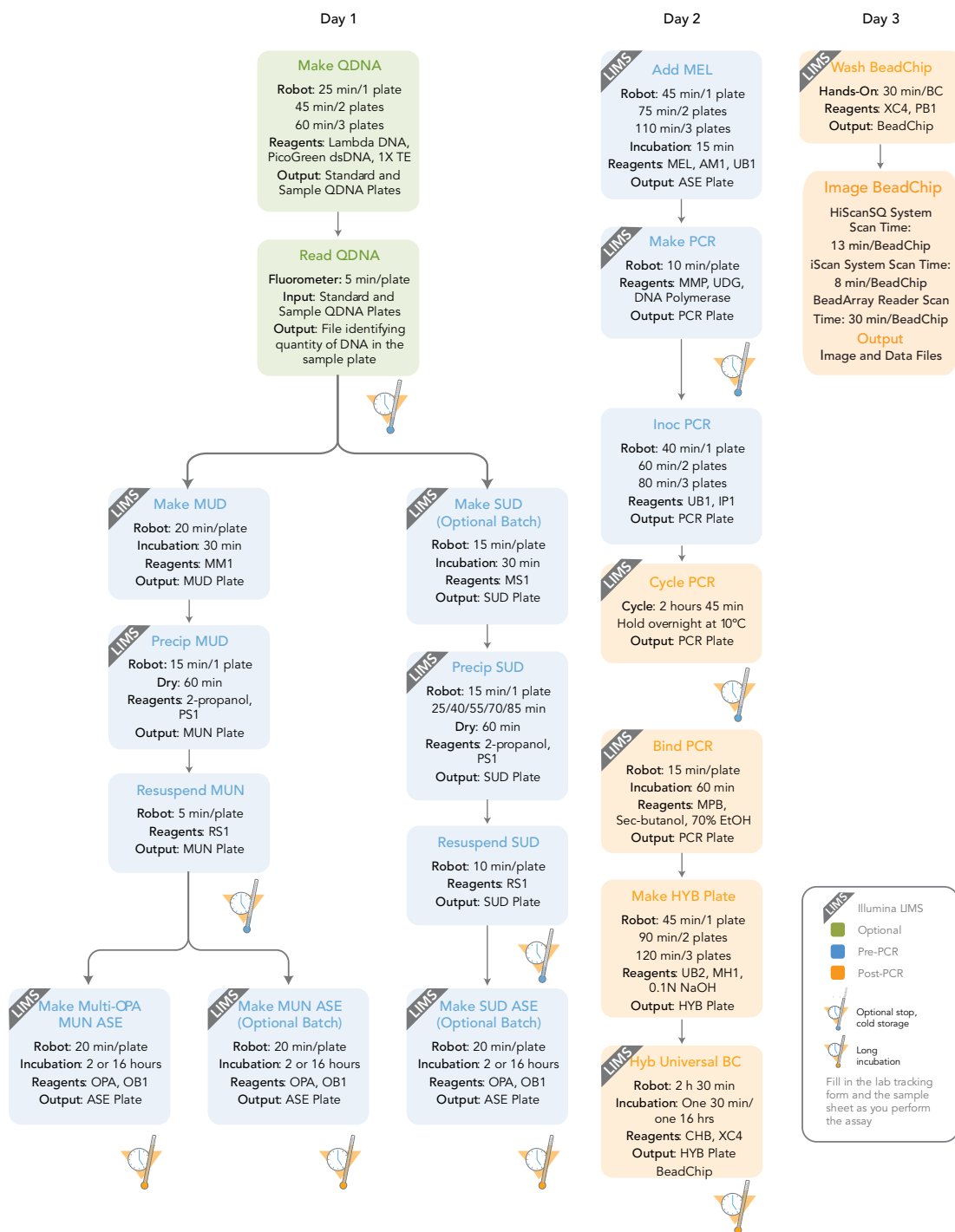


GoldenGate® Genotyping Assay, Automated

Experienced User Card

FOR RESEARCH USE ONLY



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Quantitate DNA (Optional) (Pre-PCR)

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°C 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°C 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 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 GS#-DNA plate to be quantified.
- ▶ Hand-label the microtiter plate "Standard DNA."
- ▶ Hand-label one of the Fluotrac plates "Standard 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.**
- __ 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 QDNA Standard and Sample Plates

- __ 1 At the robot PC, select **DNA Quant | Make Quant**.
- __ 2 Select the GS#-DNA plate type (MIDI, TCY or ABGN).
- __ 3 In the Basic Run Parameters pane, enter the **Number of DNA/QNT/QDNA plate(s)** (1, 2, or 3 pairs) and the **Total samples in DNA**.
- __ 4 Vortex the GS#-DNA Sample plate at 1450 rpm for 1 minute.
- __ 5 Centrifuge the GS#-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 GS#-DNA Sample, Standard DNA, Standard QDNA, and QDNA Sample plates on the robot bed according to the robot bed map. Remove any plate seals.
- __ 9 Pour the PicoGreen dilution into half reservoir A and place it on the robot bed.
- __ 10 If you are not using Illumina LIMS, clear the **Use Barcodes** checkbox. If you are using Illumina LIMS make sure the **Use Barcodes** checkbox is selected.
- __ 11 Click **Run**.
- __ 12 (Illumina LIMS only) When prompted, log in to the Illumina LIMS database.
- __ 13 Click **OK** in the message box.
- __ 14 After the robot finishes, immediately seal all plates:
 - __ a Place foil adhesive seals over Sample QDNA and Standard QDNA plates.
 - __ b Place cap mats on GS#-DNA Sample and Standard DNA plates.
- __ 15 Store the GS#-DNA and Standard DNA plates at 2°C to 8°C or -15°C to -25°C.
- __ 16 Centrifuge the Sample QDNA Plate and Standard QDNA plates to 280 xg for 1 minute.

Read QDNA Plate

- __ 1 Turn on the spectrofluorometer.
- __ 2 At the PC, open the Illumina Fluorometry Analysis software.
- __ 3 Select **Reader Tasks | Read Quant**.
- __ 4 If you are not using Illumina LIMS, clear the **Use Barcodes** checkbox. If you are using Illumina LIMS make sure the **Use Barcodes** checkbox is selected.
- __ 5 Click **Read**.
- __ 6 (Illumina LIMS only) When prompted, log in to the Illumina LIMS database.
- __ 7 When asked if you want to read a new Standard QDNA plate, click **Yes**. Remove the plate seal and load the Standard QDNA plate in the open fluorometer tray. Click **OK**.
- __ 8 Review the data from the Standard QDNA plate. Either accept it and go on to the next step, or reject it which will stop the Read Quant process.
- __ 9 Remove the Standard QDNA plate from the spectrofluorometer tray.
- __ 10 When prompted, enter the number of Sample QDNA plates you want to read Click **OK**.
- __ 11 When prompted, hand-scan or enter the Sample QDNA plate barcode. Click **OK**.
- __ 12 When prompted, remove the plate seal from the Sample QDNA plate and load it into the spectrofluorometer tray, with well A1 at the upper left corner. Click **OK**.
- __ 13 When prompted, click **Yes** to review the raw Sample QDNA plate data.
Microsoft Excel opens automatically at the same time and displays the quantitation data for the Sample QDNA plate.
- __ 14 Do one of the following:
 - Click **Yes** to send the data to Illumina LIMS.
 - Click **No** to delete the quant data.
- __ 15 If you entered more than one Sample QDNA plate to read, repeat 11 to 14 for each additional plate.
- __ 16 Do one of the following:
 - Proceed to *Make Single-Use DNA (SUD) Plate (Pre-PCR)*.
 - Store the Sample DNA plate at 2°C to 8°C for up to one month.

Make Single-Use DNA (SUD) Plate (Pre-PCR)

In this process, the robot transfers nucleic acid activator reagent (MS1) to the SUD plate, followed by 5 μ l volume containing 250 ng sample. After the robot procedure is complete, the plate is sealed and incubated on a heat block at 95°C for 30 minutes to activate the genomic DNA. The DNA plates used to make the SUD plate must already be accessioned into Illumina LIMS and assigned to the current project. For instructions, see the *Illumina LIMS User Guide*. Fill in the lab tracking form as you work.

Estimated Time

Robot: 15 minutes per plate

Incubation: 30 minutes

Consumables

Item	Quantity	Storage	Supplied By
MS1 reagent	1 tube per SUD plate	-20°C	Illumina
Genomic DNA Plate ^a	Up to 3 plates MIDI: $\geq 10 \mu$ l per sample well TCY: $\geq 20 \mu$ l per sample well	Room temperature	User
96-well 0.2 ml skirted microplate (TCY)	1 per 96 samples		User

a. Thawed, normalized to 50 ng/ μ l, diluted in 10 mM Tris pH 8.0/1 mM EDTA, and quantitated using the PicoGreen method.

Preparation

- ▶ Preheat the heat block to 95°C (one for each SUD plate). Allow 20 minutes for it to equilibrate.
- ▶ Turn on the heat sealer to preheat it.
- ▶ Thaw each MS1 tube to room temperature.
- ▶ Prepare the robot for use.
- ▶ Accession the DNA plates into Illumina LIMS and assign them to the current project, if they are not already.
- ▶ Apply a SUD barcode label to each new 96-well TCY microplate.

Steps

Set Up the Robot

- __ 1 From the robot PC, select **Universal GG SUD PrePCR | Make SUD**.
- __ 2 In the DNA Plate Selection dialog box, click on the plate type you wish to use. Roll the mouse pointer over each picture to see a description of the plate.
- __ 3 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of DNA/SUD Plate(s)** (1, 2, 3) and the **Number of samples per DNA** plate (16, 24, 32, 48, 96).
- __ 4 Pulse centrifuge each MS1 tube to 280 xg for 1 minute.
- __ 5 Place each MS1 tube in the robot tube rack according to the robot bed map. Remove the caps.
- __ 6 Vortex each GS#-DNA plate to 1450 rpm.
- __ 7 Pulse centrifuge each GS#-DNA plate to 280 xg for 1 minute.
- __ 8 Place the GS#-DNA and SUD plates on the robot bed according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (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.
 - __ d Place the WG#-DNA plate(s) on the robot bed according to the bed map and click **OK**.

Complete the Protocol

- __ 1 When the robot finishes, heat-seal each SUD plate with a foil heat seal.



CAUTION

Make sure that all wells are completely sealed, to prevent sample loss.

- __ 2 Pulse centrifuge each sealed SUD plate to 280 xg for 1 minute.
- __ 3 Vortex each plate at 2300 rpm for 20 seconds, or until all wells are completely mixed.
- __ 4 Pulse centrifuge each plate to 280 xg for 1 minute.
- __ 5 Place each sealed SUD plate in a preheated 95°C heat block and close the lid. Incubate the plate for 30 minutes.
- __ 6 (Illumina LIMS only) Log in to Illumina LIMS.
 - __ a Select **Universal GG SUD PrePCR | Incubate SUD**.
 - __ b Using the hand-held scanner, scan each SUD plate barcode into one of the boxes.
 - __ c Click **Verify**. After incubation click **Save**.

- __ 7 [Optional] After incubation, place a weight on the plate and allow it to cool for 3 minutes.
- __ 8 Pulse centrifuge each SUD plate to 3000 xg for 2 minutes.
- __ 9 If you plan to perform the Make ASE protocol today, preheat the heat block to 70°C.
- __ 10 Do one of the following:
 - Proceed to *Precipitate SUD Plate (Pre-PCR)*.
 - Store the sealed SUD plate at 4°C for up to 12 hours.

Precipitate SUD Plate (Pre-PCR)

In this process, the robot adds PS1 reagent and 2-propanol to the SUD plate to precipitate the DNA. Fill in the lab tracking form as you work.

Estimated Time

Robot:

- 1 plate: 15 minutes
- 2 plates: 25 minutes
- 3 plates: 40 minutes

Hands-on: 30 minutes

Dry: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
PS1 reagent	Bottle	4°C	Illumina
2-propanol	Bottle	Room temperature	User

Preparation

- ▶ Prepare the robot for use.
- ▶ Bring the PS1 tube and SUD plate to room temperature.

Steps

Set Up the Robot

- 1 From the robot PC, select **Universal GG SUD PrePCR | Precip SUD**.
- 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of SUD Plate(s)** (1, 2, 3) and the **Number of samples per SUD plate** (16, 24, 32, 48, 96).
- 3 Place quarter reservoir A onto the robot bed according to the robot bed map. Dispense PS1 as follows:
 - 1 plate: 2 ml
 - 2 plates: 2.5 ml
 - 3 plates: 3 ml
- 4 Place half reservoir B onto the robot bed according to the robot bed map. Dispense 2-propanol as follows:
 - 1 plate: 9 ml
 - 2 plates: 17 ml
 - 3 plates: 25 ml


- __ 5 Place each SUD plate on the robot bed according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted.
 - __ b When prompted, hand-scan the PS1 barcode.

Complete the Protocol

- __ 1 When the robot finishes, seal each SUD plate with adhesive film.
 - __ 2 Vortex each plate at 1600 rpm for 20 seconds, or until all wells are uniformly blue.
 - __ 3 Centrifuge each SUD plate to 3000 xg for 20 minutes. A faint blue pellet should be at the bottom of each well.

Perform the next step immediately to avoid dislodging the activated DNA pellets. If any delay occurs, recentrifuge to 3000 xg for 10 minutes before proceeding.
 - __ 4 Remove each SUD plate seal and decant the supernatant by inverting the plate and smacking it down onto an absorbent pad.
 - __ 5 Tap the inverted plate onto the pad to blot excess supernatant.
 - __ 6 Do one of the following:
 - Set each plate upright and allow it to dry at room temperature for 1 hour.OR
 - Place SUD plate inverted on an absorbent pad and centrifuge at 8 xg for 1 minute.
-  **CAUTION**
Do not spin the inverted plate at more than 8 xg, or sample will be lost!
- Remove SUD plate from centrifuge and allow to dry at room temperature for 15 minutes.
- __ 7 (Illumina LIMS only) During the centrifuge, log in to Illumina LIMS.
 - __ a Select **Universal GG SUD PrePCR | Spin SUD**.
 - __ b Using the hand-held scanner, scan each SUD plate barcode into one of the boxes.
 - __ c Click **Verify** and then click **Save**.
 - __ 8 Proceed to *Resuspend SUD Plate (Pre-PCR)*.

Resuspend SUD Plate (Pre-PCR)

In this process, the robot adds RS1 reagent to the SUD plate to resuspend the sample. Fill in the lab tracking form as you work.

Estimated Time

Robot: 10 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
RS1 reagent	Bottle	Room temperature	Illumina

Preparation

- ▶ Prepare the robot for use.

Steps

Set Up the Robot

- __ 1 From the robot PC, select **Universal GG SUD PrePCR | Resuspend SUD**.
- __ 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of SUD Plate(s)** (1, 2, 3) and the **Number of samples per SUD plate** (16, 24, 32, 48, 96).
- __ 3 Place quarter reservoir A onto the robot bed according to the robot bed map. Dispense RS1 as follows:
 - 1 plate: 3.5 ml
 - 2 plates: 6 ml
 - 3 plates: 8.5 ml
- __ 4 Place each SUD plate on the robot bed according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted.
 - __ b When prompted, hand-scan the RS1 barcode.

Complete the Protocol

- _ 1 When the robot finishes, seal each SUD plate with adhesive film.
- _ 2 Vortex each plate at 2300 rpm for 20 seconds, or until all wells are uniformly blue.
- _ 3 Pulse centrifuge each plate to 280 xg for 1 minute.
- _ 4 Do one of the following:
 - To create an ASE plate from a single SUD plate, proceed to *Make SUD Allele-Specific Extension (ASE) Plate (Pre-PCR)*.
 - To create an ASE plate from multiple SUD plates or multiple OPAs, see the *GoldenGate Genotyping Assay Guide*.
 - Remove the adhesive seal from each SUD plate and heat-seal the SUD plate with a foil heat seal. Store it at 4°C for up to 24 hours or at -20°C for up to 2 months.

Make SUD Allele-Specific Extension (ASE) Plate (Pre-PCR)

In this process, the robot combines the assay oligonucleotides in the OPA reagent tube and the hybridization reagents and buffers from the OB1 reagent tube in a new 96-well ASE plate. The resuspended DNA samples are transferred from the SUD plate into the ASE plate. The ASE plate is subsequently incubated to allow the activated DNA to bind with the paramagnetic particles and anneal with the oligonucleotides. Fill in the lab tracking form as you work.

Estimated Time

Robot: 20 minutes per plate

Incubation: 2–16 hours (heat block) or 16 hours (Illumina Hybridization Oven)

Consumables

Item	Quantity	Storage	Supplied By
OPA reagent	1 tube per ASE plate	-20°C	Illumina
OB1 reagent	1 tube per ASE plate	-20°C	Illumina
96-well 0.2 ml microplate (TCY)	1 per SUD plate		User

Preparation

- ▶ Preheat the heat block to 70°C (one for each ASE plate). Allow 20 minutes for it to equilibrate.
or
Set the Illumina Hybridization Oven to program 2 (heats the oven to 70°C and holds for 99 hours or indefinitely; zero rate of decrease).
- ▶ Thaw the OPA and OB1 tubes to room temperature. Vortex to 280 rpm for 1 minute to mix.
- ▶ Prepare the robot for use.
- ▶ Accession the OPAs into Illumina LIMS, if they are not already.
- ▶ Apply an ASE barcode label to each new 96-well TCY microplate.

Steps

Set Up the Robot

- __ 1 From the robot PC, select **Universal GG SUD PrePCR | Make SUD ASE**.
- __ 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of SUD/ASE Plate(s)** (1, 2, 3) and the **Number of samples per SUD** plate (16, 24, 32, 48, 96).
- __ 3 Pulse centrifuge each OPA tube to 280 xg, until the lid is free of liquid.

- __ 4 Place the OPA and OB1 tubes in the robot tube rack according to the robot bed map. Remove the caps.
- __ 5 Pulse centrifuge the SUD plate to 280 xg for 1 minute.
- __ 6 Place the SUD and ASE plates on the robot bed according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (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 OPA and SUD plates.
 - __ d When prompted, hand-scan the appropriate barcodes.

Complete the Protocol

- __ 1 When the robot finishes, heat-seal each ASE plate with a foil heat seal.
- __ 2 Vortex each plate at 1600 rpm for 1 minute.
- __ 3 If you plan to incubate the ASE plate in a heat block:
 - __ a Place each plate on a preheated 70°C heat block and close the lid.
 - __ b Immediately reset the heat block temperature to 30°C.
 - __ c Incubate the ASE plate in the heat block for 2 hours while it cools. You may leave the plate in the heat block for up to 16 hours.
- __ 4 If you plan to incubate the ASE plate in the Illumina Hybridization Oven:
 - __ a Set the high-speed shaker inside the oven to shake at 1350 rpm for 10 seconds on, 50 seconds off, overnight.
 - __ b Place the plate on the shaker and secure it with straps.
 - __ c Press **Pulse On** to begin shaking.
 - __ d Close the oven door.
 - __ e Start program 1 for oligo hybridization.
- __ 5 (Illumina LIMS only) Log in to Illumina LIMS.
 - __ a Select **Universal GG SUD PrePCR | Hyb ASE**.
 - __ b Scan each ASE plate barcode into one of the boxes.
 - __ c Click **Verify**, then **Save**.
- __ 6 Proceed to *Add Extension and Ligation Reagents (MEL) (Pre-PCR)*.

Make Multi-Use DNA (MUD) Plate (Pre-PCR)

In this process, the robot transfers nucleic acid activator reagent (MM1) to the MUD plate, followed by a 50 µl volume containing 2 µg from up to 96 genomic DNA samples. After the robot procedure is complete, the plate is sealed and incubated at 95°C for 30 minutes to activate the genomic DNA. Fill in the lab tracking form as you work.

Estimated Time

Robot: 20 minutes per plate

Incubation: 30 minutes

Consumables

Item	Quantity	Storage	Supplied By
MM1 reagent	1 tube per MUD plate	-20°C	Illumina
10 mM Tris pH 8.0/1 mM EDTA	See instructions	Room temperature	User
Genomic DNA Plate ^a	Up to 3 plates MIDI: ≥ 57 µl per sample well TCY: ≥ 47 µl per sample well	Room temperature	User
96-well 0.2 ml skirted microplate (TCY)	1 per 96 samples		User

a. Thawed, normalized to 50 ng/µl, diluted in 10 mM Tris pH 8.0/1 mM EDTA, and quantitated using the PicoGreen method.

Preparation

- ▶ Preheat the heat block to 95°C (one for each MUD plate). Allow 20 minutes for it to equilibrate.
- ▶ Turn on the heat sealer to preheat it.
- ▶ Thaw each MM1 tube to room temperature. Vortex briefly to mix.
- ▶ Apply a MUD barcode label to each new 96-well TCY microplate.
- ▶ In Illumina LIMS, accession the DNA plates and assign them to the project.

Steps

Set Up the Robot

- __ 1 From the robot PC, select **Universal GG MUD PrePCR | Make MUD**.
- __ 2 In the DNA Plate Selection dialog box, click on the plate type you wish to use. Roll the mouse pointer over each picture to see a description of the plate.
- __ 3 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of DNA/MUD plate(s)** (1, 2, 3) and the **Total samples in DNA** plate (16, 24, 32, 48, 96).
- __ 4 Pulse centrifuge each MM1 tube to 280 xg for 1 minute.
- __ 5 Place each MM1 tube in the tube rack according to the robot bed map. Remove caps.
- __ 6 Vortex each GS#-DNA plate at 1450 rpm for 1 minute.
- __ 7 Pulse centrifuge each GS#-DNA plate to 280 xg for 1 minute.
- __ 8 Place the GS#-DNA and MUD plates on the robot bed according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (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.
 - __ d Place the WG#-DNA plate(s) on the robot bed according to the bed map and click **OK**.

Complete the Protocol

- __ 1 When the robot finishes, heat-seal each MUD plate with a foil heat seal.
- __ 2 Pulse centrifuge each MUD plate to 280 xg for 1 minute.
- __ 3 Vortex each plate at 1450 rpm for 1 minute.
- __ 4 Pulse centrifuge each MUD plate to 280 xg for 1 minute.
- __ 5 Place each sealed MUD plate in a preheated 95°C heat block and close the lid. Incubate the plate for 30 minutes.
- __ 6 [Optional] After incubation, place a weight on the plate and allow it to cool for 3 minutes.
- __ 7 Pulse centrifuge each MUD plate to 3000 xg for 2 minutes.
- __ 8 If you plan to perform the Make ASE protocol today, preheat the heat block to 70°C.
- __ 9 Log in to Illumina LIMS.
- __ 10 Select **Universal GG MUD PrePCR | Incubate MUD**.
- __ 11 Using the hand-held scanner, scan each MUD plate barcode into one of the boxes.
- __ 12 Click **Verify** and then **Save**.

__ 13 Do one of the following:

- Proceed to *Precipitate MUD Plate (Pre-PCR)*.
- Store the sealed MUD plate at 4°C for up to 12 hours.

Precipitate MUD Plate (Pre-PCR)

In this process, the robot transfers activated DNA from the MUD plate to the multi-use nucleic acid (MUN) plate for precipitation. Then, the robot adds PS1 reagent and 2-propanol to the MUN plate to precipitate the DNA. Fill in the lab tracking form as you work.

Estimated Time

Robot: 15 minutes per plate

Incubation: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
PS1 reagent	Bottle	4°C	Illumina
2-propanol	Bottle	Room temperature	User
0.8 ml, V-bottom, deep well MIDI plate	1 per MUD plate		User

Preparation

- ▶ Bring all reagents and MUD plate(s) to room temperature.
- ▶ Prepare the robot for use.
- ▶ Apply a MUN barcode to each new MIDI plate.

Steps

Set Up the Robot


- __ 1 At the robot PC, select **Universal GG MUD PrePCR | Precip MUD**.
- __ 2 In the Basic Run Parameters pane, enter the **Number of MUD/MUN plate(s)** (1, 2, 3) and **Total samples in MUD** (16, 24, 32, 48, 96).
- __ 3 Place quarter reservoir A onto the robot bed according to the robot bed map. Dispense PS1 as follows:
 - 1 plate: 5 ml
 - 2 plates: 9.5 ml
 - 3 plates: 14 ml
- __ 4 Place half reservoir B onto the robot bed according to the robot bed map. Dispense 2-propanol as follows:
 - 1 plate: 15 ml
 - 2 plates: 29 ml
 - 3 plates: 43 ml

- __ 5 Place the MUD and MUN plates on the robot bed according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted.
 - __ b When prompted, hand-scan the PS1 barcode.

Complete the Protocol

- __ 1 When the robot finishes, seal each MUN plate with a cap mat.
 - __ 2 Vortex each plate at 2000 rpm for 1 minute or until the solution is uniformly blue.
 - __ 3 Centrifuge each plate to 3000 xg for 20 minutes.
Perform the next step immediately to avoid dislodging the activated DNA pellets. If any delay occurs, recentrifuge to 3000 xg for 10 minutes before proceeding.
 - __ 4 Remove the cap mat and decant the supernatant by quickly inverting each MUN plate and tapping it firmly onto an absorbent pad.
 - __ 5 Do one of the following:
 - Set each plate upright and allow it to dry at room temperature for 1 hour.OR
 - Place SUD plate inverted on an absorbent pad and centrifuge at 8 xg for 1 minute.
-  **CAUTION**
Do not spin the inverted plate at more than 8 xg, or sample will be lost!
- Remove SUD plate from centrifuge and allow to dry at room temperature for 15 minutes.
- __ 6 Log in to Illumina LIMS.
 - __ 7 Select **Universal GG MUD PrePCR | Spin MUN**.
 - __ 8 Using the hand-held scanner, scan each MUN plate barcode into one of the boxes.
 - __ 9 Click **Verify**, then **Save**.
 - __ 10 Proceed to *Resuspend MUN (Pre-PCR)*.

Resuspend MUN (Pre-PCR)

In this process, RS1 reagent is added to the multi-use DNA (MUN) plate to resuspend the sample. One MUN plate can inoculate up to six ASE plates. Fill in the lab tracking form as you work.

Estimated Time

Robot: 5 minutes per plate

Incubation: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
RS1 reagent	Bottle	Room temperature	Illumina

Preparation

- ▶ Prepare the robot for use.

Steps

Set Up the Robot

- __ 1 At the robot PC, select **Universal GG MUD PrePCR | Resuspend MUN**.
- __ 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of MUN plates** (1, 2, 3) and **Total samples in MUN** (16, 24, 32, 48, 96).
- __ 3 Place full reservoir A onto the robot bed according to the robot bed map. Dispense RS1 as follows:
 - 1 plate: 12 ml
 - 2 plates: 22 ml
 - 3 plates: 33 ml
- __ 4 Place each MUN plate on the magnetic carriers on the robot bed, according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted.
 - __ b When prompted, hand-scan the RS1 barcode.

Complete the Protocol

- _ 1 When the robot finishes, seal each MUN plate with a cap mat.
- _ 2 Vortex each plate at 1850 rpm for 1 minute.
- _ 3 Do one of the following:
 - To create an ASE plate from one MUN plate, proceed to *Make MUN ASE (Pre-PCR)*.
 - To create an ASE plate from multiple MUN plates or multiple OPAs, see the *GoldenGate Genotyping Assay Guide*.
 - Store each MUN plate at 4°C for up to 24 hours, or at -20°C for long-term storage.

Make MUN ASE (Pre-PCR)

In this process, the assay oligonucleotides in the Illumina LIMS-accessioned OPA tubes are combined with hybridization reagents and buffers (OB1) in a 96-well ASE plate. The robot then transfers the contents of the MUN plate to the ASE plate, which is subsequently incubated to bind the activated DNA with the paramagnetic particles and anneal with the oligonucleotides. Fill in the lab tracking form as you work.

Estimated Time

Robot: 20 minutes per plate

Incubation: 2–16 hours (heat block) or 16 hours (Illumina Hybridization Oven)

Consumables

Item	Quantity	Storage	Supplied By
OPA reagent	1 tube per ASE plate	-20°C	Illumina
OB1 reagent	1 tube per ASE plate	-20°C	Illumina
96-well 0.2 ml microplate (TCY)	1 per MUN plate		User

Preparation

- ▶ Preheat the heat block to 70°C (one for each ASE plate). Allow 20 minutes for it to equilibrate.
or
Set the Illumina Hybridization Oven to program 2 (heats the oven to 70°C and holds for 99 hours or indefinitely; zero rate of decrease).
- ▶ Thaw the OPA and OB1 tubes to room temperature. Vortex to 280 rpm for 1 minute to mix.
- ▶ Prepare the robot for use.
- ▶ Accession the OPAs into Illumina LIMS, if they are not already.
- ▶ Apply an ASE barcode label to each new 96-well TCY microplate.

Steps

Set Up the Robot

- __ 1 At the robot PC, select **Universal GG MUD PrePCR | Make MUN ASE**.
- __ 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of MUN/ASE plate(s)** (1, 2, 3).
- __ 3 Pulse centrifuge the OPA reagent to 280 xg.
- __ 4 Vortex the room-temperature OB1 reagent to resuspend the beads.

- __ 5 Place the OPA and OB1 tubes into the robot tube rack according to the robot bed map. Remove the caps.
- __ 6 Vortex each MUN plate to 1450 rpm for 1 minute.
- __ 7 Pulse centrifuge each MUN plate to 280 xg.
- __ 8 Place each MUN plate on the robot bed according to the robot bed map. Remove the cap mats.
- __ 9 Place each new ASE plate on the robot bed according to the robot bed map.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (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 OPA and MUN plates.
 - __ d When prompted, hand-scan the appropriate barcodes.

Complete the Protocol

- __ 1 When the robot finishes, heat-seal each ASE plate with a foil seal.
- __ 2 Vortex each ASE plate at 1600 rpm for 1 minute.
- __ 3 If you plan to incubate the ASE plate in a heat block:
 - __ a Place each plate in a preheated 70°C heat block and close the lid.
 - __ b Immediately reset the heat block temperature to 30°C.
 - __ c Incubate the ASE plate in the heat block for 2 hours while it cools. You may leave the plate in the heat block for up to 16 hours.
- __ 4 If you plan to incubate the ASE plate in the Illumina Hybridization Oven:
 - __ a Set the high-speed shaker inside the oven to shake at 1350 rpm for 10 seconds on, 50 seconds off, overnight.
 - __ b Place the plate on the shaker and secure it with straps.
 - __ c Press **Pulse On** to begin shaking.
 - __ d Close the oven door.
 - __ e Start program 1 for oligo hybridization.

Table 1 Hybridization Oven Program 1 (Oligo Hybridization)

Heats the Oven to	Holds the Temp for	Rate of Decrease (°/min)
70°C	10 minutes	-1
60°C	5 minutes	-0.1
58°C	10 minutes	-0.1
56°C	10 minutes	-0.1

Table 1 Hybridization Oven Program 1 (Oligo Hybridization)

Heats the Oven to	Holds the Temp for	Rate of Decrease (°/min)
36°C	Time necessary to reach temperature	—
30°C	Indefinitely	0.0

- __ 5 (Illumina LIMS only) Log in to Illumina LIMS.
 - __ a Select **Universal GG MUD PrePCR| Hyb ASE**.
 - __ b Scan each ASE plate barcode into one of the boxes.
 - __ c Click **Verify**, then **Save**.
- __ 6 Proceed to *Add Extension and Ligation Reagents (MEL) (Pre-PCR)*.

Add Extension and Ligation Reagents (MEL) (Pre-PCR)

In this protocol, hybridized ASO-LSO pairs on the bound genomic DNA are extended and ligated. The robot first transfers AM1 and UB1 reagents to the ASE plate to wash away non-specifically hybridized and excess oligos from the DNA, and then transfers MEL (extension and ligation enzymes) reagent to each sample in the ASE plate.

Next, the ASE plate is incubated at 45°C for 15 minutes to allow (1) the 3' ends of the properly hybridized ASOs to extend downstream to the 5' ends of their paired LSO, and (2) the extended 3' ASO ends to ligate to the 5' LSO ends. Fill in the lab tracking form as you work.

Estimated Time

Robot:

- 1 plate: 45 minutes
- 2 plates: 1 hour, 15 minutes
- 3 plates: 1 hour, 50 minutes

Incubation: 15 minutes

Consumables

Item	Quantity	Storage	Supplied By
MEL reagent	1 tube per ASE plate	-20°C	Illumina
AM1 reagent	Bottle	4°C	Illumina
UB1 reagent	Bottle	4°C	Illumina

Preparation

- ▶ Preheat the heat block to 45°C (one for each plate) and allow it to equilibrate.
- ▶ Thaw all reagents to room temperature. Invert 10 times to mix.
- ▶ Prepare the robot for use.

Steps

Set Up the Robot

- 1 From the robot PC, select **Universal GG SUD PrePCR | Add MEL** if you're running a single-use DNA project or select **Universal GG MUD PrePCR | Add MEL** for a multi-use DNA project.
- 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of ASE plate(s)** (1, 2, 3) and the **Number of samples per ASE** (16, 24, 32, 48, 96).
- 3 Pulse centrifuge each MEL tube to 280 xg for 1 minute.
- 4 Place each MEL tube in the robot tube rack according to the robot bed map. Remove the cap.

- __ 5 Place quarter reservoir A onto the robot bed according to the robot bed map. Dispense AM1 reagent as follows:
 - 1 ASE plate: 15 ml
 - 2 ASE plates: 30 ml
 - 3 ASE plates: 45 ml
- __ 6 Place half reservoir B onto the robot bed according to the robot bed map. Dispense UB1 reagent as follows:
 - 1 ASE plate: 15 ml
 - 2 ASE plates: 30 ml
 - 3 ASE plates: 45 ml
- __ 7 Pulse centrifuge each ASE plate to 280 xg for 1 minute.
- __ 8 Place each ASE plate on the magnetic carrier on the robot bed according to the robot bed map.
- __ 9 Wait for all of the beads to be captured by the magnet, which takes about 2 minutes, and then remove the plate seal.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted.
- __ 3 When prompted, remove each ASE plate from the robot bed and seal it with an adhesive seal.
- __ 4 Vortex each sealed plate at 1600 rpm for 1 minute.
- __ 5 Return each ASE plate to the correct position on the robot bed according to the bed map. Remove the seal and then click **OK**.
- __ 6 When prompted, remove each ASE plate from the robot bed and seal it with an adhesive seal.
- __ 7 Vortex each plate at 1600 rpm for 1 minute or until all beads are completely resuspended.
- __ 8 Return each plate to the correct position on the robot bed according to the bed map. Remove the seal and then click **OK**.
- __ 9 When prompted, remove each ASE plate from the robot bed.
- __ 10 Seal each ASE plate with an adhesive seal.
- __ 11 Vortex each plate at 1725 rpm for 1 minute. Click **OK** in the message box.

Complete the Protocol

- __ 1 Place each sealed plate on the preheated 45°C heat block and close the lid. Incubate the plate for exactly 15 minutes.
- __ 2 (Illumina LIMS only) Log in to Illumina LIMS.
 - __ a Select **Universal GG SUD PrePCR | Incubate ASE** if you're running a single-use DNA project or select **Universal GG MUD PrePCR | Incubate ASE** for a multi-use DNA project.
 - __ b Scan each ASE barcode into one of the boxes.

- __ c Click **Save** when complete.
- __ d Click **Verify**.
- __ 3 While the ASE plate is incubating, perform the Make PCR process.
- __ 4 Do one of the following:
 - Proceed to *Make PCR Plate (Pre-PCR)*.
 - Store the ASE plate at 4°C for up to 1 hour.

Make PCR Plate (Pre-PCR)

In this process, the robot adds an Illumina-recommended DNA polymerase and (optionally) Uracil DNA Glycosylase (UDG) to the master mix for PCR (the MMP reagent), creating a 96-sample plate for use in the Inoc PCR process. The PCR master mix contains buffer components, dNTPs, and PCR primers. Fill in the lab tracking form as you work. Absent from the master mix is the polymerase enzyme, which is added to each tube before the process begins.

Estimated Time

Robot: 10 minutes per plate

Consumables

Item	Quantity	Storage	Supplied By
MMP reagent	1 tube per PCR plate	-20°C	Illumina
DNA Polymerase	64 µl per PCR plate	-20°C	Illumina
Uracil DNA Glycosylase (UDG, Optional)	50 ml per PCR plate	-20°C	User
96-well 0.2 ml microplate (TCY)	1 per ASE plate		User

Preparation

- ▶ Thaw the MMP reagent to room temperature. Invert 10 times to mix.
- ▶ Prepare the robot for use.
- ▶ Apply a PCR barcode label to each new 96-well TCY microplate.

Steps

Set Up the Robot

- __ 1 From the robot PC, select **Universal GG SUD PrePCR | Make PCR** if you're running a single-use DNA project or select **Universal GG MUD PrePCR | Make PCR** for a multi-use DNA project.
- __ 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of PCR plate(s)** (1 to 9) and the **Number of samples per PCR** (16, 24, 32, 48, 96).
- __ 3 Add 64 µl DNA polymerase to each MMP tube.
- __ 4 [Optional] Add 50 µl Uracil DNA Glycosylase (UDG) to each MMP tube.
- __ 5 Cap each MMP tube and invert it several times to mix the contents.
- __ 6 Pulse centrifuge each MMP tube to 280 xg for 1 minute.

- __ 7 (Illumina LIMS only) Log in to Illumina LIMS.
 - __ a Select **Universal GG SUD PrePCR | Add Enzyme** if you're running a single-use DNA project or select **Universal GG MUD PrePCR | Add Enzyme** for a multi-use DNA project.
 - __ b Enter the lot number of the DNA polymerase in the **Enzyme Lot** box.
 - __ c Enter the UDG Lot number in the **UDG Lot** box.
 - __ d Scan each MMP barcode into one of the boxes.
 - __ e Click **Verify**, then **Save**.
- __ 8 Place each MMP tube in the robot tube rack according to the robot bed map. Remove the cap.
- __ 9 Place each PCR plate on the robot bed according to the robot bed map.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted.

Complete the Protocol

- __ 1 Seal each PCR plate with clear adhesive film.
- __ 2 Do one of the following:
 - Proceed to *Inoculate PCR Plate (Pre-PCR)*.
 - Place each sealed PCR plate in a vacuum-sealed autobag and store it at -20°C for up to 30 days.

Inoculate PCR Plate (Pre-PCR)

In this process, the PCR master mix is inoculated with the extended and ligated ASO-LSO products from the Add MEL process. First, the ASE plate is placed on the robot bed, where the ligation reaction mix is washed away and the samples are resuspended in elution buffer. Next, the plate is placed on a 95°C heat block for 1 minute to trigger heat denaturation. This releases single-stranded ASO-LSO products from the bound DNAs back into solution. The ASE plate is returned to the robot bed, where the robot transfers ASO-LSO solution to a prepared PCR plate that contains PCR master mix. Fill in the lab tracking form as you work.

Estimated Time

Robot:

- 1 ASE plate: 40 minutes
- 2 ASE plates: 60 minutes
- 3 ASE plates: 80 minutes

Consumables

Item	Quantity	Storage	Supplied By
IP1 reagent	1 tube per PCR plate	-20°C	Illumina
UB1 reagent	Bottle	4°C	Illumina

Preparation

- ▶ Preheat the heat block to 95°C (one for each PCR plate).
- ▶ Thaw the IP1 reagent to room temperature. Vortex to 250 rpm to mix.
- ▶ If the PCR plate is frozen, thaw for at least 20 minutes, and then pulse centrifuge to 280 xg.
- ▶ Prepare the robot for use.

Steps

Set Up the Robot

- 1 From the robot PC, select **Universal GG SUD PrePCR | Inoc PCR** if you're running a single-use DNA project or select **Universal GG MUD PrePCR | Inoc PCR** for a multi-use DNA project.
- 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of ASE/PCR plate(s)**(1, 2, or 3 pairs) and the **Number of samples per ASE** (16, 24, 32, 48, 96).
- 3 Invert each IP1 tube 10 times to mix.
- 4 Pulse centrifuge each IP1 tube to 280 xg for 1 minute.
- 5 Place each IP1 tube in the robot tube rack according to the robot bed map. Remove the cap.

- __ 6 Place quarter reservoir A onto the robot bed according to the robot bed map. Dispense UB1 as follows:
 - 1 PCR plate: 10 ml
 - 2 PCR plates: 15 ml
 - 3 PCR plates: 20 ml
- __ 7 Place each ASE plate on the magnetic carrier on the robot bed according to the robot bed map. Remove the plate seal.
- __ 8 Place each PCR plate on the robot bed according to the robot bed map. Remove the plate seal.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted.
 - __ b When prompted, hand-scan the UB1 barcode.
- __ 3 When prompted, remove each ASE plate from the robot bed and seal it with a clear adhesive seal.
- __ 4 Vortex each sealed plate at 1900 rpm for 1 minute, or until resuspended.
- __ 5 Place each sealed plate in a 95°C heat block and incubate for exactly 1 minute.
- __ 6 Return each plate to its original position on the robot bed. Remove the seal and then click **OK**.

The robot waits for 1 minute to allow the beads to settle completely, and then inoculates the PCR plate with the denatured contents of the ASE plate.

Complete the Protocol

- __ 1 Remove the ASE and PCR plates from the robot bed. Seal the PCR plates with the appropriate PCR plate-sealing film for your thermal cycler.
- __ 2 Place the plates and the lab tracking worksheets in a transfer box for the post-PCR area.
- __ 3 Immediately proceed to *Cycle the PCR Plate (Post-PCR)*.
- __ 4 Discard the ASE plate.

Cycle the PCR Plate (Post-PCR)

This process thermal cycles the PCR plate to fluorescently label and amplify the templates generated in the pre-PCR process. Three universal primers are included in the reaction mix:

- ▶ Cy3-labelled complement to allele-specific oligo (ASO) 1
- ▶ Cy5-labelled complement to ASO 2
- ▶ Biotinylated complement to locus-specific oligo (LSO)

The fluor-labelled primers provide the signal for detection, while the biotinylated primer allows for immobilization of the PCR product. The result is highly amplified double-stranded PCR products containing a fluor-labelled strand (Cy3 or Cy5, depending on the genotype) and a biotinylated strand. If using the Thermocycler Control Software (TCCS) option package, refer to the Thermocycler Control Software User Guide for additional information on using the software with Illumina LIMS.

Estimated Time

Thermocycler: ~2 hours 45 minutes

Steps

- __ 1 Turn on the Thermocycler. (TCCS only) For Tetrad 2, make sure it is operating in auto-remote mode.
- __ 2 (TCCS only) Turn on the PC and start the Thermocycler Control Software.
- __ 3 (TCCS only) Make sure the LIMS Mode box is checked and log in to Illumina LIMS.
- __ 4 (TCCS only) Enter the name of the thermocycler, the block number, and the PCR plate barcode in the Barcode Entry fields. Click **OK**.
The PCR plate is verified in LIMS and passed to the correct thermocycler block.
- __ 5 Place the sealed plate into the Thermocycler and run the program shown in this table.



NOTE

Illumina LIMS will already select the correct program from the Thermocycler when using the TCCS.

Table 2 Thermocycler Program

	Temperature	Time
	37°C	10 minutes
	95°C	3 minutes
X 34 {	95°C	35 seconds
	56°C	35 seconds
	72°C	2 minutes
	72°C	10 minutes

Table 2 Thermocycler Program

	Temperature	Time
	4°C	5 minutes

- __ 6 (TCCS only) Press **Run**, or **Run All** if you are starting multiple plates in one transaction. The TCCS is now available to start other protocols in the remaining available blocks.
- __ 7 (TCCS only) When complete, the TCCS will automatically save the PCR info to Illumina LIMS. Click the **Stop** button to terminate the thermocycler program.
- __ 8 (Illumina LIMS only) Log in to Illumina LIMS.
 - __ a Select **Universal GG SUD PostPCR | Cycle PCR** if you're running a single-use DNA project or select **Universal GG MUD PostPCR | Cycle PCR** for a multi-use DNA project.
 - __ b Scan each PCR plate barcode into one of the boxes.
 - __ c Enter the Thermocycler and Unit numbers.
 - __ d Click **Verify**, then **Save**.
- __ 9 Do one of the following:
 - Proceed immediately to *Bind PCR Products (Post-PCR)*.
 - Store the sealed plate at room temperature in a light-protected location for processing later that day.
 - Store the sealed plate at -20°C for up to 24 hours.

Bind PCR Products (Post-PCR)

In this process, MPB reagent and sec-butanol are added to the PCR plate. The plate is incubated at room temperature to bind the biotinylated strand to paramagnetic particles, thus immobilizing the double-stranded PCR products. Fill in the lab tracking form as you work.

Estimated Time

Robot: 15 minutes per plate

Incubation: 60 minutes

Consumables

Item	Quantity	Storage	Supplied By
MPB reagent	1 tube per PCR plate	4°C	Illumina
Sec-butanol	5 ml per PCR plate	Room temperature	User

Preparation

- ▶ Bring the MPB reagent to room temperature.
- ▶ Thaw each PCR plate at room temperature for at least 15 minutes in a light-protected location.
- ▶ Prepare the robot for use.

Steps

Set Up the Robot

- __ 1 From the robot PC, select **Universal PostPCR | Bind PCR**.
- __ 2 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of PCR plate(s)** (1 to 6) and the **Number of samples per PCR** (16, 24, 32, 48, 96).
- __ 3 Vortex each MPB tube at tube vortexer speed 6 for 30 seconds or until the beads are resuspended.
- __ 4 Place each MPB tube in the robot tube rack according to the robot bed map. Remove the cap.
- __ 5 Place quarter reservoir A onto the robot bed according to the robot bed map. Dispense sec-butanol as follows:
 - 1 PCR plate: 5 ml
 - 2 PCR plates: 10 ml
 - 3 PCR plates: 15 ml
- __ 6 Centrifuge each PCR plate to 3000 xg for 30 seconds.

- __ 7 Place each PCR plate on the robot bed according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted.
 - __ b Click **Verify**.



NOTE

If you pause the robot for any reason, remove the MPB tube from the rack, re-vortex it, and return it to the rack in its original position before restarting the robot.

Complete the Protocol

- __ 1 When the robot finishes, seal each PCR plate with adhesive film.
- __ 2 Incubate each PCR plate in a light-protected location for 60 minutes.
- __ 3 (Illumina LIMS only) Log in to Illumina LIMS.
 - __ a Select **Universal PostPCR | Incubate PCR**.
 - __ b Scan each PCR plate barcode into one of the boxes.
 - __ c Click **Verify**, then **Save**.
- __ 4 Do one of the following:
 - Proceed to *Make HYB Plate*.
 - Store each sealed PCR plate at 4°C for up to 4 hours.

Make HYB Plate (Universal BeadChip) (Post-PCR)

In this process, the robot transfers the single-stranded, fluor-labelled PCR product from the PCR plate to a new HYB plate that is suitable for pairing with a Universal BeadChip.

The PCR plate is placed on a magnetic carrier on the robot, where it is washed with UB2 reagent to remove all previous reagents (PCR master mix, sec-butanol, and unbound PCR products). Next, the fluor-labeled strands are denatured from the bound biotinylated strands and transferred to a clear 96-well TCY microplate (the HYB plate).

Estimated Time

Robot:

- 1 HYB plate: 45 minutes
- 2 HYB plates: 90 minutes
- 3 HYB plates: 120 minutes

Consumables

Item	Quantity	Storage	Supplied By
MH1 reagent	1 tube per HYB plate	Room temperature	Illumina
UB2 reagent	Bottle	Room temperature	Illumina
0.1N NaOH	Bottle	4°C	User
96-well 0.2 ml microplate (TCY)	2 per PCR plate		User

Preparation

- ▶ If you plan to proceed immediately to Hyb Universal BC, then preheat the hybridization oven to 60°C.
- ▶ Make sure that all reagents are at room temperature.
- ▶ Prepare the robot for use.
- ▶ Apply a INT barcode label to each new 96-well TCY microplate.
- ▶ Apply a HYB barcode label to each 96-well TCY microplate.

Steps

Set Up the Robot

- 1 From the robot PC, select **Universal PostPCR | Make HYB**.
- 2 Click **OK** to confirm you are using the correct hyb plate type.

- __ 3 (Non-Illumina LIMS) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane, enter the **Number of PCR/HYB plate(s)** (1, 2, or 3 pairs).
- __ 4 Pulse centrifuge each MH1 tube to 280 xg.
- __ 5 Place each MH1 tube in the robot tube rack according to the robot bed map. Remove the cap.
- __ 6 Place quarter reservoir A and B onto the robot bed according to the robot bed map. Dispense 0.1N NaOH into reservoir B as follows:
 - 1 HYB plate: 10 ml
 - 2 HYB plates: 15 ml
 - 3 HYB plates: 20 ml
- __ 7 Dispense UB2 into reservoir A as follows:
 - 1 HYB plate: 10 ml
 - 2 HYB plates: 15 ml
 - 3 HYB plates: 22 ml
- __ 8 Place the PCR, INT, and HYB plates on the robot bed according to the robot bed map. Remove any plate seals.

Start the Robot

- __ 1 (Non-Illumina LIMS) At the robot PC, click **Run**. Click **OK** to confirm you are using a 96-well hyb plate.
- __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**. Click **OK** to confirm you are using a 96-well hyb plate.
 - __ a Log in when prompted.
 - __ b When prompted, hand-scan the UB2 barcode at the robot PC.
 - __ c Click **Accept**.

Complete the Protocol

- __ 1 Remove each HYB plate from the robot bed and seal with clear adhesive film. Protect the plates from light.
- __ 2 Centrifuge each HYB plate at 3000 xg for 4 minutes to remove bubbles.
- __ 3 Do one of the following:
 - Immediately proceed to *Hybridize Universal BeadChip (Post-PCR)*.
 - If you do not plan to use the HYB plate immediately in the hybridization protocol, store it at -15° to -25°C for up to 24 hours.

Hybridize Universal BeadChip (Post-PCR)

In this process, DNA samples from the HYB plate are dispensed onto the BeadChips. The BeadChips are hybridized using the Hyb Chamber. After the Hyb Chamber has been assembled, the DNA-loaded BeadChips are placed into the Hyb Chamber and the samples are ready for hybridization. The BeadChip is hybridized overnight in the Illumina Hybridization Oven, with a temperature ramp-down from 60°C to 45°C.

Estimated Time

Robot time: ~15 minutes per plate

Incubation time: One 30 minute incubation, one 16–18 hour incubation

Consumables and Equipment

Item	Quantity	Storage	Supplied By
CHB reagent	1 tube per 48 samples	Room temperature	Illumina
XC4 reagent	Bottle	Room temperature	Illumina
100% EtOH	Bottle	Room temperature	User
Hyb Chamber	1 per 4 BeadChips		Illumina
Hyb Chamber inserts	1 per BeadChip		Illumina
Hyb Chamber gaskets	1 per Hyb Chamber		Illumina
Robot BeadChip Alignment Fixtures	1 per 96 samples		Illumina
Robot Tip Alignment Guide-C (32x1 BeadChip only)	1 per 96 samples		Illumina
BeadChips (12x1)	8 per 96 samples	2°C to 8°C	Illumina
BeadChips (32x1)	3 per 96 samples	2°C to 8°C	Illumina

Preparation

- ▶ Preheat the Illumina Hybridization Oven to 60°C. Allow 30 minutes for it to equilibrate.
- ▶ If the HYB plate has been frozen, thaw it completely at room temperature for at least four hours in a light-protected drawer, and then pulse centrifuge it to 250 xg for 1 minute.
- ▶ Remove the BeadChips from cold storage (2°C–8°C) at least ten minutes before you begin the Hyb process but do not unpackage. Leave them on the benchtop (no longer than 24 hours) in their packages until you are ready to begin.

- ▶ (Illumina LIMS only) In the Illumina LIMS left sidebar, click **Universal PostPCR | Confirm BeadChips for Hyb**.
 - __ a Scan the barcode(s) of the HYB plate and scan the BeadChip barcodes. Click **Verify**.

Prepare Robot Tip Alignment Guides

- __ 1 Wash and dry the Robot Tip Alignment Guides prior to each use. See *Wash Robot Tip Alignment Guides* at the end of the Hyb Universal BC steps for washing instructions.
- __ 2 Make sure you have the correct Robot Tip Alignment Guide for the GoldenGate assay. The guide barcode should say Guide-C on it.

Steps

Assemble the Hyb Chambers

- __ 1 Place the following items on the bench top:
 - BeadChip Hyb Chamber (1 per 4 BeadChips)
 - BeadChip Hyb Chamber gasket (1 per Hyb Chamber)
 - BeadChip Hyb Chamber inserts (4 per Hyb Chamber)
- __ 2 Place the Hyb Chamber Gasket into the Hyb Chamber.
- __ 3 Add 200 µl CHB into each of the eight humidifying buffer reservoirs in the Hyb Chamber.
- __ 4 Close and lock the BeadChip Hyb Chamber lid.
- __ 5 Leave the closed Hyb Chamber on the bench at room temperature until the BeadChips are loaded with DNA sample.

Load BeadChips




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 Guides* at the end of the *Hyb Universal BC* section.

- __ 1 Remove all BeadChips from their packages.
- __ 2 For the Universal-12 BeadChip, place up to two BeadChips into each Robot BeadChip Alignment Fixture (two BeadChip version) with the barcode end aligned to the ridges on the fixture.
For the Universal-32 BeadChip, place up to three BeadChips into each vertical Robot BeadChip Alignment Fixture slot (three BeadChip version) with the barcode end aligned to the ridges on the fixture.
- __ 3 From the robot PC, select **Universal PostPCR | Hyb Universal BC**.
- __ 4 Choose the plate type that matches your HYB plate from the **Hyb Universal BC - Hyb Plate Selection** dialog box.
- __ 5 Choose the appropriate BeadChip type from the dialog box.
- __ 6 (Illumina LIMS only) Make sure the **Use Barcodes** check box is checked.

- __ 7 (Non-Illumina LIMS only) Make sure the **Use Barcodes** check box is cleared. In the Basic Run Parameters pane choose the **Number of Hyb Plates** and the **Number of DNA samples per plate**.
- __ 8 Place the Robot BeadChip Alignment Fixtures onto the robot bed according to the bed map. Push the Robot BeadChip Alignment Fixture to the upper left corner in its section of the robot bed. If you are using the Universal-32 BeadChip make sure the silver arrow is pointing to the top left when you place the Robot BeadChip Alignment Fixture on the Robot bed.

Start the Robot

- __ 1 (Non-Illumina LIMS) If you are using the Universal-32 BeadChip, place a Guide-C Robot Tip Alignment Guide on top of each Robot BeadChip Alignment Fixture. The Guide-C barcode should be on the left side of the plate. Push both the Robot Tip Alignment Guide and Robot BeadChip Alignment Fixture to the upper left corner in its section of the robot bed.
 - __ a At the robot PC, click **Run**.
 - __ b Log in when prompted.
 - __ c Click **OK** to confirm you have placed the Robot BeadChip Alignment Fixtures loaded with BeadChips on the deck.
 - __ d If you are using the Universal-32 BeadChip, click **OK** to confirm you have correctly placed the Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture.
 - __ 2 (Illumina LIMS only) Make sure the **Use Barcodes** check box is selected and click **Run**.
 - __ a Log in when prompted. The robot scans the barcodes on the HYB plate(s) and BeadChips to confirm the correct BeadChips are loaded. Once the correct BeadChips are confirmed, the robot pauses.
 - __ b If you are using the Universal-32 BeadChip, place the Guide-C Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture . The Guide-C barcode should be on the left side of the plate. Push both the Robot Tip Alignment Guide and Robot BeadChip Alignment Fixture to the upper left corner in its section of the robot bed.
-  **NOTE**
There is no Robot Tip Alignment Guide used for the Universal-12 BeadChip.
- __ c At the robot PC, click **OK** to confirm you have placed the Robot Tip Alignment Guide on top of the Robot BeadChip Alignment Fixture.
 - __ d If you are using the Universal-32 BeadChip the robot scans the barcode on the Robot Tip Alignment Guide to confirm the correct tip guide is being used.
- __ 3 The robot dispenses sample to the BeadChips.
 - __ 4 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 Guides* at the end of this section for wash instructions.

- __ 5 Discard the HYB plate if you have not done so.
- __ 6 To ensure optimal performance, immediately continue on to the *Set Up Universal BeadChip for Hyb*.

Set Up Universal BeadChip for Hyb

- __ 1 Ensure the Illumina Hybridization Oven is set to 60°C.
- __ 2 Open the Hyb Chamber.
- __ 3 Load up to four Hyb Chamber Inserts containing sample-laden BeadChips into each Hyb Chamber.
- __ 4 (Illumina LIMS only) In the Illumina LIMS left sidebar, click **Universal PostPCR | GoldenGate Prepare Hyb Chamber**.
 - __ a Scan the barcode(s) of the CHB tube(s) and scan the BeadChip barcodes. Click **Verify** and then **Save**.
- __ 5 Close and lock the BeadChip Hyb Chamber lid.

Hybridize BeadChips

- __ 1 Place the Hyb Chamber into the 60°C Illumina Hybridization Oven so that the clamps face the left and right sides of the oven. The Illumina logo on top of the Hyb Chamber should face you.
- __ 2 (Optional) Start the rocker at speed 5.
- __ 3 Incubate for exactly 30 minutes at 60°C.
- __ 4 After 30 minutes, reset the temperature to 45°C.
- __ 5 Incubate for at least 16 hours but no more than 18 hours at 45°C.
- __ 6 Proceed to *Wash & Coat BeadChip (Post-PCR)*.

Resuspend XC4 Reagent for Washing BeadChip

- __ 1 Add 335 ml 100% EtOH to the XC4 bottle. The final volume will be 350 ml.
- __ 2 Shake vigorously for 15 seconds.
- __ 3 Leave the bottle upright on the lab bench overnight.
- __ 4 Shake again to ensure that the pellet is completely resuspended. If any coating is visible, vortex at 1625 rpm until it is in complete suspension.

Wash Robot Tip Alignment Guides

- 1 Soak the Robot Tip Alignment Guides in a 1% aqueous Alconox solution (one part Alconox to 99 parts water) using a 400 ml Pyrex beaker for 5 minutes. Do not use bleach or ethanol to clean the Robot Tip Alignment Guides.
- 2 After the 5 minute soak in the 1% Alconox solution, thoroughly rinse the Robot Tip Alignment Guide(s) with DiH₂O at least three times to remove any residual detergent. Make sure the DiH₂O runs through all the tip guide channels.
- 3 Dry the Robot Tip Alignment Guides, especially the channels, using a Kimwipe or lint-free paper towels. Use a laboratory air gun to ensure they are dry. Be sure to inspect the channels, including the top and bottom of the insert. Robot Tip Alignment Guides should be completely dry and free of any residual contaminants before next use.

Wash & Coat BeadChip (Post-PCR)

In this process, the BeadChips are removed from the Hyb Chamber and washed three times with PB1 and XC4 reagents.

Estimated Time

Hands-on time: ~1 hour

Consumables and Equipment

Item	Quantity	Storage	Supplied By
PB1 reagent	Bottle	Room temperature	Illumina
XC4 reagent	Bottle	Room temperature	Illumina
Wash Dish	12 BeadChips: 3 dishes		Illumina
Wash Rack	12 BeadChips: 1 rack		Illumina
Vacuum desiccator	(1 per 12 BeadChips processed simultaneously)		Illumina
Self-locking tweezers	1		Illumina

Preparation

- ▶ If you are using the BeadArray Reader, turn it on 1–2 hours before imaging.
- ▶ If you are using the iScan or HiScan Reader, turn it on five minutes before imaging.
- ▶ If the XC4 has not already been prepared, then add 335 ml 100% EtOH to the bottle and place it on a rocker for 30–40 minutes to resuspend. When it is resuspended, fill a wash dish with the XC4 and label the dish “XC4”.
- ▶ Fill two wash dishes with PB1 (300 ml per wash dish). Label each dish “PB1”.

Steps

Wash and Coat BeadChips

- __ 1 Submerge the unloaded wash rack into the first PB1 wash dish with the locking arms facing you. This orients the wash rack so that you can safely remove the BeadChips.
- __ 2 Remove up to 3 Hyb Chambers containing BeadChips from the Hyb oven at one time. Leave any remaining Hyb Chambers in the oven. Do not open all of the Hyb Chambers at once. Only open a Hyb Chamber when you are ready to remove the seals from the BeadChips. If you have more than one Hyb Chamber to process, leave the other Hyb Chambers closed on the bench while you process the first Hyb Chamber.

No longer than five minutes should elapse from the time the first Hyb Chamber is opened until the last BeadChip is placed in the wash rack and submerged in the first PB1 wash.

If there are more than 3 Hyb Chambers worth of BeadChips to process, complete the wash and coat step for the first 12 BeadChips, replace the PB1 in the wash dishes with fresh PB1, and follow this same step.

- __ 3 With a hand on top of the first Hyb Chamber, un-snap the four clips one at a time. Start with the first clip, then un-snap the clip that is diagonally across from it and so on. Lift the lid straight up and off. Set the lid to the side on the bench.
- __ 4 Remove the first BeadChip from a Hyb Chamber insert.
- __ 5 Remove the IntelliHyb Seal from the BeadChip:
 - __ a Wearing powder-free gloves, hold the BeadChip in one hand with your thumb and forefinger on opposing edges of the BeadChip. Do not touch the sample inlets. The barcode should face 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. Do not stop and start the pulling action. Do not touch the exposed active areas.
 - __ c Discard the seal.
- __ 6 Holding the BeadChip by the barcode end, immediately and carefully slide the BeadChip into the wash rack while it is submerged in PB1.
If necessary, briefly lift the wash rack out of the wash dish to seat the BeadChip. Replace it immediately after inserting the BeadChip. Place BeadChips in every other slot.
- __ 7 Ensure that the BeadChip barcodes are correctly positioned in the wash rack, with the labels facing up and away from you. This is essential for proper handling and coating.
- __ 8 Load the wash rack with up to 12 BeadChips. Put six BeadChips above the wash rack handle and six below. Try to evenly space the BeadChips in the rack.
- __ 9 Once all BeadChips are in the wash rack, move the wash rack up and down ten times, breaking the surface of the PB1 with gentle, slow agitation.
- __ 10 Transfer the wash rack to the second PB1 wash dish and let it soak for five minutes.
- __ 11 Transfer the wash rack to the XC4 wash dish and slowly move the wash rack up and down ten times. Let it soak for five minutes.



NOTE

You can use the XC4 for two sets of 12 BeadChips processed in succession. Use fresh XC4 if you plan to process more than 24 BeadChips in succession.

Dry BeadChips

- __ 1 Prepare a clean tube rack for the wash rack by placing two folded Kimwipes under the tube rack.
- __ 2 Remove the wash rack in one smooth, rapid motion and place it directly on the prepared tube rack, making sure the barcodes *face up* and the locking arms and tab *face down*.
- __ 3 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.
- __ 4 Place the tube rack in the vacuum desiccator. Each dessicator can hold one tube rack.
- __ 5 Ensure the vacuum valve is seated tightly and securely.
- __ 6 Start the vacuum, using at least 508 mm Hg (0.68 bar).
- __ 7 Dry under vacuum for 50–55 minutes.
- __ 8 (Illumina LIMS only) Log in to Illumina LIMS.
 - __ a In the Illumina LIMS left sidebar, click **Universal PostPCR | Coat Universal BC**.
 - __ b Scan the barcode(s) of the PB1, XC4, and BeadChips and click **Verify** and then click **Save**.
- __ 9 Clean the underside of each BeadChip to remove any excess XC4 with a ProStat EtOH wipe.
- __ 10 Clean the Hyb Chambers:
 - __ a Remove the rubber gaskets from the Hyb Chambers.
 - __ b Rinse all Hyb Chamber components with DI water.
 - __ c Thoroughly rinse the humidifying buffer reservoirs.
- __ 11 Clean and dry the wash dishes:
 - __ a Rinse the PB1 wash dishes with DI water.
 - __ b Rinse the XC4 wash dish with ethanol.
 - __ c Air dry all wash dishes.
- __ 12 Proceed to *Image BeadChip (Post-PCR)*.

Image BeadChip (Post-PCR)

Proceed to scanning the BeadChip(s). See the *BeadArray Reader User Guide*, *iScan System User Guide*, or the *HiScanSQ System User Guide* for general instructions on scanning your BeadChips. For specific scan settings refer to the following table:

Table 3 Scan Settings for Universal BeadChip

BeadChip	Scanner	Scan Setting Name
Universal-12	BeadArray Reader	Universal BC (GGGT, Methylation)
Universal-12	iScan and HiScan	Universal BC (GGGT, Methylation, DASL)
Universal-32	BeadArray Reader, iScan, and HiScan	Universal X (GGGT)
Universal-32 XT	BeadArray Reader, iScan, and HiScan	Universal XT (GGGT)

