Infinium XT - ST Manual Workflow Checklist

Amplify DNA (Pre-Amp)

- ☐ 1 Add 20 µl MA1 to each well of the MSA7 plate.
- ☐ 2 Add 4 µl 0.1 N NaOH to each well.
- ☐ 3 Transfer 4 µl of the DNA sample from each well of the DNA plate to the MSA7 plate.
- ☐ 4 Vortex the MSA7 plate at 1600 rpm for 1 minute.
- ☐ 5 Centrifuge at 280 × g at room temperature for 1 minute.
- ☐ 6 Incubate at room temperature for 10 minutes.
- ☐ 7 Add 35 µl MA2 per well.
- ☐ 8 Add 35 µl RAM per well.
- ☐ 9 Vortex at 1600 rpm for 1 minute.
- ☐ 10 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 Add 25 µl FMS per well.
- ☐ 3 Vortex at 1600 rpm for 1 minute.
- ☐ 4 Centrifuge at 280 × g at room temperature for 1 minute.
- ☐ 5 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 Add 50 µl PM1 per well.
- ☐ 2 Add 155 µl 100% 2-propanol per well.
- ☐ 3 Apply fresh cap mats.
- ☐ 4 Invert the plates 10 times to mix.
- ☐ 5 Centrifuge at 3000 × g at 4°C for 20 minutes.
- ☐ 6 Remove the plates from the centrifuge and remove the cap mats.
- ☐ 7 Quickly invert the plates and drain the supernatant.
- ☐ 8 Tap firmly several times for 1 minute.
- ☐ 9 Air dry pellets for 15 minutes.

Resuspend DNA

- ☐ 1 Add 23 µl RA1 per well.
- ☐ 2 Apply foil heat seals.
- ☐ 3 Incubate for 15 minutes at 48°C.
- ☐ 4 Vortex at 1800 rpm for 1 minute.
- ☐ 5 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 Remove all BeadChips from packaging.
- ☐ 8 Place 2 BeadChips onto each XT dual Hyb insert and baseplate.
- ☐ 9 Place XT Tip Guide #1 on top of each XT dual Hyb insert and baseplate.
- ☐ 10 Dispense 15 µl DNA sample into the appropriate BeadChip sections.
- ☐ 11 Remove XT tip guide #1 and replace with XT tip guide #2. Dispense 15 µl of each DNA sample into the appropriate BeadChip sections.
- ☐ 12 Remove XT tip guide #2 and replace with XT tip guide #3. Dispense 15 µl of each DNA sample into the appropriate BeadChip sections.
- ☐ 13 Remove XT tip guide #3 and inspect the BeadChips.
- ☐ 14 Load the XT dual Hyb insert and baseplates inside the XT Hyb chambers.
- ☐ 15 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.

Extend and Stain (XStain)

- ☐ 1 Fill the water circulator.
- ☐ 2 Turn on the water circulator and set the temperature..
- ☐ 3 When the chamber rack reaches 44°C, place the Flow-Through Chamber assemblies into the chamber rack.
- ☐ 4 Into the reservoir of each Flow-Through Chamber, dispense:
  - ☐ a 150 µl RA1. Incubate for 30 seconds. Repeat 5 times.

    [_] 1 [_] 2 [_] 3 [_] 4 [_] 5 [_] 6
  - ☐ b 225 µl LX1. Repeat 1 time. Incubate for 10 minutes.

    [_] 1 [_] 2
  - ☐ c 225 µl LX2. Repeat 1 time. Incubate for 10 minutes.

    [_] 1 [_] 2
  - ☐ d 300 µl EML. Incubate for 15 minutes.
  - ☐ e 250 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat twice.

    [_] 1 [_] 2 [_] 3
  - ☐ f Incubate 5 minutes.
  - ☐ g Begin ramping the chamber rack temperature to the temperature indicated on the SML tube.
  - ☐ h 250 µl XC3. Incubate for 1 minute. Repeat twice.

    [_] 1 [_] 2 [_] 3
- ☐ 5 Wait until the chamber rack reaches the correct temperature.
- ☐ 6 If you plan to image the BeadChip immediately after the staining process, turn on the scanner.

☐ 7 Into the reservoir of each Flow-Through Chamber, dispense:

- ☐ a 250 µl SML. Incubate for 10 minutes.
- ☐ b 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.

[_] 1 [_] 2 [_] 3

- ☐ c 250 µl ATM. Incubate for 10 minutes.
- ☐ d 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.

[_] 1 [_] 2 [_] 3

- ☐ e 250 µl SML. Incubate for 10 minutes.
- ☐ f 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.

[_] 1 [_] 2 [_] 3

- ☐ g 250 µl ATM. Incubate for 10 minutes.
- ☐ h 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.

[_] 1 [_] 2 [_] 3

- ☐ i 250 µl SML. Incubate for 10 minutes.
- ☐ j 250 µl XC3. Incubate for 1 minute. Repeat twice. Wait 5 minutes.

[_] 1 [_] 2 [_] 3

☐ 8 Remove the Flow-Through Chambers from the chamber rack.
☐ 9 Set up PB1 and XC4 wash dishes.
☐ 10 Pour 310 ml PB1 into a wash dish.
☐ 11 Disassemble each XCG flow-through chamber.
☐ 12 Place BeadChips into a staining rack in the PB1 wash dish.
☐ 13 Submerge the XCG glass back plates in the DI H_2O wash basin.
☐ 14 Move the staining rack up and down 10 times.
☐ 15 Soak the BeadChips for 5 minutes.
☐ 16 Shake the XC4 bottle vigorously.
☐ 17 Pour 310 ml XC4 into a wash dish.
☐ 18 Move the staining rack to the XC4 wash dish.
☐ 19 Move the staining rack up and down 10 times.
☐ 20 Soak the BeadChips for 5 minutes.
☐ 21 Remove the staining rack.
☐ 22 Dry the BeadChips for 50–55 minutes at 675 mm Hg (0.9 bar).
☐ 23 Image the BeadChips immediately, or store them, protected from light.