Infinium XT - HT Workflow Checklist (with Illumina LIMS)

Amplify DNA (Pre-Amp)

- ☐ 1 Select **MSA7 HT Tasks | Make MSA7 HT**.
  - ☐ a Select the DNA plate type.
- ☐ 2 Add reagents to quarter reservoirs (these volumes are for 3 plates):

Reagent	Volume
MA1	9 ml
0.1 N NaOH	5 ml
MA2	13.5 ml
RAM	13.5 ml

- ☐ 3 Place the DNA plates and MSA7 plates on the robot deck.
- ☐ 4 Select **Run**.
- ☐ 5 Vortex the MSA7 plates at 1600 rpm for 1 minute.
- ☐ 6 Centrifuge at 280 × g at room temperature for 1 minute.

Incubate DNA

- ☐ 1 Select **Infinium XT | Incubate MSA7 HT**.
  - ☐ a Scan the barcode of each MSA7 plate.
- ☐ 2 Incubate the MSA7 plates for 3–24 hours at 37°C.

Fragment DNA

- ☐ 1 Centrifuge the MSA7 plates at 280 × g at room temperature for 1 minute.
- ☐ 2 Select **MSA7 HT Tasks | Fragment MSA7 HT**.
- ☐ 3 Place the MSA7 plates on the robot deck.
- ☐ 4 Add 20 ml FMS to a quarter reservoir for 6 plates.
- ☐ 5 Select **Run**.
- ☐ 6 **[Optional]** Adjust the Tecan scanner bracket to **Position B**.
- ☐ 7 Scan the barcode of the reagent bottle.
- ☐ 8 Select **OK**.
- ☐ 9 Vortex at 1600 rpm for 1 minute.
- ☐ 10 Centrifuge at 280 × g at room temperature for 1 minute.
- ☐ 11 Incubate at 37°C for 30 minutes.

SAFE STOPPING POINT

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

Precipitate DNA

- ☐ 1 Select **MSA7 HT Tasks | Precip MSA7 HT**.
- ☐ 2 Place the MSA7 plates on the robot deck.
- ☐ 3 Add PM1 to a quarter reservoir:

Reagent	Number of Plates	Volume
PM1	1	8 ml
	2	14 ml
	3	21 ml
	4	27 ml
	5	34 ml
	6	40 ml

- ☐ 4 Add 2-propanol to a full reservoir:

Reagent	Number of Plates	Volume
2-propanol	1	25 ml
	2	50 ml
	3	75 ml
	4	100 ml
	5	125 ml
	6	150 ml

- ☐ 5 Select **Run**.
  - ☐ a Scan the barcode of the reagent bottle.
  - ☐ b Select **OK**.
- ☐ 6 Invert the plates 10 times.
- ☐ 7 Select **Infinium XT HT| Spin MSA7 HT**.
  - ☐ a Scan the barcode of each MSA7 plate.
- ☐ 8 Centrifuge at 3000 × g at 4°C for 20 minutes.
- ☐ 9 Invert the plates, and drain the supernatant.
- ☐ 10 Tap the plates several times.
- ☐ 11 Air dry for 15 minutes.

SAFE STOPPING POINT

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

Resuspend DNA

- ☐ 1 Select **MSA7 HT Tasks | Resuspend MSA7 HT**.
- ☐ 2 Place the MSA7 plates on the robot deck.
- ☐ 3 Add RA1 to a quarter reservoir.

Reagent	Number of Plates	Volume
RA1	1	5 ml
	2	8 ml
	3	11 ml
	4	14 ml
	5	17 ml
	6	20 ml

- ☐ 4 Select **Run**.
  - ☐ a Scan the barcode of the reagent bottle.
  - ☐ b Select **OK**.
- ☐ 5 Apply foil heat seals to the MSA7 plates.
- ☐ 6 Incubate for 15 minutes at 48°C.
- ☐ 7 Vortex at 1800 rpm for 1 minute.
- ☐ 8 Centrifuge at 280 × g at room temperature for 1 minute.

SAFE STOPPING POINT

If you are stopping, store sealed MSA7 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 MSA7 plates at 95°C for 20 minutes.
- ☐ 2 Cool at room temperature for 30 minutes.
- ☐ 3 Centrifuge at 15001000 × g at room temperature for 1 minute.
- ☐ 4 Place the gaskets into the XT Hyb chambers.
- ☐ 5 Dispense 800 µl PB2 into each reservoir.
- ☐ 6 Close the XT Hyb chamber.
- ☐ 7 Select **Infinium XT | Confirm BeadChips for Hyb**.
- ☐ 8 Scan the barcodes.
- ☐ 9 Remove all BeadChips from packaging.
- ☐ 10 Place up to 2 BeadChips onto each XT dual Hyb insert and baseplate.
- ☐ 11 Select **MSA7 HT Tasks | Hyb Multi-BC2**.
- ☐ 12 Select the 96-sample BeadChip.
- ☐ 13 Place the XT dual Hyb insert and baseplates onto the robot deck.
- ☐ 14 Place the MSA7 plates onto the robot deck.
- ☐ 15 Click **Run**.
- ☐ 16 Place an XT tip guide #1 on each XT dual Hyb insert and baseplate.
- ☐ 17 Click **OK**.
- ☐ 18 Remove XT tip guide #1 and replace it with XT tip guide #2, then click **OK**.
- ☐ 19 Remove XT tip guide #2 and replace it with XT tip guide #3, then click **OK**.
- ☐ 20 Click **OK**.
- ☐ 21 Remove XT tip guide #3.
- ☐ 22 Inspect the BeadChips.
- ☐ 23 Load the XT dual Hyb insert and baseplates inside the XT Hyb chambers.
- ☐ 24 Select **Infinium XT | Prepare Hyb Chamber**.
- ☐ 25 Scan the barcodes.
- ☐ 26 Incubate at 48°C for 16 to 24 hours.

Prepare for Next Day

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

Wash BeadChips

- ☐ 1 Submerge the wash rack in the 1X PB1 wash.
- ☐ 2 Remove the hybridization insert and baseplates.
- ☐ 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 1X PB1 Wash.
- ☐ 8 Move the wash rack up and down for 1 minute.
- ☐ 9 Fill the XCG Flow-Through Chamber assembly tray with 1X PB1.
- ☐ 10 Place a BeadChip on a submerged XCG Flow-Through Chamber frame.
- ☐ 11 Place an XCG glass back plate onto a submerged BeadChip.
- ☐ 12 Attach XCG Flow-Through Chamber clips to each XCG Flow-Through Chamber frame.
- ☐ 13 Select **Wash BeadChip XT HT**.
- ☐ 14 Scan the BeadChip barcodes.

Extend and Stain (XStain)

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

Reagent	# BeadChips	Volume
95% formamide/1 mM EDTA	24	30 ml
	48	60 ml
RA1	24	30 ml
	48	60 ml
XC3	24	150 ml
	48	250 ml

- ☐ 6 Place the XStain plates on the robot deck.
- ☐ 7 Invert the EML tubes to mix, remove the caps, and place the EML tubes on the robot deck.
- ☐ 8 Enter the number of BeadChips.
- ☐ 9 Select **Run**.
- ☐ 10 Enter the stain temperature listed on the XStain plate.
- ☐ 11 Place the XCG Flow-Through Chamber assemblies into the chamber rack.
- ☐ 12 Select **OK**.
- ☐ 13 Remove the XCG Flow-Through Chamber assemblies from the chamber rack.
- ☐ 14 Set up PB1 and XC4 wash dishes.
- ☐ 15 Pour 310 ml PB1 into a wash dish.
- ☐ 16 Disassemble each XCG flow-through chamber.
- ☐ 17 Place BeadChips into a staining rack in the PB1 wash dish.
- ☐ 18 Submerge the XCG glass back plates in the DI H_2O wash basin.
- ☐ 19 Move the staining rack up and down 10 times.
- ☐ 20 Soak the BeadChips for 5 minutes.
- ☐ 21 Shake the XC4 bottle vigorously.
- ☐ 22 Pour 310 ml XC4 into a wash dish.
- ☐ 23 Move the staining rack to the XC4 wash dish.
- ☐ 24 Move the staining rack up and down 10 times.
- ☐ 25 Soak the BeadChips for 5 minutes.
- ☐ 26 Remove the staining rack.
- ☐ 27 Dry the BeadChips for 50–55 minutes at 675 mm Hg (0.9 bar).
- ☐ 28 Image the BeadChips immediately, or store them, protected from light.
- ☐ 29 Select **Infinium XT | Coat**.
  - ☐ a Scan the barcodes.