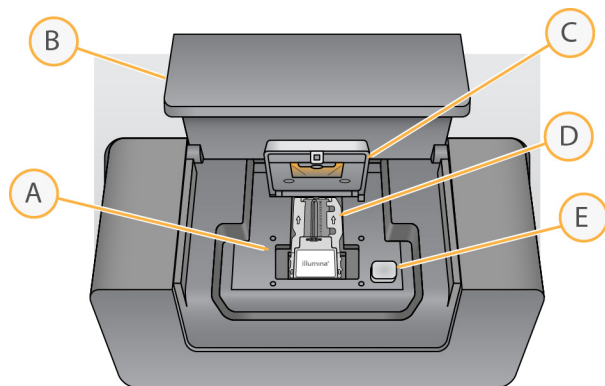
The MiSeq interface guides you through the run setup steps using the touch screen monitor. Loading run components requires access to the reagent compartment and the flow cell compartment.

Flow Cell Compartment

The flow cell compartment contains 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 clamp secures and positions the flow cell. When the flow cell clamp closes, two pins near the clamp 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.

Figure 1 Flow Cell Compartment



- A Flow Cell Stage

- B Flow Cell Compartment Door
- C Flow Cell Clamp
- D Flow Cell
- E Flow Cell Clamp Release Button

Reagent Compartment

The reagent compartment contains the reagent chiller, and positions for the wash buffer (PR2) bottle and the waste bottle. To maintain a consistent temperature, open and close the reagent chiller only when instructed.



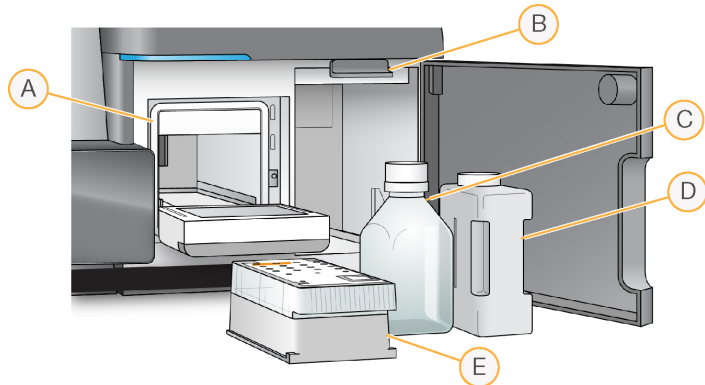
NOTE

The required temperature range of the reagent chiller is 2°C to 11°C.

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 form-fitted slots for the PR2 bottle and 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.

Figure 2 Reagent Compartment Components



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

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 is performed by MiSeq Reporter. The analysis workflow for each run is specified in the sample sheet or by the selected module.

Concept	Description
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 software (MiSeqOutput folder) or the folder populated by MiSeq Reporter (MiSeqAnalysis). For more information, see Run Folders on page 49 .
Sample Sheet	A comma-separated values file (*.csv) that contains information used 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 on analysis workflows and manifest file formats, see the *MiSeq Reporter Software Guide (document #15042295)*.

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

System Software

The instrument software suite includes integrated applications that perform sequencing runs, on-instrument analysis, and related functions.

- ▶ **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 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 analysis by RTA is complete.
- ▶ **Integrated secondary analysis software**—Performs secondary analysis. MiSeq Reporter processes base calls generated by the RTA software, 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 Software on page 8](#).





Optional software used off-instrument includes the Sequencing Analysis Viewer (SAV). For more information, see [Sequencing Analysis Viewer on page 8](#).

Status Icons

A status icon on the control software interface indicates a change in conditions during run setup or during the run. A number on the icon indicates the number of conditions for a status.

When a run status changes, the icon blinks to alert you. Select the icon to view a description of the condition. Select **Acknowledge** to clear the message, and then **Close** to close the dialog box.

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.

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.

Activity Indicators

An activity indicator icon displays the activity the instrument is currently performing.

Figure 3 Activity Indicators



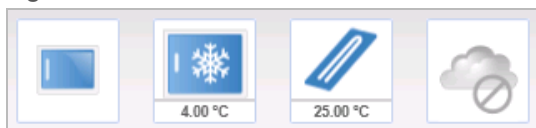
From left to right, the activity indicators represent the following activities:

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

Sensor Indicators

Sensor indicators, which appear at the base of each interface screen, represent the status of instrument components.

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)

Secondary Analysis Options

MiSeq sequencing data can be analyzed on the instrument computer using MiSeq Reporter, on a networked server using BaseSpace™ Onsite, or on the cloud using BaseSpace. These applications produce information about alignment, variants, and contig assemblies for each genome requested and for each sample of a multisample run. If performing the VeriSeq™ PGS workflow, use BlueFuse™ Multi software for analysis.

BaseSpace and BaseSpace Onsite Overview

BaseSpace is the Illumina cloud computing environment. BaseSpace Onsite provides a computing environment on a dedicated server, complete with run setup tools and analysis options.

Log in to BaseSpace or BaseSpace Onsite when you set up the sequencing run. When using BaseSpace or BaseSpace Onsite, you have the additional option to store run data locally. For more information, see [Customize System Settings on page 13](#).

When you begin your sequencing run, the icon changes to indicate that the MiSeq is connected to BaseSpace or BaseSpace Onsite and data files are being transferred to the specified location.

Figure 5 Connected to BaseSpace Icon

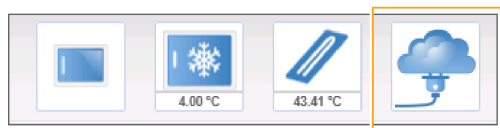


Figure 6 Connected to BaseSpace Onsite Icon



Using BaseSpace, data files are encrypted in transit, decrypted during analysis, and encrypted again when stored. Using BaseSpace Onsite, data files are encrypted in transit, decrypted during analysis, and can be optionally encrypted again when stored.

BaseSpace and BaseSpace Onsite automatically disconnect from the MiSeq at the end of the run or as soon as all RTA analysis files have finished transfer. If the internet connection is interrupted, analysis files continue to upload after the connection is restored from the point when the interruption occurred.

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

For MiSeq Reporter, several genomes are provided during installation. BaseSpace and BaseSpace Onsite only support genomes included with MiSeq Reporter.

You can connect to BaseSpace at basespace.illumina.com. Log in using your MyIllumina account login. For more information on BaseSpace, see the *BaseSpace User Guide (document # 15044182)* and the BaseSpace support pages on the Illumina website.

For more information on BaseSpace Onsite, see the *BaseSpace Onsite System Guide (document # 15049148)* and the BaseSpace OnSite support pages on the Illumina website.

MiSeq Reporter Software

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

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 Software Guide (document #15042295)*.

Sequencing During Analysis

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

With MiSeq Reporter, if a new sequencing run is started on the MiSeq before secondary analysis of an earlier run is complete, MiSeq Reporter analysis is automatically stopped.

To restart MiSeq Reporter, use the Requeue feature after the new sequencing run is complete.

After the new run completes sequencing, secondary analysis of the earlier run automatically starts again from the beginning.

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 sequencing systems, most of which use an 8-lane flow cell. Some views include drop-down lists showing lanes 1–8. Because the MiSeq flow cell has a single lane, select **All** or **Lane 1**. For more information, see the *Sequencing Analysis Viewer User Guide (document # 15020619)*.

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 message indicating how much disk space is required appears.

If prompted to make disk space available, move or delete older run folders as appropriate. For more information, see *Manage Files on page 38*. After clearing adequate disk space, select **Restart Check**.

MiSeq Reagent Kit Overview

The MiSeq Reagent Kit is a single-use reagent kit required to perform a sequencing run. It 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 a 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 [MiSeq Reagent Kits product page](#) on the Illumina website.

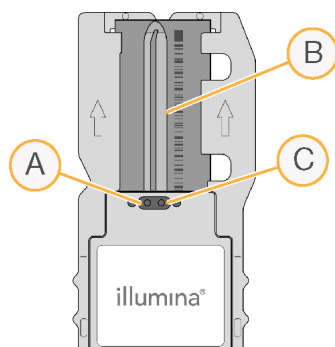
Flow Cell

The MiSeq flow cell is a single-use glass-based substrate where 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.

Libraries are loaded onto the reagent cartridge before setting up the run, and then automatically transferred to the flow cell after the run begins.

Figure 7 MiSeq Flow Cell



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

Flow Cell Cap Color

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

Flow Cell	Flow Cell Cap Color
Standard Flow Cell PGS Flow Cell	Clear
Micro Flow Cell	Green
Nano Flow Cell	Yellow

Reagent Cartridge Overview

The MiSeq reagent cartridge is a single-use consumable consisting of foil-sealed reservoirs prefilled 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**.



WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

Reserved Reservoirs

Figure 8 Reagent Cartridge with Numbered Reservoirs

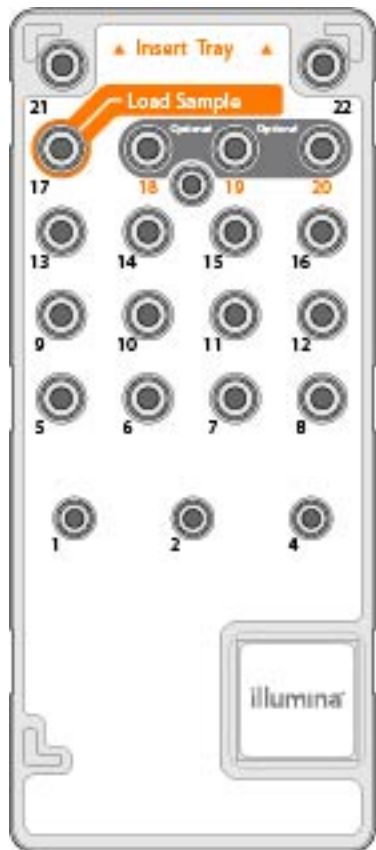


Table 1 Reagent Cartridge Reservoirs

Position	Reagent Name	Description
8	LDR	Denaturation Reagent (contains formamide)
17	Reserved	Load Sample (Reserved for sample libraries)

Position	Reagent Name	Description
18	Reserved	Reserved for custom Read 1 primer [Optional]
19	Reserved	Reserved for custom Index Read primer [Optional]
20	Reserved	Reserved for custom Read 2 primer [Optional]

**NOTE**

For more information about using custom primers on the MiSeq reagent cartridge, see the *MiSeq Custom Primers Guide* (document # 15041638).

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Start the MiSeq

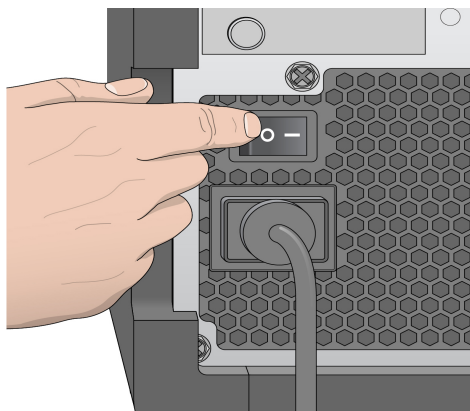
- 1 Switch the toggle power switch on the back of the instrument to the | (on) position.



NOTE

For best performance, leave the instrument on continuously. However, if the instrument must be turned off, see [Shut Down the Instrument on page 39](#). Wait a *minimum* of 60 seconds before turning the power switch back to the ON position.

Figure 9 Power Switch Location



- 2 Wait for the system to load, and then log on to the operating system. If necessary, consult your facility administrator for the user name and password.
When the operating system is loaded, the MiSeq Control Software (MCS) launches and initializes the system automatically.

Customize System Settings

- 1 From the Home screen, select **Run Options**.
- 2 Select the **Run Settings** tab.
- 3 Select **Post Run Wash** or **Maintenance Wash**.
An instrument wash is required after each run. The software requires that a wash is performed before setting up a subsequent run. The Post-Run Wash Option specifies the type of wash that is performed by default. A post-run wash takes approximately 30 minutes. A maintenance wash takes approximately 1 hour.
- 4 Enter the address of the BaseSpace Onsite server location.
The Onsite Server setting is required if you use BaseSpace Onsite.

- 5 Select **Send Instrument Performance Data to Illumina to aid technical support** to enable the Illumina Proactive monitoring service. The name of the setting in the software interface might be different from the name in this guide, depending on the version of MCS in use.
 With this setting turned on, instrument performance data are sent to Illumina. This data helps Illumina troubleshoot more easily and detect potential failures, enabling proactive maintenance and maximizing instrument uptime. For more information on the benefits of this service, see *Illumina Proactive Technical Note (document # 1000000052503)*.
 This service:
 - ▶ Does not send sequencing data.
 - ▶ Requires that the instrument be connected to a network with internet access.
 - ▶ Is turned on by default. To opt out of this service, disable the **Send Instrument Performance Data to Illumina to aid technical support** setting.
- 6 For MiSeq Reporter, select or clear **When using BaseSpace or BaseSpace Onsite, replicate analysis locally on MiSeq**.
 The Replicate Analysis Locally setting specifies analysis processing locations when using BaseSpace or BaseSpace Onsite. The setting provides the option to perform analysis both locally on the instrument and in BaseSpace or BaseSpace Onsite.
 If you select this option when using BaseSpace or BaseSpace Onsite, MiSeq Reporter launches automatically after the run and performs analysis locally.
 If you do not select this option when using BaseSpace or BaseSpace Onsite, MiSeq Reporter does not launch automatically after the run and analysis is performed in BaseSpace or BaseSpace Onsite only.
 If performing the VeriSeq PGS workflow with BlueFuse Multi, select this option.

Configure Notifications of BaseSpace Updates

- 1 From the Home screen, select **Manage Instrument**.
- 2 Select **Software Update**.
- 3 Select **Automatically check for new software updates on BaseSpace**.

Set Email Preferences

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

- 1 From the Home screen, select **Run Options**.
- 2 Select the **Email Notifications** tab.
- 3 Enter the following information:
 - ▶ **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 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.
 - ▶ **Recipient 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.

Set Default Folder Locations

Folders can be on a local network or on the instrument computer.

- 1 From the Home screen, select **Run Options**.
- 2 Select the **Folder Settings** tab.
- 3 Enter default locations for the following folders.
 - ▶ **Recipe Folder**—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 Sheet Folder**—Sets the default location for sample sheets. Sample sheets are created before library preparation and contain parameters for the run.
 - ▶ **Manifest Folder**—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* (document # 15028392).
 - ▶ **MiSeqOutput**—For MiSeq Reporter, sets the default location for analysis output files. Change 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 1*.

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 and PhiX control DNA
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 95041-714* 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 and the foil seal covering the load samples reservoir
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
MiSeq tubes	Illumina, part # MS-102-9999	Washing the template line, for use with the VeriSeq PGS workflow (optional for other workflows)
NaOCl, 5%	Sigma-Aldrich, catalog # 239305*	Washing the template line, for use with the VeriSeq PGS workflow (optional for other workflows)
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 laboratory-grade equivalent

Guidelines for Laboratory-Grade Water

Always use laboratory-grade water or deionized water to perform instrument procedures. Never use tap water. Use only the following grades of water or equivalents:

- ▶ Deionized water
- ▶ Illumina PW1
- ▶ 18 Megohms (M Ω) water
- ▶ Milli-Q water
- ▶ Super-Q water
- ▶ Molecular biology grade water

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Introduction

To perform a sequencing 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 remotely using the Sequencing Analysis Viewer (SAV). This optional application is available for download from the Illumina website.

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

Run Duration

Run duration is based on the number of cycles performed. You can perform a paired-end run up to 2 x 301 sequencing cycles plus any Index Reads with MCS v2.3 or later.

Additionally, run duration is based 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.

Number of Cycles in a Read

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

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

MiSeq Workflow



Prepare the prefilled reagent cartridge for use.



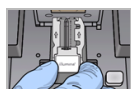
Denature and dilute libraries (does not apply to all library types). See *Preparing Libraries for Sequencing on the MiSeq* (document # 15039740).



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 or BaseSpace Onsite.



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.

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 a tile 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 base calling, filtering, and quality scoring.

Analysis

When the run is complete, 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 the *MiSeq Reporter Software on page 8*

Thaw Reagent Cartridge

Thaw the reagent cartridge using a room temperature water bath.

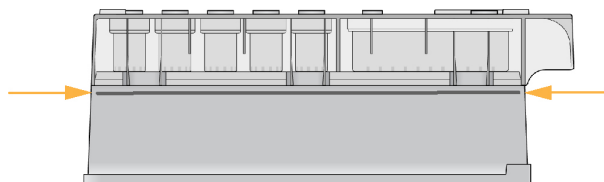


NOTE

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

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

Figure 10 Maximum Water Line



- 3 Allow the reagent cartridge to thaw in the room temperature water bath until it is thawed completely.
 - ▶ MiSeq v3 cartridges— ~ 60-90 minutes.
 - ▶ MiSeq v2 cartridges— ~ 60 minutes.
- 4 Remove the cartridge from the water bath and gently tap it on the bench to dislodge water from the base of the cartridge. Dry the base of the cartridge.

Inspect the Reagent Cartridge

- 1 Invert the reagent cartridge ten times to mix the thawed reagents, and then inspect that all positions are thawed.
- 2 Inspect the reagents in positions 1, 2, and 4 to make sure that they are fully mixed and free of precipitates.
- 3 Gently tap the cartridge on the bench to reduce air bubbles in the reagents.



NOTE

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

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

**WARNING**

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

Denature and Dilute Libraries

If necessary for your library type, denature and dilute libraries, and add optional PhiX control. See *MiSeq System Denature and Dilute Libraries Guide (document # 15039740)*. If you are performing the VeriSeq PGS workflow, see the *VeriSeq PGS Library Preparation Guide (document # 15052877)*.

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**

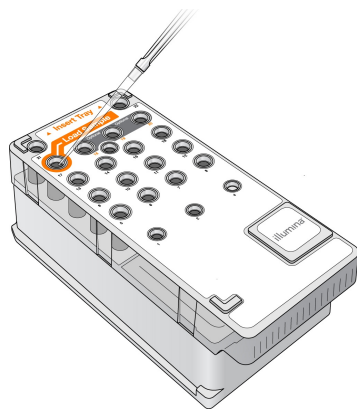
If you are using custom primers, prepare primers and set up the sample sheet as described in *MiSeq Custom Primers Guide (document # 15041638)*.

Load Sample Libraries

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

- 1 Clean the foil seal covering the reservoir labeled **Load Samples** with a low-lint lab tissue.
- 2 Pierce the foil seal with a clean 1 ml pipette.
- 3 Pipette 600 µl of prepared libraries into the reservoir **Load Samples**. Avoid touching the foil seal.

Figure 11 Load Libraries



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

Set Up a Run Using MCS

- 1 From the Home screen, select **Manage Instrument**.
- 2 Select **Reboot** to reboot the system software.

- 3 **[Optional]** From the Run Options screen, check the folder locations for MiSeqOutput, recipes, sample sheets, and manifests. For more information, see [Set Default Folder Locations on page 14](#).
- 4 From the Home screen, select **Sequence** to begin the run setup steps.
When you select **Sequence** on the Home 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 or BaseSpace Onsite Option

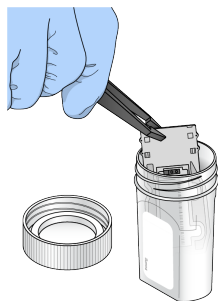
Optionally run secondary analysis using BaseSpace or BaseSpace Onsite.

- 1 From the BaseSpace Options screen, select or clear the **Use BaseSpace for storage and analysis** and **Use BaseSpace Onsite for storage and analysis** checkboxes.
- 2 Select **Next**.

Clean the Flow Cell

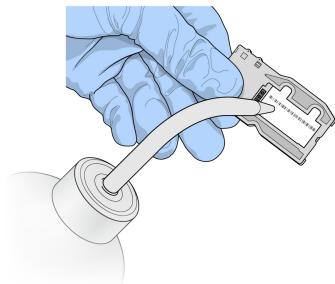
- 1 Put on a new pair of powder-free gloves.
- 2 Using plastic forceps, grip the flow cell by the base of the plastic cartridge and remove it from the flow cell container.

Figure 12 Remove Flow Cell



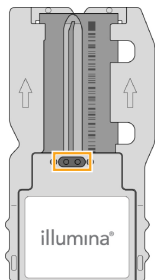
- 3 Lightly rinse the flow cell with laboratory-grade water until both the glass and plastic cartridge are thoroughly rinsed of excess salts.
Excess salts can affect flow cell seating on the instrument. If salts dry in the imaging area, imaging can also be affected.

Figure 13 Rinse Flow Cell



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

Figure 14 Flow Cell Ports and Gasket



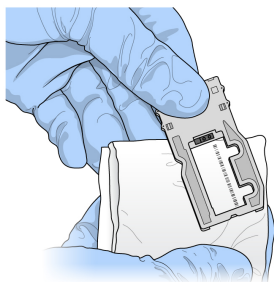
- 5 Clean the flow cell glass with an alcohol wipe. Make sure that the glass is free of streaks, fingerprints, and lint or tissue fibers.



NOTE

Do not use the alcohol wipe on the flow cell port gasket.

Figure 15 Dry Flow Cell

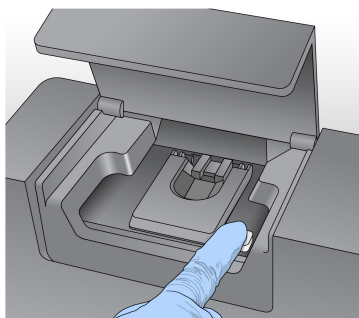


- 6 Dry excess alcohol with a lint-free lens cleaning tissue.
- 7 Make sure that the flow cell ports are free of obstructions and that the gasket is well-seated around the flow cell ports.
If the gasket appears to be dislodged, gently press it back into place until it sits securely around the flow cell ports.

Load the Flow Cell

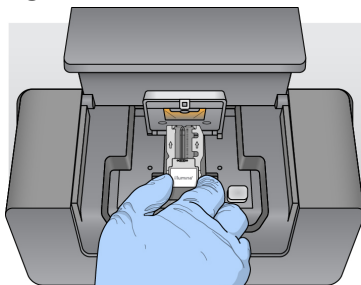
- 1 Raise the flow cell compartment door, and then press the release button to the right of the flow cell clamp.
The flow cell clamp opens.

Figure 16 Open Flow Cell Clamp



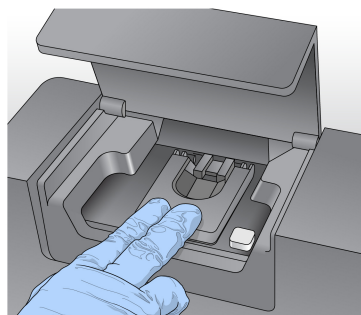
- 2 Make sure that the flow cell stage is free of lint. If lint or other debris is present, clean the flow cell stage using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol. Carefully wipe the surface of the flow cell stage until it is clean and dry.
- 3 Holding the flow cell by the edges, place it on the flow cell stage.

Figure 17 Place Flow Cell on Stage



- 4 Gently press down on the flow cell clamp to close it over the flow cell. As the flow cell clamp closes, alignment pins position the flow cell. An audible click indicates that the flow cell clamp is secure.

Figure 18 Close Flow Cell Clamp



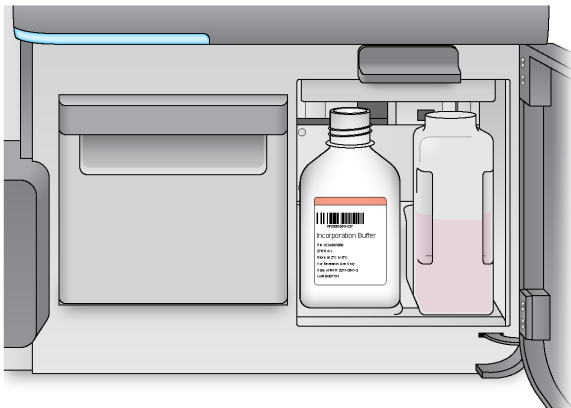
- 5 If the software does not identify the flow cell RFID, see [Resolve RFID Read Failure on page 44](#).
- 6 Close the flow cell compartment door.
- 7 Select **Next**.

Load Reagents

Load PR2 and Check the Waste Bottle

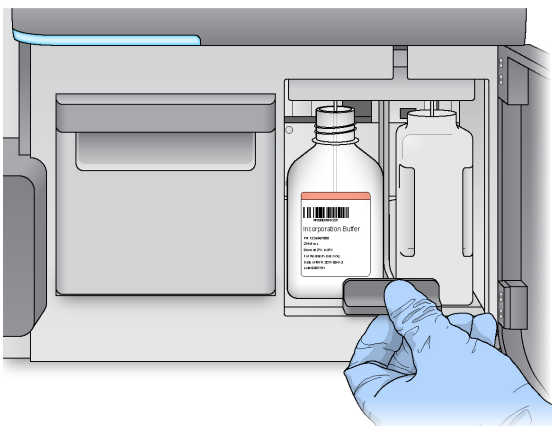
- 1 Remove the bottle of PR2 from 2° to 8°C storage. Invert to mix, and then remove the lid.
- 2 Open the reagent compartment door.
- 3 Raise the sipper handle until it locks into place.
- 4 Remove the wash bottle and load the PR2 bottle.

Figure 19 Load the PR2 Bottle



- 5 Empty the contents of the waste bottle into the appropriate waste container.
- 6 Slowly lower the sipper handle. Make sure that the sippers lower into the PR2 and waste bottles.

Figure 20 Lower Sipper Handle



- 7 If the software does not identify the RFID of the PR2 bottle, see [Resolve RFID Read Failure on page 44](#).
- 8 Select **Next**.

Load the Reagent Cartridge

- 1 Open the reagent chiller door.



NOTE

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

- 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.
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 **Home** to return to the Home screen.

Figure 21 Load Reagent Cartridge



- 3 Close the reagent chiller door.
- 4 If the software does not identify the RFID of the reagent cartridge, see [Resolve RFID Read Failure on page 44](#).
- 5 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 Home screen.
- 6 Close the reagent compartment door.
- 7 Select **Next**.

Change Sample Sheet

Use the Change Sample Sheet command for the following:

- ▶ To select a sample sheet with a name that does not match the reagent cartridge barcode number
- ▶ When the software prompts you to choose a different sample sheet on the Review screen

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.

- 1 Select **Change Sample Sheet** on the Load Reagents screen.
- 2 Select **Browse** to navigate to the sample sheet.
- 3 Select **Open**.
- 4 Select **Save and Continue**.
- 5 Select **Next**.

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 Select **Change Folder** to review the folder locations.
- 3 Modify as needed and then select **Save**.
- 4 Select **Next**.

Change Folders

To change folder locations, select **Change Folder** 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 44](#).

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

Important Notes 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 42](#).

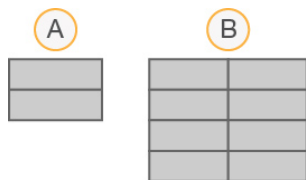


WARNING

Make sure to close all files on the MiSeq before starting a run, and do not open files during a run.

Monitor the Run

- 1 During the run, monitor run progress, intensities, and quality scores that 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 a computer independent of the instrument computer. A network connection is required.
Alternatively, if you are connected to BaseSpace, the run can be monitored 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 2 column graphic appears.



- A Indicates 2 tiles, top surface only
- B Indicates 4 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 2 greatest signals. If more than 1 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.

Template Generation

Template generation is the process where cluster positions over the entire flow cell surface are defined according to X and Y coordinate position. Real-time analysis (RTA) uses early cycles of the run for template generation.

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:

Metric	Kit	Cycle
Intensity	MiSeq Reagent Kits, v3	Cycle 1–7
	MiSeq Reagent Kits, v2	Cycle 1–4
	MiSeq Reagent Kits, v1	Cycle 1–4
Intensity and Cluster Density	MiSeq Reagent Kits, v3	Cycle 8–25
	MiSeq Reagent Kits, v2	Cycle 5–25
	MiSeq Reagent Kits, v1	Cycle 5–25
Intensity, Cluster Density, % PF, Yield, and Q-scores	MiSeq Reagent Kits, v3	Cycle 26 through run completion
	MiSeq Reagent Kits, v2	
	MiSeq Reagent Kits, v1	

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

RTA Analysis Results

The RTA analysis output from a sequencing run is a set of quality-scored base call files (*.bcl), which are generated from the raw image files. For a list of RTA files and folders, see *RTA Folders and Files on page 51*.

Perform a Post-Run Wash

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

Start the wash directly following the completion of a run. An instrument wash is required before you can set up a subsequent run. 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.



NOTE

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

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

If you are using MCS v2.5 or later, you have the option to perform a post-run wash that includes a template line wash with sodium hypochlorite solution (NaOCl). The wash takes approximately 30 minutes. See *Procedure with Template Line Wash on page 30*.



NOTE

If you are using the VeriSeq PGS workflow, perform post-run washes that include a template wash. See *Procedure with Template Line Wash on page 30*.

Consumables

- ▶ Tween 20
- ▶ Laboratory-grade water
- ▶ NaOCl (use with a post-run wash that includes a template line wash)
- ▶ MiSeq tube (part # MS-102-9999) (for post-run washes that include a template line wash)

Procedure

- 1 Prepare fresh wash solution with Tween 20 and laboratory-grade water:
 - 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:
 - 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.
Do not select **Perform optional template line wash** on the Post-Run wash screen. The template line wash requires a different procedure. See *Procedure with Template Line Wash* on page 30.
- 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

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

- 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**.

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.

Procedure with Template Line Wash

- 1 Prepare fresh wash solution with Tween 20 and laboratory-grade water:
 - 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 fresh NaOCl wash solution with laboratory-grade water:
 - a Add 36 μ l of 5% NaOCl to 864 μ l laboratory-grade water. These volumes result in a 1:25 NaOCl dilution.
 - b Add 50 μ l of the 1:25 NaOCl dilution to 950 μ l of laboratory-grade water in a MiSeq tube (part # MS-102-9999).

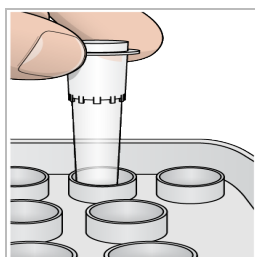


NOTE

Using the correct concentration of NaOCl is important. Make sure to check the percentage of NaOCl on the product label. If the concentration is too high, it can make cluster generation fail in subsequent runs. If 5% NaOCl is not available, make a 1 ml solution of 0.01% NaOCl in laboratory-grade water. **Do not** use NaOCl with a maintenance wash or a standby wash

- 3 Prepare the wash components with fresh wash solution:
 - 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 Insert the MiSeq tube containing 0.01% NaOCl wash solution into position 17 of the wash tray until the neck of the tube is flush with the tray. The tube displaces the Tween 20 and laboratory-grade water wash solution from position 17.

Figure 22 MiSeq Tube in Position 17 of the Wash Tray



NOTE

Make sure to insert the MiSeq tube with NaOCl into tray position 17 only. Inserting the tube in another position can make cluster generation fail in subsequent runs, and can damage the fluidic system of the MiSeq instrument.

- 5 When the run is complete, select **Start Wash**. The software automatically raises the sippers in the reagent chiller.
- 6 Select **Perform optional template line wash** on the Post-Run Wash screen.
When using the VeriSeq PGS workflow, the option **Perform optional template line wash** is pre-selected for you. The MCS tracks the type of post-run wash performed after each run. If **Perform optional template line wash** is not selected for the post-run wash, a message on the Run Review screen reminds you the next time you start a sequencing run.
- 7 Open the reagent compartment door and reagent chiller door, and slide the used reagent cartridge from the chiller.
- 8 Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
- 9 Raise the sipper handle in front of the PR2 bottle and waste bottle until it locks into place.
- 10 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.

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

**WARNING**

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

- 12 Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
- 13 Close the reagent compartment door.
- 14 Select **Next**.

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. 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.

Chapter 4 Maintenance

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Perform a Standby Wash	36
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Software Updates	39
Shut Down the Instrument	39

Maintenance Frequency

Perform the following maintenance procedures at the recommended intervals.



NOTE

If performing the VeriSeq PGS workflow, make sure to follow maintenance frequency guidelines for VeriSeq PGS. See *Maintenance Frequency for the VeriSeq PGS Workflow* on page 33.

Table 2 Maintenance During Normal Operation

Activity	Frequency
Post-Run Wash	After every run
Maintenance Wash	Monthly
Standby Wash	To prepare for idle mode (if unused for ≥ 7 days), and every 30 days the instrument remains idle
Instrument Shutdown	As needed

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

Activity	Frequency
Standby Wash	Monthly
Instrument Shutdown	As needed

Maintenance Frequency for the VeriSeq PGS Workflow

If performing the VeriSeq PGS workflow, perform the following maintenance procedures at the recommended intervals.

Table 4 Maintenance During Normal Operation

Activity	Frequency
Post-Run Wash	After every run
Maintenance Wash	Monthly
Post-Run Wash from the Perform Wash Screen	After in idle mode (unused for > 3 days)
Standby Wash	To prepare for idle mode (if unused for ≥ 7 days), and every 30 days the instrument remains idle
Instrument Shutdown	As needed

Table 5 Maintenance During Idle Mode (≥ 7 days unused)

Activity	Frequency
Standby Wash	Monthly
Instrument Shutdown	As needed

Perform a Maintenance Wash

Perform a maintenance wash every 30 days to ensure optimal performance.

The maintenance wash takes approximately 90 minutes to complete. The wash includes a series of three wash steps that thoroughly flush the system.

You can configure your instrument to perform a maintenance wash between runs. For more information, [Customize System Settings on page 13](#).

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 Home screen, select **Perform Wash**.
- 3 From the Perform Wash screen, select **Perform 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:
 - 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:
 - 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.

- a Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
 - b Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - c Close the reagent compartment door.
- 4 Select **Next**. The first wash begins.

Perform Second Wash

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:
 - 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:
 - 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:

- 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

When there are no plans to use the instrument within the next seven days, prepare the instrument and instrument fluidics lines to sit idle by performing a standby wash. Perform a standby wash every 30 days the instrument sits idle.

The standby wash takes approximately two hours to complete. The wash performs two consecutive washes that flush each position of any remaining reagents or salt accumulation. Each wash takes approximately 60 minutes.

When the standby wash is complete, the instrument is in standby mode and a message appears on the Home 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.

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 Home screen, select **Perform Wash**.
- 3 From the Wash Options screen, select **Perform 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:
 - 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:

- 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.

- a Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
 - b Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
 - c Close the reagent compartment door.
- 4 Select **Next**. The first wash begins.

Perform Second Wash

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.

Manage Files

Select the Manage Files on the Home screen to move, upload, delete files, or rename sample sheets on the instrument computer.

Delete Files

- 1 From any tab on the Manage Files screen, select **Browse** to navigate to files accessible to the instrument.
- 2 Select from the following options:
 - ▶ Select the checkbox next to individual files or folders in the list.
 - ▶ Select the checkbox to the left of the Delete button to select all files and folders in the list. This option is available for Runs, Sample Sheets, Manifests, Genomes, and Recipes.
- 3 Select **Delete**.

**NOTE**

The Delete command is available on all tabs except Bundle Logs.

Move Run Folders

The Move command *copies* the run folder to the new location and then *deletes* the folder from the old location.

- 1 From the Runs tab on the Manage Files screen, select **Browse** to navigate to files accessible to the instrument.
- 2 Select the checkbox next to individual files or folders in the list.
- 3 Select **Move**.
- 4 Select **Browse Network** and select a new location for the files or folders.
- 5 Select **OK**.

Upload Files

The Upload command is 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.

- 1 From the tab on the Manage Files screen, select **Browse** to navigate to files accessible to the instrument.
- 2 Select **Upload**.
- 3 Select **Browse Network** and browse to the location on a USB drive where the file resides.
- 4 Select **OK**.
The file is uploaded to the folder indicated in the Directory field.

Rename Sample Sheets

- 1 From the Sample Sheets tab on the Manage Files screen, select from the following options:
 - ▶ Select the checkbox next to individual sample sheets.
 - ▶ Select the checkbox to the left of the Delete button to select all sample sheets in the list.
- 2 Select **Rename**.
- 3 Select the keyboard icon and use the on-screen keyboard to rename sample sheets.
- 4 Select **Next**.
- 5 Select **Back**.

Software Updates

If your system is connected to a network with internet access, you can automatically update the instrument software from the Home screen. You can also configure the software to check automatically for BaseSpace updates. For more information, see *Configure Notifications of BaseSpace Updates* on page 14.

If your instrument is not connected to a network with internet access, you can update the software manually.

Update Software Automatically

When software updates are available, the **Update Available** button appears on the Home screen. Otherwise, this button is not visible. Make sure your MiSeq is connected to a network with internet access to enable this option.

- 1 From the Home screen, select **Update Available**.
- 2 Confirm the command to update in the dialog box.
Reboot of the instrument is required. Installation of the update begins automatically upon reboot.

Update Software Manually

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.

- 1 From the Home screen, select **Manage Instrument**.
- 2 Select **Software Update**.
- 3 Select **Browse** to navigate to the location of the installable file for the new software version.
- 4 When the path to the installable software file appears on the screen, select **Save and Update**.
- 5 Confirm the command to update in the dialog box.
Reboot of the instrument is required. Installation of the update begins automatically upon reboot.

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 34.
- 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 Home screen, select **Manage Instrument**.
- 5 Select **Shut Down**.
This command shuts down the software.
- 6 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.

Appendix A Troubleshooting

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Introduction

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

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

For problems with run quality or performance, contact Illumina Technical Support. For more information, see *Technical Assistance* on page 57.

Illumina Technical Support representatives typically request copies of run-specific files for troubleshooting purposes. You can use the Bundle Logs tab on the Manage Files screen to combine and zip the files required for troubleshooting. See *Bundle Logs for Troubleshooting* on page 41.

Bundle Logs for Troubleshooting

Bundle Logs is a feature that bundles files to send to Illumina Technical Support for troubleshooting. Use the Bundle Logs tab on the Manage Files screen to select a group of files, called a *bundle*. The bundle is zipped automatically.

The Bundle Logs feature groups the files from a run into one bundle type at a time. Repeat the Bundle Logs procedure for each run and bundle type Illumina Technical Support requests.

- 1 On the Manage Files screen, select the **Bundle Logs** tab.
- 2 Select **Browse** to navigate to the location of the MiSeqOutput folder.
- 3 Click in the blue box next to the run, and in the blue circle next to the bundle type requested by Illumina Technical Support.

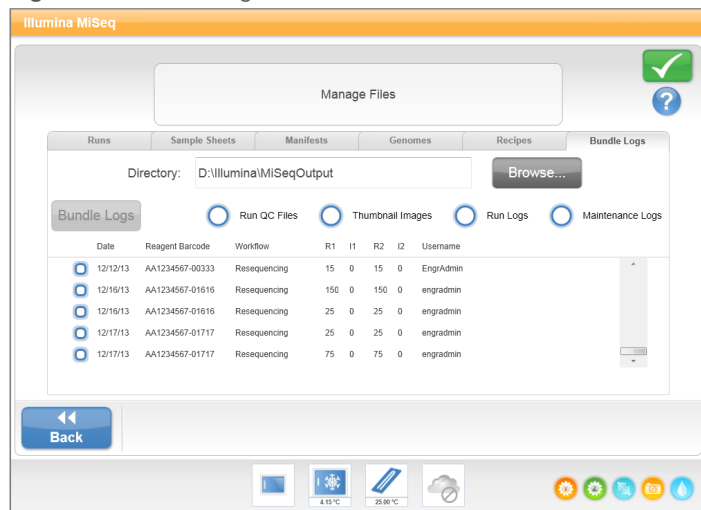
- 4 Select **Bundle Logs**.

A Bundle Files screen opens with information about the bundle, including a list of individual files the bundle contains.

For more information on the individual folders and files of the Bundle Logs feature, see *MiSeq Output and Analysis Folders Quick Reference Card (document # 15034791)*.

- 5 Select **Next**.
- 6 Navigate to a location where you want the zipped bundle files saved.
- 7 Select **Save**.
When the files finish bundling, the Bundle Logs tab reopens.
- 8 Send the zipped bundle to Illumina Technical Support.

Figure 23 Bundle Logs Tab



Perform a System Check

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 45](#).

- 1 From the Home screen, select **Manage Instrument**.
- 2 Select **System Check**.
- 3 Do one of the following:
 - ▶ Select the individual tests you want to perform.
 - ▶ Choose **Select All** to perform all tests.
- 4 Select **Next**.
When complete, the test results appear on the screen.
- 5 **[Optional]** Select **Show Details** to see a summary of the results on the software interface.
- 6 **[Optional]** Select **Export Results** to export the results in a *.csv file format to a USB drive.
- 7 Select **Done**.

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. For example, a run can be paused if you suspect that the waste bottle is full. Paused runs can be resumed.

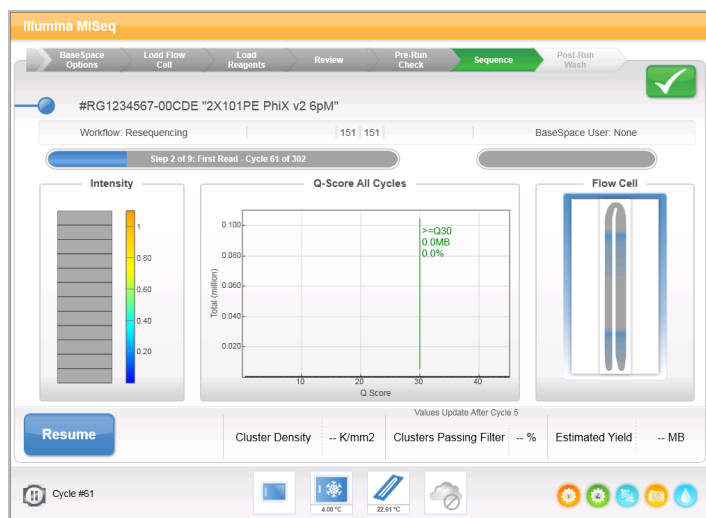
CAUTION

Do not pause a run during cluster generation or within the first eight cycles of sequencing. It is not possible to resume a run that was paused during this time. See *Run Metrics on page 28* for cycle information for MiSeq reagent cartridge kits.

When you select **Pause**, the current command is completed before pausing the run and placing the flow cell 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 24 Sequence Screen of a Paused Run

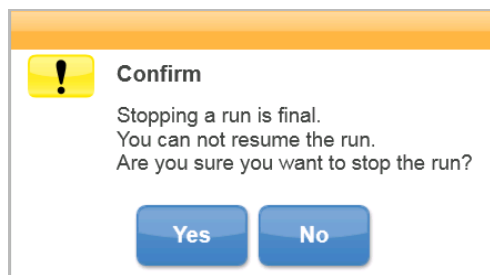


Stop a Run

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. Real-time Analysis software continues analysis for the last completed cycle.

Figure 25 Stopping a Run



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

Raise Reagent Cartridge Sippers Manually

The reagent cartridge sippers might not raise automatically if a run was interrupted unexpectedly, or if an error occurred during the run. To remove the reagent cartridge, raise the reagent cartridge sippers manually.

- 1 On the Home screen, select **Perform Wash**.
- 2 Select **Raise Sippers**.
- 3 Remove the reagent cartridge.

Resolve Run Setup Errors

If any checks in the pre-run check fail, a red icon **X** appears next to the item. A message appears on the screen that describes the error and how to correct it.

Error	Action
X Flow Rate Measured	<p>The flow rate check screen opens. Use the drop-down list or on-screen keyboard to enter the following:</p> <ul style="list-style-type: none"> • Solution: PR2 • Volume: 250 • Aspirate Rate: 2500 • Dispense Rate: 2500 <p>Select Pump. If the error persists, set the volume to pump 500 µl PR2 and repeat the process. When fluids have been pumped, select Restart Check. When the pre-run check is successful, the Start Run button becomes active. If the flow check fails again, reseal the flow cell to make sure that flow is not interrupted due to misalignment. Inspect the flow cell gasket for lint or irregularities.</p>
X Free Disk Space	<p>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.</p>
X Network Connection Active	<p>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.</p>
X Primary Analysis Ready	<p>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.</p>
X Sample Sheet Present	<p>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 Home screen. Make sure that the sample sheet file extension is *.csv. If the sample sheet is missing, create a sample sheet and copy it to the sample sheet locations specified in Run Options. When you have located a sample sheet, select Restart Check.</p>

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 retry the RFID read before proceeding. If the RFID fails a second time, select **Get Code**.
- 2 From a computer with internet access, go to my.illumina.com and select **Sign In** from the toolbar on the top of the page.
- 3 Log in to your Myllumina account.
Your name replaces the Sign In button on the toolbar.
- 4 Hover over your name and select **Account**. In the **My Tools** column, click **MiSeq Self-Service**.
- 5 On the MiSeq Self-Service page, enter the **MiSeq serial number**.
- 6 From the Type of Override Code drop-down list, select **RFID Override**.
- 7 To generate the code, select **Get Code**.
- 8 Return to the MCS interface and select **Enter Code**.
- 9 Enter the temporary bypass code using the on-screen keyboard, and then select **Next**.
- 10 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

- 11 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.

- 12 Select **Enter**.

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 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 Home screen, select **Manage Instrument**.
- 3 Select **System Check**.
- 4 Select **Conduct Volume Test**, and then select **Next**.
- 5 Fill each reservoir of the wash tray with 6 ml laboratory-grade water.
- 6 Fill the 500 ml wash bottle with 350 ml laboratory-grade water.

- 7 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 in 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.
- 8 Following the on-screen prompts, remove any droplets from the wash bottle sipper, as follows.
 - a Slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.
 - b Slowly lower the sipper handle far enough into the water to allow the surface tension to remove the droplet.
 - c Slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.
 - d Slowly lower the sipper handle completely, making sure that the sippers lower into the wash bottle and waste bottle.
- 9 Select **Next**.
When the volume test is complete, the results appear on the screen.
If the test did not pass, perform a maintenance wash. See [Perform a Maintenance Wash on page 34](#).
- 10 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
Post Run Wash that includes a Template Line Wash	25.5 ml
Standby Wash	46 ml
Maintenance Wash	51.75 ml

Configure System Settings

The MCS includes several screens that access commands to configure the system. Typically software settings are configured during MiSeq installation.

Configure IP and DNS Settings

Configure IP address and DNS server addresses if required due to a network or facility change.

- 1 From the Home screen, select **Manage Instrument**.
- 2 Select **System Settings**.
 - ▶ Select **Obtain an IP address automatically** or **Use the following IP address**.
If you select **Use the following IP address**, enter an IP address, subnet mask, and default gateway.
 - ▶ Select **Obtain DNS address automatically** or **Use the following DNS server addresses**.
If you select **Use the following DNS server addresses**, enter a preferred and alternate DNS server address.
- 3 Select **Save and Continue**.

Change System Credentials

Changing the system user name and password on the Systems Settings screen, also updates the credentials for MiSeq Reporter and BaseSpace or BaseSpace Onsite.

- 1 From the Home screen, select **Manage Instrument**.
- 2 Select **System Settings**.
- 3 Select **Save and Continue** to progress to the third screen in the series of screens.
- 4 Select **This account**.
- 5 Enter the domain name (Domain\MiSeq1, for example) and password.
- 6 Select **Save and Continue**.

Appendix B Output Files and Folders

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Run Folders

Each run on the MiSeq generates 3 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. See *RTA Folders and Files* on page 51.
- ▶ You can change the location of the output folder in the Output Folder field on the Run Options screen. For more information, see *Set Default Folder Locations* on page 14.
- ▶ **D:\Illumina\MiSeqAnalysis**—When analysis by RTA 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 49.

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.

By default, the folder name uses the following format:

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

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

MiSeqOutput Folder Contents

After RTA completes 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 except that the MiSeqOutput folder contains 2 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 Software Guide (document #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 Software Guide (document #15042295)*.

RTA Folders and 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 1 cycle, 1 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 1 cycle, 1 tile. For more information, see Flow Cell Tile Numbering on page 52 .
*.locs files	Data\ Intensities\BaseCalls\ L001	Reports the cluster coordinates. Each *.locs file represents 1 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 used for image analysis and are not copied to the Analysis folder. See Flow Cell Tile Numbering on page 52 for image file names.

Flow Cell Tiles

During the sequencing run, the single lane of the flow cell 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.

Flow Cell	MiSeq Reagent Kit	Tiles	Imaging Surface	Total Tiles Imaged
Standard Flow Cell	MiSeq Reagent Kits, v3	19 tiles	Top and bottom	38 tiles total
PGS Flow Cell	MiSeq Reagent Kit v3-PGS	19 tiles	Top and bottom	38 tiles total
Standard Flow Cell	MiSeq Reagent Kits, v2	14 tiles	Top and bottom	28 tiles total
Micro Flow Cell	MiSeq Reagent Micro Kits, v2	4 tiles	Top and bottom	8 tiles total
Nano Flow Cell	MiSeq Reagent Nano Kits, v2	2 tiles	Top only	2 tiles total

When the tiles are imaged during the sequencing run, 1 output file is generated for each tile. For more information, see [Flow Cell Tile Numbering on page 52](#).

Flow Cell Tile Numbering

When the tiles are imaged during the sequencing run, an output file is generated for each tile and named with the tile number in a four digit format. With the exception of the nano flow cell, flow cells are imaged on the top and bottom surface. The output files for each tile are located in the run folder in Data\Intensities\BaseCalls\L001.

Flow Cell	MiSeq Reagent Kit	Tiles	Imaging Surface	Image File Names
Standard Flow Cell PGS Flow Cell	MiSeq Reagent Kits, v3	1-19	Top	1101 through 1119
		1-19	Bottom	2101 through 2119
Standard Flow Cell	MiSeq Reagent Kits, v2	1-14	Top	1101 through 1114
		1-14	Bottom	2101 through 2114
Micro Flow Cell	MiSeq Reagent Micro Kits, v2	1-4	Top	1101 through 1104
		1-4	Bottom	2101 through 2104
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