Illumina Infinium LCG Quad Assay, Automated Protocol

Experienced User Card

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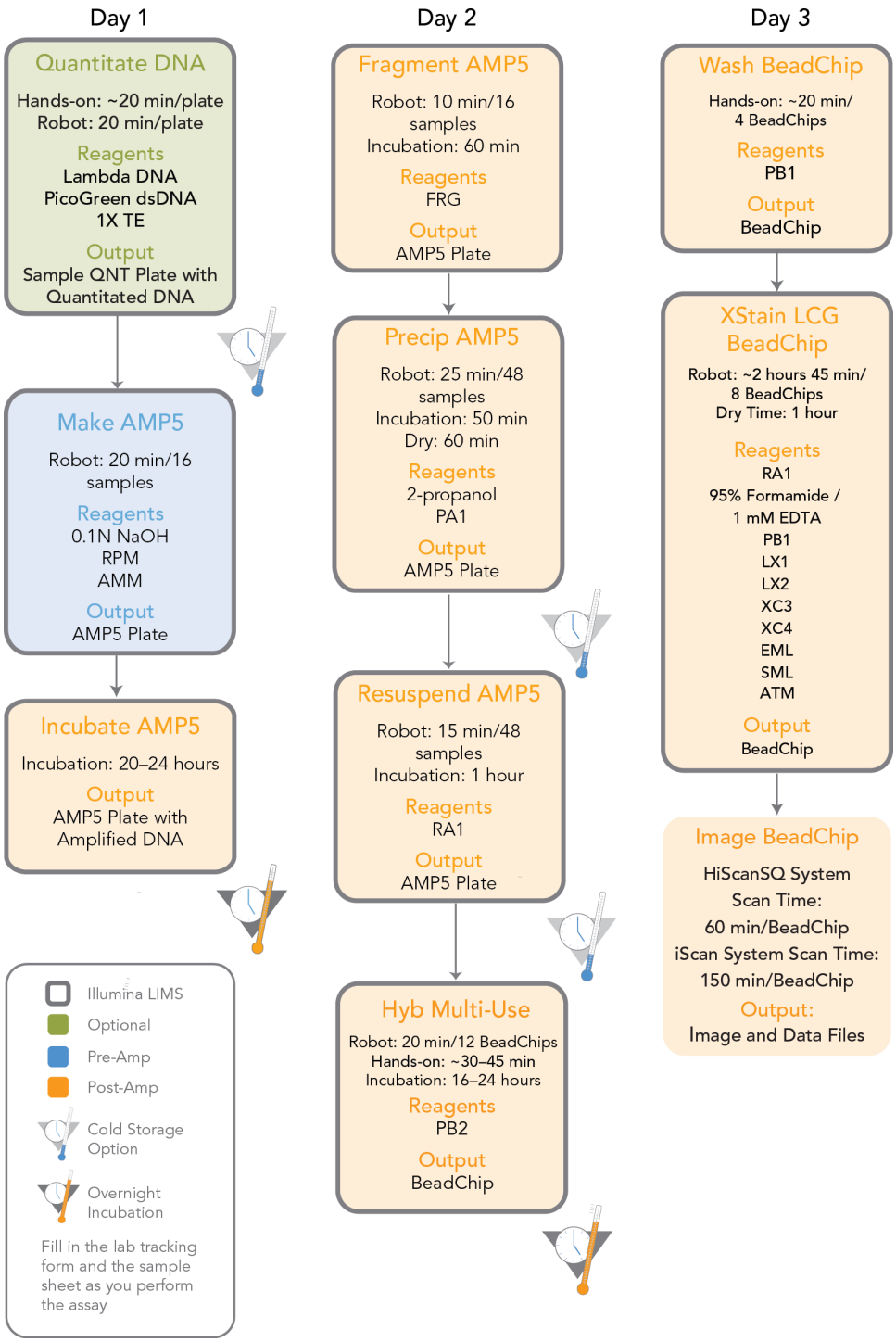


Make the AMP5 Plate (Pre-AMP)

This process creates a AMP5 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

Robot time:

- 20 minutes for 16 samples
- 30 minutes for 32 samples
- 55 minutes for 48 samples

Incubation time: ~20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
RPM	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 (per 96 samples)	2° to 8°C	User
96-well 0.8 ml microtiter plate (MIDI)	1 plate for up to 48 samples		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 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 AMP5 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 AMP5 Plate

[_] 1 If you do not already have a WG#-DNA plate, add DNA into one of the following:
- 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 DNA plate.

[_] 2 At the robot PC, select **AMP5 Tasks** | **Make AMP5**.
Alternative: select **AMP5 Tasks** | **Make Multi-AMP5** to run multiple AMP5 plates.

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

[_] 4 (Non-Illumina LIMS) Ensure that the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of DNA samples** (16, 32, or 48) that are in the plate.

[_] 5 Remove caps from the RPM and AMM 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 AMP5 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 Ensure the **Use Barcodes** check box is checked.
- [_] b Click **Run** to start the process. Login if prompted.

[_] 10 (Illumina LIMS) Select the batch you want to run, and then click **OK**.

[_] 11 (Illumina LIMS) Click **OK** to confirm the required DNAs.

[_] 12 When the robot finishes, seal the AMP5 plate with a cap mat.

[_] 13 Invert the sealed AMP5 plate at least 10 times to mix contents.

[_] 14 Centrifuge to 280 xg.

[_] 15 Record the location of DNA samples in the lab tracking worksheet.

[_] 16 If you are using Illumina LIMS:

- [_] a In the Illumina LIMS left sidebar, click **Infinium LCG Quad** | **Incubate AMP5**.
- [_] b Scan the barcode of the AMP5 plate and click **Save**. Illumina LIMS records the data and queues the plate for the next step.

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

[_] 18 Proceed to *Fragment the AMP5 Plate (Post-AMP)*.

Fragment the AMP5 Plate (Post-AMP)

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

Estimated Time

Robot time:

- 10 minutes for 16 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 FRG tubes to room temperature. Gently invert at least 10 times to mix contents.
- Remove the AMP5 plate from the Illumina Hybridization Oven.
- If you plan to Resuspend the AMP5 plate today, remove the RA1 from the freezer to thaw.

Steps to Fragment the AMP5 Plate

[_] 1 Pulse centrifuge the AMP5 plate to 280 xg.

[_] 2 Remove the cap mat.

[_] 3 At the robot PC, select **AMP5 Tasks** | **Fragment AMP5**.

[_] 4 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the **Basic Run Parameters** pane, change the value for **Number of AMP5 plate(s)** 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 AMP5 plate on the robot bed according to the bed map.

[_] 6 Place FRG 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 the **Use Barcodes** check box is checked.

[_] 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 AMP5 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 xg.

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

[_] 14 Do one of the following:

- Proceed to *Precipitate the AMP5 Plate (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 AMP5 plate at -15° to -25°C for more than 24 hours.

Precipitate the AMP5 Plate (Post-AMP)

PA1 and 2-propanol are added to the AMP5 plate to precipitate the DNA samples.

Estimated Time

Robot time:

- 15 minutes for 16 samples

Incubation and dry time: 2 hours

Consumables

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

Preparation

- Preheat the heat block to 37°C.
- If you froze the AMP5 plate overnight, thaw it to room temperature, then pulse centrifuge to 280 xg.
- Thaw PA1 to room temperature. Gently invert at least 10 times to mix contents.

Steps to Precipitate the AMP5 Plate (Post-AMP)

[_] 1 At the robot PC, select **AMP5 Tasks | Precip AMP5**.

[_] 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the **Basic Run Parameters** pane, change the value for **Number of AMP5 plate(s)** 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 AMP5 plate to 280 xg.

[_] 4 Remove the cap mat and place the AMP5 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 PA1 as follows:

- For 16 samples: 1 tube
- For 32 samples: 2 tubes
- For 48 samples: 3 tubes

[_] 6 Place a full reservoir in the reservoir frame, according to the robot bed map, and add 2-propanol as follows:
- For 16 samples: 20 ml
- For 32 samples: 30 ml
- For 48 samples: 40 ml

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

[_] 8 (Illumina LIMS) At the robot PC:
- [_] a Ensure the **Use Barcodes** check box is checked.
- [_] b Click **Run** to start the process. Log in if prompted.

[_] 9 When prompted, remove the AMP5 plate from the robot bed. Do not click **OK** in the message box yet.

[_] 10 Seal the AMP5 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 xg.

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

[_] 14 Remove the cap mat and place the AMP5 plate back on the robot bed according to the bed map.

[_] 15 At the robot PC, click **OK**.

[_] 16 When prompted, seal the plate with a new, dry cap mat.

[_] 17 Invert the plate at least 10 times to mix contents thoroughly.

[_] 18 Incubate at 4°C for 30 minutes.

[_] 19 Centrifuge to 3,000 xg at 4°C for 20 minutes. Immediately remove the AMP5 plate from centrifuge.

[_] 20 Remove the cap mat and discard it.

[_] 21 Over an absorbent pad, decant the supernatant by quickly inverting the AMP5 plate. Drain liquid onto the absorbent pad and then smack the plate down, avoiding the liquid that was just drained onto the pad.

[_] 22 Tap firmly several times for 1 minute or until all wells are devoid of liquid.

[_] 23 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.

[_] 24 If you are using Illumina LIMS:
- [_] a In the Illumina LIMS left sidebar, click **Infinium LCG Quad | Spin AMP5**.
- [_] b Scan the barcode of the AMP5 plate and click **Verify** and then click **Save**. Illumina LIMS records the centrifugation step and queues the plate for the next step.

[_] 25 Do one of the following:
- Proceed to *Resuspend the AMP5 Plate (Post-AMP)*.

- If you do not plan to proceed to the next step immediately, seal the AMP5 plate with a new cap mat and store at -15° to -25°C for no more than 24 hours.

Precipitate the AMP5 Plate (Post-AMP)

Resuspend the AMP5 Plate (Post-AMP)

RA1 is added to the AMP5 plate to resuspend the precipitated DNA samples.

Estimated Time

Robot time:

- 15 minutes for 48 samples

Incubation time: 1 hour

Consumables

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

Preparation

- If you stored the AMP5 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.
- Preheat the heat sealer. Allow 20 minutes.
- Thaw RA1 to room temperature. Invert several times to re-dissolve solution.

Steps to Resuspend the AMP5 Plate

[_] 1 At the robot PC, select **AMP5 Tasks** | **Resuspend AMP5**.

[_] 2 (Non-Illumina LIMS) In the **Basic Run Parameters** pane, change the value for **Number of AMP5 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 AMP5 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 ml for 16 samples
- 7 ml for 32 samples
- 9 ml for 48 samples

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

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

[_] a Ensure the **Use Barcodes** check box is checked.

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

[_] 7 Click **OK** in the message box. Remove the AMP5 plate from the robot bed.

[_] 8 Apply a foil seal to the AMP5 plate by firmly holding the heat sealer block down for 3 full seconds.

[_] 9 Immediately remove the AMP5 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 xg.

[_] 13 Do one of the following:

- Proceed to *Hybridize Multi 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 AMP5 plate at -15° to -25°C for no more than 24 hours. Store at -80°C if storing for 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

Robot time:

- 4x1 LCG BeadChip: ~20 minutes for 12 BeadChips (48 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 16 Samples)	Storage	Supplied By
PB2	2 tubes	Room temperature	Illumina
BeadChips	4	4°C	Illumina
Hyb Chambers	1		Illumina
Hyb Chamber gaskets	1		Illumina
Hyb Chamber inserts	4		Illumina
Robot BeadChip Alignment Fixtures	2		Illumina
Robot Tip Alignment Guide-F	2		Illumina
1% aqueous Alconox solution	As needed		User

Preparation

- Preheat the heat block to 95°C.
- Preheat the Illumina Hybridization Oven to 48°C.

Prepare the Robot Tip Alignment Guide

[_] 1 Ensure that you have the correct Robot Tip Alignment Guide for the Infinium assay you are running. The barcode should say **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 Multi BeadChip* steps for washing instructions.

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

Assemble the Hybridization Chambers

[_] 1 Place the resuspended AMP5 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 AMP5 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 AMP5 plate to 280 xg. Remove the foil seal.

[_] 6 (Illumina LIMS) In the Illumina LIMS left sidebar, click **Infinium LCG Quad | Confirm BeadChips for Hyb**.

- [_] a Scan the barcode of the AMP5 plate and click **Verify**.

Load BeadChips

[_] 1 Remove all BeadChips from their ziplock 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 **AMP5 Tasks | Hyb Multi-BC2 AMP5**.

[_] 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 AMP5 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 AMP5 plate to 280 xg.

[_] 8 Place the AMP5 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 Ensure the **Use Barcodes** check box is checked.
- [_] 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. The Guide-F barcode should be 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 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 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. Ensure DNA sample covers all of the sections of each bead stripe. Record any sections that are not completely covered.

Set up Multi BeadChip for Hybridization

[_] 1 Ensure 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 keep parallel to the lab bench at all times. 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 sidebar click **Infinium LCG Quad** | **Infinium Prepare Hyb Chamber**.

- [_] a Scan the barcode(s) of the PB2 tube(s) and scan the BeadChip barcodes. Click **Verify**, and then click **Save**.

[_] 6 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.

[_] 7 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.

[_] 8 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.

[_] 9 Incubate at 48°C for at least 16 hours but no more than 24 hours.

[_] 10 Proceed to *Wash the BeadChip (Post-AMP)* after the overnight incubation.

Resuspend XC4 Reagent for XStain LCG 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.

Wash the Robot Tip Alignment Guide

For optimal performance, the Robot Tip Alignment Guides should be washed and dried after every run.

[_] 1 Soak the tip guide inserts in a 1% aqueous Alconox solution (one 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 three 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 should be completely dry and free of any residual contaminates before next use.

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 8 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.

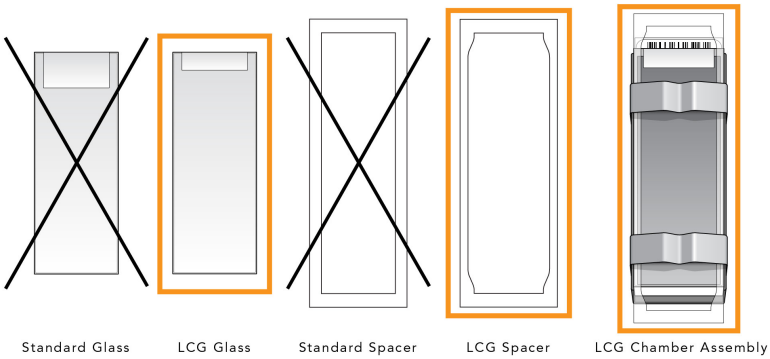
NOTE
Keep the Flow-Through Chambers in a horizontal position on the lab bench until all assembled Flow-Through Chambers are ready to be loaded into the Chamber Rack. Do not place the Flow-Through Chambers in the Chamber Rack until all BeadChips are prepared in Flow-Through Chambers.

[_] 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 LCG Quad 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 If you are using Illumina LIMS:

[_] a In the Illumina LIMS left sidebar, click **Infinium LCG Quad | 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 *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 the preparation is complete.

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

Following hybridization, RA1 reagent is used to wash away unhybridized and non-specifically hybridized DNA sample. LX1 and LX2 are added to condition the BeadChip surface for the extension reaction. EML reagents are dispensed into the Flow-Through Chambers to perform single-base extension of 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, the Flow-Through Chambers are disassembled. The BeadChips are washed in the PB1 reagent, and then coated with XC4 reagent and dried.

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	-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 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	-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 and set it to 44°C using the Circulator Manager in the automation control software.
- 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. 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. Ensure 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 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 If you are using Illumina LIMS:

[_] a In the Illumina LIMS left sidebar, click **Infinium LCG Quad | 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 one 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 *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 LCG Quad

BeadChip	Scan Setting Name
HumanOmni5-4	Infinium LCG

Image BeadChip (Post-AMP)