Infinium LCG Assay, Manual Protocol

Experienced User Card

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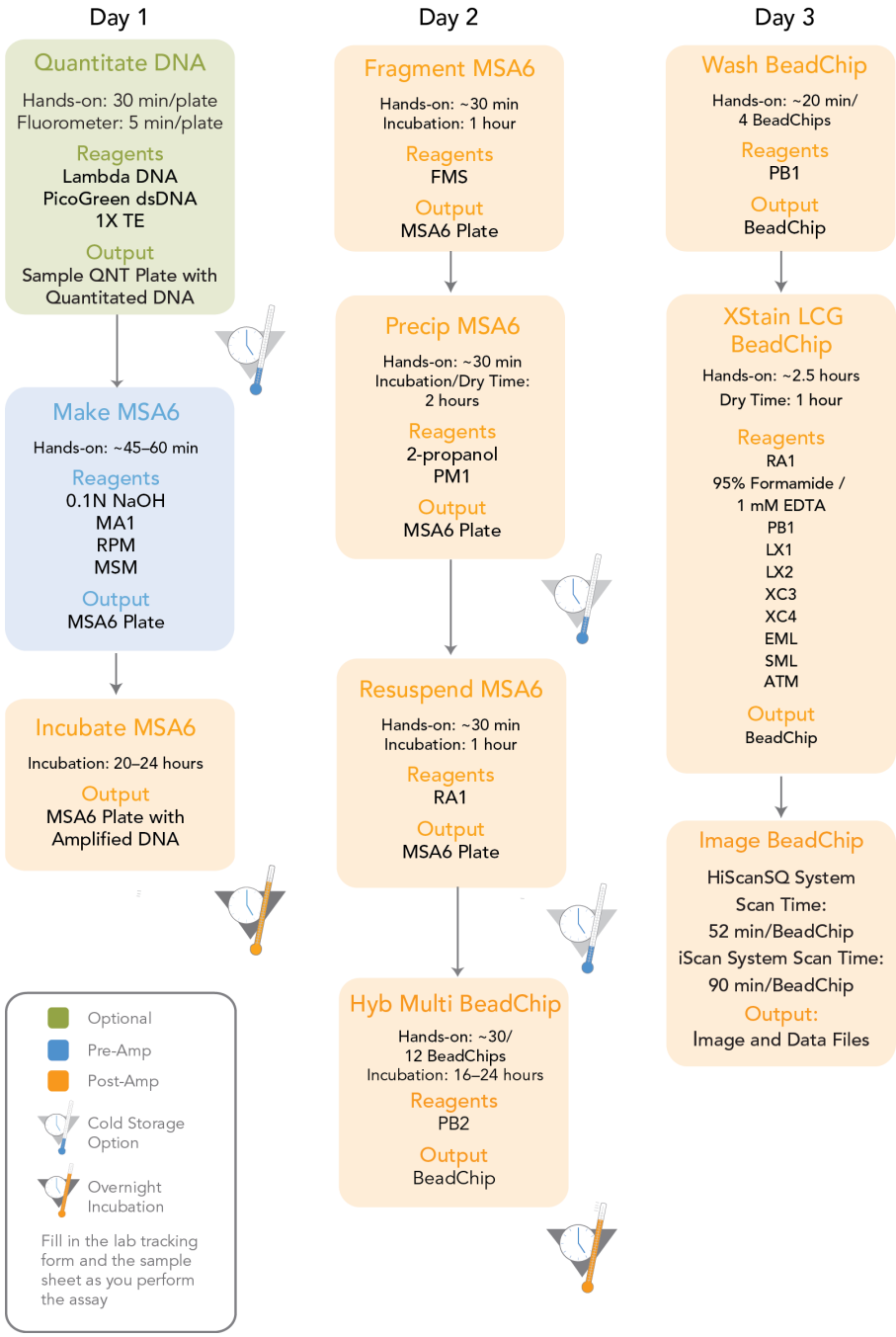


Make the MSA6 Plate (Pre-Amp)

This process creates an MSA6 plate for DNA amplification. MA1 is first added to the MSA6 plate, followed by the DNA samples. Next, the 0.1N NaOH is added to denature the DNA samples. The RPM reagent neutralizes the sample. Lastly, MSM (Multi-Sample Amplification Master Mix) is added to the plate.

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)	Room temperature	Illumina
RPM	2 tubes (per 96 samples)	-25°C to -15°C	Illumina
MSM	2 tubes (per 96 samples)	-25°C to -15°C	Illumina
0.1N NaOH	15 ml (per 96 samples)	2° to 8°C	User
96-well 0.8 ml microplate (midi plate)	1 plate		User
DNA plate with DNA samples	1 plate	-25°C to -15°C	User

Preparation

- Preheat the Illumina Hybridization Oven in the post-amp area to 37°C and allow the temperature to equilibrate.
- Apply an MSA6 barcode label to a new midi plate.
- Thaw MA1, RPM, and MSM tubes to room temperature.
- Thaw DNA samples to room temperature.
- In the sample sheet, enter the Sample_Name and Sample_Plate for each Sample_Well.

Steps to Make the MSA6 Plate

[_] 1 If you do not already have a DNA plate, add DNA into either a:
- Midi plate: 20 µl to each DNA plate well
- TCY plate: 10 µl to each DNA plate well

[_] 2 Apply a barcode label to the new DNA plate.

[_] 3 Dispense 20 µl MA1 into the MSA6 plate wells.

[_] 4 Transfer 4 µl of the DNA sample, from the DNA plate to the corresponding wells in the MSA6 plate.

[_] 5 On the lab tracking form, record the original DNA sample ID for each well in the MSA6 plate.

[_] 6 Dispense 4 µl 0.1N NaOH into each well of the MSA6 plate that contains MA1 and sample.

[_] 7 Seal the MSA6 plate with the 96-well cap mat.
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, make sure that all 96 caps are securely seated.

[_] 8 Vortex the plate at 1600 rpm for 1 minute.

[_] 9 Pulse centrifuge at 280 × g.

[_] 10 Incubate for 10 minutes at room temperature.

[_] 11 Carefully remove the cap mat.
When you remove a cap mat, set it aside, upside down, in a safe location for use later in the protocol.

[_] 12 Dispense 68 µl RPM into each well of the MSA6 plate containing sample.

[_] 13 Dispense 75 µl MSM into each well of the MSA6 plate containing sample.

[_] 14 Reseal the MSA6 plate with the cap mat.
When you place the cap mat back on the plate, be sure to match it to its original plate and orient it correctly.

[_] 15 Vortex the sealed MSA6 plate at 1600 rpm for 1 minute.

[_] 16 Pulse centrifuge at 280 × g.

[_] 17 Incubate in the Illumina Hybridization Oven for 20–24 hours at 37°C.

[_] 18 Proceed to *Fragment DNA (Post-Amp)*.

Fragment DNA (Post-Amp)

This process enzymatically fragments the amplified DNA samples. An endpoint fragmentation is used to prevent overfragmentation.

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)	-25°C to -15°C	Illumina

Preparation

[_] 1 Preheat the heat block with the midi plate insert to 37°C.

[_] 2 Thaw FMS tubes to room temperature.

[_] 3 Gently invert the FMS tubes at least 10 times to mix contents.

[_] 4 Remove the MSA6 plate from the Illumina Hybridization Oven.

Steps to Fragment the MSA6 Plate

[_] 1 Pulse centrifuge the plate to 280 × g.

[_] 2 Carefully remove the cap mat.

[_] 3 Add 50 µl FMS to each well containing sample.

[_] 4 Seal the MSA6 plate with the 96-well cap mat.

[_] 5 Vortex the plate at 1600 rpm for 1 minute.

[_] 6 Pulse centrifuge the plate to 280 × g.

[_] 7 Place the sealed plate on the 37°C heat block for 1 hour.

[_] 8 Do either of the following:

- Continue to the next step, *Precipitate DNA (Post-Amp)*. Leave plate in 37°C heat block until setup is complete. Do not leave the plate in the 37°C heat block for longer than 2 hours.
- If you do not plan to proceed to the next step within the next 4 hours, store the sealed MSA6 plate at -25°C to -15°C for no more than 24 hours.

SAFE STOPPING POINT
Now is a good stopping point in the process.

Precipitate DNA (Post-Amp)

Add PM1 and 2-propanol to the MSA6 plate to precipitate the DNA samples.

Estimated Time

Hands-on time: ~30 minutes

Incubation and dry time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PM1	2 tubes (per 96 samples)	2°C to 8°C	Illumina
100% 2-propanol	40 ml (per 96 samples)	Room temperature	User

Preparation

[_] 1 If frozen, thaw MSA6 plate to room temperature, then pulse centrifuge at 280 × g.

[_] 2 Preheat heat block to 37°C.

[_] 3 Thaw PM1 to room temperature. Gently invert at least 10 times to mix contents.

[_] 4 Remove the 96-well cap mat.

Steps to Precipitate the MSA6 Plate

[_] 1 Add 100 µl PM1 to each MSA6 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 Pulse centrifuge at 280 × g for 1 minute.

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

[_] 6 Carefully remove the cap mat and discard it.

[_] 7 Add 300 µl 100% 2-propanol to each well containing sample.

[_] 8 Carefully seal the MSA6 plate with a new, ***dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.

[_] 9 Invert the plate at least 10 times to mix contents thoroughly.

[_] 10 Incubate at 4°C for 30 minutes.

[_] 11 Centrifuge at 3000 × g at 4°C for 20 minutes. Immediately remove the MSA6 plate from centrifuge.

[_] 12 Remove the cap mat and discard it.

[_] 13 Quickly invert the MSA6 plate and drain the liquid onto an absorbent pad to decant the supernatant. Then smack the plate down on a dry area of the pad, avoiding the liquid that was drained onto the pad.

[_] 14 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

[_] 15 Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.
You can expect to see blue pellets at the bottoms of the wells.

[_] 16 Do either of the following:
- Continue to the next step, *Resuspend DNA (Post-Amp).*
- If you do not plan to proceed to the next step immediately, seal the MSA6 plate with a new cap mat and store it at -25°C to -15°C.

SAFE STOPPING POINT
Now is a good stopping point in the process.

Resuspend DNA (Post-Amp)

Add RA1 to the MSA6 plate to resuspend the precipitated DNA samples.

Estimated Time

Hands-on time: ~30 minutes

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	46 µl per sample well	-25°C to -15°C	Illumina

NOTE

Pour out only 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 BeadChip** step.

Preparation

[_] 1 If you stored the MSA6 plate at -25°C to -15°C, thaw it to room temperature. Remove the cap mat and discard it.

[_] 2 Preheat the Illumina Hybridization Oven to 48°C.

[_] 3 Turn on the heat sealer to preheat. Allow 20 minutes.

[_] 4 Thaw RA1 to room temperature. Invert several times to redissolve the solution.

Steps to Resuspend the MSA6 Plate

[_] 1 Add 46 µl RA1 to each well of the MSA6 plate containing a DNA pellet.
Reserve any leftover reagent for the Hybridization and XStain steps.

[_] 2 Apply a foil heat seal to the MSA6 plate by firmly and evenly holding the heat sealer sealing block down for 3 seconds.

[_] 3 Immediately remove the MSA6 plate from the heat sealer and forcefully roll the rubber plate sealer over the plate until you can see all 96 well indentations through the foil.
Repeat application of the heat sealer if all 96 wells are not defined.

[_] 4 Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.

[_] 5 Vortex the plate at 1800 rpm for 1 minute.

[_] 6 Pulse centrifuge to 280 × g.

[_] 7 Do either of the following:

- Continue to the next step, *Hybridize to BeadChip (Post-Amp)*. If you plan to do so immediately, it is safe to leave the MSA6 plate at room temperature for up to 1 hour.
- If you do not plan to proceed to the next step immediately, store the sealed MSA6 plate at -25°C to -15°C for no more than 24 hours. Store RA1 at -25°C to -15°C.

SAFE STOPPING POINT

Now is a good stopping point in the process.

Hybridize to BeadChip (Post-Amp)

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:

- 8x1 LCG BeadChip: ~40 minutes for 12 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 96 Samples)	Storage	Supplied By
PB2	3 tubes	Room temperature	Illumina
BeadChips	12		Illumina
Hyb Chambers	3		Illumina
Hyb Chamber gaskets	3		Illumina
Hyb Chamber inserts	12		Illumina
EtOH	330 ml		User

Preparation

[_] 1 If frozen, thaw MSA6 plate to room temperature, and then pulse centrifuge the MSA6 plate to 280 × g.

[_] 2 Preheat the heat block to 95°C.

[_] 3 Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.

Assemble the Hybridization Chambers

[_] 1 Place the resuspended MSA6 plate on the heat block to denature the samples at 95°C for 20 minutes.

[_] 2 Remove the BeadChips from 2°C to 8°C storage, leaving the BeadChips in their plastic bags and mylar packages until you are ready to begin hybridization.

[_] 3 During the 20-minute incubation, prepare the Hyb Chambers.

- [_] a Place the BeadChip Hyb Chamber gaskets into the BeadChip Hyb Chambers.
- [_] b Dispense 400 µl PB2 into the humidifying buffer reservoirs in the Hyb Chambers.
- [_] c After you fill the Hyb Chamber reservoirs with PB2, place the lid on the Hyb Chamber right away to prevent evaporation. It is not necessary to lock the lid.
- [_] d Leave the closed Hyb Chambers on the bench at room temperature until the BeadChips are loaded with DNA sample. Load BeadChips into the Hyb Chamber within 1 hour.

NOTE
You can also prepare the Hyb Chambers later, during the 30-minute cool down.

[_] 4 After the 20-minute incubation, remove the MSA6 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.

[_] 5 After the 30-minute cool down, pulse centrifuge the MSA6 plate to 280 × g. Remove the foil seal.

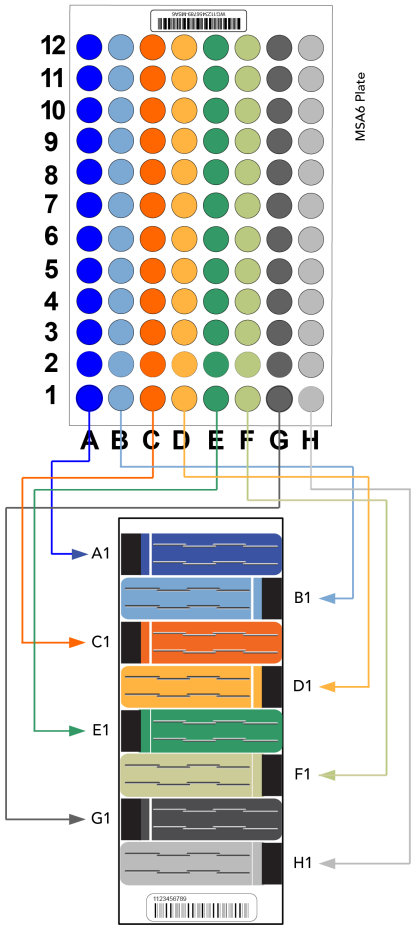
Load BeadChip

[_] 1 Just before loading DNA samples, remove all BeadChips from their plastic bags and mylar 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 35 µl of each DNA sample onto the appropriate BeadChip section, according to the chart.
Make sure that the pipette tip is in the sample inlet before dispensing.

Figure 1 Distributing Sample in the MSA6 Plate



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

[_] 5 In the sample sheet Sentrix ID column, record the BeadChip sample ID and position. See the Sample Section Naming Diagram in the lab tracking form.

[_] 6 Inspect all sections of the BeadChip to make sure that the DNA sample covers all areas of each bead stripe.

[_] 7 Record any sections that are not completely covered on the lab tracking form.

Set up BeadChip for Hybridization

[_] 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 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 so that the lid is secure and even on the base (no gaps).

NOTE
Keep the Hyb Chamber steady and level when moving it or transferring it to the Illumina Hybridization Oven.

[_] 4 Place the Hyb Chamber in the 48°C Illumina Hybridization Oven with the clamps on the left and right sides of the oven and the Illumina logo facing you.

OVERNIGHT INCUBATION
Incubate at 48°C for at least 16 hours but no more than 24 hours.

[_] 5 Proceed to *Wash BeadChips (Post-Amp)* after the overnight incubation.

Resuspend XC4 Reagent for XStain BeadChip

[_] 1 Add 330 ml 100% EtOH to the XC4 bottle, for a final volume of 350 ml.
Each XC4 bottle has enough solution to process up to 24 BeadChips.

[_] 2 Shake the XC4 bottle vigorously to ensure complete resuspension. After it is resuspended, use XC4 at room temperature.
You can store it at 2°C to 8°C for 2 weeks if unused.

Wash BeadChips (Post-Amp)

Prepare the BeadChips for the staining process.

Estimated Time

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

Consumables

Item	Quantity	Storage	Supplied By
PB1	550 ml for 1 alignment fixture 700 ml for 2 alignment fixtures 850 ml for 3 alignment fixtures	Room temperature	Illumina
Multisample BeadChip alignment fixture	1 (per 8 BeadChips)		Illumina
Te-Flow LCG flow-through chambers, with black frames, LCG spacers, LCG 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

Preparation

[_] 1 Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 30 minutes before opening.

[_] 2 While the Hyb Chamber is cooling:

- [_] a Fill 2 wash dishes with PB1 (200 ml per wash dish). Label each dish "PB1".
- [_] b Fill the Multi-Sample BeadChip Alignment Fixture with 150 ml PB1.
- [_] c Separate the clear plastic spacers from the white backs.
- [_] d Clean the glass back plates if necessary.

Steps to Wash BeadChips

[_] 1 Attach the wire handle to the rack and submerge the wash rack in the wash dish containing 200 ml PB1.

[_] 2 Remove the Hyb Chamber inserts from the Hyb Chambers.

[_] 3 Remove each BeadChip from the Hyb Chamber insert.

[_] 4 Remove the cover seal from each BeadChip.

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

[_] a Using powder-free gloved hands, hold the BeadChip securely and by the edges in one hand. Avoid contact with the sample inlets. Make sure that the barcode is facing up and closest to you, and that the top side of the BeadChip is angled slightly away from you.

[_] b Remove the entire seal in a single, continuous motion. 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 touch the exposed arrays.

[_] 5 Immediately and carefully slide each BeadChip into the wash rack, making sure that the BeadChip is submerged in the PB1.

[_] 6 Repeat steps 4 through 5 until all BeadChips (a maximum of 8) are transferred to the submerged wash rack.

[_] 7 After 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.

[_] 8 Move the wash rack to the other wash dish containing clean PB1. Make sure the BeadChips are submerged.

[_] 9 Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

[_] 10 When you remove the BeadChips from the wash rack, inspect them for remaining residue.

[_] 11 For each additional set of 8 BeadChips:

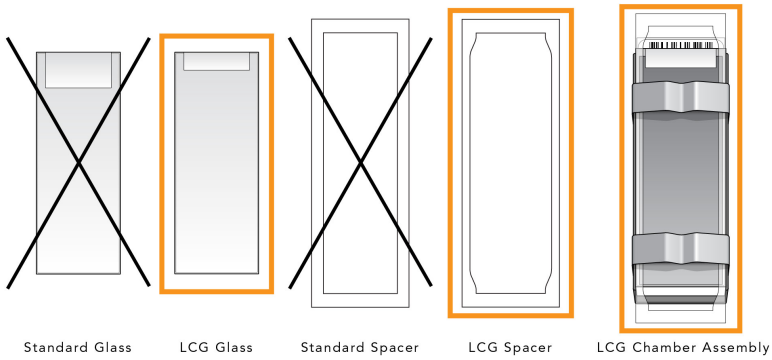
[_] a Assemble the flow-through chambers for the first 8 BeadChips, as described in *Assemble Flow-Through Chambers* on page 16.

[_] b Repeat the wash steps in this section to wash the next set of 8 BeadChips.

Assemble Flow-Through Chambers

NOTE
Confirm that you are using the correct Infinium LCG glass back plates and spacers before assembling the flow-through chambers. Refer to the following image for the correct flow-through chamber components.

Figure 2 Correct LCG Back Plates and Spacers



[_] 1 If you have not done so, fill the BeadChip alignment fixture with 150 ml PB1.
If you plan to process more than 4 BeadChips, this 150 ml of PB1 can be reused for an additional set of 4 BeadChips. Use 150 ml of fresh PB1 for every additional set of 8 BeadChips.

[_] 2 For each BeadChip to be processed, place a black frame into the BeadChip alignment fixture prefilled with PB1.

[_] 3 Place each BeadChip to be processed into a black frame, aligning its barcode with the ridges stamped onto the alignment fixture.

NOTE
Inspect the surface of each BeadChip for residue left by the seal. Use a pipette tip to remove any residue under buffer and be careful not to scratch the bead area.

[_] 4 Place a clear LCG spacer onto the top of each BeadChip. Use the alignment fixture grooves to guide the spacers into proper position.

NOTE
Be sure to use the clear plastic spacers, not the white ones.

[_] 5 Place the alignment bar onto the alignment fixture.
The groove in the alignment bar fits over the tab on the alignment fixture.

[_] 6 Place a clean LCG glass back plate on top of the clear spacer covering each BeadChip. The plate reservoir is at the barcode end of the BeadChip, facing inward to create a reservoir against the BeadChip surface.

[_] 7 Attach the metal clamps to the flow-through chambers as follows:

- [_] a Gently push the glass back plate against the alignment bar with one finger.
- [_] b Place the first metal clamp around the flow-through chamber so that the clamp is approximately 5 mm from the top edge.
- [_] c Place the second metal clamp around the flow-through chamber at the barcode end, approximately 5 mm from the reagent reservoir.

[_] 8 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.

[_] 9 ***Immediately*** wash the Hyb Chamber reservoirs with DiH_2O and scrub them with a small cleaning brush, ensuring that no PB2 remains in the Hyb Chamber reservoir.

[_] 10 Proceed to the next step, *Extend and Stain (XStain) BeadChip (Post-Amp)*.

CAUTION
Place all assembled flow-through chambers on the lab bench in a horizontal position while you perform the preparation steps for XStain BeadChip. Do not place the flow-through chambers in the chamber rack until all necessary steps are completed.

Extend and Stain (XStain) BeadChip (Post-Amp)

In this process, you use RA1 reagent to wash away unhybridized and nonspecifically hybridized DNA sample. LX1 and LX2 are added to condition the BeadChip surface for the extension reaction. Dispense EML reagent into the flow-through chambers to extend the primers hybridized to DNA on the BeadChip. This reaction incorporates labeled nucleotides into the extended primers. 95% formamide/1 mM EDTA is added to remove the hybridized DNA. After neutralization using the XC3 reagent, the labeled extended primers undergo a multilayer staining process on the chamber rack. Next, you disassemble the flow-through chambers and wash the BeadChips in the PB1 reagent, coat them with XC4, and then dry them.

Estimated Time

Hands-on time: ~2 hours and 45 minutes 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	-25°C to -15°C	Illumina
LX1	2 tubes (per 8 BeadChips)	-25°C to -15°C	Illumina
LX2	2 tubes (per 8 BeadChips)	-25°C to -15°C	Illumina
EML	2 tubes (per 8 BeadChips)	-25°C to -15°C	Illumina
XC3	50 ml for 1–8 BeadChips 100 ml for 9–16 BeadChips 150 ml for 17–24 BeadChips	Room temperature	Illumina
SML (Make sure that all SML tubes indicate the same stain temperature on the label)	2 tubes (per 8 BeadChips)	-25°C to -15°C	Illumina
ATM	2 tubes (per 8 BeadChips)	-25°C to -15°C	Illumina
PB1	310 ml for 1–8 BeadChips 285 ml for 9–24 BeadChips	Room temperature	Illumina

Item	Quantity	Storage	Supplied By
XC4	310 ml for 1–8 BeadChips 285 ml for 9–24 BeadChips	Room temperature	Illumina
Alconox Powder Detergent	As needed	Room temperature	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	-25°C to -15°C	User

Preparation

[_] 1 Place all reagent tubes in a rack in the order you plan to use them. If frozen, allow them to thaw to room temperature, and then gently invert the reagent tubes at least 10 times to mix contents.

[_] 2 Make sure that the water circulator is filled to the appropriate level.

[_] 3 Turn on the water circulator. Set it to a temperature that brings the Chamber Rack to 44°C at equilibrium.

[_] 4 Remove bubbles trapped in the Chamber Rack.

[_] 5 Test several locations on the Chamber Rack, using the Illumina Temperature Probe. All locations must be at 44°C ± 0.5°C.

Single-Base Extension

CAUTION
The remaining steps must be performed without interruption.

NOTE
If you are processing more than 8 BeadChips, complete the reagent dispensing step for each reagent for the first set of 8 BeadChips. Then continue the same reagent dispensing steps for the second set of 8 BeadChips. Finally, move to the last set of 8 BeadChips before you start the incubation time.

Steps marked with an asterisk (*) indicate when to follow this reagent dispensing method.

[_] 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.
[_] 1 [_] 2 [_] 3 [_] 4 [_] 5 [_] 6

[_] b 225 µl LX1. Repeat one time*. Incubate for 10 minutes.
[_] 1 [_] 2

[_] c 225 µl LX2. Repeat one 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

[_] 3 Wait until the chamber rack reaches the correct temperature.

Stain BeadChip

NOTE
If you are processing more than 8 BeadChips, complete the reagent dispensing step for each reagent for the first set of 8 BeadChips. Then continue the same reagent dispensing steps for the second set of 8 BeadChips. Finally, move to the last set of 8 BeadChips before you start the incubation time.

Steps marked with an asterisk (*) indicate when to follow this reagent dispensing method.

[_] 1 If you plan to image the BeadChip immediately after the staining process, turn on the scanner now to allow the lasers to stabilize.

[_] 2 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

[_] 3 Immediately remove the flow-through chambers from the chamber rack and place horizontally on a lab bench at room temperature.

Wash and Coat 8 BeadChips

[_] 1 Pour 310 ml PB1 per 8 BeadChips into a wash dish.

[_] 2 Place the staining rack inside the wash dish.

[_] 3 For each BeadChip:

[_] a Use the dismantling tool to remove the 2 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. Make sure that all BeadChips are submerged.

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

[_] 5 Allow the BeadChips to soak for an additional 5 minutes.

[_] 6 Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until dissolved.

[_] 7 Pour 310 ml XC4 into a wash dish.

CAUTION
Do not let the XC4 sit for longer than 10 minutes.

[_] 8 Move the BeadChip staining rack into the XC4 dish.

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

[_] 10 Allow the BeadChips to soak for an additional 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 rack handle if it facilitates removal of the BeadChips.

[_] 13 Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 675 mm Hg (0.9 bar).

[_] 14 Make sure that the XC4 coating is dry before continuing to the next step.

[_] 15 Clean the underside of each BeadChip with a ProStat EtOH wipe or Kimwipe soaked in EtOH.

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

[_] 16 Clean and store the glass back plates and Hyb Chamber components.

[_] 17 Do either of the following:

- Proceed to *Image BeadChip (Post-Amp)*.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box at room temperature. Image the BeadChips within 72 hours.

Image BeadChip (Post-Amp)

Follow the instructions in the *iScan System User Guide* or *HiScan System User Guide* to scan your BeadChips.

Use the appropriate scan setting for your BeadChip, as outlined in the following table:

Table 1 Scan Settings for Infinium LCG

BeadChip	Scan Setting Name
HumanOmni2.5-8	Infinium LCG