Infinium HD Super Assay Automated Workflow Checklist

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 **MSA1 Tasks | Make MSA1**.
- ☐ 3 Enter the **Number of DNA plates**.
- ☐ 4 Place the MA1, MA2, and MSM tubes in the robot tube rack.
- ☐ 5 Pour 15 ml NaOH into a trough and place on the robot bed.
- ☐ 6 Place DNA and MSA1 plates on robot bed.
- ☐ 7 Select **Run**.
- ☐ 8 Enter the barcode of each DNA plate.
- ☐ 9 Place the DNA plates on the robot bed and select **OK**.
- ☐ 10 Vortex the MSA1 plate at 1600 rpm for 1 minute.
- ☐ 11 Centrifuge at 280 × g at 22°C for 1 minute.
- ☐ 12 Remove the cap mat, place the MSA1 plate on the robot bed, and select **OK**.
- ☐ 13 When complete, select **OK**.
- ☐ 14 Remove and seal the MSA1 plate.
- ☐ 15 Vortex the MSA1 plate at 1600 rpm for 1 minute.
- ☐ 16 Centrifuge at 280 × g for 1 minute.

Incubate DNA

- ☐ 1 Incubate the MSA1 plate for 20–24 hours at 37°C.

Fragment DNA

- ☐ 1 Pulse centrifuge the MSA1 plate at 280 × g.
- ☐ 2 Select **MSA1 Tasks | Fragment MSA1**.
- ☐ 3 Place the MSA1 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 Place 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 **MSA1 Tasks | Precip MSA1**.
- ☐ 2 Pulse centrifuge the sealed plate at 280 × g.
- ☐ 3 Place the MSA1 plate on the robot bed.
- ☐ 4 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
- ☐ 5 Select **Run**.
- ☐ 6 Remove the MSA1 plate from the robot bed. Do not select **OK**.
- ☐ 7 Vortex at 1600 rpm for 1 minute.
- ☐ 8 Incubate on the heat block for 5 minutes.
- ☐ 9 Pulse centrifuge at 280 × g for 1 minute.
- ☐ 10 Set the centrifuge at 4°C.
- ☐ 11 Place the MSA1 plate on the robot bed.
- ☐ 12 Select **OK**.
- ☐ 13 Remove the MSA1 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 MSA1 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 **MSA1 Tasks | Resuspend MSA1**.
- ☐ 2 Place the MSA1 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 Remove the MSA1 plate from the robot deck.
- ☐ 6 Apply a foil seal to the MSA1 plate.
- ☐ 7 Incubate in the Illumina Hybridization Oven for 1 hour.
- ☐ 8 Vortex at 1800 rpm for 1 minute.
- ☐ 9 Make sure that the pellets are resuspended.
- ☐ 10 Pulse centrifuge at 280 × g.

SAFE STOPPING POINT

If you are stopping, store sealed MSA1 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 to BeadChip

- ☐ 1 Incubate the MSA1 plate 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 **[Illumina LIMS]** Select Select **Infinium HD Super | Confirm for Hyb**.
- ☐ 9 **[Illumina 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 MSA1 plate at 280 × g.
- ☐ 14 Place the MSA1 plate onto the robot deck.
- ☐ 15 Select **Run**.
- ☐ 16 Place each robot tip alignment guide on top of each robot BeadChip alignment fixture.
- ☐ 17 To start the run, select **OK**.
- ☐ 18 When complete, select **OK**.
- ☐ 19 Remove the robot BeadChip alignment fixtures.
- ☐ 20 Make a record of any sections of BeadChip stripes without complete DNA sample coverage.
- ☐ 21 Place each BeadChip in a hybridization chamber insert.
- ☐ 22 Place the lid on the chamber and secure with the metal clamps.
- ☐ 23 **[Illumina LIMS]** Select **Infinium HD Super | Prepare Hyb Chamber**.
  - ☐ a Scan the barcodes.
- ☐ 24 Incubate at 48°C for 16–24 hours.

Prepare for Next Day

- ☐ 1 Add 330 ml 100% EtOH to the XC4 bottle.
- ☐ 2 Resuspend XC4 by adding 100% EtOH and place the bottle on its side on a rocker until BeadChips are ready for coating. Alternatively, leave the bottle upright on the lab bench overnight.
- ☐ 3 Soak the tip guide inserts in a 1% aqueous Alconox solution.
- ☐ 4 Rinse the tip guides with DiH_2O at least 3 times.
- ☐ 5 Dry the tip guide.

Wash BeadChips

This step prepares the BeadChips for the staining process.

- ☐ 1 Submerge the wash rack in the PB1 wash.
- ☐ 2 Remove the hybridization chamber inserts.
- ☐ 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 PB1Wash.
- ☐ 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 for up to 8 BeadChips.
- ☐ 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 Select **XStain Tasks | XStain HD BeadChip.**
- ☐ 2 If imaging immediately after staining, turn on the scanner.
- ☐ 3 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

- ☐ 4 Invert the XC1, XC2, TEM, STM, and ATM tubes to mix. Remove the caps, and place on the robot deck.
- ☐ 5 Enter the number of BeadChips.
- ☐ 6 Select **Run**.
- ☐ 7 **[Non-Illumina LIMS]** Enter the stain temperature listed on the STM tube.
- ☐ 8 Place the flow-through chambers into the chamber rack.
- ☐ 9 Select **OK**.
- ☐ 10 Remove the flow-through chambers from the chamber rack.
- ☐ 11 Fill the water circulator.
- ☐ 12 Turn on the water circulator and set the temperature to 44°C.

- ☐ 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 move the staining rack up and down 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 to the XC4 wash dish.
- ☐ 24 Slowly lift the staining rack up and down 10 times.
- ☐ 25 Soak for 5 minutes.
- ☐ 26 Remove the staining rack and place it onto the tube rack.
- ☐ 27 Place the tube rack into the vacuum desiccator.
- ☐ 28 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.

Acronyms

Acronym	Definition
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
ATM	Anti-Stain Two-Color Master Mix
FMS	Fragmentation solution
MA1	Multi-Sample Amplification 1 Mix
MA2	Multi-Sample Amplification 2 Mix
MSM	Multi-Sample Amplification Master Mix
PB1	Reagent used to prepare BeadChips for hybridization
PB2	Humidifying buffer used during hybridization
PM1	Precipitation solution
RA1	Resuspension, hybridization, and wash solution
STM	Superior Two-Color Master Mix
TEM	Two-Color Extension Master Mix
XC1	XStain BeadChip solution 1
XC2	XStain BeadChip solution 2
XC3	XStain BeadChip solution 3
XC4	XStain BeadChip solution 4