Infinium LCG Assay, Automated Protocol

Experienced User Card

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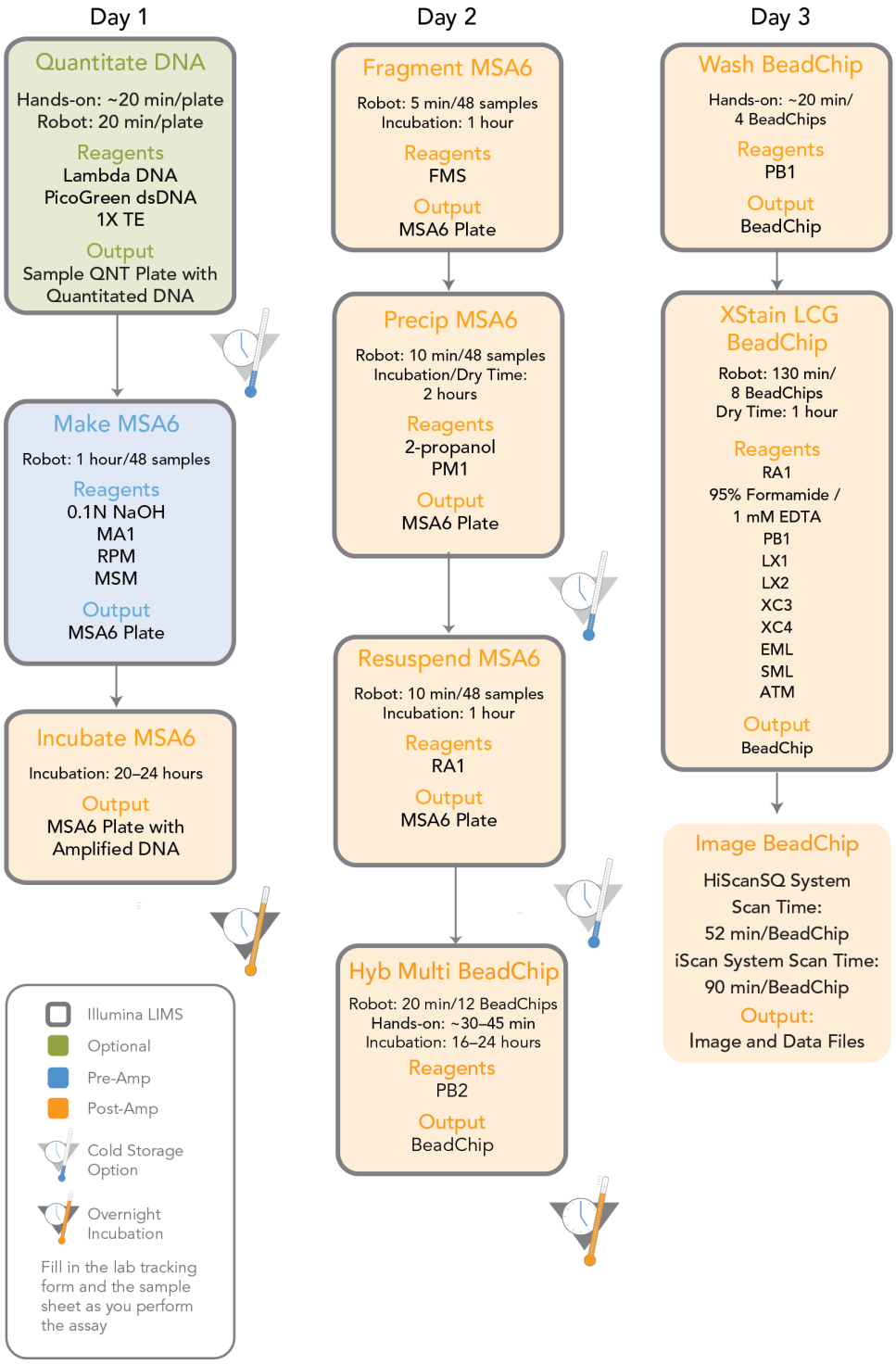


Make the MSA6 Plate (Pre-AMP)

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

Robot time:

- 30 minutes for 48 samples
- 45 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.
- In the sample sheet, enter the Sample_Name and Sample_Plate for each Sample_Well.
- 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.

Steps to Make the MSA6 Plate

[_] 1 If you do not already have a WG#-DNA plate, add DNA into either of the following, and then apply a barcode label to the new DNA plate:
- Midi plate: 20 µl to each WG#-DNA plate well
- TCY plate: 10 µl to each WG#-DNA plate well

[_] 2 At the robot PC, select **MSA6 Tasks** | **Make MSA6**.

[_] 3 Select the WG#-DNA plate type (MIDI or TCY).

[_] 4 (Non-Illumina LIMS) Make sure that the **Use Barcodes** checkbox is cleared. In the Basic Run

Parameters pane, enter the **Number of DNA samples** (48 or 96) that are in the plate.

NOTE
If you are using Illumina LIMS, click **Run** and select batches before the robot bed map displays the correct layout for the WG#-DNA plates.

[_] 5 Remove caps from MA1, RPM, and MSM tubes, then place the tubes in the robot standoff tube rack according to the bed map.

[_] 6 Add 15 ml NaOH to the quarter reservoir, then place the reservoir on the robot bed according to the bed map.

[_] 7 Place the WG#-DNA and MSA6 plates on the robot bed according to the bed map.

[_] 8 (Non-Illumina LIMS) At the robot PC, click **Run**.

[_] 9 (Illumina LIMS) At the robot PC:

- [_] a Make sure that the **Use Barcodes** checkbox is selected.
- [_] b Click **Run** to start the process. Log in if prompted.
- [_] c Select the batch you want to run, and then click **OK**.
- [_] d Click **OK** to confirm the required DNAs.

[_] 10 After the robot adds the 0.1N NaOH to the DNA in the MSA6 plate, follow the instructions at the prompt.

[_] 11 Seal the plate with a cap mat.

[_] 12 Vortex the sealed MSA6 plate at 1600 rpm for 1 minute.

[_] 13 Centrifuge at 280 × g.

[_] 14 Place the MSA6 plate back on the robot bed in its original position.

[_] 15 When the robot finishes, seal the MSA6 plate with a cap mat.

[_] 16 Vortex the sealed plate at 1600 rpm for 1 minute.

[_] 17 Centrifuge at 280 × g.

[_] 18 Record the location of DNA samples in the lab tracking worksheet.

[_] 19 If you are using Illumina LIMS:

- [_] a In the Illumina LIMS left pane, click **Infinium LCG | Incubate MSA6**.
- [_] b Scan the barcode of the MSA6 plate and click **Verify** and then click **Save**. Illumina LIMS records the data and queues the plate for the next step.

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

[_] 21 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

Robot time:

- 5 minutes for 48 samples
- 10 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. Gently invert at least 10 times to mix contents.

[_] 3 Remove the MSA6 plate from the Illumina Hybridization Oven.

[_] 4 If you plan to Resuspend the MSA6 plate today, remove the RA1 from the freezer to thaw.

Steps to Fragment the MSA6 Plate

[_] 1 Pulse centrifuge the MSA6 plate to 280 × g.

[_] 2 Remove the cap mat.

[_] 3 At the robot PC, select **MSA6 Tasks** | **Fragment MSA6**.

[_] 4 (Non-Illumina LIMS) Make sure that the **Use Barcodes** checkbox is cleared. In the **Basic Run Parameters** pane, change the value for **Number of MSA6 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.

NOTE
If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of samples.

[_] 5 Place the MSA6 plate on the robot bed according to the bed map.

[_] 6 Place FMS tubes in the robot tube rack according to the bed map. Remove the cap.

[_] 7 (Non-Illumina LIMS) At the robot PC, click **Run**.

[_] 8 (Illumina LIMS) At the robot PC:

[_] a Make sure that the **Use Barcodes** checkbox is selected.

[_] b Click **Run** to start the process. Log in if prompted.

[_] 9 When the robot finishes, click **OK** in the message box.

[_] 10 Remove the MSA6 plate from the robot bed and seal it with a cap mat.

[_] 11 Vortex at 1600 rpm for 1 minute.

[_] 12 Pulse centrifuge to 280 × g.

[_] 13 Place the sealed plate on the 37°C heat block for 1 hour.

[_] 14 Do one of the following:

- Proceed to *Precipitate DNA (Post-Amp)*. Leave plate in 37°C heat block until you have completed the preparatory steps. 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 more than 24 hours.

SAFE STOPPING POINT

Now is a good stopping point in the process.

Precipitate DNA (Post-Amp)

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

Estimated Time

Robot time:

- 10 minutes for 48 samples

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 Preheat the heat block to 37°C.

[_] 2 If you froze the MSA6 plate overnight, thaw it to room temperature, then pulse centrifuge to 280 × g.

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

Steps to Precipitate the MSA6 Plate (Post-AMP)

[_] 1 At the robot PC, select **MSA6 Tasks | Precip MSA6**.

[_] 2 (Non-Illumina LIMS) Make sure that the **Use Barcodes** checkbox is cleared. In the **Basic Run Parameters** pane, change the value for **Number of MSA6 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.

NOTE
If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of samples.

[_] 3 Pulse centrifuge the sealed MSA6 plate at 280 × g.

[_] 4 Remove the cap mat and place the MSA6 plate on the robot bed according to the bed map.

[_] 5 Place a half reservoir in the reservoir frame, according to the robot bed map, and add PM1 as follows:

- For 48 samples: 1 tube PM1
- For 96 samples: 2 tubes PM1

[_] 6 Place a full reservoir in the reservoir frame, according to the robot bed map, and add 2-propanol as follows:

- For 48 samples, dispense 20 ml 2-propanol
- For 96 samples, dispense 40 ml 2-propanol

[_] 7 (Non-Illumina LIMS) At the robot PC, click **Run**.

[_] 8 (Illumina LIMS) At the robot PC:

[_] a Make sure that the **Use Barcodes** checkbox is selected.

[_] b Click **Run** to start the process. Log in if prompted.

[_] 9 When prompted, remove the MSA6 plate from the robot bed. Do not click **OK** in the message box yet.

[_] 10 Seal the MSA6 plate with the same cap mat removed earlier.

[_] 11 Vortex the sealed plate at 1600 rpm for 1 minute.

[_] 12 Incubate at 37°C for 5 minutes.

[_] 13 Pulse centrifuge to 280 × g.

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

[_] 14 Remove the cap mat and discard it.

[_] 15 Place the MSA6 plate back on the robot bed according to the bed map.

[_] 16 At the robot PC, click **OK**.

[_] 17 When prompted, seal the plate with a new, dry cap mat.

[_] 18 Invert the plate at least 10 times to mix contents thoroughly.

[_] 19 Incubate at 4°C for 30 minutes.

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

[_] 21 Remove the cap mat and discard it.

[_] 22 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.

[_] 23 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

[_] 24 Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.
You can expect to find blue pellets at the bottoms of the wells.

[_] 25 If you are using Illumina LIMS:

[_] a In the Illumina LIMS left pane, click **Infinium LCG | Spin MSA6**.

[_] b Scan the barcode of the MSA6 plate and click **Verify** and then click **Save**. Illumina LIMS records the centrifugation step and queues the plate for the next step.

[_] 26 Do either of the following:

- Proceed to *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 at -25°C to -15°C for no more than 24 hours.

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

Resuspend DNA (Post-Amp)

RA1 is added to the MSA6 plate to resuspend the precipitated DNA samples.

Estimated Time

Robot time:

- 10 minutes for 48 samples
- 15 minutes for 96 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	Bottle (9 ml) per 96 samples	-25°C to -15°C	Illumina

Preparation

[_] 1 RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20°C to 25°C water bath. Gently mix to dissolve any crystals that might be present.

[_] 2 If you stored the MSA6 plate at -25°C to -15°C, thaw it to room temperature. Remove the cap mat and discard it.

[_] 3 Preheat the Illumina Hybridization Oven to 48°C.

[_] 4 Preheat the heat sealer. Allow 20 minutes.

[_] 5 Thaw RA1 to room temperature. Invert several times to redissolve solution.

Steps to Resuspend the MSA6 Plate

[_] 1 At the robot PC, select **MSA6 Tasks | Resuspend MSA6**.

[_] 2 (Non-Illumina LIMS) In the **Basic Run Parameters** pane, change the value for **Number of MSA6 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.

NOTE
If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of samples.

[_] 3 Place the MSA6 plate on the robot bed according to the bed map.

[_] 4 Place a quarter reservoir in the reservoir frame, according to the robot bed map, and add RA1 as follows:

- 4.5 ml for 48 samples
- 9 ml for 96 samples

[_] 5 (Non-Illumina LIMS) At the robot PC, click **Run**.

[_] 6 (Illumina LIMS) At the robot PC:

[_] a Make sure that the **Use Barcodes** checkbox is selected.

[_] b Click **Run** to start the process. Log in if prompted.

[_] 7 Click **OK** in the message box. Remove the MSA6 plate from the robot bed.

[_] 8 Apply a foil seal to the MSA6 plate by firmly holding the heat sealer block down for 3 full seconds.

[_] 9 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.

[_] 10 Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.

[_] 11 Vortex the plate at 1800 rpm for 1 minute.

[_] 12 Pulse centrifuge to 280 × g.

[_] 13 Do either of the following:

- Proceed to *Hybridize to BeadChip (Post-Amp)*. If you plan to do so immediately, it is safe to leave the RA1 at room temperature.
- 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 at -80°C if storing for 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

Robot 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
Robot BeadChip alignment fixtures	6		Illumina
Robot Tip Alignment Guide-F	6		Illumina
1% aqueous Alconox solution	As needed		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.

Prepare the Robot Tip Alignment Guide

[_] 1 Make sure that you have the correct Robot Tip Alignment Guide for the Infinium assay you are running. The barcode says **Guide-F**.

[_] 2 Wash and dry the entire one-piece Robot Tip Alignment Guide. See *Wash Robot Tip Alignment Guide* at the end of the *Hybridize to BeadChip* steps for washing instructions.

[_] 3 Place the assembled Robot Tip Alignment Guides on the lab bench until it is time to place them on the robot bed.

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 BeadChips

[_] 1 Remove all BeadChips from their plastic bags and mylar packages.
When handling the BeadChip, avoid contacting the beadstripe area and sample inlets.

[_] 2 Place BeadChips into the Robot BeadChip alignment fixtures with the barcode end aligned to the ridges on the fixture.

[_] 3 At the robot PC, select **MSA6 Tasks | Hyb Multi-BC2**.

[_] 4 Choose the appropriate BeadChip from the BeadChip Selection dialog box.

[_] 5 (Non-Illumina LIMS) In the Basic Run Parameters pane, change the value for **Number of MSA6 plates** and **Number of DNA samples per plate** to indicate the number of samples being processed.

NOTE
If you are using Illumina LIMS, you cannot change the number of DNA samples on this screen. However, the LIMS software processes the correct number of samples.

[_] 6 Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map.

[_] 7 Pulse centrifuge the MSA6 plate at 280 × g.

[_] 8 Place the MSA6 plate onto the robot bed according to the bed map. Remove the foil seal.

[_] 9 (Non-Illumina LIMS) At the robot PC, click **Run**.

[_] 10 (Illumina LIMS only) At the robot PC:

- [_] a Make sure that the **Use Barcodes** checkbox is selected.
- [_] b Click **Run** to start the process. Log in if prompted.

[_] 11 Place the Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture, with the Guide-F barcode on the left side. Push both the Robot Tip Alignment Guide and Robot BeadChip Alignment Fixture to the upper left corner in its section of the robot bed.

[_] 12 At the robot PC, click **OK** to confirm that you have placed the Robot Tip Alignment Guide on top of the Robot BeadChip alignment fixture. The robot scans the barcode on the Robot Tip Alignment Guide to confirm that the correct tip guide is being used.
The robot dispenses sample to the BeadChips.

[_] 13 Click **OK** in the message box.

[_] 14 Carefully remove the Robot BeadChip alignment fixtures from the robot bed and visually inspect all sections of the BeadChips. Make sure that DNA sample covers all the sections of each bead stripe. Record any sections that are not completely covered.

Set up BeadChip for Hybridization

[_] 1 Make sure that the Illumina Hybridization Oven is set to 48°C.

[_] 2 Carefully remove each BeadChip from the Robot BeadChip alignment fixtures when the robot finishes.

CAUTION
For optimal performance, take care to keep the Hyb Chamber inserts containing BeadChips steady and level when lifting or moving. Avoid shaking and always keep parallel to the lab bench. Do not hold by the sides near the sample inlets.

[_] 3 Carefully place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the insert.

[_] 4 Load the Hyb Chamber inserts containing loaded BeadChips inside the Illumina Hyb Chamber. Position the barcode over the ridges indicated on the Hyb Chamber.

[_] 5 (Illumina LIMS) In the Illumina LIMS left pane click **Infinium LCG** | **Infinium Prepare Hyb Chamber**.

[_] 6 Scan the barcodes of the PB2 tubes and scan the BeadChip barcodes. Click **Verify**, and then click **Save**.

[_] 7 Position the lid onto the Hyb Chamber by applying the backside of the lid first and then slowly bringing down the front end to avoid dislodging the Hyb Chamber inserts.

[_] 8 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.

[_] 9 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.

[_] 10 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 the Robot Tip Alignment Guide

For optimal performance, wash and dry the Robot Tip Alignment Guides after every run.

[_] 1 Soak the tip guide inserts in a 1% aqueous Alconox solution (1 part Alconox to 99 parts water) using a 400 ml Pyrex beaker for 5 minutes.

NOTE
Do not use bleach or ethanol to clean the tip guide inserts.

[_] 2 After the 5 minute soak in the 1% Alconox solution, thoroughly rinse the tip guides with DiH_2O at least 3 times to remove any residual detergent.

[_] 3 Dry the Robot Tip Alignment Guide using a Kimwipe or lint-free paper towels. Use a laboratory air gun to dry. Be sure to inspect the tip guide channels, including the top and bottom. Tip guides must be dry and free of any residual contaminates before next use.

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:

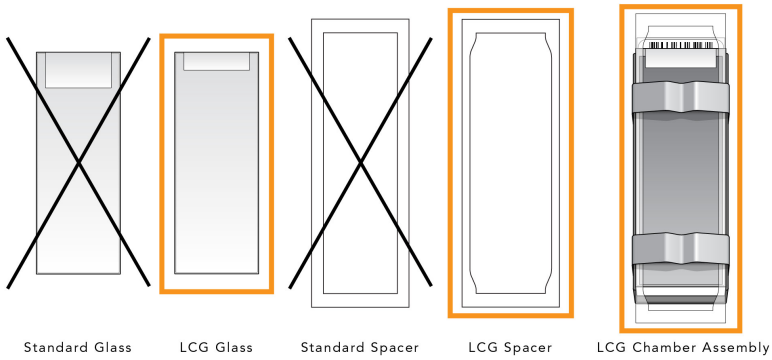
[_] a Assemble the flow-through chambers for the first 8 BeadChips, as described in *Assemble Flow-Through Chambers* on page 1.

[_] 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 1 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 If you are using Illumina LIMS:

- [_] a In the Illumina LIMS left pane, click **Infinium LCG | Wash BeadChip**.
- [_] b Scan the reagent barcodes and the BeadChip barcodes. Click **Verify** and then click **Save**. Illumina LIMS records the data and queues the BeadChips for the next step.

[_] 11 Proceed to *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 the XStain BeadChip. Do not place the flow-through chambers in the chamber rack until the preparation is complete.

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

Robot time:

- ~2 hours and 45 minutes for 8 BeadChips
- ~3 hours for 16 BeadChips
- ~3 hours and 10 minutes for 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	-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

Item	Quantity	Storage	Supplied By
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	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 RA1 is shipped frozen. Gradually warm the reagent to room temperature, preferably in a 20°C to 25°C water bath. Gently mix to dissolve any crystals that might be present.

[_] 2 Place all reagent tubes in a rack in the order in which they are used. If frozen, allow them to thaw to room temperature, and then gently invert the reagent tubes at least 10 times to mix contents.

[_] 3 Make sure that the water circulator is filled to the appropriate level.

[_] 4 Turn on the water circulator and set it to 44°C using the Circulator Manager in the automation control software.

[_] 5 Remove bubbles trapped in the Chamber Rack.

[_] 6 Test several locations on the Chamber Rack, using the Illumina Temperature Probe. All locations must be at 44°C ± 0.5°C. If the temperature on the probe is not within ± 0.5°C, contact Illumina Technical Support.

Single-Base Extension and Stain

CAUTION
The remaining steps must be performed without interruption.

[_] 1 Slide the chamber rack into column 36 on the robot bed. Make sure that it is seated properly.

[_] 2 At the robot PC, select **XStain Tasks** | **XStain LCG BeadChip**.

[_] 3 In the Basic Run Parameters pane, enter the number of BeadChips.

[_] 4 If you plan on imaging the BeadChip immediately after the staining process, turn on the iScan or HiScan now to allow the lasers to stabilize.

[_] 5 Place a quarter reservoir in the reservoir frame, according to the robot bed map, and add 95% formamide/1 mM EDTA as follows:

- 15 ml to process 8 BeadChips

- 17 ml to process 16 BeadChips
- 25 ml to process 24 BeadChips

[_] 6 Place a half reservoir in the reservoir frame, according to the robot bed map, and add RA1 in the following volumes:
- 10 ml to process 8 BeadChips
- 20 ml to process 16 BeadChips
- 30 ml to process 24 BeadChips

[_] 7 Place a full reservoir in the reservoir frame, according to the robot bed map, and add XC3 in the following volumes:
- 50 ml to process 8 BeadChips
- 100 ml to process 16 BeadChips
- 150 ml to process 24 BeadChips

[_] 8 Place each reagent tube (LX1, LX2, EML, SML, ATM) in the robot tube rack according to the bed map, and remove their caps.

[_] 9 When prompted, enter the stain temperature indicated on the SML tube.

[_] 10 Do not load the BeadChips yet.

[_] 11 When the chamber rack reaches 44°C, quickly place each flow-through chamber assembly into the first row of the chamber rack. Refer to the robot bed map for the correct layout.

[_] 12 At the robot PC, click **OK**.

[_] 13 When the robot finishes, immediately remove the flow-through chambers from the chamber rack. Place horizontally on the 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 If you are using Illumina LIMS:

[_] a In the Illumina LIMS left pane, click **Infinium LCG | Coat BC2**.

[_] b Scan the reagent barcodes and BeadChip barcodes. Click **Save**. Illumina LIMS records the data and queues the BeadChips for the next step.

[_] 18 Do either of the following:

- Proceed to *Image BeadChip (Post-Amp)*.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box inside a vacuum desiccator at room temperature. Be sure to 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