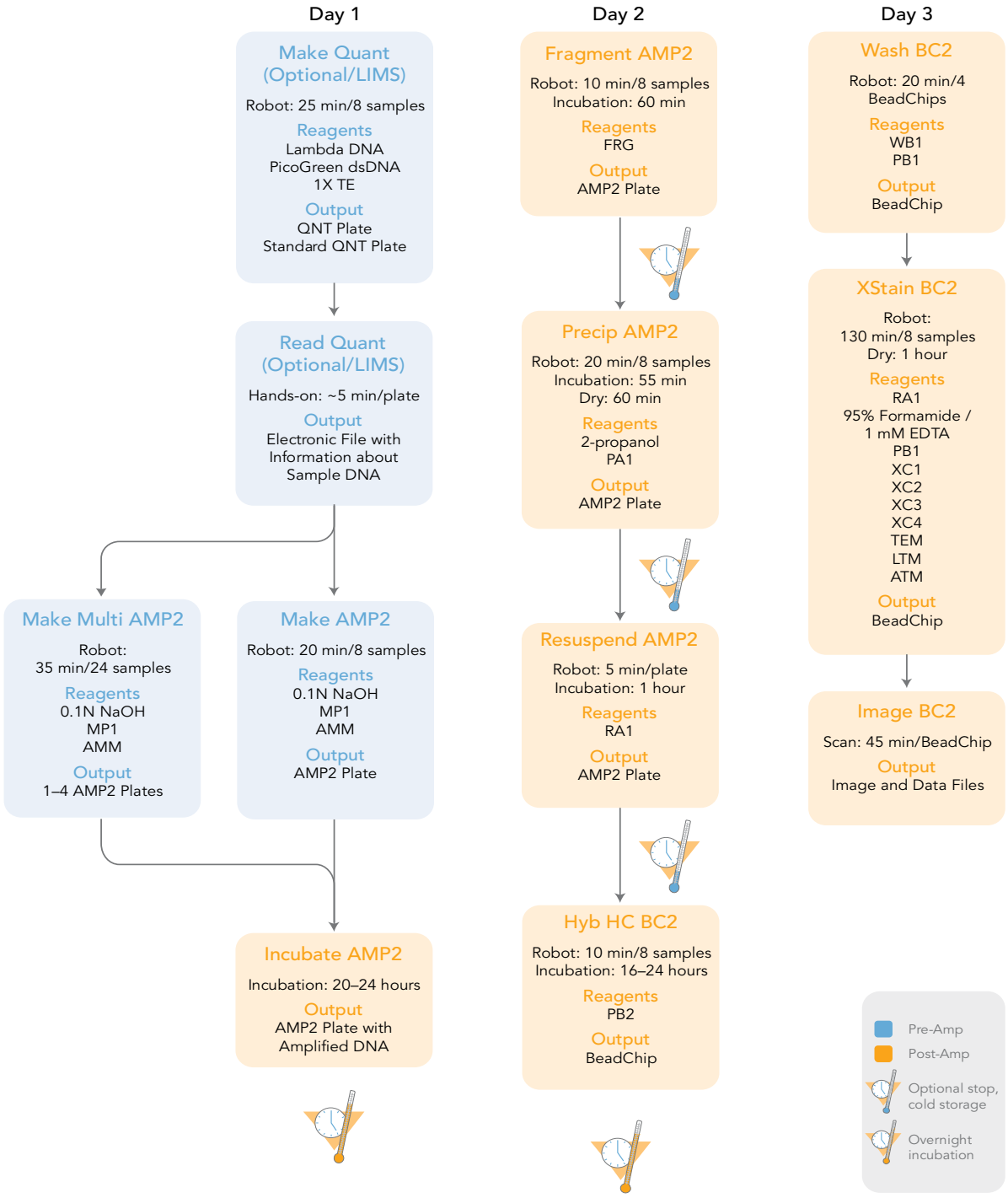
Infinium® II Assay HC Two-Sample, Automated
Experienced User Card



Make Quant (Optional/LIMS)

Quantify the DNA samples for the assay. If you already have a DNA master plate, skip this step and begin with Make AMP2.

Estimated Time

Robot time:

- 20 minutes per 8 samples (1 plate)
- 40 minutes for 16 samples (2 plates)
- 60 minutes for 24 samples (3 plates)

Consumables

Item	Quantity	Storage	Supplied By
WG#-DNA plate with 96 DNA samples, normalized to 50 ng/µl	1 to 3 plates	-20°C	User
1X TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE))		Room temperature	User
PicoGreen dsDNA			User
Black Fluotrac plate	1 plate for each WG#-DNA plate 1 plate for each Standard DNA plate	-20°C	Illumina
Standard DNA plate	1 plate	-20°C	Illumina

Preparation

[ ] For each WG#-DNA plate, place a QNT barcode label on a new black Fluotrac plate.

[ ] Label the remaining black Fluotrac plate "Standard QNT."

[ ] Remove the WG#-DNA plate(s) from the refrigerator or freezer. If frozen, thaw completely.

Steps

Set up the Robot

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

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

[ ] **3.** (Non-Infinium LIMS only) In the Basic Run Parameters pane, set the value for the **Number of DNA/QNT plates** (1, 2, or 3) and the **Number of DNA samples** (8, 16, 24, 32, 48, or 96).

NOTE

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

[ ] 4. Vortex each WG#-DNA plate:
- For MIDI plate(s): 1450 rpm (actual vortex speed) for 1 minute
- For TCY plate(s): 1250 rpm for 1 minute

[ ] 5. Centrifuge each WG#-DNA 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 plates on the robot bed according to the robot bed map. Remove any plate seals.

Prepare PicoGreen Dilution

[ ] 1. Wrap aluminum foil around a sterile plastic container to prevent light penetration.

[ ] 2. Make a 1:200 dilution of PicoGreen to 1X TE in the foil-wrapped plastic container. Mix thoroughly.

[ ] 3. Pour the mixture into a half reservoir and place it on the robot bed.

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.** Log in if prompted.

[ ] 2. After the robot finishes, immediately:
- **[ ] a.** Place foil adhesive seals over QNT and Standard QNT plates.
- **[ ] b.** Place cap mats on WG#-DNA and Standard DNA plates.

[ ] 3. Place the WG#-DNA and Standard DNA plates in the refrigerator (4°C) or freezer (-20°C).

[ ] 4. Centrifuge the QNT and Standard QNT plates to 280 xg for 1 minute.

[ ] 5. Proceed to *Read Quant*.

Read Quant

Using a fluorometer and the Infinium Fluorometry Analysis software, interpret the quantified DNA and obtain the exact concentration of DNA in the sample.

Estimated Time

Robot time: 5 minutes per plate

Steps

[ ] **1.** Turn on the fluorometer.

[ ] **2.** Open the Infinium Fluorometry Analysis software.

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

[ ] **4.** Select the **Use Barcodes** check box.

[ ] **5.** Click **Read**.

[ ] **6.** If prompted, enter your Infinium LIMS user name and password. Click **Login**.

[ ] **7.** When asked if you want to read a new Standard QNT plate, click **Yes**. Remove the plate seal and load the Standard QNT plate in the open fluorometer tray. Click **OK**. The fluorometer reads the plate data.

[ ] **8.** Review the data from the Standard QNT plate. Either accept it and go on to the next step, or reject it and read another plate.

[ ] **9.** Remove the Standard QNT plate from the fluorometer tray.

[ ] **10.** When prompted, enter the number of QNT plates that you want to read. You can read up to 3 plates. Do not include the Standard QNT plate.

[ ] **11.** Scan the first QNT barcode, load the plate in the tray, and click **OK**.

The Fluorometry Analysis screen fills in with information about the fluorescence in the wells.

Microsoft Excel opens automatically at the same time and displays the quant data for the QNT plate.

The Infinium Fluorometer Analysis software prompts you to indicate whether you wish to use the QNT data shown in the Excel file.

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

- Click **Yes** to send the data to Infinium LIMS. In Infinium LIMS, the QNT plate moves into the Make AMP2 queue.
- Click **No** to delete the quant data. You can read the quant data again for the same plate.

[ ] **13.** Proceed to *Make AMP2*.

Make AMP2

Move DNA samples into the AMP2 plate. Denature and neutralize samples, and prepare them for amplification. Incubate overnight to amplify.

Estimated Time

Robot time:

- 20 minutes per 8 samples
- 70 minutes for 48 samples

Incubation time: ~20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
0.1N NaOH	15 ml per 8–24 samples	4°C	User
WG#-DNA plate with 96 DNA samples (50 ng/µl)	1 plate	-20°C	User
MP1	1 tube per 8 samples	-20°C	Illumina
AMM	1 tube per 8 samples	-20°C	Illumina
96-well 0.8 ml microtiter plate (MIDI)	1 plate for up to 24 samples		User

Preparation

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

[ ] Thaw MP1 and AMM tubes to room temperature (22°C). Gently invert to mix, then pulse centrifuge to 280 xg.

[ ] Thaw DNA samples to room temperature (22°C).

[ ] Apply an AMP2 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 **AMP2 Sample Prep Tasks | Make AMP2**.

Alternative: Select **AMP2 Sample Prep Tasks | Make Multi AMP2** to run multiple AMP2 plates.

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

[ ] **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** (8, 16, or 24) 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 AMP2 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**.

[ ] **13.** Click **OK** in the message box. Remove the AMP2 plate from the robot bed and seal with the 96-well cap mat.

[ ] **14.** Invert the sealed AMP2 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 the AMP2 Plate*.

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.

Fragment the AMP2 Plate

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

Estimated Time

Robot time:

- 10 minutes for 8 samples
- 50 minutes for 48 samples
- 100 minutes for 96 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
FRG	1 tube per 8 samples	-20°C	Illumina

Preparation

[ ] Preheat the heat block with the MIDI plate insert to 37°C.

[ ] Thaw the FRG tube to room temperature (22°C). Invert several times to mix contents. Pulse centrifuge to 280 xg for 1 minute.

Steps

Set Up the Robot

[ ] **1.** Centrifuge the AMP2 plate to 50 xg for 1 minute.

[ ] **2.** At the robot PC, select **AMP2 Sample Prep Tasks | Fragment AMP2**.

[ ] **3.** (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP2 Plates** (1 to 4).

[ ] **4.** Place the AMP2 plate on the robot bed according to the robot bed map. Remove any plate seals.

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

[ ] **6.** 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.** Log in if prompted.

[ ] **2.** Click **OK** in the message box. Remove the AMP2 plate from the robot bed and seal with the 96-well cap mat.

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

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

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

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

- Proceed to *Precip AMP2*. Leave plate in the 37°C heat block until preparation is complete.
- Store the sealed AMP2 plate at -20°C.

Precip AMP2

Precipitate the DNA sample using PA1 and 2-propanol.

Estimated Time

Robot time:

- 20 minutes for 8 samples
- 70 minutes for 48 samples
- 140 minutes for 96 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
PA1	1 tube per 8 samples	4°C	Illumina
100% 2-propanol	1 bottle	Room temperature	User

Preparation

[ ] If you froze the AMP2 plate after fragmentation, thaw it to room temperature (22°C). Centrifuge to 280 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 (22°C). Centrifuge to 280 xg for 1 minute.

[ ] Preheat the heat sealer.

Steps

Set Up the Robot

[ ] **1.** At the robot PC, select **AMP2 Sample Prep Tasks | Precip AMP2**.

[ ] **2.** (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP2 plates** (1 to 4).

[ ] **3.** Centrifuge the sealed AMP2 plate to 50 xg for 1 minute at 22°C.

[ ] **4.** Place the AMP2 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:

- 8 samples: 1 tube
- 48 samples: 6 tubes
- 96 samples: 12 tubes

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

- 8 samples: 12 ml
- 48 samples: 74 ml
- 96 samples: 142 ml

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.** Log in if prompted.

[ ] **2.** When prompted, remove the AMP2 plate from the robot bed. Do ***not*** click **OK** in the message box yet.

[ ] **3.** Seal the AMP2 plate with the same cap mat removed earlier.

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

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

[ ] **6.** Centrifuge to 50 xg at room temperature (22°C) for 1 minute.

[ ] **7.** Return the AMP2 plate to the robot bed according to the robot bed map. Remove the plate seal.

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

[ ] **9.** Click **OK** in the message box. Remove the AMP2 plate and seal with a ***new, dry*** cap mat.

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

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

[ ] **12.** Place the sealed AMP2 plate in the centrifuge opposite another plate of equal weight.

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

[ ] **14.** Immediately remove the AMP2 plate from the centrifuge.

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

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

[ ] **16.** Decant supernatant by quickly inverting the AMP2 plate and smacking it down onto an absorbent pad.

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

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

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

- Proceed immediately to *Resuspend AMP2*.
- Heat-seal the AMP2 plate and store it at -20°C for the following day or -80°C for long-term storage.

Resuspend AMP2

Resuspend the precipitated DNA using RA1.

Estimated Time

Robot time: 5 minutes per plate
Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	Bottle (4 ml)	-20°C	Illumina

Preparation

[ ] Gradually warm the RA1 reagent to room temperature in a 20–25°C water bath. Gently mix to dissolve any crystals.

[ ] If you stored the AMP2 plate at -20°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 **AMP2 Sample Prep Tasks | Resuspend AMP2**.

[ ] **2.** (Non-Infinium LIMS only) In the Basic Run Parameters pane, enter the **Number of DNA samples** and the **Number of AMP2 plates** (1 to 4).

[ ] **3.** Place the AMP2 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:

- 4 ml for 8 samples
- 8 ml for 16 samples
- 12 ml for 24 samples

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.** Log in if prompted.

[ ] 2. When prompted, remove the AMP2 plate from the robot bed and click **OK**.

[ ] 3. Apply a foil heat seal to the AMP2 plate by firmly holding the heat sealer sealing block down for 3 seconds.

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

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

[ ] 6. Pulse centrifuge to 280 xg.

If you stored the DNA pellets at -20°C for more than 72 hours after Precip AMP2, you may need to repeat the vortexing and centrifugation until the pellets are completely resuspended.

[ ] 7. Do one of the following:

- Proceed to *Hyb HC BC2*. If you do so immediately, it is safe to leave the RA1 at room temperature.
- Store the sealed AMP2 plate and the RA1 at -20°C (-80°C if storing for more than 24 hours).

Hyb HC BC2

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:

- 8 samples: 10 minutes
- 48 samples: 30 minutes
- 96 samples: 60 minutes

Incubation time: 16–24 hours

Consumables

Item	Quantity (per 8 Samples)	Storage	Supplied By
PB2	1 tube	Room temperature	Illumina
BeadChips	4		Illumina
Hyb Chambers	1		Illumina
Hyb Chamber gaskets	1		Illumina
Hyb Chamber inserts	4		Illumina
Robot BeadChip Alignment Fixture	2		Illumina

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 BC2*.

Prepare Hyb Chambers

NOTE

Perform the Hyb Chamber assembly near the Hyb Oven to minimize the distance you need to move the BeadChip-loaded Hyb Chamber. Take care to keep the Hyb Chamber steady and level when lifting and moving. Avoid shaking and keep the Hyb Chamber parallel to the lab bench at all times.

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

[ ] **2.** Dispense 200 µl PB2 to each of the 8 humidifying buffer reservoirs in each Hyb Chamber.

[ ] **3.** Secure the lid of each Hyb Chamber. Keep on bench at room temperature (22°C) until ready to load BeadChips.

[ ] **4.** Remove the BeadChips from 4°C storage but do not unpackage.

Steps

Load the BeadChips

[ ] **1.** Place the resuspended AMP2 plate on the heat block at 95°C for 20 minutes.

[ ] **2.** Vortex the AMP2 plate at 1800 rpm.

[ ] **3.** Pulse centrifuge the AMP2 plate to 280 xg for 1 minute.

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

[ ] **5.** Slide each BeadChip into the Robot BeadChip Alignment Fixture(s) so that the barcode lines up with the ridges on the fixture.

Handle BeadChips only by the edges or by the barcode end.

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

Set Up the Robot

[ ] **1.** At the robot PC, select **AMP2 Hyb Tasks | Hyb HC BC2**.

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

You can only dispense samples from one AMP2 plate at a time.

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

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

[ ] **4.** Place the AMP2 plate onto the robot bed according to the bed map and remove the foil seal.

Start the Robot

[ ] **1.** When prompted, click **OK**.

[ ] **2.** Remove the Robot BeadChip Alignment Fixtures from the robot bed. Record any beadstripe sections that are not completely covered by DNA sample.

[ ] **3.** Apply a foil heat-seal to the AMP2 plate by firmly holding the heat sealer block down for 3 seconds.

[ ] **4.** Store the AMP2 plate at -20°C, or at -80 °C if you do not plan to use it again within 24 hours.

Set Up HC BC2 for Hyb

[ ] 1. Ensure the Illumina Hybridization Oven is set to 48°C.

CAUTION Hold the BeadChip by the ends with your thumb and forefinger (thumb at the barcode end). Avoid contacting the beadstripe area and sample inlets.

[ ] 2. Carefully remove each BeadChip from the Robot BeadChip Alignment Fixtures when the robot finishes.

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

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

[ ] 4. Load the Hyb Chamber inserts containing BeadChips into the Hyb Chambers. Position the barcode end over the ridges indicated on the Hyb Chamber.

[ ] 5. 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.

[ ] 6. 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.

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

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

[ ] 9. Place RA1 into the freezer at -20°C for use the next day.

Resuspend XC4 Reagent for XStain BC2

[ ] 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.

NOTE If the XC4 was not left to resuspend overnight, you can still proceed with the assay. Add the EtOH and put the XC4 on its side on a rocker to resuspend. Leave it there until the BeadChips are ready for coating.

[ ] 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.

[ ] 5. Proceed to *Wash BC2*.

Wash BC2

Prepare the BeadChips for the staining process.

Estimated Time

Robot time:

- 4 BeadChips: 20 minutes
- 24 BeadChips: 50 minutes

Consumables

Item	Quantity (per 8 Samples)	Storage	Supplied By
PB1	350 ml per 8 BeadChips	Room temperature	Illumina
WB1	1 bottle per 8 BeadChips	-20°C	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 (4 or 24)		Illumina
Wash Dish	4 BeadChips: 1 dish 24 BeadChips: 3 dishes		Illumina
Wash Rack	4 BeadChips: 1 rack 24 BeadChips: 3 racks		Illumina

Preparation

[ ] Thaw WB1 to room temperature (22°C) and allow to equilibrate. Ensure that the solution is completely redissolved.

[ ] Fill 1–3 wash dishes with 200 ml WB1. Label each dish.

[ ] Fill 1–3 wash dishes with 200 ml PB1. Label each dish.

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

[ ] Lay out the Te-Flow Flow-Through Chamber components (black frames, spacers, clean glass back plates, and clamps) on the bench. Separate the clear plastic spacers from the white backs.

[ ] Clean the glass back plates as directed in the *Infinium II Assay Lab Setup and Procedures Guide*.

Steps

Wash BC2

[ ] **1.** Attach the wire handle to the wash rack and submerge it in the WB1 wash dish.

[ ] **2.** For each Hyb Chamber insert:

- [ ] **a.** Remove it from the Hyb Chamber.
- [ ] **b.** Remove the BeadChip from the insert.
- [ ] **c.** Using powder-free gloves and pulling from the barcode end, remove the coverseal from the BeadChip by stripping it off in a downward direction.
- [ ] **d.** Immediately and carefully slide the BeadChip into the wash rack, making sure that the BeadChip is completely submerged in the WB1.

CAUTION To ensure no solution splatters on you, Illumina recommends removing the coverseal over an absorbent cloth or paper towels, preferably in a hood.

[ ] **3.** Repeat step 2 until all BeadChips (maximum of 8) are in the wash rack.

[ ] **4.** Move the wash rack up and down for 1 minute, breaking the surface of the WB1 with gentle, slow agitation.

[ ] **5.** Move the wash rack to the wash dish containing PB1.

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

[ ] **7.** If you are processing more than 8 BeadChips, repeat steps 2 through 6.

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

Assemble Flow-Through Chamber

[ ] **1.** For each BeadChip, place a black frame into the BeadChip Alignment Fixture. The fixture should be pre-filled with 150 ml PB1.

[ ] **2.** Place each BeadChip into a black frame. Align its barcode with the ridges stamped onto the Alignment Fixture.

[ ] **3.** Place a ***clear*** spacer onto the top of each BeadChip.

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

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

[ ] **6.** 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.

[ ] **7.** Attach the metal clamps 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 one stripe shows between it and the Alignment Bar.
- [ ] **c.** Place the second metal clamp around the Flow-Through Chamber at the barcode end, just below the reagent reservoir, so that no stripes show between the clamp and the barcode.

[ ] 8. With scissors, trim the spacer at the non-barcode end of the assembly. Slip scissors up over the barcode to trim the other end.

[ ] 9. Proceed to *XStain BC2*.

XStain BC2

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 BC2 step.

Estimated Time

Robot time:

- 2 hours and 10 minutes per 8 samples
- 2 hours and 40 minutes per 24 samples

Consumables

Item	Quantity (Per 4 BeadChips)	Storage	Supplied By
RA1	10 ml (see *Setup* for special instructions)	-20°C	Illumina
XC1	1 tube	-20°C	Illumina
XC2	1 tube	-20°C	Illumina
TEM	1 tube	-20°C	Illumina
XC3	Bottle (49 ml)	Room temperature	Illumina
LTM (Make sure that all LTM tubes indicate the same stain temperature on the label)	1 tube	-20°C	Illumina
ATM	1 tube	-20°C	Illumina
PB1	Bottle (310 ml)	Room temperature	Illumina
XC4	Bottle (310 ml)	-20°C	Illumina
Alconox Powder Detergent	as needed		Illumina
EtOH	as needed	Room temperature	Illumina
95% formamide/1 mM EDTA	30 ml	-20°C	User

Preparation

[ ] 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.

[ ] Gradually warm the RA1 reagent to room temperature (22°C) in a 20–25°C water bath. Gently mix to dissolve any crystals.

[ ] Thaw all reagent tubes to room temperature (22°C). Centrifuge the thawed reagents to 3000 xg for 1 minute.

Steps

Single-Base Extension and Stain (XStain)

The remaining steps must be performed without interruption.

[ ] **1.** Slide the Chamber Rack into column 36 on the robot bed.

[ ] **2.** At the robot PC, select **Infinium II XStain Tasks | XStain BC2**.

[ ] **3.** 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:

- 4 BeadChips: 15 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:

- 4 BeadChips: 10 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:

- 4 BeadChips: 49 ml
- 24 BeadChips: 145 ml

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

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 LTM 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**. The total wait time is 1 hour and 25 minutes.

[ ] **6.** When prompted, immediately remove the Flow-Through Chambers from the Chamber Rack. Place them horizontally on the lab bench at room temperature (22°C).

[ ] **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 4 Beadchips, dispense 310 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.

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

[ ] **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.

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

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

- For 4 Beadchips, dispense 310 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.

CAUTION Do not touch the stripes with the wipe or allow EtOH to drip onto the stripes.

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

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

- Proceed to *Image BC2*.
- Store the BeadChips in the Illumina BeadChip Slide Storage Box inside a vacuum desiccator at room temperature (22°C). Image the BeadChips within 72 hours.

Image BC2

Scan the BeadChips with the Illumina BeadArray™ Reader, which uses a laser to excite the fluorophore of the single-base extension product on the beads. The scanner records high-resolution images of the light emitted from the fluorophores. The data from these images are analyzed to determine SNP genotypes using Illumina's genotyping software.

Scan time

~45 minutes per BeadChip

Preparation

[ ] Turn on the BeadArray Reader at least one or two hours before scanning. This allows the lasers to stabilize.

Steps

[ ] **1.** Open the Illumina BeadScan application.

[ ] **2.** Click **Scan** on the Welcome screen.

[ ] **3.** Select **BeadChip** from the Docking Fixture menu.

[ ] **4.** In the Settings area, click **Edit**. The Options dialog box appears.

[ ] **5.** Click **Browse** to navigate to and select the following directories:

- Data repository directory containing your data
- Decode map directory, containing the decode data from the BeadChip CD

[ ] **6.** To save the image data in compact JPG format, select the **Compressed** check box.

[ ] **7.** Click either **Save for this Scan** or **Save for All Scans**.

[ ] **8.** Place the BeadChip(s) in the BeadArray Reader tray. Check to ensure that they are seated correctly.

[ ] **9.** Scan each BeadChip barcode. The barcode should appear on the screen in the position corresponding to the tray position.

[ ] **10.** Record the scanner ID and date of the scan in the lab tracking worksheet.

[ ] **11.** Click **Browse** (...) to open the Select Scan Settings dialog box.

[ ] **12.** Select a scan method for each BeadChip. Click **Select**.

[ ] **13.** Click **Scan**. Allow 45 minutes for the scanning process.

Tip: If the BeadArray Reader is unable to scan the fiducials, you may need to clean the BeadChips. See the *Infinium II Assay Lab Setup and Procedures Guide* for directions.

[ ] **14.** When the Welcome screen reappears, click **Open Tray**.

[ ] **15.** Remove the BeadChips.

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

- Scan the next set of BeadChips.
- Right click near the Illumina logo on the Welcome screen and select **Exit**. Close the BeadArray Reader tray and turn off the machine.