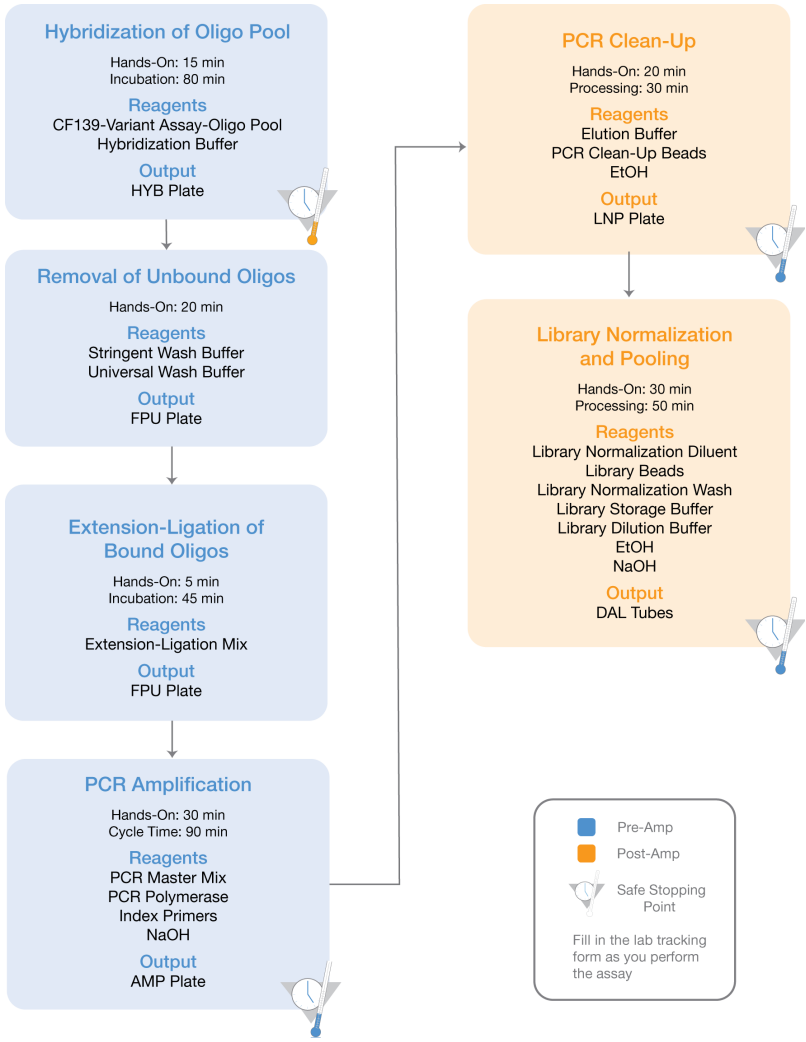
MiSeqDx Cystic Fibrosis 139-Variant Assay

Lab Tracking Form

FOR IN VITRO DIAGNOSTIC USE

Date: _____ Description: _____

Illumina Kit Lot #: _____ _____



Lab Tracking Form

Date/Time: _____ Operator: _____

Consumables

Item	Lot Number
CF 139-Variant Assay-Oligo Pool	Lot #: _____
Hybridization Buffer	Lot #: _____
Stringent Wash Buffer	Lot #: _____
Universal Wash Buffer	Lot #: _____
Filter Plate	Lot #: _____
Extension-Ligation Mix	Lot #: _____
PCR Master Mix	Lot #: _____
PCR Polymerase	Lot #: _____
0.05 N NaOH—Date Prepared: _____	10 N NaOH—Lot #: _____ Template-free Water—Lot #: _____
Elution Buffer	Lot #: _____
PCR Clean-Up Beads	Lot #: _____
80% Ethanol—Date Prepared: _____	100% Ethanol—Lot #: _____ Template-free Water—Lot #: _____
Library Normalization Diluent	Lot #: _____
Library Beads	Lot #: _____
Library Normalization Wash	Lot #: _____
Library Storage Buffer	Lot #: _____
0.1 N NaOH—Date Prepared: _____	10 N NaOH—Lot #: _____ Template-free Water—Lot #: _____
Library Dilution Buffer	Lot #: _____
10 nM PhiX Internal Control	Lot #: _____
TE Buffer	Lot #: _____
MiSeqDx Reagent Cartridge - CF 139-Variant Assay	Lot #: _____
MiSeqDx Flow Cell - CF 139-Variant Assay	Lot #: _____
MiSeqDx SBS Solution (PR2) - CF 139-Variant Assay	Lot #: _____

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Index Primers

Index Primer A (A501) Lot #: _____	Index Primer 1 (A701) Lot #: _____
Index Primer B (A502) Lot #: _____	Index Primer 2 (A702) Lot #: _____
Index Primer C (A503) Lot #: _____	Index Primer 3 (A703) Lot #: _____
Index Primer D (A504) Lot #: _____	Index Primer 4 (A704) Lot #: _____
Index Primer E (A505) Lot #: _____	Index Primer 5 (A705) Lot #: _____
Index Primer F (A506) Lot #: _____	Index Primer 6 (A706) Lot #: _____
Index Primer G (A507) Lot #: _____	Index Primer 7 (A707) Lot #: _____
Index Primer H (A508) Lot #: _____	Index Primer 8 (A708) Lot #: _____
	Index Primer 9 (A709) Lot #: _____
	Index Primer 10 (A710) Lot #: _____
	Index Primer 11 (A711) Lot #: _____
	Index Primer 12 (A712) Lot #: _____

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Acronyms

Table 1 Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay Acronyms

Acronym	Definition
AMP	AMplification Plate
CLP	CLean-up Plate
DAL	Diluted Amplicon Library
FPU	Filter Plate Unit
HYB	HYBridization Plate
LNP	Library Normalization Plate
NTC	No Template Control
PAL	Pooled Amplicon Library
SGP	StoraGe Plate

Date/Time: _____ Operator: _____

Hybridization of Oligonucleotide Pool

During this step, the cystic fibrosis oligonucleotide pool containing upstream and downstream oligonucleotides specific to the cystic fibrosis transmembrane conductance regulator (CFTR) gene is hybridized to genomic DNA samples.

Estimated Time

- Total duration: 1 hour 35 minutes
- Hands-on: 15 minutes

Preparation

[_] 1 Bring the CF 139-Variant Assay-Oligo Pool, Hybridization Buffer, genomic DNA samples, and positive control sample to room temperature.

[_] 2 Vortex the CF 139-Variant Assay-Oligo Pool and Hybridization Buffer vigorously to make sure that all precipitates have completely dissolved, then briefly centrifuge the tubes to collect liquid.

[_] 3 Set a 96-well heat block to 95°C.

[_] 4 Pre-heat an incubator to 37°C.

[_] 5 Create the sample plate according to the plate graphic printed from Illumina Worklist Manager or Local Run Manager.
Sample Sheet Name (IWM): _____ or
Run Name (Local Run Manager): _____

Procedure

[_] 1 Set out a new 96-well PCR plate (hereafter referred to as the **HYB** plate).
Plate ID: _____

[_] 2 Add 5 µl of sample or control at 50 ng/µl (250 ng total) to the appropriate wells in the **HYB** plate. Follow the generated plate layout for correct well selection.

[_] 3 Add 5 µl of the CF 139-Variant Assay-Oligo Pool to all sample wells.

[_] 4 Add 40 µl of Hybridization Buffer to each sample in the **HYB** plate. Gently pipette up and down 3–5 times to mix.

[_] 5 Seal the **HYB** plate and centrifuge 1000 × g at 20°C for 1 minute.

[_] 6 Place the **HYB** plate in the pre-heated block at 95°C and incubate for 1 minute.

Date/Time: _____ Operator: _____

[_] 7 Reduce the heat block to 40°C and continue incubating until the heat block reaches 40°C (approximately 80 minutes).
Gradual cooling is critical for proper hybridization; therefore, PCR thermal cyclers with active cooling (e.g., Peltier, thermoelectric cooled) are not recommended for this process.

Start time: _____ Stop time: _____

SAFE STOPPING POINT
After the heat block reaches 40°C, the **HYB** plate is stable holding at 40°C for 2 hours.

Comments

Date/Time: _____ Operator: _____

Removal of Unbound Oligonucleotides

This process removes unbound oligonucleotides from genomic DNA using a filter capable of size selection. Two wash steps using Stringent Wash Buffer ensure complete removal of unbound oligonucleotides. A third wash step using Universal Wash Buffer removes residual Stringent Wash Buffer and prepares samples for the extension-ligation step.

Estimated Time

- Total duration: 20 minutes
- Hands-on: 20 minutes

Preparation

[_] 1 Bring Extension-Ligation Mix, Stringent Wash Buffer, and Universal Wash Buffer to room temperature, and then vortex briefly.

[_] 2 Assemble the filter plate assembly unit (hereafter referred to as the **FPU**) in order from top to bottom: lid, filter plate, adapter collar, and MIDI plate.
Filter Plate ID: _____

[_] 3 Pre-wash the filter plate membrane as follows:

[_] a Add 45 µl of Stringent Wash Buffer to each well.

[_] b Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 5 minutes.

NOTE
Check to verify that all wells of the filter plate are draining completely. If the wash buffer does not drain completely, centrifuge again at 2400 × g at 20°C until all liquid has gone through (an additional 5–10 minutes).

CAUTION
It is critical to control the centrifuge temperature during the washing steps. If the temperature reaches 25°C or higher, the higher temperature may lead to higher stringency in primer binding. In rare cases, if samples have SNVs in primer binding regions, the higher stringency may lead to allele dropout.

Procedure

[_] 1 Remove the **HYB** plate from the heat block and centrifuge at 1000 × g at 20°C for 1 minute.

[_] 2 Transfer the entire volume (approximately 55 µl) of each sample to the corresponding wells of the filter plate.

[_] 3 Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 5 minutes.

[_] 4 Wash the filter plate as follows:

[_] a Add 45 µl of Stringent Wash Buffer to each sample well.

[_] b Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 5 minutes.

[_] 5 Repeat the wash as described in the previous step.

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NOTE
If the wash buffer does not drain completely, centrifuge again at 2400 × g at 20°C until all liquid has gone through (an additional 5–10 minutes).

[_] 6 Discard all the flow-through (containing formamide), then reassemble the **FPU**.

[_] 7 Add 45 µl of Universal Wash Buffer to each sample well.

[_] 8 Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 10 minutes.

NOTE
Make sure that all liquid has drained after centrifugation. Repeat centrifugation if necessary.

Comments

Date/Time: _____ Operator: _____

Extension-Ligation of Bound Oligonucleotides

This process connects the hybridized upstream and downstream oligonucleotides. A DNA polymerase extends from the upstream oligonucleotide through the targeted region, followed by ligation to the 5′ end of the downstream oligonucleotide using a DNA ligase. This results in the formation of products containing the targeted regions of interest flanked by sequences required for amplification.

Estimated Time

- Total duration: 50 minutes
- Hands-on: 5 minutes

Procedure

[_] 1 Add 45 µl of Extension-Ligation Mix to each sample well of the filter plate.

[_] 2 Seal the filter plate with adhesive aluminum foil, and then cover with the lid.

[_] 3 Incubate the **FPU** in the pre-heated 37°C incubator for 45 minutes.

Start time: _____ Stop time: _____

[_] 4 While the **FPU** plate is incubating, prepare the AMP (Amplification Plate) as described in the following section.

Comments

_____

_____

_____

Date/Time: _____ Operator: _____

PCR Amplification

In this step, the extension-ligation products are amplified using primers that add index sequences for sample multiplexing, as well as common adapters required for cluster generation.

Estimated Time

- Total duration: ~90 minutes
- Hands-on: 30 minutes

Preparation

[_] 1 Prepare fresh 0.05 N NaOH.

[_] 2 Determine the index primers to be used according to the plate graphic printout from Illumina Worklist Manager or Local Run Manager.

[_] 3 Bring PCR Master Mix and the appropriate index primers to room temperature. Vortex each thawed tube to mix, and then briefly centrifuge the tubes.

[_] 4 Set out a new 96-well PCR plate (hereafter referred to as the **AMP** plate).

[_] 5 Add index primers to the AMP plate as follows:

- [_] a Add 4 µl of the selected index primers [A (A501) – H (A508)] to the appropriate well in a column of the **AMP** plate.
- [_] b Discard the original white caps and apply new white caps.
- [_] c Add 4 µl of the selected index primers [1 (A701) – 12 (A712)] to the appropriate row of the **AMP** plate. ***Tips must be changed after each row to avoid index cross-contamination.***
- [_] d Discard the original orange caps and apply new orange caps.

[_] 6 Prepare the PCR Master Mix/PCR Polymerase PCR working solution as follows:

- [_] a Briefly centrifuge the PCR Polymerase tube before use to remove air bubbles.
- [_] b For 96 samples, add 56 µl of PCR Polymerase to 2.8 ml of PCR Master Mix.
- [_] c Invert the prepared PCR working solution 20 times to mix.

The PCR working solution is stable at room temperature for 10 minutes.

Procedure

[_] 1 Remove the **FPU** from the incubator, and then remove the aluminum foil seal.

[_] 2 Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 2 minutes.

[_] 3 Add 25 µl of 0.05 N NaOH to each sample well on the filter plate. Pipette the NaOH up and down 5–6 times.

[_] 4 Cover and incubate the filter plate at room temperature for 5 minutes.

[_] 5 While the filter plate is incubating, transfer 22 µl of the PCR working solution to each well of the AMP plate containing index primers.

[_] 6 Transfer samples eluted from the filter to the AMP plate as follows:

- [_] a Pipette the samples in the first column of the filter plate up and down 5–6 times.

Date/Time: _____ Operator: _____

[_] b Transfer 20 µl from the filter plate to the corresponding column of the **AMP** plate.

[_] c Gently pipette up and down 5–6 times to thoroughly combine the DNA with the PCR working solution.

[_] d Transfer the remaining columns from the filter plate to the AMP plate in a similar manner. ***Tips must be changed after each column to avoid index and sample cross-contamination.***

[_] 7 Seal the **AMP** plate and secure with a rubber roller.

[_] 8 Centrifuge at 1000 × g at 20°C for 1 minute.

[_] 9 Transfer the **AMP** plate to the post-amplification area.

[_] 10 Perform PCR using the following program on a thermal cycler:

- 95°C for 3 minutes
- 25 cycles of:
  - 95°C for 30 seconds
  - 62°C for 30 seconds
  - 72°C for 60 seconds
- 72°C for 5 minutes
- Hold at 10°C

Start time: _____ Stop time: _____

SAFE STOPPING POINT

If not proceeding immediately to PCR Clean-Up, the **AMP** plate can remain on the thermal cycler overnight, or can be stored at 2°C to 8°C up to 48 hours.

Comments

Date/Time: _____ Operator: _____

PCR Clean-Up

This process uses PCR Clean-Up Beads to purify the PCR products from the other reaction components.

Estimated Time

- Total duration: 50 minutes
- Hands-on: 20 minutes

Preparation

[_] 1 Bring the PCR Clean-Up Beads to room temperature.

[_] 2 Prepare fresh 80% ethanol from absolute ethanol.

Procedure

[_] 1 Centrifuge the AMP plate at 1000 × g at 20°C for 1 minute.

[_] 2 Set out a new MIDI plate (hereafter referred to as the **CLP** plate).
Plate ID: _____

[_] 3 Invert PCR Clean-Up Beads 10 times. Vortex vigorously and then invert 10 more times. Visually inspect solution to ensure that beads are resuspended.

[_] 4 Add 45 µl of PCR Clean-Up Beads to each well of the **CLP** plate.

[_] 5 Transfer the entire PCR product from the AMP plate to the **CLP** plate.

[_] 6 Seal the **CLP** plate and shake on a microplate shaker at 1800 rpm for 2 minutes.

[_] 7 Incubate at room temperature without shaking for 10 minutes.

[_] 8 Place the plate on a magnetic stand for a minimum of 2 minutes or until the supernatant is clear.

[_] 9 With the **CLP** plate on the magnetic stand, carefully remove and discard the supernatant.

[_] 10 With the **CLP** plate on the magnetic stand, wash the beads as follows:

- [_] a Add 200 µl of freshly prepared 80% ethanol to each sample well.
- [_] b Incubate the plate on the magnetic stand for a minimum of 30 seconds or until the supernatant is clear.
- [_] c Carefully remove and discard the supernatant.

[_] 11 Repeat the wash as described in the previous step.

[_] 12 Use a P20 multi-channel pipette set to 20 µl to remove excess ethanol.

[_] 13 Remove the **CLP** plate from the magnetic stand and air-dry the beads for 10 minutes.
Start time: _____ Stop time: _____

[_] 14 Add 30 µl of Elution Buffer to each sample.

Date/Time: _____ Operator: _____

[_] 15 Seal the **CLP** plate and shake on a microplate shaker at 1800 rpm for 2 minutes. After shaking, verify if samples were resuspended. If not, repeat this step.

[_] 16 Incubate at room temperature for 2 minutes.

[_] 17 Place the **CLP** plate on the magnetic stand for a minimum of 2 minutes or until the supernatant is clear.

[_] 18 Set out a new MIDI plate (hereafter referred to as the **LNP** plate).
Plate ID: _____

[_] 19 Transfer 20 µl of the supernatant from the **CLP** plate to the **LNP** plate.

[_] 20 [Optional] Transfer the remaining 10 µl of supernatant from the **CLP** plate to a new plate and label the plate with a run name and date. Store this plate at -25°C to -15°C until completion of the sequencing run and data analysis. The cleaned up PCR products can be used for troubleshooting efforts in the event of sample failures.

SAFE STOPPING POINT
If stopping at this point, seal the **LNP** plate and centrifuge at 1000 × g at 20°C for 1 minute. The plate is stable for up to 3 hours at 2°C to 8°C.

Comments

Date/Time: _____ Operator: _____

Library Normalization and Pooling

This process normalizes the quantity of each library to ensure equal library representation in the pooled sample. Equal volumes of normalized libraries are then combined and diluted in preparation for sequencing.

Estimated Time

- Total duration: 1 hour 20 minutes
- Hands-on: 30 minutes

Preparation

[_] 1 Prepare fresh 0.1 N NaOH by adding 30 µl of 10 N NaOH to 2970 µl of RNase/DNase-free water.

[_] 2 Bring Library Normalization Diluent, Library Beads, Library Normalization Wash, and Library Dilution Buffer to room temperature.

[_] 3 Vortex Library Normalization Diluent vigorously and ensure that all precipitates have dissolved.

[_] 4 Vortex Library Beads vigorously for 1 minute with intermittent inversion until the beads are resuspended and no pellet is found at the bottom of the tube when the tube is inverted.

Procedure

[_] 1 Mix Library Normalization Diluent and Library Beads in a fresh 15 ml conical tube as follows:

NOTE
If processing < 24 samples, use a fresh 1.5 ml tube.

[_] a For 96 samples, add 4.4 ml of Library Normalization Diluent.

[_] b Pipette Library Beads up and down 10 times to resuspend.

NOTE
It is extremely critical to completely resuspend the library bead pellet at the bottom of the tube. The use of a P1000 ensures that the beads are homogeneously resuspended and that there is no bead mass at the bottom of the tube. This is essential for achieving consistent cluster density on the flow cell.

[_] c For 96 samples, pipette 800 µl of Library Beads to the tube containing Library Normalization Diluent.

[_] d Mix by inverting the tube 15–20 times.

[_] 2 Add 45 µl of the combined Library Normalization Diluent/Library Beads working solution to each well of the **LNP** plate containing libraries.

[_] 3 Seal the **LNP** plate and shake on a microplate shaker at 1,800 rpm for 30 minutes.

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NOTE
If proceeding with sequencing on the same day, now is a good time to begin thawing the reagent cartridge. Follow the instructions to thaw the MiSeqDx Reagent cartridge in the section entitled *Prepare the Reagent Cartridge* on page 17.

Start time: _____ Stop time: _____

[_] 4 Place the plate on a magnetic stand for a minimum of 2 minutes or until the supernatant is clear.

[_] 5 With the **LNP** plate on the magnetic stand, carefully remove and discard the supernatant.

[_] 6 Remove the **LNP** plate from the magnetic stand and wash the beads with Library Normalization Wash as follows:

- [_] a Add 45 µl of Library Normalization Wash to each sample well.
- [_] b Seal the **LNP** plate and shake on a microplate shaker at 1,800 rpm for 5 minutes.
- [_] c Place the plate on the magnetic stand for a minimum of 2 minutes or until the supernatant is clear.
- [_] d Carefully remove and discard the supernatant.

[_] 7 Repeat the Library Normalization Wash procedure as described in the previous step.

[_] 8 Use a P20 multi-channel pipette set to 20 µl to remove excess Library Normalization Wash.

[_] 9 Remove the **LNP** plate from the magnetic stand and add 30 µl of 0.1 N NaOH to each well.

[_] 10 Seal the **LNP** plate and shake on a microplate shaker at 1,800 rpm for 5 minutes.

[_] 11 During the 5 minute elution, set out a new 96-well PCR plate (hereafter referred to as the **SGP** plate.)
Plate ID: _____

[_] 12 Add 30 µl Library Storage Buffer to each well to be used in the **SGP** plate.

[_] 13 After the 5-minute elution, ensure all samples in the **LNP** plate are completely resuspended. If the samples are not completely resuspended, gently pipette those samples up and down or lightly tap the plate on the bench to resuspend the beads, then shake for another 5 minutes.

[_] 14 Place the **LNP** plate on the magnetic stand for a minimum of 2 minutes.

[_] 15 Transfer the supernatant from the **LNP** plate to the **SGP** plate. Gently pipette up and down 5 times to mix.

[_] 16 Seal the **SGP** plate and then centrifuge at 1,000 × g at 20°C for 1 minute.

[_] 17 Vortex the Library Dilution Buffer and make sure that all the precipitates have dissolved completely.

[_] 18 Briefly centrifuge to collect contents.

[_] 19 Set out a fresh Eppendorf tube (hereafter referred to as the **PAL** [Pooled Amplicon Library] tube).

[_] 20 Determine the samples to be pooled for sequencing. A maximum of 48 samples can be pooled for sequencing.

[_] 21 Transfer 5 µl of each library to be sequenced from the **SGP** plate, column by column, to a PCR eight-tube strip.

Date/Time: _____ Operator: _____

[_] 22 Combine and transfer the contents of the PCR eight-tube strip into the **PAL** tube. Mix the **PAL** tube thoroughly.

[_] 23 Set out 2–3 fresh Eppendorf tubes (hereafter referred to as the **DAL** [Diluted Amplicon Library] tubes).

[_] 24 Add 585 µl of Library Dilution Buffer to the **DAL** tubes.

[_] 25 Transfer 9 µl of **PAL** to each **DAL** tube containing Library Dilution Buffer. Pipette up and down 3–5 times to rinse the tip and to make sure the transfer is complete.

SAFE STOPPING POINT

If not proceeding immediately to sequencing on the MiSeqDx, the **DAL** tubes can be stored at –25°C to –15°C for up to 14 days.

Comments

Library Sequencing

In preparation for cluster generation and sequencing, the diluted library is heat denatured prior to sequencing on the MiSeqDx. PhiX is used as an internal control for sequencing.

The flow cell is washed, dried, and loaded into MiSeqDx, samples are loaded into the reagent cartridge, the reagent cartridge is loaded into MiSeqDx, and the sequencing run is started. The MiSeqDx performs cluster generation, sequencing by synthesis, and data analysis.

Estimated Time

- Total duration: ~28 hours
- Hands-on: ~15 minutes

Prepare for Library Sequencing

[_] 1 Set a heat block suitable for 1.5 ml centrifuge tubes to 96°C.

[_] 2 In an ice bucket, prepare an ice-water bath. Chill the Library Dilution Buffer in the ice-water bath.

[_] 3 Begin thawing the MiSeqDx reagent cartridge.

Prepare the Reagent Cartridge

[_] 1 Thaw the MiSeqDx Reagent Cartridge - CF 139-Variant Assay in a water bath containing enough room temperature laboratory-grade water to submerge the base of the reagent cartridge up to the water line printed on the reagent cartridge. Do not allow the water to exceed the maximum water line.

[_] 2 Allow the reagent cartridge to thaw in the room temperature water bath for approximately 1 hour or until thawed.

[_] 3 Remove the cartridge from the water bath and gently tap it on the bench to dislodge water from the base of the cartridge. Dry the base of the cartridge. Make sure that no water has splashed on the top of the reagent cartridge.

Inspect the Reagent Cartridge

[_] 1 Invert the reagent cartridge ten times to mix the thawed reagents, and then inspect that all positions are thawed.

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

[_] 2 Inspect reagents in positions 1, 2, and 4 to make sure that they are fully mixed and free of precipitates.

[_] 3 Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

NOTE
The MiSeqDx sipper tubes go to the bottom of each reservoir to aspirate the reagents, so it is important that the reservoirs are free of air bubbles.

[_] 4 Place the reagent cartridge on ice or set aside at 2°C to 8°C (up to 6 hours) until ready to set up the run. For best results, proceed directly to loading the sample and setting up the run.

Denature and Dilute PhiX Internal Control

[_] 1 Prepare 0.1N NaOH by combining the following volumes in a conical tube:
- DNase/RNase-free water (2475 µl)
- Stock 10 N NaOH (25 µl)

[_] 2 Invert the tube several times to mix.

CAUTION
Using freshly diluted NaOH is essential in order to completely denature samples for cluster generation on the MiSeqDx.

NOTE
If PhiX is prepared the same day as Library Normalization, the same stock of 0.1N NaOH can be used.

[_] 3 Combine the following volumes to dilute the PhiX Internal Control library to 2 nM:
- 10 nM PhiX Internal Control library (2 µl)
- 1X TE Buffer (8 µl)

[_] 4 Combine the following volumes to result in a 1 nM PhiX Internal Control library:
- 2 nM PhiX Internal Control library (10 µl)
- 0.1 N NaOH (10 µl)

[_] 5 Vortex briefly to mix the 1 nM PhiX Internal Control library solution.

[_] 6 Centrifuge the 1nM PhiX Internal Control at 280 × g at 20°C for 1 minute.

[_] 7 Incubate for 5 minutes at room temperature to denature the PhiX Internal Control library solution into single strands.

[_] 8 Combine the following volumes in a new microcentrifuge tube to result in a 20 pM PhiX Internal Control library:
- Denatured PhiX Internal Control library (2 µl)
- Pre-chilled Library Dilution Buffer (98 µl)

NOTE
The denatured 20 pM PhiX Internal Control library can be stored up to 3 weeks at -25°C to -15°C as single-use aliquots.

Prepare Samples for Sequencing

[_] 1 Proceed with one **DAL** tube for sequencing.

[_] 2 If the **DAL** tube was stored frozen, thaw completely and mix by pipetting up and down.

[_] 3 Add 6 µl of 20 pM PhiX Internal Control to the **DAL** tube.

[_] 4 Pipette up and down 3–5 times to rinse the tip and ensure complete transfer.

[_] 5 Mix the **DAL** tube by vortexing the tube at top speed.

[_] 6 Centrifuge the **DAL** tube at 1000 × g at 20°C for 1 minute.

[_] 7 Incubate the **DAL** tube on a heat block at 96°C for 2 minutes.

[_] 8 After the incubation, invert the **DAL** tube 1–2 times to mix, then immediately place in the ice-water bath.

[_] 9 Keep the **DAL** tube in the ice-water bath for 5 minutes.

NOTE
Perform the heat denaturation step immediately before loading the **DAL** tube into the MiSeqDx reagent cartridge to ensure efficient template loading on the MiSeqDx flow cell.

Load Samples for Sequencing

For details on the steps outlined here, see the *MiSeqDx Instrument Reference Guide (document # 15038353)*.

[_] 1 Use a separate, clean, and empty 1 ml pipette tip to pierce the foil seal over the reservoir on the MiSeqDx Reagent Cartridge - CF 139-Variant Assay labeled **Load Samples**.

[_] 2 Pipette 600 µl of the sample DAL libraries into the **Load Samples** reservoir. Take care to avoid touching the foil seal while dispensing the sample.
Check for air bubbles in the reservoir after loading sample. If air bubbles are present, gently tap the cartridge on the bench to release the bubbles.

[_] 3 Log in to the MiSeq Operating Software (MOS).
MiSeqDx Serial Number: _____
Date of Last Preventive Maintenance: _____

[_] 4 Select **Sequence**.
A series of run setup screens open.

[_] 5 Clean the flow cell.

[_] 6 Load the flow cell.

[_] 7 Empty the waste bottle and load the MiSeqDx SBS Solution (PR2) - CF 139-Variant Assay bottle.

[_] 8 Load the reagent cartridge.

[_] 9 Confirm the run settings and results of the pre-run check.

[_] 10 Start the run.
Run ID: _____

[_] 11 Perform a post-run wash.

Comments

_____

_____

_____

MiSeqDx Cystic Fibrosis 139-Variant Assay

Lab Tracking Form

Date/Time: _____ Operator: _____

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