Infinium® HD Assay Super, Manual

Experienced User Card

Day 1

Make MSA1

Hands-on: ~45–60 min

Reagents
0.1N NaOH
MA1
MA2
MSM

Output
MSA1 Plate

Incubate MSA1

Incubation: 20–24 hours

Output
MSA1 Plate with Amplified DNA

Day 2

Fragment MSA1

Hands-on: ~30 min
Incubation: 1 hour

Reagents
FMS

Output
MSA1 Plate

Precip MSA1

Hands-on: ~30 min
Incubation/Dry Time: 2 hours

Reagents
2-propanol
PM1

Output
MSA1 Plate

Resuspend MSA1

Hands-on: ~30 min
Incubation: 1 hour

Reagents
RA1

Output
MSA1 Plate

Hyb Multi BC2

Hands-on: ~30/ 12 BeadChips
Incubation: 16–24 hours

Reagents
PB2

Output
BeadChip

Day 3

Wash BeadChip

Hands-on: ~20 min/ 4 BeadChips

Reagents
PB1

Output
BeadChip

XStain HD BeadChip

Hands-on: ~2.5 hours
Dry Time: 1 hour

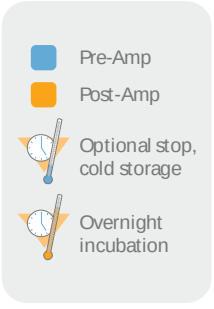
Reagents
RA1
Formamide / EDTA
PB1
XC1
XC2
XC3
XC4
TEM
STM
ATM

Output
BeadChip

Image BeadChip

Hands-on: 5 min/scan
Scan: ~3 hours/BeadChip

Output
Image and Data Files



Make MSA1

Move DNA samples into the MSA1 plate. Denature and neutralize samples, and prepare them for amplification. Incubate overnight to amplify.

Estimated Time

Hands-on time:

- 45 minutes for 48 samples
- 60 minutes for 96 samples

Incubation time: 20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
MA1	2 tubes (per 96 samples)	-20°C	Illumina
MA2	2 tubes (per 96 samples)	-20°C	Illumina
MSM	2 tubes (per 96 samples)	-20°C	Illumina
0.1N NaOH	15 ml (per 96 samples)	4°C	User
96-well 0.8 ml microtiter plate (MIDI)	1 plate		User
WG#-DNA plate with 48 or 96 DNA samples (50 ng/µl)	1 plate	-20°C	User

Preparation

[ ] Preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.

[ ] Apply an MSA1 barcode label to a new MIDI plate.

[ ] Thaw MA1, MA2, and MSM tubes to room temperature (20–24°C). Pulse centrifuge to 280 xg.

[ ] Thaw DNA samples to room temperature (20–24°C).

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Steps

[ ] **1.** If you do not already have a WG#-DNA plate, create one by adding DNA, normalized to 50 ng/µl, into either a:

- MIDI plate: 20 µl to each WG#-DNA plate well
- TCY plate: 10 µl to each WG#-DNA plate well

Apply a barcode label to the new WG#-DNA plate.

[ ] **2.** Dispense 20 µl MA1 into the MSA1 plate wells.

[ ] **3.** Transfer 4 µl DNA sample from the WG#-DNA plate to the corresponding wells in the MSA1 plate.

[ ] **4.** On the lab tracking worksheet, record the original DNA sample ID for each well in the MSA1 plate.

[ ] **5.** Dispense 4 µl 0.1N NaOH into each well of the MSA1 plate that contains MA1 and sample.

[ ] **6.** Seal the MSA1 plate with the 96-well cap mat.

CAUTION

Orient the cap mat so that A1 on the cap matches A1 on the plate. To prevent evaporation and spills, which could lead to assay variability and cross-contamination, ensure that all 96 caps are securely seated in the wells.

[ ] **7.** Vortex the plate at 1600 rpm for 1 minute.

[ ] **8.** Centrifuge to 280 xg for 1 minute.

[ ] **9.** Incubate for 10 minutes at room temperature (20–24°C).

[ ] **10.** Dispense 68 µl MA2 into each well of the MSA1 plate containing sample.

[ ] **11.** Dispense 75 µl MSM into each well of the MSA1 plate containing sample.

[ ] **12.** Seal MSA1 plate with cap mat.

[ ] **13.** Vortex the sealed MSA1 plate at 1600 rpm for 1 minute.

[ ] **14.** Pulse centrifuge to 280 xg for 1 minute.

[ ] **15.** Incubate in the Illumina Hybridization Oven for 20–24 hours at 37°C.

[ ] **16.** Proceed to *Fragment MSA1*.

Fragment MSA1

Enzymatically fragment DNA, using end-point fragmentation to avoid over-fragmentation.

Estimated Time

Hands-on time: ~30 minutes for 96 samples
Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
FMS	2 tubes (per 96 samples)	-20°C	Illumina

Preparation

[ ] Preheat the heat block with the MIDI plate insert to 37°C.
[ ] Thaw FMS tubes to room temperature (20–24°C). Gently invert to mix contents, and then pulse centrifuge to 280 xg.
[ ] Remove the MSA1 plate from the Illumina Hybridization Oven.

Steps

[ ] **1.** Centrifuge the plate to 50 xg for 1 minute.
[ ] **2.** Add 50 µl FMS to each well containing sample.
[ ] **3.** Seal the MSA1 plate with the 96-well cap mat.
[ ] **4.** Vortex the plate at 1600 rpm for 1 minute.
[ ] **5.** Centrifuge the plate to 50 xg for 1 minute at 22°C.
[ ] **6.** Place the sealed plate on the 37°C heat block for 1 hour.
[ ] **7.** Do one of the following:
- Proceed to *Precip MSA1*. Leave plate in 37°C heat block until setup is complete.
- Store the sealed MSA1 plate at -20°C if you do not plan to proceed to the next step immediately.

Precip MSA1

Precipitate the DNA sample using 2-propanol and PM1.

Hands-on time: ~30 minutes for 96 samples
Incubation and dry time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PM1	2 tubes (per 96 samples)	4°C	Illumina
100% 2-propanol	40 ml (per 96 samples)	Room temperature	User

Preparation

[ ] Preheat heat block to 37°C.

[ ] If frozen, thaw MSA1 plate to room temperature (20–24°C). Pulse centrifuge to 50 xg.

[ ] Thaw PM1 to room temperature (20–24°C). Centrifuge to 280 xg for 1 minute.

[ ] Remove the 96-well cap mat.

Steps

[ ] **1.** Add 100 µl PM1 to each MSA1 plate well containing sample.

[ ] **2.** Seal the plate with the cap mat.

[ ] **3.** Vortex the plate at 1600 rpm for 1 minute.

[ ] **4.** Incubate at 37°C for 5 minutes.

[ ] **5.** Centrifuge to 50 xg at 22°C for 1 minute.

Set centrifuge to 4°C in preparation for the next centrifuge step.

[ ] **6.** Add 300 µl 100% 2-propanol to each well containing sample.

[ ] **7.** Carefully seal the MSA1 plate with a new, ***dry*** cap mat.

[ ] **8.** Invert at least 10 times to mix contents thoroughly.

[ ] **9.** Incubate at 4°C for 30 minutes.

[ ] **10.** Centrifuge to 3,000 xg at 4°C for 20 minutes. Immediately remove the MSA1 plate from centrifuge.

Perform the next step immediately, to avoid dislodging the blue pellet. If any delay occurs, repeat the 20-minute centrifugation before proceeding.

[ ] **11.** Remove the cap mat and discard it.

[ ] **12.** Decant supernatant by quickly inverting the MSA1 plate and smacking it down onto an absorbent pad.

[ ] **13.** Tap firmly several times for 1 minute or until all wells are devoid of liquid. Do not allow supernatant to pour into other wells.

[ ] **14.** Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature (20–24°C) to air dry the pellet.

[ ] **15.** Do one of the following:

- Proceed to *Resuspend MSA1*.
- Seal the MSA1 plate with a cap mat and store it at -20°C if you do not plan to proceed to the next step immediately.

Resuspend MSA1

Resuspend the precipitated DNA using RA1.

Estimated Time

Hands-on time: ~30 minutes for 96 samples
Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	46 µl per sample well	-20°C	Illumina

Only pour out the recommended volume of RA1 needed for the suggested number of samples listed in the consumables table. Additional RA1 is used later in the **XStain HD BeadChip** step.

Preparation

[ ] Preheat the Illumina Hybridization Oven to 48°C.

[ ] Turn on the heat sealer to preheat. Allow 20 minutes.

[ ] Thaw RA1 to room temperature (20–24°C). Gently invert.

Steps

[ ] **1.** Add 46 µl RA1 to each well of the MSA1 plate containing a DNA pellet. Reserve any leftover reagent for Hyb Multi BC2 and XStain HD BeadChip.

[ ] **2.** Apply foil seal to MSA1 plate by firmly holding the heat-sealer sealing block down for 5 seconds.

[ ] **3.** Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.

[ ] **4.** Vortex the plate at 1800 rpm for 1 minute.

[ ] **5.** Pulse centrifuge to 280 xg.

If you stored the pellets at -20°C for extended periods of time after the Precip MSA1 process, you may need to repeat the vortexing and centrifugation steps until the pellets are completely resuspended.

[ ] **6.** Do one of the following:

- Proceed to *Hyb Multi BC2*. If you plan to do so immediately, it is safe to leave the MSA1 plate at room temperature.
- If you do not plan to proceed to the next step immediately, store the sealed MSA1 plate at -20°C (-80°C if storing for more than 24 hours). Store RA1 at -20°C

Hyb Multi BC2

Dispense the fragmented, resuspended DNA samples onto BeadChips. Incubate the BeadChips in the Illumina Hybridization Oven to hybridize the samples onto the BeadChips.

Estimated Time

Hands-on time:

- ~30 minutes for 12 BeadChips (48 samples)
- ~1 hour for 24 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 8 BeadChips)	Storage	Supplied By
PB2	2 tubes	Room temperature	Illumina
BeadChips	8		Illumina
Hyb Chambers	2		Illumina
Hyb Chamber gaskets	2		Illumina
Hyb Chamber inserts	8		Illumina

Preparation

[ ] Preheat the heat block to 95°C.

[ ] Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.

Steps

Prepare Hyb Chamber(s)

[ ] **1.** Place the Hyb Chamber gaskets into the Hyb Chambers.

[ ] **2.** Dispense 400 µl PB2 to each of the 8 humidifying buffer reservoirs in each Hyb Chamber.

[ ] **3.** Secure the lid of each Hyb Chamber. Keep on bench at room temperature (20–24°C) until ready to load BeadChips.

Hyb Multi BC2

[ ] **1.** Place the resuspended MSA1 plate on the heat block to denature the samples at 95°C for 20 minutes.

[ ] **2.** Remove the BeadChips from 2–8°C storage but do not unpackage.

[ ] **3.** After the 20-minute incubation, pulse centrifuge the AMP2 plate to 280 xg.

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Load BeadChip

[ ] 1. Just before loading DNA samples, remove all BeadChips from their packages.

When handling the BeadChip, avoid contacting the beadstripe area and sample inlets.

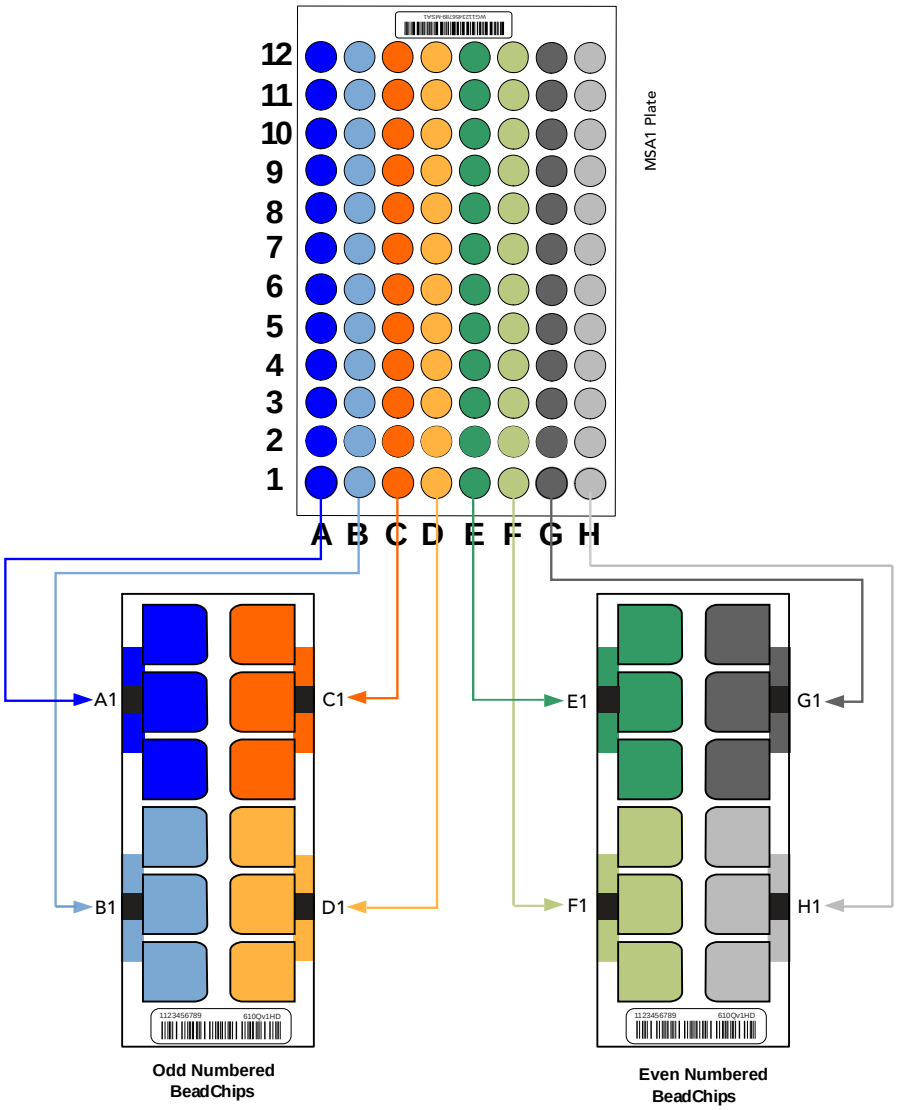
[ ] 2. Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the Hyb Chamber insert.

[ ] 3. Using a single-channel precision pipette, dispense 38 µl of each DNA sample onto the appropriate BeadChip section, according to the chart on the lab tracking worksheet and the following illustrations:

Dispense MSA1 Plate sample A1 to the A1 sample inlet of BC2#1, then MSA1 Plate sample B1 to the B1 sample inlet, C1 to C1 and D1 to D1. Next, dispense E1 through H1 into the corresponding sample inlets of BC2#2.

Follow this loading pattern for the remaining BeadChips, dispensing samples to the top left, bottom left, and then the top right, bottom right of each BeadChip.

Load samples by directly placing pipette tip to the array surface and hold the pipette directly vertical and above the array surface. Do not hold the pipette at an angle while you are loading sample. Proceed immediately to the next step as soon as all arrays have received sample.



[ ] 4. On the lab tracking worksheet, record the BeadChip barcode for each group of samples.

[ ] 5. Visually inspect all sections of the BeadChips. Ensure the DNA sample covers all of each bead stripe. Record any sections that are not completely covered.

[ ] 6. Load the Hyb Chamber inserts containing BeadChips into the Illumina Hyb Chamber. Position the barcode end over the ridges indicated on the Hyb Chamber.

[ ] 7. Place the back side of lid onto the Hyb Chamber and then slowly bring down the front end to avoid dislodging the Hyb Chamber inserts.

[ ] 8. Close the clamps on both sides of the Hyb Chamber.

NOTE

For optimal performance, keep the Hyb Chamber steady and level when lifting or moving. Avoid shaking the Hyb Chamber, keep the Hyb Chamber parallel to the lab bench while you transfer it to the Illumina Hybridization Oven.

[ ] **9.** Place the Hyb Chamber into the 48°C Illumina Hybridization Oven so that the clamps of the Hyb Chamber face the left and right side of the oven. The Illumina logo on top of the Hyb Chamber should be facing you.

[ ] **10.** If you are loading multiple Hyb Chambers, stack them on top of each other, up to 3 per stack, for a total of 6 in the Hyb Oven.

NOTE

If you are stacking multiple Hyb Chambers in the Illumina Hybridization Oven, make sure the feet of the top Hyb Chamber fit into the matching indents on top of the next Hyb Chamber. This will hold the Hyb Chambers in place while they are rocking.

[ ] **11.** (Optional) Set the rocker speed to 5.

[ ] **12.** Incubate at 48°C for at least 16 hours but no more than 24 hours.

[ ] **13.** Discard the MSA1 plate.

[ ] **14.** After the overnight incubation, proceed to *Wash BeadChip*.

Resuspend XC4 Reagent for XStain HD BeadChip

[ ] **1.** Add 330 ml 100% EtOH to the XC4 bottle.

[ ] **2.** Shake vigorously for 15 seconds.

[ ] **3.** Leave the bottle upright on the lab bench overnight.

NOTE

If the XC4 was not left to resuspend overnight, you can still proceed with the assay. Add the EtOH and put the XC4 on its side on a rocker to resuspend. Leave it there until the BeadChips are ready for coating.

[ ] **4.** Shake again to ensure that the pellet is completely resuspended. If any coating is visible, vortex at 1625 rpm until it is in complete suspension.

Wash BeadChip

Prepare the BeadChips for the staining process.

Estimated Time

Hands-on time:

- 20 minutes for 4 BeadChips
- 30 minutes for 8 BeadChips
- 40 minutes for 12 BeadChips

Consumables

Item	Quantity	Storage	Supplied By
PB1	550 ml (up to 8 BeadChips)	Room temperature	Illumina
Multi-Sample BeadChip Alignment Fixture	1 (per 4 BeadChips)		Illumina
Te-Flow Flow-Through Chambers (with black frames, spacers, glass back plates, and clamps)	1 (per BeadChip)		Illumina
Wash Dish	2 (up to 8 BeadChips)		Illumina
Wash Rack	1 (up to 8 BeadChips)		Illumina

Only pour out the recommended volume of PB1 needed for the suggested number of samples listed in the consumables table. Additional PB1 is used later in the XStain HD BeadChip step. One bottle of PB1 should be used per 8 BeadChips.

Preparation

[ ] Fill 2 wash dishes with PB1 (200 ml per wash dish). Label each dish "PB1".

[ ] Fill the BeadChip Alignment Fixture with 150 ml PB1.

[ ] Separate the clear plastic spacers from the white backs.

[ ] Clean the glass back plates as directed in the *Infinium Assay Lab Setup and Procedures Guide*.

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Steps

[ ] 1. Remove the Hyb Chamber(s) from the Illumina Hybridization Oven.

[ ] 2. Attach the wire handle to the rack and submerge the wash rack in the first wash dish containing 200 ml PB1.

[ ] 3. Remove the Hyb Chamber inserts from the Hyb Chambers.

[ ] 4. Remove BeadChips from the Hyb Chamber inserts one at a time.

[ ] 5. Remove the IntelliHyb seal from each BeadChip:

To ensure no solution splatters on you, Illumina recommends removing the seal over an absorbent cloth or paper towels, preferably in a hood.

- **[ ] a.** Using powder-free gloved hands, hold the BeadChip in one hand with your thumb and forefinger on the long edges of the BeadChip. The BeadChip may also be held with the thumb and forefinger on the short edges of the BeadChip. In either case avoid contact with the sample inlets. The barcode should be facing up and be closest to you, and the top side of the BeadChip should be angled slightly away from you.
- **[ ] b.** Remove the entire seal in a single, rapid motion by pulling it off in a diagonal direction. Start with a corner on the barcode end and pull with a continuous upward motion away from you and towards the opposite corner on the top side of the BeadChip. Do not stop and start the pulling action. Do not touch the exposed active areas.

[ ] 6. Immediately and carefully slide each BeadChip into the wash rack one at a time, making sure that the BeadChip is completely submerged in the PB1.

[ ] 7. Repeat steps 5 and 6 until all BeadChips are transferred to the submerged wash rack. The wash rack holds up to 8 BeadChips.

[ ] 8. Once all the BeadChips are in the wash rack, move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

[ ] 9. Move the wash rack to the other wash dish containing PB1. Make sure that the BeadChips are completely submerged.

[ ] 10. Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

[ ] 11. If you are processing more than 8 BeadChips:

- **[ ] a.** Complete the steps in the next section, *Assemble Flow-Through Chambers*, for the first 8 BeadChips.
- **[ ] b.** Place the assembled Flow-Through Chambers of the first 8 BeadChips on the lab bench in a horizontal position.

Keep them in a horizontal position on the lab bench until all assembled Flow-Through Chambers are ready to be loaded into the Chamber Rack. Do not place the Flow-Through Chambers in the Chamber Rack until all necessary steps are completed.

[ ] c. Repeat steps 3 through 11 from this section for any additional BeadChips.Use new PB1 for each set of 8 BeadChips.

Assemble Flow-Through Chamber

The 150 ml of PB1 used in the Flow-Through Chamber assembly can be used for up to eight BeadChips. You must use 150 ml for every additional set of eight BeadChips.

[ ] 1. If you have not done so yet, fill the BeadChip Alignment Fixture with 150 ml PB1.

[ ] 2. For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture. The Alignment Fixture should already be filled with PB1.

[ ] 3. Place each BeadChip into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture. Each BeadChip should be fully immersed in PB1.

[ ] 4. Place a clear spacer onto the top of each BeadChip. Use the Alignment Fixture grooves to guide the spacers into proper position.

[ ] 5. Place the Alignment Bar onto the Alignment Fixture.

[ ] 6. Use a laboratory air gun to quickly remove any accumulated dust from the glass back plates just before placing them onto the BeadChips.

[ ] 7. Place a clean glass back plate on top of the clear spacer covering each BeadChip. The plate reservoir should be at the barcode end of the BeadChip, facing inward to create a reservoir against the BeadChip surface.

[ ] 8. Attach the metal clamps onto each Flow-Through Chamber as follows:

- **[ ] a.** Gently push the glass back plate up against the Alignment Bar with one finger.
- **[ ] b.** Place the first metal clamp around the Flow-Through Chamber so that the clamp is ~5 mm from the top edge.
- **[ ] c.** Place the second metal clamp around the Flow-Through Chamber at the barcode end, ~5 mm from the reagent reservoir.

[ ] 9. With scissors, trim the spacer at the non-barcode end of the assembly. Slip the scissors up over the barcode to trim the other end.

[ ] **10.** Immediately wash the Hyb Chamber reservoirs with dH_2O and scrub them with a small cleaning brush, ensuring that no PB2 remains.

CAUTION

Place all assembled Flow-Through Chambers on the lab bench in a horizontal position while you perform the preparation steps for XStain HD BeadChip. Do not place the Flow-Through Chambers in the Chamber Rack until all necessary steps are completed.

[ ] **11.** Proceed to *XStain HD BeadChip*.

XStain HD BeadChip

Wash unhybridized and non-specifically hybridized DNA sample from the BeadChips. Add labelled nucleotides to extend the primers hybridized to the DNA. Stain the primers, disassemble the Flow-Through Chambers, and coat the BeadChips for protection.

Estimated Time

Hands-on time: ~3 hours for 8 BeadChips
Dry time: 55 minutes

Consumables

Item	Quantity	Storage	Supplied By
RA1	10 ml for 1–8 BeadChips 20 ml for 9–16 BeadChips 30 ml for 17–24 BeadChips	-20°C	Illumina
XC1	2 tubes (per 8 BeadChips)	-20°C	Illumina
XC2	2 tubes (per 8 BeadChips)	-20°C	Illumina
TEM	2 tubes (per 8 BeadChips)	-20°C	Illumina
XC3	49 ml for 1–8 BeadChips 97 ml for 9–16 BeadChips 145 ml for 17–24 BeadChips	Room temperature	Illumina
STM (Make sure that all STM tubes indicate the same stain temperature on the label)	2 tubes (per 8 BeadChips)	-20°C	Illumina

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Item	Quantity	Storage	Supplied By
ATM	2 tubes (per 8 BeadChips)	-20°C	Illumina
PB1	310 ml for 1–8 BeadChips 285 ml for 9–24 BeadChips	Room temperature	Illumina
XC4	310 ml for 1–8 BeadChips 285 ml for 16– 24 BeadChips	-20°C	Illumina
Alconox Powder Detergent	as needed		User
EtOH	as needed	Room temperature	User
95% formamide/1 mM EDTA	15 ml for 1–8 BeadChips 17 ml for 9–16 BeadChips 25 ml for 17–24 BeadChips	-20°C	User

Preparation

[ ] Place all reagent tubes in a rack in the order in which they will be used. If frozen, allow them to thaw to room temperature (20–24°C) and centrifuge to 3000 xg for 3 minutes.

[ ] Ensure the water circulator is filled to the appropriate level.

[ ] Turn on the water circulator. Set it to a temperature that brings the Chamber Rack to 44°C at equilibrium.

[ ] Remove bubbles trapped in the Chamber Rack.

[ ] Test several locations on the Chamber Rack, using the Illumina Temperature Probe, to ensure that it is uniformly 44°C.

Steps

The remaining steps must be performed without interruption.

Single-Base Extension

[ ] **1.** When the Chamber Rack reaches 44°C, quickly place each Flow-Through Chamber assembly into the Chamber Rack.

For 4 BeadChips, place the Flow-Through Chambers in every other position, starting at 1, in the first row of the Chamber Rack. For larger numbers of BeadChips, fill all positions in the first row, then the second and third.

[ ] **2.** Into the reservoir of each Flow-Through Chamber, dispense:

[ ] **a.** 150 µl RA1. Incubate for 30 seconds. Repeat 5 times.

[ ] **b.** 450 µl XC1. Incubate for 10 minutes.

[ ] **c.** 450 µl XC2. Incubate for 10 minutes.

[ ] **d.** 200 µl TEM. Incubate for 15 minutes.

[ ] **e.** 450 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat once.

[ ] **f.** Incubate 5 minutes.

[ ] **g.** Begin ramping the Chamber Rack temperature to the temperature indicated on the STM tube, or to 37°C if none is shown.

[ ] **h.** 450 µl XC3. Incubate for 1 minute. Repeat once.

[ ] **3.** Wait until the Chamber Rack reaches the correct temperature.

Stain BeadChip

[ ] **1.** If you plan to image the BeadChip immediately after the staining process, turn on the Illumina BeadArray™ Reader now.

[ ] **2.** Into the reservoir of each Flow-Through Chamber, dispense:

[ ] **a.** 250 µl STM and incubate for 10 minutes.

[ ] **b.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.

[ ] **c.** 250 µl ATM and incubate for 10 minutes.

[ ] **d.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.

[ ] **e.** 250 µl STM and incubate for 10 minutes.

[ ] **f.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.

[ ] **g.** 250 µl ATM and incubate for 10 minutes.

[ ] **h.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.

[ ] **i.** 250 µl STM and incubate for 10 minutes.

[ ] **j.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.

[ ] **3.** Immediately remove the Flow-Through Chambers from the Chamber Rack and place horizontally on a lab bench at room temperature (20–24°C).

Wash and Coat

[ ] **1.** Pour 310 ml PB1 per 8 BeadChips into a wash dish. Cover the dish.

[ ] **2.** Place the staining rack inside the wash dish. The locking arms should face **towards** you.

CAUTION Handle the BeadChips only by the edges or the barcode end. Do not let the BeadChips dry out.

[ ] **3.** For each BeadChip:
- [ ] **a.** Use the dismantling tool to remove the two metal clamps from the Flow-Through Chamber.
- [ ] **b.** Remove the glass back plate, the spacer, and then the BeadChip.
- [ ] **c.** Immediately place each BeadChip into the staining rack that is in the wash dish with the barcode facing **away** from you. Place half of the BeadChips above the handle and half below. All chips should be completely submerged.

[ ] **4.** Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

[ ] **5.** Soak for 5 minutes. Do not leave the BeadChips in the PB1 for more than 30 minutes.

[ ] **6.** Shake the XC4 bottle vigorously to ensure complete resuspension.

[ ] **7.** Pour 310 ml XC4 into a wash dish. Do not let it sit for more than 10 minutes.

[ ] **8.** Move the BeadChip staining rack into the XC4 dish. The barcodes should face **away** from you and the locking arms **towards** you.

[ ] **9.** Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.

[ ] **10.** Soak for 5 minutes.

[ ] **11.** Lift the staining rack out of the solution and place it on a tube rack with the staining rack and BeadChips horizontal, barcodes facing up.

[ ] **12.** Remove the BeadChips from the staining rack with locking tweezers, working from top to bottom. Place each BeadChip on a tube rack to dry. Remove the staining rack handle after removing the first four BeadChips.

[ ] **13.** Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 508 mm Hg (0.68 bar).

[ ] **14.** Clean the underside of each BeadChip with a ProStat EtOH wipe.

CAUTION Do not touch the stripes with the wipe or allow EtOH to drip onto the stripes.

[ ] **15.** Clean and store the glass back plates and Hyb Chamber components.

[ ] **16.** Do one of the following:
- Proceed to *Image BeadChip*.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box inside a vacuum desiccator at room temperature (20–24°C). Image the BeadChips within 72 hours.

Image BeadChip

Scan the BeadChips with the Illumina BeadArray™ Reader, which uses a laser to excite the fluorophore of the single-base extension product on the beads. The scanner records high-resolution images of the light emitted from the fluorophores. The data from these images are analyzed to determine SNP genotypes using Illumina's genotyping analysis software.

Scan time

~3 hours per BeadChip

Preparation

[ ] Turn on the BeadArray Reader at least one or two hours before scanning.

Steps

[ ] **1.** Open the Illumina BeadScan application.

[ ] **2.** Login and click **Scan** on the Welcome screen.

[ ] **3.** Select **BeadChip** from the Docking Fixture menu.

[ ] **4.** Place the BeadChip(s) in the BeadArray Reader tray.

Non-LIMS users only:

- In the Settings area, click **Edit**.
- Click **Browse** to select the following directories:
  - *Data repository directory containing your data*
  - *Decode map directory, containing the decode data from the BeadChip CD*
- To save the image data in compact JPG format, select the **Save Compressed Images** check box.
- Click either **Save for this Scan** or **Save for All Scans**.

[ ] **5.** Scan each BeadChip barcode.

Non-LIMS users only:

- Click **Browse** (...) to open the Select Scan Settings dialog box.
- Select a scan method for each BeadChip. Click **Select**.

[ ] **6.** Click **Scan**.

[ ] **7.** When the BeadChips are finished scanning, click **Done**.

[ ] **8.** When the Welcome screen reappears, click **Open Tray**. Remove the BeadChips.

[ ] **9.** Do one of the following:

- Scan the next set of BeadChips.
- Right click near the Illumina logo on the Welcome screen and select **Exit**. Close the BeadArray Reader tray by pushing the **Up** button and turn off the machine.