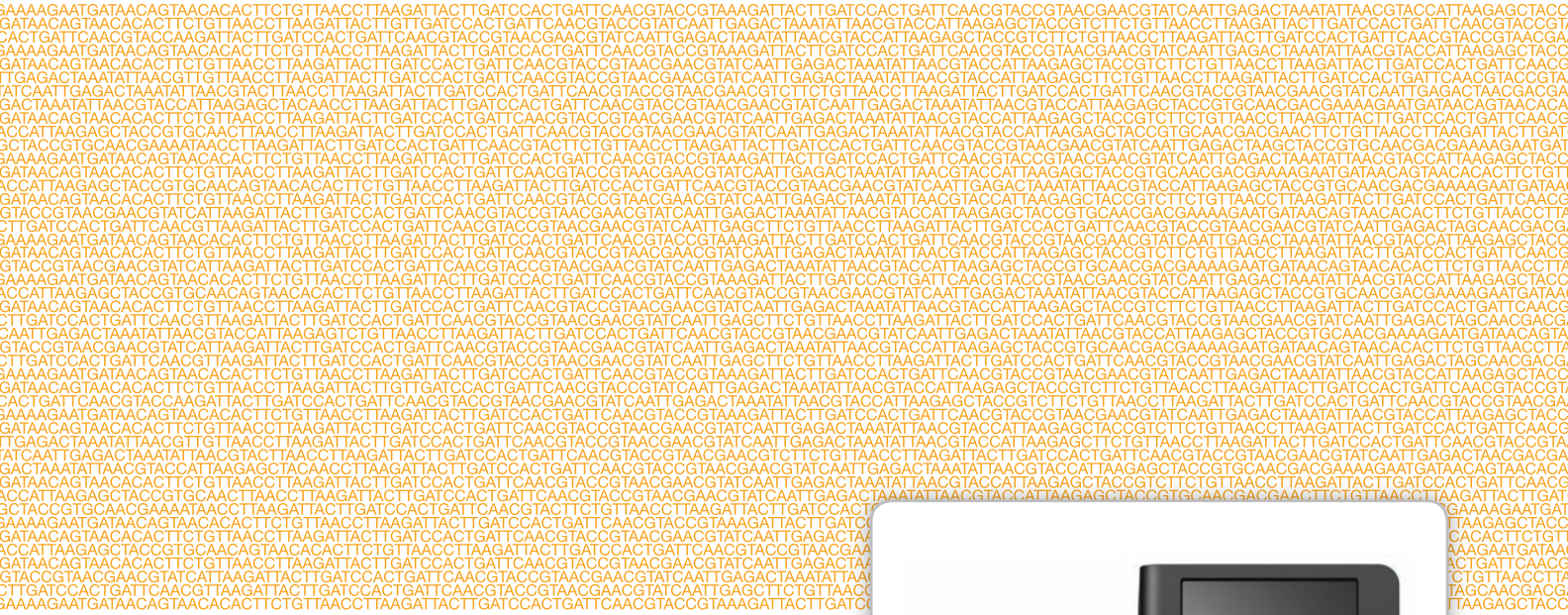




# MiSeq™ System

## User Guide



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Part # 15027617 Rev. A  
August 2011

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# Revision History

Part #	Revision	Date	Description of Change
15027617	A	August 2011	Initial release.



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# Getting Started

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## Introduction

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

### Applications

The MiSeq supports a wide range of applications, including the following:

- ▶ *De novo* sequencing
- ▶ Targeted resequencing
- ▶ Small genome sequencing
- ▶ Highly-multiplexed PCR amplicon sequencing
- ▶ Small RNA sequencing
- ▶ Library QC

### Library Preparation

You can sequence libraries prepared by any of the following Illumina sample preparation kits:

- ▶ TruSeq™ DNA Sample Preparation Kit
- ▶ TruSeq™ RNA Sample Preparation Kit
- ▶ TruSeq™ Small RNA Sample Preparation Kit
- ▶ TruSeq™ Custom Amplicon Kit
- ▶ Nextera™ Sample Preparation Kit

For more information, see *Applications and Related Consumables* on page 13.

## Features

- ▶ **Walkaway Automation**—All of the reagents required for cluster generation, sequencing, and paired-end chemistry are contained in a single reagent cartridge. After run setup, no additional hands-on time is required.
- ▶ **Prefilled Reagent Cartridge**—A specially designed single-use prefilled reagent cartridge that provides reagents for cluster generation and sequencing, including paired-end sequencing reagents. Radio-frequency identification (RFID) tracking enables positive consumable tracking.
- ▶ **Interface Controls**—MiSeq Control Software (MCS) offers a simple interface to configure and monitor your run, and perform maintenance procedures on a touch screen monitor.
- ▶ **Convenient Flow Cell Loading**—A clamping mechanism auto-positions the flow cell as you load it onto the instrument.
- ▶ **Innovative Fluidics Architecture**—Enables unmatched efficiency in chemistry cycle time during sequencing.
- ▶ **Real Time Analysis**—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.



## Related Documentation

This guide provides comprehensive information about the MiSeq System. However, other documentation is available for site preparation and safety guidelines, and as quick reference guides.

The following list describes related documentation and availability.

- ▶ ***MiSeq System Site Preparation Guide***—Provides specifications for laboratory space, electrical requirements, and environmental considerations.  
This guide is available for download from the Illumina website.
- ▶ ***MiSeq System Safety and Compliance Guide***—Provides safety-related labeling and considerations, and information about compliance certifications and labeling.  
This guide is provided with the instrument and available for download from the Illumina website.
- ▶ ***MiSeq System Quick Reference Guide***—Provides an overview of the instrument, and instructions for setting up and performing a run on the MiSeq. Use the quick reference guide after you are familiar with the contents of the *MiSeq System User Guide*.  
This guide is provided with the instrument and available for download from the Illumina website.
- ▶ ***MiSeq Reagent Preparation Guide***—Provides a description of kit contents, and instructions for preparing the reagent cartridge and sample libraries before beginning your sequencing run.  
This guide is provided with the MiSeq Reagent Kit and available for download from the Illumina website.
- ▶ ***MiSeq Sample Sheet Quick Reference Guide***—Provides information about sample sheets that is included in the *MiSeq System User Guide*, compiled into a quick reference guide.  
This guide is available for download from the Illumina website.

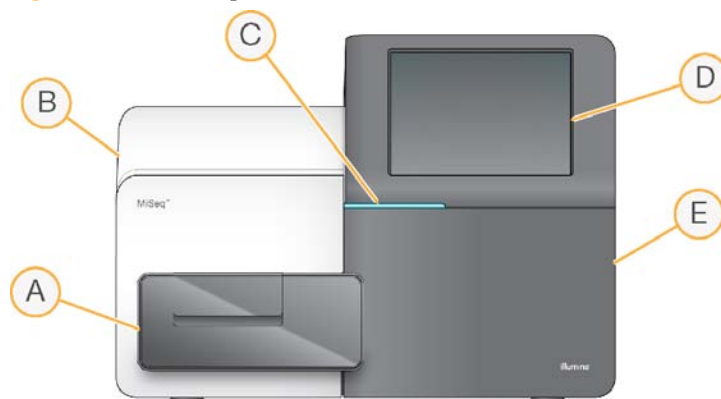
### Illumina Website

Visit the MiSeq support pages on the Illumina website (<http://www.illumina.com/systems/miseq/support.ilmn>) for access to documentation, related downloads, FAQs, and other support information.

## Components

The MiSeq System has the following main components.

Figure 1 Main Compartments



- A Flow Cell Compartment**—Contains the flow cell stage that houses the flow cell throughout the run. The flow cell stage is controlled by motors that move the stage out of the enclosed optical module for flow cell loading, and returns it when the run begins.
- B Enclosed Optics Module**—Contains optical components that enable imaging of the flow cell.
- C Status Bar**—Uses three colors to indicate instrument status. Blue indicates the instrument is running, orange indicates the instrument needs attention, and green indicates that the instrument is ready to begin the next run.
- D Touch Screen Monitor**—Enables easy run setup using the software interface and at-a-glance run monitoring.
- E Reagent Compartment**—Holds reagents used during runs, wash solutions used during instrument washes, and the waste bottle. The reagent compartment door is secured by a magnetic latch.

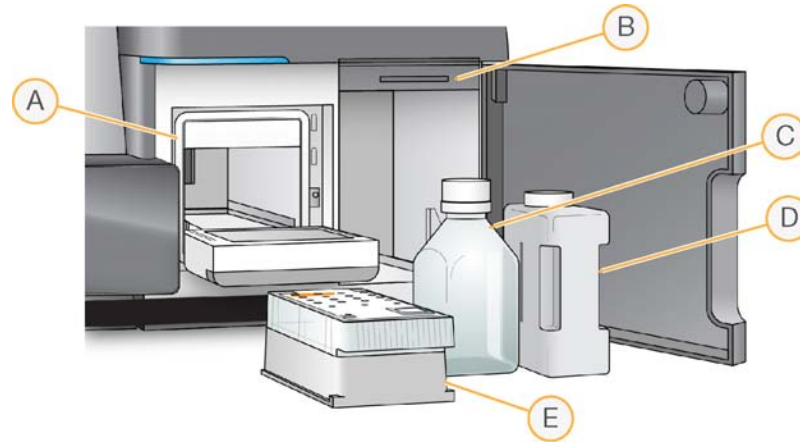
The MiSeq software interface guides you through the run setup steps using the touch screen monitor. To load run components, you need to access the reagent compartment and the flow cell compartment.

### Reagent Compartment

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

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

Figure 2 Reagent Compartment



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

**NOTE**

The reagent chiller is designed to maintain reagent temperature at 4°C. The chiller is not intended to cool down reagents; therefore, reagents must be cool before they are loaded onto the instrument.

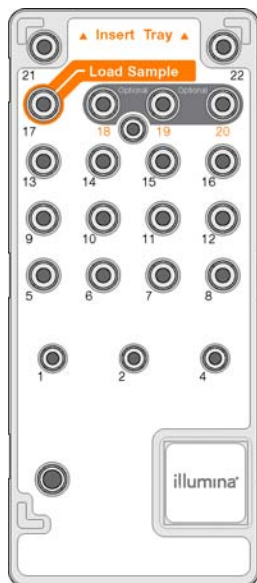
To the right of the reagent chiller are two form-fitted slots, one for the PR2 bottle and one for the waste bottle. After the bottles are in place, the sipper handle is lowered to lock the bottles in place and lower the appropriate sipper into each bottle.

Fluidics pumps inside the instrument direct reagents through the sippers, through the fluidics lines, and then through the flow cell. Reagent waste is delivered to the waste bottle throughout the process.

### Reagent Cartridge

The MiSeq reagent cartridge is a single-use consumable prefilled with cluster generation and sequencing reagents.

Figure 3 Reagent Cartridge



Each reservoir on the cartridge is numbered. The following table lists the reagent in each numbered position.

Position	Reagent Name	Description
1	IMF	Incorporation Mix
2	SRE	Scan Mix
4	CMF	Cleavage Mix
5	AMX1	Amplification Mix
6	AMX2	Read 2 Amplification Mix
7	LPM	Linearization Premix
8	LDR	Formamide
9	LMX1	Linearization Mix
10	LMX2	Read 2 Linearization Mix
11	RMF	Resynthesis Mix
12	HP10	Read 1 Primer Mix
13	HP12	Index Primer Mix
14	HP11	Read 2 Primer Mix
15	PW1	Water
16	PW1	Water
17	Sample	Your sample libraries
18	Optional	Optional use for custom sequencing primers
19	Optional	Optional use for custom sequencing primers
20	Optional	Optional use for custom sequencing primers
21	PW1	Water
22	Empty	Empty

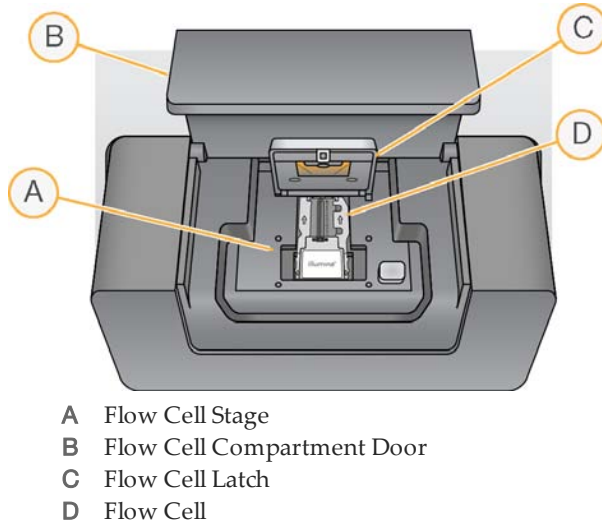
Before starting a run, your sample libraries must be loaded onto the reagent cartridge in the designated reservoir. You can sequence a single library or a pool of indexed libraries.

## Flow Cell Compartment

The flow cell compartment houses the flow cell stage, thermal station, and fluidics connections to the flow cell.

The flow cell is positioned on the flow cell stage, which moves in and out of the optics module. The flow cell is auto-positioned on the flow cell holder and held in place by the flow cell latch. When the flow cell latch closes, two pins near the latch hinge position the flow cell.

Figure 4 Flow Cell Stage



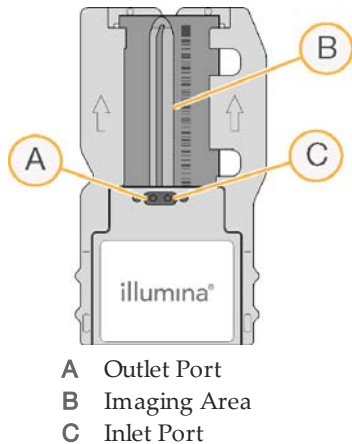
The thermal station, located beneath the flow cell stage, controls changes in flow cell temperature that are required for cluster generation and sequencing.

Fluidics connections deliver reagents to the flow cell through the inlet port, and return waste from the flow cell through the outlet port.

## Flow Cell

The MiSeq flow cell is a single-lane glass-based substrate in which clusters are generated and the sequencing reaction is performed. The single lane is divided into twelve image areas, called tiles, that are imaged by the instrument camera.

Figure 5 Flow Cell



Samples are loaded onto the reagent cartridge and then automatically transferred to the flow cell at the beginning of the cluster generation process.

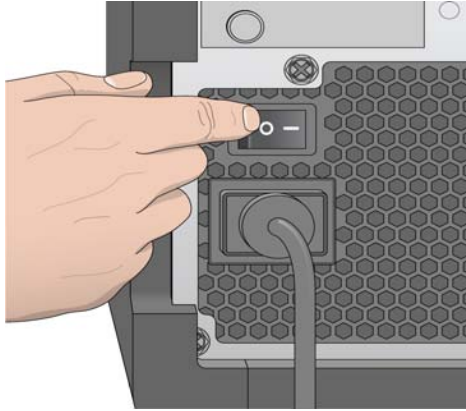
Reagents enter the flow cell through the inlet port, pass through the imaging area, and then exit the flow cell through the outlet port. Waste exiting the flow cell is delivered to the waste bottle.

## Starting the MiSeq System

Use the following instructions to start the MiSeq and instrument computer.

- 1 Reach around the right side of the instrument to locate the power switch on the back panel. It is in the lower corner directly above the power cord.

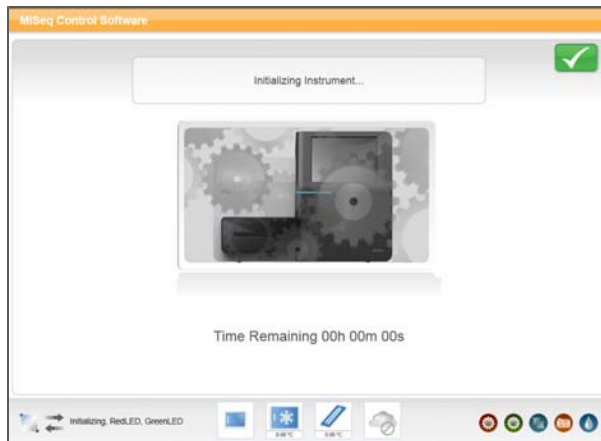
Figure 6 Power Switch Location



- 2 Turn the power switch to the ON position. The integrated instrument computer starts.
- 3 Log on to the operating system using the default user name. Wait until the operating system has completely loaded.
  - User name: sbsuser
  - Password: sbs123

When the system is ready, the MCS (MCS) launches and begins initialization.

Figure 7 MCS Initialization



After initialization is complete, the Welcome screen appears.

Figure 8 MCS Welcome Screen



- 4 To ensure adequate disk space, archive the data on the instrument computer from all previous runs to a network location. For more information, see *Manage Files* on page 22.

### Best Practice

It is best to leave the instrument on at all times. Turn off the instrument only if it will remain idle for more than ten days. For more information, see *Shutting Down the Instrument* on page 61.

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



# MiSeq Workflow

The MiSeq workflow consists of run setup steps, followed by automated cluster generation, then sequencing and primary analysis, and finally on-instrument secondary analysis.

The following steps are described in detail in *Performing a Run* on page 39.

## Setting Up Your Run



Create a sample sheet containing parameters specific to your run.



Prepare the prefilled reagent cartridge for use.



Dilute and denature your libraries, and then load your libraries onto the reagent cartridge in the designated reservoir.



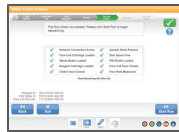
From the MCS interface, select **Sequence** to start the run set up steps.



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 Sequencing screen or on another computer using Sequencing Analysis Viewer (SAV).

## Cluster Generation

After the run is started, single DNA molecules are bound to the surface of the flow cell, and then bridge-amplified to form clusters.

## Sequencing

Following cluster generation, clusters are imaged using LED and filter combinations specific to each of the four fluorescently-labeled dideoxynucleotides. After imaging of one tile of the flow cell is complete, the flow cell is moved into place to expose the next tile and the process is repeated. Following image analysis, Real Time Analysis (RTA) launches to perform basecalling, filtering, and quality scoring.

## Analysis

When the run is complete, the MiSeq Reporter analysis software launches automatically and begins secondary analysis. You can monitor secondary analysis using an internet connection from another computer.

For more information, see *Secondary Analysis Using MiSeq Reporter* on page 66.



### NOTE

If a new sequencing run is started on the MiSeq before secondary analysis is complete, the current analysis is stopped. From the MiSeq Reporter interface, you can requeue secondary analysis for that run after the new sequencing run is complete. At that point, secondary analysis starts over from the beginning.

Alternatively, MiSeq Reporter can be run off-instrument allowing secondary analysis to occur during a subsequent sequencing run. For more information, see *Using MiSeq Reporter Off-Instrument* on page 68.

## Approximate Run Duration

Run duration depends on the number of cycles you perform. You can perform a paired-end sequencing run up to 2x150 cycles, or as few as 36 cycles for some applications.

Generally, one cycle takes about five minutes. The following table lists the approximate durations for cluster generation, sequencing, and on-instrument analysis.

Process	Read Length	Duration
Cluster Generation and Sequencing	36 Cycles (36 bp)	Less than 4 hours
	50 Cycles (50 bp)	Less than 6 hours
	Paired-End 150 Cycles (2x150 bp)	Less than 27 hours
On-Instrument Analysis and Variant Calling	Paired-End 150 Cycles (2x150 bp)	Less than 2 hours for all analysis workflows, except 16s metagenomics. 16s metagenomics completes in less than 24 hours.

## Illumina-Supplied Consumables

To perform a run on the MiSeq, you need one MiSeq Reagent Kit.

### MiSeq Reagents

The MiSeq Reagent Kit is available in the following sizes:

- ▶ **300 Cycles**—Provides kitted reagents for up to 323 cycles of sequencing on the MiSeq. This includes sufficient reagents for a 151-cycle paired-end run, plus two eight-cycle indexed reads.
- ▶ **50 Cycles**—Provides kitted reagents for up to 73 cycles of sequencing on the MiSeq. This includes sufficient reagents for a 26-cycle paired-end run, or a 51-cycle single-read run, plus two eight-cycle indexed reads.

### MiSeq Reagent Kit Contents

#### Box 1: Store at -15° to -25°C

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

#### Box 2: Store at 2° to 8°C

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

### Applications and Related Consumables

The following table lists applications supported on the MiSeq, the recommended read length for the run, and the associated Illumina-supplied consumables.

Application	Read Length	MiSeq Reagent Kit (# of cycles)	Sample Preparation Kit
TruSeq Custom Amplicon	2 x 151	300 Cycle Kit	TruSeq Custom Amplicon Library Prep
Nextera Amplicon	1 x 36	50 Cycle Kit	Nextera Sample Prep
Standard Amplicon	2 x 151	300 Cycle Kit	TruSeq DNA Sample Prep
Small Genome: <i>De novo</i>	2 x 151	300 Cycle Kit	TruSeq DNA Sample Prep
Small Genome: Resequencing	1 x 36	50 Cycle Kit or 300 Cycle Kit	TruSeq DNA Sample Prep or Nextera Sample Prep
16S Metagenomics	2 x 151	300 Cycle Kit	TruSeq DNA Sample Prep or Nextera Sample Prep

Application	Read Length	MiSeq Reagent Kit (# of cycles)	Sample Preparation Kit
LibraryQC	2 x 25	50 Cycle Kit	Open
Small RNA	1 x 36	50 Cycle Kit	TruSeq Small RNA Sample Prep
RNA-Seq	1 x 51	50 Cycle Kit	TruSeq RNA Sample Prep
TruSeq Custom Enrichment	2 x 76	300 Cycle Kit	TruSeq DNA Sample Prep

## User-Supplied Consumables

Make sure that you have all of the user-supplied consumables listed in this section before you begin your run.

**Table 1** User-Supplied Consumables

Consumable	Supplier	Purpose
1 N NaOH	General lab supplier	Denaturing sample libraries Diluting PhiX control
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 15648-981  General lab supplier	Cleaning the flow cell holder
Disposable gloves, powder-free	General lab supplier	General use
Lab tissue, low-lint	VWR, catalog # 21905-026	Cleaning the flow cell stage
Lens paper, 4 x 6 in.	VWR, catalog # 52846-001	Cleaning the flow cell
Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20	General lab supplier	Diluting PhiX control
Tweezers, square-tip plastic	McMaster-Carr, catalog # 7003A22	Removing flow cell from flow cell container
Water, laboratory-grade	General lab supplier	Washing the instrument

### Guidelines for Laboratory-Grade Water

The following list provides acceptable examples of laboratory-grade water. Never use tap water or deionized water on the instrument.

- ▶ Illumina PW1
- ▶ 18 M Ohm water
- ▶ Milli-Q water
- ▶ Super-Q water
- ▶ Molecular biology-grade water



# MiSeq Control Software

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## Introduction

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

During the run, MCS operates the flow cell stage in the optical compartment, gives commands to dispense reagents, changes temperatures of the flow cell, and captures images of clusters on the flow cell for image analysis. MCS performs the run based on parameters specified in the sample sheet. For more information, see *Sample Sheets* on page 30.

### Real Time Analysis (RTA)

After image analysis is complete, the integrated primary analysis software, Real Time Analysis (RTA), performs base calling and assigns a quality score to each base for each cycle. Quality scores can be monitored throughout the run on the MCS Sequencing screen. For more information, see *Sequencing Screen* on page 27.

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

### Sequencing Analysis Viewer (SAV)

To monitor your run in greater detail from another computer, you can use the Sequencing Analysis Viewer (SAV), an optional software application available for download from the Illumina website. For more information, see *Sequencing Analysis Viewer* on page 28.



## MiSeq Control Software Interface

The MiSeq Control Software (MCS) includes an intuitive interface that prompts you through the run setup steps, instrument setup options, and instrument washes. The interface includes a Sequencing screen that provides information about the run as it progresses.

### Welcome Screen

The interface opens to the Welcome screen when the software launches, and returns to the Welcome screen when a run is complete.

Figure 9 MCS Welcome Screen



The Welcome screen has the following options:

- ▶ **Sequence**—This option opens a series of run setup screens that guide you through the setup steps for your run. For more information, see *Run Setup Screens* on page 24.
- ▶ **Maintenance Wash**—Use this option to perform an instrument wash before a sequencing run.
- ▶ **Manage Instrument**—Provides links to **Setup Options**, **Manage Runs**, and **Perform Diagnostic**. For more information, see *Manage Instrument* on page 20.
- ▶ **About**—Provides information about instrument configuration and software versions.
- ▶ **Get Updates**—This option appears on the Welcome screen only if a software update is available. Your MiSeq must be connected to a network to enable this option. For more information, see *Get Updates* on page 20.

### Activity Indicators






In the lower-right corner of the Welcome screen are a series of icons. Each icon is an activity indicator that tells you which activity the instrument is performing. These appear throughout the interface and represent the Y-stage motor, Z-stage motor, electronics functionality, camera, and fluidics system.

Figure 10 Activity Indicators



## Status Icons

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

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

When a change in condition occurs, the icon changes to the associated image and blinks to alert you. If this happens, select the icon to open the status window, which contains a general description of the condition.

- ▶ Select any item listed to see a detailed description of the condition and instructions to resolve the condition, if applicable.
- ▶ Select **Acknowledge** to accept the message and **Close** to close the dialog box.

You can filter the types of messages that appear in the status window by selecting the icons along the top margin of the window. Selecting an icon toggles the condition to show or to hide.

## Get Updates

You can update the instrument software from the MCS Welcome screen using the Get Updates feature. Your system must be connected to a network with internet access to download updates.

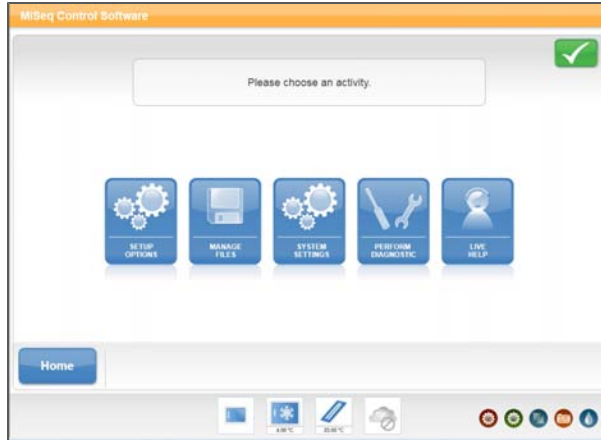
When software updates are available, the Get Updates button appears on the Welcome screen. Select **Get Updates**. A dialog box opens asking you to confirm the command, after which a reboot of the MiSeq is required. Installation of the update begins automatically upon reboot.

The Get Updates button appears on the Welcome screen only when updates are available.

## Manage Instrument

The Manage Instrument screen contains controls for setting up folder locations and wash preferences, moving and deleting files, managing recipe files and genome reference files, and performing an instrument diagnostic.

Figure 11 Manage Instrument Screen

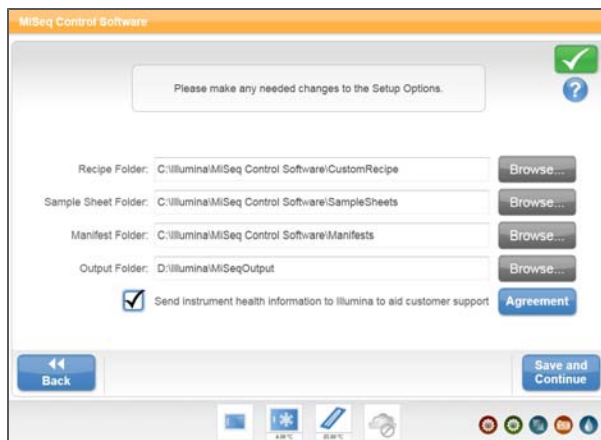


- ▶ **Setup Options**—Provides options for changing default locations of data folders. For more information, see *Setup Options* on page 21.
- ▶ **Manage Files**—Provides controls for moving and deleting files on the instrument computer. For more information, see *Manage Files* on page 22.
- ▶ **System Settings**—Provides the option to change IP Settings, machine name, or domain. For more information, see *System Settings* on page 23.
- ▶ **Perform Diagnostic**—Provides instrument diagnostic options for use by an Illumina Technical Support representative during a Live Help session. Use of this feature is not required during normal operation or for instrument maintenance.
- ▶ **Live Help**—Connects your instrument to Illumina Technical Support, allowing a representative to access your instrument computer for troubleshooting. You must have a network connection to use this feature. For more information, see *Live Help* on page 23.

## Setup Options

From Setup Options, you can set the default folder locations for recipes, sample sheets, manifests, and the output folder.

Figure 12 Setup Options



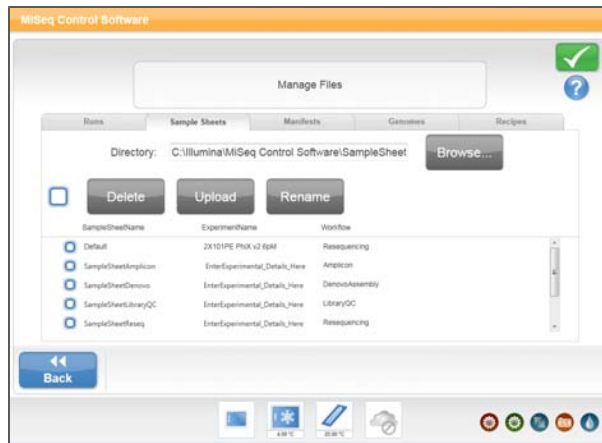
## Folder Locations

- ▶ **Recipes**—Sets the default location for recipes. Recipes are XML files that the software uses to perform the sequencing run. A recipe is created at the start of the run based on parameters that you provide in the sample sheet. After a run-specific recipe is created, it is copied to the run folder. If you pause the run and resume it later, this recipe restarts where the run was paused.
- ▶ **Sample Sheets**—Sets the default location for sample sheets. Sample sheets are files containing run parameters that are copied onto the instrument before you start your run.  
For more information, see *Sample Sheets* on page 30.
- ▶ **Manifests**—Sets the default location for manifests. Manifests are files used only with custom amplicon sequencing. They contain the amplicon sequences to which clusters are aligned during analysis. Manifests are provided when you order your custom assay (CAT). There is no need to modify the manifest.
- ▶ **MiSeqOutput**—Sets the default location for the output files. Illumina recommends that you change the default output folder location to a network location.  
For more information, see *Run Folders* on page 36.

## Manage Files

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

Figure 13 Manage Files Screen



- ▶ **Browse**—Select **Browse** to point to the appropriate output folder or folders other than the default folders containing sample sheets, manifests, genomes, or recipes.
- ▶ **Delete**—Select the checkbox next to the file or folder listed, and then select **Delete**. The Delete feature is available on all tabs.
- ▶ **Move**—Available only for runs. Select the checkbox next to the folder, and then select **Move**. A window opens and prompts you to browse to an appropriate location.
- ▶ **Select All Files**—Select the checkbox to the left of the Delete button, and then select an action, Delete or Move. The action is applied to all files or folders selected.

- ▶ **Upload Files**—Available for sample sheets, manifests, genomes, and recipes. Select **Upload**. A window opens that prompts you to browse to a location where the file resides. The file is uploaded to the folder indicated in the Directory field.
- ▶ **Rename**—Available only for sample sheets. Select the checkbox next to the file, and then select **Rename**. Use the online keyboard to rename your sample sheet.

## Available Features By Tab

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

## System Settings

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

Figure 14 System Settings



System Settings configure the following components:

- ▶ IP address and Domain Name Server (DNS) address
- ▶ Machine name
- ▶ Domain name
- ▶ MiSeq Reporter Service credentials

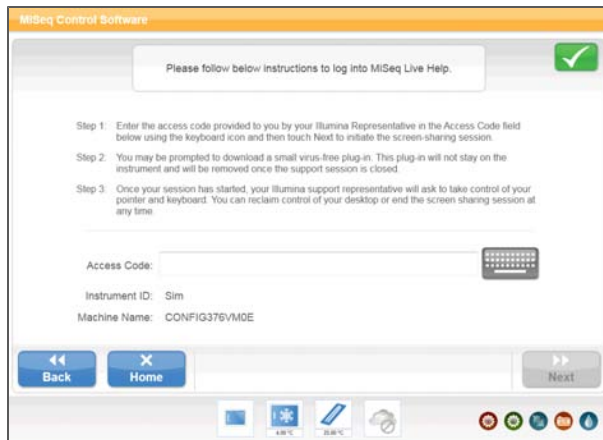
Contact your local IT representative to get information on your network settings.

## Live Help

The Live Help feature is an online assistance tool that enables a member of Illumina Technical Support to view your screen with your permission, and share control of your instrument. You have overriding control and can end the screen-sharing session at any time.

To enable a connection, you must obtain a unique connection code from Illumina Technical Support.

Figure 15 Live Help Screen



- 1 Contact Illumina Technical Support to obtain a unique access code.
- 2 Enter your unique access code using the online keyboard.
- 3 Select **Next** to begin your screen-sharing session.

## Run Setup Screens

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

## Set Cloud Option

The first screen in the run setup steps prompts you to log onto the Cloud. Use the Cloud to store data analysis files. To enable access, you need a network connection and an iCom account.

Figure 16 Cloud Option Screen

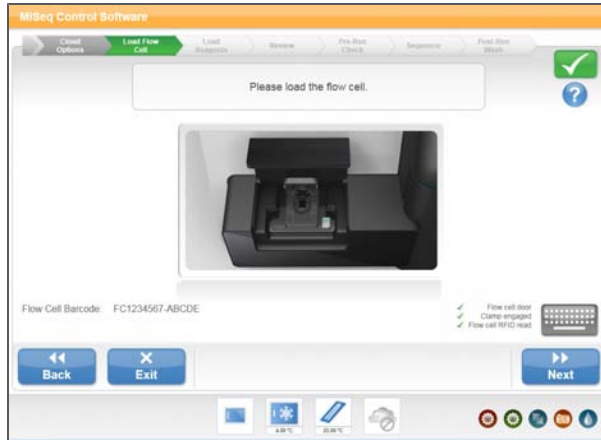


Using the Cloud feature is optional. If you do not want use it, clear the checkbox on the screen.

## Load Flow Cell

The Load Flow Cell screen prompts you to load the flow cell. When the flow cell is loaded, the software reads the RFID of the flow cell. A confirmation that the RFID was successfully read appears in the lower-right corner of the screen.

Figure 17 Load Flow Cell Screen



If for some reason the RFID is not read, select the online keyboard icon and manually enter the flow cell ID.

After the flow cell is loaded, close the flow cell latch and flow cell compartment door before proceeding. Both the latch and compartment door must be closed before beginning the run.

## Load Reagents

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

If for some reason the RFID is not read, select the online keyboard icon and manually enter the barcode number located below the barcode on the component label.

Figure 18 Load PR2 Bottle and Waste Bottle

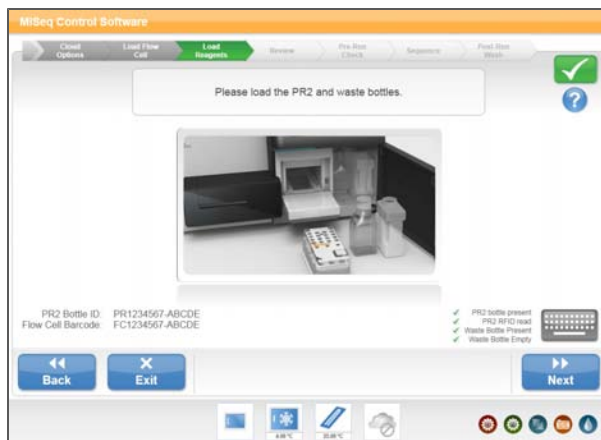
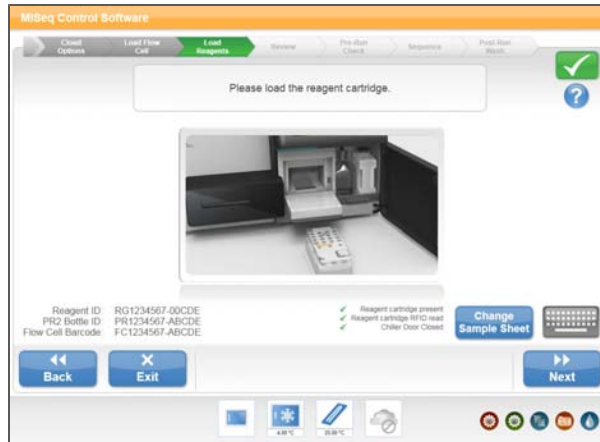


Figure 19 Load Reagent Cartridge



## Change Sample Sheet

Every run must have a unique sample sheet for the run. If the software cannot find an appropriately named sample sheet, a message appears that asks you to browse to the location of your sample sheet.

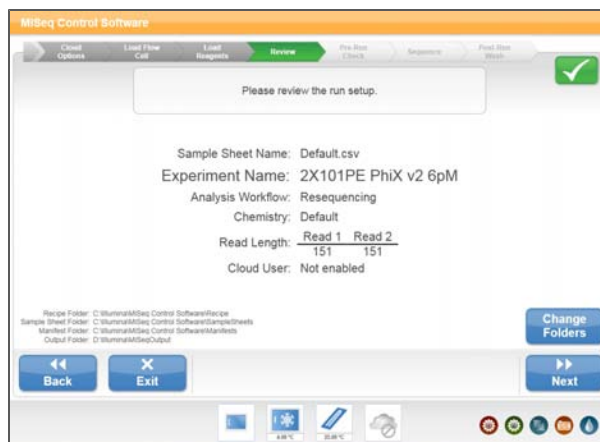
From the Load Reagents screen, you can use **Change Sample Sheet** to specify a different sample sheet for your run. A screen opens that allows you to browse to a location where you stored the sample sheet.

## Review

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

- ▶ Experiment name
- ▶ Analysis workflow
- ▶ Read length

Figure 20 Review Screen





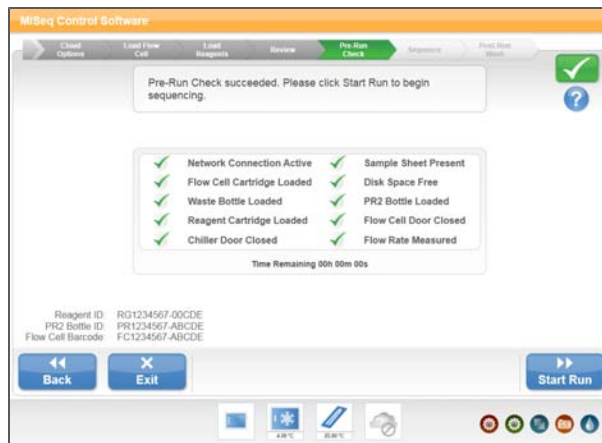
## Change Folders

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

## Pre-Run Check

The software automatically performs a pre-run check to ensure that required run components and conditions are satisfied before allowing the run to begin. If any errors occur during the pre-run check, a message appears on the screen telling you where the error occurred and what action is needed to correct it. For more information, see *Software Errors* on page 71.

Figure 21 Pre-Run Check

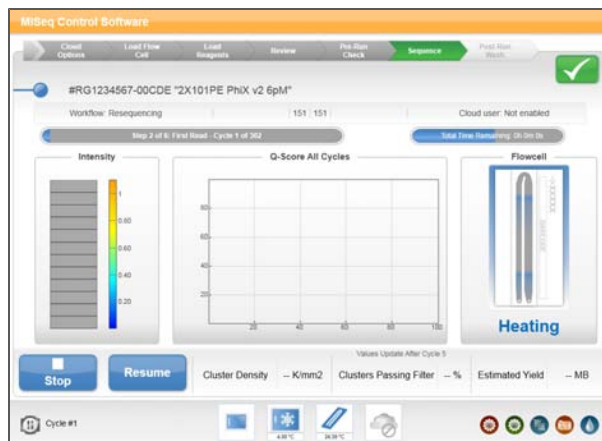


When the pre-run check is successful, the **Start Run** button becomes active. You are ready to start your run.

## Sequencing Screen

The Sequencing screen provides a visual representation of run progress, intensities, base call quality scores (Q-scores), and activity on the flow cell.

Figure 22 Sequencing Screen



- ▶ **Run Progress**—Shows run progress in a status bar and lists the number of cycles completed.
- ▶ **Intensities**—Shows the value of cluster intensities of 90% of data for each tile.
- ▶ **Q-Score All Cycle**—Shows the average percentage of bases greater than Q30. A quality score (Q-score) is a prediction of the probability of an error in base calling.

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

- ▶ **Flow Cell**—Shows the current temperature of the flow cell indicated by color. Blue indicates cooler temperatures, while orange and red indicate warmer temperatures. During imaging, imaged tiles appear dark gray. During chemistry, wavy lines indicate that reagents are pumping through the flow cell.



## NOTE

The MiSeq is sensitive to vibration. Vibrations occurring during a run will adversely impact sequencing results. Do not touch the instrument or touch screen monitor during normal run operations unless you plan to stop or pause your run.

## Sequencing Analysis Viewer

You can monitor your run in greater detail using the Sequencing Analysis Viewer, a downloadable software application that is installed onto a computer independent of the MiSeq, but with access to the same network connected to the instrument. After launching the software, you can browse to the run folder for your run.

Real Time Analysis (RTA) uses the first four cycles of the sequencing run for template generation. Template generation is the process by which cluster positions over the entire flow cell surface are defined along the X and Y axes. After the template of cluster positions has been generated, the images produced over every subsequent cycle of imaging are aligned against the template so that individual cluster intensities in all four nucleotide color channels can be extracted, and basecalls produced from the normalized cluster intensities.

After cycle 4, the Sequencing Analysis Viewer provides metrics generated by RTA and organizes the metrics into plots, graphs, and tables. Your MiSeq must be networked to use Sequencing Analysis Viewer.



## NOTE

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

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

## Stopping or Pausing a Run

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

- ▶ **Stop a run**—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.
- ▶ **Pause a run**—You might pause a run if you suspect that the waste bottle is full or to check the volume in the PR2 bottle.

### Stopping Run

You can stop a run before it has completed using the **Stop** button on the Sequencing screen.

*Stopping a run is final.* You cannot resume a run after it was stopped using the Stop button. When a run is stopped, the flow cell tray moves to the forward position. You are ready to unload run components and perform an instrument wash.

### Pausing a Run

You can temporarily interrupt a run before it has completed using the Pause button on the Sequencing screen. When you select Pause, the current command is completed, after which the run is paused and the flow cell is placed in a safe state.

#### Best Practice

Empty the waste bottle before each run and make sure that run consumables are properly loaded before starting the run.



#### NOTE

On a paused run, the flow cell is stored in safe state for a maximum of three days. After three days, the instrument flow cell temperature control will turn off.

## Sample Sheets

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

During the run setup steps, the software automatically looks for a sample sheet associated with reagent cartridge loaded onto the instrument in the previous run setup step. Therefore, Illumina recommends that you name your sample sheet with the barcode number of the reagent cartridge that you will use for your run, followed by \*.csv extension. The barcode number is located on the cartridge label directly below the barcode.

Before starting your run, copy your sample sheet to the instrument computer or to the network location indicated in Setup Options. When your run begins, the software copies your sample sheet to the root of your run folder.

At the end of your run, the sample sheet is used for secondary analysis by the MiSeq Reporter analysis software.

### Sample Sheet Template

You can open and edit the sample sheet template in Excel or in Notepad. A sample sheet template is available for download from the Illumina website at <http://www.illumina.com>. Go to the MiSeq support page and click **Downloads**. An iCom account is required.



#### NOTE

Do not use commas within a field in the sample sheet. If a comma is included within a field, your sample sheet will not be read correctly by the software.

### Illumina Experiment Designer

You can use the Illumina Experiment Designer to create your sample sheet, a wizard-based application for creating the sample sheet. The Experiment Designer guides you through the steps to create your sample sheet based on the analysis workflow for your run.

The Experiment Designer provides a feature for recording parameters for your sample plate, such as sample ID, dual indices, and other parameters applicable to your 96-well plate for Nextera, TruSeq DNA, TruSeq RNA, Small RNA, and Amplicon library preparation protocols. Using the Experiment Designer, you can import the sample plate parameters into your sample sheet. For more information, see the *Experiment Designer Online Help* integrated with the application.

The Illumina Experiment Designer can be run on any Windows platform. You can download the Experiment Designer from the Illumina website at <http://www.illumina.com>. Go to the MiSeq support page and click **Downloads**. An iCom account is required.

## Sample Sheet Parameters

The sample sheet is organized by section text indicated by [ ] brackets, such as [Header], [Reads], [Manifests], and [Data]. Section text within brackets is case-sensitive. All other fields are not case-sensitive.

Figure 23 Sample Sheet Example in Excel

Not all sections of the sample sheet are applicable to every run. Applicable sections depend on the analysis workflow used for your run.

### [Header]

The Header section of the sample sheet contains information about your run. Extra fields are ignored.

Parameter	Description
Investigator Name	Your name
Project Name	Project name of your preference
Experiment Name	Experiment name of your preference
Date	Date of your experiment
Workflow	The analysis workflow for your run. You must type the analysis workflow name exactly as shown using one of the following options: Resequencing, Amplicon, DenovoAssembly, SmallRNA, Metagenomics, or LibraryQC. If you are using the Experiment Designer, select your workflow from the drop-down menu. For more information, see <i>Analysis Workflow</i> on page 35.
Chemistry	The folder that contains recipe fragments used to build the recipe. If this field is blank, the system uses the default recipe fragments. For TruSeq RNA or TruSeq DNA libraries, this field can be blank and use the default settings. For any workflows that use dual indexing, specifically Nextera and TruSeq Custom Amplicon, the chemistry field is required. Enter "amplicon" in this field.

### [Reads]

Reads indicates the number of sequencing cycles in each read.

Parameter	Description
Number of cycles for Read 1	Read length of a target sequence. The number of cycles for the index read is indicated by the index sequence the [Data] section.
Number of cycles for Read 2	Read length of a target sequence.

## [Manifests]

This section of the sample sheet is mandatory for the Amplicon analysis workflow. You do not need to populate this section if you are using other analysis workflows.

Parameter	Description
Name of manifest file	Name of your manifest file provided by Illumina with your custom assay (CAT). The manifest file must reside in the same folder location as the sample sheet. When you enter your manifest name, omit the file extension (*.txt). More than one manifest can be specified: one manifest for your custom CAT, and one manifest for the control CAT.

## [SiteReports]

This section of the sample sheet is used by the Resequencing and Amplicon analysis workflows.

Parameter	Description
Name of SiteReport input file	Each line in the SiteReports section is the name of a SiteReport input file. This file designates positions on a given chromosome to report the genotype found at that position. You can use more than one input file for a run. The SiteReport input file must reside in the same folder location as the sample sheet. The SiteReport input file is a comma-separated value (*.csv) format file that must adhere to the following format: <ul style="list-style-type: none"> <li>• The first line are column names. Required columns are Position and Chromosome.</li> <li>• Additional user-defined columns are appended to the resulting analysis report for that position.</li> </ul> When you enter your SiteReport input file name, omit the file extension (*.csv).

## [Data]

In this section, list sample information one sample per line. There must be at least one sample in this section. Each analysis workflow requires different columns in this section. Column order is not important.

Parameter	Description
SampleID	Required. This is usually a barcode but can have any value.
Sample_Name	Required. This is used in the reporting web page.
Index	Required for multi-sample assays. Nucleotide sequence in text format. Valid characters are A, C, G, T, and N, where N matches any base.
Index2	Required for multi-sample assays with dual indexing. Nucleotide sequence in text format. Valid characters are A, C, G, T, and N, where N matches any base.

## Additional [Data] Columns for Resequencing Workflow

Parameter	Description
GenomeFolder	Required. The reference genome folder containing the FASTA files to be used in the alignment step.

## Additional [Data] Columns for Amplicon Workflow

Parameter	Description
GenomeFolder	Recommended. It is used to provide variant annotations, if available, and to set the chromosome sizes in the *.bam file output.

## Additional [Data] Columns for DenovoAssembly Workflow

Parameter	Description
GenomeFolder (or Genome)	Optional. If you populate this field, list the absolute path to the genome folder. <ul style="list-style-type: none"> <li>• If the folder listed does not exist, MiSeq Reporter combines the GenomePath configuration setting with the genome string.</li> <li>• If the path does not exist, MiSeq Reporter stops processing.</li> </ul>

## Additional [Data] Columns for SmallRNA Workflow

Parameter	Description
GenomeFolder	Optional. A string identifier for the sample. This is usually a barcode but can have any value. If provided, clusters are aligned against the full reference genome.
Contaminants	String; required. The path to the folder containing the FASTA files of contaminants.
miRNA	Required. The path to the folder containing FASTA files of mature miRNAs.
RNA	Required. The path to folder containing FASTA files of small RNAs.

## Additional [Data] Columns for Library QC Workflow

Parameter	Description
GenomeFolder	Required. The reference genome folder containing the FASTA files to be used in the alignment step.

## [Settings]

Settings are optional in the sample sheet. This section describes possible entries in the Settings section.

Parameter	Description
FilterPCRDuplicates	Setting are 0 or 1. Default is 1, no filtering. If set to 0, PCR duplicates are filtered out from subsequent analysis. Duplicates are defined as paired-end reads where both reads have the exact same alignment positions.
MinQScore	Specifies the Q-score threshold for variant calling.
OnlyGenerateFastq	Setting are 0 or 1. Default is 0. If set to 1, MiSeq Reporter generates FASTQ files and then exits the workflow. This option is provided as a shortcut if you prefer to use third-party tools to process FASTQ files.

Parameter	Description
Adapter	It is possible that some clusters will sequence beyond the sample DNA and read bases from the adapter. Specify the adapter sequence here to mask the adapter sequence. Masking the adapter sequence improves accuracy and speed during secondary analysis.



## Analysis Workflow

The analysis workflow is a procedure performed by MiSeq Reporter, the secondary analysis software on the MiSeq. Secondary analysis begins after the Real Time Analysis (RTA) software has completed base call analysis.

For more information, see *On-Instrument Analysis* on page 63.

The MiSeq supports six analysis workflows. The analysis workflow for your run is specified in the sample sheet. When you enter the analysis workflow in the sample sheet, enter the name as shown below in the Workflow column.

Workflow	Applications	Output
Targeted Resequencing Enter: <b>Resequencing</b>	Sequencing of an enriched portion of the human genome, or of a small genome such as <i>E. coli</i> . Reads are aligned against the reference and variants are noted.	Aligned reads in *.bam format. Variant calls in *.vcf format.
Amplicon Sequencing Enter: <b>Amplicon</b>	Sequencing of PCR amplicons from probes targeting particular genome positions (up to approx. 384 loci from up to approx. 96 samples). A genomic reference is recommended, but not required. Including a reference means that the genome size in the *.bam file headers is correct, and enables annotation of variants in dbSNP or within genes. Reads are aligned against a manifest file specified in the sample sheet. Two manifests can be specified: one for the control and one for the sample.	Aligned reads in *.bam format. Variant calls in *.vcf format.
<i>De novo</i> Assembly Enter: <b>DenovoAssembly</b>	Assembly of small (<20 Mb) genomes from reads without the use of a genomic reference. If a genomic reference is included, a dot-plot is generated with respect to the reference.	Contigs in FASTA format.
Small RNA Sequencing Enter: <b>SmallRNA</b>	Sequencing of miRNA. Requirements include a genomic reference, a contaminants database, an RNA database, and a mature miRNA database.	Reports on the relative abundance of each record.
Metagenomics Enter: <b>Metagenomics</b>	No genomic reference is required for metagenomics workflow. Reads are classified using a database of 16S rRNA data, which is included with the MiSeq Reporter software.	Read classifications by taxonomic group.
Library QC Enter: <b>LibraryQC</b>	Fast resequencing of a reference genome to QC the DNA library and generate per-sample statistics.	Per-sample summary statistics.

## Run Folders

Each run on the MiSeq generates a run folder on either the local drive or a network location. You can specify the location of the run folder in the MiSeqOutput field on the Setup Options screen. For more information, see *Setup Options* on page 21.

Run folders contain the data files produced during the run by Real Time Analysis (RTA), which is integrated with MCS and performs image analysis and base calling. The data files in the run folder are later used for secondary analysis. For more information, see *On-Instrument Analysis* on page 63.

### Run Folder Name

By default, the folder is named in the following format:

YYMMDD\_<InstrumentNumber>\_<Run Number>\_<Flow Cell ID>

Example: 110114\_SN106\_0016\_A90095ACXX

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

## Available Disk Space

The software checks available disk space before beginning a run. If there is not enough disk space for your run, the software pauses the run and a message alerts you. Required space for your run depends on the number of cycles in your run and the analysis workflow for your run. The required disk space for your run and the amount of disk space you must clear, is listed in the message.

If you get this message, you need to make disk space available to continue the run. Go to Manage Files and click the **Runs** tab. For more information, see *Manage Files* on page 22. When sufficient disk space is available, the run automatically resumes.

The integrated instrument computer has a storage capacity of 600 GB. A 2x150-cycle run results in approximately 4.2 GB of stored data.



# Performing a Run

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## Introduction

To perform a run on the MiSeq, you first need to prepare for the run using the following steps described in this chapter:

- ▶ Create a sample sheet and copy it to the instrument or an accessible location.
- ▶ Prepare the reagent cartridge for use.
- ▶ Denature and dilute your sample libraries.
- ▶ Load your sample libraries onto the prepared reagent cartridge.
- ▶ Set up the run using the MCS interface.

After the run begins, the MiSeq is completely automated and no other user intervention is required. The sequencing run can be monitored from the Sequencing screen or monitored remotely using the Sequencing Analysis Viewer, an optional software application that you can download from the Illumina website.

After your run is complete, perform an instrument wash.

## Setting Up the Sample Sheet

To set up a run on the MiSeq, you first need to create the sample sheet for your run. For more information, see *Sample Sheets* on page 30.

When your sample sheet is ready, copy the sample sheet to a network location connected to your instrument. If your instrument is not connected to a network, you can copy the sample sheet to the instrument using a USB flash drive.

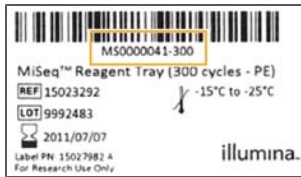
You can create the sample sheet using the sample sheet template or the Illumina Experiment Designer, a wizard-based software application available for download from the Illumina website. For more information, see *Illumina Experiment Designer* on page 30.



**NOTE**

Illumina recommends that you name your sample sheet with the barcode number of the reagent cartridge that you will use for your sequencing run. The barcode number is located on the cartridge label.

- 1 Name your sample sheet with the reagent cartridge barcode number followed by \*.csv extension.



- 2 Create your sample sheet off-instrument using the Illumina Experiment Designer or the sample sheet template. Enter the following information:
  - a Enter investigator name, project name, experiment name, and date.
  - b Enter the appropriate analysis workflow (no spaces): Resequencing, Amplicon, DenovoAssembly, SmallRNA, Metagenomics, or LibraryQC.
- 3 Enter the number of cycles for each read. To add an Index Read, include an index sequence for each sample in the [Data] section.
- 4 Complete the remaining sample sheet sections specific to your analysis workflow.

Analysis Workflow	[Manifests]	[SiteReports]	[Data]
Resequencing	Not applicable	Optional	Required: SampleID, Sample_Name, GenomeFolder Optional: Index, Index 2
Amplicon	Required	Optional	Required: SampleID, Sample_Name Optional: Index, Index 2, GenomeFolder
DenovoAssembly	Not applicable	Not applicable	Required: SampleID, Sample_Name Optional: Index, Index 2, GenomeFolder
SmallRNA	Not applicable	Not applicable	Required: SampleID, Sample_Name, Contaminants, miRNA, RNA Optional: Index, Index 2, GenomeFolder

Analysis Workflow	[Manifests]	[SiteReports]	[Data]
Metagenomics	Not applicable	Not applicable	Required: SampleID, Sample_Name Optional: Index, Index 2
LibraryQC	Not applicable	Optional	Required: SampleID, Sample_Name, GenomeFolder Optional: Index, Index 2



**NOTE**

For TruSeq Custom Amplicon sample sheets, see the *TruSeq Custom Amplicon Library Preparation Guide* for the appropriate index sequences.

For Nextera DNA sample sheets, see the *Nextera DNA Sample Preparation Guide* for the appropriate index sequences.

- 5 Copy the sample sheet to the network location specified in Setup Options, or copy it to the instrument using a USB flash drive and the Manage Files feature accessible from the Welcome screen.



## Preparing the Reagent Cartridge

- 1 Remove the reagent cartridge from  $-15^{\circ}$  to  $-25^{\circ}\text{C}$  storage.
- 2 Place the reagent cartridge in a water bath containing only enough room temperature deionized water to submerge the base of the reagent cartridge without reaching the clear cartridge cover.

Figure 24 Maximum Water Line



### NOTE

To minimize the risk of cross-contamination, do not allow the water to reach the clear cartridge cover.

- 3 Allow the reagent tray to thaw in the room temperature water bath for approximately 60 minutes or until completely thawed.
- 4 Remove the cartridge from the water bath and gently tap it on the bench to dislodge water from the base of the cartridge.
- 5 Invert the reagent cartridge ten times to mix the thawed reagents.
- 6 Visually inspect the reagent marked IMF to make sure that it is fully mixed and free of precipitates.
- 7 Gently tap the cartridge on the bench to dislodge water from the base of the cartridge and reduce air bubbles in the reagents.
- 8 Place the reagent cartridge on ice until you are ready to load your sample and set up your run.



### WARNING

**This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.**

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

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

## Preparing Your Libraries



### NOTE

*Does Not Apply to TruSeq Custom Amplicon Sequencing*—If you prepared your libraries with the TruSeq Custom Amplicon protocol, do not perform this step. The TruSeq Custom Amplicon protocol results in a ready-to-use normalized concentration of pooled libraries for the MiSeq reagent cartridge.

For all types of runs *except* TruSeq Custom Amplicon sequencing, you must denature and dilute your sample DNA for cluster generation and sequencing.

### Illumina-Supplied Consumables

- ▶ HT1 (Hybridization Buffer), pre-chilled

### User-Supplied Consumables

- ▶ 0.1 N NaOH
- ▶ Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20

## Denature DNA



### NOTE

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

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

Use the following instructions to denature the DNA with 0.1 N NaOH to a DNA concentration of 20 pM.

- 1 Combine the following volumes of sample DNA and 0.1 N NaOH in a microcentrifuge tube:
  - 2 nM sample DNA (10  $\mu$ l)
  - 0.1 N NaOH (10  $\mu$ l)
- 2 Vortex briefly to mix the sample solution.
- 3 Centrifuge the sample solution to 280 xg for one minute.
- 4 Incubate for five minutes at room temperature to denature the DNA into single strands.
- 5 Add the following volume of pre-chilled HT1 to the tube containing denatured DNA to result in a 20 pM denatured library:
  - Denatured DNA (20  $\mu$ l)
  - Pre-chilled HT1 (980  $\mu$ l)
- 6 Place the denatured DNA on ice until you are ready to proceed to final dilution.

## Dilute Denatured DNA

Use the following instructions to dilute the denatured DNA with pre-chilled HT1 to a total volume of 1,000  $\mu$ l.


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

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

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

### Denature and Dilute PhiX Control

Use the following instructions to denature and dilute the 10 nM PhiX library.

- 1 Combine the following volumes to dilute the PhiX library to 2 nM:
    - 10 nM PhiX library (2  $\mu$ l)
    - 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20 (8  $\mu$ l)
  - 2 Combine the following volumes of 2 nM PhiX library and 0.1 N NaOH in a microcentrifuge tube to result in a 1 nM PhiX library:
    - 2 nM PhiX library (10  $\mu$ l)
    - 0.1 N NaOH (10  $\mu$ l)
  - 3 Vortex briefly to mix the 1 nM PhiX library solution.
  - 4 Centrifuge the template solution to 280 xg for one minute.
  - 5 Incubate for five minutes at room temperature to denature the PhiX library into single strands.
  - 6 Add the following volume of pre-chilled HT1 to the tube containing denatured PhiX library to result in a 20 pM PhiX library.
    - Denatured PhiX library (20  $\mu$ l)
    - Pre-chilled HT1 (980  $\mu$ l)
-  **NOTE**  
The denatured 20 pM PhiX library can be stored up to three weeks at -15° to -25°C. After three weeks, cluster numbers tend to decrease.
- 7 Dilute the denatured 20 pM PhiX library to 8 pM as follows:
    - 20 pM denatured PhiX library (400  $\mu$ l)
    - Pre-chilled HT1 (600  $\mu$ l)

### Mix Sample Library and PhiX Control

- 1 Combine the following volumes of denatured PhiX control library and your denatured sample library to result in a 1% volume ratio:
  - 8 pM PhiX control library (10  $\mu$ l)
  - Denatured sample library (990  $\mu$ l)

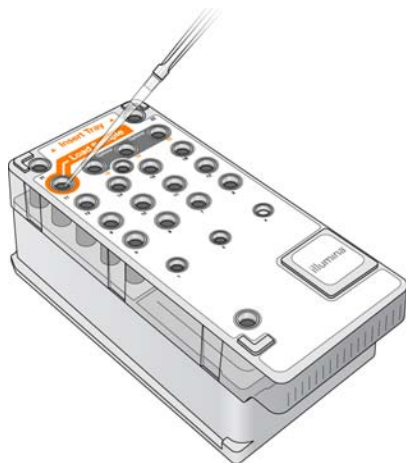
- 2 Set the combined sample library and PhiX control aside on ice until you are ready to load it onto the MiSeq reagent cartridge.

### Load Sample Libraries onto Cartridge

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

- 1 Pierce the foil over the reservoir on the reagent cartridge labeled **Load Samples**.
- 2 Pipette 600  $\mu$ l of your sample libraries into the **Load Samples** reservoir.

Figure 25 Load Libraries



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

## Clean the Flow Cell

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

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

Figure 26 Remove Flow Cell



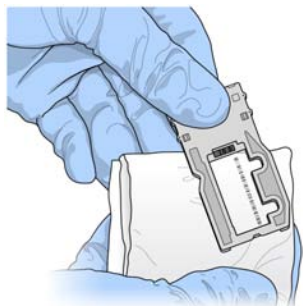
- 2 Lightly rinse the flow cell assembly with approximately 2 ml of laboratory-grade water, making sure that both the glass and plastic cartridge are thoroughly rinsed of excess salts. Excess salts can affect flow cell seating in the instrument.

Figure 27 Rinse Flow Cell



- 3 Using care around the flow cell port gaskets, thoroughly dry the flow cell and cartridge using a lint-free lens cleaning tissue. Gently pat dry in the area of the gaskets and adjacent glass.
- 4 Using an alcohol wipe, clean the flow cell glass, making sure that the glass is free of streaks and fingerprints. Avoid using the alcohol wipe on the flow cell port gaskets.

Figure 28 Dry Flow Cell



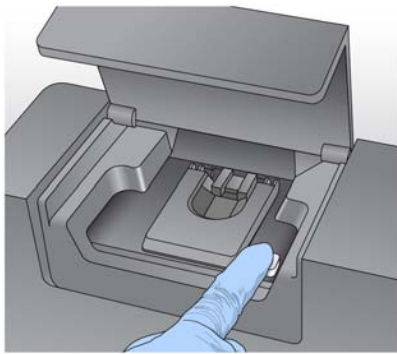
- 5 Dry any excess alcohol with a lint-free lens cleaning tissue, and visually inspect to make sure that no tissue particles are obstructing the flow cell ports and the gaskets were not at all dislodged from the flow cell ports.  
If the gaskets appear to be dislodged at all, gently press them back into place until they sit squarely around the flow cell ports.

## Loading the Flow Cell

The MCS interface will guide you through the run setup steps, starting from the Welcome screen.

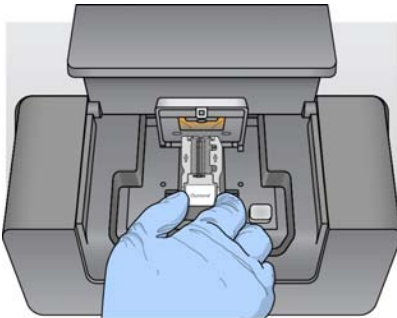
- 1 From the Welcome screen, select **Sequence**. The Cloud Option screen opens.
- 2 Set your preference for using the Cloud Option and select **Next**. The Load Flow Cell screen opens.
- 3 Put on a new pair of powder-free latex gloves.
- 4 Raise the flow cell compartment door.
- 5 Press the silver release button to the right of the flow cell latch. The flow cell latch raises to open.

Figure 29 Open Flow Cell Latch



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

Figure 30 Place Flow Cell on Stage



- 9 Gently press down on the flow cell latch to close it over the flow cell. You will hear a click when the flow cell latch is secure.

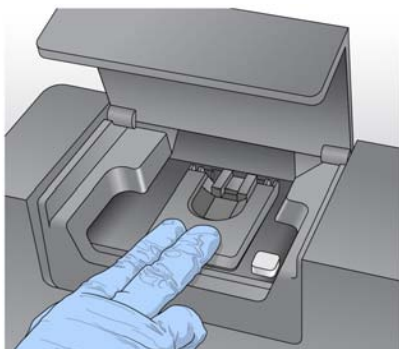


#### NOTE

As you close the flow cell latch, two alignment pins near the hinge of the flow cell

latch align and position the flow cell into place.

Figure 31 Close Flow Cell Latch



**NOTE**

The MiSeq System reads the RFID of the flow cell. If the system is unable to register the RFID of the flow cell, you can manually enter the barcode ID printed on the flow cell using the online keyboard. Select the keyboard icon in the lower right corner of the screen, enter the barcode ID, and select **Done** to return to the Load Flow Cell screen.

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



## Load Reagents

First, load the PR2 bottle and make sure that the waste bottle is empty, and then load the reagent cartridge. Check for a message on the lower-right corner of the screen that confirms the RFID was successfully read for the PR2 bottle and the reagent cartridge.



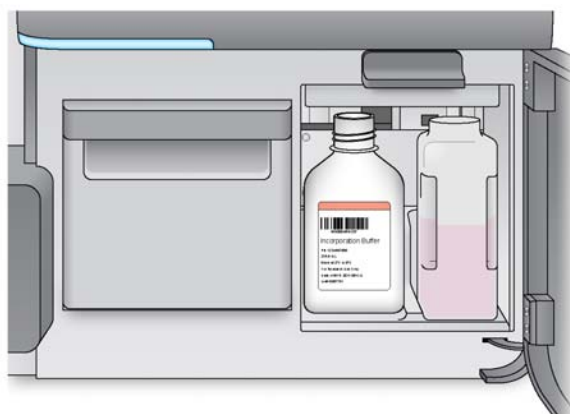
### NOTE

The MiSeq System reads the RFID of the PR2 bottle and reagent cartridge. If the system is unable to register the RFID, you can manually enter the barcode number from the label using the online keyboard. Select the keyboard icon in the lower-right corner of the screen, enter the barcode number, and select **Done** to return to the Load Reagents screen.

### Load PR2 and Check the Waste Bottle

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

Figure 32 Load the PR2 Bottle



- 5 Confirm that the PR2 RFID was successfully read.
- 6 If necessary, empty the waste bottle into the appropriate waste container.



### WARNING

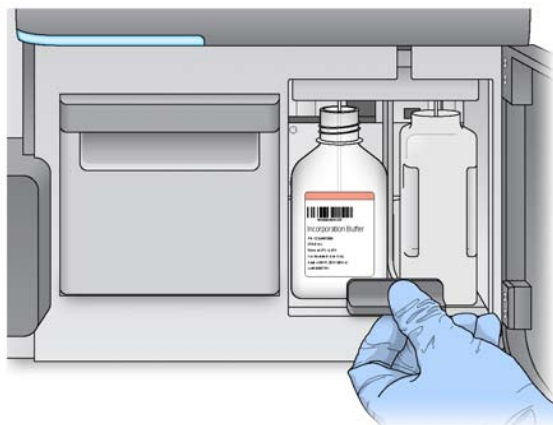
**This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.**

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

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

- 7 Slowly lower the sipper handle. Make sure that the sippers lower into the PR2 and waste bottles.

Figure 33 Lower Sipper Handle



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

### 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.

Figure 34 Load Reagent Cartridge



- 3 Close the reagent chiller door.
- 4 Confirm that the reagent cartridge RFID was successfully read.
- 5 Close the reagent compartment door.
- 6 Select **Next** on the Load Reagents screen. The Review screen opens.

## Starting the Run

After you have loaded the flow cell and reagents, the MCS interface prompts you to review run parameters and perform a pre-run check before beginning the run.

### Review Run Parameters



#### NOTE

The sample sheet name must match the reagent barcode number. A message appears if the sample sheet name and reagent barcode number are not identical, and prompts you to select a sample sheet.

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

### Review Pre-Run Check

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

If any items do not pass the pre-run check, a message appears on the screen with instructions to correct the error. For more information, see *Software Errors* on page 71.

When all items successfully pass the pre-run check, you are ready to start your run.

### Important Note Before Starting the Run



#### WARNING

**The MiSeq is sensitive to vibration. Touching the instrument after starting your run will adversely impact sequencing results.**

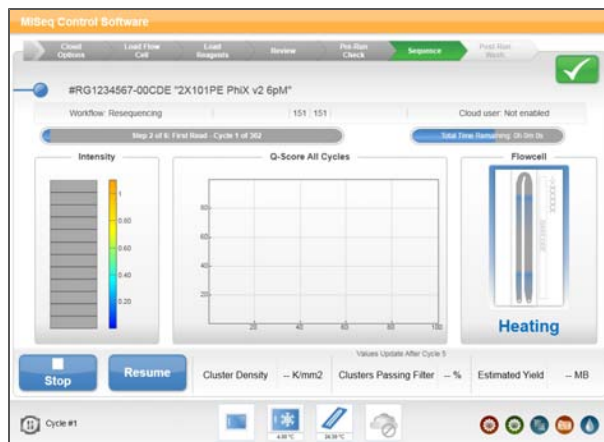
**After you select Start Run, do not open the flow cell compartment or reagent compartment doors, or touch the instrument monitor unless you plan to stop the run.**

**Wait until the run is complete before touching the monitor or opening compartment doors.**

### Monitoring the Run

When the run begins, the RTA window temporarily pops up in front of the Sequencing screen and then auto-minimizes. This is normal.

Figure 35 Sequencing Screen



During your run, use the Sequencing screen to monitor run progress, intensities, quality scores, fluidics, and imaging.

For more information, see *Sequencing Screen* on page 27.

To monitor your run in greater detail, use the Sequencing Analysis Viewer. For more information, see *Sequencing Analysis Viewer* on page 28 and the *Sequencing Analysis User Guide*.

# Post-Run Procedures

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## Introduction

Perform an instrument wash following the completion of a sequencing run. It is important to regularly wash the fluidics lines to ensure continued performance.

## Performing a Post-Run Wash

Always perform a post-run wash after completing a sequencing run. If you did not perform a wash after the last run, a reminder appears on the MiSeq software interface. The post-run wash takes approximately 20 minutes.

### Illumina-Supplied Accessories

- ▶ Wash tray
- ▶ Wash bottle, 500 ml

### User-Supplied Consumables

- ▶ Laboratory-grade water

### Procedure



#### NOTE

Leave the used flow cell on the instrument until the post-run wash is complete.

- 1 When your run is complete, select **Start Wash**.
- 2 Open the reagent compartment door.
- 3 Open the reagent chiller door and remove the used reagent cartridge from the chiller. The sippers were automatically raised at the end of the run. The reagent cartridge should slide easily from the chiller.
- 4 Prepare the wash tray by adding approximately 6 ml of laboratory-grade water to each reservoir.
- 5 Slide the wash tray into the reagent chiller until it stops. 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 Prepare the 500 ml wash bottle by adding at least 350 ml of laboratory-grade water.
- 8 Remove the PR2 bottle from the reagent compartment and replace it with the wash bottle containing laboratory-grade water.
- 9 Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.



#### WARNING

**This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.**

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

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

- 10 Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
- 11 Close the reagent compartment door.
- 12 From the Post-Run Wash screen, select **Next**. The post-run wash begins. When the post-run wash is complete, a message appears on the screen.

- 13 Select **Done**. The Welcome screen opens. You are ready to start another sequencing run.



## Unloading Components

When the post-run wash is complete you are ready to unload components from the instrument.

### Remove Wash Components

- 1 Open the reagent compartment door and raise the sipper handle to lift the sippers from the 500 ml wash bottle and waste bottle. You should feel the sipper handle lock into place.
- 2 Remove the wash bottle. Wash the wash bottle with deionized water and thoroughly dry it so it is ready for a subsequent wash. Store it upside down to protect from dust.
- 3 Remove the waste bottle and discard the contents appropriately and according to site standards.



#### WARNING

**This set of reagents contains formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact.**

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

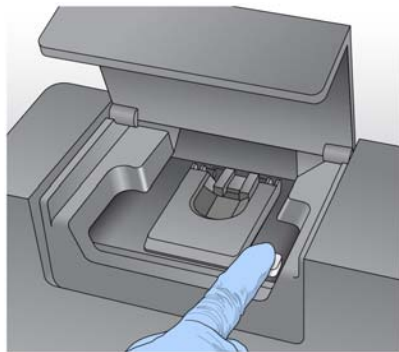
For more information, see the MSDS for this kit, at <http://www.illumina.com/msds>.

- 4 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, clean the floor of the reagent compartment of any visible spills or condensation.
- 5 Return the empty waste bottle to the reagent compartment.
- 6 Open the reagent chiller door.
- 7 Remove the wash tray. Wash the wash tray with deionized water and thoroughly dry it so it is ready for a subsequent wash. Store it upside down to protect from dust.
- 8 Close the reagent chiller door and the reagent compartment door.

### Remove the Flow Cell

- 1 Open the flow cell compartment door.
- 2 Press the release button to the right of the flow cell latch. The flow cell latch raises.

Figure 36 Flow Cell Stage



- 3 Hold the used flow cell by the edges of the flow cell cartridge near the Illumina label, and remove it from the flow cell stage.
- 4 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 holder until it is completely clean.
- 5 Gently press down on the flow cell latch to close it. You will hear a click when the flow cell latch is secure.
- 6 Close the flow cell compartment door.

## Shutting Down the Instrument

Turn off the instrument only if it will not be used for more than ten days. If the instrument will be used within the next ten days, leave the instrument on.



### 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.

Use the following procedure to safely prepare fluidics before shutting down the system.

- 1 Perform a maintenance wash to thoroughly wash the system.
- 2 Leave the used wash tray in the reagent chiller to collect any fluid that may fall back through the lines.
- 3 Empty the waste bottle and return it to the reagent compartment.
- 4 Place an empty wash bottle in the reagent compartment, lower the sipper handle, and close the reagent compartment door.
- 5 Remove the flow cell from the flow cell stage.
- 6 Using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol, carefully wipe the surface of the flow cell holder until it is completely clean and dry.
- 7 Press down on the flow cell latch until it locks into place.
- 8 Close the flow cell compartment door.
- 9 Toggle the power switch to turn off the instrument.
- 10 When you are ready to restart the instrument, load a used flow cell and perform an instrument wash prior to your next sequencing run.



# On-Instrument Analysis

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## Introduction

The MiSeq Control Software (MCS) performs image analysis and base calling using integrated Real Time Analysis (RTA). Analysis is performed during the sequencing run, which saves downstream analysis time. RTA runs automatically on the instrument.

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

### MiSeq Reporter

The MiSeq is the first instrument to provide on-instrument secondary analysis using the MiSeq Reporter software. MiSeq Reporter is a Windows-based application that processes base calls generated by RTA. The results include information about alignment, structural variants, and contig assemblies for each genome requested, and each sample if your run is a multi-sample run or an indexed run.

## Primary Analysis Results

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.

**Table 2** Contents of Analysis Folder

Key File	Subfolder	Description
RTAComplete.txt	AnalysisFolder \	A marker file generated when base call analysis is complete. The presence of this file triggers the start of secondary analysis.
SampleSheet.csv	AnalysisFolder \	This file is read and copied to the run folder before the run, and later used for secondary analysis.
RunInfo.xml	AnalysisFolder \	Identifies the boundaries of the reads (including index reads) and the quality table selected for run.
*.bcl files	AnalysisFolder \Data \ Intensities \ BaseCalls \ L001 \ CX.X	Each *.bcl file contains RTA base calling and base quality scoring results for one cycle, one tile.
*.stats files	AnalysisFolder \Data \ Intensities \ BaseCalls \ L001 \ CX.X	*.stats files contain RTA base calling statistics for a given cycle/tile.
*.filter files	AnalysisFolder \Data \ Intensities \ BaseCalls	*.filter files contain filter results per tile.
*.txt	AnalysisFolder \Data \ RTALogs	Log files from primary analysis.
*.cif files	AnalysisFolder \Data \ Intensities \ L001 \ CX.X	Each binary *.cif file contains RTA image analysis results for one cycle, one tile. These files can be used in the OLB analysis software. For more information, see the <i>Off-line Basecaller Software User Guide</i> .
*.locs files	AnalysisFolder \Data \ Intensities \ BaseCalls \ L001	Reports the cluster coordinates. There is one *.locs file for each tile..
*.jpg files	AnalysisFolder \ Thumbnail_Images \ L001 \ CX.X	Thumbnail images generated for each cycle and base, and can be used to troubleshoot a run.

## Secondary Analysis Using MiSeq Reporter

MiSeq Reporter runs on the instrument computer and uses the analysis workflow specified in the sample sheet to perform secondary analysis. For more information, see *Analysis Workflow* on page 35.

Even though MiSeq Reporter is running on the instrument, you view the analysis in progress from any web browser on another computer on the same network as your MiSeq.

### Viewing MiSeq Reporter

MiSeq Reporter launches automatically after RTA completes primary analysis. To view the MiSeq Reporter interface during analysis, use any web browser on the same network as your instrument and connect to the MiSeq HTTP service on Port 8042.

- ▶ Connect using the instrument IP address:

IP Address	HTTP Service Port	HTTP Address
10.10.10.10, for example	8042	http://10.10.10.10:8042

- ▶ Connect using the your network name:

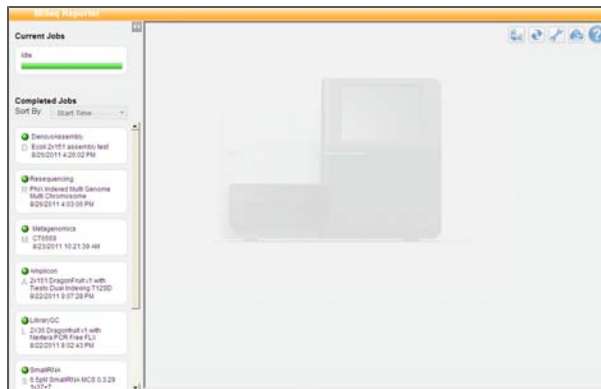
Network Name	HTTP Service Port	HTTP Address
YourNetwork, for example	8042	http://yournetwork:8042

- ▶ If MiSeq Reporter is installed locally on an off-instrument computer, connect using "localhost":

Off-Instrument	HTTP Service Port	HTTP Address
localhost	8042	http://localhost:8042

When you are linked to your MiSeq, the MiSeq Reporter interface opens.

Figure 37 MiSeq Reporter Interface



### Secondary Analysis Input Requirements

MiSeq Reporter requires the following primary analysis files to perform secondary analysis. There is no need to move or copy these files to another location before analysis begins. The software automatically accesses these files in the run folder.



File Type	Description
RTAComplete.txt	Marker file that indicates that RTA processing is complete.
SampleSheet.csv	Provided at beginning of sequencing run and contains analysis workflow and important run information.
RunInfo.xml	Identifies high-level run information.
*.bcl files	Files contain specific run and base calling information.
*.filter files	Files contain filter results for each tile.
*.locs files	Files contain the locations of all clusters on a tile.

## Pre-Installed Databases and Genomes

The MiSeq includes several pre-installed databases and genomes.

### Databases

- ▶ miRbase for human
- ▶ dbSNP for human
- ▶ refGene for human

### Genomes

- ▶ hg19 (human)
- ▶ dh10b (*E. coli*)
- ▶ *S. aureus*
- ▶ yeast
- ▶ *A. thaliana*
- ▶ mouse

You can upload and use your own reference in FASTA format. Reference FASTA files should be stored in a single folder and have a \*.fa extension.

Use the Manage Files feature in MCS to upload files. For more information, see *Manage Files* on page 22.

## Sequencing During Analysis

If a new sequencing run is started on the MiSeq before secondary analysis is complete, the current secondary analysis is stopped. From the MiSeq Reporter interface, you can requeue secondary analysis for that run after the new sequencing run is complete. At that point, secondary analysis starts over from the beginning.

For information about using MiSeq Reporter, see *MiSeq Reporter Online Help* available from the MiSeq Reporter interface.

## Using MiSeq Reporter Off-Instrument

You can install another copy of the MiSeq Reporter software on a separate computer. Doing so enables you to perform secondary analysis of run data while the MiSeq is performing a subsequent sequencing run.

### Computing Requirements

To run the MiSeq Reporter software, you will need the following computing requirements:

- ▶ 64-bit Windows OS (Vista, Windows 7, Windows Server 2008 64-bit)
- ▶ > 8 GB RAM
- ▶ > 2.2 GHz
- ▶ > 1 TB disk space

### Installation and Licensing

- ▶ You can download a second copy of the MiSeq Reporter software from the Illumina website.
- ▶ After downloading, open the software installer (setup.exe), and accept the end-user licensing agreement (EULA).
- ▶ No license key is required as this additional copy is free of charge with your MiSeq.

# Troubleshooting

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## Introduction

If an error occurs during the run setup steps, an on-screen message appears with instructions for correcting the error. Other errors are indicated by the status icon in the upper-right corner of the screen. For more information, see *Status Icons* on page 20.

For more information, visit <http://www.illumina.com/support.ilmn> or contact Illumina Technical Support.

## Software Errors

This section provides an overview of possible errors using MCS. For most errors, the action you need to take appears on the screen and the run is not impacted.

Occurs During	Error	Action
Upon starting the software.	Initialization failed.	The option to re-initialize appears on the screen. Select <b>Re-initialize</b> .
Pre-Run Check	Sample sheet not found.	Check the folder location for the sample sheet in Setup Options on the Welcome screen.  If the sample sheet is missing, create one and copy it to the sample sheet locations specified in Setup Options.  When you finished, select <b>Restart Check</b> .
Pre-Run Check	Improperly formatted sample sheet.	You might have missing information or syntax errors in your sample sheet. Correct the errors listed on the screen, and then select <b>Restart Check</b> .
Pre-Run Check	Fluidics check failed.	Select <b>Pump</b> . When fluids have been pumped, select <b>Restart Check</b> .
Pre-Run Check	Disk space low.	If disk space is low, a message appears indicating how much disk space is required, along with the Manage Runs button. Select <b>Manage Runs</b> and clear the required space from the instrument computer.
Pre-Run Check	Empty waste bottle.	Open the reagent compartment door, raise the sipper handle, and empty the waste bottle in an appropriate waste container.
Pre-Run Check	Network disconnected.	Make sure the network cable is plugged into the instrument.  If the network connection is not resolved, reboot the instrument by turning off the power switch. Wait at least 60 seconds, and then turn the instrument on using the power switch.
At end of run	Data files not copied.	Two options appear on the screen: Copy Data and Delete Data <ul style="list-style-type: none"> <li>• <b>Copy Data</b>—Allows you to select an output folder and copy files.</li> <li>• <b>Delete Data</b>—Removes temporary files from the instrument computer.</li> </ul>



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## Technical Assistance

For technical assistance, contact Illumina Customer Support.

**Table 3** Illumina General Contact Information

<b>Illumina Website</b>	<a href="http://www.illumina.com">http://www.illumina.com</a>
<b>Email</b>	<a href="mailto:techsupport@illumina.com">techsupport@illumina.com</a>

**Table 4** Illumina Customer Support Telephone Numbers

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

### MSDSs

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

### Product Documentation

If you require additional product documentation, you can obtain PDFs from the Illumina website if PDFs are available. Go to <http://www.illumina.com/support/documentation.ilmn>. When you click on a link, you will be asked to log in to iCom. After you log in, you can view or save the PDF. To register for an iCom account, please visit <https://icom.illumina.com/Account/Register>.

