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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 six plates, each containing up to 96 samples.

Estimated Time

Hands-on time: ~20 minutes per plate, plus 10 minutes to prepare the PicoGreen
Spectrofluorometer read time: ~5 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See Instructions	2° to 8°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

Preparation

- [] Thaw PicoGreen to room temperature for 60 minutes in a light-impermeable container.
- [] Hand-label the microtiter plate "Standard DNA."
- [] Hand-label one of the Fluotrac plates "Standard QNT."
- [] Hand-label the other Fluotrac plate "Sample QDNA." 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.
- [] **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 an adhesive seal.

Dilute PicoGreen



CAUTION

PicoGreen reagent degrades quickly in the presence of light. Do not use glass containers for PicoGreen reagent.

- [] 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.
 Use 115 µl PicoGreen and 23 ml 1X TE for 1 plate, 215 µl PicoGreen and 43 ml 1X TE for 2 plates, and so on up to 6 plates.
- [] 2. Cap the foil-wrapped bottle and vortex to mix.

Create Standard QDNA Plate with Diluted PicoGreen

- [] 1. Pour the PicoGreen/1X TE dilution into a clean reagent reservoir.
- [] 2. Using a multichannel pipette, transfer 195 µl PicoGreen/1X TE dilution into each well of columns 1 and 2 of the Fluotrac plate labelled "Standard QDNA".
- [] 3. Add 2 µl of each stock Lambda DNA dilution from the Standard DNA plate to columns 1 and 2 of the Standard QDNA Fluotrac plate.
- [] 4. Immediately cover the plate with an adhesive aluminum seal.

Prepare Sample QDNA Plate with PicoGreen and DNA

- [] 1. Using a multichannel pipette, transfer 195 µl PicoGreen/1X TE dilution into each well of columns 1 and 2 of the Fluotrac plate labelled "Sample QDNA".
- [] 2. Add 2 µl of DNA sample to all 96 wells of the Sample QDNA plate.
- [] 3. Immediately cover the plate with an adhesive aluminum seal.
- [] 4. Proceed to *Read QDNA Plate With LIMS* if you are running Infinium LIMS or *Read QDNA Plate Without LIMS* and you are not running Infinium LIMS.

Read QDNA Plate With LIMS

- [] 1. Turn on the spectrofluorometer.
- [] 2. Open the GTS Fluorometry Analysis software.
- [] 3. Select **Reader Tasks | Read QDNA**.
- [] 4. Select the **Use Barcodes** check box. Click **Read**.
- [] 5. When prompted, log in to the Infinium LIMS database.
- [] 6. When asked if you want to read a new Standard QNT plate, click **Yes**. Load the Standard QNT plate in the open drawer of the fluorometer.
- [] 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 (1, 2, or 3). Click **OK**.



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- [] 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 quant data for the QDNA plate.
 - Click **Yes** to send the data to Infinium LIMS. In Infinium LIMS, the QNT plate moves into the Make MSA3 queue.
- [] 13. QNTDo one of the following:
 - Proceed to *Make MSA3*.
 - Store the quantitated DNA at 2° to 8°C for up to one month.

Read QDNA Plate Without LIMS

- [] 1. Turn on the spectrofluorometer. At the PC, open the SoftMax Pro program.
- [] 2. Load the Illumina QDNA.ppr file from the installation CD that came with your system.
- [] 3. Select **Protocols | GTS_QDNA**.
- [] 4. Place the Standard QDNA Fluotrac Plate into the spectrofluorometer.
- [] 5. Click the blue arrow next to **Illumina QDNA | SQDNA_STD**.
- [] 6. Click **Read**.
- [] 7. When the software finishes reading the data, remove the plate from the drawer.
- [] 8. Click the blue arrow next to **Standard Curve** to view the standard curve graph.
- [] 9. Place the first Sample QDNA plate in the spectrofluorometer.
- [] 10. Click the blue arrow next to **SQDNA** and click **Read**.
- [] 11. When the software finishes reading the plate, remove the plate from the drawer.
- [] 12. Repeat steps 10 through 12 to quantitate all Sample QDNA plates.
- [] 13. Once all plates have been read, click **File | Save** to save the output data file (*.pda).
- [] 14. When you have saved the *.pda file, click **File | Import/Export | Export** and export the file as a *.txt file.
- [] 15. Do one of the following:
 - Proceed to *Make MSA3*.
 - Store the quantitated DNA at 2° to 8°C for up to one month.

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Make MSA3

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

Estimated Time

Hands-on time:

- 45 minutes for 48 samples
- 60 minutes 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 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.
- [] Apply MSA3 barcode label to a new MIDI plate.
- [] Thaw MA1, MA2, and MSM tubes to room temperature. Pulse centrifuge to 280 xg.
- [] Thaw DNA samples to room temperature.

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- Steps
- [] 1. If you do not already have a WG#-DNA plate, create one by adding DNA, normalized to 50 ng/μl, into either a:
 - MIDI plate: 20 μl to each WG#-DNA plate well
 - TCY plate: 10 μl to each WG#-DNA plate well
 Apply a barcode label to the new WG#-DNA plate.
 - [] 2. Dispense 20 μl MA1 into the MSA3 plate wells.
 - [] 3. Transfer 4 μl DNA sample, from the WG#-DNA plate to the corresponding wells in the MSA3 plate.
 - [] 4. On the lab tracking form, record the original DNA sample ID for each well in the MSA3 plate.
 - [] 5. Dispense 4 μl 0.1N NaOH into each well of the MSA3 plate that contains MA1 and sample.
 - [] 6. Seal the MSA3 plate with the 96-well cap mat.
 - [] 7. Vortex the plate at 1600 rpm for 1 minute.
 - [] 8. Centrifuge to 280 xg for 1 minute.
 - [] 9. Incubate for 10 minutes at room temperature.
 - [] 10. Dispense 34 μl MA2 into each well of the MSA3 plate containing sample.
 - [] 11. Dispense 38 μl MSM into each well of the MSA3 plate containing sample.
 - [] 12. Reseal the MSA3 plate with the cap mat.
 - [] 13. Vortex the sealed MSA3 plate at 1600 rpm for 1 minute.
 - [] 14. Pulse centrifuge to 280 xg for 1 minute.
 - [] 15. Incubate in the Illumina Hybridization Oven for 20–24 hours at 37°C.
 - [] 16. Proceed to *Fragment MSA3*.



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Fragment MSA3

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

Estimated Time Hands-on time: ~30 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 tube to room temperature. Gently invert to mix contents, and then pulse centrifuge to 280 xg.
- [] Remove the MSA3 plate from the Illumina Hybridization Oven.
- [] Remove the cap map.
- [] Preheat the heat block with the MIDI plate insert to 37°C.

Steps

- [] **1.** Centrifuge the plate to 50 xg for 1 minute.
- [] **2.** Add 25 µl FMS to each well containing sample.
- [] **3.** Seal the MSA3 plate with the 96-well cap mat.
- [] **4.** Vortex the plate at 1600 rpm for 1 minute.
- [] **5.** Centrifuge the plate to 50 xg for 1 minute at 22°C.
- [] **6.** Place the sealed plate on the 37°C heat block for 1 hour.
- [] **7.** Do one of the following:
 - Proceed to *Precip MSA3*. Leave the plate in the 37°C heat block until setup is complete.
 - Store the sealed MSA3 plate at -15° to -25°C if you do not plan to proceed to the next step immediately.

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Precip MSA3

Precipitate the DNA sample using 2-propanol and PM1.

Hands-on time: ~30 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 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.
- [] Remove the 96-well cap mat.

Steps

- [] 1. Add 50 µl PM1 to each MSA3 plate well containing sample.
- [] 2. Seal the plate with the cap mat.
- [] 3. Vortex the plate at 1600 rpm for 1 minute.
- [] 4. Incubate at 37°C for 5 minutes.
- [] 5. Centrifuge to 50 xg at 22°C for 1 minute.



NOTE

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

- [] 6. Add 155 µl 100% 2-propanol to each well containing sample.
- [] 7. Carefully seal the MSA3 plate with a new, **dry** cap mat.
- [] 8. Invert at least 10 times to mix contents thoroughly.
- [] 9. Incubate at 4°C for 30 minutes.
- [] 10. Centrifuge to 3,000 xg at 4°C for 20 minutes. Immediately remove the MSA3 plate from the centrifuge.
 Perform the next step immediately, to avoid dislodging the blue pellet. If any delay occurs, repeat the 20-minute centrifugation before proceeding.
- [] 11. Remove the cap mat and discard it.
- [] 12. Decant supernatant by quickly inverting the MSA3 plate and smacking it down onto an absorbent pad.



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- [] **13.** Tap firmly several times for 1 minute or until all wells are devoid of liquid. Do not allow supernatant to pour into other wells.
- [] **14.** Leave the uncovered, inverted plate on the tube rack for 1 hour at room temperature to air dry the pellet.
- [] **15.** Do one of the following:
 - Proceed to *Resuspend MSA3*.
 - Seal the MSA3 plate with a cap mat and store it at -15° to -25°C if you do not plan to proceed to the next step immediately.



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Resuspend MSA3

Resuspend the precipitated DNA using RA1.

Estimated Time Hands-on time: ~30 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

Preparation

- [] If you stored the MSA3 plate at -15° to -25°C, thaw it to room temperature. Remove the cap mat and discard it.
- [] Preheat the Illumina Hybridization Oven to 48°C.
- [] Turn on the heat sealer to preheat. Allow 20 minutes.
- [] Thaw RA1 to room temperature. Gently invert to re-dissolve solution.

Steps

- [] **1.** Add 23 µl RA1 to each well of the MSA3 plate containing a DNA pellet. Reserve any leftover reagent for XStain HD BeadChip.
- [] **2.** Apply a foil seal to MSA3 plate by firmly holding the heat-sealer sealing block down for 5 seconds.
- [] **3.** Place the sealed plate in the Illumina Hybridization Oven and incubate for 1 hour at 48°C.
- [] **4.** Vortex the plate at 1800 rpm for 1 minute.
- [] **5.** Pulse centrifuge to 280 xg.
If you stored the pellets at -15° to -25°C for extended periods of time after the Precip MSA3 process, you may need to repeat the vortexing and centrifugation steps until the pellets are completely resuspended.
- [] **6.** Do one of the following:
 - Proceed to *Hyb Multi BC2*. If you plan to do so immediately, it is safe to leave the MSA3 plate 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

Hands-on time:

- 24x1 HD BeadChips: ~1 hour for 4 BeadChips (96 samples)
- 12x1 HD BeadChips: ~1 hour 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

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

Preparation

- [] Preheat the heat block to 95°C.
- [] Preheat the Illumina Hybridization Oven to 48°C and set the rocker speed to 5.

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Steps *Prepare Hyb Chamber(s)*

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

Hybridize Sample to Multi BeadChip

- [] 1. Place the resuspended MSA3 plate on the heat block to denature the samples at 95°C for 20 minutes.
- [] 2. Remove the BeadChips from 2°–8°C storage but do not unpackage.
- [] 3. 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.
- [] 4. After the 30-minute cool down, pulse centrifuge the MSA3 plate to 280 xg.

Load BeadChip

- [] 1. Just before loading DNA samples, remove all BeadChips from their packages.



CAUTION

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

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

For the 24x1 HD BeadChip:

- [] 1. Using a multi-channel precision pipette, dispense 12 µl of each DNA sample onto the appropriate BeadChip section, according to the chart on the lab tracking form and the illustrations below:



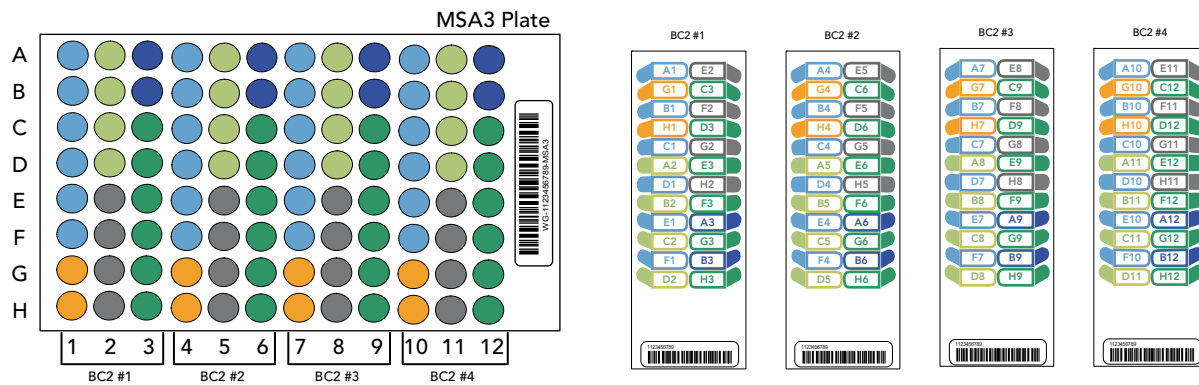
CAUTION

Load samples by directly placing pipette tip to the array surface and hold the pipette straight up above the array surface. Do not hold the pipette at an angle while you are loading sample. Proceed immediately to the next step as soon as all arrays have received sample.

- [] 2. Load samples A1–F1 from the MSA3 plate into the left side BeadChip inlet ports A1–F1 (every other inlet port on the left side of the BeadChip).
- [] 3. Load samples in G1 and H1 from the MSA3 plate into the left side BeadChip inlet ports G1 and H1.
- [] 4. Load samples A2–D2 from the MSA3 plate into the left side BeadChip inlet ports A2–D2.
- [] 5. Load samples E2–H2 from the MSA3 plate into the right side BeadChip inlet ports E2–H2.
- [] 6. Load samples A3 and B3 from the MSA3 plate into the right side BeadChip inlet ports A3 and B3.

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- [] 7. Load samples C3–H3 from the MSA3 plate into the right side BeadChip inlet ports C3–H3 (every other inlet port on the right side of the BeadChip).
- [] 8. Continue loading samples by following the color-coded sections shown in the following graphic for any additional BeadChips and until all samples are loaded.



- [] 9. On the lab tracking form, record the BeadChip barcode for each group of samples.
- [] 10. Visually inspect all sections of the BeadChips. Ensure the DNA sample entirely covers each bead stripe. Record any sections that are not completely covered.
- [] 11. To ensure optimal performance, after you have visually inspected all sections of the BeadChips, place the Hyb Chamber inserts inside the Hyb Chamber immediately as described in the next section, *Set up Multi BeadChip for Hyb*

For the 12x1 HD BeadChip:

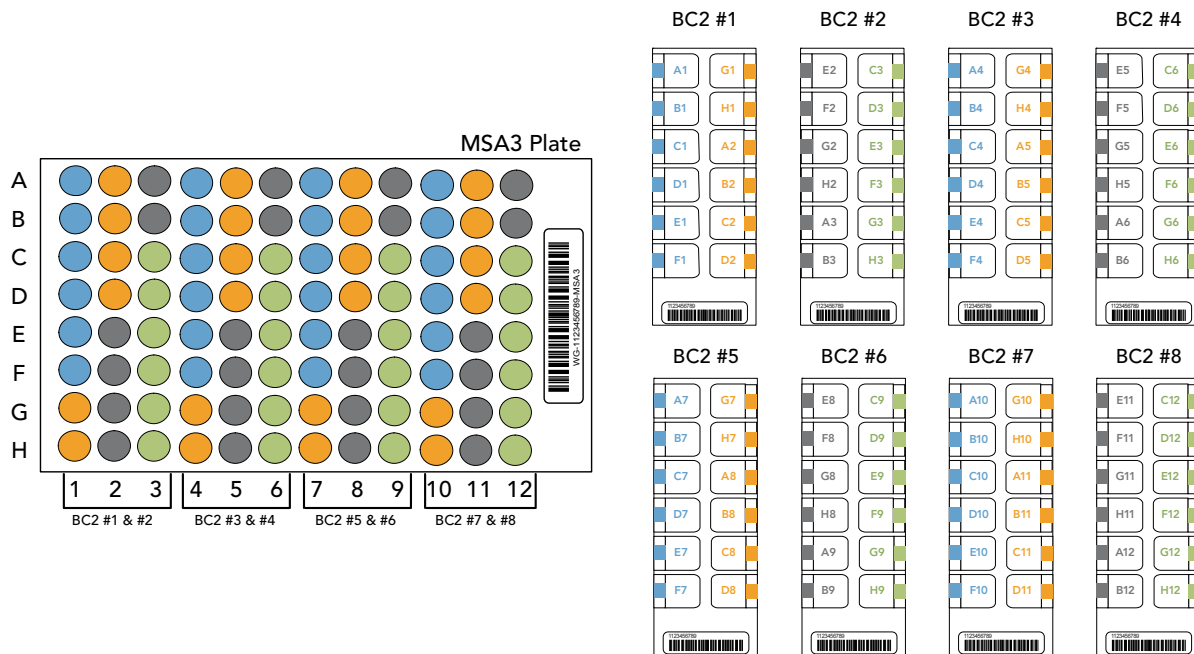
- [] 1. Using a multi-channel precision pipette, dispense 15 μ l of each DNA sample onto the appropriate BeadChip section, according to the chart on the lab tracking form and the following illustrations:


CAUTION

Load samples by directly placing pipette tip to the array surface and hold the pipette straight up above the array surface. Do not hold the pipette at an angle while you are loading sample. Proceed immediately to the next step as soon as all arrays have received sample.

- [] 2. Load samples A1–F1 from the MSA3 plate into the left side of the BeadChip.
- [] 3. Load samples in G1 and H1 from the MSA3 plate into the top two inlets of the right side of the BeadChip.
- [] 4. Load samples A2–D2 into the remaining four inlets on the right side of the BeadChip.
- [] 5. Continue loading samples by following the color-coded sections on the lab tracking form until all samples are loaded.

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- [] 6. On the lab tracking form, record the BeadChip barcode for each group of samples.
- [] 7. Visually inspect all sections of the BeadChips. Ensure the DNA sample entirely covers each bead stripe. Record any sections that are not completely covered.
- [] 8. To ensure optimal performance, after you have visually inspected all sections of the BeadChips, place the Hyb Chamber inserts inside the Hyb Chamber immediately as described in the next section, *Set up Multi BeadChip for Hyb*.



CAUTION

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

- [] 9. Load the Hyb Chamber inserts containing BeadChips into the Illumina Hyb Chamber. Position the barcode end over the ridges indicated on the Hyb Chamber.
- [] 10. Place the back side of lid onto the Hyb Chamber and then slowly bring down the front end to avoid dislodging the Hyb Chamber inserts.
- [] 11. Close the clamps on both sides of the Hyb Chamber.



NOTE

For optimal performance, keep the Hyb Chamber steady and level when lifting or moving. Avoid shaking the Hyb Chamber, and keep the Hyb Chamber parallel to the lab bench while you transfer it to the Illumina Hybridization Oven.

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- [] **12.** Place the Hyb Chamber into the 48°C Illumina Hybridization Oven so that the clamps of the Hyb Chamber face the left and right sides of the oven. The Illumina logo on top of the Hyb Chamber should be facing you.



If you are stacking multiple Hyb Chambers in the Illumina Hybridization Oven, make sure the feet of the top Hyb Chamber fit into the matching indents on top of the next Hyb Chamber. This will hold the Hyb Chambers in place while they are rocking. You can stack up to 3 Hyb Chambers per row for a maximum total of 6 Hyb Chambers in a Hyb Oven.

- [] **13.** Incubate at 48°C for at least 16 hours but no more than 24 hours.
- [] **14.** Discard the MSA3 plate.

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.



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.

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.

- [] **4.** After the overnight incubation, proceed to *Wash BeadChip*.

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Wash BeadChip | Prepare the BeadChips for the staining process.

Estimated Time Hands-on 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

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 as directed in the *Infinium Assay Lab Setup and Procedures Guide*.

Steps

- [] **1.** Attach the wire handle to the rack and submerge the wash rack in the first wash dish containing 200 ml PB1.
- [] **2.** Remove the Hyb Chamber inserts from the Hyb Chambers.
- [] **3.** Remove BeadChips from the Hyb Chamber inserts one at a time.
- [] **4.** Remove the IntelliHyb Seal from each BeadChip:
 - [] **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.

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- [] **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.
- [] **5.** Immediately and carefully slide each BeadChip into the wash rack one at a time, making sure that the BeadChip is completely submerged in the PB1.
- [] **6.** Repeat steps 4 through 5 for each BeadChip. The wash rack holds a max of 8 BeadChips.
- [] **7.** Once all the BeadChips are in the wash rack, move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.
- [] **8.** Move the wash rack to the other wash dish containing PB1. Make sure that the BeadChips are completely submerged.
- [] **9.** Move the wash rack up and down for 1 minute, breaking the surface of the PB1 with gentle, slow agitation.

Assemble Flow-Through Chamber



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 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 onto each Flow-Through Chamber 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.

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- [] c. Place the second metal clamp around the Flow-Through Chamber at the barcode end, about 5 millimeters from the reagent reservoir.
- [] 9. With scissors, trim the spacer at the non-barcode end of the assembly. Slip the scissors up over the barcode to trim the other end.
- [] 10. Immediately wash the Hyb Chamber reservoirs with dH₂O and scrub them with a small cleaning brush, ensuring that no PB2 remains.
- [] 11. Proceed to *XStain HD BeadChip*.



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 the preparation is complete.

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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

Hands-on time: ~3 hours for 8 BeadChips

Dry time: 1 hour

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

- [] Ensure the water circulator is filled to the appropriate level.
- [] 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.

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- [] Test several locations on the Chamber Rack, using the Illumina Temperature Probe, to ensure that it is uniformly 44°C.
- [] Place all reagent tubes in a rack in the order in which they will be used. If frozen, allow them to thaw to room temperature and centrifuge to 3000 xg for 3 minutes.
- [] Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.

Steps The remaining steps must be performed without interruption.

Single-Base Extension

- [] **1.** When the Chamber Rack reaches 44°C, quickly place each Flow-Through Chamber assembly into the Chamber Rack.
For 4 BeadChips, place the Flow-Through Chambers in every other position, starting at 1, in the first row of the Chamber Rack. For larger numbers of BeadChips, fill all positions in the first row, then the second and third.
- [] **2.** Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.
- [] **3.** Into the reservoir of each Flow-Through Chamber, dispense:
 - [] **a.** 150 µl RA1. Incubate for 30 seconds. Repeat 5 times.
 - [] **b.** 450 µl XC1. Incubate for 10 minutes.
 - [] **c.** 450 µl XC2. Incubate for 10 minutes.
 - [] **d.** 200 µl TEM. Incubate for 15 minutes.
 - [] **e.** 450 µl 95% formamide/1 mM EDTA. Incubate for 1 minute. Repeat once.
 - [] **f.** Incubate 5 minutes.
 - [] **g.** Begin ramping the Chamber Rack temperature to the temperature indicated on the STM tube, or to 37°C if none is shown.
 - [] **h.** 450 µl XC3. Incubate for 1 minute. Repeat once.
- [] **4.** Wait until the Chamber Rack reaches the correct temperature.

Stain BeadChip

- [] **1.** If you plan to image the BeadChip immediately after the staining process, turn on the Illumina BeadArray Reader or iScan now.
- [] **2.** Into the reservoir of each Flow-Through Chamber, dispense:
 - [] **a.** 250 µl STM and incubate for 10 minutes.
 - [] **b.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
 - [] **c.** 250 µl ATM and incubate for 10 minutes.
 - [] **d.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
 - [] **e.** 250 µl STM and incubate for 10 minutes.
 - [] **f.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
 - [] **g.** 250 µl ATM and incubate for 10 minutes.
 - [] **h.** 450 µl XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.

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- [] i. 250 μ l STM and incubate for 10 minutes.
- [] j. 450 μ l XC3 and incubate for 1 minute. Repeat once, and then wait 5 minutes.
- [] 3. Immediately remove the Flow-Through Chambers from the Chamber Rack and place horizontally on a lab bench at room temperature.

Wash and Coat

- [] 1. Pour 310 ml PB1 per 8 BeadChips into a wash dish. Cover the dish.
- [] 2. Place the staining rack inside the wash dish. The locking arms should face **towards** you.
- [] 3. For each BeadChip:
 - [] a. Use the dismantling tool to remove the two metal clamps from the Flow-Through Chamber.
 - [] b. Remove the glass back plate, the spacer, and then the BeadChip.
 - [] c. Immediately place each BeadChip into the staining rack that is in the wash dish with the barcode facing **away** from you. 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.
- [] 6. Shake the XC4 bottle vigorously to ensure complete resuspension. If necessary, vortex until completely dissolved.
- [] 7. Pour 310 ml XC4 into a wash dish. Do not let it 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.
- [] 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. 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**.
You can click any barcode to view an image of the corresponding BeadChip.
- [] 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 BeadChip or **Infinium HD-HT.scst** for the 24x1 HD BeadChip and click **Open**.



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- [] 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** 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. 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.