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Performing a MiSeq Next Generation Sequencing Run

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1.0 Purpose

1.1 This protocol describes the procedures for use on the Illumina MiSeq sequencing platform.

2.0 Definitions

2.1 MiSeq SBS Solution (PR2) – a buffered saline solution

3.0 Critical Equipment

3.1 Illumina MiSeq

3.2 -20°C ± 5°C Freezer

3.3 2°C - 8°C Refrigerator

3.4 Water Bath

4.0 Materials

4.1 Pipettes and aerosol barrier tips

4.2 Tween-20 (Sigma-Aldrich, Cat. #P7949)

4.3 DNase-free, RNase-free water

4.4 MiSeq Reagent Kit v2 300 cycles (Illumina, Cat# MS-102-2002, , MS-103-1001, or MS-103,1002)

4.5 Kimwipes (Fisher brand Cat#06-666)

4.6 MiSeq tube (for bleach wash) (part # 15054695)

4.7 Sodium Hypochlorite (household strength –typically 6-9% bleach)

5.0 Safety Precautions

5.1 Adhere to the safety guidelines provided in the Biosafety in Microbiological and Biomedical Laboratories and follow all established site-specific safety procedures, including wearing proper personal protective equipment (PPE).

6.0 MiSeq Procedures

6.1 Prepare the Reagent Cartridge

6.1.1 Remove the reagent cartridge from -25° to -15°C storage.

6.1.2 Place the reagent cartridge in a water bath containing enough room temperature water to submerge the base of the reagent cartridge up to the water line printed on the cartridge. Do not allow the water to exceed the maximum water line.

6.1.3 Allow the reagent cartridge to thaw in the room temperature water bath for approximately 20 minutes, then place in a 4°C refrigerator until ready to load the samples. The cartridge can also be placed in a water/ice bath until ready to use.

6.2 Cleaning the Flow Cell

6.2.1 Using gloved fingers, grip the flow cell by the base of the plastic case and remove it from the flow cell container.

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

6.2.3 Thoroughly dry the flow cell and cartridge using a lint-free lens cleaning tissue. Gently pat dry around the gasket and adjacent glass.

6.2.4 Visually inspect to make sure that the flow cell ports are free of obstructions and that the gasket is well seated around the flow cell ports. If the gasket appears to be dislodged, gently press it back into place until it sits securely around the flow cell ports.

6.3 Operate MiSeq to Start a Run

6.3.1 Prepare the loading sample as described in LP-309 (Preparation of the DNA Libraries for Loading onto the MiSeq) and then follow the steps below.

6.3.2 Click on **Sequence** to open a series of run setup screens that guide users through the run setup steps.

6.3.3 Select **Sample Sheet** on the Run Setup Option screen and browse to select the sample sheet file containing the run parameters, sample names and indices. Select **Next** to proceed to load the flow cell.

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

6.3.5 Holding the flow cell by the edges of the flow cell cartridge, place the flow cell on the flow cell stage.

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

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

6.3.7 Confirm that the flow cell RFID was successfully read.

6.3.8 Select **Next**.

6.3.9 Open the reagent compartment door.

6.3.10 Check the waste bottle and empty, if necessary.

6.3.11 Remove the bottle of MiSeq SBS Solution (PR2) from 2° to 8°C storage. Gently invert the bottle to mix the MiSeq SBS Solution (PR2) bottle. Open the reagent compartment door. Raise the sipper handle until it locks into place. Place the MiSeq SBS Solution (PR2) bottle in the indentation to the right of the reagent chiller. Lower sipper handle.

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

6.3.13 Select **Next**

6.4 Loading Sample to Cartridge

6.4.1 Remove the cartridge from the water bath or 4°C refrigerator and gently tap it on the bench to dislodge water from the base of the cartridge. Dry the base of the cartridge. Make sure that no water has splashed on the top of the reagent cartridge.

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

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

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

NOTE: The MiSeq 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.

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

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

6.4.5 Pipette 600 µl of the denatured diluted amplicon libraries/HT1/PhiX mix into the Load Samples reservoir. Check for air bubbles in the reservoir after loading sample.

6.4.6 Open the reagent chiller door and slide the reagent cartridge into the reagent chiller until the cartridge stops

NOTE: Do not leave the reagent chiller door open for extended periods of time. Use a Kimwipe to blot any accumulated condensation in the base of the reagent chiller before loading the reagent cartridge.

6.4.7 Close the reagent chiller door.

6.4.8 Confirm that the RFID was successfully read.

6.4.9 Select **Next.**

6.4.10 Review the run parameters:

- Experiment Name
- Analysis Workflow
- Read Length

6.4.11 Select **Next.**

6.4.12 The system performs a check of all run components, disk space, and network connections before starting the run. If any items do not pass the pre-run check, a message appears on the screen with instructions on how to correct the error.

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

6.4.14 After selecting **Start Run**, do not open the flow cell compartment or the reagent compartment doors, or touch the instrument monitor except to pause the run.

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

6.5 Monitoring the Run

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

6.5.2 When the run is complete, the **Next** button appears. Review the results on the Sequencing screen before proceeding.

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

6.6 Performing a Post-Run Bleach Wash and Reagent Disposal

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

6.6.2 When the run is complete, select **Start Wash**. The software automatically raises the sippers in the reagent chiller.

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

6.6.3 Select **Perform optional template line wash** on Post-Run Wash screen.

6.6.4 Prepare **fresh** sodium hypochlorite wash solution with laboratory-grade water as follows:

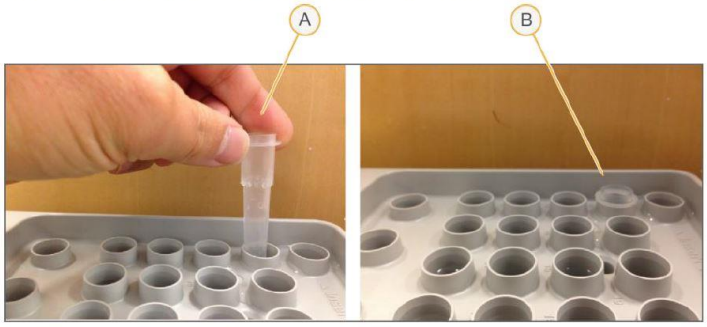
- Add 30 µl of 6% sodium hypochlorite to 870 µl laboratory-grade water. These volumes result in a 1:30 sodium hypochlorite dilution.
- Add 50 µl of the 1:30 sodium hypochlorite dilution to 950 µl of laboratory-grade water in an Illumina-supplied MiSeq tube (part # 15054695).

NOTE: Make sure to check the percentage of sodium hypochlorite on the product label. Using the correct concentration of sodium hypochlorite is important. If the concentration is too high, it can make cluster generation fail in subsequent runs. If 6% sodium hypochlorite is not available, make a 1 mL solution of 0.01% sodium hypochlorite in laboratory-grade water.

6.6.5 Insert the MiSeq tube containing 0.01% sodium hypochlorite wash solution into **position 17** of the wash tray until the neck of the tube is flush with the tray.

NOTE: Make sure to insert the MiSeq tube with sodium hypochlorite 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.

Figure 1. Inserting the 0.01% Sodium Hypochlorite MiSeq Tube



6.6.6 Prepare fresh 0.5% Tween wash solution with Tween 20 and laboratory-grade water, as follows:

- Add 5 mL of 100% Tween 20 to 45 mL laboratory-grade water. These volumes result in 10% Tween 20.
- Add 25 mL of 10% Tween 20 to 475 mL laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.
- Invert five times to mix.

6.6.7 Prepare the wash components with fresh Tween 20 and laboratory-grade water wash solution, as follows:

- Add 6 mL 0.5% Tween 20 wash solution to each reservoir of the wash tray except **position 17**.
- Add 500 mL 0.5% Tween 20 wash solution to the 500 mL wash bottle.

6.6.8 Remove the waste bottle and pour the solution into the PR2 bottle.

6.6.9 Slide out the used reagent cartridge from the chiller. Label the waste to discard in accordance with local waste disposal.

6.6.10 Remove the PR2 bottle and replace it with the wash bottle containing 0.5% Tween 20.

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

6.6.12 Load the wash tray containing the 0.5% Tween 20 wash buffers and 0.01% sodium hypochlorite in position 17.

6.6.13 Close the reagent compartment door.

6.6.14 Select **Next**. The post-run wash will begin.

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

6.7 Performing a Maintenance Wash

6.7.1 Perform a maintenance wash every month to ensure optimal performance. The maintenance wash includes a series of three wash steps using a wash solution of DNase-free, RNase-free water mixed with Tween 20. Allow approximately 90 minutes to complete the wash.

6.7.2 Make sure that a used flow cell is loaded on the instrument.

6.7.3 From the Welcome screen, select **Perform Wash**.

6.7.4 From the Perform Wash screen, select **Maintenance Wash**. The software automatically raises the sippers in the reagent chiller.

6.7.5 **Perform the First and Second Washes**

6.7.6 Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows:

6.7.6..1 Add 5 mL of 100% Tween 20 to 45 mL DNase-free, RNase-free water (10% Tween 20)

6.7.6..2 Add 25 mL of 10% Tween 20 to 475 mL DNase-free, RNase-free water (0.5% Tween 20)

6.7.6..3 Invert several times to mix.

6.7.6..4 Prepare the wash components with fresh wash solution as follows:

6.7.6..5 Add 6 mL 0.5% Tween 20 wash solution to each reservoir of the wash tray.

6.7.6..6 Add 500 mL 0.5% Tween 20 wash solution to the 500 mL wash bottle.

6.7.6..7 Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.

6.7.6..8 Load the wash tray and wash bottle into the instrument:

6.7.6..9 Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.

6.7.7 Select **Next**. The first wash begins.

6.7.8 After the first wash is completed, repeat section 6.7.5 for the second wash.

6.8 Perform the Third Wash

6.8.1 Prepare the wash components with fresh laboratory-grade water as follows:

6.8.1..1 Add 6 mL laboratory-grade water to each reservoir of the wash tray.

6.8.1..2 Add 350 mL laboratory-grade water to the 500 mL wash bottle.

6.8.2 Load the wash tray and wash bottle onto the instrument:

6.8.2..1 Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.

6.8.2..2 Load the wash tray and wash bottle onto the instrument.

6.8.2..3 Slowly lower the sipper handle, making sure that the sippers lower into the wash bottle and waste bottle.

6.8.3 Select **Next**. The third wash begins.

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

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

7.0 Related Procedures

7.1 LP-309 - MiSeq Library Loading Preparation

8.0 References

8.1 Illumina MiSeq® System User Guide (Document # 15027617 Rev. v05 August 2019) https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/miseq-system-guide-for-local-run-manager-15027617-05.pdf

9.0 Attachments – N/A