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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 QDNA 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 TE to well B1.
- 3.** Add 100 µl 1X TE to wells C, D, E, F, G, and H of column 1.

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- [] 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 **GTS Pre-PCR Tasks | DNA Prep | Make QDNA.**
- [] 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.**
- [] 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. If you are not using LIMS, clear the **Use Barcodes** checkbox.
- [] 10. Click **Run**. Click **OK** in the message box.
- [] 11. 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.
- [] 12. Store the WG#-DNA and Standard DNA plates at 2° to 8°C or -15° to -25°C.
- [] 13. Centrifuge the Sample QNT Plate and Standard QNT plates to 280 xg for 1 minute.

Read QNT Plate

- [] 1. Turn on the spectrofluorometer. At the PC, open the Infinium Fluorometry Analysis program.
- [] 2. Select **Reader Tasks | Read QDNA.**
- [] 3. If you are not using LIMS, clear the **Use Barcodes** checkbox.
- [] 4. Click **Read**.
- [] 5. When prompted, log in to the Illumina LIMS database.



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- [] **6.** 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**.
- [] **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, enter the number of Sample QNT plates you want to read. Click **OK**.
- [] **10.** When prompted, hand-scan or enter the Sample QNT plate barcode. Click **OK**.
- [] **11.** When prompted, remove the plate seal from the Sample QNT plate and load it into the spectrofluorometer tray. Click **OK**.
- [] **12.** When prompted, click **Yes** to review the raw Sample QNT plate data. Microsoft Excel opens automatically at the same time and displays the quantitation data for the Sample QNT plate.
- [] **13.** Do one of the following:
 - Click **Yes** to send the data to Illumina LIMS.
 - Click **No** to delete the quant data.
- [] **14.** If you entered more than one Sample QNT plate to read, repeat steps 10 to 13 for each additional plate.
- [] **15.** Do one of the following:
 - Proceed to *Make MSA3*.
 - Store the Sample DNA plate at 2° to 8°C for up to one month.



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Make MSA3

Transfer DNA samples into the MSA3 plate. Denature and neutralize samples, and prepare them for amplification. Incubate overnight to amplify.

Estimated Time

Robot time:

- 30 minutes for 48 samples
- 1 hour for 96 samples

Incubation time: ~20–24 hours

Consumables

Item	Quantity	Storage	Supplied By
MA1	1 tube (per 96 samples)	-15° to -25°C	Illumina
MA2	1 tube (per 96 samples)	-15° to -25°C	Illumina
MSM	1 tube (per 96 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		User
WG#-DNA plate with 48 or 96 DNA samples (50 ng/μl)	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.
- [] On the Sample Sheet, enter the Sample_Name (optional) and Sample_Plate for each Sample_Well.
- [] Apply an MSA3 barcode label to a new MIDI.
- [] Thaw MA1, MA2, and MSM tubes to room temperature. Pulse centrifuge to 280 xg.
- [] Thaw DNA samples to room temperature.

Steps

- [] **1.** If you do not already have a WG#-DNA plate, create one by adding DNA into either a:
 - MIDI plate: 20 μl to each WG#-DNA plate well
 - TCY plate: 10 μl to each WG#-DNA plate wellApply a barcode label to the new WG#-DNA plate.
- [] **2.** At the robot PC, select **MSA3 Tasks | Make MSA3**.

- [] 3. Select the WG#-DNA plate type (MIDI or TCY).
- [] 4. (Non-Illumina LIMS only) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of DNA samples** (48 or 96) that are in the plate.



NOTE

(Non-Illumina LIMS only) If you are running 48 or less samples enter 48 in the Basic Run Parameters. Enter 96 if you are running 49–96 samples.



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. Remove caps from MA1, MA2, and MSM tubes, then place the tubes in the robot tube rack according to the bed map.
- [] 6. Vortex the sealed WG#-DNA plate at 1600 rpm for 1 minute.
- [] 7. Centrifuge to 280 xg for 1 minute.
- [] 8. Add 15 ml NaOH to the quarter reservoir, then place the reservoir on the robot bed according to the bed map.
- [] 9. Place the WG#-DNA and MSA3 plates on the robot bed according to the bed map.
- [] 10. (Non-Illumina LIMS only) At the robot PC, click **Run**.
- [] 11. (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - [] a. Log in when prompted.
 - [] b. Select the batch you want to run and click **OK**.
 - [] c. Click **OK** to confirm the required DNAs.
- [] 12. When prompted, remove the MSA3 plate and seal it with a cap mat.
- [] 13. Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.
- [] 14. Centrifuge to 280 xg for 1 minute at 22°C.
- [] 15. Place the MSA3 plate back on the robot bed according to the bed map and click **OK** to start the ten minute incubation.
- [] 16. When the robot finishes, seal the MSA3 plate with a cap mat.
- [] 17. Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.
- [] 18. Centrifuge to 280 xg for 1 minute.
- [] 19. (Non-Illumina LIMS only) Record the location of DNA samples in the lab tracking form.
- [] 20. (Illumina LIMS only) In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Incubate MSA3**.
- [] 21. Scan the barcode of the MSA3 plate and click **Verify** and then **Save**.
- [] 22. Incubate in the Illumina Hybridization Oven for 20–24 hours at 37°C.
- [] 23. Proceed to *Fragment MSA3*.

Fragment MSA3

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

Estimated Time

Robot time:

- 5 minutes for 48 samples
- 10 minutes for 96 samples

Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
FMS	1 tube (per 96 samples)	-15° to -25°C	Illumina

Preparation

- [] Preheat the heat block with the MIDI plate insert to 37°C.
- [] Thaw FMS tubes to room temperature (22°C). Gently invert to mix contents, and then pulse centrifuge to 280 xg.
- [] Remove the MSA3 plate from the Illumina Hybridization Oven.
- [] Remove the cap mat.
- [] If you plan to Resuspend MSA3 today then remove the RA1 from the freezer to thaw.

Steps

- [] 1. Centrifuge the MSA3 plate to 50 xg for 1 minute.
- [] 2. At the robot PC, select **MSA3 Tasks | Fragment MSA3**.
- [] 3. (Non-Illumina LIMS only) In the Basic Run Parameters pane, change the value for **Number of DNA samples** to reflect 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.

- [] 4. Place the MSA3 plate on the robot bed according to the bed map. Remove the plate seal. If you have not already done so, remove the cap mat from the plate.
- [] 5. Place the FMS tube in the robot tube rack according to the bed map. Remove the cap.
- [] 6. (Non-Illumina LIMS) At the robot PC, click **Run**.
- [] 7. (Illumina LIMS only) Make sure the Use Barcodes check box is selected and click **Run**. Log in if prompted.
- [] 8. When the robot finishes, seal the MSA3 plate with a cap mat.



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- [] **9.** Vortex at 1600 rpm for 1 minute.
- [] **10.** Centrifuge to 50 xg for 1 minute at 22°C.
- [] **11.** Place the sealed plate on the 37°C heat block for 1 hour.
- [] **12.** Do one of the following:
 - Proceed to *Precip MSA3*. Leave plate in 37°C heat block until you have completed the preparatory steps.
 - Store the sealed MSA3 plate at -15° to -25°C if you do not plan to proceed to the next step immediately.

Precip MSA3

Precipitate the DNA sample using 2-propanol and PM1.

Estimated Time

Robot time:

- 10 minutes for 48 samples
- 20 minutes for 96 samples

Incubation and dry time: 2 hours

Consumables

Item	Quantity	Storage	Supplied By
PM1	1 tube (per 96 samples)	2° to 8°C	Illumina
100% 2-propanol	30 ml (per 96 samples)	Room temperature	User

Preparation

- Preheat the heat block to 37°C.
- If frozen, thaw MSA3 plate to room temperature. Pulse centrifuge to 50 xg.
- Thaw PM1 to room temperature. Centrifuge to 280 xg for 1 minute.

Steps

- 1. At the robot PC, select **MSA3 Tasks | Precip MSA3**.
- 2. (Non-Illumina LIMS only) Make sure the Use Barcodes check box is cleared. In the Basic Run Parameters pane, change the value for **Number of MSA3 plate(s)** and **Number of DNA samples per plate** to reflect the number of DNA 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. Remove the cap mat and place the MSA3 plate on the robot bed according to the bed map.
- 4. Place a half reservoir in the reservoir frame, according to the robot bed map, and add PM1 as follows:
 - For 96 samples, dispense 1 PM1 tube
- 5. Place a full reservoir in the reservoir frame, according to the robot bed map, and add 2-propanol as follows:
 - For 96 samples, dispense 30 ml 2-propanol
- 6. (Non-Illumina LIMS) At the robot PC, click **Run**.



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- [] **7.** (Illumina LIMS only) At the robot PC, make sure the Use Barcodes check box is selected and click **Run**. Log in if prompted.
- [] **8.** When prompted, remove the MSA3 plate from the robot bed. Do not click **OK** in the message box yet.
- [] **9.** Seal the MSA3 plate with the same cap mat removed earlier.
- [] **10.** Vortex the sealed plate at 1600 rpm for 1 minute.
- [] **11.** Incubate at 37°C for 5 minutes.
- [] **12.** Centrifuge to 50 xg at room temperature for 1 minute.
- [] **13.** Remove the cap mat and place the MSA3 plate back on the robot bed according to the bed map.
- [] **14.** In preparation for the 4°C spin, set centrifuge to 4°C.
- [] **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.** (Illumina LIMS only) In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Spin MSA3**.
 - [] **a.** Scan the barcode of the MSA3 plate and click **Verify** and then **Save**.
- [] **20.** Centrifuge to 3000 xg at 4°C for 20 minutes.
- [] **21.** Immediately remove the MSA3 plate from the centrifuge. Remove the cap mat and discard it.

Perform the next step immediately to avoid dislodging the blue pellet. If any delay occurs, repeat steps 20 through 21 before proceeding.
- [] **22.** Over an absorbent pad appropriate for 2-propanol disposal, decant the supernatant by quickly inverting the MSA3 plate and smacking it down.
- [] **23.** Tap the plate firmly on the pad several times over a period of 1 minute or until all wells are completely devoid of liquid.
- [] **24.** Leave the uncovered plate inverted on the tube rack for 1 hour at room temperature to air dry the pellet.
- [] **25.** Do one of the following:
 - Proceed to *Resuspend MSA3*.
 - If you do not plan to proceed to the next step immediately, seal the MSA3 plate with a new cap mat and store at -15° to -25°C.

Resuspend MSA3

Resuspend the precipitated DNA using RA1.

Estimated Time Robot time: 15 minutes for 96 samples
Incubation time: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RA1	7 ml per 96 samples	-15° to -25°C	Illumina



Only pour out the recommended volume of RA1 needed for the suggested number of samples listed in the consumables table. Additional RA1 is used later in the **XStain HD BeadChip** step.

Preparation

- [] 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

Set Up the Robot

- [] 1. At the robot PC, select **MSA3 Tasks | Resuspend MSA3**.
- [] 2. (Non-Illumina LIMS only) Make sure the Use Barcodes check box is cleared. In the Basic Run Parameters pane, change the value for **Number of MSA3 plate(s)** and **Number of DNA samples per plate** to reflect the number of DNAs being processed.



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 MSA3 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:
 - 7 ml for 96 samples
- [] 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. (Non-Illumina LIMS) At the robot PC, click **Run**.
- [] 2. (Illumina LIMS only) At the robot PC, make sure the Use Barcodes check box is selected and click **Run**. Log in if prompted.
- [] 3. Click **OK** in the message box. Remove the MSA3 plate from the robot bed.
- [] 4. Apply a foil seal to the MSA3 plate by firmly holding the heat sealer block down for 5 seconds.
- [] 5. Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.
- [] 6. Vortex the plate at 1800 rpm for 1 minute.
- [] 7. Pulse centrifuge to 280 xg.



NOTE

If you stored the DNA pellets at -15° to -25°C for extended periods of time, you may need to re-vortex and centrifuge until the pellets are completely resuspended.

- [] 8. Do one of the following:
 - Proceed to *Hyb Multi BC2*. 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 MSA3 plate at -15° to -25°C (-80°C if storing for more than 24 hours). Store RA1 at -15° to -25°C.



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Hyb Multi BC2

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:

- 24x1 HD BeadChip: ~40 minutes for 4 BeadChips (96 samples)
- 12x1 HD BeadChip: ~40 minutes for 8 BeadChips (96 samples)

Incubation time: 16–24 hours

Consumables

24x1 HD BeadChip

Item	Quantity (per 96 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 Fixtures	2		Illumina
Robot Tip Alignment Guide-D (One-piece guide)	2		Illumina
1% aqueous Alconox solution	As needed		User

Consumables

12x1 HD BeadChip

Item	Quantity (per 96 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 Fixtures	4		Illumina

Item	Quantity (per 96 Samples)	Storage	Supplied By
Robot Tip Alignment Guide-B (One-piece guide)	4		Illumina
Robot Tip Alignment Guide-B inserts (for multi-piece guide)	8		Illumina
Robot Tip Alignment Guide-B frames (for multi-piece guide)	4		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 Robot Tip Alignment Guide(s)

There are two versions of the Guide-B Robot Tip Alignment Guide (both used with the 12x1 HD BeadChip only). There is a multi-piece that includes two plastic inserts and a metal frame per set and a plastic one-piece. The Guide-D Robot Tip Alignment Guide is used with the 24x1 HD BeadChip only.

- [] **1.** Make sure you have the correct Robot Tip Alignment Guide for the Infinium HD Ultra Assay. The barcode should say "Guide B" (if using 12x1 HD BeadChips) or "Guide D" (if using 24x1 HD BeadChips) on it.
- [] **2.** Wash and dry the multi-piece Robot Tip Alignment Guide inserts or the entire one-piece Robot Tip Alignment Guide. See *Wash Robot Tip Alignment Guide* 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.

Prepare Hyb Chamber(s)



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 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°–8°C storage but do not unpackage.

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- [] 6. (Illumina LIMS only) In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Confirm BeadChips/UAMs for Hyb.**
- [] 7. Scan the barcode of the MSA3 plate and all the BeadChips you plan to hybridize with the plate. Click **Verify**.

Steps



NOTE

Make sure the Robot Tip Alignment Guide inserts are washed and thoroughly dried before you begin the following steps. See *Wash Robot Tip Alignment Guide* at the end of the *Hybridize Multi BeadChip* section.

- [] 1. Place the resuspended MSA3 plate on the heat block to denature the samples at 95°C for 20 minutes.
- [] 2. After the 20-minute incubation, remove the MSA3 plate from the heat block and place it on the benchtop at room temperature for 30 minutes.
- [] 3. After the 30-minute cool down, pulse centrifuge the MSA3 plate to 280 xg.
- [] 4. Remove all BeadChips from their packages.



CAUTION

When handling the BeadChip, avoid contacting the beadstripe area and sample inlets.

- [] 5. Place two BeadChips into each Robot BeadChip Alignment Fixture with the barcode end aligned to the ridges on the fixture.
- [] 6. At the robot PC, select **MSA3 Tasks | Hyb Multi BC2**.
- [] 7. Choose the appropriate BeadChip type from the dialog box.
- [] 8. (Non-Illumina LIMS only) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, change the value for the **Number of MSA3 plate(s)** and **Number of DNA samples per plate** to reflect the number of DNA 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.

- [] 9. Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map.
- [] 10. Remove the foil seal.
- [] 11. Place the MSA3 plate onto the robot bed according to the bed map.
- [] 12. (Non-Illumina LIMS) At the robot PC, click **Run**.
- [] 13. (Illumina LIMS only) Make sure the Use Barcodes check box is selected and click **Run**. Log in if prompted.
- [] 14. (Illumina 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.

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- [] 15. Place the Robot Tip Alignment Guide with the correct inserts loaded on top of the Robot BeadChip Alignment Fixture. The Guide-B (12x1 HD BeadChip) or Guide-D (24x1 HD BeadChip) barcode should be upside down and facing away from you. Push both the Robot Tip Alignment Guide and Robot BeadChip Alignment Fixture to the upper left corner in its section of the robot bed. See *Prepare Robot Tip Alignment Guide(s)* for the correct multi-piece Robot Tip Alignment Guide preparation instructions.
- [] 16. At the robot PC, click **OK** to confirm you have placed the correct Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture.
- [] 17. (Illumina LIMS only) The robot scans the barcode on the Robot Tip Alignment Guide to confirm the correct tip guide is being used.



NOTE

In the rare occasion the robot scanner fails to read a barcode, use the hand scanner attached to the PC to manually scan the barcode.

- [] 18. The robot dispenses sample to the BeadChips.
- [] 19. 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.



NOTE

For optimal performance, the Robot Tip Alignment Guide should be washed and dried after every run. See *Wash Robot Tip Alignment Guide* at the end of this section for wash instructions.

- [] 20. Discard the MSA3 plate if you have not done so.
- [] 21. To ensure optimal performance, immediately after the robot has finished delivering samples to all BeadChips, remove each Robot BeadChip Alignment Fixture from the robot bed and visually inspect all sections of the BeadChips. Next, place the Hyb Chamber inserts inside the Hyb Chamber as described in the next section, *Set Up Multi BeadChip for Hyb*.

Set Up Multi 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.



CAUTION

For optimal performance, keep the BeadChips steady and level when lifting or moving. Avoid contacting the sample inlets when handling the BeadChips.

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- [] 3. Position the lid onto the Hyb Chamber by applying the back side of the lid first and then slowly bringing down the front end to avoid dislodging the Hyb Chamber inserts.
- [] 4. Secure the lid by closing down the clamps on both sides of the Hyb Chamber.
- [] 5. 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.
- [] 6. Incubate at 48°C for at least 16 hours but no more than 24 hours.
- [] 7. On the lab tracking form, record the start and stop times.
- [] 8. After the overnight hybridization, 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.



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.

Wash Robot Tip Alignment Guide

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

- [] 1. This first step applies to the multi-piece Robot Tip Alignment Guide only. Hold the tip guide upside down with the barcode side facing down. Remove tip guide inserts from the underside of the tip guide frame by using thumb pressure on the beveled, barcode end and pushing the insert down and out. The metal frame does not need to be washed.

The remaining steps apply to both the multi-piece (once the plastic inserts are removed) and the one-piece Robot Tip Alignment Guides.

- [] 2. Soak in a 1% aqueous Alconox solution (one part Alconox to 99 parts water) using a 400 ml Pyrex beaker for 5 minutes.
- [] 3. After the 5 minute soak in the 1% Alconox solution, thoroughly rinse with DiH₂O at least three times to remove any residual detergent. Make sure the DiH₂O runs through all the tip guide channels.
- [] 4. Dry the inserts or one-piece Robot Tip Alignment Guide, especially the tip guide channels, 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 guide inserts and frames should be completely dry and free of any residual contaminants before next use.

Wash BeadChip

Prepare the BeadChips for the staining process.

Estimated Time

- ~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 4 BeadChips)		Illumina
Te-Flow Flow-Through Chambers (with black frames, spacers, 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



CAUTION

Only pour out the recommended reagent volume needed for the suggested number of BeadChips listed in the Consumables table of each section. Some reagents are used later in the protocol.

Preparation

- [] Remove each Hyb Chamber from the Illumina Hybridization Oven. Let cool on the benchtop for 25 minutes prior to opening.
- [] 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 BeadChips

- [] 1. (Illumina LIMS only) In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Wash BeadChip**.
 - [] a. Scan the barcode(s) of the PB1 and scan the BeadChip barcodes. Click **Verify** and then **Save**.
- [] 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.

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- [] 5. Remove the IntelliHyb seal from each BeadChip as follows:
 - [] a. Using powder-free gloved hands, 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, slow, consistent 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 for each BeadChip to be processed. 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.

Assemble Flow-Through Chambers



NOTE

The 150 ml of PB1 used in the Flow-Through Chamber assembly can be used for up to eight BeadChips. You must use 150 ml of new PB1 for every additional set of eight BeadChips.

- [] 1. If you have not done so yet, fill the Multi-Sample 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 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 Whoosh duster or laboratory air gun to quickly remove any accumulated dust from the glass back plates just before placing them onto the BeadChips.

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- [] 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 mm from the top edge.
 - [] c. Place the second metal clamp around the Flow-Through Chamber at the barcode end, about 5 mm 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 DiH₂O and scrub them with a small cleaning brush, ensuring that no PB2 remains.
- [] 11. Proceed to *XStain HD BeadChip*.



CAUTION

Place all assembled Flow-Through Chambers on the lab bench in a horizontal position while you perform the preparation steps for XStain HD BeadChip. Do not place the Flow-Through Chambers in the Chamber Rack until all necessary steps are completed.

XStain HD BeadChip

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

Estimated Time

Robot time:

- 2 hours and 15 minutes for 4 BeadChips
- 2 hours and 40 minutes for 8 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
XC1	1 tube (per 4 BeadChips)	-15° to -25°C	Illumina
XC2	1 tube (per 4 BeadChips)	-15° to -25°C	Illumina
TEM	1 tube (per 4 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
STM (Make sure that all STM tubes indicate the same stain temperature on the label)	1 tube (per 4 BeadChips)	-15° to -25°C	Illumina
ATM	1 tube (per 4 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 to be used in the assay in a tube rack. If frozen, thaw to room temperature and centrifuge to 3000xg for 3 minutes.
- [] Ensure the water circulator is filled to the appropriate level.

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- [] Turn on the water circulator. Set it to a temperature that brings the Chamber Rack to 44°C at equilibrium.
- [] Remove bubbles trapped in the Chamber Rack.
- [] Test several locations on the Chamber Rack, using the Illumina Temperature Probe, to ensure that it is uniformly 44°C.

Steps



CAUTION

The remaining steps must be performed without interruption.

- [] 1. Slide the Chamber Rack into column 28 on the robot bed. Ensure that it is seated properly.
- [] 2. At the robot PC, select **XStain Tasks | Infinium II Chemistry | XStain HD BeadChip**.
- [] 3. In the Basic Run Parameters pane, enter the number of BeadChips.

Single-Base Extension and Stain

- [] 1. 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 1–8 BeadChips
 - 17 ml to process 9–16 BeadChips
 - 25 ml to process 17–24 BeadChips
- [] 2. Place a half reservoir in the reservoir frame, according to the robot bed map, and add RA1 as follows:
 - 10 ml to process 1–8 BeadChips
 - 20 ml to process 9–16 BeadChips
 - 30 ml to process 17–24 BeadChips
- [] 3. Place a full reservoir in the reservoir frame, according to the robot bed map, and add XC3 as follows:
 - 50 ml to process 1–8 BeadChips
 - 100 ml to process 9–16 BeadChips
 - 150 ml to process 17–24 BeadChips
- [] 4. Place each reagent tube (XC1, XC2, TEM, STM, ATM) in the robot tube rack according to the bed map, and remove their caps.
 - 1 tube to process 1–4 BeadChips
 - 2 tubes to process 5–8 BeadChips
- [] 5. Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.

Wash and Coat

- [] 1. (Illumina LIMS only) In the Illumina LIMS left sidebar, click **Infinium HD Ultra | Coat BC2**.
 - [] a. Scan the barcode(s) of the PB1, XC4, and BeadChips and click **Verify** and then **Save**.

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- [] 2. Dispense PB1 into a wash dish as follows, and then cover the dish:
 - 310 ml for 1–8 Beadchips
 - 300 ml for 9–16 Beadchips
 - 285 ml for 17–24 Beadchips
- [] 3. Submerge the unloaded staining rack into the wash dish with the locking arms and tab facing **towards** you.



CAUTION

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

- [] 4. 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. Place half of the BeadChips above the handle and half below. All chips should be completely submerged.
- [] 5. Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.
- [] 6. Soak for 5 minutes.



CAUTION

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

- [] 7. Dispense XC4 into a wash dish as follows:
 - 310 ml for 1–8 Beadchips
 - 300 ml for 9–16 Beadchips
 - 285 ml for 17–24 Beadchips

Do not let the XC4 sit for more than 10 minutes.
- [] 8. Move the BeadChip staining rack into the XC4 dish. The barcodes should face **away** from you and the locking arms **towards** you.
- [] 9. Slowly move the staining rack up and down 10 times, breaking the surface of the reagent.
- [] 10. Soak for 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 staining rack handle after removing the first four BeadChips.
- [] 13. Dry the BeadChips in the vacuum desiccator for 50–55 minutes at 508 mm Hg (0.68 bar).
- [] 14. Clean the underside of each BeadChip with a ProStat EtOH wipe.



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- [] 15. Clean and store the glass back plates and Hyb Chamber components.
- [] 16. Do one of the following:
 - 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.

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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:

Bead Types	24x1 HD Format			12x1 HD Format	
	3K–20K	20K–40K	40K–60.8K	60.8K–100K	100K–200K
Stripes per Sample Section	1	2	3	2	4
Scan time per BeadChip	7 min	13 min	24 min	20 min	35 min




NOTE

Scan times may be shorter depending on the complexity of the Bead Pool loaded onto the 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 iScan Control Software application.
- [] 2. Let the iScan Reader warm up for at least 5 minutes before beginning a scan. It is fine to use the iScan Control Software during this time.
- [] 3. For each BeadChip, download the decode content from iCom or copy the contents of the DVD provided with the BeadChip (if purchased) into the Decode folder. The folder name should be the BeadChip barcode (for example, 4264011131).
- [] 4. Double-click the iScan Control Software icon  on the desktop.
- [] 5. (Non-Illumina LIMS) Set the **LIMS** dropdown list to **None** and enter your Windows user name.
- [] 6. (Illumina LIMS only) Select the **LIMS** server from the dropdown list and enter your LIMS user name and password.
- [] 7. 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**.
You can click any barcode to view an image of the corresponding BeadChip.



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- [] 2. If the Scan Setting field beside each BeadChip does not contain "Infinium HD" (12x1 HD BeadChip) or "Infinium HD-HT" (24x1 HD BeadChip), click **Settings**.
- [] 3. Select **Infinium HD.scst** for the 12x1 HD or **Infinium HD-HT.scst** for the 24x1 HD BeadChip and click **Open**.
- [] 4. If you want to change the image format (*.jpg or *.tif), click the Menu button and select **Tools | Options**.
- [] 5. Click any BeadChip section to remove it from the scan. The section will no longer be highlighted blue.
- [] 6. If you want to remove an entire BeadChip from the scan, delete the barcode from the Setup window.
- [] 7. To begin scanning the BeadChips, click **Scan**.
- [] 8. 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.
- [] 9. When you finish reviewing the data, click **Done** (Non-Illumina LIMS only) or **Submit to LIMS** (Illumina LIMS only) to return to the Start window.

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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 warm-up for the BeadArray Reader (first use of the day only)

Scanning:

	24x1 HD Format			12x1 HD Format	
Bead Types	3K–20K	20K–40K	40K–60.8K	60.8K–100K	100K–200K
Stripes per Sample Section	1	2	3	2	4
Scan time per BeadChip	58 min	115 min	171 min	96 min	180 min



NOTE

Scan times may be shorter depending on the complexity of the Bead Pool loaded onto the BeadChip.

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. (Illumina LIMS only) 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. For each BeadChip, download the decode content from iCom or copy the contents of the DVD provided with the BeadChip (if purchased) into the Decode folder. The folder name should be the BeadChip barcode (for example, 4264011131).
- [] 4. For each BeadChip:
 - [] a. Place the BeadChip into the BeadArray Reader tray.



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- b.** If either the **Sentrix Type** or **Scan Settings** are not correct, click **Browse (...)** to open the Select Scan Settings dialog box.
- c.** Select **Infinium HD.scst** for the 12x1 HD BeadChip or **Infinium HD-HT.scst** for the 24x1 HD BeadChip 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 red or green dots in the closeup image to see the relative intensity and the XY position.

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.