Illumina Infinium LCG Quad Assay, Automated Protocol

Experienced User Card

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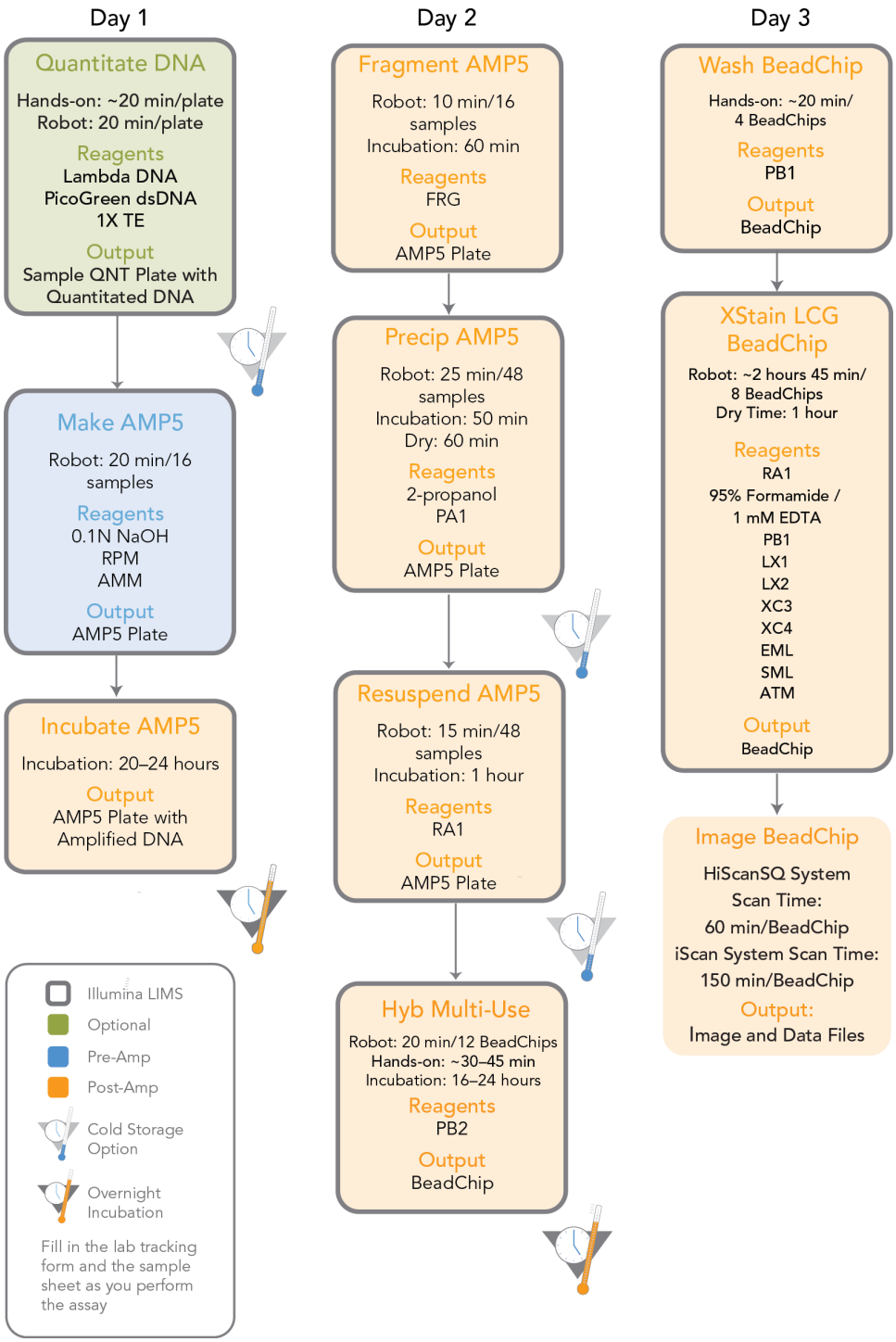

Make the AMP5 Plate (Pre-AMP)

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.