Infinium HD Methylation Assay Manual 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.

Create the BCD Plate

- ☐ 1 If frozen, thaw BCD samples to room temperature and vortex to mix.
- ☐ 2 Apply a BCD barcode label to a new 0.8 ml midi plate or a new 0.2 ml TCY plate.
- ☐ 3 Transfer the BCD to the plate as follows
  - Midi plate: 20 µl BCD sample to each well (requires ≥ 1000 ng input in bisulfite conversion)
  - TCY plate: 10 µl BCD sample to each well (requires ≥ 500 ng input in bisulfite conversion)

Amplify DNA

- ☐ 1 Add 20 µl MA1 to each well.
- ☐ 2 Transfer 4 µl DNA sample from the BCD plate to the MSA4 plate.
- ☐ 3 Add 4 µl 0.1N NaOH in to each well.
- ☐ 4 Seal the MSA4 plate with the 96-well cap mat.
- ☐ 5 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
- ☐ 6 Incubate at room temperature for 10 minutes.
- ☐ 7 Add 68 µl RPM in to each well.
- ☐ 8 Add 75 µl MSM in to each well.
- ☐ 9 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.

Incubate DNA

- ☐ 1 Incubate the MSA4 plate for 20–24 hours at 37°C.

Fragment DNA

- ☐ 1 Pulse centrifuge the plate at 280 × g.
- ☐ 2 Add 50 µl FMS to the MSA4 plate.
- ☐ 3 Vortex at 1600 rpm for 1 minute, and then centrifuge at 280 × g for 1 minute.
- ☐ 4 Incubate at 37° C for 1 hour.

SAFE STOPPING POINT

If you are stopping, seal the plate, and store at -25°C to -15°C for up to 24 hours.

Precipitate DNA

- ☐ 1 Add 100 µl PM1 to the MSA4 plate.
- ☐ 2 Reseal with the cap mat.
- ☐ 3 Vortex the plate at 1600 rpm for 1 minute.
- ☐ 4 Incubate at 37° C for 5 minutes.
- ☐ 5 Pulse centrifuge at 280 × g for 1 minute.
- ☐ 6 Set the centrifuge at 4°C.
- ☐ 7 Remove and discard the cap mat.
- ☐ 8 Add 300 µl 100% 2-propanol to each well.
- ☐ 9 Apply fresh cap mats.
- ☐ 10 Invert the plate 10 times to mix.
- ☐ 11 Incubate in a refrigerator set at 4°C for 30 minutes.
- ☐ 12 Centrifuge at 3000 × g at 4°C for 20 minutes.
- ☐ 13 Immediately remove the plate from the centrifuge.
- ☐ 14 Make sure that a blue pellet is present.
- ☐ 15 Remove and discard the cap mat.
- ☐ 16 Quickly invert the plate and drain the supernatant.
- ☐ 17 Firmly tap until all wells are free of liquid.
- ☐ 18 Place the plate on the tube rack for 1 hour at room temperature.
- ☐ 19 Make sure that a blue pellet is still present.

SAFE STOPPING POINT

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Resuspend DNA

- ☐ 1 Add 46 µl RA1 per well.
- ☐ 2 Apply a foil heat seal.
- ☐ 3 Incubate at 48°C for 1 hour.
- ☐ 4 Vortex at 1800 rpm for 1 minute.
- ☐ 5 Pulse centrifuge at 280 × g.

SAFE STOPPING POINT

If you are stopping, seal the plate, and store at -25°C to -15°C for up to 24 hours.

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 to the top and bottom wells.
- ☐ 6 Immediately cover the chamber with the lid.
- ☐ 7 Remove the BeadChips from packaging.
- ☐ 8 Place each BeadChip into an insert.
- ☐ 9 Transfer 26 µl each sample to the BeadChip.
- ☐ 10 Wait for the DNA to disperse.
- ☐ 11 Inspect the loading port for excess liquid.
- ☐ 12 If excess liquid is not present, add leftover sample.
- ☐ 13 Store RA1 at -25°C to -15°C.
- ☐ 14 Load the inserts into the hybridization chamber.
- ☐ 15 Place the lid on the chamber and secure with the metal clamps.
- ☐ 16 Place the chamber into the preheated Illumina Hybridization Oven.
- ☐ 17 Incubate at 48°C for 16–24 hours.
- ☐ 18 Store RA1 at -25°C to -15°C.

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.

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 Turn on the water circulator and set the temperature to 44°C.
- ☐ 3 When the chamber rack reaches 44°C, place the flow-through chamber assemblies into the chamber rack.
- ☐ 4 Fill the reservoir of each flow-through chamber as follows.
  - ☐ a 150 µl RA1. Incubate for 30 seconds. Repeat 4 times.

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  - ☐ b 450 µl XC1. Incubate for 10 minutes.
  - ☐ c 450 µl XC2. Incubate for 10 minutes.
  - ☐ d 200 µl TEM. Incubate for 15 minutes.
  - ☐ e 450 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat once.

    [_] 1 [_] 2
  - ☐ f Incubate 5 minutes.
  - ☐ g Set the the chamber rack temperature to the temperature indicated on the STM tube.
  - ☐ h 450 µl XC3. Incubate for 1 minute. Repeat once.

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- ☐ 5 Wait for the chamber rack to reach the correct temperature.
- ☐ 6 If imaging the BeadChip immediately after the staining process, turn on the scanner.
- ☐ 7 Fill the reservoir of each flow-through chamber as follows.
  - ☐ a 250 µl STM. Incubate for 10 minutes.
  - ☐ b 450 µl XC3. Incubate for 1 minute. Repeat once.

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- ☐ c Wait 5 minutes.
- ☐ d 250 µl ATM. Incubate for 10 minutes.
- ☐ e 450 µl XC3. Incubate for 1 minute. Repeat once.

[_] 1 [_] 2

- ☐ f Wait 5 minutes.
- ☐ g 250 µl STM. Incubate for 10 minutes.
- ☐ h 450 µl XC3. Incubate for 1 minute. Repeat once.

[_] 1 [_] 2

- ☐ i Wait 5 minutes.
- ☐ j 250 µl ATM. Incubate for 10 minutes.
- ☐ k 450 µl XC3. Incubate for 1 minute. Repeat once.

[_] 1 [_] 2

- ☐ l Wait 5 minutes.
- ☐ m 250 µl STM. Incubate for 10 minutes.
- ☐ n 450 µl XC3. Incubate for 1 minute. Repeat once.

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- ☐ o Wait 5 minutes.

☐ 8 Remove the flow-through chambers from the chamber rack.
☐ 9 Set up two top-loading wash dishes labeled PB1 and XC4.
☐ 10 Add 310 ml PB1 to the PB1 wash dish.
☐ 11 Submerge the staining rack in the wash dish.
☐ 12 Leave the staining rack in the wash dish.
☐ 13 Disassemble each flow-through chamber.
☐ 14 Place the BeadChips into the submerged staining rack.
☐ 15 Slowly move the staining rack up and down 10 times.
☐ 16 Soak for 5 minutes.
☐ 17 Vigorously shake the XC4 bottle.
☐ 18 Add 310 ml XC4 to the XC4 wash dish and cover.
☐ 19 Transfer the staining rack to the XC4 wash dish.
☐ 20 Slowly lift the staining rack up and down 10 times.
☐ 21 Soak for 5 minutes.
☐ 22 Remove the staining rack and place it onto the tube rack.
☐ 23 Place the tube rack into the vacuum desiccator.
☐ 24 Dry the BeadChips for 50–55 minutes at 675 mm Hg (0.9 bar).

SAFE STOPPING POINT

Store the BeadChips in the Illumina BeadChip Slide Storage Box at room temperature. Scan within 72 hours.