Infinium HD Methylation Assay Automated Workflow Checklist

Convert DNA

- ☐ 1 Follow the instructions in the Zymo EZ DNA Methylation Kit to denature the genomic DNA and add conversion reagent.
- ☐ 2 Incubate in a thermal cycler using the following settings for 16 cycles:
  - 95°C for 30 seconds
  - 50°C for 1 hour
- ☐ 3 Hold DNA at 4°C for 10 minutes until cleanup.
- ☐ 4 Use the instructions in the Zymo EZ DNA Methylation Kit to cleanup the conversion reagent.

SAFE STOPPING POINT

If you are stopping, heat-seal the plate, and store at -25°C to -15°C for up to 30 days.

Amplify DNA

- ☐ 1 Add BCD DNA into either of the following to create a BCD plate:
  - Midi plate: 20 µl to each BCD well
  - TCY plate: 10 µl to each BCD well
- ☐ 2 Select **MSA4 Tasks | Make MSA4**.
- ☐ 3 Select the BCD plate type.
- ☐ 4 Enter the **Number of DNA plates**.
- ☐ 5 Place the MA1, RPM, and MSM tubes in the robot tube rack.
- ☐ 6 Pour 15 ml NaOH into a trough and place on the robot bed.
- ☐ 7 Place BCD and MSA4 plates on robot bed.
- ☐ 8 Select **Run**.
- ☐ 9 Enter the barcode of each BCD plate.
- ☐ 10 Place the BCD plates on the robot bed and select **OK**.
- ☐ 11 Vortex the MSA4 plate at 1600 rpm for 1 minute.
- ☐ 12 Centrifuge at 280 × g.
- ☐ 13 Remove the cap mat, place the MSA4 plate on the robot bed, and select **OK**.
- ☐ 14 When complete, select **OK**.
- ☐ 15 Remove and seal the MSA4 plate.
- ☐ 16 Invert the MSA4 10 times to mix.
- ☐ 17 Centrifuge at 280 × g.

Incubate DNA

- ☐ 1 **[LIMS]** Select **Infinium HD Methylation | Incubate MSA4**
  - ☐ a Scan the barcodes.
- ☐ 2 Incubate the MSA4 plate for 20–24 hours at 37°C.

Fragment DNA

- ☐ 1 Pulse centrifuge the MSA4 plate at 280 × g.
- ☐ 2 Select **MSA4 Tasks | Fragment MSA4**.
- ☐ 3 Place the MSA4 plate on the robot bed.
- ☐ 4 Place FMS tubes in the robot tube rack.
- ☐ 5 Select **Run**.
- ☐ 6 When complete, select **OK**.
- ☐ 7 Remove the plate and seal with a cap mat.
- ☐ 8 Vortex at 1600 rpm for 1 minute.
- ☐ 9 Pulse centrifuge at 280 × g.
- ☐ 10 Incubate on the 37°C heat block for 1 hour.

SAFE STOPPING POINT

If you are stopping, seal the plate(s), and store at -25°C to -15°C.

Precipitate DNA

- ☐ 1 Select **MSA4 Tasks | Precip MSA4**.
- ☐ 2 Place the MSA4 plate on the robot bed.
- ☐ 3 Place a half reservoir in the frame, and add PM1 as follows:
  - For 48 samples, add 1 tube PM1
  - For 96 samples, add 2 tubes PM1
- ☐ 4 Place a full reservoir in the frame, and add 2-propanol as follows:
  - For 48 samples, add 20 ml 2-propanol
  - For 96 samples, add 40 ml 2-propanol
- ☐ 5 Select **Run**.
- ☐ 6 Remove the MSA4 plate from the robot bed. Do not select **OK**.
- ☐ 7 Vortex at 1600 rpm for 1 minute.
- ☐ 8 Incubate at 37° C on the heat block for 5 minutes.
- ☐ 9 Centrifuge at 280 × g for 1 minute.
- ☐ 10 Set the centrifuge at 4°C.
- ☐ 11 Place the MSA4 plate on the robot bed.
- ☐ 12 Select **OK**.
- ☐ 13 Remove the MSA4 plate from the robot bed and seal.
- ☐ 14 Invert 10 times to mix.
- ☐ 15 Incubate at 4°C for 30 minutes.
- ☐ 16 Place in the centrifuge.
- ☐ 17 Centrifuge at 3000 × g for 20 minutes.
- ☐ 18 Remove MSA4 plate.
- ☐ 19 Make sure that a blue pellet is present.
- ☐ 20 Remove and discard the cap mat.
- ☐ 21 Quickly invert the plate and drain the supernatant.
- ☐ 22 Firmly tap until all wells are free of liquid.
- ☐ 23 Place the plate on a tube rack for 1 hour at room temperature.
- ☐ 24 Make sure that a blue pellet is still present.

SAFE STOPPING POINT

If you are stopping, seal the plate(s), and store at -25°C to -15°C.

Resuspend DNA

- ☐ 1 Select **MSA4 Tasks | Resuspend MSA4**.
- ☐ 2 Place the MSA4 plate on the robot bed.
- ☐ 3 Place a quarter reservoir in the frame, and add RA1 as follows:
  - For 48 samples, add 4.5 ml RA1
  - For 96 samples, add 9 ml RA1
- ☐ 4 Select **Run**.
- ☐ 5 Select **OK**.
- ☐ 6 Remove the MSA4 plate from the robot deck.
- ☐ 7 Apply a foil seal to the MSA4 plate.
- ☐ 8 Incubate at 48°C for 1 hour.
- ☐ 9 Vortex at 1800 rpm for 1 minute.
- ☐ 10 Make sure that the pellets are resuspended.
- ☐ 11 Pulse centrifuge at 280 × g.

SAFE STOPPING POINT

If you are stopping, store sealed MSA4 plate(s) at 2°C to 8°C for up to 24 hours. If more than 24 hours, store at -25°C to -15°C.

Store sealed RA1 at -25°C to -15°C. If RA1 will be used the next day, seal it, and store it overnight at 4°C.

Hybridize DNA to the BeadChip

- ☐ 1 Incubate the MSA4 plate at 95° C on the heat block for 20 minutes.
- ☐ 2 Cool at room temperature for 30 minutes.
- ☐ 3 Pulse centrifuge at 280 × g.
- ☐ 4 Place the gasket into the hybridization chamber.
- ☐ 5 Add 400 µl PB2 into each reservoir.
- ☐ 6 Place the hybridization chamber insert into the hybridization chamber.
- ☐ 7 Immediately cover the chamber with the lid.
- ☐ 8 **[LIMS]** Select Select **Infinium HD Methylation | Confirm for Hyb**.
- ☐ 9 **[LIMS]** Scan the barcodes.
- ☐ 10 Remove all BeadChips from packaging.
- ☐ 11 Place BeadChips into the robot BeadChip alignment fixtures.
- ☐ 12 Select **task name on UI Tasks | Hyb-Multi BC2**.
- ☐ 13 Place the robot BeadChip alignment fixtures onto the robot deck.
- ☐ 14 Pulse centrifuge the MSA4 plate at 280 × g.
- ☐ 15 Place the MSA4 plate onto the robot deck.
- ☐ 16 Select **Run**.
- ☐ 17 Place each robot tip alignment guide on top of each robot BeadChip alignment fixture.
- ☐ 18 To start the run, select **OK**.
- ☐ 19 When complete, select **OK**.
- ☐ 20 Remove the robot BeadChip alignment fixtures.
- ☐ 21 Place each BeadChip in a hybridization chamber insert.
- ☐ 22 Place the lid on the chamber and secure with the metal clamps.
- ☐ 23 **[LIMS]** Select **Infinium HD Methylation | Prepare Hyb Chamber**.
  - ☐ a Scan the barcodes.
- ☐ 24 Incubate at 48°C for 16–24 hours.

Prepare for Next Day

- ☐ 1 Add 330 ml fresh 100% EtOH to the XC4 bottle.
- ☐ 2 Leave the bottle upright on the lab bench overnight.
- ☐ 3 Soak the robot tip alignment guides in 1% aqueous Alconox solution.
- ☐ 4 Rinse and dry the robot tip alignment guides.

Wash BeadChips

- ☐ 1 Submerge the wash rack in the PB1 wash.
- ☐ 2 Remove the hybridization insert.
- ☐ 3 Remove the BeadChips.
- ☐ 4 Remove the cover seals from the BeadChips.
- ☐ 5 Place the BeadChips into the submerged wash rack.
- ☐ 6 Move the wash rack up and down for 1 minute.
- ☐ 7 Move the wash rack to the next PB1 Wash.
- ☐ 8 Move the wash rack up and down for 1 minute.
- ☐ 9 Confirm that you are using the correct Infinium glass back plates and spacers.
- ☐ 10 Fill the BeadChip alignment fixture with 150 ml PB1.
- ☐ 11 For each BeadChip, place one black frame into the BeadChip alignment fixture.
- ☐ 12 Place each BeadChip into a black frame.
- ☐ 13 Place a ***clear*** spacer onto the top of each BeadChip.
- ☐ 14 Place the alignment bar onto the alignment fixture.
- ☐ 15 Place a clean glass back plate on top of each clear spacer.
- ☐ 16 Secure each flow-through chamber assembly with metal clamps.
- ☐ 17 Remove the assembled flow-through chamber from the alignment fixture.
- ☐ 18 Trim the spacers from each end of the assembly.
- ☐ 19 Leave assembled flow-through chambers on the lab bench.
- ☐ 20 Wash the hybridization chamber reservoirs with DI H_2O.

Extend and Stain BeadChips

- ☐ 1 Fill the water circulator.
- ☐ 2 Select **Robot QC Tasks | Circulator Manager** to set to 44°C.
- ☐ 3 Select **XStain Tasks | XStain HD BeadChip.**
- ☐ 4 Turn on the iScan systems.
- ☐ 5 Add the following reagents to reservoirs:

Reagent	# BeadChips	Volume
95% formamide/1 mM EDTA	1–8	15 ml
	9–16	17 ml
	17–24	25 ml
RA1	1–8	10 ml
	9–16	20 ml
	17–24	30 ml
XC3	1–8	50 ml
	9–16	100 ml
	17–24	150 ml

- ☐ 6 Invert the XC1, XC2, TEM, STM, and ATM tubes to mix. Remove the caps, and place on the robot deck.
- ☐ 7 Enter the number of BeadChips.
- ☐ 8 Select **Run**.
- ☐ 9 **[Non-LIMS]** Enter the stain temperature listed on the STM tube.
- ☐ 10 Place the flow-through chambers into the chamber rack.
- ☐ 11 Select **OK**.
- ☐ 12 Remove the flow-through chambers from the chamber rack.
- ☐ 13 Select **OK**.
- ☐ 14 Set up two top-loading wash dishes labeled PB1 and XC4.
- ☐ 15 Add 310 ml PB1 to the PB1 wash dish.
- ☐ 16 Submerge the staining rack in the wash dish.
- ☐ 17 Leave the staining rack in the wash dish.
- ☐ 18 Disassemble each flow-through chamber.
- ☐ 19 Place the BeadChips into the submerged staining rack.
- ☐ 20 Slowly lift the staining rack 10 times.
- ☐ 21 Soak for 5 minutes.
- ☐ 22 Vigorously shake the XC4 bottle.
- ☐ 23 Add 310 ml XC4 to the XC4 wash dish and cover.
- ☐ 24 Transfer the staining rack from the PB1 to the XC4.
- ☐ 25 Slowly lift the staining rack 10 times.
- ☐ 26 Soak for 5 minutes.
- ☐ 27 Remove the staining rack and place it onto the tube rack.
- ☐ 28 Dry each BeadChip as follows.
  - ☐ a Grip the BeadChip by the barcode end.
  - ☐ b Place onto a tube rack with the barcode facing up and toward you.
- ☐ 29 Place the tube rack into the vacuum desiccator.
- ☐ 30 Dry the BeadChips for 50–55 minutes at 675 mm Hg (0.9 bar).
- ☐ 31 **[LIMS]** Select **Infinium HD Methylation | Coat BC2**.
  - ☐ a Scan the barcodes.