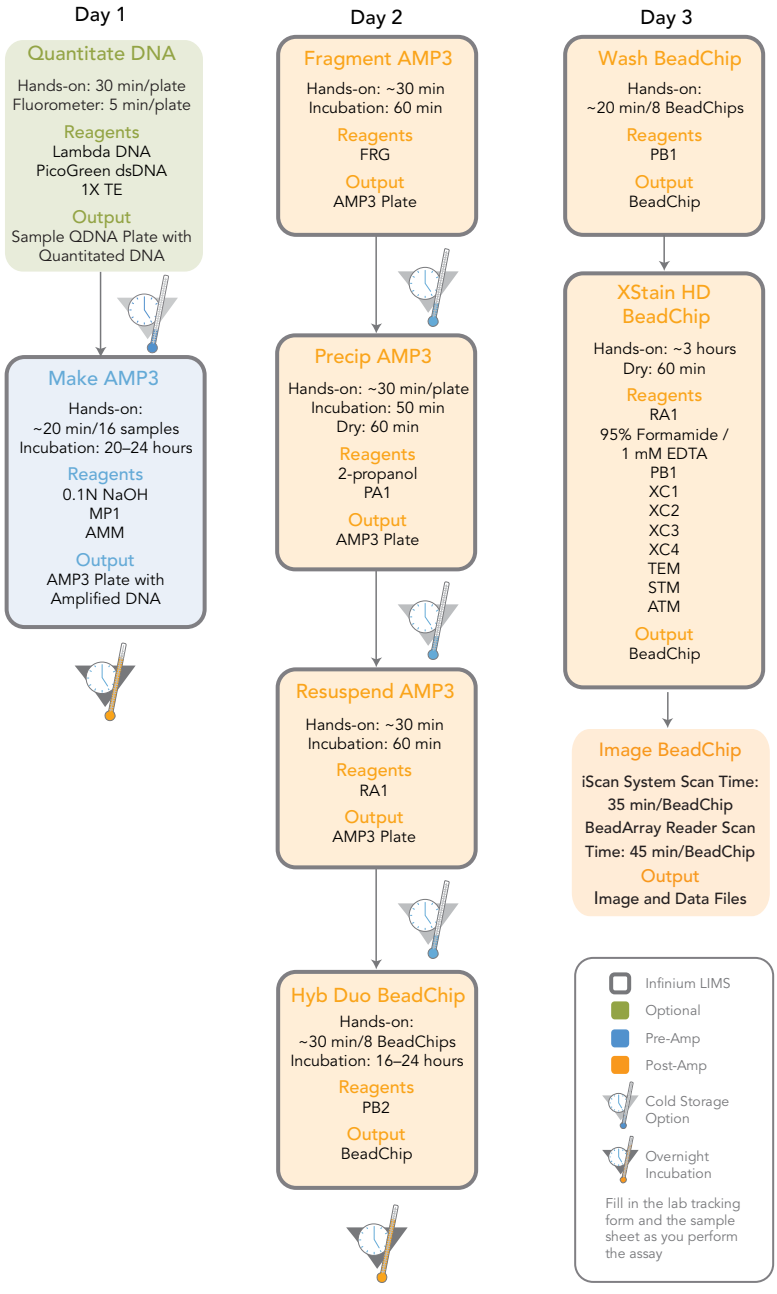
Experienced User Card



Quant DNA (Optional)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples. You can quantitate up to six plates, each containing up to 96 samples.

Estimated Time

Hands-on time: ~20 minutes per plate, plus 10 minutes to prepare the PicoGreen
Spectrofluorometer read time: ~5 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See Instructions	2 to 8°C	User
1X TE	See Instructions	Room temperature	User
Lambda DNA	See Instructions	2 to 8°C	User
96-well 0.65 ml microtiter plate	1 per 96 samples		User
Fluotrac 200 96-well flat-bottom plate	1 per Std DNA plate 1 per Sample DNA plate		User

Preparation

[ ] Thaw PicoGreen to room temperature for 60 minutes in a light-impermeable container.

[ ] Hand-label the microtiter plate "Standard DNA."

[ ] Hand-label one of the Fluotrac plates "Standard QDNA." Hand-label the other Fluotrac plate "Sample QDNA." In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

Steps

Make Standard DNA Plate

[ ] **1.** Add stock Lambda DNA to well A1 in the plate labelled "Standard DNA" and dilute it to 75 ng/µl in a final volume of 233.3 µl. Pipette up and down several times.

[ ] **2.** Add 66.7 µl 1X TEto well B1.

[ ] **3.** Add 100 µl 1X TE to wells C, D, E, F, G, and H of column 1.

[ ] **4.** Transfer 133.3 µl of Lambda DNA from well A1 into well B1. Pipette up and down several times.

[ ] **5.** Change tips. Transfer 100 µl from well B1 into well C1. Pipette up and down several times.

[ ] **6.** Repeat for wells D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.** Well H1 serves as the blank 0 ng/µl Lambda DNA.

[ ] **7.** Cover the Standard DNA plate with an adhesive seal.

Dilute PicoGreen

[ ] **1.** Prepare a 1:200 dilution of PicoGreen into 1X TE, using a sealed 100 ml or 250 ml Nalgene bottle wrapped in aluminum foil.

Use 115 µl PicoGreen and 23 ml 1X TE for 1 plate, 215 µl PicoGreen and 43 ml 1X TE for 2 plates, and so on up to 6 plates.

[ ] **2.** Cap the foil-wrapped bottle and vortex to mix.

Create Standard QDNA Plate with Diluted PicoGreen

[ ] **1.** Pour the PicoGreen/1X TE dilution into a clean reagent reservoir.

[ ] **2.** Using a multichannel pipette, transfer 195 µl PicoGreen/1X TE dilution into each well of columns 1 and 2 of the Fluotrac plate labelled "Standard QDNA".

[ ] **3.** Add 2 µl of each stock Lambda DNA dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA Fluotrac plate.

[ ] **4.** Immediately cover the plate with an adhesive aluminum seal.

Prepare Sample QDNA Plate with PicoGreen and DNA

[ ] **1.** Using a multichannel pipette, transfer 195 µl PicoGreen/1X TE dilution into each well of columns 1 and 2 of the plate labelled "Sample QDNA".

[ ] **2.** Add 2 µl of DNA sample to all 96 wells of the Sample QDNA plate.

[ ] **3.** Immediately cover the plate with an adhesive aluminum seal.

Read QDNA Plate

[ ] **1.** Turn on the spectrofluorometer. At the PC, open the SoftMax Pro program.

[ ] **2.** Load the Illumina QDNA.ppr file from the installation CD that came with your system.

[ ] **3.** Select **Assays | Nucleic Acids | Illumina QDNA**.

[ ] **4.** Place the Standard QDNA Fluotrac Plate into the spectrofluorometer.

[ ] **5.** Click the blue arrow next to **Lambda Standard** then click **Read**.

[ ] **6.** When the software finishes reading the data, remove the plate from the drawer.

[ ] **7.** Click the blue arrow next to **Standard Curve** to view the standard curve graph.

[ ] **8.** Place the first Sample QDNA plate in the spectrofluorometer.

[ ] **9.** Click the blue arrow next to **QDNA#1** then click **Read**.

[ ] **10.** When the software finishes reading the plate, remove the plate from the drawer.

[ ] **11.** Repeat steps 8 through 10 to quantitate all Sample QDNA plates.

[ ] **12.** Once all plates have been read, click **File | Save** to save the output data file (*.pda).

[ ] **13.** When you have saved the *.pda file, click **File | Import/Export | Export** and export the file as a *.txt file.

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

- Proceed to *Make AMP3*.
- Store the quantitated DNA at 2 to 8°C for up to one month.

Experienced User Card

Make AMP3

Move DNA samples into AMP3 plate. Denature and neutralize samples, and prepare them for amplification. Incubate overnight to amplify.

Estimated Time

Hands-on time: ~20 minutes per 16 samples
Incubation time: ~20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
MP1	1 tube per 16 samples	-15 to -25°C	Illumina
AMM	1 tube per 16 samples	-15 to -25°C	Illumina
0.1N NaOH	15 ml for 16–48 samples	2 to 8°C	User
96-well 0.8 ml microtiter plate (MIDI)	1 plate per 48 samples		User
WG#-DNA plate with up to 96 DNA samples (50 ng/ l)	1 plate for up to 48 samples	-15 to -25°C	User

Preparation

[ ] Preheat the Illumina Hybridization Oven in the post-amp area to 37°C.
[ ] Apply an AMP3 barcode label to a new MIDI plate.
[ ] Thaw MP1 and AMM tubes to room temperature. Gently invert to mix, then pulse centrifuge to 280 xg.
[ ] Thaw DNA samples to room temperature.

Steps

[ ] **1.** If you do not already have a WG#-DNA plate, create one by adding DNA, normalized to 50 ng/ l, into either a:
- MIDI plate: 40 µl to each WG#-DNA plate well
- TCY plate: 30 µl to each WG#-DNA plate well

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

[ ] **2.** Vortex the WG#-DNA plate at 1600 rpm (actual vortex speed) for 1 minute.
[ ] **3.** Centrifuge to 280 xg for 1 minute.
[ ] **4.** Transfer 8 µl DNA sample, into each well in the following AMP3 plate columns:
- Column 1 (8 samples)
- Columns 1 and 3 (16 samples)
- Columns 1, 3, 5, 7, 9, and 11 (48 samples)

[ ] **5.** Dispense 8 µl 0.1N NaOH into each well that contains DNA.
[ ] **6.** Incubate for 10 minutes at room temperature.
[ ] **7.** Dispense 135 µl MP1 into each well containing sample.

[ ] **8.** Dispense 150 µl AMM into each well containing sample.

[ ] **9.** Seal the plate with a cap mat.

[ ] **10.** Invert the sealed plate at least 10 times to mix contents.

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

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

[ ] **13.** Proceed immediately to *Fragment AMP3*.

This is the end of Pre-Amp. You may now remove these Experienced User Cards from the Pre Amp area and take them elsewhere. Do not return with them into the Pre-Amp area at any time.

Fragment AMP3

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

Estimated Time

Hands-on time: ~30 minutes per 48 samples
Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
FRG	1 tube per 16 samples	-15 to -25°C	Illumina

Preparation

[ ] Preheat the heat block with the MIDI plate insert to 37°C.

[ ] Thaw the FRG tube to room temperature. Invert several times to mix contents. Pulse centrifuge to 280 xg for 1 minute.

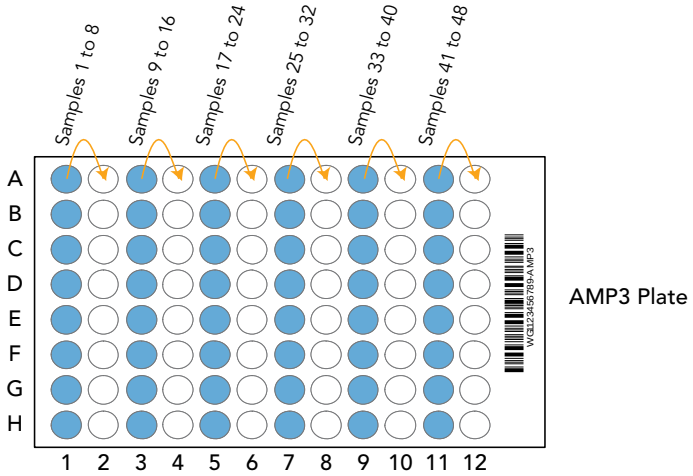
Steps

[ ] **1.** Centrifuge the plate to 50 xg for 1 minute.

[ ] **2.** Thoroughly pipette-mix all wells containing sample to evenly distribute precipitate.

[ ] **3.** Split the sample into 1 additional well, for a total of 2 wells per sample. Each well should contain 150 µl.

For example, move 150 µl sample from A1 into A2.



[ ] **4.** Dispense 50 µl FRG to each well containing sample.

[ ] **5.** Seal the AMP3 plate with the cap mat.

[ ] **6.** Vortex the AMP3 plate at 1600 rpm for 1 minute.

[ ] **7.** Centrifuge the plate to 50 xg for 1 minute at 22°C.

[ ] **8.** Incubate the sealed plate on the 37°C heat block for 1 hour.

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

- Proceed to *Precip AMP3*. Leave the plate in 37°C heat block until setup is complete.
- Store the sealed AMP3 plate at -15 to -25°C if you do not plan to proceed to the next step immediately.

Precip AMP3

Precipitate the DNA sample using 2-propanol and PA1.

Estimated Time

Hands-on time: ~30 minutes per 48 samples
Incubation time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PA1	1 tube per 16 samples	2 to 8°C	Illumina
100% 2-propanol	12 ml per 16 samples 40 ml per 48 samples	Room temperature	User

Preparation

- [ ] If frozen, thaw AMP3 plate to room temperature. Pulse centrifuge to 50 xg.
- [ ] Thaw PA1 to room temperature. Centrifuge to 280 xg for 1 minute.
- [ ] Preheat the heat block to 37°C, if it is not already.
- [ ] Turn on the heat sealer.
- [ ] Set the centrifuge to 4°C.

Steps

- [ ] **1.** Dispense 100 µl PA1 to each well containing sample.
- [ ] **2.** Seal the plate with the cap mat.
- [ ] **3.** Vortex the plate at 1600 rpm for 1 minute.
- [ ] **4.** Centrifuge to 50 xg at 22°C for 1 minute.
- [ ] **5.** Incubate at 37°C for 5 minutes.
- [ ] **6.** Add 300 µl 100% 2-propanol to each well containing sample.
- [ ] **7.** Seal the plate with a new, dry cap mat.
- [ ] **8.** Invert at least 10 times to mix contents.
- [ ] **9.** Incubate at 4°C for 30 minutes.
- [ ] **10.** Place the sealed AMP3 plate in the centrifuge opposite another plate of equal weight.
- [ ] **11.** Centrifuge to 3000 xg at 4°C for 20 minutes.

  Perform the next steps immediately, to avoid dislodging the blue pellet. If any delay occurs, repeat the 20-minute centrifugation.
- [ ] **12.** Remove the cap mat.
- [ ] **13.** Decant the supernatant by quickly inverting the AMP3 plate and smacking it down onto an absorbent pad.
- [ ] **14.** Tap the plate firmly on the pad several times over a period of 1 minute or until all wells are completely devoid of liquid.

[ ] **15.** Place the inverted, uncovered plate on a tube rack for 1 hour at room temperature to air dry the pellet.

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

- Proceed to *Resuspend AMP3*.
- Heat-seal the AMP3 plate and store it at -15 to -25°C for up to 24 hours or -80°C for long-term storage.

Resuspend AMP3

Resuspend the precipitated DNA using RA1.

Estimated Time

Hands-on time: ~30 minutes per 48 samples
Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	9 ml per 48 samples	-15 to -25°C	Illumina

Preparation

- [ ] Gradually warm the RA1 reagent to room temperature. Gently mix to dissolve crystals.
- [ ] If you stored the AMP3 plate at -15 to -25°C, thaw it to room temperature.
- [ ] Preheat the Illumina Hybridization Oven to 48°C.
- [ ] Turn on the heat sealer.

Steps

- [ ] **1.** Add 46 µl RA1 to each well of the AMP3 plate containing a DNA pellet.
- [ ] **2.** Heat-seal the AMP3 plate with a foil seal.
- [ ] **3.** Incubate it in the Illumina Hybridization Oven 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 -15 to -25°C for more than 72 hours after the Precip AMP3 process, you may need to repeat steps 3 to 5 until the pellets are completely resuspended.
- [ ] **6.** Do one of the following:
  - Proceed to *Hyb Duo BeadChip*. If you plan to do so immediately, it is safe to leave the RA1 at room temperature.
  - Store the sealed AMP3 plate and the RA1 at -15 to -25°C (-80°C if storing for more than 24 hours).

Hyb Duo BeadChip

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 8 BeadChips (16 samples)
- ~45 minutes for 16 BeadChips (32 samples)
- ~1 hour for 24 BeadChips (48 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 16 samples)	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.

[ ] If you plan to perform the XStain process tomorrow, begin thawing the XC4 reagent. For instructions, see *Resuspend XC4 Reagent for XStain HD BeadChip*.

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.** Place the Hyb Chamber inserts into the Hyb Chambers.

[ ] **4.** Secure the lid of each Hyb Chamber. Keep on bench at room temperature until ready to load BeadChips.

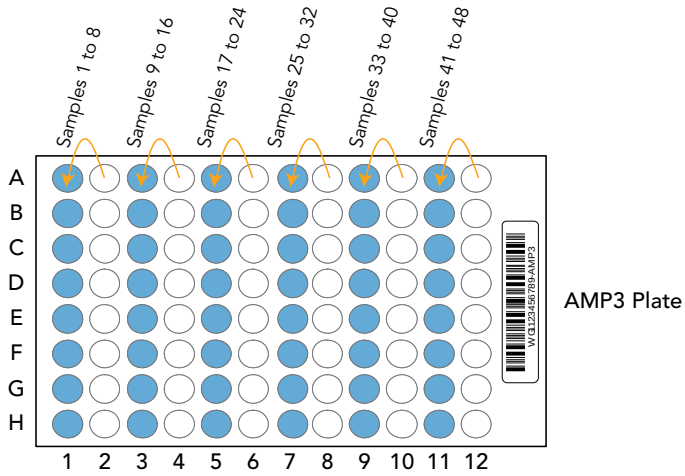
Hyb Duo BeadChip

[ ] **1.** Place the resuspended AMP3 plate on the heat block to denature the samples at 95°C for 20 minutes.

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

[ ] **3.** After the 20-minute incubation, pulse centrifuge the AMP3 plate to 280 xg.

[ ] **4.** Combine the two separate wells back into the original well. For example, move 46 µl sample from the A2 well back into A1.



Load BeadChip

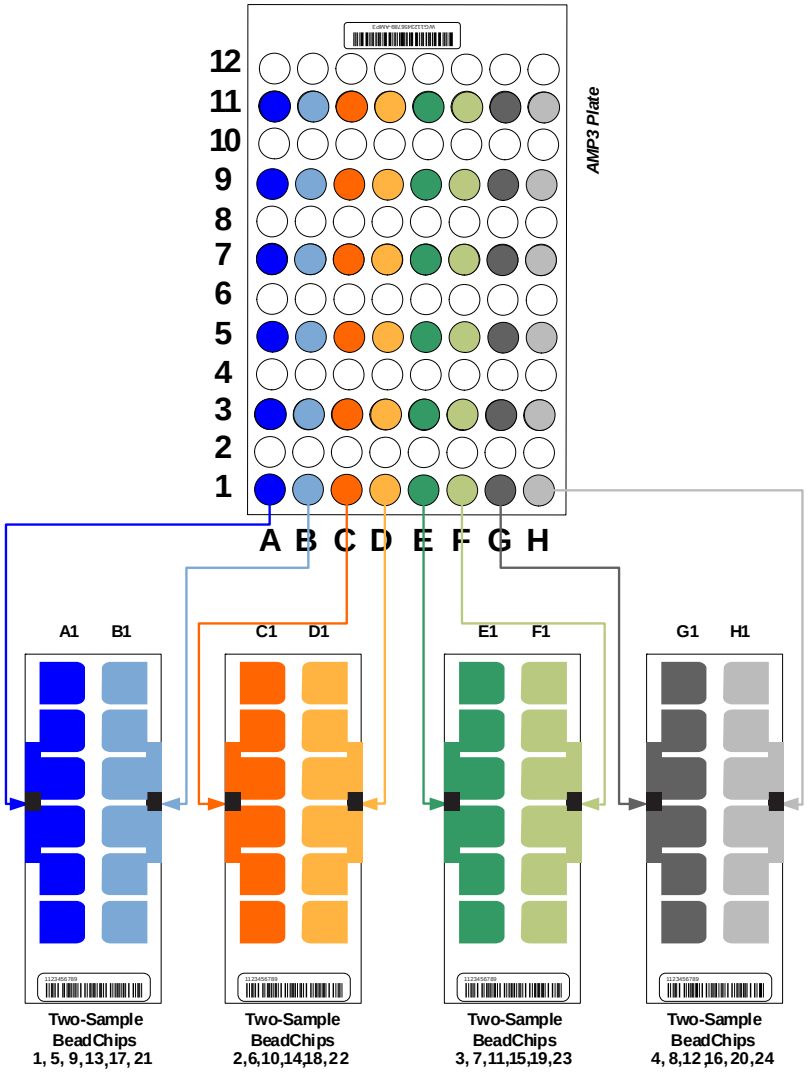
[ ] **1.** Just before loading DNA samples, remove all BeadChips from their packages.

[ ] **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. Make sure you place each BeadChip into the Hyb Chamber insert prior to loading the DNA sample.

[ ] **3.** Dispense 84 µl of each DNA sample into the appropriate BeadChip inlet port according to the lab tracking form.

- [ ] **a.** Load samples in the A1 and B1 wells of the AMP3 plate into the first BeadChip.
- [ ] **b.** Load samples in C1 and D1 into the second BeadChip.
- [ ] **c.** Load samples in E1 and F1 into the third BeadChip.
- [ ] **d.** Load samples in G1 and H1 into the fourth BeadChip.

Repeat the same pattern to transfer sample from column 3 to BeadChips 5–8, and from column 5 to BeadChips 9–12.



[ ] **4.** Visually inspect all sections of the BeadChips to ensure the DNA sample covers all of each bead stripe. Record any sections that are not completely covered.

Set Up Duo BeadChip for Hyb

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

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

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

[ ] **4.** 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.

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

[ ] **6.** Start the rocker (optional).

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

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.

[ ] **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.

[ ] **5.** Proceed to *Wash BeadChip*.

Wash BeadChip

Prepare the BeadChips for the staining process.

Estimated Time

Hands-on time:

- 20 minutes per 8 BeadChips (16 samples)
- 30 minutes per 16 BeadChips (32 samples)
- 50 minutes per 24 BeadChips (48 samples)

Consumables

Item	Quantity (per 8 BeadChips)	Storage	Supplied By
PB1	550 ml	Room temperature	Illumina
Multi-Sample BeadChip Alignment Fixture	1		Illumina
Te-Flow Flow-Through Chambers (with black frames, spacers, glass back plates, and clamps)	1 per BeadChip		Illumina
Wash Dish	8 BeadChips: 2 dishes 24 BeadChips: 6 dishes		Illumina
Wash Rack	8 BeadChips: 1 rack 24 BeadChips: 3 racks		Illumina

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 according to the directions in the *Infinium Assay Lab Setup and Procedures Guide*.

Steps

Wash BeadChip

[ ] **1.** Remove each Hyb Chamber 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 as follows:
- [ ] **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 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 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 eight BeadChips.
- [ ] **b.** Place the assembled Flow-Through Chambers of the first eight BeadChips on the lab bench in a horizontal position.
- [ ] **c.** Repeat steps 3 through 11 from this section for any additional BeadChips. Use new PB1 for each set of eight BeadChips.

Assemble Flow-Through Chambers

[ ] 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.

[ ] 3. Place each BeadChip to be processed 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 to the Flow-Through Chambers 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 about 5 millimeters from the top edge.
- **[ ] c.** Place the second metal clamp around the Flow-Through Chamber at the barcode end, about 5 millimeters from the reagent reservoir.

[ ] 9. Using scissors, trim the ends of the clear plastic spacers from the Flow-Through Chamber assembly. Slip 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.

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

Experienced User Card

XStain HD BeadChip

Wash unhybridized and non-specifically hybridized DNA sample from the BeadChips. Add labeled 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 8–24 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	-15 to -25°C	Illumina
XC1	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
XC2	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
TEM	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
XC3	50 ml for 1–8 BeadChips 100 ml for 9–16 BeadChips 150 ml for 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)	-15 to -25°C	Illumina
ATM	2 tubes (per 8 BeadChips)	-15 to -25°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 9– 24 BeadChips	-15 to -25°C	Illumina
Alconox Powder Detergent	as needed	Room temperature	User
EtOH	as needed	Room temperature	User

Item	Quantity	Storage	Supplied By
95% formamide/1 mM EDTA	15 ml for 1–8 BeadChips 17 ml for 9–16 BeadChips 25 ml for 17–24 BeadChips	-15 to -25°C	User

Preparation

[ ] Gradually warm the RA1 reagent to room temperature. Gently mix to dissolve crystals.

[ ] 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.

[ ] 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 and centrifuge to 3000 xg for 3 minutes.

Steps

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 iScan or 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.

Wash and Coat

[ ] **1.** Dispense PB1 into a wash dish as follows, and then cover the dish:
- For 8 Beadchips, dispense 310 ml
- For 16 Beadchips, dispense 300 ml
- For 24 BeadChips, dispense 285 ml

[ ] **2.** Place the staining rack inside the wash dish. The locking arms and tab 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:
- Use the dismantling tool to remove the two metal clamps from the Flow-Through Chamber.
- Remove the glass back plate, the spacer, and then the BeadChip.
- Immediately place each BeadChip into the staining rack that is in the PB1 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.

CAUTION Do not leave the BeadChips in the PB1 for more than 30 minutes.

[ ] **6.** Dispense XC4 into a wash dish as follows:
- For 8 Beadchips, dispense 310 ml
- For 16 Beadchips, dispense 300 ml
- For 24 BeadChips, dispense 285 ml

Do not let it sit for more than 10 minutes.

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

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

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

[ ] **10.** Lift the staining rack out of the solution and place it horizontally on a tube rack, with the BeadChip barcodes facing ***up***.

[ ] **11.** 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 before removing the BeadChips below it.

[ ] **12.** Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 508 mm Hg (0.68 bar). Each desiccator can hold 8 BeadChips.

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

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

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

- Proceed to *Image BeadChip on iScan System* or *Image BeadChip on BeadArray Reader*.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box inside a vacuum desiccator at room temperature. Image the BeadChips within 72 hours.

Image BeadChip on iScan System

The iScan Reader uses a laser to excite the fluor of the single-base extension product on the beads of the BeadChip sections. Light emissions from these fluors are then recorded in high-resolution images of the BeadChip sections. Data from these images are analyzed using Illumina's GenomeStudio Genotyping Module.

Estimated Time Scanning: 35 minutes per BeadChip

Preparation

[ ] On the lab tracking form, record the following for each BeadChip:
- Scanner ID
- Scan date

Steps

[ ] **1.** Turn on the iScan Reader, boot up the iScan PC, and start the GenomeScan application.

Starting Up the iScan System

[ ] **1.** Turn on the iScan Reader and the attached PC.

[ ] **2.** Let the iScan Reader warm up for at least 5 minutes.

[ ] **3.** For each BeadChip, copy the mini-CD provided with the BeadChip into the Decode folder. The folder name should be the BeadChip barcode (for example, 4264011131).

[ ] **4.** Double-click the GenomeScan icon on the desktop.

[ ] **5.** Set the **LIMS** dropdown list to **None** and enter your Windows user name.

[ ] **6.** Click **Start**.

Loading BeadChips and Starting the Scan

[ ] **1.** Load the BeadChips into their carrier and place the carrier into the iScan Reader tray. Click **Next**.

[ ] **2.** Click any BeadChip section to remove it from the scan. The section will no longer be highlighted blue.

[ ] **3.** If you want to remove an entire BeadChip from the scan, delete the barcode from the Setup window.

[ ] **4.** To begin scanning the BeadChips, click **Scan**.

[ ] **5.** At the end of the scan, a Review window appears. If any stripes fail to scan successfully, click **Rescan** to automatically rescan all failed areas.

[ ] **6.** When you finish reviewing the data, click **Done** to return to the Start window.

Image BeadChip on BeadArray Reader

The Illumina BeadArray Reader uses a laser to excite the fluor of the hybridized single-stranded product on the beads of the BeadChip sections. Light emissions from these fluors are then recorded in high-resolution images of the BeadChip sections. Data from these images are analyzed using Illumina's GenomeStudio Genotyping Module.

Estimated Time

1–2 hours warmup for the BeadArray Reader (first use of the day only)
45 minutes to scan each BeadChip using BeadScan 3.2 FastScan settings

Preparation

[ ] If this is the first time the BeadArray Reader is being used today, follow the steps described in this section.

[ ] On the lab tracking form, record the following for each BeadChip:

- Scanner ID
- Scan date

Initializing the BeadArray Reader (Daily)

If this is the first time the scanner is being used today, follow these steps.

[ ] **1.** Locate the power switch on the lower-left side of the BeadArray Reader back panel and turn it to the ON position.

[ ] **2.** Wait for the ready indicator to stop flashing.

[ ] **3.** Open the **BeadScan** software.

[ ] **4.** Log in and click **Scan**.

Imaging BeadChip

When the BeadArray Reader is initialized, follow these steps to perform the scanning process.

[ ] **1.** From the **Docking Fixture** listbox, select BeadChip.

[ ] **2.** Check the Data Repository path and the Decode Map path in the Settings area.

[ ] **3.** Copy the decode map (*.dmap) files for each BeadChip from the BeadChip CD to the Decode Map path directory.

[ ] **4.** For each BeadChip:

- [ ] **a.** Place the BeadChip into the BeadArray Reader tray.
- [ ] **b.** If either the **Sentrix Type** or **Scan Settings** are not correct, click **Browse** (...) to open the Select Scan Settings dialog box.
- [ ] **c.** Select the appropriate scan method and click **Select**.

[ ] **5.** Make sure that the BeadChips are properly seated in the BeadArray Reader tray.

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

Scanning Process

BeadScan begins the BeadArray Reader Tilt and Align processes.

Once the Tilt and Align processes are complete, the Scan process begins. Hover over any of the green dots in the closeup image to see the relative intensity and the XY position. The red value should be at or close to zero, because this is a one-color assay.

As the BeadArray Reader scans, the front panel blue Scanning indicator lights flash in sequence.

Experienced User Card

When the BeadArray Reader finishes scanning, a green message screen appears if the scan was successful, or a red message if it completed with any warnings.

If Scan is Successful

[ ] **1.** Click **OK** on the Scan Completed message to view the next screen.

[ ] **2.** Click **Done** in the Review pane.

[ ] **3.** When the application returns to the Welcome screen, click **Open Tray**. The BeadArray Reader tray, loaded with the scanned BeadChips, will eject.

[ ] **4.** Remove the BeadChips from the tray.

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

[ ] If you have more BeadChips to scan, repeat the scanning process.

[ ] If this is the last use of the day:

- [ ] **a.** Wipe the BeadArray Reader tray with a lint-free, absorbent towel. Pay particular attention to the tray edges where reagent may have wicked out.
- [ ] **b.** Close the tray.
- [ ] **c.** Turn the power switch at the back of the scanner to the **OFF** position.
- [ ] **d.** Shut down the BeadArray Reader BeadScan software. To exit, right-click near the Illumina logo and click **Exit**.

If Scan is not Successful

Re-scan the array. For more information, refer to the Illumina BeadArray Reader *User Guide*.

If the scanner was unable to locate the alignment fiducials (focus points), you may need to clean the edges of the BeadChip before re-scanning.