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Procedure for Operation and Maintenance of the Illumina MiSeq for Whole Genome Sequencing

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1 Works for me dx.doi.org/10.17504/protocols.io.bja6kihe

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ABSTRACT

This procedure outlines the procedures for operation and maintenance of an Illumina MiSeq Sequencer for Whole Genoem Sequencing.

This document applies to all laboratory personnel in the Division of Microbiology (DM) as well as laboratories in the GenomeTrakr Network.

Complete in order:

1. DNA Extraction (Manual DNA Extraction *or* Automated DNA Extraction using the Qiacube)
- Step-by-step procedures to obtain high quality DNA from isolates in TSB for whole genome sequencing

2. DNA Quantitation
- Quantitation of extracted DNA using the Qubit Flourometer

3. Library Preparation for WGS (Library Preparation using Illumina DNA Prep *or* Library Preparation using Illumina Nextera XT)
- Library preparation using NexteraXT *or* Illumina DNA Prep (previously Nextera DNA Flex)

4. Sequencing using Illumina MiSeq (Included SOP)

5. Data Quality Checks and NCBI Submission

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GUIDELINES

Sequencing Reagent Kits contain 2 components:

Box 1 of 2 (Store at -15^{O}C to -25^{O}C, away from light)

- MiSeq Cartridge
- Hybridization Buffer (HT1)

Box 2 of 2 (Store at 2 to 8^{O}C)

- Incorporation Buffer (PR2)
- Flow Cell

Abbreviations:

- **CD**: Cluster Density
- **DM**: Division of Microbiology
- **gDNA**: Genomic DNA
- **GT**: GenomeTrakr
- **HT1**: Hybridization Buffer
- **LRM**: Local Run Manager
- **MCS**: MiSeq Control Software
- **MRS**: MiSeq Reporter Software
- **NaOH**: Sodium Hydroxide
- **PI:** Principal Investigator
- **PR2**: Incorporation Buffer
- **QC**: Quality Control
- **RFID**: Radio-Frequency Identification
- **SAV**: Sequencing Analysis Viewer
- **UPS:** Uninterrupted Power Supply
- **WGS:** Whole Genome Sequencing

MATERIALS

NAME	CATALOG #	VENDOR
Ethanol (100%, Molecular Biology Grade)	BP2818500	Fisher Scientific
Sodium Hydroxide 1N	S2770-100ml	Sigma Aldrich
Ethanol lab-grade 70%	BP82031GA	Fisher Scientific
Water molecular-grade	BP24701	Fisher Scientific
Sodium Hypochlorite 4-4.99%	239305-25ML	Sigma Aldrich
Tween 20	P9416-50ML	Sigma Aldrich
Miseq v2 Micro Sequencing Reagent Kit (300 cycles)	MS-103-1002	Illumina, Inc.
MiSeq v2 Sequencing Reagents (500 cycles)	MS-102-2003	Illumina, Inc.
MiSeq v3 Sequencing Reagents (600 cycles)	MS-102-3003	Illumina, Inc.

MATERIALS TEXT

Supplies:

- Illumina MiSeq Wash Tray
- Lint-free wipes (Fisher Scientific, Cat# 06-666 or equivalent)
- Lens Paper (Fisher Scientific, Cat# 11-996 or equivalent)
- MiSeq Disposable Wash Tubes for template line wash (Illumina, Cat# MS-102-9999)
- Serological pipets (10ml, 50ml)
- Sterile pipette tips, filtered (various sizes)

Equipment:

- MiSeq Desktop Sequencer
- Pipettes
- Heat Block
- Ice Bucket
- Pipet-aid

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- Microcentrifuge
- Microplate centrifuge or equivalent
- UPS battery backup

SAFETY WARNINGS

Chemical Safety Warning: Take proper precautions, and wear appropriate PPE when handling potentially hazardous chemicals. Ensure that chemicals, spent containers, and unused contents are disposed of in accordance with governmental safety standards.

- The MiSeq Reagent Cartridges contain formamide (GHS classification Category 1B for reproductive toxicity). Personal injury can occur through inhalation, ingestion, and/or skin and eye contact. Review the SDS for additional information and take proper precautions when handling and disposing of waste. Ensure waste is disposed of in accordance with governmental safety standards.
- Sodium Hydroxide (NaOH) is corrosive (GHS Category 1A and 1, GHS Category 3 for acute hazards to aquatic environment); take precaution when handling, storing and disposing of NaOH in the laboratory.

Starting the Illumina MiSeq Desktop Sequencer

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

2 If the MiSeq is networked, this is the point where the username and password would be entered.

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

Preparing the Reagent Cartridge for the MiSeq

4 Plan for sufficient time to allow complete thawing of the cartridge. The Cartridge and HT1 are contained in the same box and must be thawed on ice or at 2-8^{O}C.

See the table below for guidance regarding reagent kit thawing and storage:

Method of Thawing	Time Required to Thaw	Thawed Cartridge Storage Life (2-8°C)	A cartridge should not be re-frozen after thawing. It is recommended to use within 24 hours.
Room Temperature in water bath (opened)	~1 hour	24 hours	
2-8°C (unopened)	8-12 hours	3 days	

5 Once thawed, invert the reagent cartridge 10 times to mix, and then visually inspect to verify that all reagents are completely thawed and free of participates.

6 Gently tap the cartridge on the bench to dislodge air bubbles in the bottom of the wells, and wipe the cartridge off with a paper towel.

7 Place the thawed reagent cartridge and HT1 on ice or at 2-8^{O}C until the sample is ready to be loaded. (The cartridge contains photosensitive reagents and should be kept in the dark)

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Setting up the run on the MiSeq

8 Prior to setting up the run, ensure that there is enough free disk space (100GB) on the instrument. If there is less than 100GB available, proceed to *Instrument Mainentance* section to free up disk space.

9 From the MCS home screen, select **Sequence**

10 Select a run option:
- **Local Run Manager**: Select this option if the run has previously been set up in the LRM Software
- **Sample Sheet**: Select this option if a sample sheet has been generated for the run. The sample sheet will be sent to the LRM for validation and automatic run creation
- **Manual**: Create a run manually but selecting all parameters of the run. No secondary analysis will be performed if this option is selected.

11 Enter BaseSpace email and password if applicable (Note: DM does not use BaseSpace). Select **Next**.

12 Select the Local Run Manager run or select the sample sheet for the run depending on the option used in Step 10.

13 Proceed immediately to *Preparing and Loading the Flow Cell* section.

Preparing and Loading the Flow Cell

14 The flow cell and Incorporation buffer (PR2) are located in Box 2 of 2 and stored at 2-8^{o}C.

15 While wearing clean, powder-free gloves, carefully remove the flow cell from the container, by gripping the base of the plastic end.

16 Lightly rinse the flow cell with molecular-grade water, making sure to rinse both sides of the glass and plastic casing. It is very critical to ensure all excess salts are removed. If salts dry in the imaging area, imaging can be affected during the run.

17 Pat dry the flow cell using a lint-free wipe, using care around the black port gasket.

18 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 fibers. An alcohol wipe can also be used for this step.

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

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19 Dry any excess ethanol with the lens paper and visually inspect to make sure that the flow cell is free of smudges or debris and dry. Inspect the port gasket to ensure it 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.

20 Raise the flow cell compartment door and gently press the release button to open the clamp and remove the previously used flow cell.

21 Holding the flow cell by the edges, place it on the flow cell stage.

22 Gently press down on the flow cell clamp to close it over the flow cell (it will click when closed fully). Close the compartment door. The software should identify the flow cell RFID.

23 Select **Next**.

Loading the Incorporation Buffer

24 Gently invert the bottle of PR2 to mix, and then remove the lid.

25 Open the reagent compartment door.

26 Raise the sipper handle until it locks into place.

27 Remove the wash bottle and load the PR2 bottle. Re-cap the wash bottle and set aside for future use.

28 Empty the contents of the waste bottle into the appropriate chemical waste container.

29 Lower the sipper handle, make sure the sipper lowers into the PR2 and waste bottles.

30 The software will identify the RFID of the PR2 bottle.

Note: If the RFID is not read by the system for any step, the software prompts you through the steps to obtain a temporary bypass code and proceed with setting up the run. For more information, see “Resolving RFID Read Failure” in the MiSeq System User Guide.

31 Select **Next**.

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Loading the Sample into the Cartridge and Loading into the Instrument

32 Remove the thawed reagent cartridge, ensuring that it has been thoroughly dried and the reagents have been mixed by inversion.

33 Tap the reagent cartridge on a hard surface to collect contents at the bottom of the reservoirs.

34 Use a clean 1000 µl pipette tip to pierce the foil seal over the reservoir labeled **Load Sample**.

35 Transfer **600 µl** of the denatured DNA library into the **Load Sample** reservoir, take care to avoid droplets on the foil seal as sample is being transferred.

36 Check the bottom of the cartridge to ensure there are no bubbles present at the bottom of the reservoirs. If so, tap the cartridge gently on the tabletop to release the bubbles.

37 Make sure the MCS screen states **Load Reagent Cartridge** to ensure the sippers in the chiller have been raised.

38 Open the reagent chiller door, remove the wash tray, and dry bottom of chiller compartment if necessary. (Humidity fluctuations in the laboratory can cause condensation to pool in the chiller)

39 Hold the reagent cartridge by the end with the Illumina label and slide it into the chiller until it stops.

Note: Do not leave the chiller door open for extended periods of time

40 Close the chiller door and check the screen to confirm the software has identified the RFID of the reagent cartridge.

41 Close the reagent compartment door.

42 Select **Next**. Review run parameters and ensure that they are correct. Select **Next** to start the Pre-Run Check.

Note: The system performs a check of all run components, disk space, and network connections prior to a run start. If any part of the pre-run check fails, a message will appear with general instructions describing the error and details how to correct it. For more information, see “Resolving Run Setup Errors” in the MiSeq System User Guide. If all items successfully pass the pre-run check, the system is ready to start the run.

43 Select **Start Run**. Once the run has started, do not open the instrument or touch the instrument monitor unless to pause or stop the run.

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Note: The MiSeq is sensitive to vibration, performing tasks that cause vibration during a run could cause the run to fail or adversely impact sequencing results.

Post-Run Washes and Reagent Disposal

44 Post-Run washes are required at the conclusion of every run. It is recommended by FDA-GenomeTrakr to also perform a post-run wash (Tween only) at the beginning of each run.

45 Once the run is complete, select **Start Wash** option at the bottom of the MCS.

46 Check the box for **Perform optional template line wash** to proceed with a Postrun bleach wash. The purpose of the bleach wash is to reduce the rate of carryover of nucleic acids to proceeding runs.

47 Prepare fresh wash solution with Tween-20 and laboratory grade water:

47.1 **10 % Tween-20**: Add **5 µl** of Tween-20 to **45 µl** of molecular grade water.

47.2 **0.5% Tween-20 Wash**: Add **25 µl** of 10% Tween-20 to **475 µl** of molecular grade water.

47.3 Invert five times to mix

47.4 Preparing stock solutions of 10% and 0.5% tween are not advised, these stocks can grow mold and become contaminated.

48 Prepare a fresh dilution of molecular-grade sodium hypochlorite (NaOCl – Bleach), using molecular-grade water.

48.1 **0.2% NaOCl**: Add **36 µl** of 5% NaOCl to **864 µl** of molecular-grade water.

48.2 **0.01% NaOCl**: Add **50 µl** of the 0.2% NaOCl to **950 µl** of molecular-grade water.

48.3 Add the entire 1.0mL of the 0.01% NaOCl to the wash tube.

Note: Using the correct concentration of NaOCl is important. If the concentration is too high, it can

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cause cluster generation failures in subsequent runs. It is recommended to purchase the NaOCl from Sigma as listed in the reagents section. Use of Clorox is not advised due to the variability in concentration of NaOCl. Template line washes should not be performed with maintenance or standby washes.

49 Insert the MiSeq wash tube with the 0.01% NaOCl solution into position 17 of the wash tray until the neck of the tube is flush with the tray. The tube must be put into position 17 *only*. Inserting the tube in other positions can cause cluster generation failure and can damage the fluidic system.

50 Add at least **6 µl** of the 0.5% Tween-20 Wash solution in to all other reservoirs of the wash tray.

51 Add **350 µl** of 0.5% Tween-20 Wash solution to the 500 ml wash bottle.

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

53 Slide the wash tray into the chiller until it stops, then close the chiller door.

54 Raise the sipper handle fully and remove the used PR2 bottle and replace with the wash bottle. Discard the residual buffer in chemical waste and the used PR2 bottle.

55 Remove waste bottle and discard of the contents in a chemical waste receptacle that is appropriate for formamide. Return the waste bottle to the reagent compartment.

56 Lower the sipper handle and close the reagent compartment door.

57 Select **Next**

58 Once the post-run bleach wash is complete, select Done on the screen to return to the MiSeq Control Software home screen. Leave the used flow cell, wash tray, and wash bottle on the instrument. The sippers will remain in the down position, they should remain in the wash solution to prevent drying out and air from entering the fluidics.

OPTIONAL: A second-post run wash can be performed with Tween-20 wash solution only to clear out any excess NaOCl from the lines.

Instrument Maintenance

59 Maintenance during Normal Operation:

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Activity	Frequency
Post-Run Wash (with template line wash)	After every run
Post-Run Wash from the Perform Wash Screen	Once a week if no run is performed (<7 days)
Maintenance Wash	Every 30 days
Instrument Reboot	Weekly (and after data deletion)
Instrument Shutdown	As Needed

60 Maintenance during Idle Operation: (Instrument unused for >14 days):

Activity	Frequency
Standby Wash	To prepare for idle mode, and every 30 days the instrument remains idle
Post-Run Wash from the Perform Wash Screen	Once a week
Instrument Shutdown	As Needed

61 **Perform a Post-Run Wash (without bleach):**

61.1 It is a good practice to run a Post-Run wash prior to starting a run to ensure that the fluidic lines are flushed and there are no air bubbles that could affect the run. A post-run wash should also be performed once a week if the instrument is sitting idle.

61.2 Make sure a flow cell is loaded on the instrument. From the MCS home screen, select **Perform Wash**. From the Perform Wash screen, select **Perform Post-Run Wash.**

61.3 Prepare fresh 0.5% Tween-20 wash solution and add **6 µl** to each reservoir in the wash tray, and **350 µl** to the wash bottle.

61.4 Load the wash tray and wash bottle onto the instrument.

61.5 Select **Next**, the wash will begin.

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

62 **Perform a Maintenance Wash:**

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62.1 Maintenance washes are performed every 30 days to ensure optimal performance. The wash takes approximately 90 minutes to complete. The wash includes a series of three washes that thoroughly flush the system.

Note: The MCS may lock down the instrument if a maintenance wash is not performed and will not allow a run to start until it is performed.

62.2 Make sure a flow cell is loaded on the instrument. From the MCS home screen, select **Perform Wash**. From the Perform Wash screen, select **Perform Maintenance Wash.**

62.3 Prepare fresh 0.5% Tween-20 wash solution and add **6 µl** to each reservoir in the wash tray, and **350 µl** to the wash bottle.

62.4 Load the wash tray and wash bottle onto the instrument.

62.5 Select **Next**, the first wash will begin.

62.6 Always use fresh wash solution for each wash step. Reusing wash solution from previous washes can return waste into the fluidics.

62.7 Fill the second wash tray with fresh 0.5% Tween-20 wash solution.

62.8 When the instrument prompts to, remove the first wash tray and wash bottle and replace with the second wash tray and wash bottle.

62.9 Select **Next**, the second wash will begin.

62.10 Dispose of the waste from the first wash tray and wash bottle.

62.11 Fill the first tray with fresh 0.5% Tween-20 wash solution.

62.12 When the instrument prompts to, remove the second wash tray and wash bottle and replace with the first wash tray and wash bottle.

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62.13 Select **Next**, the final wash will begin.

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

63 Instrument Reboot:

63.1 The instrument should be rebooted weekly and after file deletion to free up virtual memory.

63.2 On the MCS home screen, select **Manage Instrument** then **Reboot**. It will take approximately 10 minutes for the system to reboot and the MCS to restart.

63.3 Alternatively, the instrument can be power cycled by selecting the **Manage Instrument** menu option and then **Shut Down**. Once the instrument has shut down, reach behind the right-hand side of the instrument and flip the power switch located near the power cord. Allow for at least 1 minute before turning the power back on.

64 Management of Disk Space on the Data (D:\) Drive:

64.1 The MiSeq requires 100GB of free disk space on the D:\ drive before starting a sequencing run. **Data should be cleared only after any data transfer, and if no troubleshooting is necessary for that run.** The following outlines which
files can be deleted and their locations:

64.2 Navigate to Data (D:/Illumina/MiSeqOutput/(most recent run folder)
- Within this folder, select the Images and Thumbnail_images folders and delete

64.3 Navigate to D:/Illumina/MiSeqAnalysis/
- Delete any run folders excluding the most recent folder

Note: It is suggested to reboot the instrument following data deletion in order to free virtual memory in the system.

65 Perform a Standby Wash:

65.1 If the instrument is not to be used for an extended period of time (>14 days), the instrument should be prepared for idle mode by performing a standby wash. The standby wash takes approximately 2 hours to complete.

The standby wash consists of two 60 minutes washes with the 0.5% Tween-20 wash solution.

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65.2

65.3 After the initial standby wash, a standby wash needs to be performed every 30 days while the instrument remains idle.

65.4 To bring an instrument out of idle mode, a maintenance wash must be performed before a run can be initiated.

Appendix 1: Addition of PhiX Control

66 PhiX is a balanced genome of a bacteriophage and recommended by Illumina as an internal control to assess how well the sequencing instrument is performing. A PhiX run is recommended after any instrument repair or service. PhiX can also be spiked into a final library as a control or to improve sequencing of low-diversity libraries.

67 Diluted Denatured PhiX Library Preparation:

67.1 The denatured PhiX control should be diluted to 20 pM, when used with v3 chemistry and 12.5 pM, when used with v2 chemistry for optimal cluster density.

67.2 Prepare 0.2 N NaOH and place on ice until ready to use.

67.3 Combine **2 µl** of 10 nM PhiX library from the stock and **3 µl** of EBT to obtain 4 nM PhiX library in a new 1.5 ml microcentrifuge tube.

67.4 Combine **5 µl** of 4 nM PhiX Library and **5 µl** of 0.2 N NaOH and mix well.

67.5 **5 µl** Incubate for **00:05:00** at room temperature to denature the PhiX library.

67.6 **IMMEDIATELY** add **990 µl** of pre-chilled HT1 to the tube containing the 10 µl of denatured PhiX to obtain 20 pM of denatured Phix library.

67.7 Label, date, and initial the tube. Denatured 20 pM PhiX libraries may be stored up to 3 weeks at -15^{O}C to -25^{O}C.

67.8 If you are using a MiSeq Reagent v3 Kit, no further dilution is required. If you are using a v2 kit, then remove 375 µl from the 20 pM PhiX dilution and add 375 µl of HT1 back into this tube. The result is 1 ml of 12.5 pM PhiX library.

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68 **PhiX Spike-in Control:**

68.1 A stock of diluted PhiX can be prepared and stored for subsequent use.

68.2 Prepare a 20 pM of denatured PhiX as described in steps 61.1 - 61.7.

68.3 Dilute the denatured 20 pM PhiX library to 8 pM by adding **40 µl** of library to **60 µl** of pre-chilled HT1.

68.4 Determine the percentage of PhiX (N%) to add to run. If troubleshooting a library preparation, then 1 – 10% is appropriate. If using PhiX routinely in every run, then 1% is recommended, for low-diversity libraries use of a 5% spike-in is recommended.

68.5 For a 1% PhiX spike, add 10 µl of 8 pM PhiX to 990 µl of the final denatured 10-20 pM sample library. For other percentages, combine N x 10 µl of 8 pM PhiX with (1000 – (N x 10) µl of sample library where N = the percentage of spike-in.

Appendix 2: MiSeq Sample Sheet Setup

69 **Exporting a MiSeq Sample Sheet from the GenomeTrakr Flex Library Workbook:**

69.1 Ensure that the SampleSheet tab is properly populated. (Do not modify the Indices tab, the index sequences are populated from this tab)

69.2 Save as a .csv or export the tab as a .csv:

To Save As:

- Open the File menu option and select **Save As**
- Change **Save as type**: to **CSV (Comma delimited)**
- Navigate to the desired file folder location and save the file using the laboratory Plate ID as the filename
- Click **OK** to save only the active sheet, and **Yes** in the following window to keep using the CSV format.

OR

To export as .csv:

- Open the File menu option and select **Export**
- Select **Change File Type**
- Choose **CSV (Comma delimited)**
- Save and select **OK** to save only the active sheet

69.3 Transfer this file to the Sample Sheet folder on the MiSeq, or a shared network drive that can be accessed by the MiSeq.

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69.4 Open the Local Run Manager software.

69.5 Select **+Create Run**.

69.6 Select **Import Sample Sheet.**

69.7 Verify the information that is populated is correct.

69.8 Select **Save Run.**

70 **Manually Entering Run Information into Local Run Manager:**

70.1 Select **+Create Run.**

70.2 Select **Generate Fastq.**

70.3 Enter information for the following required fields:
- **Run Name**
- **Library Prep Kit** – (Nextera XT or Nextera DNA CD Indexes – 96 Indexes Plated)
- **Read Type** – Paired End
- **Read Length** – 151 or 251 (Fill in both R1 and R2)
- **Adapter Trimming** – ON

70.4 Enter the # of rows to be added to the sample sheet.

NOTE: The two required fields are Sample ID and Index Well

70.5 Select **Save Run.**

Forms

71 MiSeq Maintenance Records:

MiSeq Wash Chart Jan to June.pdf **MiSeq Wash Chart July to Dec.pdf**

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Reference Documents

72 MiSeq System Guide (August 2019)

MiSeq Denature and Dilute Libraries Guide (February 2019)

Local Run Manager Software Guide v1 (January 2020)

Local Run Manager Software Guide v2 (January 2020)

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