Infinium HTS Methylation Assay Automated Workflow Checklist

illumina

Convert DNA

1. Add 130µl of Lightning Conversion Reagent to 20 µl of a DNA sample in a conversion plate.
2. Incubate in a thermal cycler using the following settings for 16 cycles:
   - 98°C for 8 minutes
   - 54°C for 1 hour
3. Hold DNA at 4°C for 10 minutes until cleanup.
4. Use the instructions in the Zymo EZ-96 DNA Methylation-Lightning MagPrep 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
   - TCY plate: 10 µl BCD sample to each well

Amplify DNA

1. Add DNA into either of the following to create a DNA plate:
   - Midi plate: 20 µl to each DNA well
   - TCY plate: 10 µl to each DNA well
2. Select **MSA4 Tasks | Make MSA4**.
3. Select the DNA 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 DNA and MSA4 plates on robot bed.
8. Select **Run**.
9. Place the DNA plates on the robot bed and select **OK**.
10. Vortex the sealed MSA4 plate at 1600 rpm for 1 minute.
11. Centrifuge at 280 × g.
12. Remove the cap mat, place the MSA4 plate on the robot bed, and select **OK**.
13. When complete, select **OK**.
14. Remove and seal the MSA4 plate.
15. Centrifuge at 280 × g.
16. Invert the MSA4 plate 10 times to mix.

Incubate DNA

1. **[LIMS]** Select
   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 HTS 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. Place the robot BeadChip alignment fixtures onto the robot deck.
13. Pulse centrifuge the MSA4 plate at 280 × g.
14. Place the MSA4 plate onto the robot deck.
15. Select **Run**.
16. Enter the number of BeadChips and the number of MSA4 plates.
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 HTS 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. Vigorously shake to resuspend.
3. Leave the bottle upright on the lab bench overnight.
4. Soak the robot tip alignment guides in 1% aqueous Alconox solution.
5. 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 LCG 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 LCG BeadChip.**
4. If imaging the BeadChip immediately after the staining process, turn on the scanner.
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 LX1, LX2, EML, SML, and ATM tubes to mix. Remove the caps, and place on the robot deck.
7. Enter the number of BeadChips.
8. Select **Run**.
9. Enter the stain temperature listed on the SML 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. Set up two top-loading wash dishes labeled PB1 and XC4.
14. Add 310 ml PB1 to the PB1 wash dish.
15. Submerge the staining rack in the wash dish.
16. Leave the staining rack in the wash dish.
17. Disassemble each flow-through chamber.
18. Place the BeadChips into the submerged staining rack.
19. Slowly lift the staining rack 10 times.
20. Soak for 5 minutes.
21. Vigorously shake the XC4 bottle.
22. Add 310 ml XC4 to the XC4 wash dish and cover.
23. Transfer the staining rack from the PB1 to the XC4.
24. Slowly lift the staining rack 10 times.
25. Soak for 5 minutes.
26. Remove the staining rack and place it onto the tube rack.
27. 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.

28. Place the tube rack into the vacuum desiccator.
29. Dry the BeadChips for 50–55 minutes at 675 mm Hg (0.9 bar).
30. **[LIMS]** Select **Infinium HTS Methylation | Coat BC2**.
    c. Scan the barcodes.