Infinium HTS Extra Workflow Checklist (no Illumina LIMS)

Amplify DNA (Pre-Amp)

- ☐ 1 Select **MSA8 Tasks | Make MSA8**.
  - ☐ a Select the WG#-DNA plate type.
- ☐ 2 Add reagents to quarter reservoirs (these volumes are for 3 plates only):

Reagent	Volume
MA1	9 ml
0.1 N NaOH	5 ml
MA2	13.5 ml
MSM	15 ml

- ☐ 3 Place the WG#-DNA plates and MSA8 plates on the robot deck.
- ☐ 4 Select **Run**.
- ☐ 5 Vortex the MSA8 plates at 1600 rpm for 1 minute.
- ☐ 6 Centrifuge the MSA8 plates at 280 × g at room temperature for 1 minute.

Incubate DNA

- ☐ 1 Incubate the MSA8 plates for 20–24 hours at 37°C.

Fragment DNA

- ☐ 1 Centrifuge the MSA8 plates at 280 × g at room temperature for 1 minute.
- ☐ 2 Select **MSA8 Tasks | Fragment MSA8**.
- ☐ 3 Place six MSA8 plates on the robot deck.
- ☐ 4 Add 20 ml FMS to a quarter reservoir.
- ☐ 5 Select **Run**.
- ☐ 6 Select **OK**.
- ☐ 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 plates, and store at -25°C to -15°C.

Precipitate DNA

- ☐ 1 Select **MSA8 Tasks | Precip MSA8**.
- ☐ 2 Place six MSA8 plates on the robot deck.
- ☐ 3 Add 40 ml PM1 to a quarter reservoir.
- ☐ 4 Add 150 ml 2-propanol to a full reservoir.
- ☐ 5 Select **Run**.
  - ☐ a Select **OK**.
- ☐ 6 Invert the plates 10 times.
- ☐ 7 Centrifuge at 3000 × g at 4°C for 20 minutes.
- ☐ 8 Invert the plates, and drain the supernatant.
- ☐ 9 Tap the plates several times.
- ☐ 10 Air dry for 15 minutes.

SAFE STOPPING POINT

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

Resuspend DNA

- ☐ 1 Select **MSA8 Tasks | Resuspend MSA8**.
- ☐ 2 Place six MSA8 plates on the robot deck.
- ☐ 3 Add 20 ml RA1 to a quarter reservoir.
- ☐ 4 Select **Run**.
  - ☐ a Select **OK**.
- ☐ 5 Apply foil heat seals to the MSA8 plates.
- ☐ 6 Incubate for 15 minutes at 48°C.
- ☐ 7 Vortex at 1800 rpm for 1 minute.
- ☐ 8 Centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, store sealed MSA8 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 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 MSA8 plates at 95°C for 20 minutes.
- ☐ 2 Cool at room temperature for 30 minutes.
- ☐ 3 Centrifuge at 1500 × g at room temperature for 1 minute.
- ☐ 4 Place the gasket into the hyb chamber.
- ☐ 5 Dispense 400 µl PB2 into each reservoir.
- ☐ 6 Place the hyb chamber insert into the hyb chamber.
- ☐ 7 Close the hyb chamber.
- ☐ 8 Remove all BeadChips from packaging.
- ☐ 9 Place BeadChips into the robot BeadChip alignment fixtures.
- ☐ 10 Select **MSA8 Tasks | Hyb**.
  - ☐ a Select the 24-sample BeadChip.
  - ☐ b Enter the **Number of MSA8 plates**.
- ☐ 11 Place the robot BeadChip alignment fixtures onto the robot deck.
- ☐ 12 Place the MSA8 plates onto the robot deck.
- ☐ 13 Select **Run**.
- ☐ 14 Place each robot tip alignment guide on top of each robot BeadChip alignment fixture.
- ☐ 15 Select **OK**.
- ☐ 16 Select **OK**.
- ☐ 17 Remove the robot BeadChip alignment fixtures.
- ☐ 18 Place each BeadChip in a hyb chamber insert.
- ☐ 19 Close the hyb chamber.
- ☐ 20 Incubate at 48°C for 16 to 24 hours.

Prepare for Next Day

- ☐ 1 Soak the robot tip alignment guides in 1% aqueous Alconox solution.
- ☐ 2 Rinse and dry the robot tip alignment guides.
- ☐ 3 Add 330 ml 100% EtOH to the XC4 bottle and shake.

Wash BeadChips

- ☐ 1 Submerge the wash rack in the 1X PB1 wash.
- ☐ 2 Remove the hyb chamber inserts.
- ☐ 3 Inspect the BeadChips.
- ☐ 4 Remove BeadChips from the hyb chamber inserts.
- ☐ 5 Remove the cover seals from the BeadChips.
- ☐ 6 Place the BeadChips into the submerged wash rack.
- ☐ 7 Move the wash rack up and down for 1 minute.
- ☐ 8 Move the wash rack to the next 1X PB1 Wash.
- ☐ 9 Move the wash rack up and down for 1 minute.
- ☐ 10 Fill the multi-sample BeadChip alignment fixture with 150 ml 1X PB1.
- ☐ 11 Place black frames into the multi-sample BeadChip alignment fixture.
- ☐ 12 Place BeadChips into black frames.
- ☐ 13 Inspect the BeadChip. Remove excess residue.
- ☐ 14 Place a clear LCG spacer onto each BeadChip.
- ☐ 15 Place the alignment bar onto the multi-sample BeadChip alignment fixture.
- ☐ 16 Place LCG glass back plates on top of the clear spacers.
- ☐ 17 Attach the metal clamps to the flow-through chambers.
- ☐ 18 Trim the excess ends of the clear plastic spacers.
- ☐ 19 Return the flow-through chamber to the multi-sample BeadChip alignment fixture.

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 LCG BeadChip HT**.
- ☐ 4 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

- ☐ 5 Place the XStain plates on the robot deck.
- ☐ 6 Invert the EML tubes to mix, remove the caps, and place the EML tubes on the robot deck.
- ☐ 7 Enter the number of BeadChips.
  - ☐ a Select **Run**.
  - ☐ b Enter the stain temperature listed on the XStain plate.
  - ☐ c Select **OK**.
- ☐ 8 Place the LCG flow-through chambers into the chamber rack.
- ☐ 9 Select **OK**.
- ☐ 10 While the XStain task runs, wash the hyb chamber humidifying buffer reservoirs.
- ☐ 11 Remove the LCG flow-through chambers from the chamber rack.
- ☐ 12 Set up PB1 and XC4 wash dishes.
- ☐ 13 Pour 310 ml PB1 into a wash dish.

- ☐ 14 Disassemble each LCG flow-through chamber.
- ☐ 15 Place BeadChips into a staining rack in the PB1 wash dish.
- ☐ 16 Submerge the LCG glass back plates in the DI H_2O wash basin.
- ☐ 17 Move the staining rack up and down 10 times.
- ☐ 18 Soak the BeadChips for 5 minutes.
- ☐ 19 Shake the XC4 bottle vigorously.
- ☐ 20 Pour 310 ml XC4 into a wash dish.
- ☐ 21 Move the staining rack to the XC4 wash dish.
- ☐ 22 Move the staining rack up and down 10 times.
- ☐ 23 Soak the BeadChips for 5 minutes.
- ☐ 24 Remove the staining rack.
- ☐ 25 Dry the BeadChips for 50–55 minutes at 675 mm Hg (0.9 bar).
- ☐ 26 Turn on the iScan™ systems.
- ☐ 27 Image the BeadChips immediately, or store them, protected from light.