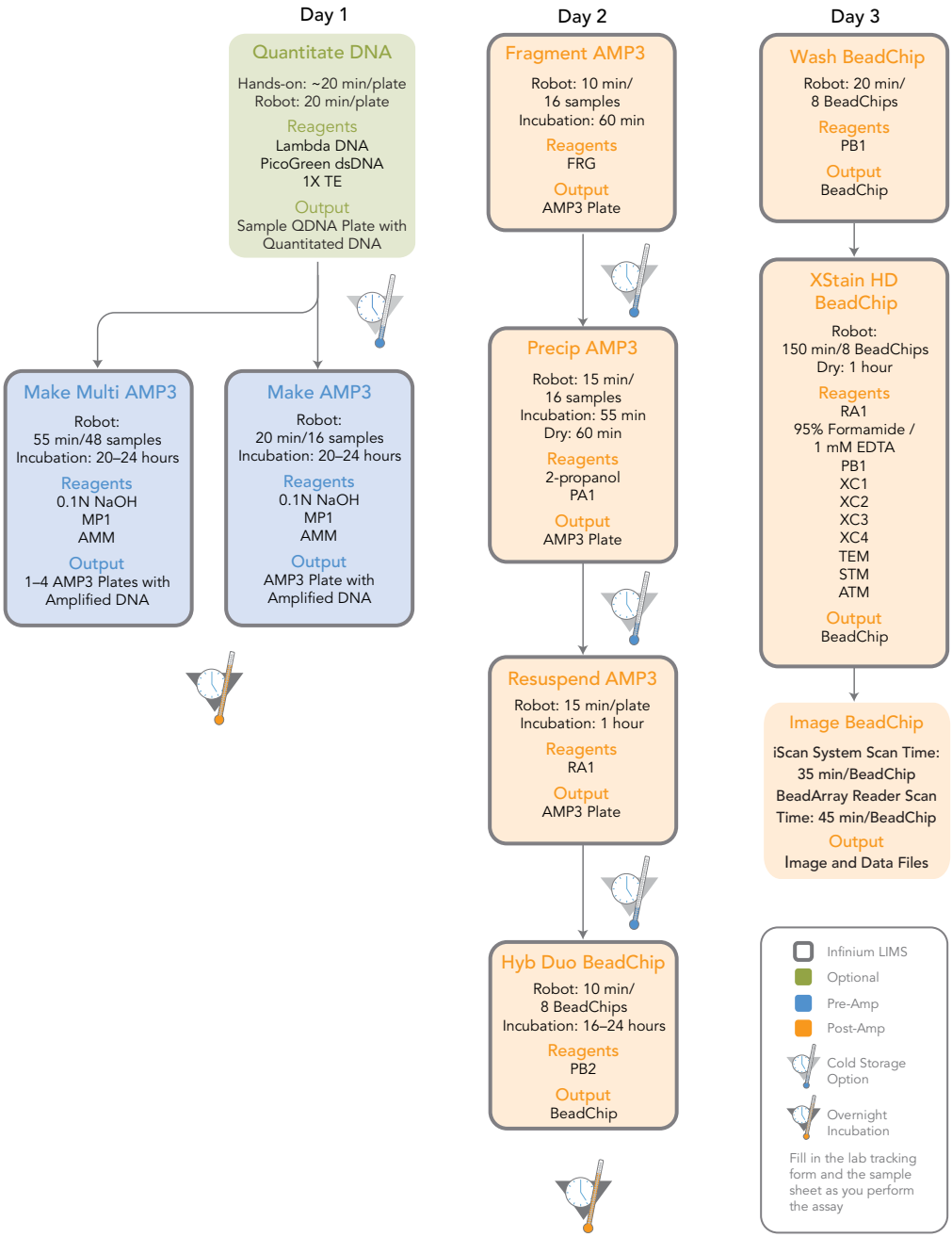
Infinium® HD Assay Gemini, Automated Protocol

Experienced User Card



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Quant DNA (Optional)

This process uses the PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples. You can quantitate up to three plates, each containing up to 96 samples

Estimated Time

Hands-on time: ~20 minutes per plate
Robot: 20 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See Instructions	-15 to -25°C	User
1X TE (10 mM Tris-HCl pH8.0, 1 mM EDTA (TE))	See Instructions	Room temperature	User
Lambda DNA	See Instructions	2 to 8°C	User
96-well 0.65 ml microtiter plate	1 per 96 samples		User
Fluotrac 200 96-well flat-bottom plate	1 per Std DNA plate 1 per Sample DNA plate		User
WG#-DNA plate with DNA samples	8, 16, 24, 32, 48, or 96 DNA samples in 1 to 3 plates	2 to 8°C	User

Preparation

- [ ] Thaw PicoGreen to room temperature in a light-impermeable container.
- [ ] Prepare the robot for use.
- [ ] Thaw the sample DNA plates to room temperature.
- [ ] Apply a QNT barcode label to a new Fluotrac plate for each WG#-DNA plate to be quantified.
- [ ] Hand-label the microtiter plate “Standard DNA.”
- [ ] Hand-label one of the Fluotrac plates “Standard QNT.”
- [ ] In the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.

Steps

Make Standard DNA Plate

- [ ] **1.** Add stock Lambda DNA to well A1 in the plate labelled “Standard DNA” and dilute it to 75 ng/µl in a final volume of 233.3 µl. Pipette up and down several times.
- [ ] **2.** Add 66.7 µl 1X TEto well B1.
- [ ] **3.** Add 100 µl 1X TE to wells C, D, E, F, G, and H of column 1.

[ ] **4.** Transfer 133.3 µl of Lambda DNA from well A1 into well B1. Pipette up and down several times.

[ ] **5.** Change tips. Transfer 100 µl from well B1 into well C1. Pipette up and down several times.

[ ] **6.** Repeat for wells D1, E1, F1, and G1, changing tips each time. **Do not transfer from well G1 to H1.** Well H1 serves as the blank 0 ng/µl Lambda DNA.

[ ] **7.** Cover the Standard DNA plate with cap mat.

Dilute PicoGreen

[ ] **1.** Prepare a 1:200 dilution of PicoGreen into 1X TE, using a sealed 100 ml or 250 ml Nalgene bottle wrapped in aluminum foil.

[ ] **2.** Cap the foil-wrapped bottle and vortex to mix.

Create Standard QNT Standard and Sample Plates

[ ] **1.** At the robot PC, select **DNA Quant | Make Quant**.

[ ] **2.** Select the WG#-DNA plate type (MIDI, TCY or ABGN).

[ ] **3.** In the Basic Run Parameters pane, enter the **Number of DNA/QNT plates** (1, 2, or 3 pairs) and the **Number of DNA Samples**.

The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

[ ] **4.** Vortex each plate for 1 minute.

[ ] **5.** Centrifuge the WG#-DNA Sample plate to 280 xg for 1 minute.

[ ] **6.** Vortex the Standard DNA plate at 1450 rpm for 1 minute.

[ ] **7.** Centrifuge the Standard DNA plate to 280 xg for 1 minute.

[ ] **8.** Place the WG#-DNA Sample, Standard DNA, Standard QNT, and QNT Sample plates on the robot bed according to the robot bed map. Remove any plate seals.

[ ] **9.** Pour the PicoGreen dilution into half reservoir A and place it on the robot bed.

[ ] **10.** If you are not using LIMS, clear the **Use Barcodes** checkbox.

[ ] **11.** Click **Run**. Click **OK** in the message box.

[ ] **12.** After the robot finishes, immediately seal all plates:

- [ ] **a.** Place foil adhesive seals over Sample QNT and Standard QNT plates.
- [ ] **b.** Place cap mats on WG#-DNA Sample and Standard DNA plates.

[ ] **13.** Store the WG#-DNA and Standard DNA plates at 2 to 8°C or -15 to -25°C.

[ ] **14.** Centrifuge the Sample QNT Plate and Standard QNT plates to 280 xg for 1 minute.

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Read QNT Plate

[ ] **1.** Turn on the spectrofluorometer. At the PC, open the Infinium Fluorometry Analysis program.

[ ] **2.** Select **Reader Tasks | Read Quant**.

[ ] **3.** If you are not using LIMS, clear the **Use Barcodes** checkbox.

[ ] **4.** Click **Read**.

[ ] **5.** When prompted, enter the number of Sample QNT plates you want to read (1, 2, or 3). Do not include the Standard QNT plate in this number. Click **OK**.

[ ] **6.** Remove the seal from the Standard QNT plate, place the plate in the spectrofluorometer tray, and click **OK**.

[ ] **7.** Review the data from the Standard QNT plate. Either accept it and go on to the next step, or reject it, which will stop the Read Quant process.

[ ] **8.** Remove the Standard QNT plate from the spectrofluorometer tray.

[ ] **9.** When prompted, hand-scan the Sample QNT plate barcode. Click **OK**.

[ ] **10.** When prompted, remove the plate seal from the Sample QNT plate and load it into the spectrofluorometer tray, with well A1 at the upper left corner. Click **OK**.

[ ] **11.** When prompted, click **Yes** to review the raw Sample QNT plate data.

[ ] **12.** If you entered more than one Sample QNT plate to read, repeat steps 9 to 11 for each additional plate.

[ ] **13.** Do one of the following:

- Proceed to *Make AMP3*.
- Store the Sample DNA plate at 2 to 8°C for up to one month.

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Make AMP3

Move DNA samples into the AMP3 plate. Denature and neutralize samples, and prepare them for amplification. Incubate overnight to amplify.

Estimated Time

Robot time:

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

Incubation time: ~20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
0.1N NaOH	15 ml per 16–48 samples	2 to 8°C	User
WG#-DNA plate with up to 96 DNA samples (50 ng/ l)	1 plate	-15 to -25°C	User
MP1	1 tube per 16 samples	-15 to -25°C	Illumina
AMM	1 tube per 16 samples	-15 to -25°C	Illumina
96-well 0.8 ml microtiter plate (MIDI)	1 plate for up to 48 samples		User

Preparation

[ ] Preheat the Illumina Hybridization Oven in the post-amp area to 37°C.

[ ] Thaw MP1 and AMM tubes to room temperature. Gently invert to mix, then pulse centrifuge to 280 xg.

[ ] Thaw DNA samples to room temperature.

[ ] Apply an AMP3 barcode to a new MIDI plate.

Steps

[ ] **1.** If you do not already have a WG#-DNA plate, dispense DNA into either a:

- 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 WG#-DNA plate.

[ ] **2.** At the robot PC, select **AMP3 Tasks | Make AMP3**.

Alternative: Select **AMP3 Tasks | Make Multi AMP3** to run multiple AMP3 plates.

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

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[ ] **4.** (Non-Infinium LIMS only) Make sure 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 the caps. Place the MP1 and AMM tubes in the robot tube rack according to the robot bed map.

[ ] **6.** Place a quarter reservoir on the robot bed according to the bed map, and add 15 ml 0.1N NaOH.

[ ] **7.** Vortex the sealed WG#-DNA plate at 1600 pm for 1 minute.

[ ] **8.** Centrifuge to 280 xg for 1 minute at 22°C.

[ ] **9.** Place the AMP3 and WG#-DNA plates on the robot bed according to the bed map. Remove all plate seals.

[ ] **10.** Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

[ ] **11.** (Non-Infinium LIMS only) At the robot PC, click **Run**.

[ ] **12.** (Infinium LIMS only) Make sure the **Use Barcodes** check box is checked and click **Run**.

- [ ] **a.** Log in when prompted.
- [ ] **b.** Select the batches you want to run and click **OK**.

The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

[ ] **13.** Click **OK** in the message box. Remove the AMP3 plate from the robot bed and seal with the 96-well cap mat.

[ ] **14.** Invert the sealed AMP3 plate at least 10 times to mix the contents.

[ ] **15.** Pulse centrifuge to 280 xg.

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

[ ] **17.** Proceed immediately to *Fragment AMP3*.

This is the end of Pre-Amp. You may now remove these Experienced User Cards from the Pre Amp area and take them elsewhere. Do not return with them into the Pre-Amp area at any time.

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Fragment AMP3

Enzymatically fragment DNA, using end-point fragmentation to avoid over-fragmentation.

Estimated Time

Robot time:

- 10 minutes per 16 samples
- 15 minutes per 32 samples
- 35 minutes per 48 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 the FRG tube to room temperature. Invert several times to mix contents. Pulse centrifuge to 280 xg for 1 minute.

For instructions on preparing the robot for use in a protocol see the *Infinium Assay Lab Setup and Procedures Guide*.

Steps

Set Up the Robot

[ ] 1. Centrifuge the AMP3 plate to 50 xg for 1 minute.

[ ] 2. At the robot PC, select **AMP3 Tasks | Fragment AMP3**.

[ ] 3. (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP3 Plates**.

The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

[ ] 4. Remove any plate seals. Place the AMP3 plate on the robot bed according to the robot bed map.

[ ] 5. Remove the caps. Place the FRG tubes in the robot tube rack according to the robot bed map.

[ ] 6. Make sure to properly place all items on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

[ ] 7. At the robot PC:

[ ] a. If you are not running Infinium LIMS, clear the **Use Barcodes** check box.

[ ] b. Click **Run** to start the process.

[ ] 8. Click **OK** in the message box. Remove the AMP3 plate from the robot bed and seal with the 96-well cap mat.

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

[ ] **10.** Centrifuge to 50 xg for 1 minute at 22°C.

[ ] **11.** Incubate on the heat block for 1 hour at 37°C.

[ ] **12.** Do one of the following:

- Proceed to *Precip AMP3*. Leave plate in the 37°C heat block until preparation is complete.
- Store the sealed AMP3 plate at -15 to -25°C.

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Precip AMP3

Precipitate the DNA sample using PA1 and 2-propanol.

Estimated Time

Robot time:

- 15 minutes per 16 samples
- 20 minutes per 32 samples
- 30 minutes per 48 samples

Dry time: 1 hour

Consumables

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

Preparation

[ ] If you froze the AMP3 plate after fragmentation, thaw it to room temperature. Centrifuge to 50 xg for 1 minute.

[ ] Preheat the heat block to 37°C.

[ ] In preparation for the 4°C spin, set the centrifuge to 4°C.

[ ] Thaw PA1 to room temperature. Centrifuge to 280 xg for 1 minute.

[ ] Preheat the heat sealer.

Steps

Set Up the Robot

[ ] **1.** At the robot PC, select **AMP3 Tasks | Precip AMP3**.

[ ] **2.** (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP3 plates** (1 to 4).

The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

[ ] **3.** Centrifuge the sealed AMP3 plate to 50 xg for 1 minute at 22°C.

[ ] **4.** Place the AMP3 plate on the robot bed according to the robot bed map. Remove the plate seal.

[ ] **5.** Place a half reservoir in the reservoir frame, according to the robot bed map, and add PA1 as follows:

- 16 samples: 1 tube
- 32 samples: 2 tubes
- 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:

- 16 samples: 20 ml
- 32 samples: 30 ml
- 48 samples: 40 ml

Start the Robot

[ ] **7.** When prompted, remove the AMP3 plate from the robot bed. Do ***not*** click **OK** in the message box yet.

[ ] **8.** Seal the AMP3 plate with the same cap mat removed earlier.

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

[ ] **10.** Incubate at 37°C for 5 minutes.

[ ] **11.** Centrifuge to 50 xg at room temperature for 1 minute.

[ ] **12.** Return the AMP3 plate to the robot bed according to the robot bed map. Remove the plate seal and discard it.

[ ] **13.** At the robot PC, click **OK** to restart the run.

[ ] **14.** Click **OK** in the message box. Remove the AMP3 plate and seal with a ***new, dry*** cap mat.

[ ] **15.** Invert each plate at least 10 times to mix contents thoroughly.

[ ] **16.** Incubate for 30 minutes at 4°C.

[ ] **17.** Place the sealed AMP3 plate in the centrifuge opposite another plate of equal weight.

[ ] **18.** Centrifuge to 3000 xg for 20 minutes at 4°C.

[ ] **19.** Immediately remove the AMP3 plate from the centrifuge.

Perform the next step immediately to avoid dislodging the blue pellet.

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

[ ] **21.** Decant supernatant by quickly inverting the AMP3 plate and smacking it down onto an absorbent pad.

[ ] **22.** Tap the plate firmly on the pad several times over a period of 1 minute or until all wells are completely devoid of liquid.

[ ] **23.** Place the inverted, uncovered plate on a tube rack for 1 hour at 22°C to air dry the pellet.

[ ] **24.** Do one of the following:

- Proceed immediately to *Resuspend AMP3*.
- Heat-seal the AMP3 plate and store it at -15 to -25°C for the following day or -80°C for long-term storage.

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Resuspend AMP3

Resuspend the precipitated DNA using RA1.

Estimated Time

Robot time: 15 minutes per 48 samples
Incubation time: 1 hour

Consumables

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

Preparation

- [ ] Gradually warm the RA1 reagent to room temperature. Gently mix to dissolve any crystals.
- [ ] If you stored the AMP3 plate at -15 to -25°C, thaw it to room temperature.
- [ ] Preheat the Illumina Hybridization Oven to 48°C.
- [ ] Preheat the heat sealer.

Steps

Set Up the Robot

[ ] **1.** At the robot PC, select **AMP3 Tasks | Resuspend AMP3**.

[ ] **2.** (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP3 plates** (1 to 4).

The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed. All barcodes must face to the right.

[ ] **3.** Place the AMP3 plate on the robot bed according to the robot bed map. Remove the plate seal.

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

- 16 samples: 4 ml
- 32 samples: 7 ml
- 48 samples: 9 ml

Start the Robot

[ ] **5.** When prompted, remove the AMP3 plate from the robot bed and click **OK**.

[ ] **6.** Apply a foil heat seal to the AMP3 plate by firmly holding the heat sealer sealing block down for 3 seconds.

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

[ ] **8.** Vortex the sealed plate at 1800 rpm for 1 minute.

[ ] **9.** Pulse centrifuge to 280 xg.

[ ] **10.** Do one of the following:

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- Proceed to *Hyb Duo BeadChip*. If you do so immediately, it is safe to leave the RA1 at room temperature.
- Store the sealed AMP3 plate and the RA1 at -15 to -25°C (-80°C if storing for more than 24 hours).

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Hyb Duo BeadChip

Dispense the fragmented, resuspended DNA samples onto BeadChips. Each BeadChip can hold two samples. Incubate the BeadChips in the Illumina Hybridization Oven to hybridize the samples onto the BeadChips.

Estimated Time

Robot time:

- 10 minutes per 8 BeadChips (16 samples)
- 30 minutes per 16 BeadChips (32 samples)
- 60 minutes per 24 BeadChips (48 samples)

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 16 Samples)	Storage	Supplied By
PB2	2 tubes	Room temperature	Illumina
BeadChips	8		Illumina
Hyb Chambers	2		Illumina
Hyb Chamber gaskets	2		Illumina
Hyb Chamber inserts	8		Illumina
Robot BeadChip Alignment Fixture	4		Illumina
Robot Tip Alignment Guide	4		Illumina
1% aqueous Alconox	As needed		User

Preparation

[ ] Preheat the heat block to 95°C.

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

[ ] If you plan to perform the XStain process tomorrow, begin thawing the XC4 reagent. For instructions, see *Resuspend XC4 Reagent for XStain HD BeadChip*.

Steps

Prepare Robot Tip Alignment Guides

[ ] **1.** Wash and dry the Robot Tip Alignment Guides prior to each use. See *Wash Robot Tip Alignment Guides* at the end of the Hyb Duo BeadChip steps (page 18) for washing instructions.

[ ] **2.** Make sure you have the correct Robot Tip Alignment Guide for the Gemini assay. The guide barcode should say Guide-A on it.

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Prepare Hyb Chambers

[ ] **1.** Place the Hyb Chamber gaskets into the Hyb Chambers.

[ ] **2.** Dispense 400 µl PB2 to each of the 8 humidifying buffer reservoirs in each Hyb Chamber.

[ ] **3.** Place the Hyb Chamber inserts into the Hyb Chambers.

[ ] **4.** Secure the lid of each Hyb Chamber. Keep on bench at room temperature until ready to load BeadChips.

[ ] **5.** Remove the BeadChips from 2 to 8°C storage but do not unpackage.

Load the BeadChips

[ ] **1.** Place the resuspended AMP3 plate on the heat block at 95°C for 20 minutes.

[ ] **2.** Pulse centrifuge the AMP3 plate to 280 xg for 1 minute.

[ ] **3.** Remove all BeadChips from their packages.

[ ] **4.** Place two BeadChips into each Robot BeadChip Alignment Fixture so that the barcode lines up with the ridges on the fixture.

[ ] **5.** Stack the Robot BeadChip Alignment Fixtures and carry them to the robot.

Set Up the Robot

[ ] **1.** At the robot PC, select **AMP3 Tasks | Hyb Duo BeadChip**.

[ ] **2.** (Non-LIMS only) In the Basic Run Parameters pane, enter the **Number of BeadChips**.

You can only dispense samples from one AMP3 plate at a time.

[ ] **3.** Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map.

[ ] **4.** Place the AMP3 plate onto the robot bed according to the bed map and remove the foil seal.

[ ] **5.** Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

[ ] **1.** At the robot PC, click **Run**.

[ ] **2.** (Infinium LIMS only) The robot scans the barcodes on the BeadChips to confirm the correct BeadChips are loaded. Once the correct BeadChips are confirmed, the Robot pauses.

[ ] **3.** Place a Robot Tip Alignment Guide on top of each Robot BeadChip Alignment Fixture. The Guide-A barcode should be upside down and facing away from you when properly placed on the Robot BeadChip Alignment Fixture.

[ ] **4.** At the robot PC, click **OK** to confirm you have placed a Robot Tip Alignment Guide on top of each Robot BeadChip Alignment Fixture.

[ ] **5.** (Infinium LIMS only) The Robot scans the barcode on the Robot Tip Alignment Guide to confirm the correct guide is being used.

[ ] **6.** The robot dispenses sample to the BeadChips.

[ ] **7.** Carefully remove each Robot Tip Alignment Guide from the robot bed and set it to the side. Next, 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.

[ ] **8.** If you are running 12 BeadChips or fewer, discard the AMP3 plate and proceed to *Set Up Duo BeadChip for Hyb*.

[ ] **9.** If you are running 13 BeadChips or more, the Infinium Robot Control application prompts you to place a new set of BeadChips on the robot deck. Before you place the next set of samples on the Robot bed, immediately assemble the Hyb Chambers for the first set of 12 BeadChips as described in the *Set Up Duo BeadChip for Hyb* steps. Leave the assembled Hyb Chambers of the first 12 BeadChips on the lab bench in a horizontal position.

[ ] **10.** Replace the Robot Tip Alignment Guides used for the first set of BeadChips with a new set of Robot Tip Alignment Guides that have been washed and dried.

[ ] **11.** After you have completed the *Set Up Duo BeadChip for Hyb* steps for the first set of 12 BeadChips, place the new set of BeadChips on the robot bed according to the bed map and click **OK.**

[ ] **12.** When prompted, place a new set of Robot Tip Alignment Guides that have been washed and dried on top of each Robot BeadChip Alignment Fixture and click **OK** to start the next run.

Set Up Duo BeadChip for Hyb

[ ] **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. Place each BeadChip in a Hyb Chamber insert, orienting the barcode end so that it matches the barcode symbol on the insert.

[ ] **3.** Place the back side of the lid onto the Hyb Chamber and then slowly bring down the front end. Close the clamps on both sides.

[ ] **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 sides of the oven.

[ ] **5.** If you are loading multiple Hyb Chambers, stack them on top of each other, up to 3 per stack, for a total of 6 in the Hyb Oven.

[ ] **6.** (Optional) Set the rocker speed to 5 and start the rocker.

[ ] **7.** Incubate the Hyb Chamber(s) in the Illumina Hybridization Oven for 16–24 hours at 48°C.

[ ] **8.** Proceed to *Wash BeadChip*.

Resuspend XC4 Reagent for XStain HD BeadChip

[ ] **1.** Add 330 ml 100% EtOH to the XC4 bottle.

[ ] **2.** Shake vigorously for 15 seconds.

[ ] **3.** Leave the bottle upright on the lab bench overnight.

[ ] **4.** Shake again to ensure that the pellet is completely resuspended. If any coating is visible, vortex at 1625 rpm until it is in complete suspension.

Wash Robot Tip Alignment Guides

[ ] **1.** Soak the Robot Tip Alignment Guides in a 1% aqueous Alconox solution (one part Alconox to 99 parts water) using a 400 ml Pyrex beaker for 5 minutes.

[ ] **2.** After the 5 minute soak in the 1% Alconox solution, thoroughly rinse the Robot Tip Alignment Guides with DiH_2O at least three times to remove any residual detergent. Make sure the DiH_2O runs through all the tip guide channels.

[ ] **3.** Dry the Robot Tip Alignment Guides, especially the channels, using a Kimwipe or lint-free paper towels. Use a laboratory air gun to ensure they are dry. Be sure to inspect the channels, including the top and bottom of the insert. Robot Tip Alignment Guides should be completely dry and free of any residual contaminates before next use.

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Wash BeadChip

Prepare the BeadChips for the staining process.

Estimated Time

Hands-on time:

- 20 minutes per 8 BeadChips (16 samples)
- 30 minutes per 16 BeadChips (32 samples)
- 50 minutes per 24 BeadChips (48 samples)

Consumables

Item	Quantity (per 8 BeadChips)	Storage	Supplied By
PB1	550 ml	Room temperature	Illumina
Multi-Sample BeadChip Alignment Fixture	1		Illumina
Te-Flow Flow-Through Chambers (with Black Frames, Spacers, Glass Back Plates, and Clamps)	1 per BeadChip		Illumina
Wash Dish	8 BeadChips: 2 dishes 24 BeadChips: 6 dishes		Illumina
Wash Rack	8 BeadChips: 1 rack 24 BeadChips: 3 racks		Illumina

Preparation

[ ] Fill 2 wash dishes with PB1 (200 ml per wash dish). Label each dish "PB1".

[ ] Fill the BeadChip Alignment Fixture with 150 ml PB1.

[ ] Separate the clear plastic spacers from the white backs.

[ ] Clean the glass back plates according to the directions in the *Infinium Assay Lab Setup and Procedures Guide*.

Steps

Wash BeadChip

[ ] **1.** Remove each Hyb Chamber from the Illumina Hybridization Oven.

[ ] **2.** Attach the wire handle to the rack and submerge the wash rack in the first wash dish containing 200 ml PB1.

[ ] **3.** Remove the Hyb Chamber inserts from the Hyb Chambers.

[ ] **4.** Remove BeadChips from the Hyb Chamber inserts one at a time.

[ ] **5.** Remove the IntelliHyb seal from each BeadChip as follows:

[ ] **a.** Wearing powder-free gloves, hold the BeadChip in one hand with your thumb and forefinger on the long edges of the BeadChip. The BeadChip may also be held with the thumb and forefinger on the short edges of the BeadChip. In either case 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, rapid motion by pulling it off in a diagonal direction. 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 stop and start the pulling action. Do not touch the exposed active areas.

[ ] **6.** 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.

[ ] **7.** Repeat steps 5 and 6 until all BeadChips are transferred to the submerged wash rack. The wash rack holds up to 8 BeadChips.

[ ] **8.** 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.

[ ] **9.** Move the wash rack to the other wash dish containing PB1. Make sure the BeadChips are completely submerged.

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

[ ] **11.** If you are processing more than 8 BeadChips:

[ ] **a.** Complete the steps in the next section, *Assemble Flow-Through Chambers*, for the first eight BeadChips.

[ ] **b.** Place the assembled Flow-Through Chambers of the first eight BeadChips on the lab bench in a horizontal position.

[ ] **c.** Repeat steps 3 through 11 from this section for any additional BeadChips. Use new PB1 for each set of eight BeadChips.

Assemble Flow-Through Chambers

[ ] **1.** If you have not done so yet, fill the BeadChip Alignment Fixture with 150 ml PB1.

[ ] **2.** For each BeadChip to be processed, place a black frame into the Multi-Sample BeadChip Alignment Fixture.

[ ] **3.** Place each BeadChip to be processed into a black frame, aligning its barcode with the ridges stamped onto the Alignment Fixture. Each BeadChip should be fully immersed in PB1.

[ ] **4.** Place a clear spacer onto the top of each BeadChip. Use the Alignment Fixture grooves to guide the spacers into proper position.

[ ] **5.** Place the Alignment Bar onto the Alignment Fixture.

[ ] **6.** Use a laboratory air gun to quickly remove any accumulated dust from the glass back plates just before placing them onto the BeadChips.

[ ] 7. Place a clean 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.

[ ] 8. 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 about 5 millimeters from the top edge.
- **[ ] c.** Place the second metal clamp around the Flow-Through Chamber at the barcode end, about 5 millimeters from the reagent reservoir.

[ ] 9. 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.

[ ] 10. Immediately wash the Hyb Chamber reservoirs with dH_2O and scrub them with a small cleaning brush, ensuring that no PB2 remains.

[ ] 11. Proceed to *XStain HD BeadChip*.

Experienced User Card

XStain HD BeadChip

Wash unhybridized and non-specifically hybridized DNA sample from the BeadChips. Add labelled nucleotides to extend the primers hybridized to the DNA. Stain the primers, disassemble the Flow-Through Chambers, and coat the BeadChips for protection.

You can process up to 24 BeadChips at a time during the XStain HD BeadChip step.

Estimated Time

Robot time:

- 2 hours and 30 minutes per 8 BeadChips (16 samples)
- 2 hours and 40 minutes per 16 BeadChips (32 samples)
- 2 hours and 50 minutes per 24 BeadChips (48 samples)

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
XC1	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
XC2	2 tubes (per 8 BeadChips)	-15 to -25°C	Illumina
TEM	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 24 BeadChips	Room temperature	Illumina
STM (Make sure that all STM 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	-15 to -25°C	Illumina

Experienced User Card

Item	Quantity	Storage	Supplied By
Alconox Powder Detergent	as needed		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

- [ ] RA1 is shipped frozen. Gradually warm the RA1 reagent to room temperature. Gently mix to dissolve any crystals that may be present.
- [ ] Place all reagent tubes to be used in the assay in a tube rack; if frozen, thaw to room temperature and centrifuge to 3000 xg for 3 minutes.
- [ ] Ensure the water circulator is filled to the appropriate level.
- [ ] Remove bubbles trapped in the Chamber Rack.
- [ ] Turn on the water circulator. Set it to a temperature that brings the Chamber Rack to 44°C at equilibrium. Test several locations on the Chamber Rack with the Illumina Temperature Probe to ensure that it is uniformly 44°C.

For instructions on preparing the robot for use in a protocol and ensuring that the Chamber Rack is properly installed on the post-amplification robot bed, see the *Infinium Assay Lab Setup and Procedures Guide*.

Steps

Single-Base Extension and Stain (XStain)

CAUTION The remaining steps must be performed without interruption.

- [ ] **1.** Slide the Chamber Rack into column 28 on the robot bed.
- [ ] **2.** At the robot PC, select **XStain Tasks | Infinium II Chemistry | XStain HD BeadChip**.
- [ ] **3.** (Non–LIMS only) In the Basic Run Parameters pane, enter the **Number of BeadChips** (up to 24).

  The robot PC updates the Required Run Item(s) and the bed map to show the correct position of items on the robot bed.
- [ ] **4.** Place a quarter reservoir in the reservoir frame according to the robot bed map, and add 95% formamide/1 mM EDTA as follows:
  - 8 BeadChips: 15 ml
  - 16 BeadChips: 20 ml
  - 24 BeadChips: 25 ml

[ ] **5.** Place a half reservoir in the reservoir frame according to the robot bed map, and add RA1 as follows:
- 8 BeadChips: 10 ml
- 16 BeadChips: 20 ml
- 24 BeadChips: 30 ml

[ ] **6.** Place a full reservoir in the reservoir frame according to the robot bed map, and add XC3 as follows:
- 8 BeadChips: 50 ml
- 16 BeadChips: 100 ml
- 24 BeadChips: 150 ml

[ ] **7.** Place the XC1, XC2, TEM, STM, and ATM tubes in the robot tube rack according to the robot bed map. Remove the caps.

[ ] **8.** Make sure that all items are placed properly on the robot bed, that all caps and seals have been removed, and that all the barcodes face to the right.

Start the Robot

[ ] **1.** At the robot PC:
- [ ] **a.** If you are not running Infinium LIMS, clear the **Use Barcodes** check box.
- [ ] **b.** Click **Run** to start the process.
- [ ] **c.** When prompted , enter the stain temperature. The correct temperature is indicated on the STM tube. If no temperature is listed, enter 37°C.

[ ] **2.** When prompted, wait for the Chamber Rack to reach 44°C. Do not load the BeadChips or click **OK** yet.

[ ] **3.** When the temperature probe registers 44°C, click **OK**.

[ ] **4.** Quickly place each Flow-Through Chamber into the Chamber Rack according to the robot bed map.

[ ] **5.** At the robot PC, click **OK**.

[ ] **6.** When prompted, immediately remove the Flow-Through Chambers from the Chamber Rack. Place them horizontally on the lab bench at room temperature.

[ ] **7.** Click **OK** to finish the process.

Wash and Coat

[ ] **1.** Dispense PB1 into a wash dish as follows, and then cover the dish:
- For 8 Beadchips, dispense 310 ml
- For 16 Beadchips, dispense 300 ml
- For 24 BeadChips, dispense 285 ml

[ ] **2.** Place the staining rack inside the wash dish. The locking arms and tab should face ***towards*** you.

CAUTION Handle the BeadChips only by the edges or the barcode end. Do not let the BeadChips dry out.

Experienced User Card

[ ] 3. For each BeadChip:

- Use the dismantling tool to remove the two metal clamps from the Flow-Through Chamber.
- Remove the glass back plate, the spacer, and then the BeadChip.
- Immediately place each BeadChip into the staining rack that is in the PB1 wash dish, with the barcode facing ***away*** from you. Place half of the BeadChips above the handle and half below. All chips should be completely submerged.

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

[ ] 5. Soak for 5 minutes.

CAUTION	Do not leave the BeadChips in the PB1 for more than 30 minutes.

[ ] 6. Dispense XC4 into a wash dish as follows:

- For 8 Beadchips, dispense 310 ml
- For 16 Beadchips, dispense 300 ml
- For 24 BeadChips, dispense 285 ml

Do not let it sit for more than 10 minutes.

[ ] 7. Move the BeadChip staining rack into the XC4 dish. The barcodes should face **away** from you and the locking arms **towards** you.

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

[ ] 9. Soak for 5 minutes.

[ ] 10. Lift the staining rack out of the solution and place it horizontally on a tube rack, with the BeadChip barcodes facing ***up***.

[ ] 11. 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 staining rack handle before removing the BeadChips below it.

[ ] 12. Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 508 mm Hg (0.68 bar). Each desiccator can hold 8 BeadChips.

[ ] 13. Clean the underside of the BeadChip with a ProStat EtOH wipe.

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

- Proceed to *Image BeadChip on iScan System* or *Image BeadChip on BeadArray Reader*.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box inside a vacuum desiccator at room temperature. Image the BeadChips within 72 hours.

Experienced User Card

Image BeadChip on iScan System

The iScan Reader uses a laser to excite the fluor of the single-base extension product on the beads of the BeadChip sections. Light emissions from these fluors are then recorded in high-resolution images of the BeadChip sections. Data from these images are analyzed using Illumina's GenomeStudio Genotyping Module.

Estimated Time

Scanning: 35 minutes per BeadChip

Preparation

[ ] On the lab tracking form, record the following for each BeadChip:

- Scanner ID
- Scan date

Steps

[ ] **1.** Turn on the iScan Reader, boot up the iScan PC, and start the GenomeScan application.

Starting Up the iScan System

[ ] **1.** Turn on the iScan Reader and the attached PC.

[ ] **2.** Let the iScan Reader warm up for at least 5 minutes.

[ ] **3.** For each BeadChip, copy the mini-CD provided with the BeadChip into the Decode folder. The folder name should be the BeadChip barcode (for example, 4264011131).

[ ] **4.** Double-click the GenomeScan icon  on the desktop.

[ ] **5.** Set the **LIMS** dropdown list to **None** and enter your Windows user name.

[ ] **6.** Click **Start**.

Loading BeadChips and Starting the Scan

[ ] **1.** Load the BeadChips into their carrier and place the carrier into the iScan Reader tray. Click **Next**.

[ ] **2.** Click any BeadChip section to remove it from the scan. The section will no longer be highlighted blue.

[ ] **3.** If you want to remove an entire BeadChip from the scan, delete the barcode from the Setup window.

[ ] **4.** To begin scanning the BeadChips, click **Scan**.

[ ] **5.** At the end of the scan, a Review window appears. If any stripes fail to scan successfully, click **Rescan** to automatically rescan all failed areas.

[ ] **6.** When you finish reviewing the data, click **Done** to return to the Start window.

Experienced User Card

Image BeadChip on BeadArray Reader

The Illumina BeadArray Reader uses a laser to excite the fluor of the hybridized single-stranded product on the beads of the BeadChip sections. Light emissions from these fluors are then recorded in high-resolution images of the BeadChip sections. Data from these images are analyzed using Illumina's GenomeStudio Genotyping Module.

Estimated Time

1–2 hours warmup for the BeadArray Reader (first use of the day only)

45 minutes to scan each BeadChip using BeadScan 3.2 FastScan settings

Preparation

[ ] If this is the first time the BeadArray Reader is being used today, follow the steps described in this section.

[ ] On the lab tracking form, record the following for each BeadChip:

- Scanner ID
- Scan date

Initializing the BeadArray Reader (Daily)

If this is the first time the scanner is being used today, follow these steps.

[ ] **1.** Locate the power switch on the lower-left side of the BeadArray Reader back panel and turn it to the ON position.

[ ] **2.** Wait for the ready indicator to stop flashing.

[ ] **3.** Open the **BeadScan** software.

[ ] **4.** Log in and click **Scan**.

Imaging BeadChip

When the BeadArray Reader is initialized, follow these steps to perform the scanning process.

[ ] **1.** From the **Docking Fixture** listbox, select BeadChip.

[ ] **2.** Check the Data Repository path and the Decode Map path in the Settings area.

[ ] **3.** Copy the decode map (*.dmap) files for each BeadChip from the BeadChip CD to the Decode Map path directory.

[ ] **4.** For each BeadChip:

- [ ] **a.** Place the BeadChip into the BeadArray Reader tray.
- [ ] **b.** If either the **Sentrix Type** or **Scan Settings** are not correct, click **Browse** (...) to open the Select Scan Settings dialog box.
- [ ] **c.** Select the appropriate scan method and click **Select**.

[ ] **5.** Make sure that the BeadChips are properly seated in the BeadArray Reader tray.

[ ] **6.** Click **Scan**.

Scanning Process

BeadScan begins the BeadArray Reader Tilt and Align processes.

Once the Tilt and Align processes are complete, the Scan process begins. Hover over any of the green dots in the closeup image to see the relative intensity and the XY position. The red value should be at or close to zero, because this is a one-color assay.

Experienced User Card

As the BeadArray Reader scans, the front panel blue Scanning indicator lights flash in sequence.

When the BeadArray Reader finishes scanning, a green message screen appears if the scan was successful, or a red message if it completed with any warnings.

If Scan is Successful

[ ] **1.** Click **OK** on the Scan Completed message to view the next screen.

[ ] **2.** Click **Done** in the Review pane.

[ ] **3.** When the application returns to the Welcome screen, click **Open Tray**. The BeadArray Reader tray, loaded with the scanned BeadChips, will eject.

[ ] **4.** Remove the BeadChips from the tray.

[ ] **5.** Do one of the following:

[ ] If you have more BeadChips to scan, repeat the scanning process.

[ ] If this is the last use of the day:

- [ ] **a.** Wipe the BeadArray Reader tray with a lint-free, absorbent towel. Pay particular attention to the tray edges where reagent may have wicked out.
- [ ] **b.** Close the tray.
- [ ] **c.** Turn the power switch at the back of the scanner to the **OFF** position.
- [ ] **d.** Shut down the BeadArray Reader BeadScan software. To exit, right-click near the Illumina logo and click **Exit**.

If Scan is not Successful

Re-scan the array. For more information, refer to the Illumina BeadArray Reader *User Guide*.

If the scanner was unable to locate the alignment fiducials (focus points), you may need to clean the edges of the BeadChip before re-scanning.