

MiSeq FGx™ Instrument Reference Guide

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Revision History

Document #	Date	Description of Change
VD2018006 Rev. A	June 2018	Updated document number and other document numbers listed in this guide to Verogen document numbers. Updated technical support information with Verogen contacts. Updated the location of SDS information on the Verogen website. Added information about MiSeq FGx reagent kits, including kit contents, storage requirements, kit component overviews, flow cell types, and software version compatibility. Added instructions to prepare the reagent cartridge and denature and dilute libraries for a sequencing run.
15050524 Rev. C	February 2015	Corrected formatting errors.
15050524 Rev. B	January 2015	Updated the name of the reagent kit from MiSeq ForenSeq Sequencing Kit to MiSeq FGx Reagent Kit.
15050524 Rev. A	October 2014	Initial release.

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Getting Started

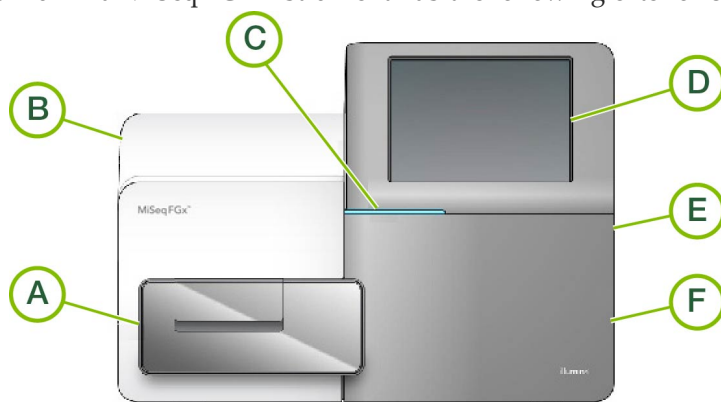
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Intended Use

The Illumina MiSeq FGx™ is a sequencing instrument that measures fluorescence signals of labeled nucleotides through the use of instrument-specific reagents and flow cells, imaging hardware, and data analysis software. The MiSeq FGx, as part of the Verogen MiSeq FGx Forensic Genomics Solution, is intended for targeted sequencing of DNA for forensic casework, database, and other human identification applications. The MiSeq FGx also features a Research Use Only (RUO) mode that works with all Illumina RUO library preparation kits compatible with the MiSeq.

Components

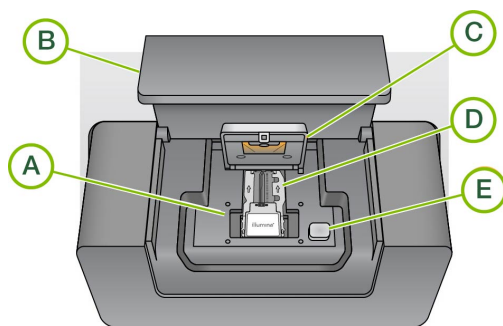
The Illumina MiSeq FGx instrument has the following exterior components:



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

The MiSeq FGx interface guides users through the run setup steps using the touch screen monitor.

Flow Cell Compartment

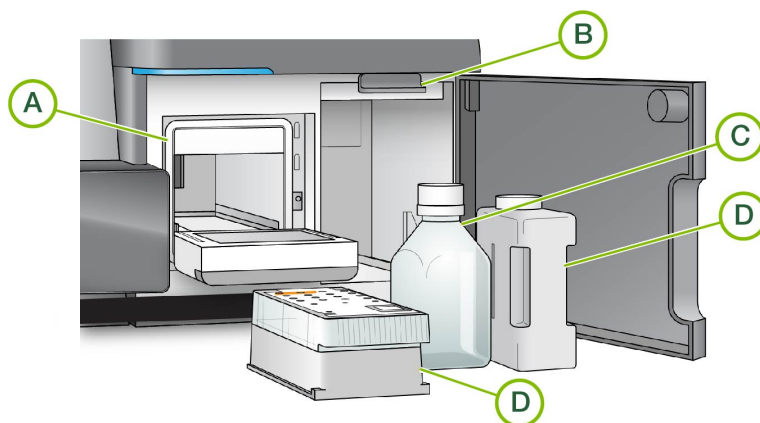


- A Flow Cell Stage
- B Flow Cell Compartment Door
- C Flow Cell Latch
- D Flow Cell
- E Flow Cell Latch Release Button

The flow cell compartment houses the flow cell stage, thermal station, and fluidics connections to the flow cell. The flow cell stage holds the flow cell and the flow cell latch secures and positions the flow cell. When the flow cell latch closes, two pins near the latch hinge auto-position the flow cell.

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

Reagent Compartment



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

The reagent compartment contains the reagent chiller, and positions for the 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 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.

Starting the MiSeq FGx

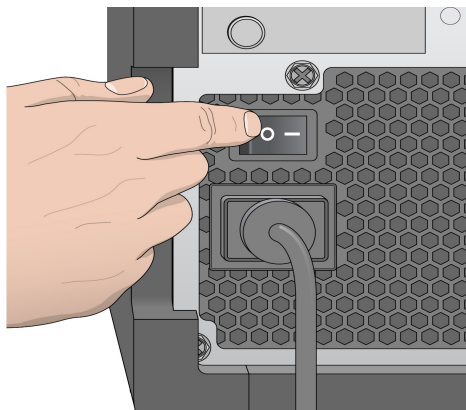


NOTE

Verogen recommends that you leave the instrument on continuously. However, if the instrument must be turned off follow the shutdown procedure described in *Shutting Down the Instrument* on page 65. Wait a **minimum** of 60 seconds before turning the power switch back to the ON position.

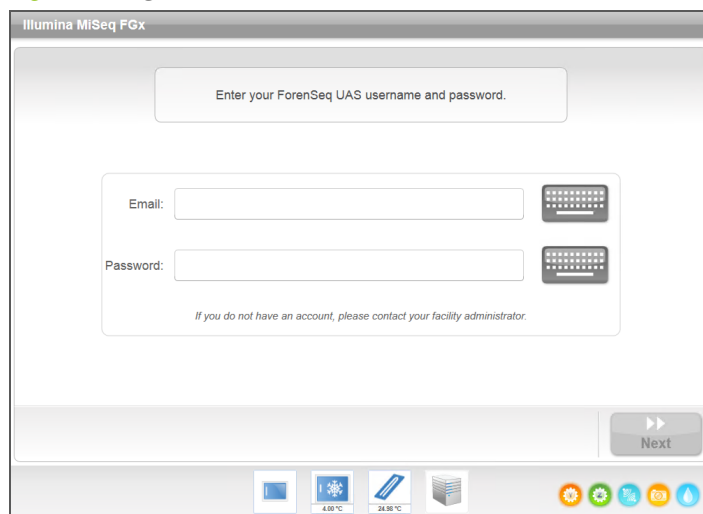
- 1 If the MiSeq FGx is not already on, reach around the right side of the instrument to locate the power switch on the back panel. It is in the lower corner directly above the power cord.

Figure 1 Power Switch Location



- 2 Turn the power switch to the **ON** position. The integrated instrument computer starts.
- 3 Log in to the operating system.
- 4 Wait until the operating system has finished loading. The MiSeq FGx Control Software launches and initializes the instrument automatically.
- 5 After the initialization step is complete, log in using your ForenSeq Universal Analysis Software user name and password.

Figure 2 Log In Screen



- 6 Click **Next**. The Welcome screen opens. See *Welcome Screen* on page 15.

MiSeq FGx Reagent Kit Overview

The MiSeq FGx Reagent Kit is a single-use reagent kit required to perform a sequencing run. Each kit includes a kit-specific flow cell type and all the reagents required for performing a run.

The flow cell, SBS Solution (PR2) bottle, and reagent cartridge provided in the kit use radio-frequency identification (RFID) for accurate consumable tracking and compatibility. Always use the reagent cartridge associated with your flow cell type. If the reagent cartridge is not compatible, a message appears during run setup that prompts you to load a compatible reagent cartridge.

Kit Type	Catalog #
MiSeq FGx Reagent Kit	TG-143-1001
MiSeq FGx Reagent Micro Kit	TG-143-1002

Each kit type includes one kit-specific MiSeq FGx flow cell, either standard or micro.

Kit Type	Flow Cell Type	Kit Size
MiSeq FGx Reagent Kit	Standard flow cell	600 cycles
MiSeq FGx Reagent Micro Kit	Micro flow cell	600 cycles

Reagent Kit Contents and Storage Requirements

All MiSeq FGx reagent kits are packaged in two boxes. As soon as you receive your kit, promptly store the kit components at the indicated temperature.

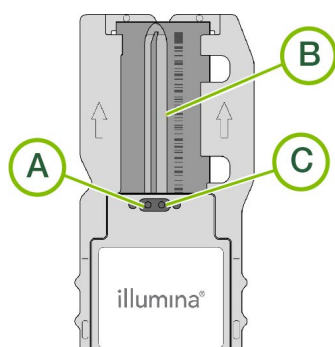
Table 1 Box 1: Store at -25°C to -15°C

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

Table 2 Box 2: Store at 2°C to 8°C

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

Flow Cell Overview



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

The MiSeq FGx 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.

Flow Cell Cap Color

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

Flow Cell Type	Flow Cell Cap Color
MiSeq FGx Standard Flow Cell	Clear cap
MiSeq FGx Micro Flow Cell	Green cap

Flow Cell Tiles

Flow Cell Type	Tiles	Imaging Surface	Total Tiles Imaged
MiSeq FGx Standard Flow Cell	19 tiles	Top	19 tiles total
MiSeq FGx Micro Flow Cell	8 tiles	Top and bottom	8 tiles total

MiSeq FGx Control Software Version Requirements

Each kit type requires a compatible version of MiSeq FGx Control Software to read the RFID and provide compatible run modes during run setup.

Kit Type	Required Software Version	Compatible Run Mode
MiSeq FGx Reagent Kit	MiSeq FGx Control Software v1.0, or later	Forensic Genomics Research Use Only
MiSeq FGx Reagent Micro Kit	MiSeq FGx Control Software v1.3, or later	Forensic Genomics Research Use Only
MiSeq Reagent Kits v1/v2/v3	MiSeq FGx Control Software v1.0, or later	Research Use Only

Reagent Cartridge Overview

The MiSeq FGx reagent cartridge is a single-use consumable consisting of foil-sealed reservoirs prefilled with clustering and sequencing reagents sufficient for sequencing one flow cell.

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

Figure 3 Reagent Cartridge with Numbered Reservoirs

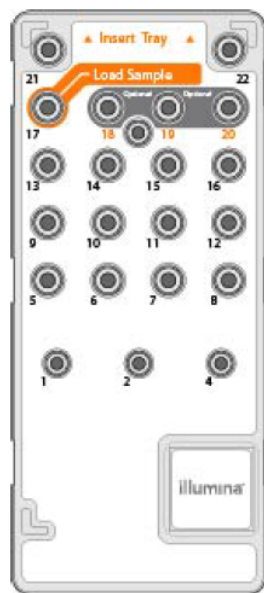


Table 3 Reagent Cartridge Numbered Positions

Numbered Position	Reagent Name	Description
8	LDR	Denaturation Reagent (contains formamide)
17	Reserved	Load Sample (reserved for sample libraries)
18	Reserved	Reserved for custom Read 1 primer [Optional]
19	Reserved	Reserved for custom Index Read primer [Optional]
20	Reserved	Reserved for custom Read 2 primer [Optional]



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 the risk exposure. Handle reagents as chemical waste and discard in accordance with the applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at www.verogen.com/sds.

User-Supplied Consumables

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

Consumable	Supplier	Purpose
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	VWR, catalog # 95041-714* General lab supplier	Cleaning the flow cell holder
Disposable gloves, powder-free	General lab supplier	General use
Lab tissue, low-lint	VWR, catalog # 21905-026*	Cleaning the flow cell stage and the foil seal covering the load samples reservoir
Lens paper, 4 x 6 in.	VWR, catalog # 52846-001*	Cleaning the flow cell
Microcentrifuge tubes	General lab supplier	Denaturing and diluting sample libraries and control DNA
MiSeq wash tube	Verogen, part # MS-102-9999	Washing the instrument
Sodium hypochlorite	General lab supplier	Washing the instrument
Tween 20	Sigma-Aldrich, catalog # P7949	Washing the instrument
Tweezers, square-tip plastic (optional)	McMaster-Carr, catalog # 7003A22*	Removing flow cell from flow cell shipping container
Water, nuclease-free	General lab supplier	Washing the instrument

* or equivalent

Guidelines for Nuclease-free Water

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

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

MiSeq FGx Software

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Introduction

The software described in this chapter is used to configure, run, and analyze data from the MiSeq FGx instrument.

- ▶ **MiSeq FGx Control Software**—Controls the instrument operation, including various configuration settings. This software is installed and runs on the instrument. See *MiSeq FGx Control Software* on page 15.
- ▶ **Real-Time Analysis (RTA)**—Performs image processing 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. This software is installed and runs on the instrument.
- ▶ **ForenSeq Universal Analysis Software**—Performs user authentication and analysis such as demultiplexing, alignment, variant calling, and report generation. The specific functions that are supported vary by application. This software is installed off-instrument on a stand-alone server. The output is viewed through a web browser. See the *ForenSeq Universal Analysis Software Guide (document # VD2018007)* for information on analyzing data.
- ▶ For RUO runs, BaseSpace and MiSeq Reporter are available to perform additional analyses. See *Analysis Software Options for RUO Runs* on page 35.

MiSeq FGx Control Software

During the run, MiSeq FGx Control Software operates the flow cell stage, dispenses reagents, controls flow cell temperatures, and captures images of clusters on the flow cell. The control software performs the run according to parameters specified in the ForenSeq Universal Analysis Software.

Welcome Screen

The MiSeq FGx Control Software interface opens to the Welcome screen when the software launches.

Figure 4 Welcome Screen



- ▶ **Sequence**—This option opens a series of run setup screens that guide users through the run setup steps. See *Run Setup Screens* on page 24.
- ▶ **Perform Wash**—Provides options to start the following types of instrument washes: a maintenance wash, standby wash, or a post-run wash. See *Perform Wash Screen* on page 17.
- ▶ **Manage Files**—Provides controls for moving, deleting, and uploading files on the instrument computer. See *Manage Files Screen* on page 18.
- ▶ **Run Options**—Provides options for the post-run wash, changing default locations of data folders, and specifying email notification preferences. See *Run Options Screen* on page 19.
- ▶ **Manage Instrument**—Provides options to go to system settings, perform a systems check, update software manually, and reboot or shut down the instrument. See *Manage Instrument Screen* on page 22.

Activity Indicators

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

Figure 5 Activity Indicators



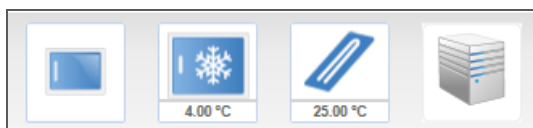
From left to right, the activity indicators represent the following activities:

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

Sensor Indicators

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

Figure 6 Sensor Indicators







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

- ▶ Flow cell compartment door in the closed or open positions
- ▶ Temperature of the reagent chiller in °C
- ▶ Temperature of the flow cell in °C
- ▶ Status of server connection

Status Icons

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

Status Icon	Status Name	Description
	Status OK	No change. System is normal.
	Attention	Important information. Action is recommended.
	Warning	Warnings do not stop a run. However, some warnings require action before proceeding.
	Error	Errors usually stop a run and generally require action before proceeding with the run.

When a change in condition occurs, the icon changes to the associated image and blinks to capture attention. 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.

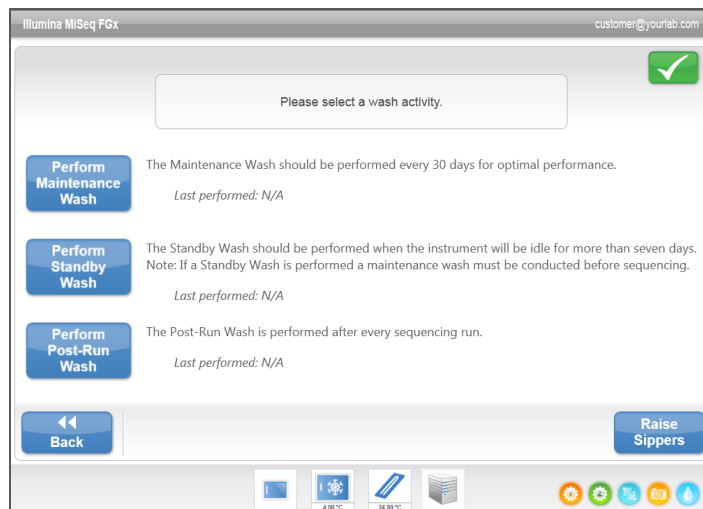
Messages in the status window can be filtered by selecting the icons along the top margin of the window. Selecting an icon toggles the condition to show or hide.

Perform Wash Screen

The Perform Wash screen enables users to perform the following types of washes, as well as see the date of the most recent wash performed:

- ▶ **Maintenance Wash**—The maintenance wash consists of three consecutive wash cycles that thoroughly flush the system. Perform a maintenance wash at least every 30 days. See *Procedure* on page 60.
- ▶ **Standby Wash**—The standby wash properly prepares the fluidics lines for sitting idle and consists of two consecutive wash cycles. Perform a standby wash if you expect the instrument to remain idle for up to seven days. See *Performing a Standby Wash* on page 63.
- ▶ After the instrument has been placed in an idle state, a maintenance wash must be performed before a sequencing run can be initiated.
- ▶ **Post-Run Wash**—The post-run wash is the standard instrument wash performed between sequencing runs and consists of a single wash cycle. To perform a post-run wash at a time other than directly following a run, use the command on the Perform Wash screen to initiate the wash.
- ▶ The MiSeq FGx instrument can be configured to perform a maintenance wash, rather than a post-run wash, between runs. For more information, see *Run Options Screen* on page 19.

Figure 7 Perform Wash Screen



The Perform Wash screen also includes a command that raises the sippers, so the reagent cartridge can be removed from the instrument in the case of an error or unexpected run interruption.

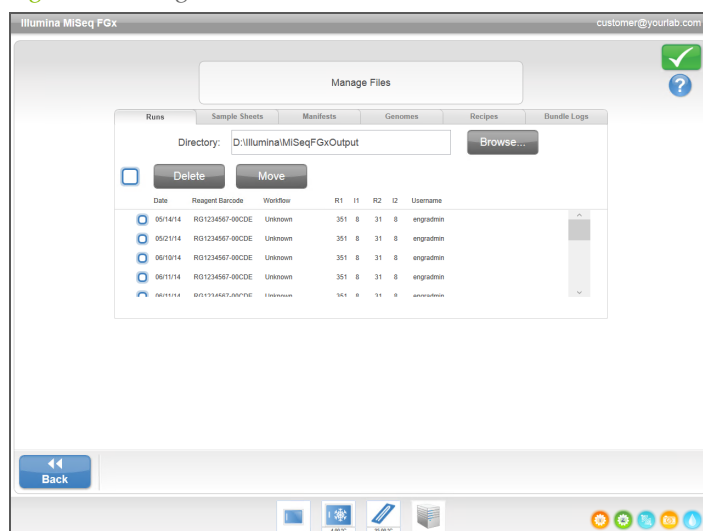
**CAUTION**

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

Manage Files Screen

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

Figure 8 Manage Files Screen



Manage Files Options

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

Tab	Features	Use
Runs	Delete or Move	For forensic or RUO mode.
Sample Sheets	Delete, Upload, or Rename	For RUO mode.
Manifests	Delete or Upload	For RUO mode.
Genomes	Delete or Upload	For RUO mode.
Recipes	Delete or Upload	For RUO mode.
Bundle logs	Bundle Logs	For Verogen Technical Support troubleshooting only.

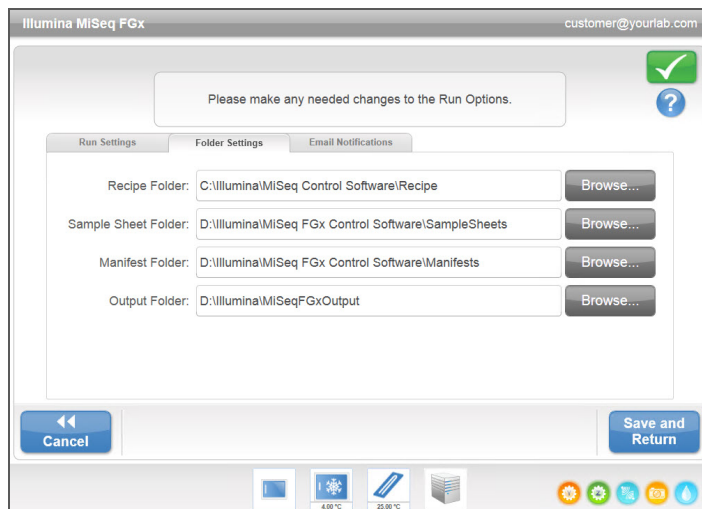
* ForenSeq manifest, genome and recipe files are pre-loaded for forensic kits and should not be changed.

- ▶ **Delete**—Select the checkbox next to the file or folder listed, and then select **Delete**. The Delete feature is available on all tabs. Admin user access level is required to delete run files.
- ▶ **Move**—Available only for run folders. Select the checkbox next to the folder name, select **Move**, and then browse to an appropriate location. **Move** *copies* the run folder to the new location and then *deletes* the folder from the old location.
- ▶ **Select All Files**—Select the checkbox to the left of the Delete button, and then select an action: Delete or Move. The action is applied to all files or folders.
- ▶ **Upload Files**—If the MiSeq FGx is not connected to a network, use this feature to upload sample sheet or manifest files to the instrument computer from a USB drive. Select **Upload** and browse to the location on a USB drive where the sample sheet resides. The file is uploaded to the folder indicated in the Directory field.
- ▶ **Rename**—Select the checkbox next to the sample sheet file, and then select **Rename**. Use the on-screen keyboard to rename the sample sheet.
- ▶ **Bundle Logs**—This command combines and zips groups of files for use by Verogen Technical Support for troubleshooting purposes.

Run Options Screen

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

Figure 9 Run Settings Tab on Run Options Screen



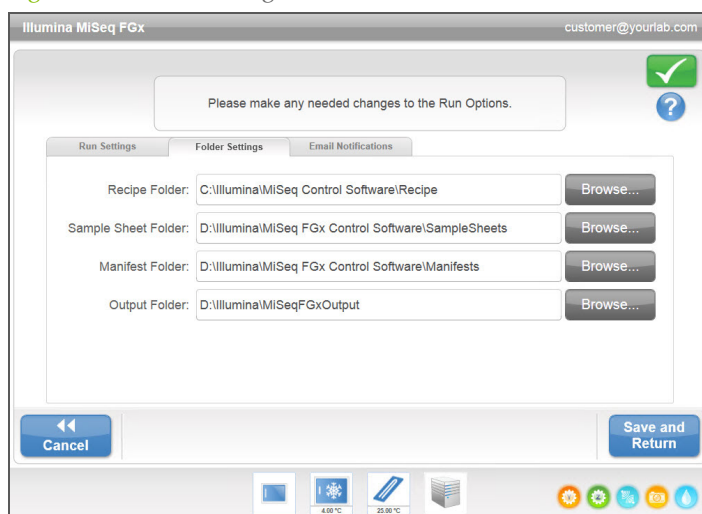
Run Settings Tab

- ▶ **Post-Run Wash Option**—An instrument wash is required after each run. The software will not proceed to the run setup steps for a subsequent run until a wash has been performed. This setting determines what type of wash will be performed by default after a run is configured here. A post-run wash takes about 30 minutes and a maintenance wash takes about 1 hour.

Folder Settings Tab

The MiSeq FGx requires access to various types of files stored in folders on a local network or on the instrument computer. The options on the Folder Settings tab tell the MiSeq FGx where those folders are located.

Figure 10 Folder Settings Tab



- ▶ **Recipes**—Custom recipes are not supported for ForenSeq kits.
- ▶ **Sample Sheets**—Sets the default location for sample information to the ForenSeq Universal Analysis Software. Sample information is created before library preparation and contains the parameters for the run.
- ▶ **Manifests**—ForenSeq kits use manifest files that are preloaded.

- ▶ **MiSeqFGxOutput**—Sets the default location for analysis output files. Verogen recommends changing the default output folder to a network location for sharing and long-term storage.

Email Notifications Tab

The MiSeq FGx instrument can be configured to send an email notification when RTA analysis is complete, when ForenSeq Universal Analysis Software analysis is complete, or if a critical MiSeq FGx software error occurs. Email notification is only available if the instrument is connected to a network with access to the internet.

Figure 11 Email Notifications Tab

- ▶ **Local SMTP email server address**—Use the on-screen keyboard to enter the local SMTP email server address. If necessary, contact the facility administrator for this information.
- ▶ **Sender email address**—Use the on-screen keyboard to enter the sender email address. This address can be the email address of an Admin user 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.

Manage Instrument Screen

The Manage Instrument screen contains controls for system settings, troubleshooting, manual software updates, rebooting, or shutting down the instrument software.

Figure 12 Manage Instrument Screen

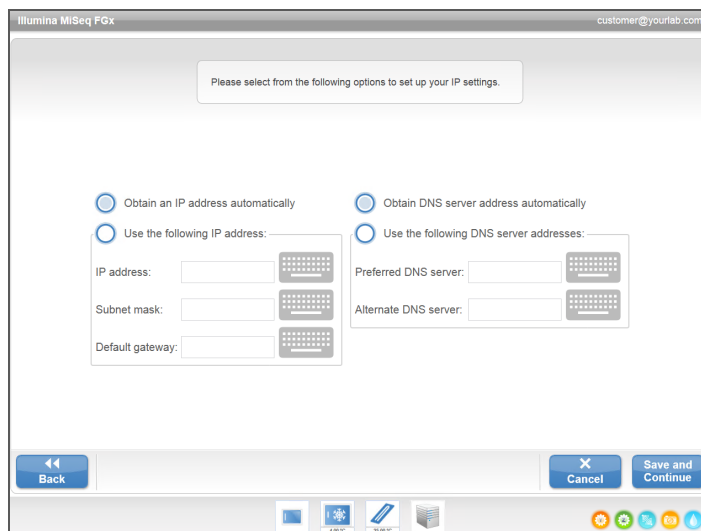


- ▶ **System Settings**—Provides the option to change IP Settings, machine name, or domain. See *System Settings Screen* on page 23.
- ▶ **System Check**—Provides troubleshooting options for checking the operational status of instrument components. See *System Check Screen* on page 23.
- ▶ **Manual Update**—Provides the option to update software on the instrument computer manually. See *Manual Update Screen* on page 24.
- ▶ **Reboot**—Use the Reboot command to reboot the system software. There is no requirement to reboot the software as part of regular maintenance.
- ▶ **Shut Down**—Use the Shut Down command to shut down the control software and Windows on the instrument computer. See *Shutting Down the Instrument* on page 65.
- ▶ **Minimize to Windows**—Provides quick access to the instrument operating system and any folders on the instrument computer when the MiSeq FGx is running in kiosk mode as opposed to Windows mode. Admin or SuperUser user access level is required to use this feature.

System Settings Screen

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

Figure 13 System Settings



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

System Check Screen

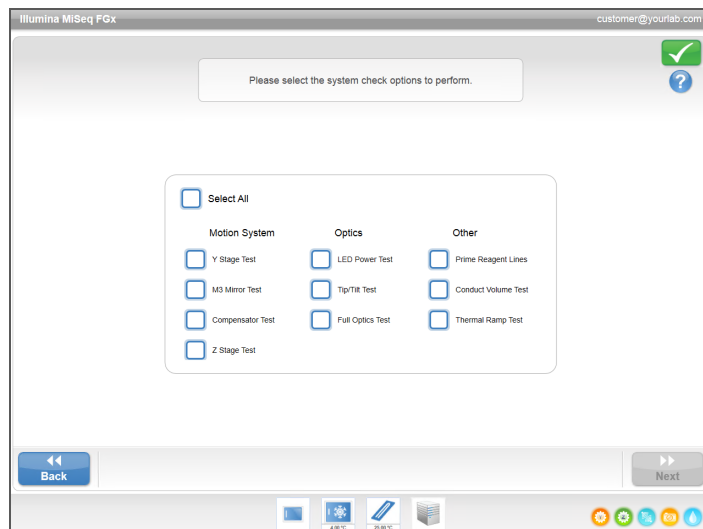
The System Check screen is used to evaluate performance of various instrument functions, usually during troubleshooting. Use of this feature is not required during normal operation or for instrument maintenance.

Some system checks, such as the Volume Test, can be performed before contacting Verogen Technical Support. 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 *Performing a Volume Test* on page 74.

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

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

Figure 14 System Check Options



Manual Update Screen

The Manual Update feature is used to update instrument control software and analysis software. This feature is not available for the MiSeq FGx. All software updates are performed by Verogen personnel to maintain proper system configuration.

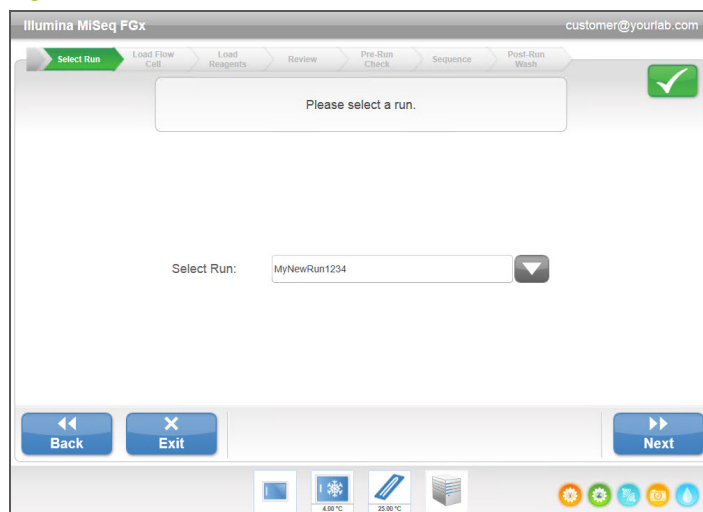
Run Setup Screens

After selecting **Sequence** on the Welcome screen, select a run type. When you select Forensic Genomics, a series of run setup screens open in the following order: Select Run, Load Flow Cell, Load Reagents, Review, and Pre-Run Check. When you select a Research Use Only Run, a different series of run setup screens opens. See *Run Setup Screens for an RUO Run* on page 28.

Select A Run Screen

Select a run for sequencing using the drop-down menu. Runs are set up in the ForenSeq Universal Analysis Software.

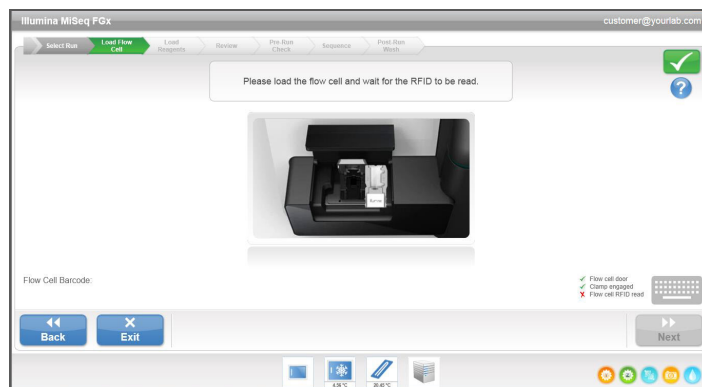
Figure 15 Select a Run Screen



Load Flow Cell Screen

The Load Flow Cell screen appears when it is time to load the flow cell. After loading the flow cell, close the flow cell latch and flow cell compartment door. Both the latch and compartment door must be closed before beginning the run.

Figure 16 Load Flow Cell Screen



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

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, or SBS Solution) to fail. For more information, see *Resolving RFID Read Failure* on page 70.

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

Load Reagents Screen

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

Figure 17 Load SBS Solution (PR2) Bottle and Waste Bottle

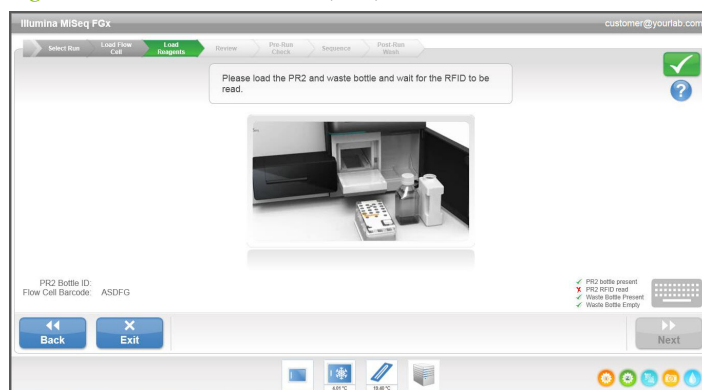
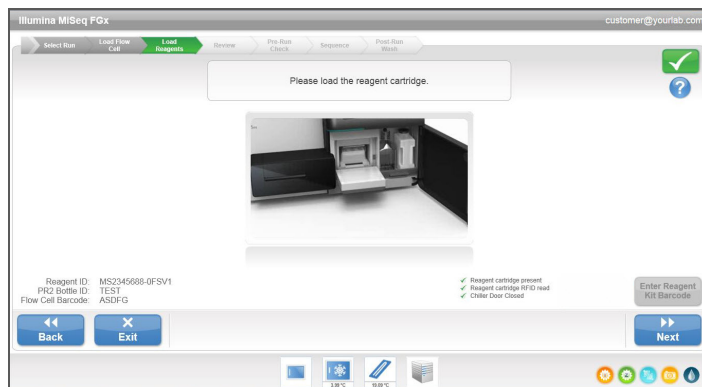


Figure 18 Load Reagent Cartridge



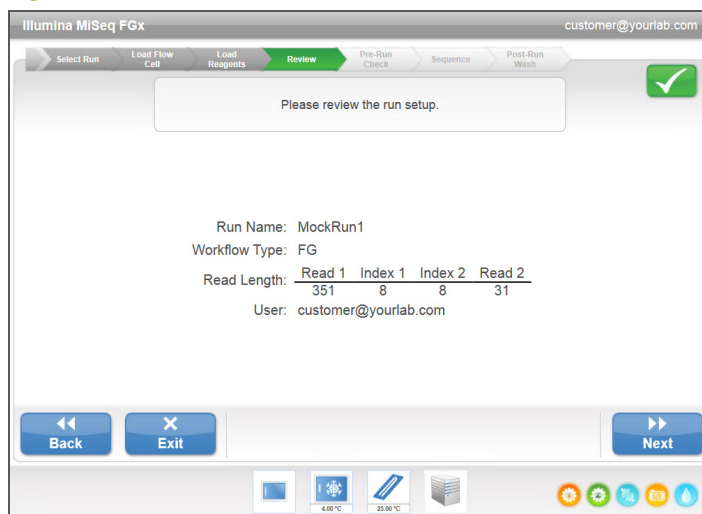
If the RFID cannot be read, identifying information can be entered manually. However, the software allows only one of the three RFID-labeled components (flow cell, reagent cartridge, or SBS Solution) to fail. For more information, see *Resolving RFID Read Failure* on page 70.

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

Review Screen

The Review screen confirms that MiSeq FGx Control Software received the run information from the ForenSeq Universal Analysis Software.

Figure 19 Review Screen

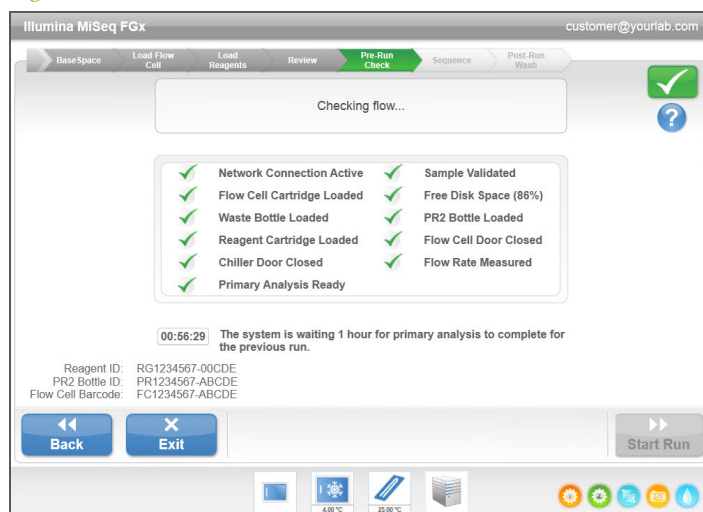


Pre-Run Check Screen

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

For more information, see *Resolving Run Setup Errors* on page 69.

Figure 20 Pre-Run Check Screen



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

Sequencing Screen

The Sequencing screen opens when the run begins. This screen provides a visual representation of the run in progress, including intensities and quality scores (Q-scores).

- ▶ **Run Progress**—Shows run progress in a status bar and lists the number of cycles completed.
- ▶ **Intensity**—Shows the number of tiles and surfaces being imaged, and shows the value of cluster intensities of the 90th percentile for each tile.
 - ▶ The standard flow cell is imaged on the top surface only. A single-column graphic appears.
 - ▶ The micro flow cell is imaged on the top and bottom surfaces. A dual-column graphic appears.
- ▶ **Flow Cell**—Shows the current temperature and status of the flow cell.
 - ▶ Temperature—Blue indicates cooler temperatures. Orange and red indicate warmer temperatures.
 - ▶ Imaging—Dark gray indicates the tile has been imaged.
 - ▶ Chemistry—Wavy lines indicate that reagents are pumping through the flow cell.
- ▶ **Cluster Density (K/mm²)**—Shows the number of clusters per square millimeter for the run. Optimally, expect a cluster density range of 400-1650 K/mm².
- ▶ **Clusters Passing Filter (%)**—Shows the percentage of clusters passing filter based on the Illumina chastity filter, which measures quality. This data appears only after cycle 25.



NOTE

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

- ▶ **Phasing (%)**—See *Number of Cycles in a Read* on page 33 and the Run Metrics Tab section in the *ForenSeq Universal Analysis Software Guide* (document # VD2018007).
- ▶ **Prephasing (%)**—See *Number of Cycles in a Read* on page 33 and the Run Metrics Tab section in the *ForenSeq Universal Analysis Software Guide* (document # VD2018007).
- ▶ **Read 1**—For information, see the Run Metrics Tab section in the *ForenSeq Universal Analysis Software Guide* (document # VD2018007).

- ▶ **Index 1**—For information, see the Run Metrics Tab section in the *ForenSeq Universal Analysis Software Guide* (document # VD2018007).
- ▶ **Read 2**—For information, see the Run Metrics Tab section in the *ForenSeq Universal Analysis Software Guide* (document # VD2018007).
- ▶ **Index 2**—For information, see the Run Metrics Tab section in the *ForenSeq Universal Analysis Software Guide* (document # VD2018007).

Run Setup Screens for an RUO Run

After selecting **Sequence** on the Welcome screen, select a run type. When you select Research Use Only Run, a series of run setup screens open in the following order: BaseSpace Option, Load Flow Cell, Load Reagents, Review, and Pre-Run Check. When you select Forensic Genomics, a different series of run setup screens opens. See *Run Setup Screens* on page 24.

BaseSpace Option Screen

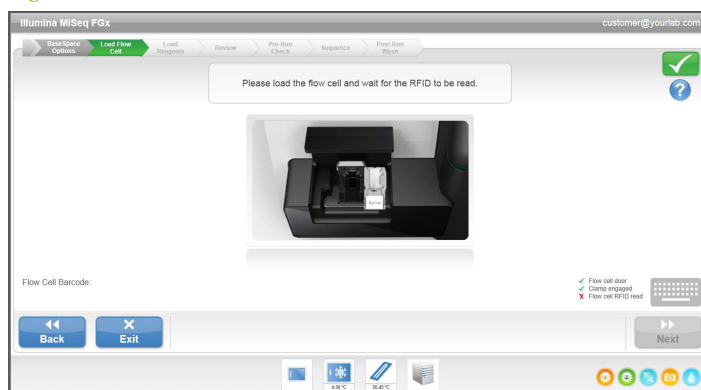
If performing analysis of an RUO run on BaseSpace, select **Use BaseSpace for storage analysis**. When you select the BaseSpace option, run information transfers to BaseSpace. Enter your Myllumina account information.

Figure 21 BaseSpace Option Screen

Load Flow Cell Screen

The Load Flow Cell screen appears when it is time to load the flow cell. After loading the flow cell, close the flow cell latch and flow cell compartment door. Both the latch and compartment door must be closed before beginning the run.

Figure 22 Load Flow Cell Screen



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

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, or SBS Solution) to fail. For more information, see *Resolving RFID Read Failure* on page 70.

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

Load Reagents Screen

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

Figure 23 Load SBS Solution (PR2) Bottle and Waste Bottle

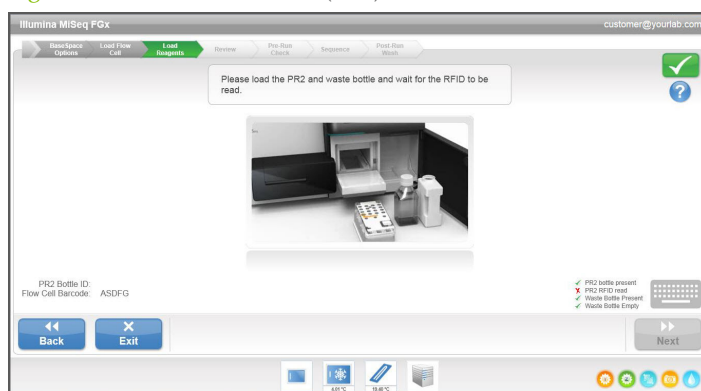
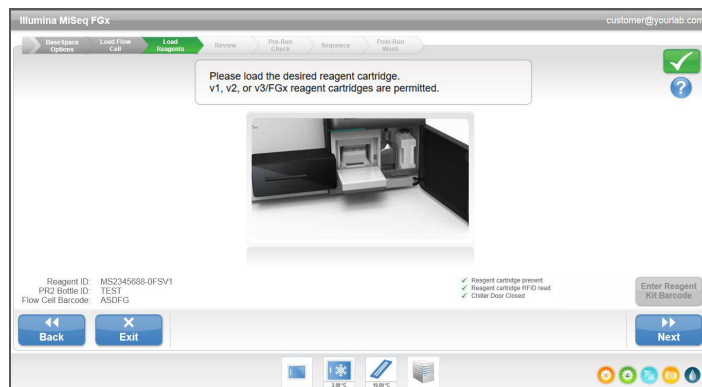


Figure 24 Load Reagent Cartridge



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, or SBS Solution) to fail. For more information, see *Resolving RFID Read Failure* on page 70.

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

Change Sample Sheet

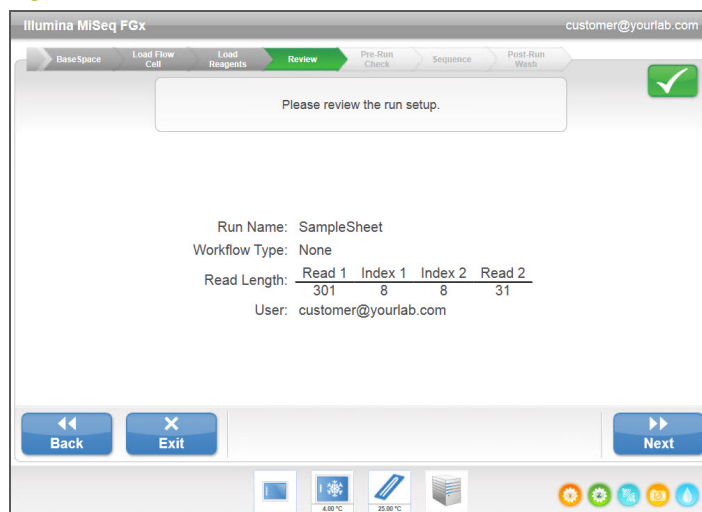
A sample sheet is required for an RUO run. By default, the software searches for a sample sheet file with a name that matches the barcode number of the reagent cartridge loaded on the instrument. If no sample sheet is found, a window opens so you can browse to the sample sheet.

Select the **Change Sample Sheet** button on the Load Reagents screen and browse to the appropriate sample sheet.

Review Screen

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

Figure 25 Review Screen

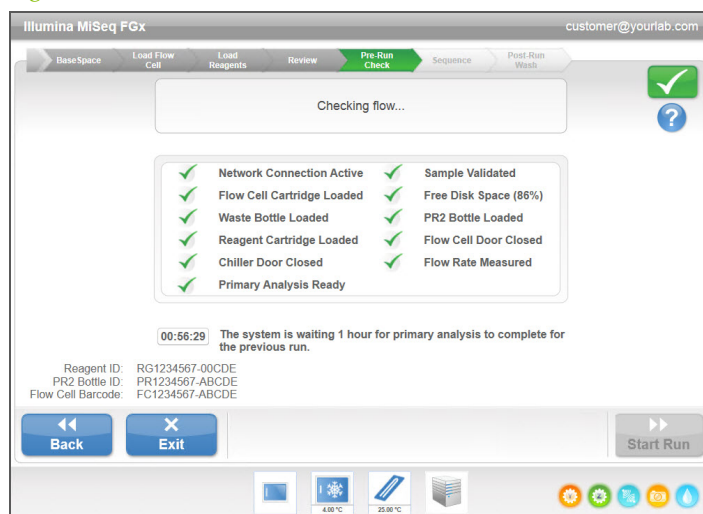


Pre-Run Check Screen

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

For more information, see *Resolving Run Setup Errors* on page 69.

Figure 26 Pre-Run Check Screen



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

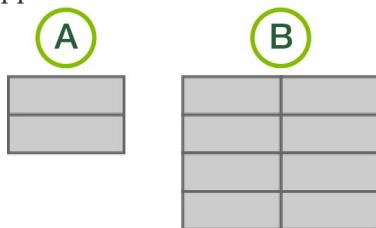
Sequencing Screen for RUO

During the RUO run, monitor run progress, intensities, and quality scores that appear on the Sequencing screen. The Sequencing screen is view-only.

To monitor the run in greater detail, use the Sequencing Analysis Viewer (SAV) installed on another computer independent of the instrument computer. A network connection is required. See *Sequencing Analysis Viewer User Guide* (part # 15020619).

If you are connected to BaseSpace, you can monitor your run using SAV in BaseSpace.

- ▶ **Run Progress**—Shows run progress in a status bar and lists the number of cycles completed.
- ▶ **Intensity**—Shows the value of cluster intensities of the 90th percentile for each tile. The graphic in the Intensity area represents the number of tiles and number of surfaces being imaged:
 - ▶ For flow cells imaged on the top surface only, a single-column graphic appears.
 - ▶ For flow cells imaged on the top and bottom surfaces, a dual-column graphic appears.



- A Indicates two tiles, top surface only
- B Indicates four tiles, top and bottom surface

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

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

- ▶ **Cluster Density (K/mm²)**—Shows the number of clusters per square millimeter for the run.
- ▶ **Clusters Passing Filter (%)**—Shows the percentage of clusters passing filter based on the Illumina chastity filter, which measures quality. This data appears only after cycle 25.

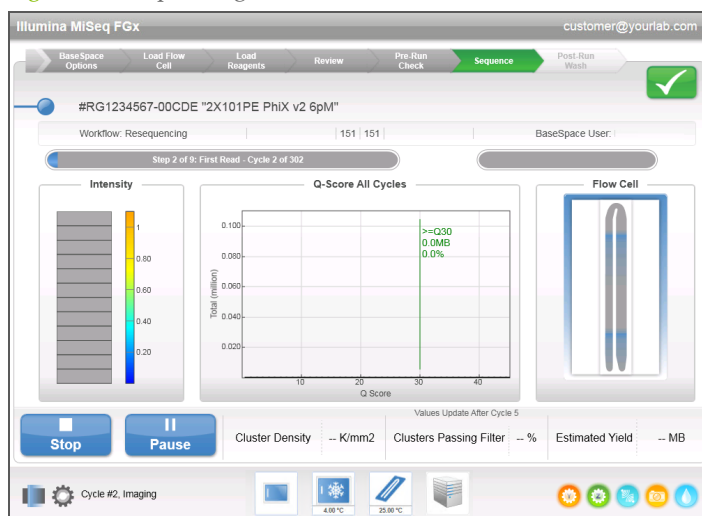


NOTE

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

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

Figure 27 Sequencing Screen for an RUO Run



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. Real-Time Analysis (RTA) uses the first seven cycles of the sequencing run for template generation for MiSeq FGx and v3 RUO kits, and the first four cycles for v1 and v2 RUO kits.

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 Folders

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

- ▶ **D:\Illumina\MiSeqFGxTemp**—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 MiSeq FGx Control Software and RTA. There is no need to access the Temp folder. Contents of this folder are deleted after seven days.
- ▶ **D:\Illumina\MiSeqFGxOutput**—RTA copies files from the Temp folder to the Output folder. As base call and base call quality score 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. The location of the output folder can be changed in the Output Folder field on the Run Options screen. For more information, see *Run Options Screen* on page 19.
- ▶ **D:\Illumina\MiSeqFGxAnalysis**—When RTA analysis is complete, ForenSeq Universal Analysis Software accesses the Analysis folder on the instrument local drive to begin further analysis. All files written to the Analysis folder are copied to the Output folder. For more information, see the *ForenSeq Universal Analysis Software Guide* (document # VD2018007).

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.

Run Duration

Run duration depends on the number of cycles performed. A Forensic Genomics run on the MiSeq FGx performs 8 + 8 indexed sequencing run of 351 + 31 cycles using one MiSeq FGx sequencing kit.

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 350 + 30-cycle run performs a 351-cycle read and a 31-cycle read for a total of 382 cycles. At the end of the run, 380 cycles are analyzed.

If performing a Research Use Only run, see the appropriate MiSeq Reagent Kit guide for RUO.

Required Disk Space

The integrated instrument computer has approximately 550 GB of storage capacity.

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

If prompted to make disk space available, go to the Welcome screen and select **Manage Files**. From the Manage Files screen, select the **Runs** tab. Move or delete older run folders

as appropriate. For more information, see *Manage Files Screen* on page 18. After clearing adequate disk space, select **Restart Check**.

Pausing or Stopping a Run

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

Pausing a Run

A run can be paused during sequencing to empty the waste bottle or check the volume remaining in the SBS Solution (PR2) bottle, for example, and then resumed to continue sequencing.



NOTE

Do *not* pause a run during cluster generation or within the first eight cycles of sequencing. It is not possible to resume a run that was paused during this time. If you use the MiSeq FGx instrument in RUO mode, see *Run Metrics* on page 53 for cycle information.

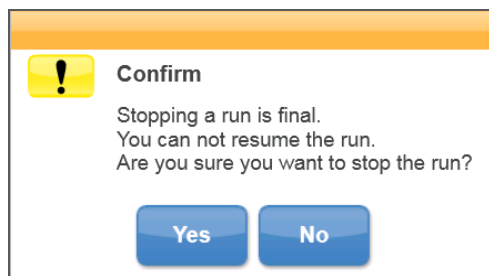
To pause a run from the Sequencing screen, select **Pause**. The current command is completed, and then the run is paused, the flow cell is placed in a safe state, and the button changes to **Resume**. At this point the waste bottle can be emptied, for example. Select **Resume** to continue with the run.

Stopping a Run

A run can be stopped during sequencing if necessary (for example, if the run was set up incorrectly, if the data quality is bad, or if there is a hardware error.)

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. RTA analysis continues for the last completed cycle.

Figure 28 Stopping a Run



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

If you stop a run, you cannot use reagents remaining in the reagent cartridge for a subsequent run. A new run requires a new reagent cartridge.

Analysis Software Options for RUO Runs

After RTA analysis is complete, MiSeq FGx sequencing data from a Forensic Genomics run require analysis using the ForenSeq Universal Analysis Software.

When performing an RUO run on the MiSeq FGx, sequencing data can be analyzed on the cloud using BaseSpace or on the instrument computer using MiSeq Reporter.

BaseSpace Overview

BaseSpace is the Illumina cloud computing environment. Using BaseSpace to store and analyze your run data provides the following benefits:

- ▶ Eliminates the need for onsite storage and computing
- ▶ Enables web-based data management and analysis
- ▶ Allows a new sequencing run to be started while data are being analyzed
- ▶ Provides tools for global collaboration and sharing

You can log in to BaseSpace when you set up the sequencing run. For more information, see *BaseSpace Option Screen* on page 28. When you begin your sequencing run, data files are encrypted in transit, decrypted during analysis, and encrypted again when stored.

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

When the last base call file is uploaded to BaseSpace, additional analysis of your data begins. The same analysis workflows are supported on BaseSpace as with on-instrument analysis using MiSeq Reporter. BaseSpace only supports the same genomes that are provided with MiSeq Reporter.

You can connect to BaseSpace at basespace.com. Log in using your MyIllumina account login. For more information about using BaseSpace, see the BaseSpace online help on the Illumina website: www.illumina.com/help/BaseSpaceHelp/BaseSpaceHelp.htm.

MiSeq Reporter Overview

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

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

When secondary analysis is complete, a file named *CompletedJobInfo.xml* is written to the run folder. For more information, see the *MiSeq Reporter Software Guide (part # 15042295)*.

Sequencing During Analysis

If you choose MiSeq Reporter for additional analysis of an RUO run, the MiSeq FGx instrument computing resources are dedicated to either sequencing or analysis. If a new sequencing run is started on the MiSeq FGx before secondary analysis of an earlier run is complete, a confirmation dialog box appears. After confirming the sequencing run, secondary analysis stops.

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

Performing a Run

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MiSeq FGx Workflow

Make sure that the sample libraries have already been loaded onto the reagent cartridge before setting up the run. These steps apply to any assay protocol.



Prepare the reagent cartridge, denature and dilute libraries, and then load the pooled library into the designated reservoir.



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

[Optional] Connect to BaseSpace if performing an RUO run.



Wash and thoroughly dry the flow cell.

Follow the software prompts to load the flow cell.



Follow the software prompts to load the SBS Solution (PR2) bottle, make sure that the waste bottle is empty, and load the reagent cartridge. For an RUO run, select a sample sheet.



Review run parameters and pre-run check results.

Select **Start Run**.



As desired, monitor the run from the Sequencing screen.



Perform a post-run wash using nuclease-free water mixed with sodium hypochlorite.

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 base calling, filtering, and quality scoring.

Analysis

When the run is complete, the ForenSeq Universal Analysis Software automatically performs further analysis. Analysis is monitored using an internet connection from another computer. See the *ForenSeq Universal Analysis Software Guide* (document # VD2018007).

Prepare the Reagent Cartridge



NOTE

Make sure that the cartridge used is compatible with the selected run mode and your software version.

Table 4 Kit Compatibility for Forensic Genomics Run Mode

Run Mode	Compatible Kit Types	Required Software Version
Forensic Genomics	MiSeq FGx Reagent Kit	MiSeq FGx Control Software v1.0, or later
	MiSeq FGx Reagent Micro Kit	MiSeq FGx Control Software v1.3, or later

Table 5 Kit Compatibility for Research Use Only Run Mode

Run Mode	Compatible Kit Types	Required Software Version
Research Use Only	MiSeq Reagent Kit v1/v2/v3	MiSeq FGx Control Software v1.0, or later
	MiSeq FGx Reagent Kit	MiSeq FGx Control Software v1.3, or later
	MiSeq FGx Reagent Micro Kit	MiSeq FGx Control Software v1.3, or later

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

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

Figure 29 Maximum Water Line



- 3 Allow the reagent cartridge to thaw in the room temperature water bath for approximately 60–90 minutes or until it has thawed completely.
- 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 10 times to mix the thawed reagents, and then visually inspect that all positions are thawed.



NOTE

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

- 2 Visually inspect the reagents in positions 1, 2, and 4 to make sure that they are fully mixed and free of precipitates.

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



NOTE

The MiSeq FGx 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 6 hours) until ready to set up the run. For best results, proceed directly to loading the sample and setting up the run.

Denature and Dilute Libraries

This process dilutes the libraries in HT1 (Hybridization Buffer), adds HSC (Human Sequencing Control), and heat denatures the libraries in preparation for sequencing.



NOTE

Perform this process immediately before loading the library onto the reagent cartridge to ensure efficient template loading on the flow cell.

Consumables

- ▶ ForenSeq DNA Signature Prep Kit contents:
 - ▶ HP3 (2N-NaOH)
 - ▶ HSC (Human Sequencing Control)
- ▶ 1.5 ml microcentrifuge tubes (2)
- ▶ MiSeq FGx Reagent Kit contents:
 - ▶ HT1 (Hybridization Buffer)
 - ▶ Reagent cartridge
- ▶ Nuclease-free water

Preparation

- 1 Prepare the following consumables.

Item	Storage	Instructions
HP3	-25°C to -15°C	Thaw at room temperature.
HSC	-25°C to -15°C	Thaw at room temperature.
HT1	-25°C to -15°C	Thaw at room temperature.
Reagent cartridge	-25°C to -15°C	Thaw at room temperature.

- 2 Preheat the microheating system to 96°C.
- 3 Prepare either of the following:
 - ▶ Remove a tube benchtop cooler from -25°C to -15°C storage or ice bucket.
 - ▶ Prepare an ice-water bath by combining 3 parts ice and 1 part nuclease-free water.
- 4 Label tubes with a marker as follows:
 - ▶ HSC mixture
 - ▶ DNL to indicate Denatured Normalized Libraries.

Procedure

- 1 Create an HSC denaturation reaction in the HSC mixture tube.
 - ▶ HSC (2 µl)
 - ▶ HP3 (2 µl)
 - ▶ Nuclease-free water (36 µl)
- 2 Vortex and then centrifuge briefly.
- 3 Incubate at room temperature for 5 minutes.
- 4 Add 591 µl HT1 to the DNL tube.
- 5 Transfer 7 µl from the PNL tube to the DNL tube. Pipette to mix.

- 6 Cap the PNL tube and store at -25°C to -15°C for up to 30 days. Exceeding 30 days in storage results in a significant reduction of cluster density.
- 7 Transfer 2 µl HSC mixture to the DNL tube. Pipette to mix. Do not store HSC mixture long term, which results in a significant reduction of cluster density.
- 8 Vortex and then centrifuge briefly.
- 9 Place on the 96°C microheating system for 2 minutes.
- 10 Invert the tube several times to mix.
- 11 Immediately place in the ice-water bath or on the -25°C to -15°C benchtop cooler for 5 minutes.
- 12 Immediately load the entire contents onto the reagent cartridge.

Load Sample Libraries onto Cartridge



NOTE

If you are performing an RUO run, follow sample loading instructions in *Load Sample Libraries onto Cartridge for RUO* on page 44.

After you have prepared the MiSeq FGx reagent cartridge for use, you are ready to load your samples.

- 1 Use a low-lint lab tissue to clean the foil seal covering the reservoir labeled **Load Samples**.
- 2 Use a clean 1 ml pipette tip to pierce the foil seal covering the reservoir labeled **Load Samples**.

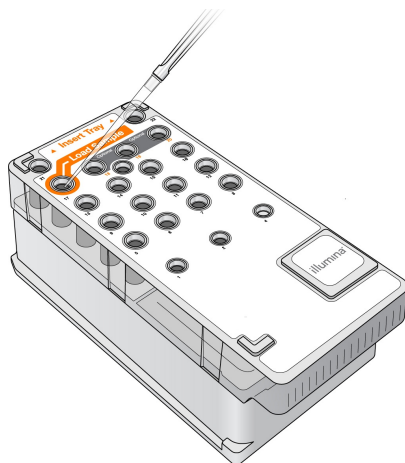


NOTE

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

- 3 Pipette 600 μ l of the **DNL** sample libraries into the **Load Samples** reservoir. Avoid touching the foil seal as you dispense the sample.
- 4 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 30 Load Libraries



- 5 Proceed directly to the run setup steps using the MiSeq FGx Control Software interface.

Load Sample Libraries onto Cartridge for RUO



NOTE

If necessary for your library type, denature and dilute libraries, and add optional PhiX control. See the *Illumina MiSeq System Denature and Dilute Libraries Guide* (part # 15039740).

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



NOTE

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

After you have prepared the MiSeq reagent cartridge for use, you are ready to load your samples.

- 1 Use a low-lint lab tissue to clean the foil seal covering the reservoir labeled **Load Samples**.
- 2 Use a clean 1 ml pipette tip to pierce the foil seal covering the reservoir labeled **Load Samples**.

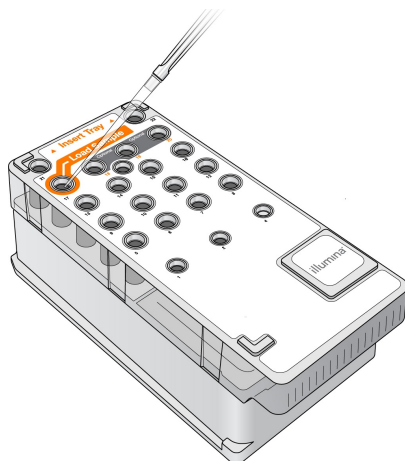


NOTE

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

- 3 Pipette 600 µl of prepared libraries into the **Load Samples** reservoir. Avoid touching the foil seal as you dispense the sample.
- 4 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 31 Load Libraries

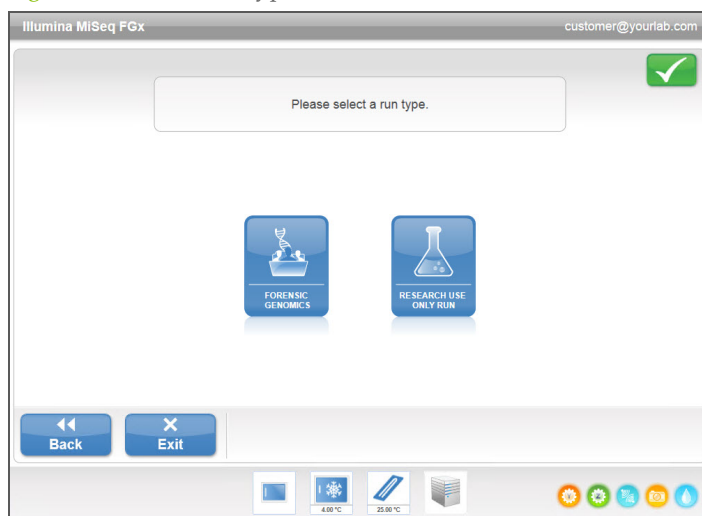


- 5 Proceed directly to the run setup steps using the MiSeq FGx Control Software interface.

Log In and Select Run Type

- 1 From the log in screen, enter your ForenSeq Universal Analysis Software user name and password, and then select **Next**.
- 2 From the Welcome screen, select **Sequence**.
- 3 When the Select Run Type screen appears, select **Forensic Genomics** or **Research Use Only Run**.

Figure 32 Select Run Type



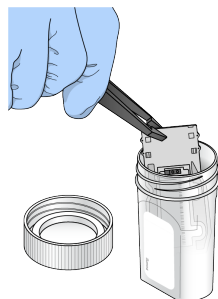
- 4 Follow the prompts to load the flow cell and reagents and set up the run (described in the following sections).

Cleaning the Flow Cell

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

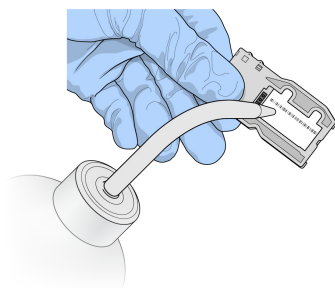
- 1 Put on a new pair of powder-free gloves.
- 2 Using plastic forceps, grip the flow cell by the base of the plastic cartridge and remove it from the flow cell container.

Figure 33 Remove Flow Cell



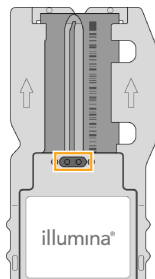
- 3 Lightly rinse the flow cell with nuclease-free water, making sure that both the glass and plastic cartridge are thoroughly rinsed of excess salts. Excess salts can affect flow cell seating on the instrument. If salts dry in the imaging area, imaging can also be affected.

Figure 34 Rinse Flow Cell



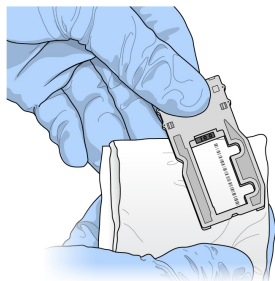
- 4 Using care around the black flow cell port gasket (outlined in orange in the following illustration), thoroughly dry the flow cell and cartridge using a lint-free lens cleaning tissue. Gently pat dry in the area of the gasket and adjacent glass.

Figure 35 Flow Cell Ports and Gasket



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

Figure 36 Dry Flow Cell



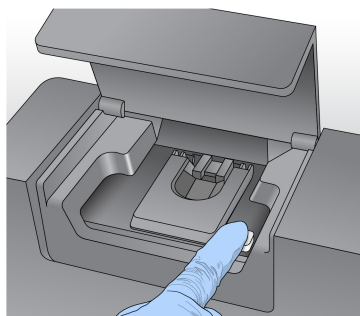
- 6 Dry any excess alcohol with a lint-free lens cleaning tissue. Visually inspect to make sure that the flow cell ports are free of obstructions and that the gasket is well-seated around the flow cell ports.
If the gasket appears to be dislodged, gently press it back into place until it sits securely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

Loading the Flow Cell

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

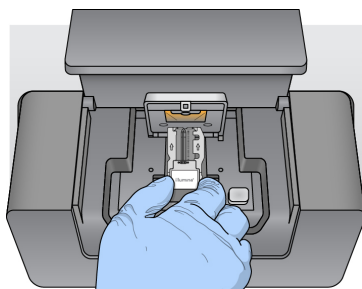
- 1 Raise the flow cell compartment door, and then press the release button to the right of the flow cell latch. The flow cell latch opens.

Figure 37 Open Flow Cell Latch



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

Figure 38 Place Flow Cell on Stage



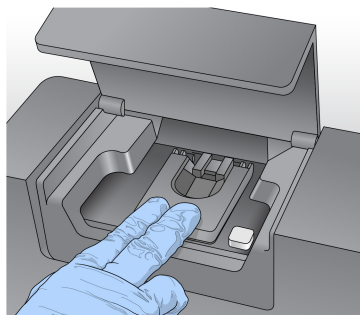
- 4 Gently press down on the flow cell latch to close it over the flow cell.



NOTE

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

Figure 39 Close Flow Cell Latch



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



NOTE

If the RFID 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, or SBS Solution) to fail. For more information, see *Resolving RFID Read Failure* on page 70.

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

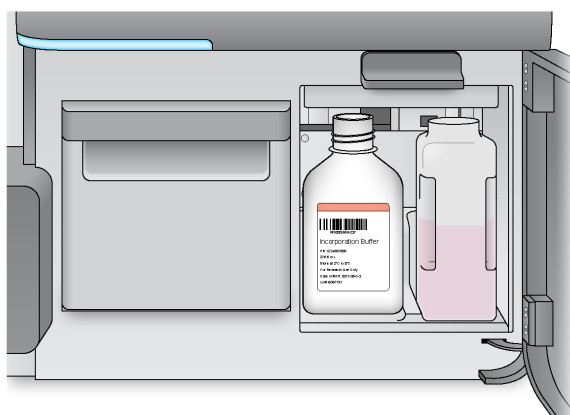
Loading Reagents

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

Load SBS Solution (PR2) and Check the Waste Bottle

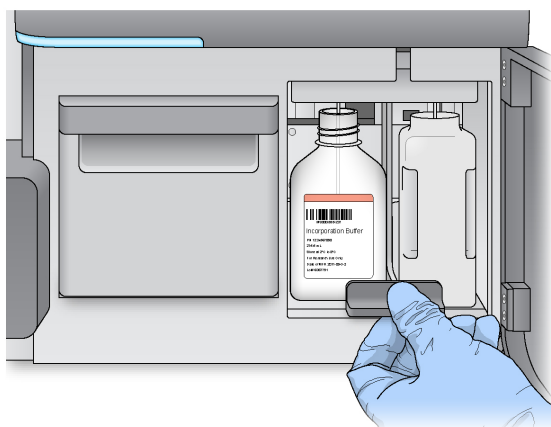
- 1 Remove the bottle of SBS Solution (PR2) from 2° to 8°C storage. Gently invert the bottle to mix, and then remove the lid.
- 2 Open the reagent compartment door.
- 3 Raise the sipper handle until it locks into place.
- 4 Place the SBS Solution (PR2) bottle in the indentation to the right of the reagent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

Figure 40 Load the SBS Solution (PR2) Bottle



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

Figure 41 Lower Sipper Handle



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

**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, or SBS Solution) to fail. For more information, see *Resolving RFID Read Failure* on page 70.

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

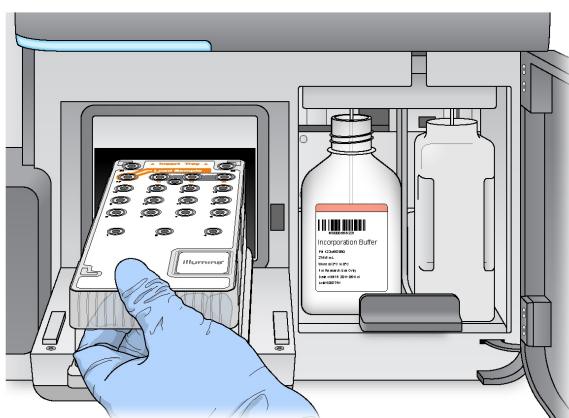
Load the Reagent Cartridge

**NOTE**

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

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

Figure 42 Load Reagent Cartridge



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

**NOTE**

If that 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, or SBS Solution) to fail. For more information, see *Resolving RFID Read Failure* on page 70.

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

Starting the Run

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

Review Run Parameters

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

Review Pre-Run Check

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

If any items do not pass the pre-run check, a message appears on the screen with instructions on how to correct the error. For more information, see *Resolving Run Setup Errors* on page 69.

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

Important Note Before Starting the Run



WARNING

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

After selecting **Start Run**, do not open the flow cell compartment or the reagent compartment doors, or touch the instrument monitor except to pause the run. For more information, see *Pausing a Run* on page 34.

Monitoring the Run

- 1 During the run, monitor run progress, intensities, and quality scores that appear on the Sequencing screen. The Sequencing screen is view-only. For more information, see *Sequencing Screen* on page 27. For an RUO run, see *Sequencing Screen for RUO* on page 31.
- 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.

Run Metrics

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

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

Maintenance Procedures

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Introduction

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

Regular instrument washes ensure continued performance in the following ways:

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

Maintenance Frequency

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

Table 6 Maintenance During Normal Operation

Activity	Daily	Monthly	As Needed
Post-Run Wash	After every run		
Maintenance Wash		X	
Standby Wash			To prepare for idle (≥ 7 days unused)
Instrument Shutdown			X

Table 7 Maintenance During Idle (≥ 7 days unused)

Activity	Daily	Monthly	As Needed
Standby Wash		X	
Instrument Shutdown			X

Preventive Maintenance

One preventive maintenance is recommended per calendar year. If you are not under a service contract, please contact your account manager or Verogen technical support to arrange for a billable preventive maintenance service.

Performing a Post-Run Wash

Always perform an instrument wash after completing a sequencing run. Follow the software prompts to load the wash components and perform the wash. The post-run wash takes approximately 30 minutes.



NOTE

The post-run wash contains sodium hypochlorite. **Do not** use sodium hypochlorite for a maintenance wash or a standby wash.

The software cannot proceed to the run setup steps for a subsequent run until a wash has been performed.



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)
- ▶ Sodium hypochlorite
- ▶ Nuclease-free water
- ▶ MiSeq wash tube (Verogen, part # MS-102-9999)

Procedure

- 1 Prepare fresh wash solution with Tween 20 and nuclease-free, as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml nuclease-free water. These volumes result in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 475 ml nuclease-free water. These volumes result in a 0.5% Tween 20 wash solution.
 - c Invert several times to mix.
- 2 Prepare fresh sodium hypochlorite wash solution with nuclease-free water, as follows:
 - a Add 30 μ l of 6% sodium hypochlorite to 870 μ l nuclease-free water. These volumes result in a 1:30 sodium hypochlorite dilution.
 - b Add 50 μ l of the 1:30 sodium hypochlorite dilution to 950 μ l of nuclease-free water in a MiSeq wash tube (part # MS-102-9999).

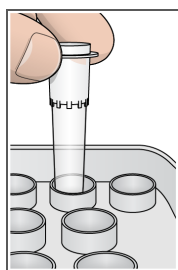
NOTE

Check the percentage of sodium hypochlorite on the product label. Using the correct concentration of sodium hypochlorite is important. If the concentration is too high, it can make cluster generation fail in subsequent runs. If 6% sodium hypochlorite is not available, make a 1 ml solution of 0.01% sodium hypochlorite in nuclease-free water.

 - c Mix the sodium hypochlorite dilution and water mixture well, pipetting up and down with a P1000 micropipette.
- 3 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.

- 4 Insert the MiSeq wash tube containing .01% sodium hypochlorite 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 nuclease-free wash solution from position 17.

Figure 43 MiSeq Wash Tube in Position 17 of the Wash Tray



NOTE

Inserting the MiSeq wash tube into any position other than position 17 can make cluster generation fail in a subsequent run, and damage the MiSeq FGx fluidics system.

- 5 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.
- 6 Open the reagent compartment door and reagent chiller door, and slide the used reagent cartridge from the chiller.
- 7 Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
- 8 Raise the sipper handle in front of the SBS Solution (PR2) bottle and waste bottle until it locks into place.
- 9 Remove the SBS Solution (PR2) bottle and replace it with the wash bottle.



NOTE

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

- 10 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 the risk exposure. Handle reagents as chemical waste and discard in accordance with the applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at www.verogen.com/sds.

- 11 Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
- 12 Close the reagent compartment door.
- 13 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. A small amount of wash solution remains in the MiSeq wash tube in position 17.

**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.

Performing a Maintenance Wash

Perform a maintenance wash every 7 days to ensure optimal performance. The maintenance wash includes a series of three wash steps using a wash solution of nuclease-free water mixed with Tween 20. Allow approximately 90 minutes to complete the wash.

User-Supplied Consumables


- ▶ Tween 20 (Sigma-Aldrich, catalog # P7949)
- ▶ Nuclease-free water

Procedure

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

Perform First Wash

- 1 Prepare fresh wash solution with Tween 20 and nuclease-free water as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml nuclease-free water. These volumes result in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 475 ml nuclease-free 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 SBS Solution (PR2) bottle and waste bottle until it locks into place, and replace the SBS Solution (PR2) bottle with the wash bottle.



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

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

Perform Second Wash



NOTE

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

- 1 Prepare fresh wash solution with Tween 20 and nuclease-free water, as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml nuclease-free water. These volumes result in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 475 ml nuclease-free 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 nuclease-free water, as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml nuclease-free water. These volumes result in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 475 ml nuclease-free 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.

Performing a Standby Wash

If there are no plans to use the instrument within the next seven days, prepare the instrument to sit idle by performing a standby wash. A standby wash performs two consecutive washes that flush each position of any remaining reagents or salt accumulation. Each wash takes approximately 60 minutes. Allow approximately 2 hours to complete the standby wash.

When the standby wash is complete, the instrument is in standby mode and a message appears on the Welcome screen stating the status of the instrument. When the instrument is in standby mode, a maintenance wash must be performed before a sequencing run can be initiated. See *Maintenance Frequency* on page 56 for information on when to perform washes.



NOTE

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

User-Supplied Consumables

- ▶ Tween 20 (Sigma-Aldrich, catalog # P7949)
- ▶ Nuclease-free water

Procedure

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

Perform First Wash

- 1 Prepare fresh wash solution with Tween 20 and nuclease-free water as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml nuclease-free water. These volumes result in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 475 ml nuclease-free 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 SBS Solution (PR2) bottle and waste bottle until it locks into place, and replace the SBS Solution (PR2) bottle with the wash bottle.

**NOTE**

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

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

Perform Second Wash

**NOTE**

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

- 1 Prepare fresh wash solution with Tween 20 and nuclease-free water, as follows:
 - a Add 5 ml 100% Tween 20 to 45 ml nuclease-free water. These volumes result in 10% Tween 20.
 - b Add 25 ml 10% Tween 20 to 475 ml nuclease-free 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**

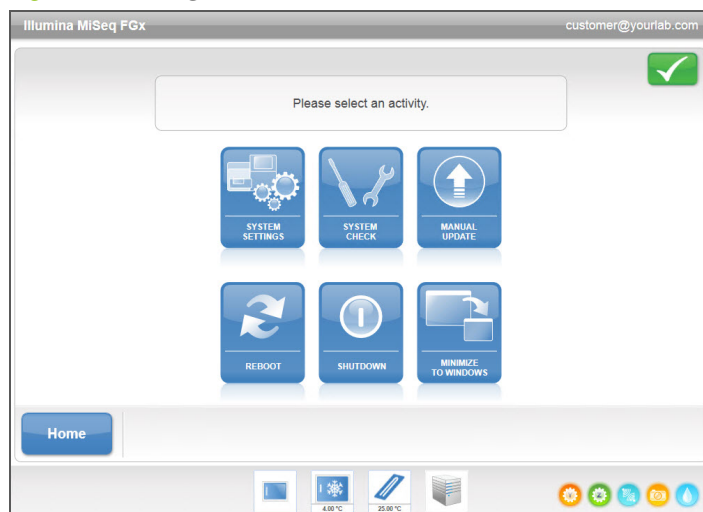
The sippers remain in the down position, which is normal. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Shutting Down the Instrument

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

- 1 Perform a maintenance wash. For more information, see *Procedure* on page 60.
- 2 Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
- 3 Close the reagent compartment door.
- 4 From the Manage Instrument screen, select **Shut Down**. This command shuts down the software.

Figure 44 Manage Instrument Screen



- 5 Toggle the power switch to the OFF position.



NOTE

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

Troubleshooting

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Introduction

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

For technical questions, visit the MiSeq FGx support pages on the Verogen website for access to frequently asked questions.

For problems with run quality or performance, contact Verogen Technical Support. For more information, see *Technical Assistance* on page 79.

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

- ▶ SampleSheet.csv if using the instrument in RUO mode
- ▶ RunParameters.xml
- ▶ RunInfo.xml
- ▶ Interop folder

Resolving Run Setup Errors

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

Error	Action
✖ Flow Rate Measured	<p>The flow rate check screen opens. Use the drop-down list or on-screen keyboard to enter the following:</p> <ul style="list-style-type: none"> • Solution: PR2 • Volume: 250 • Aspirate Rate: 2500 • Dispense Rate: 2500 <p>Select Pump. If the error persists, set the volume to pump 500 µl SBS Solution (PR2) and repeat the process. When fluids have been pumped, select Restart Check. When the pre-run check is successful, the Start Run button becomes active. If the flow check fails again, reseal the flow cell to make sure that flow is not interrupted due to misalignment. Inspect the flow cell gasket for lint or irregularities.</p>
✖ Free Disk Space	<p>If disk space is low, a message appears indicating how much disk space is required. Use the Manage Files feature to clear the required space from the instrument computer.</p>
✖ Network Connection Active	<p>Make sure that the network cable is plugged into the instrument.</p> <p>If the network connection is not restored, select Reboot on the Manage Instrument screen to reboot the software.</p> <p>If the connection is still not restored, select Shut Down on the Manage Instrument screen, and then turn off the instrument using the power switch. Wait at least 60 seconds, and then turn on the instrument and start the software.</p>
✖ Primary Analysis Ready	<p>RTA analysis from the previous run is not complete. The default time to allow RTA analysis to complete is 1 hour, and a countdown appears on the screen. The options are to wait 1 hour or select Terminate Analysis. ForenSeq Universal Analysis Software analysis stops for any incomplete cycles.</p>
✖ Sample Sheet Present	<p>If the sample sheet is not named with the reagent cartridge ID for the run, the instrument cannot locate the appropriate sample sheet automatically. Browse to the sample sheet for the run.</p> <p>If the sample sheet is named with the reagent cartridge ID for the run, make sure that the sample sheet is located in the default sample sheet folder. Check the default folder location in Run Options on the Welcome screen.</p> <p>Make sure that the sample sheet file extension is *.csv.</p> <p>If the sample sheet is missing, create one and copy it to the sample sheet locations specified in Run Options.</p> <p>After locating a sample sheet, select Restart Check.</p>

Resolving RFID Read Failure

RFID failures are triggered under the following conditions:

- ▶ The component loaded is not part of a forensic genomics kit.
- ▶ For an RUO run, the component loaded is not part of the kit identified by the sample sheet.
- ▶ There is a technical failure with the RFID tag in the component.

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



NOTE

A 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 occurs, contact Verogen Technical Support.

Flow Cell RFID

- 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, open the flow cell compartment door and remove the flow cell carefully.
- 3 Check the flow cell and the flow cell stage for debris or salts that can stop the RFID read.
- 4 Reseat the flow cell. Make sure that the latch brings the flow cell into proper alignment.
- 5 Close the flow cell compartment door.
- 6 If the RFID continues to fail, select the keyboard icon to open the on-screen keyboard.
- 7 Enter the barcode number of the flow cell, which is located on the flow cell container label directly below the barcode. Select **Done**.
- 8 Select **Next** to proceed to the next run setup step.

SBS Solution (PR2) Bottle RFID

- 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, open the reagent compartment door. Raise the sipper handle and remove the SBS Solution (PR2) bottle.
- 3 Check the bottle and the reagent compartment for debris or salts that can stop the RFID read.
- 4 Replace the SBS Solution (PR2) bottle and slowly lower the sipper handle until it locks into place. Make sure that the sippers lower into the SBS Solution (PR2) and waste bottles.
- 5 If the RFID continues to fail, select the keyboard icon to open the on-screen keyboard.
- 6 Enter the barcode number of the SBS Solution (PR2) bottle, which is located on the bottle label directly below the barcode. Select **Done**.
- 7 Select **Next** to proceed to the next run setup step.

Reagent Cartridge RFID

- 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, open the reagent chiller door and remove the cartridge.
- 3 Check the reagent cartridge and reagent chiller for debris or salts that can stop the RFID read.
- 4 Replace the reagent cartridge and close the reagent chiller door.
- 5 If the RFID continues to fail, select **Enter Reagent Kit Barcode**.
- 6 Enter the reagent kit barcode number, which is located on the kit label directly below the barcode.
- 7 Enter the part number of the reagent cartridge, which is located to the right of **REF** on the reagent kit label.

Figure 45 Enter Reagent Information

Illumina MiSeq FGx customer@yourlab.com

BaseSpace Options Load Flow Cell **Load Reagents** Review Pre-Run Check Sequence Post-Run Wash

Valid bypass code has been entered.
Please enter the reagent kit barcode and part number.
Allowed format for barcode is 'AA1234567-12345'

Reagent Kit Barcode:

Reagent Kit Part Number:

Back Next

4.00 °C 24.00 °C

- 8 Select **Next** to return to the Load Reagents screen.
- 9 Select **Next** to proceed to the next run setup step.

Preventing Reboots During a Run

If the MiSeq FGx restarts in the middle of a run, it might mean the Windows Update software on the network is configured to automatically install software updates. (This setting should have been turned off during installation.) Contact the local IT department for help to disable automatic updates to the Windows operating system running in the background on the MiSeq FGx instrument.



NOTE

A restart terminates the run and it cannot be recovered. The run has to be started again from the beginning.

Troubleshooting Flow Rate Error

The flow rate is the speed in which fluids pass through the fluidics system ($\mu\text{l}/\text{min}$). It is measured before each run during the pre-run check. If the system is unable to measure the flow rate, pump a volume of 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 μl**
 - ▶ Aspirate Rate: **2500 $\mu\text{l}/\text{min}$**
 - ▶ Dispense Rate: **2500 $\mu\text{l}/\text{min}$**
- 2 Select **Pump**.

Figure 46 Measure Flow Rate



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

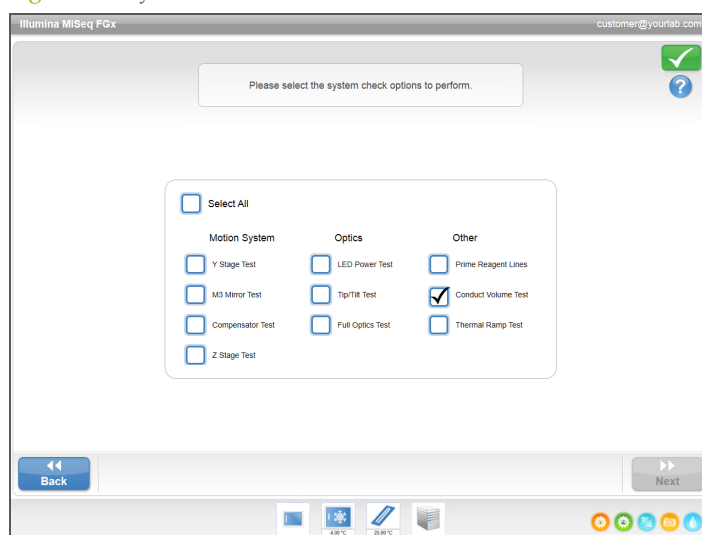
Performing a Volume Test

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 nuclease-free water and a used flow cell must be in place. Follow the onscreen prompts to perform the test.

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

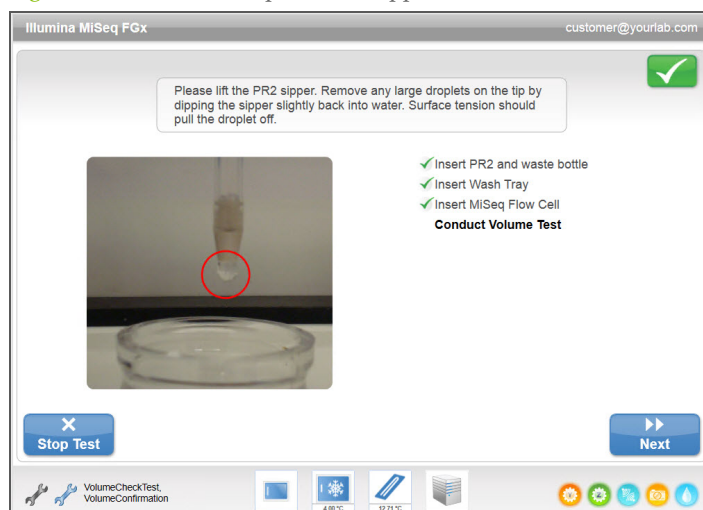
Figure 47 System Check Screen



- 4 Fill each reservoir of the wash tray with 6 ml nuclease-free water.
- 5 Fill the 500 ml wash bottle with 350 ml nuclease-free water.
- 6 Load the wash tray and wash bottle onto the instrument.
 - a Open the reagent compartment door and reagent chiller door, and slide the wash tray into the reagent chiller until it stops. Close the reagent chiller door.
 - b Raise the sipper handle until it locks into place, and load the wash bottle.
 - c Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.
 - d Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.
- 7 Following the on-screen prompts, remove any droplets from the wash bottle sipper, as follows:
 - a When prompted, slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.
 - b When prompted, slowly lower the sipper handle far enough into the water to allow the surface tension to remove the droplet.

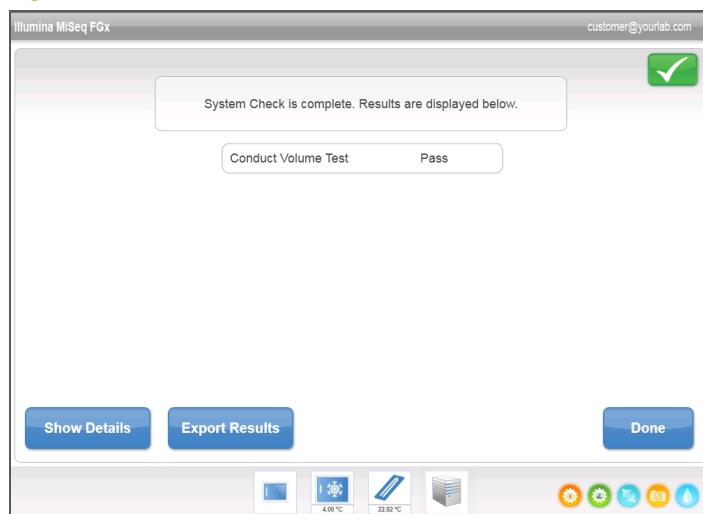
- c When prompted, slowly raise the sipper handle and check the wash bottle sipper for the presence of a large water droplet.
- d When prompted, slowly lower the sipper handle completely, making sure that the sippers lower into the wash bottle and waste bottle.

Figure 48 Remove Droplet from Sipper



- 8 Select **Next**. The volume test begins. When the volume test is complete, the results appear on the screen.

Figure 49 Volume Test Results



If the test did not pass, perform a maintenance wash. See *Procedure* on page 60.

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

Measuring Expected Wash Volumes

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

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

Wash Type	Expected Wash Volume
Post Run Wash	25.5 ml
Standby Wash	46 ml
Maintenance Wash	17.25 ml

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Technical Assistance

For technical assistance, contact Verogen Technical Support.

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Safety data sheets (SDSs)

- ▶ For MiSeq FGx sequencing kit safety data sheets, visit www.verogen.com/sds.
- ▶ For Research Use Only (RUO) sequencing reagent and Illumina library preparation kit safety data sheets, visit support.illumina.com/sds.

Product documentation—Available for download in PDF from the Verogen website. Go to www.verogen.com/support select the appropriate document.

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