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C-SOP-601: Operation of the Illumina MiSeq for Whole Genome Sequencing (WGS)

Mihir Kekre[1]

[1]The Centre for Genomic Pathogen Surveillance, Oxford, United Kingdom

1 *Works for me* This protocol is published without a DOI.

Mihir Kekre
Centre for Genomic Pathogen Surveillance, Oxford Big Data In...

ABSTRACT

The Illumina® MiSeq™ system combines proven sequencing by synthesis (SBS) technology with a revolutionary workflow that lets you go from DNA to analysed data in as few as 48 hours. The MiSeq integrates cluster generation, sequencing, and data analysis on a single instrument.

This method describes the operation and maintenance of a MiSeq instrument used to perform whole-genome sequencing on human and non-human (bacterial, viral etc.) short-fragment libraries and the preliminary run performance analysis of the data generated as a result.

This protocol has been adapted from the technology and methods developed by Illumina Inc.

PROTOCOL CITATION

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Sequencing, MiSeq, WGS, Whole genome sequencing, bacterial sequencing, pathogen WGS, illumina sequencing, short read , SBS, sequencing by synthesis

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GUIDELINES

Storage:

1. MiSeq Reagent Kit:

Box 1 of 2:

Store at -15°C to -25°C, away from light

- MiSeq Cartridge
- Hybridisation Buffer (HT1)

Box 2 of 2:

Store at 2-8°C

- Incorporation Buffer (PR2)
- Flow Cell

2. Remaining materials:

Refer to manufacturer's instructions.

MATERIALS TEXT

1. Quantified (and/or pooled) double-stranded, amplified and indexed DNA libraries

Equipment:
2. Illumina MiSeq

3. Single- and multi-channel pipettes (P10, P200, P1000) with compatible, sterile filtered tips.

4. Heat block

5. Ice trough

6. Tabletop tube spinner (Sigma-Aldrich MyFuge, combi-rotor, Cat no. Z681725 or equivalent)

7. UPS battery backup (for the MiSeq instrument)

Labware and Reagents:
8. Ethanol, 100%, Molecular Biology Grade

9. Sodium Hydroxide, 1.0 N

10. Nuclease-free water

11. Sodium Hypochlorite, 4.99%

12. Tween 20

13. MiSeq reagent kit (any one of the following):
Miseq v2 Nano Sequencing Reagent Kit, 300 cycles (Illumina, Inc., Cat no. MS-103-1001)
Miseq v2 Nano Sequencing Reagent Kit, 500 cycles (Illumina, Inc., Cat no. MS-103-1003)
Miseq v2 Micro Sequencing Reagent Kit, 300 cycles (Illumina, Inc., Cat no. MS-103-1002)
Miseq v2 Sequencing Reagent Kit, 300 cycles (Illumina, Inc., Cat no. MS-102-2002)
MiSeq v2 Sequencing Reagents, 500 cycles (Illumina, Inc., Cat no. MS-102-2003)
MiSeq v3 Sequencing Reagents, 600 cycles (Illumina, Inc., Cat no. MS-102-3003)

14. MiSeq Wash Tray

15. Lint-free wipes (ThermoFisher Scientific, Cat no. 06-666 or equivalent)

16. Lens Paper (ThermoFisher Scientific, Cat no. 11-996 or equivalent)

17. MiSeq Disposable Wash Tubes for template line wash (Illumina Inc., Cat no. MS-102-9999)

18. Serological pipets (10ml, 50ml) and pipet-buoy

19. Illumina PhiX control (Illumina Inc., Cat no. FC-110-3001)

20. Hybridisation Buffer, HT1 (Illumina Inc., part of the MiSeq reagent kit)

21. Tris-HCl (ph 7.0)

22. 0.1M TE (low-TE)

23. 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20

24. Quantified, size-selected and amplified dsDNA libraries (tagged with Illumina-compatible adaptors and indexes)

25. 2.0ml microcentrifuge tubes

26. Tweezers, square-tip, plastic (McMaster-Carr, Cat no. 7003A22)

SAFETY WARNINGS

Biosafety warning:

This document describes handling of DNA and associated products, and does not describe best practices for handling of biological infectious material. Handling biological material should be carried out in accordance with local health and safety standards. Handling and discarding used reagents such as chemical waste should be in accordance with applicable regional, national, and local laws and regulations.

Chemical Safety Warning:
The MiSeq Reagent Cartridges contain formamide (GHS classification Category 1B for reproductive toxicity), an aliphatic amide that is a potential reproductive toxin. Personal injury can occur through inhalation, ingestions, skin and eye contact. See the Illumina kit SDS for additional information and take proper precautions when handling the cartridges and MiSeq waste. Ensure spent containers and unused contents are disposed of in accordance with local governmental safety standards.

- Ethanol is flammable (GHS Flammability Category 2); take precautions when handling, storing and disposing of ethanol in the laboratory.
- Sodium hydroxide is corrosive (GHS Category 1A and 1, GHS Category 3 for acute hazards to aquatic environment); take precautions when handling, storing and disposing of sodium hydroxide in the laboratory.

Waste generated from a sequencing run on the MiSeq 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. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

DISCLAIMER:

> This protocol has been adapted from the technology and methods developed by Illumina Inc.

ABSTRACT

The Illumina® MiSeq™ system combines proven sequencing by synthesis (SBS) technology with a revolutionary workflow that lets you go from DNA to analysed data in as few as 48 hours. The MiSeq integrates cluster generation, sequencing, and data analysis on a single instrument.

This method describes the operation and maintenance of a MiSeq instrument used to perform whole-genome sequencing on human and non-human (bacterial, viral etc.) short-fragment libraries and the preliminary run performance analysis of the data generated as a result.

This protocol has been adapted from the technology and methods developed by Illumina Inc.

Before Starting

1 Prior to initiating the protocol, ensure that all active workbenches are cleaned with 80% ethanol, all relevant personal protective clothing is worn and the work area is prepared according to local GLP guidelines for molecular methods.

Create an organised bench space by clearing away all clutter in order to maximize work efficiency and to avoid unnecessary movements that will minimise exposure of sterile materials to airborne and liquid contaminants.

Prepare a large bucket of ice to store reagents and samples temporarily during use.

2 An overview of the MiSeq instrument, software and reagent kit components can be found on pg. 3-11 of the MiSeq Systems Guide.

3 Prepare a fresh aliquot of **sodium hydroxide (NaOH)** by combining the reagents below:

- **200 µl** of 1.0 N NaOH
- **800 µl** of Nuclease-free water

The result is a 1 mL of 0.2N NaOH.

Use the fresh dilution within 8 hours of preparation.

4 ⚠

Remove the **HT1 buffer** from the deep freezer (**-20 °C**) storage and thaw it at room temperature.

Store at **4 °C** until ready for use.

Buffer HT1 has a high salt concentration and can take long periods to thaw out completely. Defrost this reagent sufficiently ahead of the run.

5 **For best results, step 5 and its sub-steps should be performed 2 hours prior to sample loading and run set up.**

Allow adequate time for thawing of the reagent cartridge. Table 1 details the conditions needed to ensure optimal defrosting.

A	B	C
Method of thawing	**Thawing time**	**Shelf-life of thawed cartridge (at 4ºC or on ice)**
Ambient temperature water bath (opened)	1.5 hours	up to 24 hours
2-8ºC (sealed)	12 hours	up to 4 days

Table 1.
Note: A cartridge should not be refrozen after the first and only thaw cycle.

5.1 Remove the reagent cartridge from the **-20 °C** storage.

5.2 ⚠

Place the reagent cartridge in a water bath containing enough room temperature deionized/MilliQ water to submerge the base of the cartridge.

Do NOT allow the water to exceed the maximum water line printed on the cartridge (as shown below).

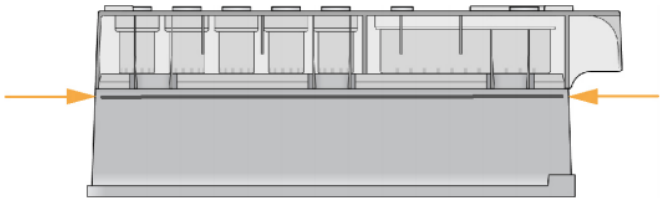

Figure 1.

5.3 Allow the reagent cartridge to completely thaw in the room temperature water bath. 2h 30m

- MiSeq v3 cartridges— ~ **01:30:00** .
- MiSeq v2 cartridges— ~ **01:00:00** .

5.4 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.

5.5

Invert the reagent cartridge ten times to mix the thawed reagents, and then inspect that all positions are thawed.

5.6

Inspect the reagents in positions 1, 2, and 4 to make sure that they are fully mixed and free of precipitates or icy blocks.

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

> The MiSeq's 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.

5.8 Set aside the thawed reagent cartridge at **4 °C** until the run is ready for setup.

Instrument Initialisation

6 Power on the instrument using the power switch located on the back of the instrument.

7

If the MiSeq is networked, enter your Illumina BaseSpace account username and password. If you do not have an account, sign up to register for one here.

8 After the MiSeq computer has booted up, the MiSeq Control Software (MCS) will load automatically. Wait for the machine to complete initialisation.

Library Denaturation and Dilution (Standard Method) 1m 10s

9 Follow the steps most appropriate for your library and the version of MiSeq reagent kit that is in use (as detailed in Table 2)

For the NexteraTM DNA Flex Library Prep Kit, see dilute and denature directions in the Nextera DNA Flex Library Prep Reference Guide (document # 1000000025416).

Chemistry	Compatible Denature and Dilute Steps
MiSeq Reagent Kit v3	**4 nM library**—Results in a 6–20 pM loading concentration.
MiSeq Reagent Kit v2	**4 nM library**—Results in a 6–20 pM loading concentration. **2 nM library**—Results in a 6–10 pM loading concentration.

Table 2.

Step 9 includes a Step case.
Denature and Dilute 4 nM Library
Denature and Dilute 2 nM Library

step case

Denature and Dilute 4 nM Library

10 Combine the following reagents in a microcentrifuge tube:
- **5 µl** of the 4nM library sample or pool.
- **5 µl** of 0.2N NaOH

11 1m 10s

Vortex for **00:00:10** and centrifuge in a tabletop tube spinner for **00:01:00** .

12 5m

Incubate on the bench at **Room temperature** for **00:05:00** .

13 Add **990 µl** of **chilled HT1 buffer** to the tube containing the denatured library sample.

The resulting solution will now be **1 mL** of a **20pM denatured library**.

14 Dilute the 20pM library to the desired concentration using the volumes in Table 3a below:

Concentration	6 pM	8 pM	10 pM	12 pM	15 pM	20 pM
20 pM library	180 µl	240 µl	300 µl	360 µl	450 µl	600 µl
Prechilled HT1	420 µl	360 µl	300 µl	240 µl	150 µl	0 µl

Table 3a.

Invert several times to mix and pulse centrifuge the solution.

This is now your library **loading concentration**.

For bacterial WGS at 300-cycle chemistry, we recommend diluting the denatured 20pM library down to either **10pM** depending on the cluster metrics required.

PhiX (Control) Denaturation and Dilution 1m 10s

15 To spike PhiX as a sequencing performance control into the run, use Table 4 and the subsequent steps to dilute and denature the PhiX stock.

Chemistry	Final PhiX Concentration
MiSeq Reagent Kit v3	Dilute the denatured PhiX control to 20 pM, which produces an optimal cluster density using v3 reagents.
MiSeq Reagent Kit v2	Dilute the denatured PhiX control to 12.5 pM, which produces an optimal cluster density using v2 reagents.

Table 4.

16

Combine the following reagents in a microcentrifuge tube:

- **2 µl** of the 10nM PhiX library.
- **3 µl** of 10 mM Tris-Cl, pH 8.5 with 0.1% Tween 20

Always prepare a fresh dilution of PhiX and use within 12 hours.

The resulting solution is a **4nM PhiX library**.

17

Combine the following reagents in a microcentrifuge tube:

- **5 µl** of the 4nM PhiX library.
- **5 µl** of 0.2N NaOH

18 Vortex for **00:00:10** and centrifuge in a tabletop tube spinner for **00:01:00** . 1m 10s

19 Incubate on the bench at **Room temperature** for **00:05:00** . 5m

20 Add **990 µl** of **chilled HT1 buffer** to the tube containing the denatured library sample.

The resulting solution will now be **1 mL** of a **20pM denatured PhiX library**.

Invert several times to mix.

21 If using a **MiSeq Reagent v3 kit**, **no further dilution is required.**
If using a MiSeq Reagent v2,

Combine the following in a 2.0 mL microcentrifuge tube:

- **375 µl** of the 20pM denatured PhiX library.
- **225 µl** of chilled buffer HT1.

The resulting solution is **600 µl** of a **12.5pM denatured PhiX library**.

Invert several times to mix.

22 For most libraries, a low concentration of **PhiX control spike-in of 1%** is adequate as a sequencing control. For low diversity libraries, increase the PhiX control spike-in to at least 5%.

Combine the following volumes from Table 5 to obtain your final loading spike-in of PhiX for the run.

	Most Libraries (1% Spike-In)	Low-Diversity Libraries (≥ 5% Spike-In)
Denatured and diluted PhiX	6 µl	30 µl
Denatured and diluted library (from protocol A, B, C, or D)	594 µl	570 µl

Table 5.

Set aside on ice until it is ready to load into the reagent cartridge.

Setting Up a Run on the MiSeq

23 Prior to setting up the run, ensure that there is enough free disk space (**100 GB**) on the instrument.

If there is less than 100 GB is available, check the *Instrument Maintenance* section of the MiSeq Systems Guide.

23.1 **BaseSpace Users:** It is recommended to maintain a duplicate copy of the run folder locally on the instrument as a backup. Doing the following will ensure that a run folder will be generated in the Data drive file folders, in addition to being streamed to BaseSpace. Follow the procedure below:

23.2 From the MCS home screen, select "**Run Options**."

23.3 Under the "**Run Settings**" tab, check the "When using BaseSpace, replicate analysis locally on MiSeq" checkbox.

23.4 Select "**Save and Return**" to save these changes and return to the home screen.

24 Ensure that the Sample Sheet (and Sample Plate, if applicable) setup has been completed using **Illumina Experiment Manager (IEM)** on the instrument.

25 Open the MiSeq Control Software window, select "**Sequence**" and proceed immediately to Preparing and Loading the Flow Cell.

If using BaseSpace, on the following screen, check the box to ensure the user info fields are active and enter user

credentials to default data streaming directly to BaseSpace.

Preparing and Loading the Flow Cell

26 Retrieve the Flow Cell and Incorporation buffer (PR2) stored at **4 °C** .

27 Wearing clean powder-free gloves, and using plastic tweezers, grip the flow cell by the base of the plastic cartridge and remove it from the flow cell container (as shown below).

Figure 2.

28 ⚠

Lightly rinse the flow cell with laboratory-grade water until both the glass and plastic cartridge are **thoroughly rinsed** of excess salts.

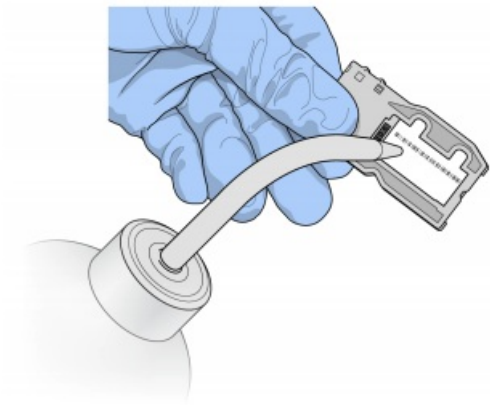

Figure 3.

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

29 ⚠

Thoroughly dry the flow cell and cartridge with a lint-free lens cleaning tissue. Gently pat dry in the area of the back port gasket and adjacent glass.

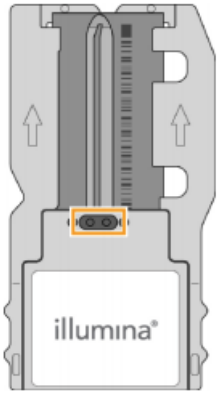

Figure 4.

Wet a clean piece of lens paper with ethanol and clean the glass portion of the flowcell. The glass must be free of streaks, lint, and tissue fibres. An alcohol wipe can also be used for this step.

Do not add ethanol directly to the flow cell, and avoid getting ethanol (or wipes) on the port gasket.

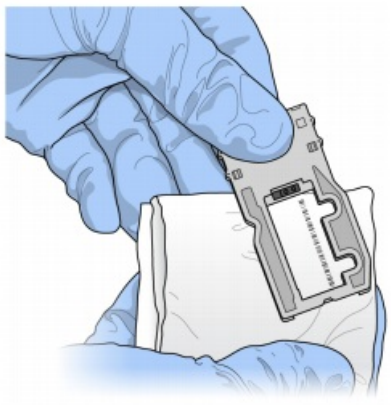

Figure 5.

30 ⚠

Dry excess alcohol with a lint-free lens cleaning tissue.

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.

31 Raise the **flow cell compartment door**, and then press the **release button** to the right of the flow cell clamp (as shown below).

The flow cell clamp opens.

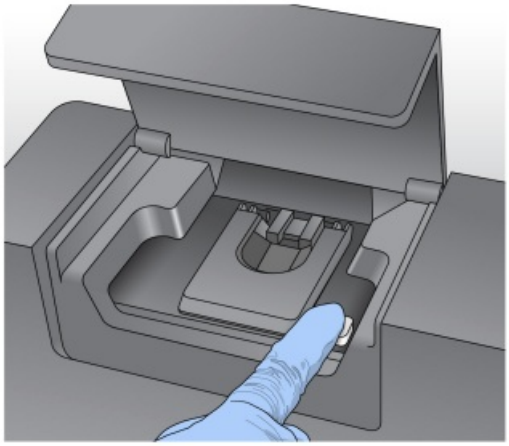

Figure 6.

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

32 Holding the flow cell by the edges, place it on the flow cell stage (as shown below).

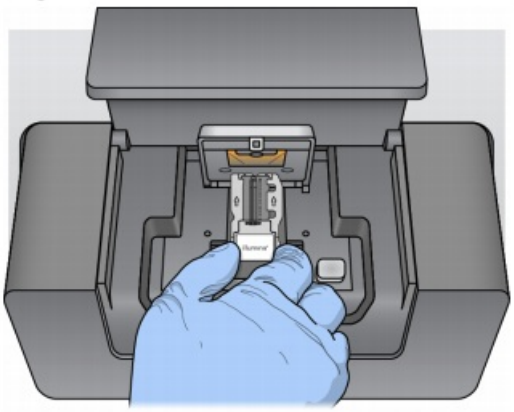

Figure 7.

33 Gently press down on the flow cell clamp to close it over the flow cell.

As the flow cell clamp closes, alignment pins position the flow cell. An audible click indicates that the flow cell clamp is secure.

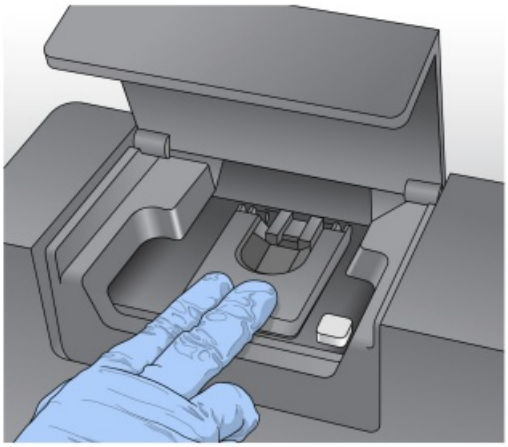

Figure 8.

34 The software will now identify and indicate the Flow Cell's RFID tag to confirm successful placement.

Close the Flow Cell compartment door and hit **Next**.

If the software does not identify the RFID, refer to pg. 44 of the MiSeq System Guide.

Incorporation Buffer (PR2) and Waste Bottle Check

35 ⚠

Open the reagent compartment door and raise the sipper handle until it locks into place.

36 Remove the bottle of PR2 from **4 °C** storage. Invert several times to mix, and then remove the lid.

37 Remove the wash bottle and load the PR2 bottle.

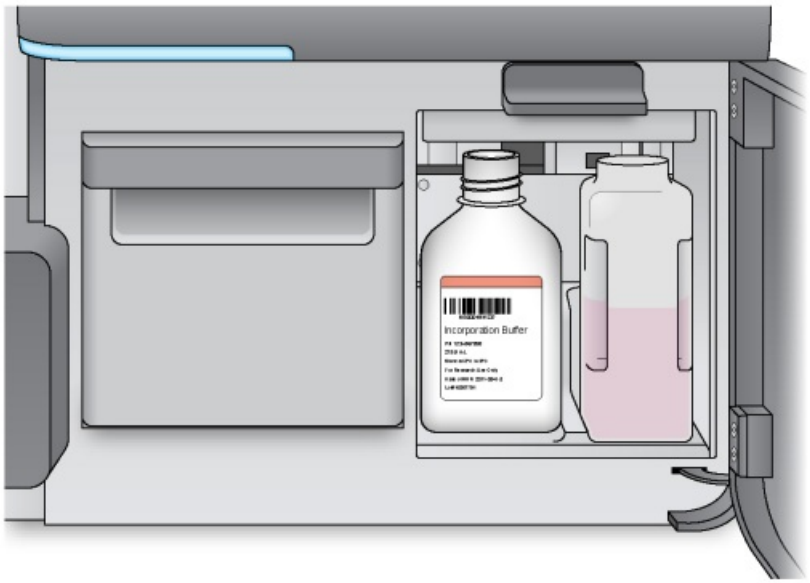

Figure 9.

38 The software will now identify and indicate the RFID tag of the PR2 bottle to confirm successful placement.

Close the reagent compartment door and hit **Next**.

If the software does not identify the RFID, refer to pg. 44 of the MiSeq System Guide.

39 Empty the contents of the waste bottle into the appropriate waste container.

Reload the empty bottle back into its appropriate slot in the compartment.

40 Slowly lower the sipper handle ensuring that the sippers lower into the PR2 and waste bottles.

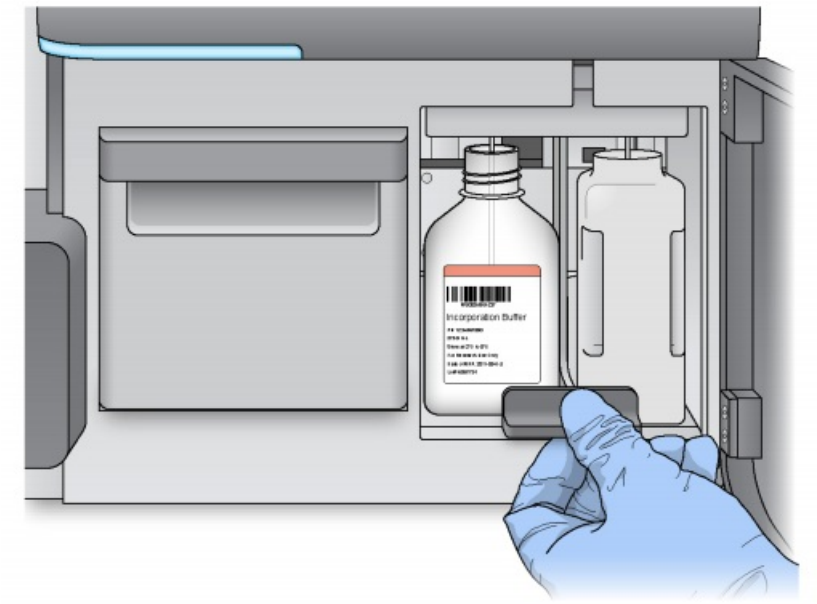

Figure 10.

Loading Sample Library onto the Cartridge

41 Remove the thawed reagent cartridge from 4°C storage (or water bath), ensuring that it has been thoroughly dried and the reagents mixed by gentle inversion.

42 Tap the reagent cartridge on a flat hard surface to gather the contents at the bottom of the reservoirs.

43 Using a clean 1000 µl pipette tip, pierce the foil seal over the reservoir labeled "**Load Sample.**"

44 ⚠

Pipette **600 µl** of the **denatured DNA library** into the "Load Sample" reservoir (as shown below).

Tap gently on a hard surface to gather all the contents to the bottom.

It is recommended that the sample be dispensed toward the bottom end of the reservoir using an extended 1000 µl pipette tip to reduce the risk of droplets forming on the sides.

Avoid depositing droplets on the foil seal while the sample is loaded.

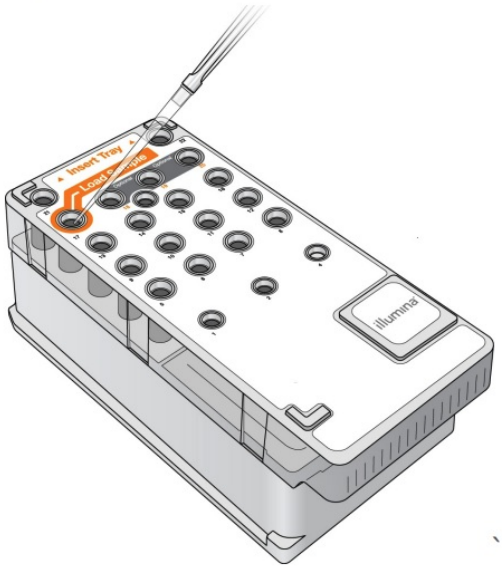

Figure 11.

Loading the Reagent Cartridge onto the Instrument

45 On the instrument screen, confirm it says **"Load Reagent Cartridge".**

This will ensure the sippers in the chiller have been raised to **avoid snapping them** when removing the wash tray.

46 Open the reagent chiller door, remove the wash tray, and dry bottom of chiller compartment if necessary using absorbent wipes.

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

47 Hold the reagent cartridge on the end with the Illumina label, and slide the reagent cartridge into the reagent chiller until the cartridge stops (as shown below).

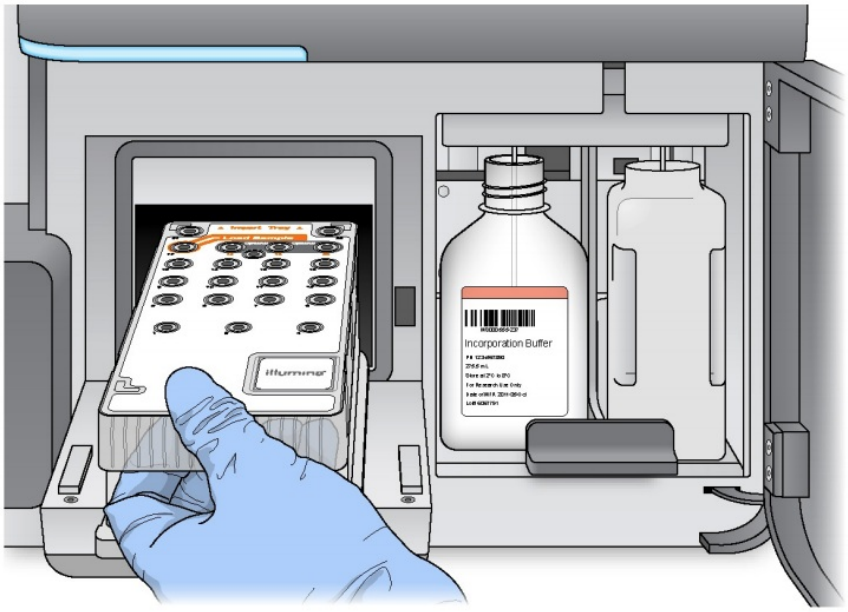

Figure 12.

Always use the reagent cartridge associated with the type of flow cell that you loaded. If the reagent cartridge is not compatible, a message appears on the screen. Select **Back** to load the appropriate reagent cartridge or **Home** to return to the Home screen.

48 Close the chiller door and confirm that the RFID of the reagent cartridge has been read successfully.

If the software does not identify the RFID, refer to pg. 44 of the MiSeq System Guide.

If the reagent cartridge is not compatible with the flow cell, a message appears. Select **Back** to load a compatible cartridge, or select **Exit** to return to the Home screen.

Also close the reagent compartment door.

Setting Up the Sample Sheet

49 Every run must have a sample sheet. By default, the software looks for a sample sheet file with a name matching the barcode number of the reagent cartridge loaded on the instrument. If a sample sheet is not found, a message appears that prompts you to browse to the location of the correct sample sheet for your run.

On the subsequent screen, select **Change Sample Sheet** and then select **Browse** to navigate to the appropriate sample sheet.

i. Use the Change Sample Sheet command for the following:
- To select a sample sheet with a name that does not match the reagent cartridge barcode number
- When the software prompts you to choose a different sample sheet on the Review screen

ii. To prevent the software from searching unsuccessfully, use the Change Sample Sheet command on the Load Reagents screen to direct the software to the appropriate sample sheet.

50 Select the desired sample sheet and click **OK**.

Hit **Save and Continue**, and then **Next**. Your sample sheet is now successfully loaded.

Refer to the MiSeq Sample Sheet: Quick Reference Guide for details on sheet design.

Starting the Run

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

52 **Review Run Parameters:**

1. Review Experiment Name, Analysis Workflow, and Read Length. These parameters are specified in the sample sheet.

If IDT Illumina Unique Dual Indexes are used, the Index Reads need to be changed to 10 cycles (instead of 8 cycles).

2. Select Change Folder to review the folder locations.

To change folder locations, select Change Folder and browse to a preferred location. Using this option from the Review screen changes folder locations for the current run only.

3. Modify 1. and 2. as needed and then select **Save,** and then **Next**.

53 **Review Pre-Run Check:**

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

When all items successfully pass the pre-run check, select **Start 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, refer to pg. 44 of the MiSeq System Guide.

Once the run has started, do NOT open the flow cell or reagent compartment doors, neither should the instrument monitor be engaged unless the run is to be stopped or paused.

Image capture on the MiSeq is sensitive to vibration. Performing tasks that cause vibration near/on the instrument during a run could cause the run to halt or adversely impact sequencing results.

Post-Run Wash and Reagent Disposal 20m

50m

54 ⚠

The **post-run wash** is the standard instrument wash performed between sequencing runs. **Always perform an instrument wash after completing a 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 crystallisation in the fluidics lines and sippers

- Prevents cross-contamination from the previous run

Follow the on-screen prompts to load the wash components and perform the wash.

The post-run wash takes approximately **00:20:00** .

If you are using MCS v2.5 or later, you have the option to perform a post-run wash that includes a **template line wash** with sodium hypochlorite solution (NaOCl). The wash takes approximately **00:30:00** .

Maintenance and Standby washes are detailed on pg. 34 and 36 of the MiSeq System Guide.

Start the wash directly following the completion of a run.

An instrument wash is required before you can set up a subsequent run.

To perform a post-run wash at a time other than directly following a run, use the command on the Perform Wash screen to initiate the wash.

Leave the used flow cell (from a previous run) on the instrument. It is mandatory that a flow cell be loaded on the instrument when performing an instrument wash.

55 While the MiSeq run is underway:

1. Prepare a fresh wash solution of Tween 20 with nuclease-free water:

a. Add **5 mL** 100% Tween 20 to **45 mL** nuclease-free water. This results in a **10% Tween 20**.

b. Add **25 mL** 10% Tween 20 to **475 mL** nuclease-free water. This results in a **0.5% Tween 20 wash solution**.

c. Invert five times to mix.

2. Prepare fresh sodium hypochlorite (NaOCl) wash solution with nuclease-free water:

a. Add **36 µl** of 5% NaOCl to **864 µl** nuclease-free water. This results in a 1:25 NaOCl dilution.

b. Add **50 µl** of the 1:25 NaOCl dilution to **950 µl** of nuclease-free water in a MiSeq Disposable Wash Tube.

3. Prepare the wash components with fresh wash solution:

a. Add **6 mL** wash solution to each reservoir of the wash tray.

b. Add **350 mL** wash solution to the 500 ml wash bottle.

Using the correct concentration of NaOCl is important. Make sure to check the percentage of NaOCl on the product label. If the concentration is too high, it can make cluster generation fail in subsequent runs.

If 5% NaOCl is not available, make a 1 ml solution of 0.01% NaOCl in laboratory-grade water. Do not use NaOCl with a maintenance wash or a standby wash

56 Insert the MiSeq Wash Tube containing 0.01% NaOCl wash solution into **position 17** of the MiSeq wash tray.

The tube displaces the Tween 20 and laboratory-grade water wash solution from position 17.

Figure 13.

Ensure that the neck of the tube is flush with the tray.

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

57 Once the run is complete, select the "**Start Wash**" option at the bottom right of the screen. The software automatically raises the sippers in the reagent chiller.

Tick the "**Perform Optional Template Line Wash**" checkbox to proceed with a Post-Run bleach wash. Bleach, or sodium hypochlorite is used to reduce carry-over of nucleic acid from previous runs.

If Perform optional template line wash is not selected for the post-run wash, a message on the Run Review screen reminds you the next time you start a sequencing run.

58 Open the reagent compartment door and reagent chiller door, and remove the used reagent cartridge from the chiller.

59 Slide in the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.

60 Raise the sipper handle in front of the PR2 bottle and waste bottle until it locks into place.

Remove the PR2 bottle and replace it with the wash bottle.

Discard the PR2 bottle after each run. Do not reuse any remaining PR2 buffer.

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

62 Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.

63 Close the reagent compartment door.

Select **Next.**

When the wash is complete:
- Leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.
- The sippers remain in the down position. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Monitoring Run Performance

64 During and after the run, monitor run progress, intensities, and quality scores that appear on the Sequencing screen.

Alternatively, if you are connected to BaseSpace, the run can be monitored real-time using SAV in BaseSpace or using the BaseSpace Hub on mobile.
- **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 Sequencing screen is view-only. To monitor the run in greater detail, use the Sequencing Analysis Viewer (SAV) installed on a computer independent of the instrument computer. A network connection is required.

For more details, refer to the Sequence Analysis Viewer (SAV) Software User Guide (15020619) and pg. 26-28 of the MiSeq System Guide.

It may take up to 1 hour for the MiSeq to generate *fastq* files post-run as the Real-Time Analysis Software lags behind the sequencing run.

If *fastq* data is unavailable for a given sample(s) due to incorrect index assignment or sample sheet errors, the run can be re-queued for analysis using either the MiSeq Reporter (MSR) or BaseSpace Re-Queue tutorial

Exporting Data from the MiSeq

65 Data is found on the following path:
D:\ → Illumina → MiSeq Output → Run folder (choose the most recent run folder) → Data → Intensities → BaseCalls.

Within the BaseCalls folder, the raw read data is in a ***fastq.gz* format**.

There will be 2 files per isolate for paired reads (R1 and R2). This data can be copied onto an external hard drive and moved to a computer workstation.

Alternatively, the MiSeq instrument can also be connected to a local network share drive to directly transfer files from the instrument.

This section is for users unable to stream to BaseSpace or in need of a secondary data repository (i.e. local server) for local data analysis or storage

Instrument Shutdown & Reboot 10m

66 The instrument should be rebooted **weekly** and after a file deletion to free any virtual memory. 10m

On the MCS home screen, select **Manage Instrument** then push "**Reboot**". It will take approximately **00:10:00** for the system to reboot and restart the MiSeq Control software.

67 Alternatively, the instrument can be power cycled by selecting **Manage Instrument** on the home screen and then "**Shut Down**". 10m

Once the instrument has shut down (noted by a distinct click sound), reach behind the right-hand side of the instrument and flip the power switch located near the power cord.

Let the instrument power down for at least **00:10:00** and then flip the switch back on. The instrument will boot up the MCS as normal.

Instrument Maintenance

68 The following maintenance protocols are to be carried out at the recommended intervals.

A	B	C	D	E
Monthly Wash Schedule Scenarios				
	Week 1	**Week 2**	**Week 3**	**Week 4**
Example 1	PTL (post-run) M (end of week)	PTL (post-run)	PTL (post-run)	PTL (post-run)
Example 2	P (idle, no run)	PTL (post-run) W	P (idle, no run)	M, W (end of week)
Example 3	P (idle, no run)	P (idle, no run)	P (idle, no run) PTL (post-run)	M, W (end of week)
Example 4	P (idle, no run)	PTL (post-run)	P (idle, no run)	PTL (post-run)
Example 5	P (idle, no run)	PTL (post-run)	M, W (end of week)	P (idle, no run) PTL (post-run)

Table 6. Options for monthly wash schedules, by week, and by varying combination of run schedules. Maintenance wash (M), Post-Run wash w/ Tween 20 (P), Post-Run template-line wash w/ bleach (PTL), Water wash (W)

Protocols for the **post-run wash, post-run (w/ template line) bleach wash, maintenance wash and standby wash** are detailed in the MiSeq System Guide.

Additional Information and Troubleshooting

69 Illumina MiSeq Workflow Overview

MiSeq System Denature and Dilute Libraries Guide (15039740)

MiSeq System Guide (1000000061014)
"Troubleshooting Appendix" - pg. 41 onwards

MiSeq Sample Sheet: Quick Reference Guide (15028392)
"Creating a New MiSeq Sample Sheet" (pg. 17-18)

MiSeq Performance Specifications

Sequence Analysis Viewer (SAV) Software User Guide (15020619)

MiSeq Reporter (MSR) Software Guide (15042295)
"Re-queuing a Run for Analysis" (pg. 19-20)

Illumina Experiment Manager (IEM) Software Guide (15031335)

"The five Quality metrics every NGS user should know", Perkin-Elmer Horizon Discovery.