Infinium XT - HT Workflow Checklist (no Illumina LIMS)

Amplify DNA (Pre-Amp)

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

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

- ☐ 5 Place WG#-DNA source and MSA7 plates on the robot bed.
- ☐ 6 Click **Run**.
- ☐ 7 Vortex the MSA7 plates at 1600 rpm for 1 minute.
- ☐ 8 Centrifuge at 280 × g at room temperature for 1 minute.

Incubate DNA

- ☐ 1 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 Enter the **Number of MSA7plates**.
- ☐ 4 Place the MSA7 plates on the robot bed.
- ☐ 5 Add 20 ml FMS to a quarter reservoir for 6 plates.
- ☐ 6 Click **Run**.
- ☐ 7 Vortex at 1600 rpm for 1 minute.
- ☐ 8 Centrifuge at 280 × g at room temperature for 1 minute.
- ☐ 9 Incubate at 37°C for 30 minutes.

SAFE STOPPING POINT

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

Precipitate DNA

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

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

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

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

- ☐ 6 Click **Run**.
- ☐ 7 Click **OK**.
- ☐ 8 Invert the plates 10 times.
- ☐ 9 Centrifuge at 3000 × g at 4°C for 20 minutes.
- ☐ 10 Invert the plates and drain the supernatant.
- ☐ 11 Tap firmly several times for 1 minute.
- ☐ 12 Air dry for 15 minutes.

SAFE STOPPING POINT

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

Resuspend DNA

- ☐ 1 Select **MSA7 HT Tasks | Resuspend MSA7 HT**.
- ☐ 2 Enter the **Number of MSA7 plates**.
- ☐ 3 Place the MSA7 plates on the robot bed.
- ☐ 4 Add RA1 to a quarter reservoir:

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

- ☐ 5 Click **Run**.
- ☐ 6 Click **OK**.
- ☐ 7 Apply foil heat seals to the MSA7 plates.
- ☐ 8 Incubate for 15 minutes at 48°C.
- ☐ 9 Vortex at 1800 rpm for 1 minute.
- ☐ 10 Centrifuge at 280 × g at room temperature for 1 minute.

SAFE STOPPING POINT

If you are stopping, store sealed MSA7 plates at 2°C to 8°C for up to 24 hours. If more than 24 hours, store at -25°C to -15°C. Store RA1 at -25°C to -15°C. If RA1 will be used the next day, 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 280 × 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 Remove all BeadChips from packaging.
- ☐ 8 Place up to 2 BeadChips onto each XT dual Hyb insert and baseplate.
- ☐ 9 Select **MSA7 HT Tasks | Hyb Multi-BC2**.
- ☐ 10 Select the 96-sample BeadChip.
- ☐ 11 Enter the **Number of MSA7 plates**.
- ☐ 12 Place the XT dual Hyb insert and baseplates onto the robot bed.
- ☐ 13 Place the MSA7 plates onto the robot bed.
- ☐ 14 Place an XT tip guide #1 on top of each XT dual Hyb insert and baseplate.
- ☐ 15 Click **Run**, then click **OK**.
- ☐ 16 Remove XT tip guide #1 and replace it with XT tip guide #2, then click **OK**.
- ☐ 17 Remove XT tip guide #2 and replace it with XT tip guide #3, then click **OK**.
- ☐ 18 Click **OK**.
- ☐ 19 Remove XT tip guide #3.
- ☐ 20 Inspect the BeadChips.
- ☐ 21 Load the XT dual Hyb insert and baseplates inside the XT Hyb chambers.
- ☐ 22 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 XT tip guides in 1% aqueous Alconox solution.
- ☐ 4 Rinse and dry the XT tip guides.

Wash BeadChips

- ☐ 1 Submerge the wash rack in the 1X PB1 wash.
- ☐ 2 Remove the XT dual Hyb 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.

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 bed.
- ☐ 7 Invert the EML tubes to mix, remove the caps, and place on the robot bed.
- ☐ 8 Enter the number of BeadChips.
- ☐ 9 Click **Run**.
- ☐ 10 Enter the stain temperature listed on the XStain plate.
- ☐ 11 Place the XCG Flow-Through Chamber assemblies into the chamber rack.
- ☐ 12 Click **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 protected from light.