

## Amplify DNA

- 1 Add 20 µl MA1 to each well.
- 2 Transfer 4 µl DNA sample from the DNA plate to the MSA3 plate.
- 3 Add 4 µl 0.1N NaOH in to each well.
- 4 Seal the MSA3 plate with the 96-well cap mat.
- 5 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.
- 6 Incubate at room temperature for 10 minutes.
- 7 Add 34 µl MA2 in to each well.
- 8 Add 38 µl MSM in to each well.
- 9 Vortex at 1600 rpm for 1 minute, and then pulse centrifuge at 280 × g.

## Incubate DNA

- 1 Incubate the MSA3 plate for 20–24 hours at 37°C.

## Fragment DNA

- 1 Pulse centrifuge the plate at 50 × g.
- 2 Add 25 µl FMS to the MSA3 plate.
- 3 Vortex at 1600 rpm for 1 minute, and then centrifuge at 50 × g at 22°C for 1 minute.
- 4 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. For more than 24 hours, store at -80°C.

Store RA1 at -25°C to -15°C.

## Precipitate DNA

- 1 Add 50 µl PM1 to the MSA3 plate.
- 2 Reseal with the cap mat.
- 3 Vortex the plate at 1600 rpm for 1 minute.
- 4 Incubate on the heat block for 5 minutes.
- 5 Pulse centrifuge at 280 × g for 1 minute.
- 6 Set the centrifuge at 4°C.
- 7 Remove and discard the cap mat.
- 8 Add 155 µl 100% 2-propanol to each well.
- 9 Apply fresh cap mats.
- 10 Invert the plate 10 times to mix.
- 11 Incubate in a refrigerator set at 4°C for 30 minutes.
- 12 Centrifuge at 3000 × g at 4°C for 20 minutes.
- 13 Immediately remove the plate from the centrifuge.
- 14 Make sure that a blue pellet is present.
- 15 Remove and discard the cap mat.
- 16 Quickly invert the plate and drain the supernatant.
- 17 Firmly tap until all wells are free of liquid.
- 18 Place the plate on the tube rack for 1 hour at room temperature.
- 19 Make sure that a blue pellet is still present.

### SAFE STOPPING POINT

If you are stopping, seal the plate, and store at -25°C to -15°C for up to 24 hours. For more than 24 hours, store at -80°C.

Store RA1 at -25°C to -15°C.

## Resuspend DNA

- 1 Add 23 µl RA1 per well.
- 2 Apply a foil heat seal.
- 3 Incubate for 1 hour.
- 4 Vortex at 1800 rpm for 1 minute.
- 5 Pulse centrifuge at 280 × g.

### SAFE STOPPING POINT

If you are stopping, seal the plate, and store at -25°C to -15°C for up to 24 hours. For more than 24 hours, store at -80°C.

Store RA1 at -25°C to -15°C.

## Hybridize DNA to the BeadChip

- 1 Incubate the MSA3 plate on the heat block for 20 minutes.
- 2 Cool at room temperature for 30 minutes.
- 3 Pulse centrifuge at 280 × g.
- 4 Place the gasket into the hybridization chamber.
- 5 Add 400 µl PB2 to the top and bottom wells.
- 6 Immediately cover the chamber with the lid.
- 7 Remove the BeadChips from packaging.
- 8 Place each BeadChip into an insert.
- 9 Using either a single-channel pipette or an adjustable spacer multichannel precision pipette, transfer 14 µl each sample to the BeadChip.
- 10 Wait for the DNA to disperse.
- 11 Inspect the loading port for excess liquid.
- 12 If excess liquid is not present, add leftover sample.
- 13 Store RA1 at -25°C to -15°C.
- 14 Load the inserts into the hybridization chamber.
- 15 Place the lid on the chamber and secure with the metal clamps.
- 16 Place the chamber into the preheated Illumina Hybridization Oven.
- 17 Incubate at 48°C for 16–24 hours.
- 18 Store RA1 at -25°C to -15°C.

## Prepare for Next Day

- 1 Add 330 ml fresh 100% EtOH to the XC4 bottle.
- 2 Vigorously shake to resuspend.
- 3 Leave the bottle upright on the lab bench overnight.

## Wash BeadChips

- 1 Submerge the wash rack in the PB1 wash.
- 2 Remove the hybridization insert.
- 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 PB1 Wash.
- 8 Move the wash rack up and down for 1 minute.
- 9 Confirm that you are using the correct Infinium LCG glass back plates and spacers.
- 10 Fill the BeadChip alignment fixture with 150 ml PB1.
- 11 For each BeadChip, place one black frame into the BeadChip alignment fixture.
- 12 Place each BeadChip into a black frame.
- 13 Place a *clear* spacer onto the top of each BeadChip.
- 14 Place the alignment bar onto the alignment fixture.
- 15 Place a clean glass back plate on top of each clear spacer.
- 16 Secure each flow-through chamber assembly with metal clamps.
- 17 Remove the assembled flow-through chamber from the alignment fixture.
- 18 Trim the spacers from each end of the assembly.
- 19 Leave assembled flow-through chambers on the lab bench.

- 20 Wash the hybridization chamber reservoirs with DI H<sub>2</sub>O.

## Extend and Stain BeadChips

- 1 Fill the water circulator.
- 2 Turn on the water circulator and set the temperature to 44°C.
- 3 When the chamber rack reaches 44°C, place the flow-through chamber assemblies into the chamber rack.
- 4 Fill the reservoir of each flow-through chamber as follows.
- 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 Set the 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 for the chamber rack to reach the correct temperature.
- 6 If imaging the BeadChip immediately after the staining process, turn on the scanner.
- 7 Fill the reservoir of each flow-through chamber as follows.
- a 250 µl SML. Incubate for 10 minutes.
- b 250 µl XC3. Incubate for 1 minute. Repeat twice.
- 1  2  3
- c Wait 5 minutes.
- d 250 µl ATM. Incubate for 10 minutes.
- e 250 µl XC3. Incubate for 1 minute. Repeat twice.
- 1  2  3
- f Wait 5 minutes.
- g 250 µl SML. Incubate for 10 minutes.
- h 250 µl XC3. Incubate for 1 minute. Repeat twice.
- 1  2  3
- i 250 µl ATM. Incubate for 10 minutes.
- j 250 µl XC3. Incubate for 1 minute. Repeat twice.
- 1  2  3
- k Wait 5 minutes.
- l 250 µl SML. Incubate for 10 minutes.
- m 250 µl XC3. Incubate for 1 minute. Repeat twice.
- 1  2  3
- n Wait 5 minutes.
- 8 Remove the flow-through chambers from the chamber rack.
- 9 Set up two top-loading wash dishes labeled PB1 and XC4.
- 10 Add 310 ml PB1 to the PB1 wash dish.
- 11 Submerge the staining rack in the wash dish.
- 12 Leave the staining rack in the wash dish.
- 13 Disassemble each flow-through chamber.
- 14 Place the BeadChips into the submerged staining rack.
- 15 Slowly move the staining rack up and down 10 times.
- 16 Soak for 5 minutes.
- 17 Vigorously shake the XC4 bottle.
- 18 Add 310 ml XC4 to the XC4 wash dish and cover.
- 19 Transfer the staining rack to the XC4 wash dish.
- 20 Slowly lift the staining rack up and down 10 times.
- 21 Soak for 5 minutes.
- 22 Remove the staining rack and place it onto the tube rack.
- 23 Place the tube rack into the vacuum desiccator.
- 24 Dry the BeadChips for 50–55 minutes at 675 mm Hg (0.9 bar).

**SAFE STOPPING POINT**

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