Infinium CytoSNP 850K Checklist

Amplify DNA

- ☐ 1 Preheat the Illumina Hybridization Oven to 37°C.
- ☐ 2 Add 20 µl MA1 to the midi plate wells to prepare the MSA1 plate.
- ☐ 3 Transfer 4 µl DNA sample (50 ng/µl) to the MSA1 plate.
- ☐ 4 Add 4 µl 0.1 N NaOH to each well.
- ☐ 5 Seal the MSA1 plate with a 96-well cap mat.
- ☐ 6 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
- ☐ 7 Incubate for 10 minutes at room temperature.
- ☐ 8 Remove the sealing mat and set aside upside down in a safe location.
- ☐ 9 Add 68 µl MA2 to each well.
- ☐ 10 Add 75 µl MSM to each well.
- ☐ 11 Reseal with the cap mat.
- ☐ 12 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
- ☐ 13 Incubate in the Illumina Hybridization Oven for 20–24 hours.

Fragment DNA

- ☐ 1 Preheat the heat block containing the midi plate insert to 37°C.
- ☐ 2 Remove the MSA1 plate from the hybridization oven.
- ☐ 3 Pulse centrifuge the MSA1 plate to 280 × g.
- ☐ 4 Remove the sealing mat and set aside upside down in a safe location.
- ☐ 5 Add 50 µl FMS to each well.
- ☐ 6 Reseal with the cap mat.
- ☐ 7 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
- ☐ 8 Incubate on the heat block for 1 hour.

SAFE STOPPING POINT

If you are stopping, seal the plate, and store at -25°C to -15°C for up to 24 hours.

Precipitate DNA

- ☐ 1 Leave the MSA1 plate on the heat block until preparation is complete. If frozen, thaw at room temperature then pulse centrifuge at 280 × g.
- ☐ 2 Preheat a heat block to 37°C.
- ☐ 3 Add 100 µl PM1 to each sample well.
- ☐ 4 Reseal with the cap mat.
- ☐ 5 Vortex at 1600 rpm for 1 minute.
- ☐ 6 Incubate on the heat block for 5 minutes.
- ☐ 7 Pulse centrifuge at 280 × g.
- ☐ 8 Set the centrifuge at 4°C.
- ☐ 9 Add 300 µl 100% 2-propanol to each well.
- ☐ 10 Seal with a ***new, dry*** cap mat.
- ☐ 11 Invert 10 times to mix.
- ☐ 12 Incubate in a refrigerator set at 4°C for 30 minutes.
- ☐ 13 Place in the centrifuge opposite another plate of equal weight.
- ☐ 14 Centrifuge at 3000 × g for 20 minutes.
  - ▶ When centrifuging is complete, proceed ***immediately*** to the next step.
  - ▶ If a delay occurs, repeat the 20 minute centrifuge.
- ☐ 15 Remove the plate from centrifuge.
- ☐ 16 Remove and discard the cap mat.
- ☐ 17 Hold the plate over an absorbent pad and do as follows.
  - ☐ a Quickly invert.
  - ☐ b Drain liquid onto the absorbent pad, and then smack the plate down.
- ☐ 18 Firmly tap until all wells are free of liquid.
- ☐ 19 Place the plate on a tube rack for 1 hour at room temperature.
- ☐ 20 Make sure that a blue pellet is present.

SAFE STOPPING POINT

If you are stopping, seal the MSA1 plate with a new sealing mat and store at -25°C to -15°C for up to 24 hours.

Resuspend DNA

- ☐ 1 If you plan to proceed immediately from this procedure to the subsequent procedure, preheat the heat block to 95°C.
- ☐ 2 Preheat the Illumina Hybridization Oven to 48°C.
- ☐ 3 Preheat the heat sealer for 10 minutes.
- ☐ 4 Invert thawed RA1 several times to dissolve.
- ☐ 5 Add 46 µl RA1 to each well.
- ☐ 6 Apply a foil heat seal.
- ☐ 7 Firmly roll the rubber plate sealer over the plate.
- ☐ 8 Incubate in the Illumina Hybridization Oven for 1 hour.
- ☐ 9 Vortex at 1800 rpm for 1 minute, and then pulse centrifuge at 280 × g. Repeat as needed to resuspend the pellets.

SAFE STOPPING POINT

If you are stopping, store the sealed MSA1 plate at -25°C to -15°C and RA1 at 2°C to 8°C for up to 24 hours.

Alternatively, store the MSA1 plate at -85°C to -65°C for up to one week. For more than 24 hours, store RA1 at -25°C to -15°C.

Hybridize DNA to the BeadChip

- ☐ 1 If frozen, thaw the MSA1 plate at room temperature, and then pulse centrifuge at 280 × g.
- ☐ 2 Preheat the heat block to 95°C.
- ☐ 3 Preheat the Illumina Hybridization Oven to 48°C.
- ☐ 4 Place on the heat block for 20 minutes.
- ☐ 5 Place the hybridization chambers, hybridization chamber gaskets, and hybridization chambers inserts on the benchtop.
- ☐ 6 Place the gasket onto the chamber, and press into place.
- ☐ 7 Add 200 µl PB2 to the top and bottom reservoirs of each BeadChip.
- ☐ 8 Immediately cover the chamber with the lid.
- ☐ 9 Leave on the benchtop at room temperature until the BeadChips are loaded with DNA.
- ☐ 10 When the incubation is complete, transfer the MSA1 plate to the benchtop and cool at room temperature for 30 minutes.
- ☐ 11 Pulse centrifuge at 280 × g.
- ☐ 12 Remove the BeadChips from all packaging.
- ☐ 13 Place each BeadChip into an insert.
- ☐ 14 Remove the foil seal from the MSA1 plate.
- ☐ 15 Transfer 26 µl each sample to the BeadChip.
- ☐ 16 Wait for the DNA to disperse.
- ☐ 17 Inspect the loading port for excess liquid.
- ☐ 18 If excess liquid is not present, add leftover sample.
- ☐ 19 Load the inserts containing BeadChips into the hybridization chamber.
- ☐ 20 Place the lid on the chamber and close the four clamps.

- ☐ 21 Place the chamber into the preheated Illumina Hybridization Oven.
- ☐ 22 Incubate at 48°C for 16–24 hours.
- ☐ 23 Store RA1 at 2°C to 8°C.
- ☐ 24 Discard the MSA1 plate.
- ☐ 25 Add 330 ml fresh 100% EtOH to the XC4 bottle.
- ☐ 26 Vigorously shake XC4.
- ☐ 27 **[Optional]** Store at 2°C to 8°C.

Wash BeadChips

- ☐ 1 Remove each hybridization chamber from the hybridization oven. Allow to cool for 25 minutes before opening.
- ☐ 2 Prepare the following items:
  - ▶ Fill two wash dishes with 200 ml PB1 each and label them accordingly.
  - ▶ Using a graduated cylinder, fill the Multi-Sample BeadChip Alignment Fixture with 150 ml PB1.
- ☐ 3 Remove the Te-Flow flow-through chamber components from storage.
- ☐ 4 Attach the wire handle to the wash rack, and then submerge into a wash dish containing 200 ml PB1.
- ☐ 5 Remove a BeadChip from the hybridization chamber, and then remove the cover seal.
- ☐ 6 Slide the BeadChip into the wash rack.
- ☐ 7 Repeat steps 5 and 6 until all BeadChips are transferred.
- ☐ 8 Lift the wash rack up and down for 1 minute.
- ☐ 9 Move the wash rack to the other wash dish containing 200 ml clean PB1, submerging the BeadChips.
- ☐ 10 Repeat step 8 in the new wash dish.
- ☐ 11 Remove BeadChips from the wash rack and inspect them for residue.
- ☐ 12 If residue is present, scrape it away from the bead sections.
- ☐ 13 For each BeadChip, place one black frame into the BeadChip alignment fixture.
- ☐ 14 Place each BeadChip into a black frame.
- ☐ 15 Place a ***clear*** spacer onto the top of each BeadChip.
- ☐ 16 Place the alignment bar onto the alignment fixture.
- ☐ 17 Place a clean glass back plate on top of each clear spacer.
- ☐ 18 Secure each flow-through chamber assembly with metal clamps.
- ☐ 19 Remove the assembled flow-through chamber from the alignment fixture.
- ☐ 20 Trim the spacers from each end of the assembly.
- ☐ 21 Leave the assembled flow-through chambers in PB1 in the alignment fixture until ready to load onto the chamber rack in *Extend and Stain BeadChips* on page 4.
  - ▶ Do not place on absorbent paper.
  - ▶ Do not place in the chamber rack until instructed to do so.
- ☐ 22 Wash the hybridization chamber reservoirs with deionized water.

Extend and Stain BeadChips

- ☐ 1 Turn on the water circulator and set it to a temperature that brings the chamber rack to 44°C at equilibrium.
- ☐ 2 Confirm the temperature.
- ☐ 3 Remove bubbles trapped in the chamber rack.
- ☐ 4 Test the temperature in three locations.
- ☐ 5 Place each flow-through chamber assembly into the chamber rack.
- ☐ 6 Fill the reservoir of each flow-through chamber as follows.
  - ☐ a Add 150 µl RA1 and incubate for 30 seconds. Repeat five times.
  - ☐ b Add 450 µl XC1. Incubate for 10 minutes.
  - ☐ c Add 450 µl XC2. Incubate for 10 minutes.
  - ☐ d Add 200 µl TEM. Incubate for 15 minutes.
  - ☐ e Add 450 µl 95% formamide/1 mM EDTA and incubate for 1 minute. Repeat one time.
  - ☐ f Incubate for 5 minutes.
  - ☐ g Set the chamber rack temperature to the temperature indicated on the STM tube ± 0.5°C.
  - ☐ h Add 450 µl XC3 and incubate for 1 minute. Repeat one time.
- ☐ 7 Wait for the chamber rack to reach the appropriate temperature.
- ☐ 8 Fill the reservoir of each flow-through chamber as follows.
  - ☐ a 250 µl STM. Incubate for 10 minutes.
  - ☐ b 450 µl XC3 and incubate for 1 minute. Repeat one time.
  - ☐ c Wait 5 minutes.
  - ☐ d 250 µl ATM. Incubate for 10 minutes.
  - ☐ e 450 µl XC3 and incubate for 1 minute. Repeat one time.
  - ☐ f Wait 5 minutes.
  - ☐ g 250 µl STM. Incubate for 10 minutes.
  - ☐ h 450 µl XC3 and incubate for 1 minute. Repeat one time.
  - ☐ i Wait 5 minutes.
  - ☐ j 250 µl ATM. Incubate for 10 minutes.
  - ☐ k 450 µl XC3 and incubate for 1 minute. Repeat one time.
  - ☐ l Wait 5 minutes.
  - ☐ m 250 µl STM. Incubate for 10 minutes.
  - ☐ n 450 µl XC3 and incubate for 1 minute. Repeat one time.
  - ☐ o Wait 5 minutes.
- ☐ 9 Immediately remove the flow-through chambers and place in reserved alignment fixtures submerged in PB1 at room temperature.
- ☐ 10 Gather the following equipment:
  - Kimwipes, large
  - Staining rack
  - Self-locking tweezers
  - Tube rack
  - Vacuum desiccator
  - Vacuum hose
  - Wash dishes (2)
- ☐ 11 During the procedure, prevent dust or lint from entering the wash dishes.
- ☐ 12 Wash the tube racks and wash dishes thoroughly after each use.
- ☐ 13 Place a clean tube rack on top of several layers of Kimwipes or an absorbent pad.
- ☐ 14 Prepare another clean tube rack that fits the internal dimensions of vacuum desiccator for removal of the BeadChips. Allow one rack per eight BeadChips.
- ☐ 15 Set up two top-loading wash dishes labeled PB1 and XC4.
- ☐ 16 To indicate fill volume of each wash dish:
  - ☐ a Add 310 ml water.
  - ☐ b Mark the water level on the side.
  - ☐ c Empty the water.
- ☐ 17 Add 310 ml PB1 to the PB1 wash dish.
- ☐ 18 Submerge the staining rack in the wash dish so that the locking arms and tab ***face you***.
- ☐ 19 Leave the staining rack in the wash dish.
- ☐ 20 ***Using the dismantling tool***, remove the two metal clamps from a flow-through chamber.
- ☐ 21 Lift the glass back plate straight up to remove.
- ☐ 22 Remove the spacer, avoiding contact with the BeadChip stripes.
- ☐ 23 Remove the BeadChip from the black frame.
- ☐ 24 Repeat steps 20–23 to disassemble each flow-through chamber.
- ☐ 25 Place the BeadChips into the submerged staining rack.
- ☐ 26 Slowly lift the staining rack 10 times.
- ☐ 27 Soak for 5 minutes.
- ☐ 28 Vigorously shake the XC4 bottle. If necessary, vortex until dissolved.
- ☐ 29 Add 310 ml to the XC4 wash dish and cover.
- ☐ 30 Transfer the staining rack from the PB1 to the XC4.
- ☐ 31 Slowly lift the staining rack 10 times.
- ☐ 32 Soak for 5 minutes.
- ☐ 33 Remove the staining rack and place it onto the tube rack.
- ☐ 34 Dry each BeadChip as follows.
  - ☐ a Grip the BeadChip by the barcode end.
  - ☐ b Place onto a tube rack with the barcode facing up and toward you.

- ☐ 35 Place the tube rack into the vacuum desiccator.
- ☐ 36 Remove the red plug from the three-way valve.
- ☐ 37 Start the vacuum, using at least 675 mm Hg (0.9 bar).
- ☐ 38 Dry for 50–55 minutes.
- ☐ 39 Release the vacuum.
- ☐ 40 Return the desiccator to storage.
- ☐ 41 Touch the BeadChip ***borders*** (not the stripes) to make sure that the barcode sidesare dry.
- ☐ 42 Clean the back of each BeadChip using a Kimwipe sprayed with 70% EtOH.

SAFE STOPPING POINT

Store the BeadChips in the Illumina BeadChip Slide Storage Box at room temperature. Scan within 72 hours.