GoldenGate® Genotyping Assay, Manual

Experienced User Card

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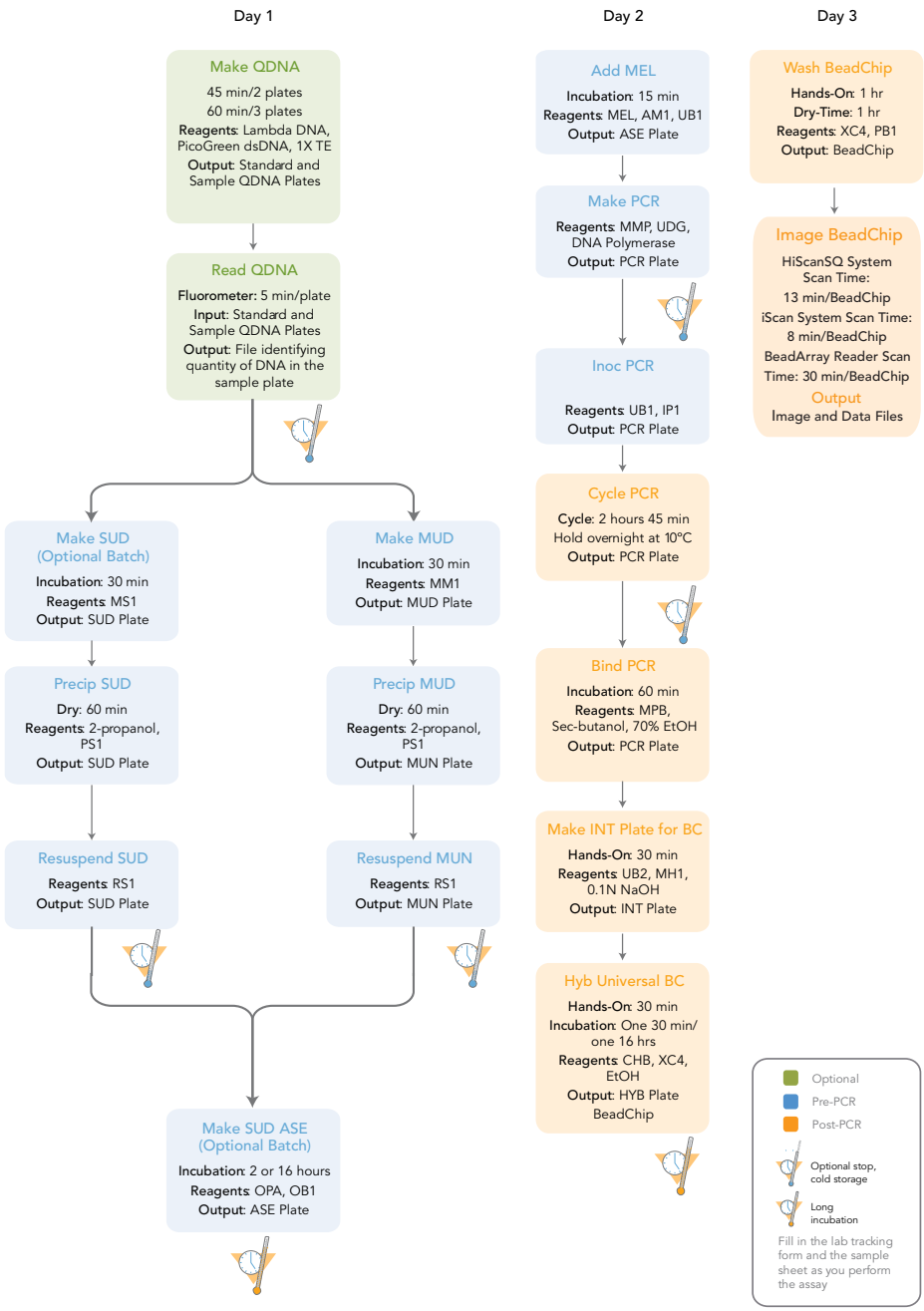

Make DNA Quantitation Plate (Optional) (Pre-PCR)

This process uses the Quant-iT PicoGreen dsDNA quantitation reagent to quantitate double-stranded DNA samples for the GoldenGate Genotyping Assay.

Estimated Time

Hands-on: 20 minutes per plate, plus 10 minutes to prepare the PicoGreen

Consumables

Item	Quantity	Storage	Supplied By
PicoGreen dsDNA quantitation reagent	See instructions	-20ºC	User
1X TE	See instructions	Room temperature	User
Lambda DNA	See instructions	-20ºC	User
96-well 0.65 ml microplate (MIDI)	1 per 96 samples		User
Fluotrac 200 (96-well black flat-bottom) plate	2 per 96 samples		User

Preparation

- Remove PicoGreen reagent from freezer and thaw at room temperature for 60 minutes in a light-impermeable container.
- Label a 96-well MIDI plate “Standard QDNA.”
- Label a 96-well black flat-bottom plate “Standard QDNA.”
- Label a 96-well black flat-bottom plate “Sample QDNA.”

Steps

Make Standard QDNA MIDI Plate

__ 1 Place stock Lambda DNA in well A1 of the Standard QDNA MIDI plate and dilute it to 75 ng/µl in a final volume of 233.3 µl.

__ 2 Add 66.7 µl 1X TE to well B1 of the same plate.

__ 3 Add 100 µl 1X TE to wells C, D, E, F, G, and H of column 1 of the same plate.

__ 4 Pipette the contents of A1 up and down 10 times to mix.

__ 5 Transfer 133.3 µl of Lambda DNA from well A1 into well B1, and then pipette the contents of well B1 up and down 10 times.

__ 6 Change pipette tips. Transfer 100 µl from well B1 into well C1, and then pipette the contents of well C1 up and down 10 times.

__ 7 Change pipette tips. Transfer 100 µl from well C1 into well D1, and then pipette the contents of well D1 up and down 10 times.

__ 8 Change pipette tips. Transfer 100 µl from well D1 into well E1, and then pipette the contents of well E1 up and down 10 times.

__ 9 Change pipette tips. Transfer 100 µl from well E1 into well F1, and then pipette mix the contents of well F1 up and down 10 times.

__ 10 Change pipette tips. Transfer 100 µl from well F1 into well G1, and then pipette the contents of well G1 up and down 10 times.

__ 11 **Do not transfer solution from well G1 to well H1.** Well H1 serves as the blank 0 ng/µl Lambda DNA.

__ 12 Cover the plate with a cap mat.

__ 13 Do one of the following:

- Proceed to *Prepare Standard QDNA Fluotrac Plate with PicoGreen Dilution*.
- Store the plate at 4°C for future use.

Prepare Standard QDNA Fluotrac Plate with PicoGreen Dilution

__ 1 Prepare a 1:200 dilution of PicoGreen to 1X TE, using the kit supplies and a sterile 100 ml plastic container wrapped in aluminum foil.

__ 2 Cap the sterile plastic container and vortex to mix.

__ 3 Pour the PicoGreen/1X TE dilution into a sterile reservoir.

__ 4 Using an 8-channel pipette, transfer 195 µl PicoGreen/1X TE dilution into each well of columns 1 and 2 of the Standard QDNA Fluotrac plate.

__ 5 Add 2 µl of each stock Lambda DNA dilution from column 1 of the original Standard QDNA MIDI plate into the corresponding wells of columns 1 and 2 in the Standard QDNA Fluotrac plate.

__ 6 Pipette mix the contents of the new Standard QDNA plate.

__ 7 Immediately cover the plate with an aluminum adhesive seal.

Prepare Sample QDNA Fluotrac plate with PicoGreen and DNA

__ 1 Transfer 195 µl of the PicoGreen/1X TE dilution that you made earlier into each well of the new black flat-bottom plate labelled “Sample QDNA”.

__ 2 Add 2 µl sample DNA to each well of the Sample QDNA plate.

__ 3 Pipette mix the contents of the Sample QDNA plate.

__ 4 Immediately cover the new plate with an aluminum adhesive seal.

__ 5 Proceed to *Read QDNA Plate (Optional)*.

Read QDNA Plate (Optional) (Pre-PCR)

This process uses the Gemini XS or XPS Fluorometer to provide DNA-specific quantitation. Illumina recommends using a fluorometer, because fluorometry provides DNA-specific quantitation, whereas spectrophotometry may also measure RNA and yield values that are too high.

Estimated Time

Fluorometer: 5 minutes per plate

Steps

__ 1 Turn on the fluorometer.

__ 2 At the PC, open the SoftMax Pro program.

__ 3 Load the Illumina QDNA.ppr file.

__ 4 Place the Standard QDNA Plate into the fluorometer loading rack with well A1 in the upper-left corner.

__ 5 Click the blue arrow next to **Lambda Standard**.

__ 6 Click **Read** in the SoftMax Pro interface.

__ 7 Remove the Standard QDNA Plate from the drawer.

__ 8 Click the blue arrow next to **Standard Curve** to view the standard curve graph.If the standard curve is acceptable, continue with the sample plate. Otherwise, click **Standard Curve** again.

__ 9 Place the Sample QDNA plate in the reader with well A1 in the upper left corner.

__ 10 Click the blue arrow next to **QDNA#1** and click **Read**.

__ 11 Remove the plate from the drawer.

__ 12 Repeat steps 11 through 13 for all sample plates that you want to quantitate.

__ 13 Once all plates have been read, click **File** | **Save** to save the output data file (*.pda).

__ 14 Proceed to *Make SUD Plate*.

Read QDNA Plate (Optional) (Pre-PCR)

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

This process activates sufficient DNA of each individual sample to be used ***once*** in the GoldenGate Genotyping Assay.

Estimated Time

Hands-on: ~15 minutes

Incubation: 30 minutes

Consumables

Item	Quantity	Storage	Supplied By
10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE)	See instructions	Room temperature	User
MS1 reagent	1 tube per SUD plate	-20ºC	Illumina
DNA samples and controls	96 or 384	-20ºC	User
96-well 0.2 ml skirted microplate	96 samples per plate		User

Preparation

- Preheat the heat block to 95°C.
- Turn on the heat sealer to preheat it. Allow 15 minutes.
- Thaw the MS1 reagent tube to room temperature. Vortex to mix the contents, and pour the entire tube into a new, non-sterile reservoir.
- Thaw the DNA samples and controls to room temperature and vortex to mix the contents.
- Apply a SUD barcode label to a new microplate.

Steps

__ 1 Normalize DNA samples to 50 ng/µl with 10 mM Tris-HCl pH 8.0, 1 mM EDTA.

__ 2 Add 5 µl MS1 reagent to each well of the SUD plate.

__ 3 Using an 8-channel pipette, transfer 5 µl normalized DNA sample to each well of the SUD plate. Change tips between column dispenses.

__ 4 Apply a microplate foil heat seal to the SUD plate and seal it with the heat sealer (3 seconds).

__ 5 Pulse centrifuge the SUD plate to 250 xg.

__ 6 Vortex at 2300 rpm for 20 seconds.

__ 7 Pulse centrifuge to 250 xg.

__ 8 Place the SUD plate in the preheated heat block and close the lid.

__ 9 Incubate the SUD plate at 95°C for exactly 30 minutes.

__ 10 Pulse centrifuge the plate to 250 xg.

__ 11 If you plan to perform the Make ASE protocol today, then immediately set the heat block to 70°C.

__ 12 Proceed to *Precip SUD Plate*.

Precipitate SUD Plate (Pre-PCR)

In this process, PS1 and 2-propanol are added to the SUD plate to precipitate the DNA and remove excess DNA activation reagent MS1.

Estimated Time

Hands-on: ~30 minutes

Drying: 15 minutes

Consumables

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

Preparation

- Pour 1 ml PS1 into a reagent reservoir.
- Pour 2 ml 2-propanol into a second reservoir.

NOTE

To record information about your assay such as operator information, start and stop times, and barcodes, use the lab tracking form. This form can be filled out and saved online, or printed and filled in by hand. Go to http://www.illumina.com/documentation to download the lab tracking form.

Steps

__ 1 Remove the heat seal from the heated SUD plate.

__ 2 Add 5 µl PS1 reagent to each well of the SUD plate.

__ 3 Seal the SUD plate with clear adhesive film.

CAUTION

To avoid contaminating the pipette tips, place the tips against the top edge of the well. If you suspect the tips are contaminated with the contents of the well, discard them and use new tips.

__ 4 Pulse centrifuge the plate to 250 xg.

__ 5 Vortex at 2300 rpm for 20 seconds or until the solution is uniformly blue.

__ 6 Remove the film and add 15 µl 2-propanol to each well of the SUD plate.

__ 7 Seal the SUD plate with clear adhesive film.

__ 8 Vortex at 1600 rpm for 20 seconds or until the solution is uniformly blue.

__ 9 Centrifuge the sealed 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.

__ 10 Remove the SUD plate seal and decant the supernatant by inverting the SUD plate and smacking it down onto an absorbent pad.

CAUTION

Do not tilt the plate, as this can cause cross-contamination between wells. Tap the plate firmly enough to decant all the supernatant; tapping lightly will not work as well.

__ 11 Tap the inverted plate onto the pad to blot excess supernatant.

__ 12 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.

__ 13 Do one of the following:

- Proceed to *Resuspend SUD Plate*.
- Seal the plate with adhesive film and store at -20°C for up to 24 hours.

Resuspend SUD Plate (Pre-PCR)

In this process, RS1 is added to the SUD plate to resuspend the DNA.

Estimated Time

Hands-on: ~15 minutes

Consumables

Item	Quantity	Storage	Supplied By
RS1 reagent	Bottle	Room temperature	Illumina

Preparation

- Pour 1.2 ml RS1 into a reagent reservoir.

Steps

__ 1 Add 10 µl RS1 reagent to each well of the SUD plate.

__ 2 Seal the SUD plate with microplate clear adhesive film.

__ 3 Pulse centrifuge to 250 xg.

__ 4 Vortex at 2300 rpm for 1 minute or until the blue pellet is completely dissolved.

__ 5 Do one of the following:
- Proceed immediately to *Make ASE Plate*.
- Store the SUD plate at 4°C overnight or at -20ºC for one month.

Resuspend SUD Plate (Pre-PCR)

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

In this process, the nucleic acid activator reagent (MM1) is transferred to the MUD plate, followed by a 50 µl volume containing 2 µg from up to 96 genomic DNA samples. 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.

NOTE
MS1 reagent is photosensitive. Store it and thaw it away from light.

- Prepare the robot for use.
- Apply a MUD barcode label to each new 96-well TCY microplate.
- Pour MM1 tube contents into a sterile trough.

Steps

__ 1 Add 40 µL MM1 reagent to each well of the GS#-MUD plate.

__ 2 Add 40 µL normalized DNA sample to each well of the MUD plate.

__ 3 Pipette mix DNA sample and MM1 reagent in the MUD plate.

__ 4 Apply the microplate foil heat seal to the MUD plate and heat-seal it with the heat sealer.

__ 5 Pulse centrifuge sealed plate to 250 xg.

NOTE
It's important to centrifuge MUD plate to 250 xg before the 95°C incubation to prevent wells from drying out during the incubation.

__ 6 Heat MUD plate at 95°C for 30 minutes in the preheated heat block.

__ 7 Using the heat block cover, cover the MUD plate to reduce condensation on the plate seal.

__ 8 Remove the MUD plate from the heat block and pulse centrifuge to 250 xg to remove condensation from the walls of each well.

__ 9 Proceed to *Precip MUD*.

Precipitate MUD Plate (Pre-PCR)

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

Estimated Time

Hands-on: ~30 minutes

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.
- Pour 5 mL GS#-PS1 into a new sterile trough.
- Pour 13 mL 2-propanol into another sterile trough.
- Apply a MUN barcode to each new MIDI plate.

Steps

__ 1 Carefully remove heat seal from heated MUD plate, taking care to avoid splashing from the wells.

__ 2 Add 40 µL PS1 reagent to each well of the MUN plate.

__ 3 Transfer the entire contents (80 µL) from each well of the heated MUD plate to the corresponding well of the MUN plate.

__ 4 Using the cap mat, seal the MUN plate.

__ 5 Pulse centrifuge to 250 xg to collect the contents to the bottom of the wells.

__ 6 Vortex at 2000 rpm for 20 seconds.

__ 7 Pulse centrifuge to 250 xg.

__ 8 Remove the cap mat and add 120 µL 2-propanol to each well of the MUN plate.

__ 9 Using the cap mat, seal the MUN plate and vortex at 1600 rpm for 20 seconds.

__ 10 Centrifuge sealed MUN plate at 3000 xg for 20 minutes.

__ 11 Remove MUN plate from centrifuge.

__ 12 Remove cap mat from MUN plate.

__ 13 Decant supernatant by inverting the MUN plate over an absorbent pad. Press inverted plate onto absorbent pad to blot off excess supernatant.

__ 14 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.

__ 15 Proceed to *Resuspend MUN*.

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

Hands-on: ~30 minutes

Consumables

Item	Quantity	Storage	Supplied By
RS1 reagent	Bottle	Room temperature	Illumina

Preparation

- Pour 12 mL RS1 into a sterile trough.

Steps

__ 1 Add 100 µL RS1 reagent to each well of the MUN plate.

__ 2 Using the cap mat, seal MUN plate and vortex at 2000 rpm for 1 minute to resuspend the blue pellet.

__ 3 Verify that the blue pellet in each well of the MUN plate has been dissolved back into solution. If not, repeat step 2.

__ 4 MUN sample plate activation is complete. Either seal and store at 4°C, or proceed immediately to Make ASE Plate.

__ 5 Proceed to *Make ASE Plate*.

Resuspend MUN (Pre-PCR)

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

This process combines the biotinylated gDNAs from the SUD plate with query oligos, hybridization reagents, and paramagnetic particles in an Allele Specific Extension (ASE) plate. The ASE plate is placed in a heat block and the query oligos for each sequence target of interest are allowed to anneal to the biotinylated gDNA samples. The gDNA is simultaneously captured by paramagnetic particles. The resulting ASE plate is ready for the extension and ligation of the hybridized oligos on the bound gDNAs.

Estimated Time

Hands-on: ~20 minutes

Incubation: 2–16 hours

Consumables

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

Preparation

- Preheat the heat block to 70°C.
- Turn on the heat sealer to preheat it. Allow 15 minutes.
- Thaw the OPA reagent tube to room temperature. Vortex the tube, and then pulse centrifuge to 250 xg. Pour the contents of the OPA tube into a reagent reservoir.
- Thaw the OB1 reagent tube to room temperature. Vortex the tube to completely resuspend the beads. Pour the contents of the OB1 tube into a second reagent reservoir.Do not centrifuge the OB1 tube.
- Apply an ASE barcode label to a new microplate.

Steps

__ 1 Pulse centrifuge the SUD plate to 250 xg.

__ 2 Add 10 µl OPA reagent to each well of the ASE plate.

__ 3 Add 30 µl OB1 reagent to each well of the ASE plate.

__ 4 Carefully remove the heat seal from the SUD plate.

__ 5 Transfer 10 µl of biotinylated sample from each well of the SUD plate (where 10 µl is the entire volume) to the corresponding well of the ASE plate.

__ 6 Using a microplate heat seal, heat-seal the ASE plate (3 seconds).

__ 7 Pulse centrifuge the ASE plate to 250 xg.

__ 8 Vortex the ASE plate at 1600 rpm for 1 minute or until all beads are completely resuspended.

__ 9 Place the sealed ASE plate on the 70°C heat block and close the lid.

__ 10 Immediately reset the temperature to 30°C.

__ 11 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.

__ 12 Proceed to *Add MEL*.

Add Master Mix for Extension & Ligation (MEL) (Pre-PCR)

In this process, AM1 and UB1 reagents are added to the ASE plate to wash away non-specifically hybridized and excess oligos. An enzymatic extension and ligation master mix (MEL) is added to each DNA sample. The extension and ligation reaction occurs at 45°C. Work quickly during the washes to prevent the beads from drying out.

Estimated Time

Hands-on: ~30 minutes

Incubation: 15 minutes

Consumables

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

Preparation

- Remove the ASE plate from the heat block.
- Preheat the heat block to 45°C for about one hour.
- Thaw the MEL tube to room temperature.
- Pour 11 ml AM1 into a reagent reservoir. Add 10 ml for each additional plate.
- Pour 11 ml UB1 into a second reagent reservoir. Add 10 ml for each additional plate.
- Pour the thawed MEL tube contents into a third reagent reservoir.

Steps

__ 1 Centrifuge the ASE plate to 250 xg.

__ 2 Place the ASE plate on the raised-bar magnetic plate for approximately 2 minutes, or until the beads are completely captured.

__ 3 Carefully remove the heat seal from the ASE plate.

__ 4 Using an 8-channel pipette with new tips, remove and discard all the liquid (50 µl) from the wells. Leave the beads in the wells.

__ 5 Visually inspect pipette tips after removing solution from each column to ensure no beads have been removed. If beads are visible in pipette tips, return the solution to the same wells, allow magnet to re-collect beads, and change the pipette tips.

__ 6 With the ASE plate on the raised-bar magnetic plate, use an 8-channel pipette with new tips to add 50 µl AM1 to each well of the ASE plate.

__ 7 Seal the ASE plate with microplate clear adhesive film.

__ 8 Vortex the ASE plate at 1600 rpm for 20 seconds or until all beads are resuspended.

__ 9 Place the ASE plate on the raised-bar magnetic plate for approximately 2 minutes, or until the beads are completely captured.

__ 10 Remove the seal from the ASE plate.

__ 11 Using the same 8-channel pipette with the same tips, remove all AM1 reagent from each well. Leave the beads in the wells.

__ 12 Repeat steps 6 through 11 once.

__ 13 Remove the ASE plate from the raised-bar magnetic plate.

__ 14 Using an 8-channel pipette with new tips, add 50 µl UB1 to each well of the ASE plate.

__ 15 Place the ASE plate onto the raised-bar magnetic plate for approximately 2 minutes, or until the beads are completely captured.

__ 16 Using the same 8-channel pipette with the same tips, remove all UB1 reagent from each well. Leave the beads in the wells.

__ 17 Repeat steps 13 through 16 once.

__ 18 Remove the ASE plate from the raised-bar magnetic plate.

__ 19 Using an 8-channel pipette with new tips, add 37 µl MEL to each well of the ASE plate.

__ 20 Seal the plate with microplate clear adhesive film.

__ 21 Vortex the plate at 1600 rpm for 1 minute or until the beads are resuspended.

__ 22 Incubate the ASE plate on the preheated 45°C heat block for exactly 15 minutes.

__ 23 During the incubation, perform the *Make PCR Plate* procedure.

__ 24 Proceed to *Make PCR Plate*. Leave the ASE plate at room temperature if you proceed immediately, or store it at 4°C for up to 1 hour.

Make PCR Plate (Pre-PCR)

This process adds the Illumina-recommended DNA Polymerase and optional Uracil DNA Glycosylase (UDG) to the master mix for PCR (MMP reagent) and creates a 96-well plate for the Inoc PCR process.

Estimated Time

Hands-on: ~15 minutes

Consumables

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

Preparation

- Thaw the MMP tube to room temperature.
- Apply a PCR barcode label to a new microplate.

Steps

__ 1 Add 64 µl DNA Polymerase to the MMP tube.

__ 2 [Optional] Add 50 µl Uracil DNA Glycosylase to the MMP tube.

__ 3 Invert the tube several times to mix the contents, and then pour the contents of the tube into a reagent reservoir.

__ 4 Using an 8-channel pipette, add 30 µl of the mixture into each well of the PCR plate.

__ 5 Seal the PCR plate with microplate clear adhesive film.

__ 6 Pulse centrifuge to 250 xg, and then place the PCR plate in a light-protected location.

__ 7 Proceed to *Inoc PCR Plate*.

Inoculate PCR Plate (Pre-PCR)

This process uses the template formed in the extension and ligation process in a PCR reaction. This PCR reaction uses three universal primers (MMP reagent): two are labeled with fluorescent dyes and the third is biotinylated. The biotinylated primer allows capture of the PCR product and elution of the strand containing the fluorescent signal. The eluted samples are transferred from the ASE plate to the PCR plate.

Estimated Time

Hands-on: ~30 minutes

Consumables

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

Preparation

- Remove the ASE plate from the heat block.
- Reset the heat block to 95°C.
- Pour 6 ml UB1 into a reagent reservoir.
- Thaw the IP1 reagent to room temperature. Pour the contents of the tube into a reagent reservoir.

Steps

__ 1 Place the ASE plate on the raised-bar magnetic plate for approximately 2 minutes, or until the beads are completely captured.

__ 2 Remove the microplate clear adhesive film from the ASE plate.

__ 3 Using an 8-channel precision pipette, remove and discard the supernatant (~50 µl) from all wells of the ASE plate. Leave the beads in the wells.

__ 4 Visually inspect pipette tips after removing solution from each column to ensure no beads have been removed. If beads are visible in pipette tips, return the solution to the same wells, allow magnet to re-collect beads, and change the pipette tips.

__ 5 Leaving the plate on the magnet and using an 8-channel precision pipette with new tips, add 50 µl UB1 to each well of the ASE plate.

__ 6 Leave the ASE plate on the raised-bar magnetic plate for approximately 2 minutes, or until the beads are completely captured.

__ 7 Remove and discard the supernatant (~50 µl) from all wells of the ASE plate. Leave the beads in the wells.

__ 8 Remove the plate from the magnet.

__ 9 Using an 8-channel precision pipette with new tips, add 35 µl IP1 to each column of the ASE plate.

__ 10 Seal the plate with microplate clear adhesive film.

__ 11 Vortex at 1800 rpm for 1 minute, or until all the beads are resuspended.

__ 12 Place the plate on the 95°C heat block for 1 minute.

__ 13 Place the ASE plate back onto the raised-bar magnetic plate for 2 minutes or until the beads have been completely captured.

__ 14 Using an 8-channel pipette with new tips, transfer 30 µl supernatant from each well in the first column of the ASE plate to the first column of the PCR plate.

__ 15 Repeat for each column of the ASE plate. Change tips between column dispenses.

__ 16 Seal the PCR plate with the appropriate PCR plate-sealing film for your thermocycler.

__ 17 Immediately transfer the PCR plate to the thermocycler.

__ 18 Proceed to *Cycle PCR*.

This concludes the Pre-PCR processes for the manual GoldenGate Genotyping Assay. If you remove materials such as experienced user cards from the Pre-PCR lab, do not return with them into the Pre-PCR lab at any time.

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.

Estimated Time

Thermal Cycle: ~2 hours 45 minutes

Preparation

Steps

__ 1 Place the sealed plate into the thermocycler and run the thermocycler program shown in this table.

Table 1 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
	4°C	5 minutes

__ 2 Do one of the following:

- Proceed immediately to *Bind PCR*. Store the PCR plate at room temperature in a light-protected drawer.
- Seal and store the PCR plate at -20°C overnight.

Cycle the PCR Plate (Post-PCR)

Bind PCR Products (Post-PCR)

In this process, MPB reagent is added to the PCR plate and the solution is transferred to a filter plate. The filter plate is incubated at room temperature to bind the biotinylated strand to paramagnetic particles, thus immobilizing the double-stranded PCR products.

Estimated Time

Hands-on: ~30 minutes

Incubation: 1 hour

Consumables

Item	Quantity	Storage	Supplied By
MPB reagent	1 tube per PCR plate	4°C	Illumina
0.45 µM clear Styrene filter plate with lid	1 per PCR plate		User

Preparation

- Vortex the MPB tube several times or until the beads are well resuspended. Pour the contents of the tube into a non-sterile reagent reservoir.
- Write the PCR plate barcode number in the space provided on a "Filter Plate: GS _____ __-PCR" label. Apply the label to the top surface of the filter plate, adjacent to column 12.

Steps

__ 1 Pulse centrifuge the PCR plate to 250 xg.

__ 2 Place new tips onto an 8-channel pipette.

__ 3 Add 20 µl resuspended MPB into each well of the PCR plate.

__ 4 Set an 8-channel pipette to 85 µl to allow space for bubbles, and attach new tips.

__ 5 Pipette the solution in the PCR plate up and down several times to mix the beads with the PCR product.

__ 6 Transfer the mixed solution into the first column of the filter plate. There should be about 70 µl fluid in each well.

__ 7 Repeat step 6 for each column of the PCR plate. Change tips between column dispenses.

__ 8 Cover the filter plate with its lid and store it at room temperature, protected from light, for 60 minutes.

__ 9 Proceed to *Make INT Plate for BeadChip*.

Bind PCR Products (Post-PCR)

Make INT Plate for BeadChip (Post-PCR)

In this step, the single-stranded fluor-labeled PCR product from the filter plate is washed and then eluted into an intermediate (INT) plate. The product from this plate is hybridized to the BeadChip.

Estimated Time

Hands-on time: ~30 minutes

Consumables

Item	Quantity	Storage	Supplied By
0.1N NaOH	Bottle	2°C to 8°C	User
UB2 reagent	Bottle	Room temperature	Illumina
MH1 reagent	1 tube per INT plate	Room temperature	Illumina
96-well V-bottom plate	2 per filter plate		User
Filter plate adapter	1 per filter plate		User

Preparation

- Apply a INT barcode label to a new 96-well V-bottom plate.
- Using a serological pipette, transfer 6 ml UB2 into a sterile reservoir.
- Pour 4 ml 0.1N NaOH into a second sterile reservoir.
- Pour the contents of an MH1 tube into a third sterile reservoir.

Steps

__ 1 Place the filter plate adapter on an empty, unlabelled 96-well V-bottom plate (waste plate).

__ 2 Place the filter plate containing the bound PCR products onto the filter plate adapter.

__ 3 Centrifuge to 1000 xg for 5 minutes at 25°C.
Using a multichannel pipette with new tips, add 50 µl UB2 to each well containing sample of the filter plate. Dispense slowly to avoid disturbing the beads

__ 4 Replace the filter plate lid.

__ 5 Centrifuge to 1000 xg for 5 minutes at 25°C.

__ 6 Using a multichannel pipette with new tips, add 30 µl MH1 to each well of the INT plate.

__ 7 Replace the waste plate with the INT plate. Orient the INT plate so that well A1 of the filter plate matches well A1 of the INT plate.

__ 8 Using a multichannel pipette with new tips, add 30 µl 0.1N NaOH to each occupied well of the filter plate.

__ 9 Replace the filter plate lid.

__ 10 Centrifuge immediately to 1000 xg for 5 minutes at 25°C. At the end, no beads should be visible in the wells of the INT plate.

__ 11 Discard the filter plate. Save the adapter for later use in other protocols.

__ 12 Gently mix the contents of the INT plate by moving it from side to side without splashing.

__ 13 Seal the INT plate with a 96-well cap mat. Store the plate in the dark until ready to dispense sample onto a BeadChip.

__ 14 Do one of the following:

- Proceed to *Hyb Universal BC*.
- If you do not plan to use the INT 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 INT 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

Hands-on time: ~30 minutes

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	-15°C to -25°C	Illumina
100% EtOH	Bottle	Room temperature	User
Hyb Chamber	1 per 4 BeadChips		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
Hyb Chamber inserts	1 per BeadChip		
Hyb Chamber gaskets	1 per Hyb Chamber		

Preparation

- Preheat the Illumina Hybridization Oven to 60°C. Allow 30 minutes for it to equilibrate.
- If the INT plate has been frozen, thaw it completely at room temperature 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.

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.

Prepare BeadChips for Hybridization

CAUTION
Do not unpackage BeadChips unless you are ready to begin hybridization.

__ 1 Remove all the BeadChips from their packages.

__ 2 Place each BeadChip in a Hyb Chamber Insert.

Load Sample

__ 1 Pipette samples up and down in the INT plate to mix.

__ 2 For the Universal-12 BeadChip, use a single or multi-channel precision pipette to add 15 µl sample from the INT plate onto the center of each inlet port.
For the Universal-32 BeadChip, use a multi-channel precision pipette to add 15 µl sample from the INT plate onto the center of each inlet port.

__ 3 Open the Hyb Chamber. Load four Hyb Chamber Inserts containing sample-laden BeadChips into each Hyb Chamber.

__ 4 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 BeadChip*.

Hybridize Universal BeadChip (Post-PCR)

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 (8 BeadChips).

__ 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 Release the vacuum by turning the handle very slowly.

__ 9 Touch the borders of the chips (***do not touch the stripes***) to ensure that the etched, bar-coded side of the BeadChips are dry to the touch.

__ 10 Clean the underside of each BeadChip to remove any excess XC4 with a ProStat EtOH wipe.

__ 11 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.

__ 12 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.

__ 13 Proceed to *Image BeadChip (Post-PCR)*.

Wash & Coat 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 2 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)

Image BeadChip (Post-PCR)