MiSeq FGx Sequencing System

Reference Guide

VEROGEN PROPRIETARY
Document # VD2018006 Rev. F
February 2021

Revision History

Document #	Date	Description of Change
VD2018006 Rev. F	February 2021	Added a target cluster density of 700–1400 K/mm² for ForenSeq Kintelligence libraries.
VD2018006 Rev. E	February 2021	Refreshed the format of the guide: • Added an introduction to the guide and a chapter on system configuration. • Renamed, consolidated, and moved some sections as needed to improve continuity. • Replaced the workflow table with a diagram. • Updated the style of several graphics and added captions. • Updated fonts, table styles, and other visual elements. Added a list of user-supplied equipment. Added deionized water to the list of user-supplied consumables. Added descriptions of the metrics that appear on the Sequencing screen. Updated the following product names: • BaseSpace Sequence Hub • MiSeq FGx Sequencing System • Universal Analysis Software Updated wash instructions: • Added information on wash tray maintenance. • Added requirement to perform a maintenance wash after shutting down the instrument for more than seven days. • Updated the frequency of washes. • Updated the post-run wash to use only sodium hypochlorite and nuclease-free water. • Updated the final wash of a maintenance or standby wash to use only nuclease-free water. • Removed requirement to fill position 17 with nuclease-free water for a post-run wash. • Removed incorrect wash volume of 17.25 ml. • Clarified information on the standby wash and standby mode. • Increased standby wash duration to 2.5 hours. Updated instructions on signing in and selecting a run. Updated the safety data sheet (SDS) link to verogen.com/documentation. Updated the ForenSeq trademark to registered trademark. Replaced the 600 µl loading volume with an instruction to load the entire volume of denatured and diluted libraries. Removed droplet removal steps from the volume test instructions. Removed duplicate entries of run setup errors. Removed references to the MiSeq RUO Reagent Kit v1, which is discontinued.
VD2018006 Rev. D	August 2020	Updated the guide name to *MiSeq FGx Sequencing System Reference Guide*. Updated the following catalog numbers: • MiSeq FGx Reagent Kit to Verogen part # 15066817 • MiSeq FGx Reagent Micro Kit to Verogen part # 20021681 Updated phone numbers for Verogen contact information. Removed denature and dilute instructions, which are provided in the library prep reference guides.

Document #	Date	Description of Change
VD2018006 Rev. C	June 2019	Added information on signing in to the ForenSeq Universal Analysis Software. Referenced the *ForenSeq Universal Analysis Software Guide (VD2019002)*. Corrected ForenSeq product marking to trademark (™).
VD2018006 Rev. B	August 2018	Updated trademark to registered trademark for ForenSeq and MiSeq FGx product names.
VD2018006 Rev. A	June 2018	Added reagent kit information, including kit contents, storage requirements, flow cell types, and software version. Added instructions to prepare the reagent cartridge and denature and dilute libraries for a run. Updated the document number for this guide to VD2018006. Updated other document numbers listed in this guide to Verogen document numbers. Updated the location of safety data sheets (SDSs) to the Verogen website. Updated technical support information with Verogen contacts.
15050524 Rev. C	February 2015	Corrected formatting errors.
15050524 Rev. B	January 2015	Updated the reagent kit name to MiSeq FGx Reagent Kit.
15050524 Rev. A	October 2014	Initial release

Table of Contents

System Overview

Introduction

The Verogen MiSeq FGx® Sequencing System provides a fully validated, next-generation sequencing (NGS) solution for forensics genomics. Built with the high resolution and unmatched accuracy of sequencing-by-synthesis (SBS) technology, the system can transform a broad range of samples into high-quality data.

Intended Use

The MiSeq FGx System is an instrument that uses dedicated reagents and flow cells, imaging hardware, and analysis software to measure fluorescence. A component of the Verogen MiSeq FGx Forensic Genomics Solution, the MiSeq FGx System is intended for targeted sequencing of DNA for forensic casework, databasing, and other human identification applications. The system also features a Research Use Only (RUO) mode that can sequence MiSeq-compatible libraries generated with Illumina RUO library prep kits.

Compatibility of Run Components

Use the MiSeq FGx Control Software (MFCS) version and run mode compatible with your reagent kit. For more information on these components, see *System Software* on page 10 and *MiSeq FGx Reagent Kits* on page 14.

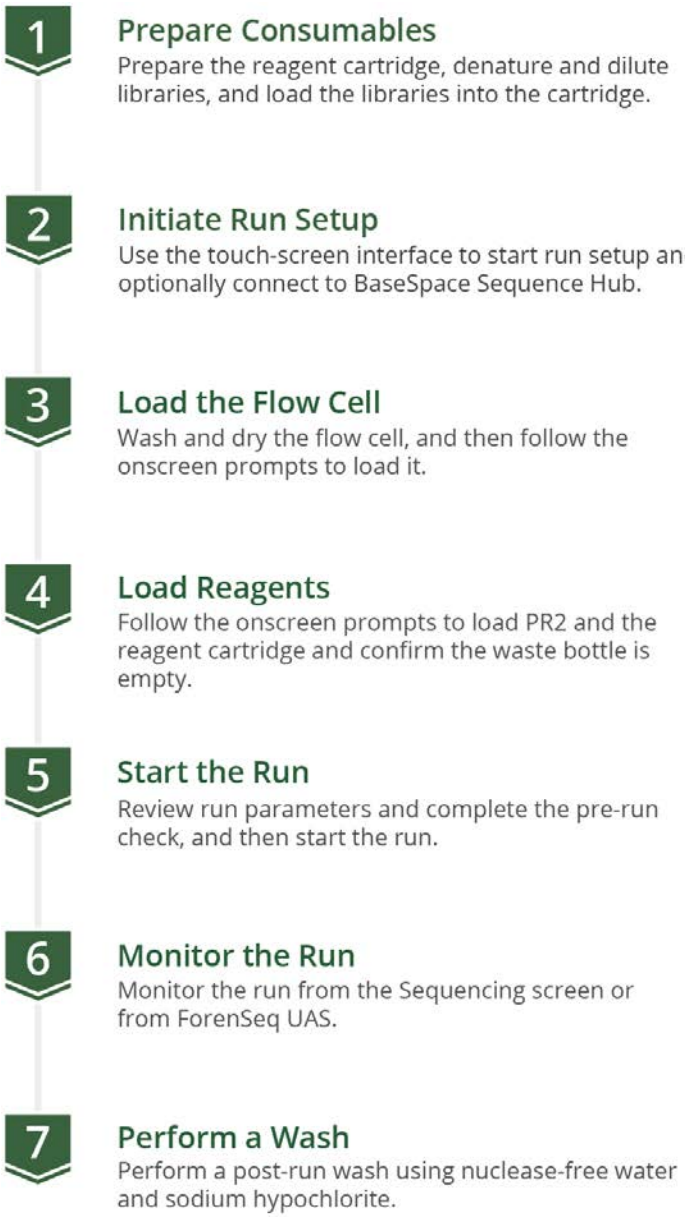
Table 1 Software and kit compatibility

Reagent Kit	Control Software	Run Mode
MiSeq FGx Reagent Kit	MFCS v1.0, or later	Forensic Genomics
	MFCS v1.3, or later	Research Use Only
MiSeq FGx Reagent Micro Kit	MFCS v1.3, or later	Forensic Genomics Research Use Only
MiSeq Reagent Kit v2	MFCS v1.0, or later	Research Use Only
MiSeq Reagent Kit v3	MFCS v1.0, or later	Research Use Only

Sequencing Steps

The following diagram summarizes the steps to set up and perform a sequencing run on the MiSeq FGx System. For sequencing instructions, see *Performing a Run* on page 18.

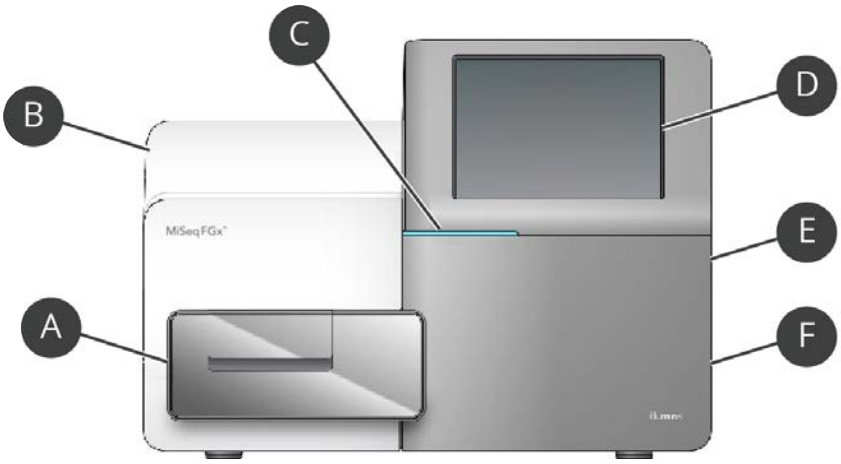
Figure 1 Overview of sequencing steps



Instrument Hardware

Externally, the MiSeq FGx System is equipped with a status bar, touch-screen monitor, USB port, optics module, and flow cell and reagent compartments.

Figure 2 External components

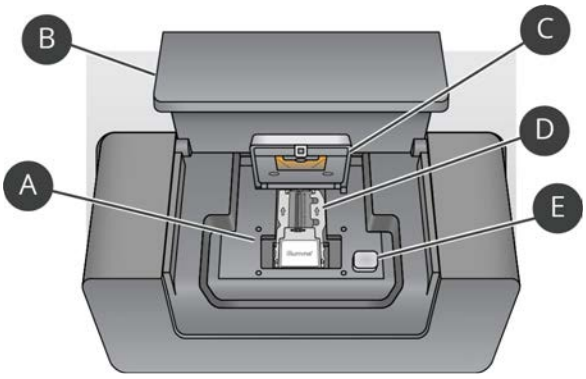


A **Flow cell compartment**–Contains the flow cell stage to hold the flow cell. Motors move the stage into the optical module for sequencing.
B **Optics module**–Encloses the optical components that image the flow cell.
C **Status bar**–Indicates system status as ready to sequence (green), processing (blue), or needs attention (orange).
D **Touch-screen monitor**–Displays the user interface for system configuration and run setup.
E **USB port**–Facilitates data transfer through the instrument computer.
F **Reagent compartment**–Holds reagents at proper temperatures, wash solutions, and the waste bottle. A magnetic latch secures the door.

Flow Cell Compartment

The flow cell compartment includes the flow cell stage, thermal station, and fluidics connections. A latch secures the flow cell onto the stage. When the latch is closed, two pins automatically position the flow cell for a run. The thermal station beneath the flow cell stage controls the temperature.

Figure 3 Components of the flow cell compartment

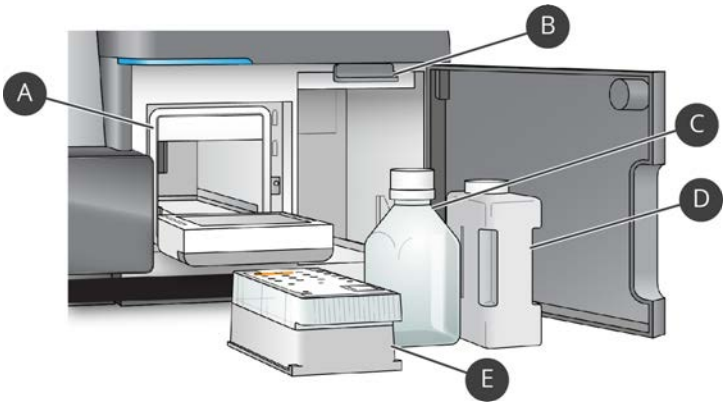


A Flow cell stage
B Compartment door
C Flow cell latch
D Loaded flow cell
E Latch release button

Reagent Compartment

The reagent compartment holds consumables for runs and washes. A reagent chiller holds the reagent cartridge during a run and the wash tray during a wash. Slots next to the reagent chiller are form-fitted for the PR2 bottle and the waste bottle.

A sipper handle locks the bottles in place and lowers the appropriate sipper into each bottle. Depending on the process, the software also automatically lowers the sippers into reagent cartridge reservoirs. Reagents are pumped through the sippers and fluidics tubes, and then to the flow cell. Reagent waste is delivered to the waste bottle throughout the process.



A Reagent chiller
B Sipper handle (raised position)
C PR2 bottle
D Waste bottle
E Reagent cartridge

System Software

The system includes the following built-in software to configure and operate the instrument. For information on the post-sequencing analysis software, see *Analysis Options* on page 12.

- **MiSeq FGx Control Software (MFCS)**–Controls instrument operations and provides an interface for configuring the system and setting up a run. During a run, MFCS operates the flow cell stage, dispenses reagents, controls the flow cell temperature, and images clusters.
- **Real-Time Analysis (RTA)**–Performs primary analysis, a process that calls bases during a run and assigns a quality score to each base for each cycle. Images are temporarily stored in the run folder for RTA processing and automatically deleted when image analysis is complete.

Control Software Interface

The MFCS interface displays screens for monitoring instrument activity and sensors, acknowledging alerts, and operating the instrument.

Welcome Screen

The Welcome screen displays information and functions for MFCS. From this screen, you can initiate the following tasks:

- **Sequence**–Sign in to Universal Analysis Software (UAS) and open a series of screens that guide you through run setup.
- **Perform Wash**–Initiate a post-run wash, maintenance wash, or standby wash to clean the instrument.
- **Manage Files**–Access controls for moving, deleting, and uploading files on the instrument computer.
- **Run Options**–Access settings for the post-run wash, default folder locations, and email notifications.
- **Manage Instrument**–Access system settings, system checks, and manual software updates with options to reboot or shut down the system and minimize windows.

Status Icons

The top-right corner of the Welcome screen displays a status icon that signals any change in conditions during run setup or the run. When a change in condition occurs, the icon changes to the associated image and blinks. When an icon is blinking, select it to open the status window and review a general description of the condition.

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

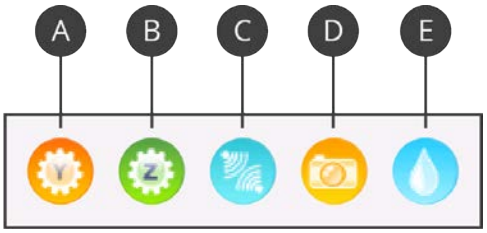
Icon	Name	Description
	Status OK	No change. System is normal.
	Attention	Important information. Action is recommended.

Icon	Name	Description
	Warning	Warnings do not stop a run, but require action to proceed.
	Error	Errors usually stop a run and require action to proceed.

Filter messages in the status window by selecting the icons along the top margin of the window. Selecting an icon shows or hides the condition.

Activity Indicators

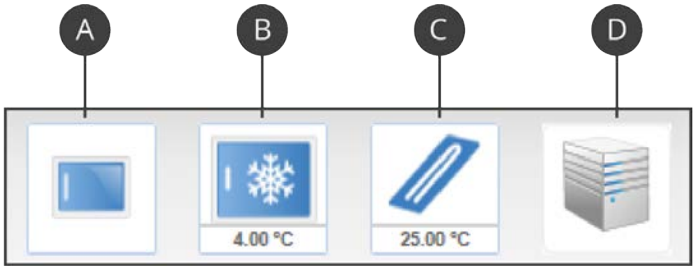
The lower-right corner of each MFCS screen displays a series of activity indicators. Each activity indicator is an icon that shows which task the instrument is performing.



A Moving the Y-stage
B Moving the Z-stage
C Activating electronics functionality
D Using the camera
E Pumping liquid through the fluidics system

Sensor Indicators

The bottom of each MFCS screen displays sensor indicators that show the status of an instrument component.



A Flow cell compartment door in the closed or open position
B Reagent chiller temperature in degrees Celsius (°C)
C Flow cell temperature in degrees Celsius
D Status of the server connection

Perform Wash Screen

On the Perform Wash screen, you can initiate washes and see when the last wash of each type was performed. For information on wash types and instructions on performing a wash, see *Maintenance* on page 27.

The Perform Wash screen also includes a command to raise the sippers. If an error occurs or the run is interrupted, use this command to remove the reagent cartridge from the instrument.

Warning: Always close the reagent chiller door after loading the wash tray and before starting a wash. Doing so keeps your hands away from the sippers when they lower, preventing potential injury.

Manage Instrument Screen

The Manage Instrument screen displays the following buttons. From this screen, you can access the following options and commands:

- **System Settings**–Options to change IP settings, the instrument name, or the domain.
- **System Check**–Options for checking the operational status of instrument components for troubleshooting purposes.
- **Manual Update**–Command to manually update the system software.
- **Reboot**–Command to reboot the system software as needed. A reboot is not necessary for regular maintenance.
- **Shut Down**–Command to shut down MFCS and Windows.
- **Minimize to Windows**–Quick access to the instrument operating system (OS) and folders on the instrument computer when the system is in kiosk mode. This command requires administrator or super user access.

Analysis Options

During sequencing, RTA performs base calling and assigns quality scores. After a run is complete, analyze the data in analysis software compatible with your run mode.

Table 2 Compatible analysis software

Run Mode	Analysis Software
Forensic Genomics	UAS
Research Use Only	BaseSpace Sequence Hub MiSeq Reporter

BaseSpace Sequence Hub and MiSeq Reporter are Illumina applications described in the following sections. UAS is an integrated Verogen application installed off-instrument on a dedicated server. For more information, visit the Universal Analysis Software page on the Verogen website.

BaseSpace Sequence Hub

BaseSpace Sequence Hub is a cloud computing environment where you can store and analyze run data. Eliminating the need for onsite storage and computing, BaseSpace Sequence Hub also provides tools for collaboration and sharing.

Connect to BaseSpace Sequence Hub at basespace.illumina.com. After the run starts, data files are encrypted in transit, decrypted during analysis, and encrypted again for storage. BaseSpace Sequence Hub automatically disconnects from the MiSeq FGx System at the end of a run or after RTA files are uploaded. If the internet connection is interrupted, file upload resumes from the point of interruption after the connection is restored.

After the last base call (BCL) file is uploaded to BaseSpace Sequence Hub, analysis begins. BaseSpace Sequence Hub supports the same analysis workflows as MiSeq Reporter and supports only the genomes provided with MiSeq Reporter. For more information, visit BaseSpace Sequence Hub Support on the Illumina website.

MiSeq Reporter

MiSeq Reporter is a Windows service application that runs on the instrument computer and processes RTA-generated files. The software is viewed from the web browser of another computer that is connected to the same network as MiSeq Reporter. MiSeq Reporter starts analysis after RTA analysis is complete. When MiSeq Reporter analysis is complete, the file CompletedJobInfo.xml is written to the run folder. For more information, visit MiSeq Reporter Support on the Illumina website.

When using MiSeq Reporter to analyze data, the system dedicates instrument computing resources to either sequencing or analysis. If you start a new run before analysis of a previous run is complete, the software prompts for confirmation of the new run. Analysis stops after confirmation. Use the MiSeq Reporter Requeue feature to restart analysis after the new run is complete. Analysis starts from the beginning.

Run Folders

Each run generates the following folders.

Folder	Directory	Purpose	Description
Temp	D:\Illumina\MiSeqFGxTemp	A working area for MFCS and RTA.	When the run starts, the software writes the temp folder to the local drive of the instrument computer. After seven days, the software deletes the folder contents. Accessing the temp folder is unnecessary.
Output	D:\Illumina\MiSeqFGxOutput	Contains output files from the run.	RTA copies files from the temp folder to the output folder. As BCL files are generated, RTA copies all files except focus and thumbnail images back to the temp folder and populates the analysis folder. You can change the location of the output folder.
Analysis	D:\Illumina\MiSeqFGxAnalysis	Contains the output files for analysis purposes.	After RTA completes, UAS accesses files in the analysis folder to analyze the sequencing data. All files written to the analysis folder are copied to the output folder.

Root Folder Name

Each run folder has the same root folder name. The root folder name uses the following format, which includes the run date, instrument and run numbers, and flow cell barcode number. Each time the instrument performs a run, the run number increments by one.

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

MiSeq FGx Reagent Kits

Performing a sequencing run requires one MiSeq FGx Reagent Kit or MiSeq FGx Reagent Micro Kit. Both kits are single-use, 600-cycle, and dedicated to the MiSeq FGx System.

Each kit includes reagents and a standard or micro flow cell. The flow cell, reagent cartridge, and PR2 bottle use radio-frequency identification (RFID) for consumable tracking and compatibility. Always match the flow cell lot number to the reagent cartridge lot number.

Kit Name	Flow Cell Type	Reagent Cartridge Type
MiSeq FGx Reagent Kit	Standard	Standard
MiSeq FGx Reagent Micro Kit	Micro	Micro

Contents and Storage

Two boxes comprise each reagent kit. When you receive your kit, promptly store components at the indicated temperatures.

Table 3 MiSeq FGx Reagent Kit

Box	Consumable	Quantity	Storage Temperature
1	Hybridization Buffer (HT1), 5 ml tube	1	-25°C to -15°C
	Standard reagent cartridge	1	-25°C to -15°C
2	SBS Solution (PR2), 500 ml bottle	1	2°C to 8°C
	Standard flow cell (clear cap) immersed in storage buffer	1	2°C to 8°C

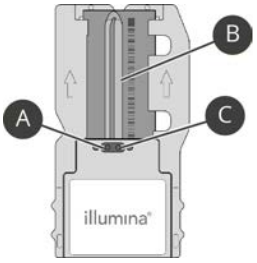
Table 4 MiSeq FGx Reagent Micro Kit

Box	Consumable	Quantity	Storage Temperature
1	Hybridization Buffer (HT1), 5 ml tube	1	-25°C to -15°C
	Micro reagent cartridge	1	-25°C to -15°C
2	SBS Solution (PR2), 500 ml bottle	1	2°C to 8°C
	Micro flow cell (green cap) immersed in storage buffer	1	2°C to 8°C

Flow Cell

The MiSeq FGx flow cell is a single-lane, glass-based substrate on which clusters are generated and the sequencing reaction is performed. Reagents enter the flow cell through an inlet port, move through the flow cell lane, and then exit through an outlet port. Waste is delivered to the waste bottle.

Figure 4 Flow cell components



A Outlet port
B Flow cell lane (imaging area)
C Inlet port

Tiling Scheme

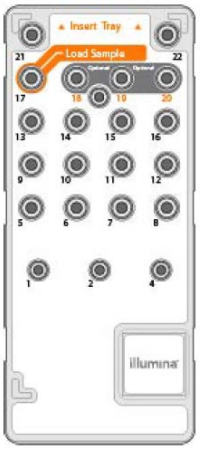
During a run, the flow cell lane is imaged in small areas called tiles. The number of tiles in a lane depends on the flow cell type.

Flow Cell Type	Tiles	Imaging Surface	Total Tiles Imaged
Standard	19	Top	19
Micro	8	Top and bottom	8

Reagent Cartridge

The reagent cartridge is prefilled with clustering and sequencing reagents with sufficient volumes for one run. All reagent reservoirs are sealed with foil and numbered. Reservoir 8 is filled with LDR, a denaturation reagent that contains formamide.

Figure 5 Cartridge with numbered reservoirs



Reserved Reservoirs

The following reagent reservoirs are reserved for libraries and optional custom primers. For more information, see *Custom Primers* on page 18.

Position	Description
17	Labeled **Load Sample** and reserved for libraries
18	Reserved for custom Read 1 primer
19	Reserved for custom Index Read primer
20	Reserved for custom Read 2 primer

User-Supplied Consumables and Equipment

Performing a sequencing run or wash on the MiSeq FGx System requires purchasing the consumables and equipment listed in this section. Make sure that all items are available before starting a run or wash.

Consumables

Consumable	Supplier	Purpose
Alcohol prep pads	VWR, catalog # 95041-714[1]	Cleaning the flow cell holder
Gloves, disposable, powder-free	General lab supplier	General use
Lab tissue, low-lint	VWR, catalog # 21905-026[2]	Cleaning the flow cell stage and sample seal
Lens paper, 4 x 6 in	VWR, catalog # 52846-001[2]	Cleaning the flow cell
MiSeq Disposable Wash Tubes	Verogen, part # MS-102-9999	Washing the instrument
One of the following kits: • MiSeq FGx Reagent Kit • MiSeq FGx Reagent Micro Kit	The equivalent supplier: • Verogen, part # 15066817 • Verogen, part # 20021681	Provides the reagents and flow cell for a run
Sodium hypochlorite	General lab supplier	Post-run washes
Tween 20	Sigma-Aldrich, catalog # P7949	Washing the instrument
Water, deionized	General lab supplier	Thawing reagents
Water, nuclease-free	General lab supplier	Washing the instrument
[Optional] Tweezers, plastic square tip	McMaster-Carr, catalog # 7003A22[2]	Handling the flow cell

[1] Or equivalent pads (70% isopropyl or 70% ethanol)

[2] Or equivalent

Guidelines for Nuclease-Free Water

Always use nuclease-free water to perform instrument procedures. Do not use tap water or deonized water.

The following reagents are considered nuclease-free water:

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

Equipment

- 1000 µl micropipettes
- Freezer, -25°C to -15°C, frost-free
- Ice bucket
- Refrigerator, 2°C to 8°C
- Test tube brushes

Performing a Run

Sequencing Overview

Cluster generation, sequencing, and analysis comprise sequencing on the MiSeq FGx System. The system automatically performs each of these steps during a run.

- **Cluster generation**–Single DNA molecules are bound to the surface of the flow cell, and then bridge-amplified to form clusters on the surface of the flow cell.
- **Sequencing**–A combination of LED and filters specific to each of the four fluorescently labeled dideoxynucleotides image the clusters. After imaging of one flow cell tile is complete, the flow cell is positioned to expose the next tile. This process is repeated until all tiles are imaged. After image analysis, the software performs base calling, filtering, and quality scoring.
- **Analysis**–If your system is configured for UAS, UAS automatically starts analyzing sequencing data after the run is complete. Monitoring analysis from another computer requires an internet connection. For more information, see *Analysis Options* on page 12.

Prerequisites

Before proceeding, review the following prerequisites for sequencing:

- Make sure that you have the required consumables and equipment. For a list, see *User-Supplied Consumables and Equipment* on page 16.
- Make sure you are using the compatible flow cell, reagent cartridge, and MFCS version for your run mode. For requirements, see *Compatibility of Run Components* on page 6.

Custom Primers

Custom primers are an option for Research Use Only runs sequencing libraries prepared with third-party kits. Specify the use of custom primers in the sample sheet, and then load them into the reserved reservoirs of the reagent cartridge. For instructions, download the *MiSeq System Custom Primers Guide (document # 15041638)* from the Illumina website.

Denaturing and Diluting Libraries

Denature and dilute libraries ***after*** preparing the reagent cartridge. The denature and dilute process dilutes libraries to the appropriate loading concentration, adds a sequencing control, and divides the DNA into single strands.

Instructions to denature and dilute libraries vary by library prep kit:

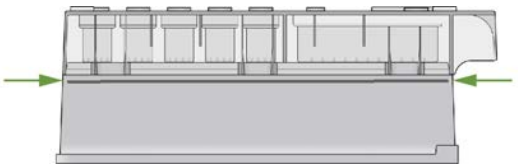
- If you are performing a Forensic Genomics run and sequencing libraries prepared with a Verogen kit, follow the denature and dilute instructions in the reference guide for your kit.
- If you are performing an RUO run and sequencing libraries prepared with a third-party kit, consult the kit documentation for denature and dilute instructions. For some kits, you can follow instructions in the Illumina *MiSeq System Denature and Dilute Libraries Guide (document # 15039740)*. Other kits result in ready-to-use libraries that do not require denaturing and diluting.

Prepare the Reagent Cartridge

Preparing the reagent cartridge for a run requires thawing and mixing reagents and inspecting the cartridge to ensure readiness. Thorough thawing and mixing is critical for proper sequencing. Thawing takes about 1 hour.

Thaw Reagents

1. Remove the reagent cartridge from -25°C to -15°C storage.
2. Remove the HT1 tube from the underside of the cartridge.
3. Prepare a water bath with room-temperature deionized water. Use enough water to submerge to the fill line printed on the side of the cartridge.



4. Place the reagent cartridge in the water bath. Do not allow the water to exceed the fill line.
5. Thaw for ~60-90 minutes. Allow to thaw completely.
6. Remove from the water bath and gently tap on the bench to dislodge water from the base.
7. Dry the base and make sure that no water has splashed on the top.

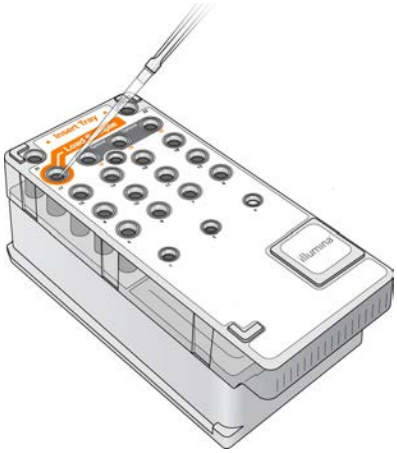
Mix and Inspect Reagents

1. Invert the reagent cartridge 10 times to mix reagents.
2. Inspect all positions to make sure reagents are completely thawed.
3. Inspect positions 1, 2, and 4 to make sure that reagents are fully mixed and free of precipitates.
4. Gently tap on the bench to remove bubbles, which interfere with reagent aspiration.
5. Place on ice or set aside at 2°C to 8°C for up to 6 hours. For best results, proceed immediately.
6. Denature and dilute libraries per instructions in the applicable library prep guide.

Load Libraries

1. Using a low-lint lab tissue, clean the foil seal covering the Load Samples reservoir.
2. Using a clean 1 ml pipette tip, pierce the foil seal covering the Load Samples reservoir. Do not pierce any other positions.

   Sippers automatically pierce the other positions during the run.
3. Add the entire volume of denatured and diluted libraries to the Load Samples reservoir. Avoid touching the foil seal as you dispense.



4. Inspect the Load Samples reservoir for bubbles.
5. If bubbles are present, gently tap the reagent cartridge on the bench to release them.
6. Proceed immediately to run setup.

Sign In and Select a Run

1. On the MFCS sign-in screen, select your analysis software:
   - **1.3**–For runs set up in UAS v1.3 to analyze ForenSeq® DNA Signature Prep libraries.
   - **2.0**–For runs set up in UAS v2.0, or later, to analyze all other compatible library types.
2. Sign in to UAS:
   a. In the User field, enter your user name for UAS.
   b. In the Password field, enter your password for UAS.
3. Select **Next**.
4. On the Welcome screen, select **Sequence**.
5. Select a run mode:
   - **Forensic Genomics**–Sequence libraries prepared with a Verogen kit.
   - **Research Use Only**–Sequence libraries prepared with a third-party kit.
6. [Forensic Genomics] On the Select Run screen, in the Select Run list, select a run created in UAS.

7. [Research Use Only] On the BaseSpace Option screen, do as follows.
   a. [Optional] Select the **Use BaseSpace for storage and analysis** checkbox to connect the run to BaseSpace Sequence Hub.
   b. In the Email field, enter the email address for your MyIllumina account.
   c. In the Password field, enter the password for your MyIllumina account.
8. Select **Next**.

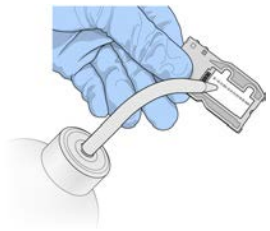
   The software initiates a series of run setup screens prompting you to load consumables and start the run. A Help icon in the corner of each screen opens a video animating the steps to load each consumable.

Clean and Load the Flow Cell

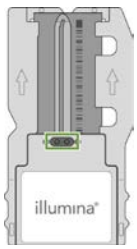
1. Put on a new pair of powder-free gloves.
2. Using plastic forceps, grip the flow cell by the base of the plastic cartridge and remove the flow cell from the container.

3. Lightly rinse the flow cell with nuclease-free water. Make sure that both the glass and plastic are thoroughly rinsed.

   Excess salts can affect flow cell seating on the instrument and imaging.

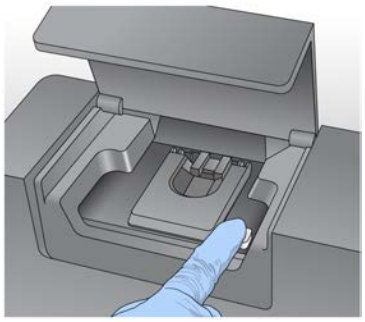


4. Thoroughly dry the flow cell with a lint-free lens cleaning tissue.
   - Gently pat dry the area of the gasket and adjacent glass.
   - Use care around the black port gasket (indicated in orange).

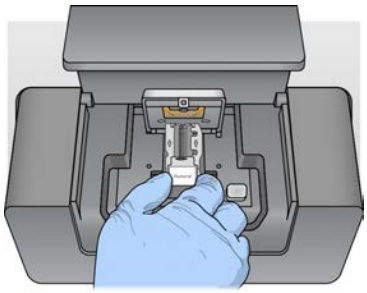


5. Clean the flow cell glass with an alcohol wipe.
   - Avoid using the alcohol wipe on the port gasket.
   - Make sure the glass is free of streaks, fingerprints, lint, and tissue fibers.

6. Dry any excess alcohol with a lint-free lens cleaning tissue.
7. Inspect the flow cell to make sure the ports are free of obstructions and that the gasket is well seated around the ports.
8. If the gasket is dislodged, gently press it back into place so it sits securely around the ports.
9. When the Load Flow Cell screen appears, open the flow cell latch as follows.
   a. Raise the flow cell compartment door.
   b. Press the release button to the right of the flow cell latch.

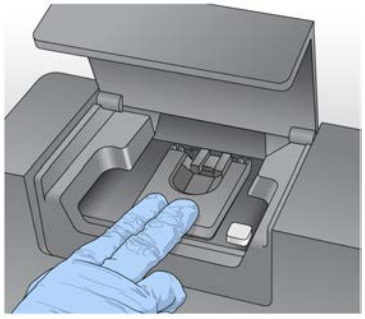


10. Make sure that the flow cell stage is free of lint or other debris.
11. If lint or other debris is present, do as follows.
    a. Clean the stage with an alcohol wipe or lint-free tissue moistened with ethanol or isopropanol.
    b. Carefully wipe the surface of the stage until it is clean and dry.
12. Holding the flow cell by the cartridge edges, place the flow cell onto the flow cell stage.



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

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



14. Check the lower-right corner of the screen to confirm that the software successfully read and recorded the RFID.
15. If the software cannot read the RFID, manually enter the identifying information.

    The software allows one RFID-labeled component (flow cell, PR2, or reagent cartridge) to fail. For more information, see *RFID Read Failures* on page 39.
16. Close the flow cell compartment door, and then select **Next**.

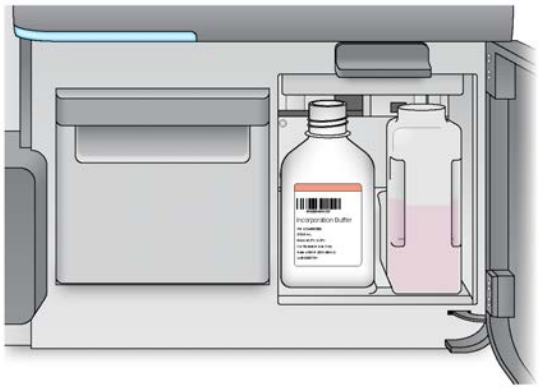
    The Load Reagents screen appears.

Load Reagents

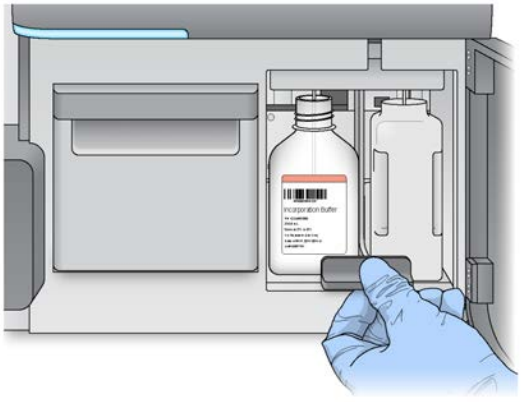
Loading reagents is a two-step process: load the PR2 bottle and confirm the waste bottle is empty, and then load the reagent cartridge.

Load PR2 and Check Waste

1. Remove PR2 from 2°C to 8°C storage.
2. Gently invert to mix, and then remove the lid.
3. Open the reagent compartment door, and then raise the sipper handle until it locks into place.
4. Place PR2 in the recess to the right of the reagent chiller.



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 so that the sippers lower into the PR2 and waste bottles.



7. Check the lower-right corner of the screen to confirm that the software successfully read and recorded the RFID.
8. If the software cannot read the RFID, manually enter the identifying information.

   The software allows one RFID-labeled component (flow cell, PR2, or reagent cartridge) to fail. For more information, see *RFID Read Failures* on page 39.
9. Select **Next**.

Load the Reagent Cartridge

1. Open the reagent chiller door. Avoid leaving it open for an extended period of time.
2. Holding the reagent cartridge on the end with the Illumina label, slide it into the reagent chiller until it stops.

3. Close the reagent chiller door.
4. Check the lower-right corner of the screen to confirm that the software successfully read and recorded the RFID.
5. If the software cannot read the RFID, manually enter the identifying information.

   The software allows one RFID-labeled component (flow cell, PR2, or reagent cartridge) to fail. For more information, see *RFID Read Failures* on page 39.
6. Close the reagent compartment door, and then select **Next**.

Change the Sample Sheet

For a Research Use Only run, the software automatically searches for a sample sheet with a file name that matches the reagent cartridge barcode number. If the software cannot locate the sample sheet, you must change it.

1. On the Load Reagents screen, select **Change Sample Sheet**.
2. Browse to and select the applicable sample sheet.

Review Run Parameters

1. On the Review screen, review the run name, workflow type, and read length.

   MFCS receives these run parameters from UAS for a Forensic Genomics run and the sample sheet for a Research Use Only run.
2. [Research Use Only] Review folder locations on the lower-left corner of the screen.

   a. To make changes, select **Change Folders**.

   b. When changes are complete, select **Save**, and then select **Next**.
3. Select **Next** to start the pre-run check.

   The Pre-Run Check screen appears.

Review the Pre-Run Check

The pre-run check evaluates run components, disk space, and network connections to make sure the system is ready to start the run. For help troubleshooting pre-run check errors, see *Run Setup Errors* on page 38.

1. Wait for the pre-run check to complete.
2. If any items do not pass, follow the instructions displayed on the onscreen message to correct the error.
3. When all items successfully pass, select **Start Run**.
   - Do not open the compartment doors or otherwise touch the instrument.
   - Do not touch the instrument monitor except to pause the run.

Monitor the Run

Metrics appear after sequencing starts and remain visible throughout the run. The following table shows when each metric appears. For details, see *Run Status and Metrics* on page 43.

Metric*	MiSeq Reagent Kit	Sequencing Cycle
Intensity	All	1
Cluster Density	MiSeq FGx Reagent Kit	8
	MiSeq Reagent Kit v3 (RUO)	8
	MiSeq Reagent Kit v2 (RUO)	5

Metric*	MiSeq Reagent Kit	Sequencing Cycle
%PF, Yield, and Q-scores	All	26

* Metrics vary for Forensic Genomics and Research Use Only runs.

1. On the Sequencing screen, monitor run progress and metrics.
2. When the Next button appears, review the results.
3. When you are ready to exit the Sequencing screen, select **Next**.

   After selecting Next, returning to the Sequencing screen is not possible.
4. Leave the used flow cell on the instrument for the post-run wash.

   You must complete a post-run wash before the next run can start. For instructions, see *Post-Run Wash* on page 28.

Pause the Run

The MiSeq FGx System is designed to complete a run without intervention, but you can pause and then resume a run if necessary. However, ***do not*** pause a Forensic Genomics run during cluster generation or the first eight cycles of sequencing. Resuming a run paused during these processes is not possible.

1. On the Sequencing screen, select **Pause**.

   The software completes the current process, pauses the run, and places the flow cell in a safe state.
2. Complete any desired tasks.

   For example, empty the waste bottle or check the volume of the PR2 bottle.
3. Select **Resume** to continue the run.

Stop the Run

If necessary, you can stop a run. For example, you might stop a run that was set up incorrectly, indicates poor data quality, or experiences a hardware error.

Stopping a run is final. A stopped run cannot be resumed and you cannot use reagents remaining in the reagent cartridge. A new run requires a new cartridge.

1. On the Sequencing screen, select **Stop**.
2. In the confirm dialog box, select **Yes** to stop the run.

   The software ends the current process and moves the flow cell stage to the forward position. RTA continues for the last completed cycle.
3. Proceed to the post-run wash. See *Post-Run Wash* on page 28 for instructions.

Maintenance

Maintenance Procedures

The following tables show the recommended frequency for maintenance during normal operation and idle periods when the instrument is unused for at least seven days. In addition to these procedures, Verogen recommends one annual preventive maintenance. Visit the Preventative Maintenance page on the Verogen website for more information.

Table 5 Maintenance for normal operation

Activity	Frequency
Post-run wash	After every run
Maintenance wash	Weekly

Table 6 Maintenance for idle periods

Activity	Frequency
Maintenance wash	• Leaving standby mode • Before instrument shutdown • After a shutdown lasting > 7 days
Standby wash	• To prepare for standby mode • Monthly during standby mode
Instrument shutdown	• As needed

Fluidics System

Regular washes maintain the fluidics system and ensure continued performance in the following ways:

- Flush any remaining reagents from the fluidics tubes and sippers.
- Prevent salt accumulation and crystallization in the fluidics tubes and sippers.
- Prevent cross-contamination from the previous run.

Wash Tray Maintenance

Check the inside of the wash tray before each use, preferably using a flashlight to inspect each hole. If you see residue, scrub the inside of the wash tray with a test tube brush and rinse thoroughly with nuclease-free water to remove all residue. After rinsing, store the wash tray upside down.

Manual Software Updates

To maintain proper system configuration, Verogen personnel perform all software updates. Therefore, the Manual Update feature used to update the control and analysis software is not available on the MiSeq FGx System.

Post-Run Wash

Perform a post-run wash after each sequencing run. The wash takes approximately 30 minutes. Until the wash is performed, the software cannot start a new run.

> You can configure the system to require a maintenance wash after each run instead of a post-run wash. For instructions, see *Configure the Wash Type* on page 35.

A post-run wash requires a wash solution of 0.01% sodium hypochlorite. Prepare fresh 0.01% hypochlorite for each wash and ***use within one day***. The post-run wash is the only wash that uses sodium hypochlorite.

Prepare Wash Components

1. Check the sodium hypochlorite product label for a concentration of 6%.
   Higher concentrations can fail cluster generation in subsequent runs.
2. If 6% sodium hypochlorite is not available, do as follows.
   a. In a MiSeq wash tube, use nuclease-free water to prepare 1 ml 0.01% sodium hypochlorite.
   b. Mix thoroughly with a 1000 µl micropipette.
   c. Proceed to step 6.
3. Combine the following volumes to prepare 900 µl 1:30 sodium hypochlorite:
   - 6% sodium hypochlorite (30 µl)
   - Nuclease-free water (870 µl)
4. In a MiSeq wash tube, combine the following volumes to prepare 1 ml 0.01% sodium hypochlorite:
   - 1:30 sodium hypochlorite (50 µl)
   - Nuclease-free water (950 µl)
5. Mix thoroughly with a 1000 µl micropipette.
6. Add 6 ml nuclease-free water to each reservoir of the wash tray ***except position 17***.
7. Add 350 ml nuclease-free water to the 500 ml wash bottle.

8. Insert the MiSeq wash tube into position 17 of the wash tray until the tube neck is flush with the tray.

Perform a Post-Run Wash

1. On the Welcome screen, select **Perform Wash**.
2. On the Perform Wash screen, select **Perform Post-Run Wash**.
   The software raises the sippers in the reagent chiller.
3. Wait several seconds to make sure the sippers are fully raised.
4. Open the reagent compartment door and the reagent chiller door.
5. Slide the used reagent cartridge from the chiller.
6. Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
7. Raise the sipper handle in front of the PR2 and waste bottles until it locks into place.
8. Replace the PR2 bottle with the wash bottle.
9. Discard the PR2 bottle. Do not use any remaining solution.
10. Remove the waste bottle and discard the contents appropriately.

> Warning: This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. **For complete environmental, health, and safety information, see the safety data sheets (SDS) at verogen.com/documentation.**

11. Return the waste bottle to the reagent compartment.
12. Slowly lower the sipper handle so that the sippers lower into the wash and waste bottles.
13. Close the reagent compartment door.
14. Select **Next** to start the wash.
15. When the wash is complete, leave the used flow cell, wash tray, and wash bottle in the instrument.
    A small amount of wash solution remains in the MiSeq wash tube.
16. Leave the sippers in the down position in the unused wash solution and wash bottle to prevent the sippers from drying out and air from entering the system.

Maintenance Wash

Perform a maintenance wash every seven days during normal operation, before shutting down the instrument, and when resuming normal operation after an idle period. A maintenance wash takes approximately 90 minutes.

A series of three washes comprise a maintenance wash. The first two washes require a wash solution of 0.5% Tween 20 and the third wash uses nuclease-free water. Preparing 0.5% Tween requires a 10% dilution of stock Tween 20. After diluting, you can use the 10% Tween 20 for up to seven days. Prepare the second dilution, 0.5% Tween 20, daily. Do not use 0.5% Tween 20 that is more than one day old.

Initiate a Maintenance Wash

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

Perform the First Wash

1. Combine the following volumes to prepare 50 ml 10% Tween 20:
   - Tween 20 (5 ml)
   - Nuclease-free water (45 ml)
2. Combine the following volumes to prepare 500 ml 0.5% Tween 20:
   - 10% Tween 20 (25 ml)
   - Nuclease-free water (475 ml)
3. Invert several times to mix.
4. Add 6 ml 0.5% Tween 20 to each reservoir of the wash tray.
5. Add 350 ml 0.5% Tween 20 to the 500 ml wash bottle.
6. Open the reagent compartment door and the reagent chiller door.
7. Slide the used reagent cartridge from the chiller.
8. Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
9. Raise the sipper handle in front of the PR2 and waste bottles until it locks into place.
10. Replace the PR2 bottle with the wash bottle.
11. Discard the PR2 bottle. Do not use any remaining solution.
12. Remove the waste bottle and discard the contents appropriately.

Warning: This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. **For complete environmental, health, and safety information, see the safety data sheets (SDS) at verogen.com/documentation.**

13. Return the waste bottle to the reagent compartment.
14. Slowly lower the sipper handle so that the sippers lower into the wash and waste bottles.
15. Close the reagent compartment door.
16. Select **Next** to start the first wash.

Perform the Second Wash

1. Combine the following volumes to prepare 50 ml 10% Tween 20:
   - Tween 20 (5 ml)
   - Nuclease-free water (45 ml)
2. Combine the following volumes to prepare 500 ml 0.5% Tween 20:
   - 10% Tween 20 (25 ml)
   - Nuclease-free water (475 ml)
3. Invert several times to mix.
4. When the first wash is complete, remove the wash tray and wash bottle. Discard the remaining wash solution. Reusing wash solution can return waste to the fluidics system.
5. Add 6 ml 0.5% Tween 20 to each reservoir of the wash tray.
6. Add 350 ml 0.5% Tween 20 to the 500 ml wash bottle.
7. Load the wash tray and wash bottle as follows.
   a. Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
   b. Load the wash bottle, and then slowly lower the sipper handle so the sippers enter the wash and waste bottles.
   c. Close the reagent compartment door.
8. Select **Next** to start the second wash.

Perform the Final Wash

1. When the second wash is complete, remove the wash tray and wash bottle. Discard the remaining wash solution.
2. Add 6 ml nuclease-free water to each reservoir of the wash tray.
3. Add 350 ml nuclease-free water 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, and then close the reagent chiller door.
   b. Load the wash bottle, and then slowly lower the sipper handle so the sippers enter the wash and waste bottles.

c. Close the reagent compartment door.

5. Select **Next** to start the final wash.
6. When the wash is complete, do as follows.
   - Leave the used flow cell, wash tray, and wash bottle in the instrument.
   - Leave the sippers in the down position in the unused wash solution and wash bottle.

   Leaving the sippers down prevents them from drying out and keeps air out of the system.

Standby Mode

Enter standby mode to prepare the instrument to sit idle for at least seven days. To enter standby mode, perform a standby wash to flush each position of any residual reagents and accumulated salts. When the wash is complete, the instrument automatically enters standby mode. Leaving standby mode requires a maintenance wash.

Two washes comprise a standby wash, taking approximately 2.5 hours total. The first wash requires a wash solution of fresh 0.5% Tween 20. The second wash uses nuclease-free water to remove residual wash solution so the instrument does not remain idle with 0.5% Tween 20 in the fluidics system.

Initiate a Standby Wash

1. Make sure that a used flow cell is loaded on the instrument.
2. On the Welcome screen, select **Perform Wash**.
3. On the Perform Wash screen, select **Standby Wash**.

   The software raises the sippers in the reagent chiller.

Perform the First Wash

1. Combine the following volumes to prepare 50 ml 10% Tween 20:
   - Tween 20 (5 ml)
   - Nuclease-free water (45 ml)
2. Combine the following volumes to prepare 500 ml 0.5% Tween 20:
   - 10% Tween 20 (25 ml)
   - Nuclease-free water (475 ml)
3. Invert several times to mix.
4. Add 6 ml 0.5% Tween 20 to each reservoir of the wash tray.
5. Add 350 ml 0.5% Tween 20 to the 500 ml wash bottle.
6. Open the reagent compartment door and the reagent chiller door.
7. Slide the used reagent cartridge from the chiller.
8. Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.
9. Raise the sipper handle in front of the PR2 and waste bottles until it locks into place.
10. Replace the PR2 bottle with the wash bottle.
11. Discard the PR2 bottle. Do not use any remaining solution.

12. Remove the waste bottle and discard the contents appropriately.

    Warning: This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. **For complete environmental, health, and safety information, see the safety data sheets (SDS) at verogen.com/documentation.**

13. Return the waste bottle to the reagent compartment.
14. Slowly lower the sipper handle so that the sippers lower into the wash and waste bottles.
15. Close the reagent compartment door.
16. Select **Next** to start the first wash.

Perform the Second Wash

1. When the first wash is complete, remove the wash tray and wash bottle. Discard the remaining wash solution.
2. Add 6 ml nuclease-free water to each reservoir of the wash tray.
3. Add 350 ml nuclease-free water 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, and then close the reagent chiller door.
   b. Load the wash bottle, and then slowly lower the sipper handle so the sippers enter the wash and waste bottles.
   c. Close the reagent compartment door.
5. Select **Next** to start the second wash.
6. When the wash is complete, do as follows.
   - Leave the used flow cell, wash tray, and wash bottle in the instrument.
   - Leave the sippers in the unused wash solution and wash bottle, which prevents the sippers from drying out and air from entering the system.

   The Welcome screen indicates that the instrument is in standby mode. The next time you set up a run, the software prompts you to perform a maintenance wash.

Repeat the Standby Wash

Repeat the standby wash every 30 days the instrument remains in standby mode.

1. On the Welcome screen, select **Sequence**.
2. When prompted, select **Start Wash**.

   This command initiates a maintenance wash. For instructions, see *Maintenance Wash* on page 30.
3. When the maintenance wash is complete, repeat the standby wash to reenter standby mode.

Turning the System On and Off

Verogen recommends leaving on the instrument continuously. If you must turn off the instrument, use the following instructions to shut down Windows and prepare the fluidics system.

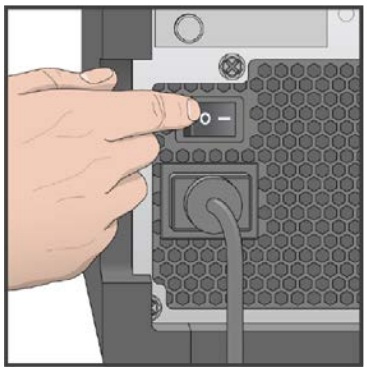
If the system remains shut down for more than seven days, perform a maintenance wash upon restart.

Shut Down the System

1. Perform a maintenance wash. See *Maintenance Wash* on page 30.
2. Remove the waste bottle and discard the contents appropriately.
3. Return the waste bottle to the reagent compartment, and then close the reagent compartment door.
4. On the Manage Instrument screen, select **Shutdown**.
   This command shuts down the software.
5. Reach around the right side of the instrument and turn the power switch to the OFF position.
   The power switch is in the lower corner of the back panel, directly above the power cord.
6. Wait at least 60 seconds before returning the power switch to the ON position.

Start the System

1. If the instrument is not already on, do as follows.
   a. Reach around the right side of the instrument to locate the power switch, which is in the lower corner of the back panel, directly above the power cord.
   b. Turn the power switch to the ON position to start the instrument computer.



2. Log in to the OS.
3. Wait for the OS to finish loading.
   MFCS automatically launches and initializes the system.
4. After initialization, enter your UAS user name (email) and password to sign in, and then select **Next**.
   The Welcome screen appears.

System Configuration

Specify Run Settings

The Run Options screen provides options to specify the default settings for a run. The screen is divided into separate tabs for runs, folders, and emails.

Configure the Wash Type

After each run, the system requires a post-run wash by default. However, you can configure the system to instead require a maintenance wash. For information on each wash type, see *Maintenance* on page 27.

1. On the Welcome screen, select **Run Options**.
2. On the Run Settings tab, select **Post-Run Wash** or **Maintenance Wash**.
3. When you are finished, select **Save and Return**.

Set Folder Locations

The MiSeq FGx System requires access to folders that reside on a local network or the instrument computer. Options on the Folder Settings tab indicate folder locations.

1. On the Welcome screen, select **Run Options**.
2. On the Run Options screen, select the Folder Settings tab.
3. For each of the following fields, select **Browse**, and then navigate to the desired directory.
   - **Recipe Folder**—The default location for recipes. Custom recipes are not supported for Verogen library prep kits.
   - **Sample Sheet Folder**—The default location for the sample information specified before library prep and run parameters.
   - **Manifest Folder**—The default location for manifest files. Verogen library prep kits use preloaded manifests.
   - **Output Folder**—The default location for analysis output files. Change the default output folder to a network location to facilitate sharing and long-term storage.
4. When you are finished, select **Save and Return**.

Set Up Email Notifications

You can configure the system to notify you via email when selected events occur. Setting up these notifications requires that the system is connected to a network with internet access.

1. On the Welcome screen, select **Run Options**.
2. On the Run Options screen, select the **Email Notifications** tab.

3. Use the onscreen keyboards to complete the following fields:
   - **Local SMTP email server address**–Enter the address of the local Simple Mail Transfer Protocol (SMTP) server. If necessary, contact your administrator for this information.
   - **Sender address**–Enter the sender email address, which must have the same domain name as the server email address. A sender can be an administrator account used to send notifications.
   - **Recipient addresses**–Enter the email address of each notification recipient, separating each address with a comma.
4. To send a test email to recipients, select **Test**.
5. Select any of the following checkboxes to specify which events trigger a notification:
   - **Primary analysis is complete**–Sends a notification when RTA completes analysis.
   - **On-instrument analysis is complete**–Sends a notification when UAS completes analysis.
   - **A critical MiSeq Software error occurs**–Sends a notification when the system software experiences an error.
6. When you are finished, select **Save and Return**.

Manage Files

The Manage Files screen provides options to move, upload, and delete files on the instrument computer. The screen is divided into separate tabs for run files, sample sheets, manifest files, genomes, recipes, and bundle logs.

- The Runs tab contains files for Forensic Genomics and Research Use Only runs. All other tabs apply only to Research Use Only runs.
- The Bundle Logs tab is reserved for Verogen Technical Support to zip files for troubleshooting purposes.
- The Manifest, Genomes, and Recipe tabs include files preloaded for Verogen library kits. Do not change these files.

Locate a File

1. On the Welcome screen, select **Manage Files**.
2. On the Manage Files screen, select the **Runs** tab.
3. Select **Browse**, and then navigate to any file accessible to the instrument.

Delete Files

1. On the Welcome screen, select **Manage Files**.
2. On the Manage Files screen, select the applicable tab.

   Deleting files from the Runs tab requires administrator privileges.
3. Select the checkbox next to the name of each file or folder you want to delete. To delete all files, select the checkbox to the left of the Delete button.
4. Select **Delete**.

Move Run Folders

1. On the Welcome screen, select **Manage Files**.
2. On the Manage Files screen, select the **Runs** tab.

3. Select the checkbox next to the name of each run folder you want to move. To move all run folders, select the checkbox to the left of the Delete button.
4. Select **Move**, and then browse to the desired location.

   The software copies the folders to the new location and deletes them from the old location.

Rename a Sample Sheet

1. On the Welcome screen, select **Manage Files**.
2. On the Manage Files screen, select the **Sample Sheets** tab.
3. Select the checkbox next to the sample sheet.
4. Select **Rename**, and then use the onscreen keyboard to enter a new name.

Upload a Sample Sheet or Manifest

Use the file upload feature to upload sample sheets or manifests from a USB drive to a system that is not connected to network.

1. On the Welcome screen, select **Manage Files**.
2. On the Manage Files screen, select the Sample Sheet or Manifests tab.
3. Select **Upload**, and then browse to the USB location of the sample sheet or manifest.

   The software uploads the file to the directory indicated on the Folder Settings tab. For information on specifying directories, see *Set Folder Locations* on page 35.

Update System Settings

System settings are configured when the instrument is installed and started for the first time. If a network or facility change requires a change to the settings, contact your facility administrator for help.

Troubleshooting

Resolving System Issues

This chapter describes common system issues and how to resolve them before contacting Verogen Technical Support. Most errors produce an onscreen prompt with instructions to correct the issue. For problems with run quality or performance, contact Verogen Technical Support. See *Technical Support* on page 49.

Your Verogen Technical Support representative might request copies of run-specific files to assist with troubleshooting. The following files reside at the root level of the run folder:

- InterOp folder
- RunInfo.xml
- RunParameters.xml
- SampleSheet.csv (for Research Use Only mode)

Run Setup Errors

The Not Ready icon (red X) appears next to items that fail a pre-run check. An onscreen message appears to describe the error and how to correct it. The following sections detail the possible errors and corrective actions.

Flow Rate Measured

1. On the Flow Rate Check screen, enter the following values:
   - Solution: **PR2**
   - Volume: **250**
   - Aspirate Rate: **2500**
   - Dispense Rate: **2500**
2. Select **Pump**.
3. If the error persists, set the volume to pump 500 µl PR2 and repeat the process. When fluids have been pumped, select **Restart Check**.

   When the pre-run check is successful, the Start Run button becomes active.
4. If the flow check fails again, reseat the flow cell to correct misalignment and restore flow. Inspect the flow cell gasket for lint or irregularities.

Free Disk Space

The instrument computer has a storage capacity of approximately 550 GB. Before starting a run, the software checks available disk space. If disk space is insufficient, a message appears. The message indicates how much disk space the run requires and how much disk space to clear so the run can proceed.

1. On the Welcome screen, select **Manage Files**.
2. On the Manage Files screen, select the **Runs** tab.
3. Move or delete run folders as appropriate.
4. Select **Restart Check**.

Network Connection Active

1. Make sure that the network cable is plugged into the instrument.
2. If the network connection is not restored, on the Manage Instrument screen, select **Reboot** to restart the software.
3. If the connection is still not restored, do as follows.
    a. On the Manage Instrument screen, select **Shutdown**.
    b. Turn the power switch to the OFF position.
    c. Wait at least 60 seconds.
    d. Turn on the instrument and start the software.

Primary Analysis Ready

The default time to allow RTA to complete primary analysis is one hour, and a countdown is visible on the screen. When RTA has not completed analysis from the previous run, you can wait 1 hour or terminate analysis. UAS stops analysis for any incomplete cycles.

Sample Sheet Present

If the software cannot locate a sample sheet, try the following actions and restart the check.

- The software cannot automatically locate a sample sheet that is not named with the reagent cartridge ID for the run. Browse to the appropriate sample sheet.
- If the sample sheet is named with the reagent cartridge ID for the run, make sure it is located in the default sample sheet folder. Check the default folder location on the Welcome screen in Run Options.
- Make sure that the sample sheet file extension is .csv.
- If the sample sheet is missing, create a sample sheet and copy it to the sample sheet locations specified in Run Options.

RFID Read Failures

The following conditions trigger an RFID read failure:

- The component is not part of a forensic genomics kit.

- For a Research Use Only run, the component is not part of the kit indicated in the sample sheet.
- The component contains an RFID tag with a technical failure. The following sections list the steps to resolve a technical failure.

The software allows one RFID-labeled component (flow cell, PR2, or reagent cartridge) to fail. If the software cannot read the RFID for multiple components, run setup cannot proceed and you must contact Verogen Technical Support.

Resolve a Flow Cell RFID Issue

1. Open and then close the flow cell compartment door to retry the RFID read.
2. If the RFID read fails again, carefully remove the flow cell.
3. Check the flow cell and the flow cell stage for debris or salts that can stop an 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 read continues to fail, do as follows.

    a. Select **Keyboard** to display the onscreen keyboard.
    b. Enter the flow cell barcode number, and then select **Done**.

    The barcode number is printed directly below the barcode on the flow cell container label.
7. Select **Next** to proceed with run setup.

Resolve a PR2 RFID Issue

1. Raise and then lower the reagent sipper handle to retry the RFID read.
2. If the RFID read fails again, raise the sipper handle and remove the PR2 bottle.
3. Check the bottle and the reagent compartment for debris or salts that can stop an RFID read.
4. Replace the PR2 bottle.
5. Slowly lower the sipper handle until it locks into place. Make sure that the sippers lower into the PR2 and waste bottles.
6. If the RFID read continues to fail, do as follows.

    a. Select **Keyboard** to display the onscreen keyboard.
    b. Enter the barcode number of the PR2 bottle, and then select **Done**.

    The barcode number is printed directly below the barcode on the bottle label.
7. Select **Next** to proceed to the next run setup step.

Resolve a Reagent Cartridge RFID Issue

1. Open and then close the reagent chiller door to retry the RFID read.
2. If the RFID read fails again, remove the reagent cartridge.
3. Check the reagent cartridge and reagent chiller for debris or salts, which can stop the RFID read.
4. Replace the reagent cartridge, and then close the reagent chiller door.

5. If the RFID read continues to fail, select **Enter Reagent Kit Barcode**.
6. In the Reagent Kit Barcode field, enter the reagent kit barcode number.
   The barcode number is printed directly below the barcode on the reagent kit label.
7. In the Reagent Kit Part Number field, enter the reagent cartridge part number.
   The part number is printed to the right of REF on the reagent kit label.
8. Select **Next** to return to the Load Reagents screen.
9. Select **Next** to proceed with run setup.

System Checks

A system check evaluates the performance of instrument components for troubleshooting purposes. Normal operation and instrument maintenance does not require a system check.

When a system check is complete, the results appear onscreen with the following commands:

- Show Details displays a summary of results.
- Export Results saves the summary to a USB drive as a .csv file.

Perform a Volume Test

A fluidics tube obstruction can hinder reagent delivery and affect sequencing results. If you suspect an obstruction, perform a volume test, which is a type of system check you can perform before contacting Verogen Technical Support. A volume test checks the health of the fluidics system by estimating the volume between two bubbles as they pass the sensors.

1. Make sure that a used flow cell is loaded on the instrument.
2. On the Welcome screen, select **Manage Instrument**.
3. Select **System Check**.
4. Select the **Conduct Volume Test** checkbox, and then select **Next**.
5. Add 6 ml nuclease-free water to each reservoir in the wash tray.
6. Add 350 ml nuclease-free water to the 500 ml wash bottle.
7. Load the wash tray and wash bottle into the instrument:
   a. Slide the wash tray into the reagent chiller until it stops.
   b. Close the reagent chiller door.
   c. Raise the sipper handle until it locks into place.
   d. Load the wash bottle.
   e. Remove the waste bottle and discard the contents appropriately.
   f. Return the waste bottle to the reagent compartment.
   g. Slowly lower the sipper handle. Make sure that the sippers lower into the wash and waste bottles.
8. Select **Next** to start the volume test.
   When the test is complete, results appear on the screen.

9. If the test does not pass, perform a maintenance wash and repeat the volume test.
   For wash instructions, see *Maintenance Wash* on page 30.

Check the Flow Rate

The system measures the flow rate, which is the speed at which fluids pass through the fluidics system (µl/min), during a pre-run check. If the system cannot measure the flow rate, pump PR2 through the system and check the flow rate again.

1. In the Solution list, select **[PR2, 3]**.
2. For the remaining fields, select **Keyboard**, and then enter the following values:

Field	Value
Volume	**250**
Aspirate Rate	**2500**
Dispense Rate	**2500**

3. Select **Pump**.
4. When pumping is complete, select **Restart Check**.
5. If the error persists, set the volume to pump 500 µl PR2 and repeat pumping one time.
6. If second pumping attempt does not resolve the error, contact Verogen Technical Support.

Measure Wash Volumes

Measure wash volumes to confirm that wash fluidics are performing efficiently.

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

Wash Type	Expected Wash Volume (ml)
Post-run wash	25.5
Maintenance wash	17.25
Standby wash	46

Mid-Run Reboot

If the MiSeq FGx System restarts during a run, the Windows OS might be configured to automatically install software updates. Contact your IT department for help turning off automatic updates.

> Automatic updates are supposed to be turned off during installation.

After a reboot occurs, you must restart the run. A restart terminates the run and it cannot be recovered.

Run Status and Metrics

Run Progress

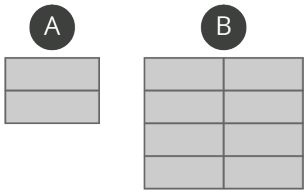
A status bar tracks run progress and indicates the number of run steps and cycles completed. The number of cycles performed in a run affects run duration, so more cycles increase run time. For details, see *Number of Cycles in a Read* on page 44.

Intensity

Intensity shows the number of flow cell tiles and surfaces being imaged with the value of cluster intensities of the 90th percentile for each tile.

- The standard flow cell is imaged on the top surface only, so the graphic has one column.
- The micro flow cell is imaged on the top and bottom surfaces, so the graphic has two columns.

Figure 6 Flow cell tiles



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

Run Quality Metrics

During a run, the Sequencing screen displays the following run quality metrics under Quality Information: cluster density, clusters passing filter, phasing, and prephasing.

Cluster Density

Cluster density shows the number of clusters per square millimeter (K/mm²). The target cluster density range for ForenSeq mtDNA libraries is 400–1650 K/mm². For ForenSeq Kintelligence libraries, it is 700–1400 K/mm².

Clusters Passing Filter

Clusters passing filter shows the percentage of clusters that passed the quality filter. This metric is based on the Illumina chastity filter, which measures quality and can detect low-quality base calls. Data appear after cycle 25.

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 multiple base calls have a chastity value < 0.6 in the first 25 cycles, reads do not pass filter.

Phasing and Prephasing

Phasing and prephasing metrics appear on the Sequencing screen for a Forensic Genomics run only. Phasing shows the percentage of molecules in a cluster that fall behind the current cycle in Read 1. Prephasing shows the percentage of molecules in a cluster that jump ahead of the current cycle in Read 1. For both metrics, lower percentages indicate higher quality run statistics.

Read and Index Quality Metrics

Read and index quality metrics appear under Quality Information on the Sequencing screen. These metrics appear for a Forensic Genomics run only and indicate the status of each read and overall quality.

Reads in a Run

A sequencing run completes up to four reads. Read 1 and Read 2 sequence the DNA template strands, and the Index 1 Read and Index 2 Read sequence the index adapters.

- **Read 1**—Read 1 sequencing primer is annealed to the template strand during cluster generation. RTA evaluates the first 50 cycles for quality.
- **Index 1 Read**—The Read 1 product is removed and the Index 1 sequencing primer is annealed to the same template strand as in Read 1. After index read preparation, the Index 1 Read is performed. RTA evaluates all eight cycles for quality.
- **Index 2 Read**—The Index 1 Read product is removed and the template anneals to the P5 primer grafted to the flow cell surface. The run proceeds through seven chemistry-only cycles without any imaging, followed by eight cycles of sequencing. RTA evaluates all eight cycles for quality.
- **Read 2**—The Index 2 Read product is extended to copy the original template strand. The original template strand is then removed and the Read 2 sequencing primer is annealed.

Number of Cycles in a Read

For Forensic Genomics runs, the number of cycles performed in a read is one more cycle than the number of cycles analyzed. Phasing and prephasing calculations require the extra cycle.

For example: A 350-cycle, paired-end run with 30 extra cycles performs one 351-cycle read and one 31-cycle read for a total of 382 cycles. At the end of the run, the software analyzes 380 cycles.

The number of cycles in a Research Use Only run depends on the reagent kit.

Template Generation

Template generation is a process that uses X and Y coordinates to define cluster positions on the flow cell surface. After defining a template of cluster positions, the software aligns the images produced over subsequent imaging cycles against the template. Individual cluster intensities in the four nucleotide color channels are extracted and base calls are produced from the normalized cluster intensities.

The number of template generation cycles depends on the run mode and reagent kit:

- For Forensic Genomics runs and Research Use Only runs using v3 kits, UAS uses the first seven cycles for template generation.
- For Research Use Only runs using v2 kits, UAS uses the first four cycles for template generation.

Flow Cell Status

Under Flow Cell, a graphic depicts the flow cell temperature and imaging status:

- Blue indicates cooler temperatures.
- Orange and red indicate warmer temperatures.
- Dark gray indicates a tile that has been imaged.

Quality Score

A quality score (Q-score) predicts the probability of an incorrect base call. The following table shows the probability of an incorrect base call for each Q-score.

Q-Score	Error Probability
Q40	1 in 10,000
Q30	1 in 1000
Q20	1 in 100
Q10	1 in 10

The software calculates Q-scores after cycle 25. On the Sequencing screen of a Research Use Only run a graph under Q-Score All Cycles displays the average percentage of bases greater than Q30. Q30 is a Q-score measurement.

Estimated Yield

The estimated yield metric shows the projected number of bases called for a run in megabases (Mb). These data appear after cycle 25 of a Research Use Only run.

Index

T

U

V

W

Technical Support

For technical assistance, contact Verogen Technical Support.

	Contact Information
Address	11111 Flintkote Avenue San Diego CA 92121 USA
Website	www.verogen.com
Email	techsupport@verogen.com
Telephone	+1.833.837.6436 toll-free (North America) +1.858.285.4101 tel +44 (0) 208 054 8706 (United Kingdom)

Safety data sheets (SDS)–Available for download from verogen.com/documentation.

Product documentation–Available for download from verogen.com/documentation.

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Verogen

+1.833.837.6436 toll-free
(North America)

+1.858.285.4101
(outside North America)

techsupport@verogen.com

www.verogen.com