

# MiSeqDx Instrument

## Reference Guide for MOS v4

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# Revision History

<b>Document #</b>	<b>Date</b>	<b>Description of Change</b>
Document # 1000000157953 v00	November 2021	Initial release to support MOS v4.0 and Local Run Manager v3.0.

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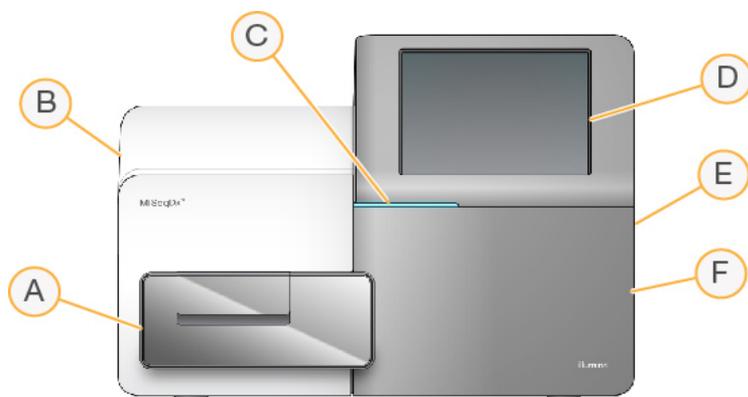
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# Overview

## Components

The MiSeqDx has the following exterior components:



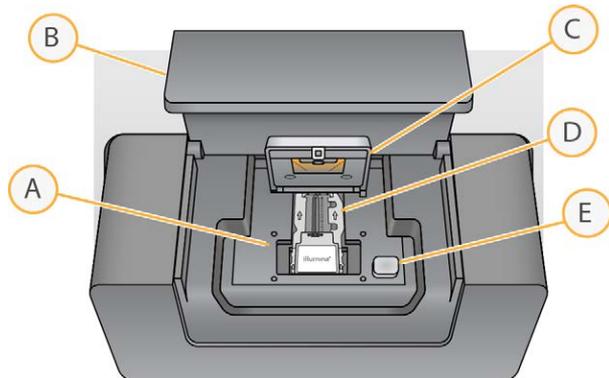
- A. **Flow cell compartment**—Contains the flow cell stage that houses the flow cell throughout the run. Flow cell stage motors move the stage out of the enclosed optical module for flow cell loading and return the stage when the run begins.
- B. **Enclosed optics module**—Contains optical components 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 port**—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 MiSeqDx interface guides you through the run setup steps using the touch screen monitor.

Loading run

components requires access to the reagent compartment and the flow cell compartment.

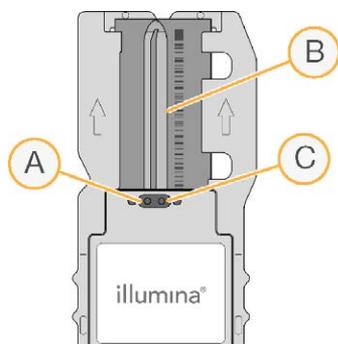
## Flow Cell Compartment



- A. Flow cell stage
- B. Flow cell compartment door
- C. Flow cell latch
- D. Flow cell
- E. Flow cell latch release button

The flow cell compartment houses the flow cell stage, thermal station, and fluidics connections to the flow cell. The flow cell stage holds the flow cell and the flow cell latch secures and positions the flow cell. When the flow cell latch closes, two pins near the latch hinge position the flow cell automatically. The thermal station, located beneath the flow cell stage, controls changes in flow cell temperature required for cluster generation and sequencing.

## Flow Cell



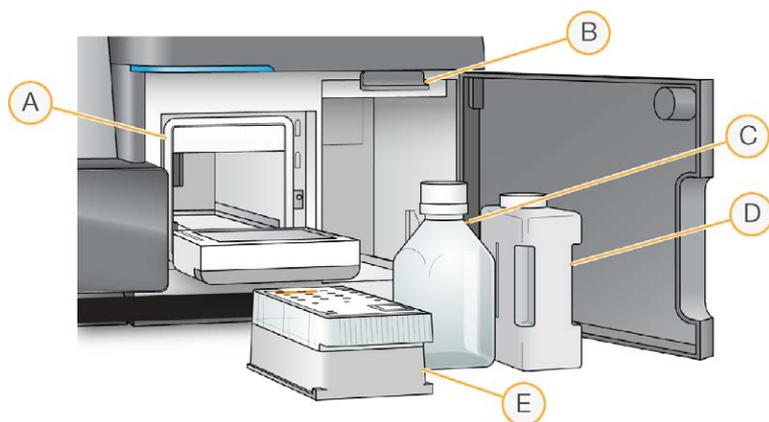
- A. Outlet port
- B. Imaging area
- C. Inlet port

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

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

During the sequencing run, the single lane is imaged in small imaging areas called tiles.

## Reagent Compartment



- A. Reagent chiller
- B. Sipper handle (shown in raised position)
- C. MiSeqDx SBS Solution (PR2) bottle
- D. Waste bottle
- E. Reagent cartridge

The reagent compartment contains the reagent chiller, and positions for the MiSeqDx SBS Solution (PR2) bottle and the waste bottle.

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

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

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

## MiSeqDx Software

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

- **MiSeq Operating Software (MOS)**—Controls instrument operation. The MiSeq Operating Software (MOS) 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. The software is installed and runs on the instrument.
- During the run, MOS operates the flow cell stage, dispenses reagents, controls flow cell temperatures, and captures images of clusters on the flow cell. MOS performs the run according to the parameters specified in the Local Run Manager software.
- **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 RTA analysis is complete.
- **Local Run Manager software**—An on instrument integrated solution used to create a run, monitor status, analyze sequencing data, and view results. Local Run Manager also tracks sample information and controls user permissions. The software runs on the instrument computer and is viewed through a web browser. See [Local Run Manager Software on page 5](#).

## Status Icons

Whenever the instrument is initializing or launched, a status icon on the control software interface indicates a change in conditions. 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.
	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.

## Sensor Indicators

Three sensor indicators at the base of each interface screen represent the status of an instrument component.

Figure 1 Sensor Indicators



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

- Temperature of the reagent chiller in °C
- Temperature of the flow cell in °C

## Local Run Manager Software

Local Run Manager software is an on instrument integrated solution for recording samples for a run, specifying run parameters, monitoring status, analyzing sequencing data, and viewing results.

In addition, Local Run Manager controls user authentication, granting various access level permissions to users. Permissions are saved in a database file, which the MiSeqDx references. Local Run Manager can also monitor the sequencing run. For more information, see the *Local Run Manager v3 Software Reference Guide for MiSeqDx (document # 200003931)*.

## Sequencing During Analysis

The MiSeqDx instrument computing resources are dedicated to either sequencing or analysis.

With Local Run Manager, if a new sequencing run is started on the MiSeqDx before secondary analysis of an earlier run is complete, a confirmation dialog box appears. After confirming you want the new sequencing run to start, secondary analysis of the earlier run is stopped until the new run completes sequencing.

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

## Required Disk Space

The integrated instrument computer has approximately 650 GB of storage capacity.

Before starting a run, the software checks available disk space. If there is not enough disk space for the run, a software prompt appears. The message indicates how much disk space is required for the run and how much disk space must be cleared before the run can proceed.

If prompted to make disk space available, move or delete older run folders as appropriate.

## Software Restriction Policies

Windows Software Restriction Policies (SRP) use rules to allow only specified software to run. For the MiSeqDx, SRP rules are based on certificates, file names, file extensions, and directories.

By default, SRP is turned on to prevent unwanted software from running on the control computer. Only the sbsadmin user can turn off SRP.

An IT representative or system administrator can add and remove rules to customize the security level. If the system is added to a domain, the local Group Policy Object (GPO) might automatically modify the rules and turn off SRP.

For information on configuring SRP, see [Illumina Instrument Control Computer Security and Networking](#).



### CAUTION

Turning off the SRP prevents the protection it provides. Changing the rules overrides the default protections.

## Antivirus Software

An antivirus software of your choice is highly recommended to protect the instrument control computer against viruses. You will need to turn off Windows Software Restriction Policies (SRP) temporarily while you install the antivirus software.

For information on configuring antivirus software and SRP, see [Illumina Instrument Control Computer Security and Networking](#).

## Sequencing Mode

When you boot into the instrument, the Windows **Choose an operating system** screen displays. This screen lets you select the operating system sequencing mode to boot into—Research (RUO) or Diagnostic (Dx). If you wait 10 seconds, the default mode is automatically selected. You can change the default mode and the default boot mode timer at any time.

- After you have selected a mode, you must reboot the system to switch mode. See [Reboot System Software on page 7](#).
- When you switch between RUO mode and Dx mode, you are prompted to perform a post-run wash. The wash state is not retained between modes.

To use the reboot feature, Windows Administrator level access or reboot to research mode permission for a regular user is required.



## CAUTION

Restore Factory OS (RUO/Dx) is intended for Illumina field service use only. This option permanently deletes all information on the C drive and restores the operating system to its original state. Selecting this option requires an Illumina field service engineer to restore the system. After it is started, the restore process cannot be canceled. Only select this option if recommended by Illumina field service.

### To change the default boot mode:

1. Select **Change defaults or choose other options**.
2. On the Options screen, select **Choose a default operating system**.
3. Select the preferred boot option.
4. Select the Back arrow to return to the **Options** screen.

### To change the default boot mode timer:

1. Select **Change defaults or choose other options**.
2. On the Options screen, select **Change the timer**.
3. On the Change the timer screen, select the desired timer.
4. Select the Back arrow to return to the **Options** screen.



## WARNING

In Windows 10, if a user reboots the system, only that user will be able to log in to the system after boot up.

## Reboot System Software

Use the Reboot command to reboot the system software. There is no requirement to reboot the software as a part of regular maintenance.

- From the Main Menu, select **Reboot**.

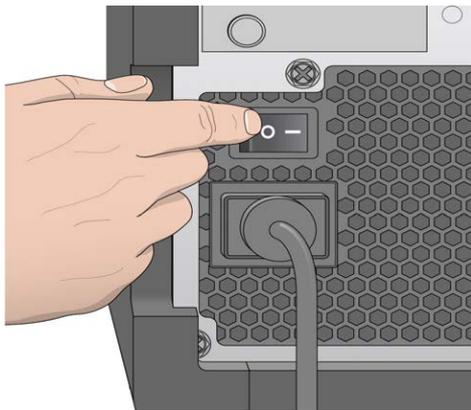
# Getting Started

## Start the MiSeqDx

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 32](#). Wait a *minimum* of 60 seconds before turning the power switch back to the ON position.

Figure 2 Power Switch Location



2. Wait for the system to load, and then select the operating system and log in. If necessary, consult your facility administrator for the user name and password.  
For more information on operating system and sequencing mode options, see [Sequencing Mode on page 6](#).  
When the operating system is loaded, the MiSeq Operating Software (MOS) launches and initializes the system automatically. Note that if RUO mode is selected, MCS launches automatically.
3. For Local Run Manager, if user management is enabled, log in using your Local Run Manager user name and password, and select **Next**.

## Set Post-Run Wash Option

An instrument wash is required after each run. The software requires that a wash is performed before setting up a subsequent run. The Post-Run Wash Option specifies which type of wash is performed by default. A post-run wash takes about 30 minutes. A maintenance wash takes about 90 minutes.

1. From the Main Menu, select **System Settings**.
2. Select the Run Settings tab.

3. Select **Post Run Wash** or **Maintenance Wash**.

## Set Automatic Start Run Option

The MiSeqDx can be configured to start the sequencing run automatically after a successful automatic check. Local Run Manager Administrator level access is required to configure this feature.

1. From the Main Menu, select **System Settings**.
2. Select the Run Settings tab.
3. Select the **Start run after pre-run check. Do not prompt for confirmation.** checkbox.  
If this setting is disabled, start the run manually after the pre-run check.

## Turn On Illumina Proactive Support

1. From the Main Menu, select **System Settings**.
2. Select the Proactive tab.
3. Select **Turn on Illumina Proactive Support** to enable the Illumina Proactive monitoring service.  
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 off by default. To opt in to this service, enable the **Turn on Illumina Proactive Support** option.
4. From the drop-down list, select the hosting region the instrument should connect to.

## Set Email Preferences

The MiSeqDx can be configured to send an email notification when RTA analysis completes, when on-instrument secondary analysis completes, or if a critical MiSeqDx software error occurs. Typically, this configuration is done during MiSeqDx installation. Local Run Manager Admin access level is required to use this feature.

1. From the Main Menu, select **System Settings**.
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. The sender email address must have the same domain name as the email server address.
- **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 Output Folder Location

The MiSeqDx Output folder sets the default location for analysis output files. Folders can be on a local network or on the instrument computer. Change the default Output folder to a network location for sharing or long-term storage.

Local Run Manager Admin user access level is required to configure this feature.

1. From the Main Menu, select **System Settings**.
2. Select the Run Settings tab.
3. In the Output Folder field, enter the path to the folder location.

Make sure to enter the full UNC path, such as `\\YourServer\Path\OutputFolder`.



### WARNING

If you use a mapped drive such as `Z:\OutputFolder`, analysis of the sequencing run does not complete.

## Required Consumables

### Sequencing Consumables

The sequencing consumables required to run the MiSeqDx are provided separately as part of an *in vitro* diagnostic kit.

### User-Supplied Consumables

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

Consumable	Purpose
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	Cleaning the flow cell glass and stage

Consumable	Purpose
Lab tissue, low-lint	Cleaning the flow cell stage
Lens paper, 4 x 6 in	Cleaning the flow cell
MiSeq tubes	Washing the template line (optional)
NaOCl, 5%	Washing the template line (optional)
Tween 20	Washing the instrument
Tweezers, square-tip plastic (optional)	Removing flow cell from flow cell shipping container
Water, laboratory-grade	Washing the instrument

## 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 $\Omega$ ) water
- Milli-Q water
- Super-Q water
- Molecular biology grade water

## Storage and Handling

Element	Specification
Temperature	Transportation and Storage: -10°C to 40°C (14°F to 104°F) Operating Conditions: 19°C to 25°C (66°F to 77°F)
Humidity	Transportation and Storage: Non-condensing humidity Operating Conditions: 30–75% relative humidity (non-condensing)

# Sequencing

## Introduction

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

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

## Run Duration

Run duration is based on the number of cycles performed. Depending on the version of MiSeqDx reagents, the MiSeqDx can perform a paired-end sequencing run up to 2 x 301 sequencing cycles.

## Number of Cycles in a Read

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

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

## Cluster Generation

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

## Sequencing

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

## Analysis

When the run is complete, the Local Run Manager analysis software launches automatically to perform secondary analysis.

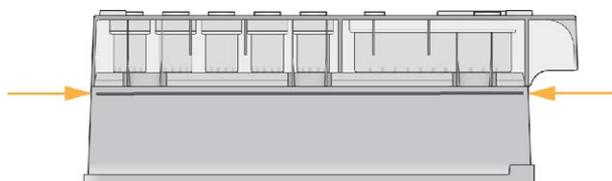
Secondary analysis can be monitored using an internet connection from another computer, provided the computer is on the same network connection as the MiSeqDx. See the *Local Run Manager v3 Software Reference Guide for MiSeqDx (document # 200003931)*.

## Prepare the Reagent Cartridge

The following instructions describe how to thaw the reagent cartridge using a room temperature water bath.

1. Thaw the reagent cartridge in a water bath containing enough room temperature deionized water to submerge the base of the reagent cartridge up to the water line printed on the reagent cartridge. Do not allow the water to exceed the maximum water line.
1. Remove the reagent cartridge from  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  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 3 Maximum Water Line



3. Allow the reagent cartridge to thaw in the room temperature water bath until it is thawed completely.  
Thawing times range from approximately 60 to 90 minutes depending on the type of reagent cartridge. Refer to the assay package insert for more information.
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. Make sure that no water has splashed on the top of the reagent 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 reagents in positions 1, 2, and 4 to make sure that they are fully mixed and free of precipitates.

**NOTE** It is critical that the reagents in the cartridge are thoroughly thawed and mixed to ensure proper sequencing.

3. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

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

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

## Load Sample Libraries onto Cartridge

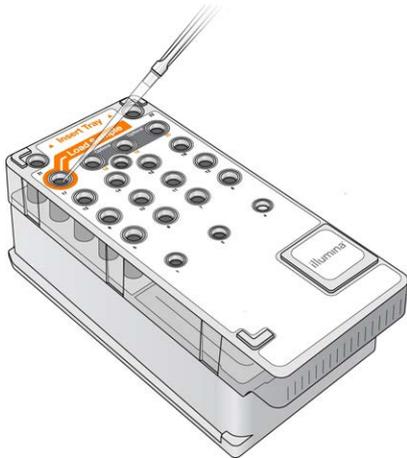
When the reagent cartridge is fully thawed and ready for use, you are ready to load samples into the cartridge.

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

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

2. Pipette 600 µl prepared diluted amplicon library (DAL) sample libraries into the **Load Samples** reservoir. Avoid touching the foil seal.
3. Check for air bubbles in the reservoir after loading sample. If air bubbles are present, gently tap the cartridge on the bench to release the bubbles.

Figure 4 Load Libraries



4. Proceed directly to the run setup steps using the MiSeq Operating Software (MOS) interface.

## Log In and Follow Sequencing Prompts

1. From the Home screen, select **Sequence**.
2. If the log in screen opens, enter the appropriate user credentials, and then select **Next**. Select **Sequence** again after logging in.

3. Select a run from the list.
4. [Optional] Select **Preview Samples** to see a list of samples in the run.
5. Select **Next**.
6. Follow the prompts to load the flow cell and reagents and set up the run (described in the following sections).

## Clean the Flow Cell

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

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 5 Remove Flow Cell



3. Lightly rinse the flow cell with laboratory-grade water, making sure that both the glass and plastic cartridge are thoroughly rinsed of excess salts.

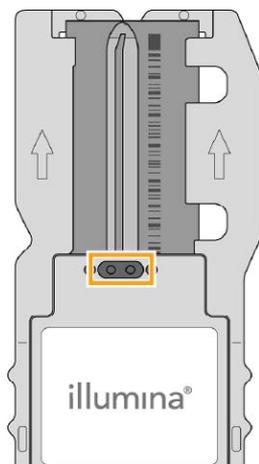
Excess salts can affect flow cell seating on the instrument. If salts dry in the imaging area, imaging can also be affected.

Figure 6 Rinse Flow Cell



4. Using care around the black flow cell port gasket (outlined in the following illustration), 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.

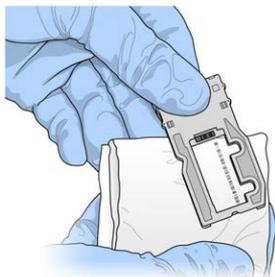
Figure 7 Flow Cell Ports and Gasket



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 8 Dry Flow Cell

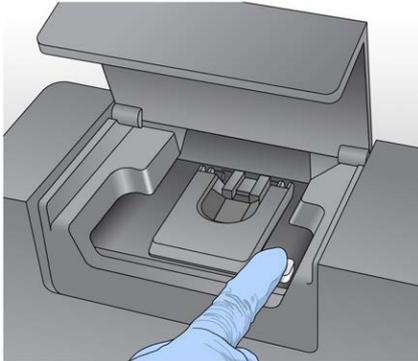


6. Dry excess alcohol with a lint-free lens cleaning tissue.
7. Make sure that the flow cell ports are free of obstructions and that the gasket is well seated around the flow cell ports.  
If the gasket appears to be dislodged, gently press it back into place until it sits securely around the flow cell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

## Load the Flow Cell

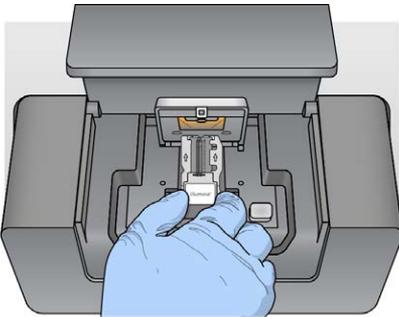
1. Raise the flow cell compartment door, and then press the release button to the right of the flow cell latch. The flow cell latch opens.

Figure 9 Open Flow Cell Latch



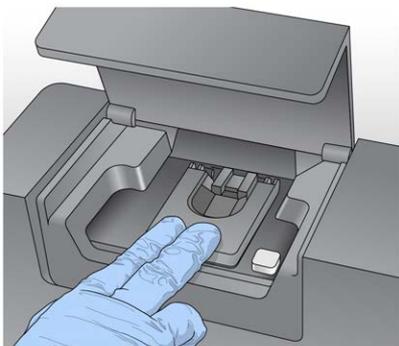
2. Make sure that the flow cell stage is free of lint. If lint or other debris is present, clean the flow cell stage using an alcohol wipe or a lint-free tissue moistened with ethanol or isopropanol. Carefully wipe the surface of the flow cell stage until it is clean and dry.
3. Holding the flow cell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

Figure 10 Place Flow Cell on Stage



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

Figure 11 Close Flow Cell Latch



5. If the software does not identify the flow cell RFID, see [Resolve RFID Read Failure on page 37](#).

**NOTE** If the RFID cannot be read, identifying information can be entered manually. However, the software allows only one of the three RFID-labeled components (flow cell, reagent cartridge, MiSeqDx SBS Solution (PR2)) to fail on an in vitro diagnostic run. For more information, see [Resolve RFID Read Failure on page 37](#).

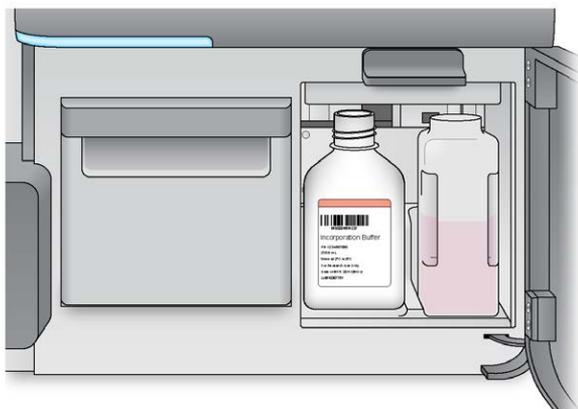
6. Close the flow cell compartment door.
7. Select **Next**.

## Load Reagents

### Load MiSeqDx SBS Solution (PR2) and Check the Waste Bottle

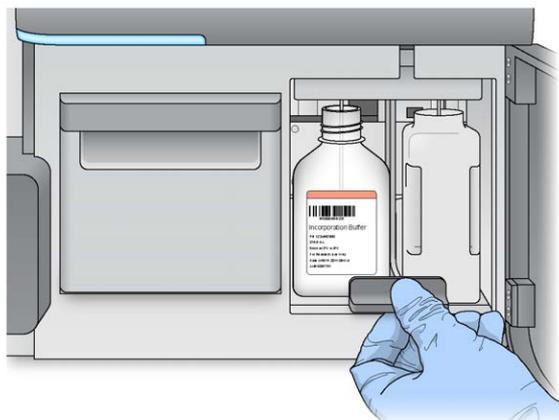
1. Remove the bottle of MiSeqDx SBS Solution (PR2) from 2° to 8°C storage. Invert to mix and then remove the lid.
2. Open the reagent compartment door.
3. Raise the sipper handle until it locks into place.
4. Remove the wash bottle and load the MiSeqDx SBS Solution (PR2) bottle.

Figure 12 Load the MiSeqDx SBS Solution (PR2) Bottle.



5. Empty the contents of the waste bottle into the appropriate container.
6. Slowly lower the sipper handle. Make sure that the sippers lower into the MiSeqDx SBS Solution (PR2) and waste bottles.

Figure 13 Lower Sipper Handle



7. If the software does not identify the RFID of the MiSeqDx SBS Solution (PR2) bottle, see [Resolve RFID Read Failure on page 37](#).

**NOTE** If the RFID cannot be read, identifying information can be entered manually. However, the software allows only one of the three RFID-labeled components (flow cell, reagent cartridge, MiSeqDx SBS Solution (PR2)) to fail on an in vitro diagnostic run. For more information, see [Resolve RFID Read Failure on page 37](#).

8. Select **Next**.

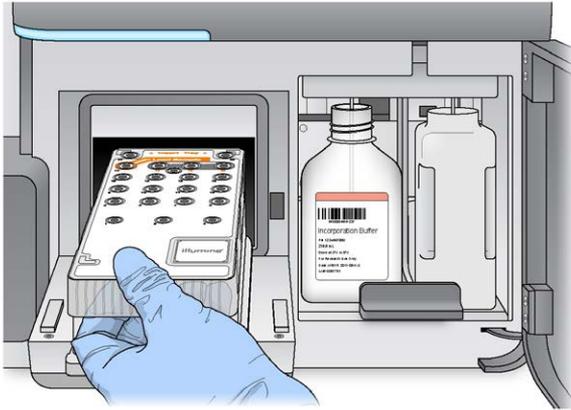
## Load the Reagent Cartridge

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.

Figure 14 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 37](#).

**NOTE** If the RFID cannot be read, identifying information can be entered manually. However, the software allows only one of the three RFID-labeled components (flow cell, reagent cartridge, MiSeqDx SBS Solution (PR2)) to fail on an in vitro diagnostic run. For more information, see [Resolve RFID Read Failure on page 37](#).

5. To start the run, select from the following options.
  - If the system is not configured to start automatically after a successful check, select **Start Run**.
  - If the system is configured to start automatically after a successful check, the sequencing run begins automatically. You do not have to be present. However, if any errors occur during the check, the run does not begin automatically.

**NOTE** If the reagent chiller temperature is out of range, it can prevent the sequencing run from starting. See [Resolve Reagent Chiller Temperature Errors on page 41](#).

## Important Note Before Starting the Run



### WARNING

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

After loading the reagent cartridge and closing the reagent compartment door, do not open the flow cell compartment or the reagent compartment doors. Do not touch the instrument monitor except to pause the run. For more information, see [Pause a Run on page 35](#).

**WARNING**

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

## Monitor the Run

During a run, monitor run detail using the Sequencing screen on the instrument. The Sequencing screen is view-only.

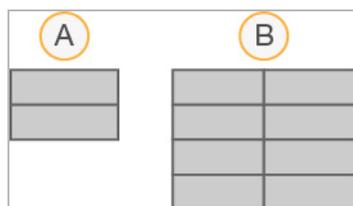
You can also use Local Run Manager to monitor a run remotely if the instrument is connected to the same network.

Local Run Manager shows run progress and sequencing information (Total Clusters, % Clusters PF, Read 1 and Read 2 %>= Q30, and Last Scored Cycle). For more information, see [Local Run Manager Software on page 5](#).

- On the instrument Sequencing screen, monitor run progress, intensities, and quality scores that appear.
  - 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 90<sup>th</sup> percentile for each tile.

The graphic in the Intensity area represents the number of tiles being imaged.

- If the flow cell is imaged on the top surface only, a single-column graphic appears.
- If the flow cell is imaged on the top surface and bottom surface, a two column graphic appears.



A. Indicates two tiles, top surface only

B. Indicates four tiles, top and bottom surface

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

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

- **Cluster Density (K/mm<sup>2</sup>)**—Shows the number of clusters per square millimeter for the run. Optimally, expect a cluster density of 800K/mm<sup>2</sup>.

**NOTE** The chastity of a base call is the ratio of the intensity of the greatest signal divided by the sum of the two greatest signals. If more than one base call has a chastity value of less than 0.6 in the first 25 cycles, reads do not pass the quality filter.

- **Estimated Yield (Mb)**—Shows the projected number of bases called for the run, measured in megabases. This data appears only after cycle 25.
2. When the run is complete, the Next button appears. Review the results on the Sequencing screen before proceeding.

**NOTE** The Sequencing screen remains viewable until Next is selected. After you select Next, it is not possible to return to the Sequencing screen.

3. Select **Next** to exit the Sequencing screen and proceed to a post-run wash.

## Template Generation

Real-Time Analysis (RTA) uses the first four cycles of the sequencing run for template generation. Template generation is the process by which cluster positions over the entire flow cell surface are defined according to X and Y coordinate position.

After the template of cluster positions is generated, images produced over every subsequent cycle of imaging are aligned against the template. Individual cluster intensities in all four nucleotide color channels are extracted and base calls are produced from the normalized cluster intensities.

## Run Metrics

Run metrics appear on the Sequencing screen at different points in a run. During cluster generation steps, no metrics appear.

After sequencing begins, the following metrics appear at the indicated cycles:

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

## 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 sequencing 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 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.

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

You can optionally 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 24](#).

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

### User-Supplied Consumables

- Tween 20 (Sigma-Aldrich, catalog # P7949)
- Laboratory-grade water
- 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, 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 several times to mix.
2. Prepare the wash components with fresh 0.5% Tween 20 wash solution, as follows:
  - a. Add 6 ml wash solution to each reservoir of the wash tray.
  - b. Add 350 ml wash solution to the 500 ml wash bottle.

3. From the post-run wash screen, select **Start Wash**. The software automatically raises the sippers in the reagent chiller. Wait several seconds to make sure that the sippers are fully raised before continuing.  
*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 24](#).
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 MiSeqDx SBS Solution (PR2) bottle and waste bottle until it locks into place.
7. Remove the MiSeqDx SBS Solution (PR2) bottle and replace it with the wash bottle.

**NOTE** Discard the MiSeqDx SBS Solution (PR2) bottle after each run. Do not reuse any remaining MiSeqDx SBS Solution (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 safety data sheet (SDS) at [support.illumina.com/sds.html](http://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**. The post-run wash begins.

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

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

## Procedure with Template Line Wash

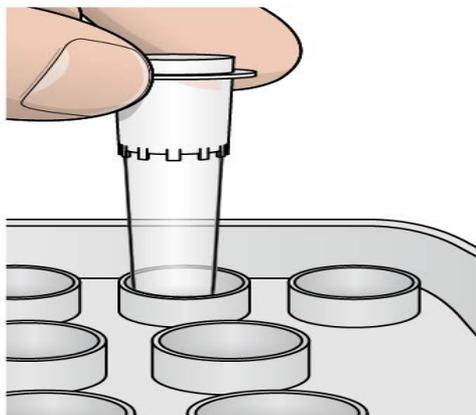
1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows.

- a. Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. These volumes result in 10% Tween 20.
  - b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
  - c. Invert five times to mix.
2. Prepare fresh NaOCl wash solution with laboratory-grade water as follows.
    - a. Add 36  $\mu$ l 5% NaOCl to 864  $\mu$ l laboratory-grade water. These volumes result in a 1:25 NaOCl dilution.
    - b. Add 50  $\mu$ l 1:25 NaOCl dilution to 950  $\mu$ l 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, 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. 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 15 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 MiSeqDx 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.
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.
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 safety data sheet (SDS) at [support.illumina.com/sds.html](http://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**. The post-run wash begins.

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

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

# Maintenance

## Maintenance Frequency

Perform the maintenance activities described in this chapter at the intervals shown in the following tables.

Table 1 Maintenance During Normal Operation

Activity	Monthly	As Needed
Maintenance Wash	X	
Standby Wash		To prepare for idle (≥ 7 days unused)
Instrument Shutdown		X

Table 2 Maintenance During Idle (≥ 7 days unused)

Activity	Monthly	As Needed
Standby Wash	X	
Instrument Shutdown		X

## Preventive Maintenance

Illumina recommends one preventive maintenance per calendar year. If you are not under a service contract, contact your Territory Account Manager or Illumina Technical Support to arrange for a billable preventive maintenance service.

## 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 using a wash solution of laboratory-grade water mixed with Tween 20.

You can configure your instrument to perform a maintenance wash, rather than a post-run wash, between runs. See [Set Post-Run Wash Option on page 8](#).

### User-Supplied Consumables

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



## CAUTION

Always close the reagent chiller door after loading the wash tray and before starting a wash. This step prevents potential injury that could occur if your hands are in the path of the sippers when they lower.

## 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 **Maintenance Wash**. The software automatically raises the sippers in the reagent chiller.

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

## Perform First Wash

1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:
  - a. Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. These volumes result in 10% Tween 20.
  - b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
  - c. Invert several times to mix.
2. Prepare the wash components with fresh 0.5% Tween 20 wash solution, as follows:
  - a. Add 6 ml wash solution to each reservoir of the wash tray.
  - b. Add 350 ml wash solution to the 500 ml wash bottle.
3. Load the wash tray and wash bottle onto the instrument:
  - a. Open the reagent compartment door and reagent chiller door, and slide the used reagent cartridge or wash tray from the chiller.
  - b. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
  - c. Raise the sipper handle in front of the MiSeqDx SBS Solution (PR2) bottle and waste bottle until it locks into place, and replace the MiSeqDx SBS Solution (PR2) bottle with the wash bottle.

**NOTE** Discard the MiSeqDx SBS Solution (PR2) bottle after each run. Do not reuse any remaining MiSeqDx SBS Solution (PR2).

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

### **Perform Second Wash**

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

### **Perform Final Wash**

1. Prepare fresh wash solution with Tween 20 and laboratory-grade water, as follows:
  - a. Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. These volumes result in 10% Tween 20.
  - b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
  - c. Invert several times to mix.

2. When the second wash is complete, remove the wash tray and wash bottle, and discard the remaining wash solution.
3. Refill the wash components with fresh 0.5% Tween 20 wash solution, as follows:
  - a. Add 6 ml wash solution to each reservoir of the wash tray.
  - b. Add 350 ml wash solution to the 500 ml wash bottle.
4. Load the wash tray and wash bottle, as follows:
  - a. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
  - b. Load the wash bottle and slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
  - c. Close the reagent compartment door.
5. Select **Next**. The final wash begins.

## After the Wash

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

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

## Perform a Standby Wash

If there are no plans to use the instrument within the next 7 days, prepare the instrument to sit idle by performing a standby wash. The standby wash prepares the fluidics lines for sitting idle and performs two consecutive washes that flush each position of any remaining reagents or salt accumulation. Each wash takes approximately 60 minutes. Allow approximately two hours to complete the standby wash.

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.

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

### User-Supplied Consumables

- Tween 20 (Sigma-Aldrich, catalog # P7949)
- Laboratory-grade water or deionized water (for guidelines on laboratory-grade water, see the *MiSeqDx Site Prep Guide (document # 15070066)*)

## 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 **Standby Wash**. The software automatically raises the sippers in the reagent chiller.

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

### Perform First Wash

1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:
  - a. Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. These volumes result in 10% Tween 20.
  - b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
  - c. Invert several times to mix.
2. Prepare the wash components with fresh 0.5% Tween 20 wash solution, as follows:
  - a. Add 6 ml wash solution to each reservoir of the wash tray.
  - b. Add 350 ml wash solution to the 500 ml wash bottle.
3. Load the wash tray and wash bottle onto the instrument:
  - a. Open the reagent compartment door and reagent chiller door, and slide the used reagent cartridge or wash tray from the chiller.
  - b. Slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
  - c. Raise the sipper handle in front of the MiSeqDx SBS Solution (PR2) bottle and waste bottle until it locks into place, and replace the MiSeqDx SBS Solution (PR2) bottle with the wash bottle.

**NOTE** Discard the MiSeqDx SBS Solution (PR2) bottle after each run. Do not reuse any remaining MiSeqDx SBS Solution (PR2).

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

## Perform Second Wash

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 several times to mix.
2. When the first wash is complete, remove the wash tray and wash bottle, and discard the remaining wash solution.
3. Refill the wash components with fresh 0.5% Tween 20 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.

## 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 [Procedure on page 28](#).
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 Main Menu, select **Shut Down Instrument**.

This command shuts down the instrument software.

5. Toggle the power switch to the OFF position.

**NOTE** If the instrument is turned off, wait a *minimum* of 60 seconds before turning the power switch back to the ON position.

# Troubleshooting

## 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 MiSeqDx support pages on the Illumina website. The support pages provide access to documentation, downloads, and frequently asked questions. For access to support bulletins sign in to your MyIllumina account.

For problems with run quality or performance, contact Illumina Technical Support. For more information, see [Technical Assistance on page 48](#).

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

## 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. Select the box next to the run.
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.

## Perform System Check

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 40](#).



### CAUTION

The Tip/Tilt and Full Optics tests require a special flow cell and should only be performed by an Illumina technician.

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

## Pause or Stop a Run

The MiSeqDx 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.

When you select **Pause**, the current command is completed before pausing the run and placing the flow cell in a safe state.



### CAUTION

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

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**.

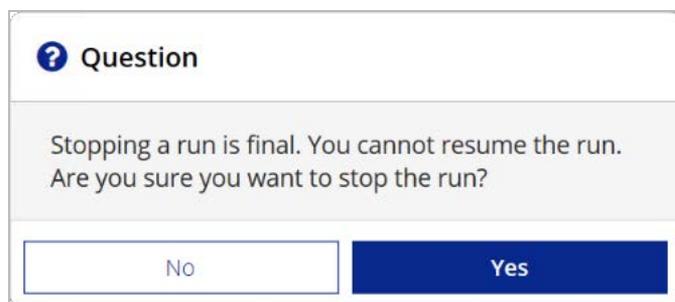
## Stop a Run

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

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

To stop a run from the Sequencing screen, select **Stop**. When a run is stopped, the current command is not completed and the flow cell stage moves to the forward position. Primary analysis continues for the last completed cycle.

Figure 16 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
<b>✘ Flow Rate Measured</b>	<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"> <li>• Solution: <b>PR2</b></li> <li>• Volume: <b>250</b></li> <li>• Aspirate Rate: <b>2500</b></li> <li>• Dispense Rate: <b>2500</b></li> </ul> <p>Select <b>Pump</b>. If the error persists, set the volume to pump 500 µl MiSeqDx SBS Solution (PR2) and repeat the process. When fluids have been pumped, select <b>Restart Check</b>.</p> <p>When the pre-run check is successful, the <b>Start Run</b> button becomes active.</p> <p>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>
<b>✘ Free Disk Space</b>	<p>If disk space is low, a message appears indicating how much disk space is required. Use the <b>Manage Files</b> feature to clear the required space from the instrument computer.</p>
<b>✘ Network Connection Active</b>	<p>Make sure that the network cable is plugged into the instrument.</p> <p>If the network connection is not restored, select <b>Reboot</b> on the Manage Instrument screen to reboot the software.</p> <p>If the connection is still not restored, select <b>Shut Down</b> 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>
<b>✘ Primary Analysis Ready</b>	<p>Primary analysis from the previous run is not complete. The default time to allow primary analysis to complete is one hour, and a countdown appears on the screen. The options are to wait one hour or select <b>Terminate Analysis</b>. Secondary analysis stops for any incomplete cycles.</p>

## Resolve RFID Read Failure

RFID failures are triggered if:

- The component loaded is not part of an *in vitro* diagnostic kit.
- The component loaded is not part of the kit identified by the Local Run Manager module.
- There is a technical failure with reading the RFID tag on the component.

The following steps can be used to resolve RFID failures resulting from a technical failure.

**NOTE** A diagnostics run is allowed one RFID read failure. If the RFID of two consumables cannot be read, the software cannot proceed to the next run setup step. If this error occurs, contact Illumina Technical Support.

### Flow Cell

1. Always retry the RFID read before proceeding. To do so, open and then close the flow cell compartment door.
2. If the RFID fails a second time, select **Get Code**.  
Contact Illumina Technical Support to obtain a temporary RFID bypass code. A temporary bypass code expires in seven days.
3. Enter the temporary bypass code using the on-screen keyboard.
4. Select **Next**.
5. Enter the following information:
  - Barcode number of the flow cell, which is on the flow cell container label directly below the barcode
  - Flow cell part number
6. Select **Next** to proceed to the Load Flow Cell screen.
7. Select **Next** to proceed to the next run setup step.

### MiSeqDx SBS Solution (PR2) Bottle

1. Always retry the RFID read before proceeding. To do so, raise and then lower the reagent sipper handle.
2. If the RFID fails a second time, select **Get Code**.  
Contact Illumina Technical Support to obtain a temporary RFID bypass code. A temporary bypass code expires in seven days.
3. Enter the temporary bypass code using the on-screen keyboard.
4. Select **Next**.
5. Enter the following information:
  - Barcode number of the MiSeqDx SBS Solution (PR2) bottle, which is on the MiSeqDx SBS Solution (PR2) bottle label directly below the barcode
  - MiSeqDx SBS Solution (PR2) bottle part number
6. Select **Next** to proceed to the Load Reagents screen.
7. Select **Next** to proceed to the next run setup step.

### Reagent Cartridge

1. Always retry the RFID read before proceeding. To do so, open and then close the reagent chiller door.

2. If the RFID fails a second time, select **Get Code**.  
Contact Illumina Technical Support to obtain a temporary RFID bypass code. A temporary bypass code expires in seven days.
3. Enter the temporary bypass code using the on-screen keyboard.
4. Select **Next**.
5. Enter the following information:
  - Reagent kit barcode number, which is on the kit label directly below the barcode
  - Reagent kit part number
6. Select **Next** to return to the Load Reagents screen.
7. Select **Next** to proceed to the next run setup step.

## Prevent Reboots During a Run

If the MiSeqDx restarts during a run, it may mean that the Windows Update software on the network is configured to install software updates automatically. This setting should have been turned off during installation. Contact the local IT department for help with disabling automatic updates to the Windows operating system running in the background on the MiSeqDx.

## Troubleshoot Flow Rate Error

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

1. Use the drop-down list or on-screen keyboard to enter the following information:
  - Solution: **PR2**
  - Volume: **250  $\mu\text{l}$**
  - Aspirate Rate: **2500  $\mu\text{l}/\text{min}$**
  - Dispense Rate: **2500  $\mu\text{l}/\text{min}$**
2. Select **Pump**.
3. When the pump step is complete, select **Restart Check**.
4. If the error persists, set the volume to pump 500  $\mu\text{l}$  MiSeqDx SBS Solution (PR2) and repeat the process one more time. Contact Illumina Technical Support if the second attempt does not resolve the error.

## Perform a Volume Test

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

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

1. Make sure that a used flow cell is loaded on the instrument.
2. From the Main Menu, select **System Check**.
3. Select **Conduct Volume Test**, and then select **Next**.
4. Fill each reservoir of the wash tray with 6 ml laboratory-grade water.
5. Fill the 500 ml wash bottle with 350 ml laboratory-grade water.
6. Load the wash tray and wash bottle onto the instrument.
  - a. Open the reagent compartment door and reagent chiller door, and slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
  - b. Raise the sipper handle until it locks into place, and load the wash bottle.
  - c. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
  - d. Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
7. Following the on-screen prompts, remove any droplets from the wash bottle sipper, as follows:
  - a. When prompted, slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.
  - b. When prompted, slowly lower the sipper handle far enough into the water to allow the surface tension to remove the droplet.
  - c. When prompted, slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.
  - d. When prompted, slowly lower the sipper handle completely, making sure that the sippers lower into the wash bottle and waste bottle.
8. Select **Next**. The volume test begins.

When the volume test is complete, the results appear on the screen.  
If the test did not pass, perform a maintenance wash. See [Procedure on page 28](#).
9. When the maintenance wash is complete, repeat the volume test.

## Resolve Reagent Chiller Temperature Errors

The required temperature range of the reagent chiller is 2°C to 11°C. A sensor indicator shows the temperature of the reagent chiller. See [Sensor Indicators on page 5](#).

If you receive an error message that the chiller is not in the specified temperature range, contact Illumina Technical Support.

If the chiller temperature is out of range, it can prevent the sequencing run from starting. If you receive the error message during a sequencing run, allow the run to complete.

For more information on the reagent chiller, see [Reagent Compartment on page 3](#).

## Resolve Local Run Manager Analysis Errors

For troubleshooting information related to analysis errors, contact Illumina Technical Support. The *Local Run Manager v3 Software Reference Guide for MiSeqDx (document # 200003931)* includes instructions on how to requeue analysis.

## Configure System Settings

The MOS includes tabs that access commands to configure the system.

- IP and DNS settings are configured on the IP tab. Windows Administrator level access is required to use this feature.
- Network and start-up settings are configured on the following tabs:
  - Network Credentials—Windows Administrator level access is required to use this feature.
  - Start-Up Options—Local Run Manager Admin level access is required to use this feature.

Typically, these system settings are configured during MiSeqDx installation.

## Configure IP and DNS Settings

Configure IP address and DNS server addresses if necessary due to a network or facility change. Windows Administrator level access is required to configure this feature.

1. From the Main Menu, select **System Settings**.
2. Select the IP tab, and then select from the following options to set up the IP address:
  - **Obtain an IP address automatically**—Select this option to obtain the IP address using Dynamic Host Configuration Protocol (DHCP) server.

**NOTE** Dynamic Host Configuration Protocol (DHCP) is a standard network protocol used on IP networks for dynamically distributing network configuration parameters.

- **Use the following IP address**—Select this option to connect the instrument to another server manually as follows. Contact your network administrator for the addresses specific to your facility.
    - Enter IP address. The IP address is a series of four numbers separated by a dot, similar to 168.62.20.37, for example.
    - Enter the subnet mask, which is a subdivision of the IP network.
    - Enter the default gateway, which is the router on the network that connects to the Internet.
3. Select from the following options to set up the DNS address:
    - **Obtain a DNS address automatically**—Reads the DNS address associated with the IP address.
    - **Use the following DNS addresses**—Connects the instrument to a server that translates domain names into IP addresses.
      - Enter the preferred DNS address. The DNS address is the server name used to translate domain names into IP addresses.
      - Enter the alternate DNS address. The alternate is used if the preferred DNS cannot translate a particular domain name to an IP address.
  4. Select **Save**.

## Configure Network and Start-Up Settings

Configure network and start-up settings on the Network Credentials tab (Windows Administrator level access is required to use this feature) and the Start-Up Options tab (Local Run Manager Admin level access is required).

1. From the Main Menu, select **System Settings**.
2. Select the Network Credentials tab, and then configure network settings as follows.
3. The machine name is assigned to the instrument computer at the time of manufacture. Typically, there is no need to change the machine name. Any changes made to the machine name on this screen can affect connectivity and require the user name and password of a network administrator. The machine name is recorded as the instrument name in Local Run Manager software output.
4. Connect the instrument computer to a domain or a workgroup as follows.
  - **For instruments connected to the Internet**—Select **Domain**, and then enter domain name associated with the internet connection at your facility.
  - **For instruments not connected to the Internet**—Select **Workgroup**, and then enter a work group name.
5. Select the Start-Up Options tab, and then select from the following options:
  - **Kiosk Mode** (recommended)—Shows the control software interface in full screen. The software is designed for use in kiosk mode.
  - **Windows Mode**—Allows access to Windows on the instrument computer. Interaction with the software interface, such as button location, might be altered in this mode.
6. Select **Save**.

# Output Folders

## Run Folders

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

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

## Root Folder Naming

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

By default, the folder name uses the following format:

YYMMDD\_<InstrumentNumber>\_<Run Number>\_A<FlowCellBarcode>

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

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# Technical Assistance

For technical assistance, contact Illumina Technical Support.

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**Safety data sheets (SDSs)**—Available on the Illumina website at [support.illumina.com/sds.html](https://support.illumina.com/sds.html).

**Product documentation**—Available for download from [support.illumina.com](https://support.illumina.com).



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