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**REVISION HISTORY:**

| Document No. | Version | Description of Revision | Effective Date |
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| MCCRD-SOP0009 | 2.0 | Formatting corrected. Signature approvals and a Revision History section, and method of deviation documentation added to beginning of document. | 10/18/2018 |
| MCCRD-SOP0009 | 1.0 | Original Release | 12/1/2014 |

## 1.0 PURPOSE/SCOPE

This Standard Operating Procedure (SOP) describes the steps necessary to configure a run, prepare reagents, and load a flow cell clustered with PDX library pools for sequencing on the Illumina HiSeq 2500. This protocol includes procedures for HiSeq maintenance and water washes, sample sheet generation, reagents preparation and loading, software run configuration, and loading a flow cell to begin sequencing. This SOP is intended for processing 8 lanes of samples per flow cell. Each HiSeq is capable of sequencing 2 flow cells simultaneously. **This SOP is for research purposes only and no clinical samples will be processed using this SOP.**

## 2.0 REFERENCE DOCUMENTATION

| Document Number/Name | Title |
|---|---|
| 15035786 v01 | HiSeq 2500 System Guide<br>http://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/hiseq2500/hiseq-2500-system-guide-15035786-01.pdf |

## 3.0 BACKGROUND

The HiSeq 2500 is an ultra-high-throughput sequencing system that allows RNA and whole exome sequencing of PDX samples using Illumina's SBS (sequencing by synthesis) technology. PDX samples are sequenced using the HiSeq v4 high output run parameters.

## 4.0 DEFINITIONS

| Abbreviation/Term | Definition |
|---|---|
| HCS | HiSeq Control Software |

## 5.0 EQUIPMENT

| Description | Model # | Vendor |
|---|---|---|
| Single Channel Pipettes (p200, 1000) | Variable | Rainin |
| Advanced High Volume Plate Stirrer | 12621-056 | VWR Scientific |
| SCIENCEWARE® Carboys with Spigot, Bel-Art, 8L | 118470020 | VWR Scientific |
| VWR® Circulus™ Magnetic Stir Bar, Red | 58947-907 | VWR Scientific |
| Argos Technologies Omega Single-Channel Pipet Controller | 03-391-253 | Fisher Scientific |
| HiSeq 2500 | HiSeq 2500 | Illumina |

## 6.0 REAGENTS/MATERIALS

| Description | Product No. | Vendor |
|---|---|---|
| HiSeq® SBS Kit v4 (250 cycles) | FC-401-4003 | Illumina |
| 200-1000 µL Aerosol Barrier Pipette Tips | 30389213 | Rainin |
| 20-200 µL Aerosol Barrier Pipette Tips | 30389240 | Rainin |
| ProClin 300 | 48912-U | Sigma-Aldrich |
| Kimwipes™ Delicate Task Wipers, 1-Ply | 06-666 | Fisher Scientific |
| Centrifuge tubes, 250 mL | 430776 | Corning |

| Description | Product No. | Vendor |
|---|---|---|
| Conical tubes, 15 mL | 430052 | Corning |
| Tween 20 | BP337-100 | Fisher Scientific |
| Lens paper 6x8 inches | 52845-009 | VWR Scientific |
| Sterile isopropanol wipes prep pads, 70% | 22-363-750 | Fisher Scientific |
| Disposable Standard Serological Pipets, 25 mL capacity | 13-678-14B | Fisher Scientific |
| Disposable Standard Serological Pipets, 10 mL capacity | 13-678-12E | Fisher Scientific |
| Forceps | 609T | Lerloy |
| Laboratory grade water 18 M Ohm | n/a | n/a |

## 7.0 AUTOMATION METHODS

The cBot is pre-programmed. The user must load the instrument with the necessary reagents and clustered flow cell(s) and select the appropriate settings to be used.

## 8.0 SAFETY

8.1 Lab coats, safety glasses, gloves must be worn at all times when handling hazardous or sensitive equipment, samples, reagents, and materials. These safety measures must also be followed when in close proximity to those who are working with these items.

8.2 The paired-end reagents contain formamide, a probable reproductive toxin. Wear protective equipment when handling. Handle used reagents as chemical waste and discard in accordance with governmental safety standards.

## 9.0 ASSAY GUIDELINES

9.1 Thaw frozen reagents in a water bath at room temperature and then store on ice during preparation. Alternatively, sequencing reagents can be thawed for about 16 hours at 4°C.

*Note:* If thawing reagents using a water bath, thaw CRM in a separate water bath. Always replace gloves after handling CRM. Once thawed, store CRM separately on ice.

*Note:* Protect IRM from light during and after thawing.

9.2 Library pool samples are loaded for clustering at a concentration of 13.25pM with a 1% PhiX spike-in. This results in a cluster density of approximately 1050 K/mm$^2$ when sequenced on a HiSeq 2500.

9.3 A maintenance wash and water wash must be performed within 24 hours of starting a sequencing run. The HCS will not allow you to start a sequencing run if the wash has not been completed within the past 24 hours.

9.4 Two flow cells may be loaded and sequenced at the same time on a single HiSeq 2500. Separate reagents must be prepared for each flow cell.

## 10.0 PROCEDURE

### 10.1 Start the HiSeq 2500

10.1.1 Start the instrument control computer.

10.1.2 If necessary, log onto the operating system.

10.1.3 Turn on the main power switch to the On position. If you are facing the front of the instrument, the power switch is on the left side.

10.1.4 Wait for the instrument drive called DoNotEject to initialize.

*Note:* Never eject this drive—it contains hardware configuration files.

10.1.5 Copy the Illumina Maintenance folder onto the computer's Desktop from drive D:

10.1.6 Right click on drives D: and E: and Quick Format them

10.1.7 Copy the Illumina Maintenance Folder from the Desktop back onto drive D:

10.1.8 If necessary, reconnect network drive where data files will be directed.

10.1.9 Start the HiSeq Control Software.

*Note:* The HCS Welcome Screen is split into 2 panels, one for flow cell A and 1 for flow cell B. You can run a set up for both flow cells in parallel using the software interface

*Note:* The HCS provides commands to being a sequencing run, wash the instrument, perform a system check, and change modes. The current mode appears at the top of the screen. When a run is complete, the software returns to the Welcome Screen.

### 10.2 Perform a maintenance wash

10.2.1 Prepare maintenance wash solution.

*Note:* The prepared maintenance wash solution can be stored for up to 30 days at room temperature.

10.2.1.1 Prepare 250 mL of 10% Tween 20 by combining 225 mL of laboratory-grade water with 25 mL Tween 20.

10.2.1.2 Place a stir bar in an empty carboy.

10.2.1.3 Combine 750 mL laboratory-grade water, 250 mL 10% Tween 20, and 1.5 mL ProClin 300 in the carboy.

10.2.1.3.1 These volumes result in a 2.5% Tween 20, 0.15% ProClin 300 solution

10.2.1.4 Place the carboy on the stir plate and stir until thoroughly mixed.

10.2.1.5 Add 4 L laboratory-grade water to the solution.

10.2.1.5.1 These volumes result in a 0.5% Tween 20, 0.03% ProClin 300 wash solution.

10.2.1.6 Stir until solution is thoroughly mixed.

10.2.1.7 Store maintenance wash solution at room temperature until ready to use.

10.2.2 Prepare the HiSeq 2500 for a maintenance wash and start the wash.

10.2.2.1 From the Welcome Screen select Wash→Maintenance

10.2.2.2 Select Yes to wash PE reagent positions.

10.2.2.3 Select Next.

10.2.2.4 Fill a 250 mL centrifuge bottle with 250 mL maintenance wash solution for each SBS reagent position in the HiSeq. Screw a funnel cap onto each bottle. 8 bottles will be needed for each side of the HiSeq.

10.2.2.5 Fill a 15 mL conical tube with 12 mL maintenance wash solution for each paired end position in the HiSeq. Do not place lids on the tubes. 10 tubes will be needed for each side of the HiSeq.

10.2.2.6 Load the bottles and tubes onto the instrument in the assigned reagent rack positions. Lower the sippers into the tubes and close the door to the reagent compartment on the HiSeq.

10.2.2.7 Empty the waste bottle.

10.2.2.8 Select the Wash solution loaded and template loading station closed checkbox, then select Next.

10.2.2.9 Remove the front and back gaskets from both flow cell positions on the HiSeq.

10.2.2.10 Wipe down the flow cell stage with an isopropanol wipe and allow to dry completely.

10.2.2.11 Place new gaskets on the front and back slots of the flow cell holders.

10.2.2.12 Load a used flow cell into both flow cell holders and engage the vacuum.

10.2.2.13 Make sure the Vacuum engaged checkbox is selected and select Next.

10.2.2.14 Select Next to start the wash.

10.2.2.15 When the wash is complete, select Return to Start.

10.3 **Perform a Water Wash.**

10.3.1 From the welcome screen, select Wash→Water

10.3.2 Select Yes to wash paired end reagent positions, then select Next.

10.3.3 Load the instrument with laboratory-grade water

10.3.3.1 Fill a 15 mL conical tube with 12 mL water for each paired end position in the HiSeq. Do not place lids on the tubes. 10 tubes will be needed for each side of the HiSeq.

10.3.3.2 Fill a 15 mL conical tube with 12 mL water for each paired end position in the HiSeq. Do not place lids on the tubes. 10 tubes will be needed for each side of the HiSeq.

10.3.3.3 Load the bottles and tubes onto the instrument in the assigned reagent rack positions. Lower the sippers into the tubes and close the door to the reagent compartment on the HiSeq.

10.3.4 Empty the waste bottle.

10.3.5 Make sure a used flow cell is loaded.

10.3.6 Select Next

10.3.7 Perform a fluidics check:

10.3.7.1 Select solution 2 from the drop-down list. Accept the default pump values.

10.3.7.2 Inspect the flow cell for bubbles passing through the lanes and leaks near the gaskets.

10.3.7.3 Separate the fluidics waste lines into 15 mL conical tubes.

10.3.7.4 Select Next to start the water wash. Approximate run time is 60 minutes.

10.3.7.5 When the wash is complete check void volume consistency across all tubes.

10.3.7.6 Return the tubing to the waste bottle.

10.4 **Create a Sample Sheet**

10.4.1 Using the Sample Sheet template csv file on the S drive as a guide, enter sample information for the flow cell.

10.4.2 The PDM IDs should be used in the Sample ID column on the Sample Sheet file.

10.4.3 Only dashes, underscores, and alphanumeric names are allowed. **No spaces are allowed.**

10.4.4 Ensure that there are not hidden empty characters on the sheet.

10.4.5 Save the file in the desired location.

10.4.6 File name should be flow cell ID.

10.5 **Enter flow cell information on the Flow Cell master file**

10.5.1 Enter sample and flow cell information on the Flow Cell Master file on the S drive.

10.6 **Enter run parameters**

10.6.1 From the HCS Welcome Screen, select Sequence→New Run.

10.6.2 Follow the on-screen prompts to configure the run

10.6.2.1 Integration Screen

10.6.2.1.1 Select None to proceed without using BaseSpace.

10.6.2.1.2 Select Next

10.6.2.2 Storage Screen

10.6.2.2.1 Select the Save to an output folder checkbox and select Browse to navigate to the desired network location.

10.6.2.2.2 Select Zip to BCL files? YES

10.6.2.2.3 Select Bin Q Scores? YES

10.6.2.2.4 In Save Auxiliary Files options select Save All Thumbnails

10.6.2.2.5 Select Next

10.6.2.3 Flow Cell Setup Screen

10.6.2.3.1 Scan the flow cell barcode of the flow cell to be sequenced.

10.6.2.3.2 Confirm that the flow cell type is HiSeq Flow Cell v4. Flow cell type should be selected automatically based on the flow cell ID.

10.6.2.3.3 Enter an experiment name.

10.6.2.3.4 Enter a user name.

10.6.2.3.5 Select Next.

10.6.2.4 Advanced Screen

10.6.2.4.1 Do not check the box next to Confirm the First Base.

10.6.2.4.2 Check the box next to each lane for Align to PhiX.

10.6.2.4.3 Select Next.

10.6.2.5 Recipe Screen

10.6.2.5.1 For Index Type select Custom

10.6.2.5.2 Select Paired End for Flow Cell Format

10.6.2.5.3 Enter the number of cycles for Read 1, Read 2 and the Index Reads

10.6.2.5.3.1 Read 1: 125

10.6.2.5.3.2 Index 1: 9 (Exome) or 7 (RNASeq)

10.6.2.5.3.3 Index 2: 0

10.6.2.5.3.4 Read 2: 125

10.6.2.6 Confirm default chemistry settings. Fields will be auto-populated depending on the selected index option type:

10.6.2.6.1 SBS: HiSeq SBS Kit v4

10.6.2.6.2 Index: HiSeq v4 Single Index

10.6.2.6.3 PE turnaround: HiSeq PE Cluster kit v4

10.6.2.7 Do not check the box next to Use Existing Recipe. Allow the software to create the recipe using the run parameters.

10.6.2.8 Sample Sheet Screen

10.6.2.8.1 Select Browse to navigate to the location where the sample sheet was stored.

10.6.2.8.2 Select Next.

10.6.2.9 Reagents Screen

10.6.2.9.1 Scan the SBS reagent kit ID. The kit ID is used to determine reagent kit type and run mode compatibility.

10.6.2.9.2 Scan the Paired-End Cluster reagent kit ID.

10.6.2.9.3 Select 250 cycles as the SBS reagent kit for the run

10.6.2.9.4 Select Prime SBS Reagents to prime reagents before starting a run. Always prime reagents before loading a new flow cell.

10.6.2.9.5 Select Next

10.6.2.10 Review Screen

10.6.2.10.1 Review the run parameters on the Review Screen to ensure no entry errors were made.

10.6.2.10.2 Select Next to proceed or Back to change parameters.

10.7 **Load and prime reagents**

10.7.1 Load SBS reagents

10.7.1.1 Invert each reagent bottle to mix.

*Note:* After handling the bottle of CRM, discard gloves and replace them.

10.7.1.2 Place each reagent bottle onto the rack in the associated number position.

10.7.1.3 Place a reagent bottle containing PW1 into position 2. This bottle is not included with the SBS reagents.

Table 1 SBS Reagent Positions

| Position | Reagent | Description |
|---|---|---|
| 1 | IRM | Incorporation Reagent Master Mix |
| 2 | PW1 | 25 ml of PW1 or laboratory-grade water |
| 3 | USM | Universal Scan Mix |
| 4 | SBS Buffer 1 (SB1) | High Salt Buffer |
| 5 | SBS Buffer 2 (SB2) | Incorporation Wash Buffer |
| 6 | SBS Buffer 2 (SB2) | Incorporation Wash Buffer |
| 7 | CRM | Cleavage Reagent Mix |
| 8 | SBS Buffer 3 (SB3) | Cleavage Buffer |

10.7.1.4 Remove the cap from each reagent bottle and replace it with a funnel cap.

*Note:* Handle the bottle of CRM last, after you have loaded all other reagents, to prevent cross-contamination. Always replace gloves after handling CRM.

10.7.1.5 Open the reagent compartment door.

10.7.1.6 Raise the sippers, slide the reagent rack into the reagent compartment, and lower the sippers.

10.7.1.7 Select the PW1 (25 mL) loaded checkbox.

10.7.2 Load Indexing Reagents

10.7.2.1 Invert each reagent tube to mix.

10.7.2.2 Remove caps and place each reagent tube onto the rack in the associated number position.

Table 3 Paired-End Flow Cells

| Position | Reagent | Description |
|---|---|---|
| 10 | FRM* | Fast Resynthesis Mix |
| 15 | FDR | Fast Denaturation Reagent (contains formamide) |
| 17 | HP12 | Index Sequencing Primer i7 |

10.7.3 Load Paired End Reagents

10.7.3.1 Invert each reagent tube to mix

10.7.3.2 Remove caps and place each reagent tube onto the rack in the associated number position.

Table 4 Paired-End Flow Cell

| Position | Reagent | Description |
|---|---|---|
| 10 | FRM* | Fast Resynthesis Mix |
| 11 | FLM2 | Fast Linearization Mix 2 |
| 13 | AMS | Fast Amplification Mix |
| 14 | FPM | Fast Amplification Premix |
| 15 | FDR* | Fast Denaturation Reagent (contains formamide) |
| 16 | HP11 | Read 2 Sequencing Primer |

*** If you loaded indexing reagents for a single-index run, FRM is already loaded in position 10.**

10.7.3.3 Place 15 mL conicals filled with PW1 in unused positions 12, 18 and 19.

10.7.3.4 Slide the reagent rack into the reagent compartment.

10.7.3.5 Lower the sippers into the paired-end reagent tubes.

10.7.3.6 Select Next.

10.7.4 Prime Reagents

*Note:* Always use a used flow cell to prime reagents.

10.7.4.1 Load a priming flow cell

*Note:* Do not remove or replace the flow cell gaskets during this step.

10.7.4.1.1 Rinse the used priming flow cell with laboratory-grade water. Dry with a lens wipe.

10.7.4.1.2 Using an alcohol wipe, wipe the surface of the flow cell holder. Do not allow alcohol to drip into the vacuum holes or around the manifolds.

10.7.4.1.3 Place the flow cell on the flow cell holder with inlet and outlet ports facing down. Make sure that the arrow on the left edge of the flow cell, which indicates flow direction, points towards the instrument.

10.7.4.1.4 Gently slide the flow cell towards the top and right guide pins until it stops.

10.7.4.1.5 Slowly move the flow cell level to position 1 to engage the vacuum. When the flow cell level is blinking green, the vacuum is engaged.

10.7.4.1.6 Wait for 5 seconds, and slowly move the flow cell level to position 2. When the flow cell level is solid green, the manifolds are in position and the flow cell is ready.

10.7.4.1.7 Make sure the Vacuum Engaged checkbox is selected and select Next.

10.7.4.2 Confirm proper flow

10.7.4.2.1 Select solution 2 (laboratory-grade water, PW1) from the drop-down list.

*Note:* Use water to confirm proper flow on a used flow cell only. Never use water to confirm proper flow on a clustered flow cell.

10.7.4.2.2 Confirm the default values:

10.7.4.2.2.1 Volume: 125

10.7.4.2.2.2 Aspirate rate: 250

10.7.4.2.2.3 Dispense rate: 2000

10.7.4.2.3 Select Pump

10.7.4.2.4 Visually inspect the flow cell for bubbles passing through the lanes and leaks near the manifold. If you see excessive bubbles, check the gaskets for obstructions. If problems persist, remove the flow cell, repeat the cleaning steps and reload the flow cell.

10.7.4.3 Position tubing and start prime

10.7.4.3.1 Remove the 8 lines of waste tubing for each flow cell from the waste container.

10.7.4.3.2 Place each waste tubing into an empty 15 mL conical, 1 conical per line. Priming waste is collected and measured after the priming step.

10.7.4.3.3 Select Start Prime

10.7.4.3.4 When priming step is complete, measure the waste in each conical. Volume in each tube should be 2 mL.

10.7.4.3.5 Return the waste tubing to the waste container.

10.7.4.3.6 Select Next

10.8 **Load a flow cell**

10.8.1 Remove the used flow cell

10.8.1.1 Slowly move the flow cell lever to position 1 to disengage the manifolds.

10.8.1.2 Slowly move the flow cell lever to position 0 to disengage the vacuum seal and release the flow cell.

10.8.1.3 Remove the used flow cell.

10.8.2 Clean the flow cell holder using an isopropanol wipe.

10.8.3 Clean the flow cell

10.8.3.1 Remove the clustered flow cell from the container using forceps

10.8.3.2 Rinse the flow cell with laboratory-grade water and dry with lens paper.

10.8.3.3 Protect the flow cell from dust until you are ready to load it onto the instrument.

10.8.4 Load the sequencing flow cell

*Note:* Do not replace the manifold gaskets. Replace the manifold gaskets before the maintenance wash.

10.8.4.1 Place the flow cell on the flow cell holder with the inlet and outlet ports facing down and the barcode on the right. Make sure that the arrow on the left edge of the flow cell points towards the instrument.

10.8.4.2 Gently slide the flow cell towards the top and right guide pins until it stops.

10.8.4.3 Slowly move the flow cell level to position 1 to engage the vacuum. When the flow cell level is blinking green, the vacuum is engaged.

10.8.4.4 Wait for 5 seconds, and slowly move the flow cell level to position 2. When the flow cell level is solid green, the manifolds are in position and the flow cell is ready.

10.8.4.5 Make sure the Vacuum Engaged checkbox is selected and select Next.

10.8.5 Confirm proper flow

10.8.5.1 Select solution 5 from the drop-down list

10.8.5.2 Enter the following default values:

10.8.5.2.1 Volume: 250

10.8.5.2.2 Aspirate Rate: 250

10.8.5.2.3 Dispense Rate: 2000

10.8.5.3 Select Pump

10.8.5.4 Visually inspect the flow cell for bubbles passing through the lanes or leaks near the manifolds. If bubbles are present, check the manifold gaskets for

obstructions and repeat the pump process using solution 6 to avoid depleting solution 5. Reduce the aspirate rate to 100, and pump another 250 uL to the flow cell.

10.8.5.5 Select Next. Ensure that the flow cell lever is green and close the flow cell compartment door.

10.8.5.6 Confirm that the checkboxes Vacuum Engaged and Door Closed are selected, and select Next

10.8.5.7 Select Start to start the sequencing run.