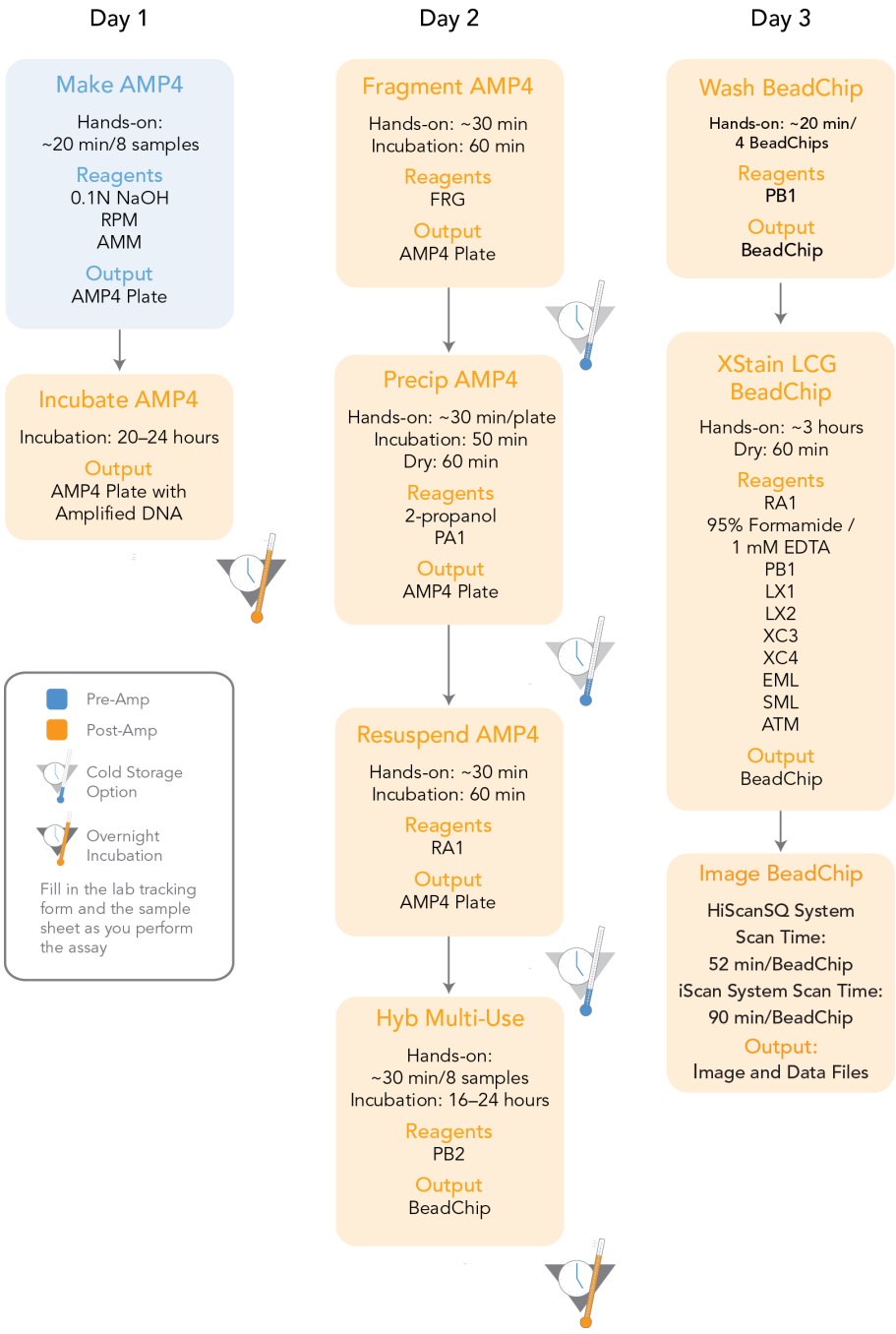
Illumina Infinium Multi-Use LCG Assay, Manual Protocol

Experienced User Card

FOR RESEARCH USE ONLY



Make the AMP4 Plate (Pre-AMP)

This process creates a AMP4 plate for DNA amplification. The DNA sample is denatured with 0.1N NaOH and then neutralized with RPM. The last reagent added is AMM (Amplification Master Mix).

Estimated Time

Hands-on time:

- ~20 minutes per 8 samples

Incubation time: 20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
RPM	1 tube (per 8 samples)	-15° to -25°C	Illumina
AMM	1 tube (per 8 samples)	-15° to -25°C	Illumina
0.1N NaOH	15 ml (per 8–24 samples)	2° to 8°C	User
96-well 0.8 ml microtiter plate (MIDI)	1 plate		User
DNA plate with DNA samples	1 plate	-15° to -25°C	User

Preparation

- Preheat the Illumina Hybridization Oven in the post-amp area to 48°C and allow the temperature to equilibrate.
- Apply an AMP4 barcode label to a new MIDI plate.
- Thaw RPM and AMM tubes to room temperature.
- Thaw DNA samples to room temperature.

Steps to Make the AMP4 Plate

[_] 1 If you do not already have a DNA plate, add DNA into either a:
- MIDI plate: 40 µl to each DNA plate well
- TCY plate: 30 µl to each DNA plate well

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

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

[_] 4 Pulse centrifuge to 280 xg.

[_] 5 Transfer 15 µl of the DNA sample, normalized to 50 ng/µl, into each well in the following AMP4 plate columns:
- Column 1 (8 samples)

- Columns 1 and 5 (16 samples)
- Columns 1, 5, and 9 (24 samples)

[_] 6 On the lab tracking form, record the original DNA sample ID for each well in the AMP4 plate.

[_] 7 Dispense 15 µl 0.1N NaOH into each well of the AMP4 plate that contains DNA.

[_] 8 Seal the AMP4 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, ensure that all 96 caps are securely seated.

[_] 9 Vortex the plate at 1600 rpm for 1 minute.

[_] 10 Pulse centrifuge to 280 xg.

[_] 11 Incubate for 10 minutes at room temperature.

[_] 12 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.

[_] 13 Dispense 270 µl RPM into each well of the AMP4 plate containing sample.

[_] 14 Dispense 300 µl AMM into each well of the AMP4 plate containing sample.

[_] 15 Seal the AMP4 plate with the 96-well 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.

[_] 16 Invert the sealed plate at least 10 times to mix contents.

[_] 17 Pulse centrifuge to 280 xg.

[_] 18 Incubate in the Illumina Hybridization Oven for 20–24 hours at 48°C.

[_] 19 Proceed to *Fragment the AMP4 Plate (Post-AMP)*.

Fragment the AMP4 Plate (Post-AMP)

This process enzymatically fragments the amplified DNA samples. An end-point fragmentation is used to prevent over-fragmentation.

Estimated Time

Hands-on time: ~30 minutes for 96 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
FRG	1 tube (per 8 samples)	-15° to -25°C	Illumina

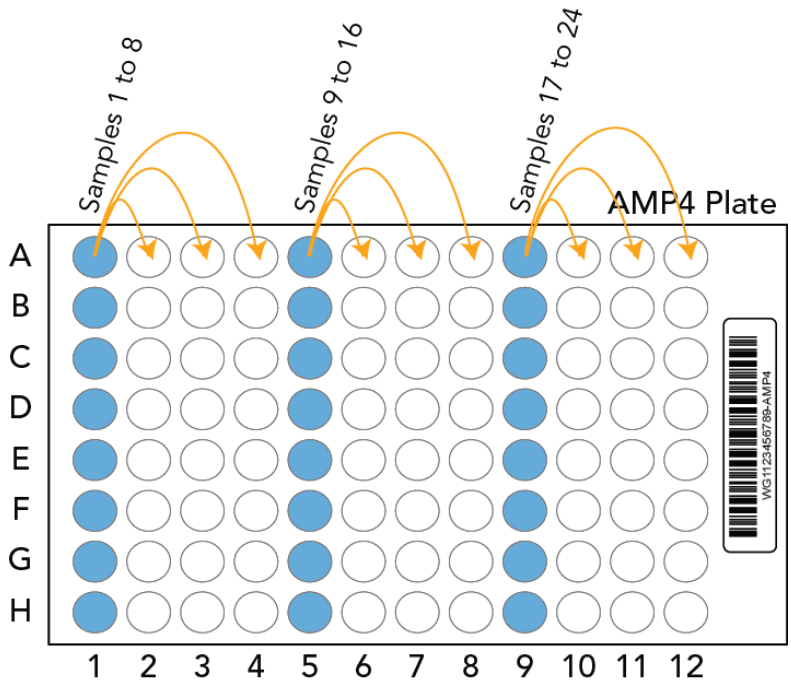
Preparation

- Preheat the heat block with the MIDI plate insert to 37°C.
- Thaw FRG tubes to room temperature. Gently invert at least 10 times to mix contents.
- Remove the AMP4 plate from the Illumina Hybridization Oven.

Steps to Fragment the AMP4 Plate

[_] 1 Pulse centrifuge the plate to 280 xg.

[_] 2 Split the sample into 3 additional wells, for a total of 4 wells per sample. Each well should contain 150 µl.
For example, move 150 µl sample from A1 into A2, A3, and A4.
Follow this pattern for rows B–H, columns 1, 5, and 9.



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

[_] 4 Seal the AMP4 plate with the 96-well cap mat.

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

[_] 6 Pulse centrifuge the plate to 280 xg.

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

[_] 8 Do one of the following:

- Continue to the next step, *Precipitate the AMP4 Plate (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 AMP4 plate at -15° to -25°C for no more than 24 hours.

Precipitate the AMP4 Plate (Post-AMP)

Add PA1 and 2-propanol to the AMP4 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
PA1	1 tubes (per 8 samples)	2° to 8°C	Illumina
100% 2-propanol	12–142 ml	Room temperature	User

Preparation

- If frozen, thaw AMP4 plate to room temperature, then pulse centrifuge the plate to 280 xg.
- Preheat heat block to 37°C.
- Thaw PA1 to room temperature. Gently invert at least 10 times to mix contents.
- Remove the 96-well cap mat.

Steps to Precipitate the AMP4 Plate

[_] 1 Add 100 µl PA1 to each AMP4 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 to 280 xg.

NOTE
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 AMP4 plate with a new, ***dry*** cap mat, taking care not to shake the plate in any way until the cap mat is fully seated.

[_] 8 Invert the plate 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 AMP4 plate from centrifuge.

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

[_] 12 Over an absorbent pad, decant the supernatant by quickly inverting the AMP4 plate. Drain liquid onto the absorbent pad and then smack the plate down, avoiding the liquid that was just drained onto the pad.

[_] 13 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

[_] 14 Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.
At this point, blue pellets should be present at the bottoms of the wells.

[_] 15 Do one of the following:
- Continue to the next step, *Resuspend the AMP4 Plate (Post-AMP)*.
- If you do not plan to proceed to the next step immediately, seal the AMP4 plate with a new cap mat and store it at -15° to -25°C for no more than 24 hours.

Resuspend the AMP4 Plate (Post-AMP)

Add RA1 to the AMP4 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	Bottle (42 µl per sample well)	-15° to -25°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

- If you stored the AMP4 plate at -15° to -25°C, thaw it to room temperature. Remove the cap mat and discard it.
- Preheat the Illumina Hybridization Oven to 48°C.
- Turn on the heat sealer to preheat. Allow 20 minutes.
- Thaw RA1 to room temperature. Invert several times to re-dissolve the solution.

Steps to Resuspend the AMP4 Plate

[_] 1 Add 42 µl RA1 to each well of the AMP4 plate containing a DNA pellet.
Reserve any leftover reagent for **Hyb Multi BeadChip** and **XStain BeadChip**.

[_] 2 Apply a foil heat seal to the AMP4 plate by firmly and evenly holding the heat sealer sealing block down for 3 seconds.

[_] 3 Immediately remove the AMP4 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 xg.

[_] 7 Do one of the following:

- Continue to the next step, *Hybridize Multi BeadChip (Post-AMP)*. If you plan to do so immediately, it is safe to leave the AMP4 plate at room temperature for up to 1 hour.

- If you do not plan to proceed to the next step immediately, store the sealed AMP4 plate at -15° to -25°C for no more than 24 hours. Store RA1 at -15° to -25°C.

Hybridize Multi 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	12		Illumina
Hyb Chamber gaskets	3		Illumina
Hyb Chamber inserts	12		Illumina
EtOH	330 ml		User

Preparation

- If frozen, thaw AMP4 plate to room temperature, and then pulse centrifuge the AMP4 plate to 280 xg.
- Preheat the heat block to 95°C.
- Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.

Assemble the Hybridization Chambers

[_] 1 Place the resuspended AMP4 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, leaving the BeadChips in their ziplock bags and mylar packages until you are ready to begin hybridization.

[_] 3 During the 20-minute incubation, prepare the Hyb Chamber(s).

[_] 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. The lid does not need to be locked down.

[_] 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 one hour.

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

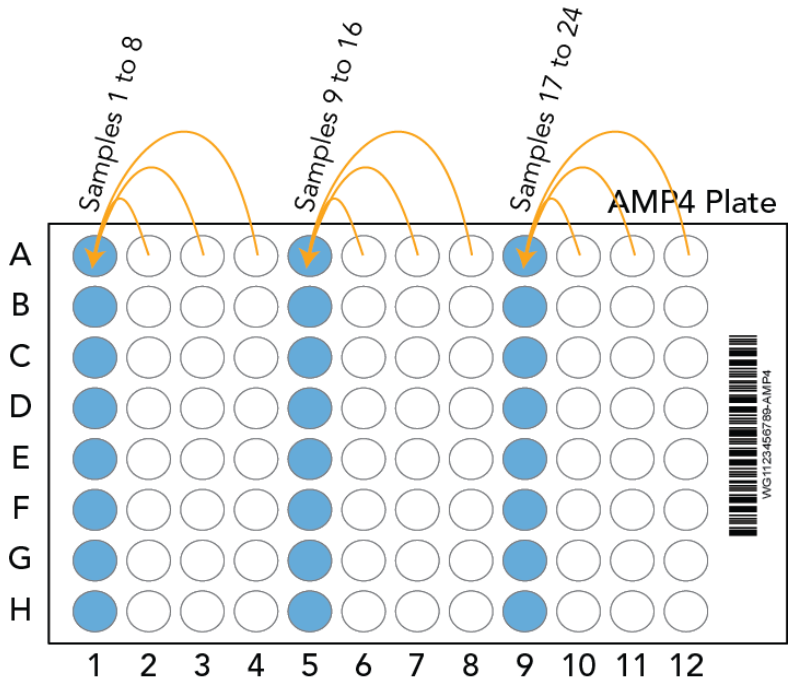
[_] 4 After the 20-minute incubation, remove the AMP4 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.

[_] 5 After the 30-minute cool down, pulse centrifuge the AMP4 plate to 280 xg. Remove the foil seal.

For the First Hybridization

Step 6 only needs to be performed for the first hybridization of the Infinium Multi-Use LCG Assay. If this is not the first hybridization proceed directly to the next section, *Load BeadChip*.

[_] 6 Combine the four separate wells back into the original well. For example, move 35 µl sample from each of the A2, A3, and A4 wells back into A1.



Load BeadChip

[_] 1 Just before loading DNA samples, remove all BeadChips from their ziplock 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 shown below.
Make sure that the pipette tip is in the sample inlet prior to dispensing.

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

[_] 5 In the sample sheet's Sentrix ID column, record the BeadChip sample ID and position. Please see the Sample Section Naming Diagram in the Lab Tracking Form.

[_] 6 Visually inspect all sections of the BeadChip to ensure 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 Multi 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 so that the clamps of the Hyb Chamber face the left and right side of the oven and the Illumina logo on top of the Hyb Chamber is facing you.

[_] 5 Incubate at 48°C for at least 16 hours but no more than 24 hours.

[_] 6 Cover the residual sample in the AMP4 plate with a foil seal.
You can store the plate indefinitely at -80°C.

[_] 7 Proceed to *Wash the BeadChip (Post-AMP)* after the overnight incubation.

Resuspend XC4 Reagent for XStain BeadChip

[_] 1 Add 330 ml 100% EtOH to the XC4 bottle.
Each XC4 bottle (350 ml) has enough solution to process up to 24 BeadChips.

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

Hybridize Multi BeadChip (Post-AMP)

Wash the BeadChip (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 (up to 24 BeadChips)	Room temperature	Illumina
Multi-Sample 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

- Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 25 minutes prior to opening.
- While the Hyb Chamber is cooling:
  - Fill two wash dishes with PB1 (200 ml per wash dish). Label each dish "PB1".
  - Fill the Multi-Sample BeadChip Alignment Fixture with 150 ml PB1.
  - Separate the clear plastic spacers from the white backs.
  - Clean the glass back plates if necessary.

Steps to Wash BeadChip

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

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

NOTE
To ensure 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. 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, 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, one at a time, making sure that the BeadChip is completely 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 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.

[_] 8 Move the wash rack to the other wash dish containing clean PB1. Make sure the BeadChips are completely 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 If you are processing more than 8 BeadChips

[_] a Assemble the Flow-Through Chambers for the first eight BeadChips, as described in the next section, and place them on the lab bench in a horizontal position.

NOTE
Keep the Flow-Through Chambers 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 BeadChips are prepared in Flow-Through Chambers.

[_] b Return to this procedure and follow the steps described above to wash the next set of eight BeadChips.

[_] c Repeat for each remaining set of eight BeadChips.

Assemble Flow-Through Chambers

NOTE
Confirm you are using the correct Infinium Multi-Use LCG Assay glass back plates and spacers before assembling the Flow-Through Chambers. Refer to the following image for the correct Flow-Through Chamber components.

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

If more than four BeadChips will be processed, this 150 ml of PB1 can be reused for an additional set of four BeadChips. You must use 150 ml of fresh PB1 for every additional set of eight BeadChips.

[_] 2 For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture pre-filled 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 should fit 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 should be 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 up 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 *Single Base Extension and Stain LCG 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.

Wash the BeadChip (Post-AMP)

Single-Base Extension and Stain BeadChip (Post-AMP)

In this process, you use RA1 reagent to wash away unhybridized and non-specifically 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 multi-layer 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: 1 hour

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
LX1	2 tubes (per 8 BeadChips)	-15° to -25°C	Illumina
LX2	2 tubes (per 8 BeadChips)	-15° to -25°C	Illumina
EML	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 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)	-15° to -25°C	Illumina
ATM	2 tubes (per 8 BeadChips)	-15° to -25°C	Illumina
PB1	310 ml for 1-8 BeadChips	Room temperature	Illumina

Item	Quantity	Storage	Supplied By
	285 ml for 9-24 BeadChips		
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	-15° to -25°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, and then gently invert the reagent tubes at least 10 times to mix contents.
- 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. All locations should 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. Then move to the last set of 8 BeadChips before you start the incubation time.

Steps marked with an asterisk (*) indicate when you should 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 once*. Incubate for 10 minutes.
[_] 1 [_] 2

[_] c 225 µl LX2. Repeat once*. 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. Then move to the last set of 8 BeadChips before you start the incubation time.

Steps marked with an asterisk (*) indicate when you should 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 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. All chips should be completely 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 completely 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 Ensure 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 one 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 *HiScanSQ 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 Multi-Use LCG

BeadChip	Scan Setting Name
HumanOmni2.5-8	Infinium LCG

Image BeadChip (Post-AMP)