

## Make SUD

| Process  | Plates Used | Reagents Used                         | Volume Required      |
|----------|-------------|---------------------------------------|----------------------|
| Make SUD | GS#-SUD     | 10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE) | Variable             |
|          |             | GS#-MS1 (tube)                        | Entire tube contents |

- Enter **Sample\_Name** (optional), and **Sample\_Plate**, for each **Sample\_Well** in the Sample Sheet.
- Preheat the heat block(s) to 95°C (may take 45 minutes).
- Thaw **GS#-MS1** to room temperature.
- Vortex to fully mix tube contents and pour into a sterile trough.
- Apply a **GS#-SUD** barcode label to a 96-well 0.2 mL plate.
- Normalize DNA samples to 50 ng/μL with TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA).
- Add 5 μL **GS#-MS1** reagent to each well of **GS#-SUD** plate.
- Transfer 5 μL normalized DNA sample to each well of the **GS#-SUD** plate.
- Apply microplate foil seal, heat-seal the **GS#-SUD** plate, and pulse-centrifuge to 250 Xg.
- Vortex at **2300 rpm\*** for 20 seconds.
- Pulse-centrifuge sealed plate to 250 Xg to prevent wells from drying out during the incubation.
- Heat **GS#-SUD** plate at 95°C for 30 min in preheated heat block, covering plate with heat block cover to reduce condensation (95°C incubation must not exceed 30 minutes).
- Remove **GS#-SUD** plate from heat block and pulse-centrifuge to 250 Xg (if proceeding to *Make ASE* immediately after DNA preparation, immediately switch heat block to 70°C).

## Precip SUD

| Process    | Plates Used | Reagents Used    | Volume Required |
|------------|-------------|------------------|-----------------|
| Precip SUD | GS#-SUD     | 2-propanol       | 2 mL            |
|            |             | GS#-PS1 (bottle) | 1 mL            |

- Carefully remove heat seal from **GS#-SUD** plate -- do not splash (use optional foil stripper).
- Add 5 μL **GS#-PS1** reagent to each well of the **GS#-SUD** plate, and seal plate with adhesive film.
- Pulse-centrifuge **GS#-SUD** plate to 250 Xg, then vortex at **2300 rpm\*** for 20 seconds.
- Remove adhesive film and add 15 μL 2-propanol to each well of **GS#-SUD** plate.
- Seal **GS#-SUD** plate with adhesive film and vortex at **1600 rpm\*** for 20 seconds.
- Inspect wells for uniform blue color, and re-vortex if non-uniform appearance is observed.
- Centrifuge sealed **GS#-SUD** plate at 3000 Xg for 20 minutes.
- Remove **GS#-SUD** plate from centrifuge, remove plate seal, and decant supernatant by inversion onto an absorbent pad. Blot off excess supernatant.
- Invert **GS#-SUD** plate on absorbent pad and centrifuge at 8 Xg for 1 minute. Do not spin the inverted plate at more than 8 Xg.
- Allow plate to dry at room temperature for 15 minutes.

\* Actual vortex speed

## Resuspend SUD

| Process       | Plates Used | Reagents Used    | Volume Required |
|---------------|-------------|------------------|-----------------|
| Resuspend SUD | GS#-SUD     | GS#-RS1 (bottle) | 1.2 mL          |

- Pour 1.2 mL **GS#-RS1** into a sterile trough.
- Add 10 µL **GS#-RS1** reagent to each well of **GS#-SUD** plate.
- Seal **GS#-SUD** plate with adhesive film, pulse centrifuge to 250 Xg.
- Vortex at **2300 rpm\*** for 1 minute to resuspend blue pellet.
- Verify that all blue pellets have been dissolved back into solution. If not, repeat vortex.
- Either heat-seal the plate and store at 4°C, or proceed immediately to *Make ASE*.



### NOTE:

For long-term storage, the activated DNA may be frozen at -20°C. If the activated DNA is stored frozen, thaw completely and vortex to mix contents before use in assay.

\* Actual vortex speed

## Make MUD

| Process  | Plates Used | Reagents Used                         | Volume Required      |
|----------|-------------|---------------------------------------|----------------------|
| Make MUD | GS#-MUD     | 10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE) | variable             |
|          |             | GS#-MM1 (tube)                        | Entire tube contents |

- Enter **Sample\_Name** (optional) and **Sample\_Plate**, for each **Sample\_Well** in the Sample Sheet.
- Preheat the heat block(s) to 95°C (~ 45 minutes).
- Thaw **GS#-MM1** to room temperature.
- Apply a **GS#-MUD** barcode label to a 96-well 0.2 mL plate.
- Normalize DNA samples to 50 ng/μL with TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA).
- Vortex **GS#-MM1** to fully mix tube contents and pour into sterile trough.
- Add 40 μL **GS#-MM1** reagent to each well of **GS#-MUD** plate.
- Add 40 μL normalized DNA sample to each well of **GS#-MUD** plate, and pipet-mix well.
- Apply microplate foil heat seal and heat-seal the **GS#-MUD** plate.
- Pulse-centrifuge sealed plate to 250 Xg to prevent wells from drying out during the incubation.
- Heat **GS#-MUD** plate at 95°C for 30 minutes in preheated heat block, covering plate with a heat block cover to reduce condensation (95°C incubation must not exceed 30 minutes) (if proceeding to *Make ASE* immediately after DNA preparation, immediately switch heat block to 70°C).
- Remove **GS#-MUD** plate from heat block and pulse centrifuge to 250 Xg.

## Precip MUD

| Process    | Plates Used | Reagents Used    | Volume Required |
|------------|-------------|------------------|-----------------|
| Precip MUD | GS#-MUD     | 2-propanol       | 13 mL           |
|            |             | GS#-PS1 (bottle) | 5 mL            |

- Apply a **GS#-MUN** barcode label to a 96-well 0.65 mL deep-well V-bottom plate.
- Carefully remove heat seal from **GS#-MUD** plate -- do not splash (use optional foil stripper).
- Add 40 μL **GS#-PS1** to each well of the **GS#-MUN** plate.
- Transfer entire contents (80 μL) from each well of **GS#-MUD** plate to corresponding well of **GS#-MUN** plate.
- Seal **GS#-MUN** plate with cap mat and pulse centrifuge to 250 Xg.
- Vortex at **2000 rpm\*** for 20 seconds.
- Pulse centrifuge to 250 Xg.
- Remove cap mat and add 120 μL 2-propanol to each well of **GS#-MUN** plate.
- Seal **GS#-MUN** plate with the cap mat and vortex at **1600 rpm\*** for 20 seconds.
- Centrifuge sealed **GS#-MUN** plate at 3000 Xg for 20 minutes.
- Remove **GS#-MUN** plate from centrifuge, remove cap mat, and decant supernatant by inversion over an absorbent pad. Blot off excess supernatant.
- Invert **GS#-MUN** plate on absorbent pad and centrifuge at 8 Xg for 1 minute. Do not spin the inverted plate at more than 8 Xg.
- Allow **GS#-MUN** plate to dry at room temperature for 15 minutes.

\* Actual vortex speed

## Resuspend MUN

| Process       | Plates Used | Reagents Used    | Volume Required |
|---------------|-------------|------------------|-----------------|
| Resuspend MUN | GS#-MUN     | GS#-RS1 (bottle) | 12 mL           |

- Pour 12 mL **GS-RS1** into a sterile trough.
- Add 100 µL **GS#-RS1** reagent to each well of **GS#-MUN** plate.
- Seal **GS#-MUN** plate with cap mat and vortex at **2000 rpm\*** for 1 minute.
- Verify that blue pellet in each well has been dissolved back into the solution. If not, re-vortex.
- Seal and store **GS#-MUN** plate at 4°C, or proceed immediately to *Make ASE*.

**NOTE:**

For long-term storage, the activated DNA may be frozen at -20°C. If the activated DNA is stored frozen, thaw completely and vortex to mix contents before use in assay.

\* Actual vortex speed

## Make ASE

| Process  | Plates Used               | Reagents Used  | Volume Required      |
|----------|---------------------------|----------------|----------------------|
| Make ASE | GS#-SUD <i>or</i> GS#-MUN | GS#-OPA (tube) | Entire tube contents |
|          |                           | GS#-OB1 (tube) | Entire tube contents |

Enter the **GS#-OPA** in the **Pool\_ID** column of the Sample Sheet.

Preheat heat block to 70°C.

Remove **GS#-OPA** tube from refrigerator.



**NOTE:**

If the **GS#-OPA** has been frozen, thaw completely and vortex contents to mix completely, then pulse centrifuge to collect the contents at the bottom of the tube.

Remove **GS#-OB1** tube from the freezer and thaw to room temperature.

Apply a **GS#-ASE** barcode label to a new 96-well 0.2 mL plate.

Record **GS#-OPA** barcode number on Sample Sheet.

Pulse centrifuge the plate containing activated DNA (**GS#-SUD** or **GS#-MUN**) to 250 Xg.

Pour entire **GS#-OPA** tube contents (1.2 mL) into trough.

Add 10 µL **GS#-OPA** to each well of the new **GS#-ASE** plate.

Vortex **GS#-OB1** and pour into trough.

Add 30 µL **GS#-OB1** to each well of the **GS#-ASE** plate.

Carefully remove heat seal from **GS#-SUD** or **GS#-MUN** plate.

Transfer 10 µL of activated DNA sample from the **GS#-SUD** or **GS#-MUN** plate to the **GS#-ASE** plate.

Heat-seal **GS#-ASE** plate and pulse-centrifuge to 250 Xg.

Vortex **GS#-ASE** plate at **1600 rpm\*** for 1 minute.

Place sealed **GS#-ASE** plate on heat block (70°C) and cover with a heat block cover to reduce condensation.

Immediately change the set temperature of the heat block to 30°C and allow the **GS#-ASE** plate to sit in the heat block until it cools to 30°C.

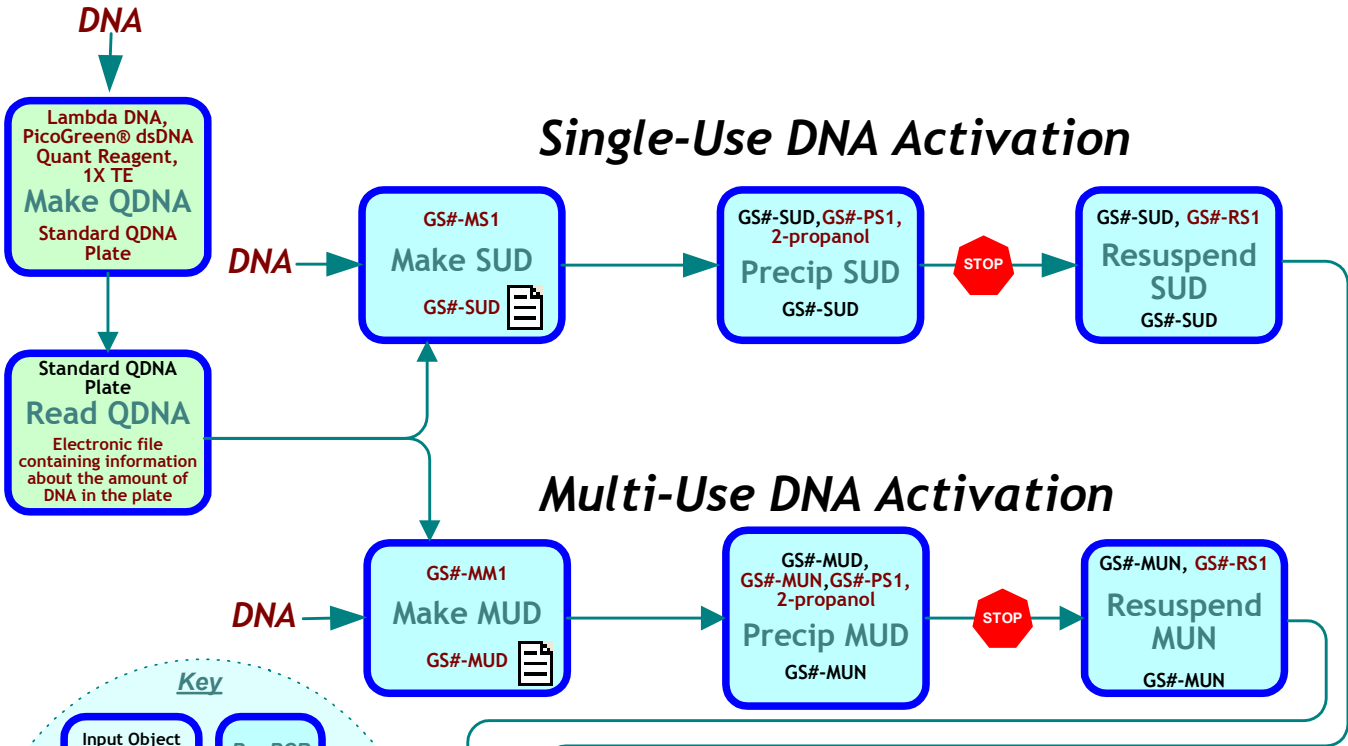


**NOTE:**

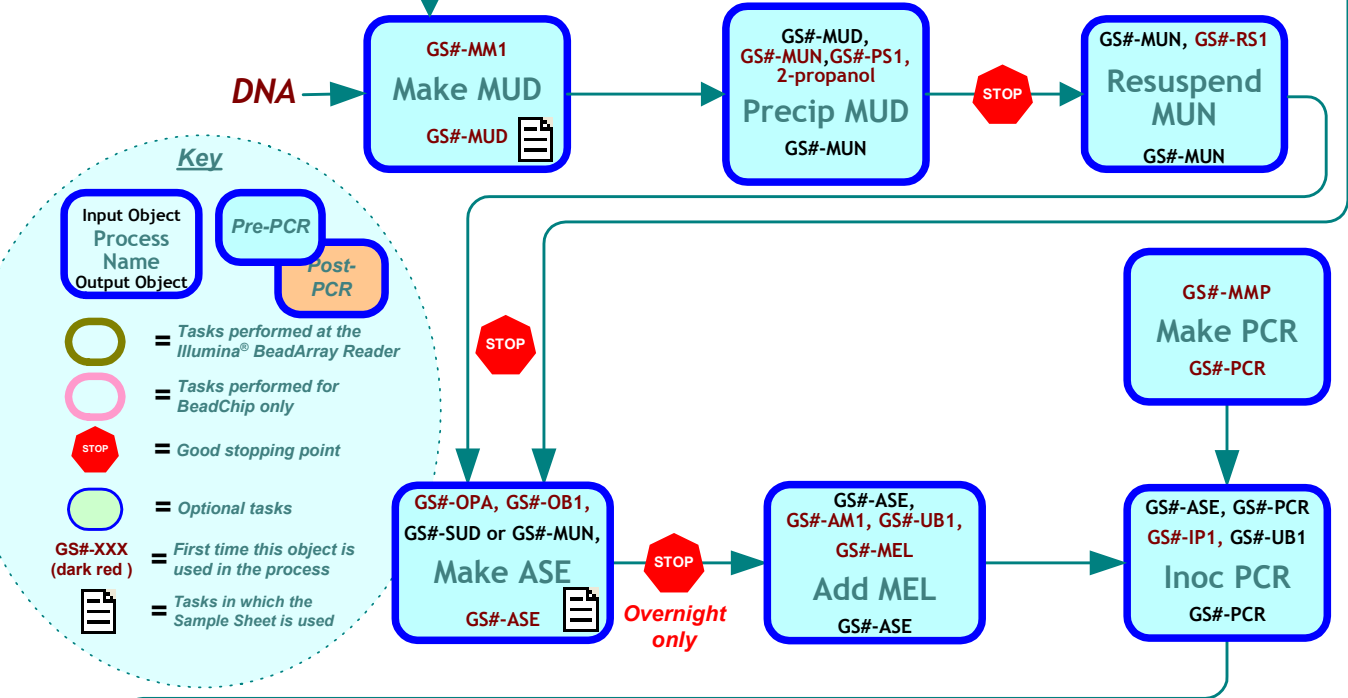
This should take approximately 2 hours. **GS#-ASE** plate may remain on the heat block at 30°C for up to 16 hours.

\* Actual vortex speed

## Single-Use DNA Activation



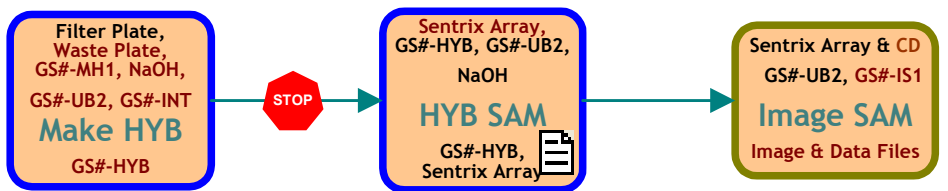
## Multi-Use DNA Activation



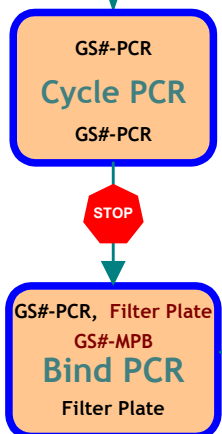
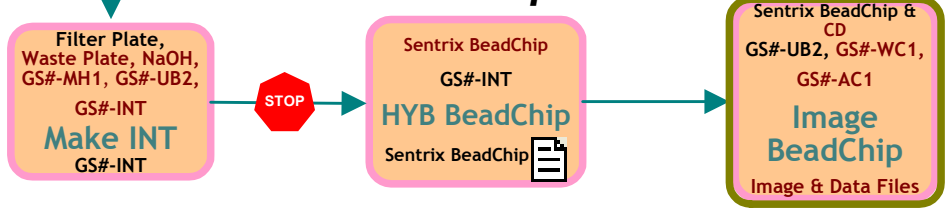
**Key**

- Input Object: [Blue box]
- Process Name: [Blue box]
- Output Object: [Blue box]
- Pre-PCR: [Blue box]
- Post-PCR: [Orange box]
- Tasks performed at the Illumina® BeadArray Reader: [Green circle]
- Tasks performed for BeadChip only: [Pink circle]
- Good stopping point: [Red octagon]
- Optional tasks: [Light blue circle]
- GS#-XXX (dark red): First time this object is used in the process
- Tasks in which the Sample Sheet is used: [Blue box with document icon]

## Sentrix® Array Matrix




## Sentrix® BeadChip

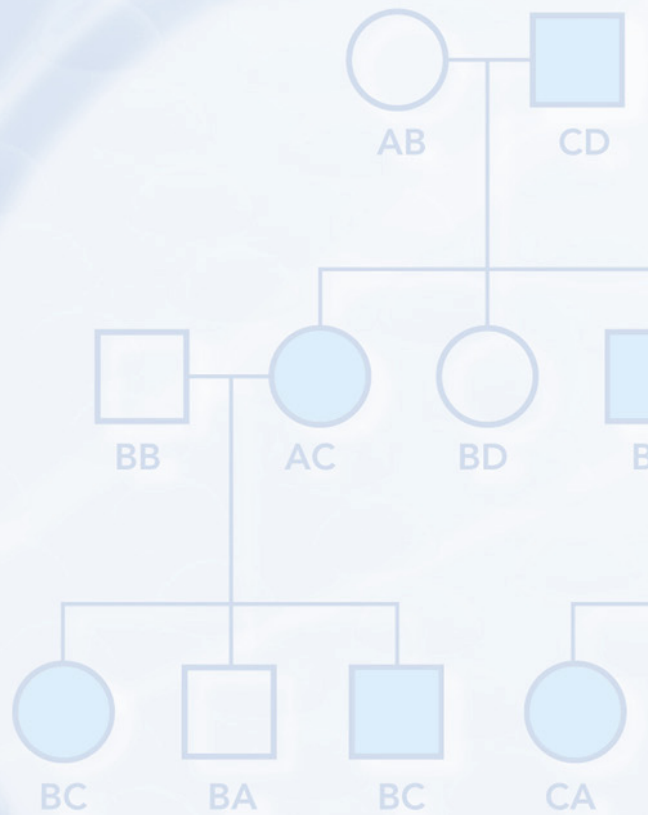
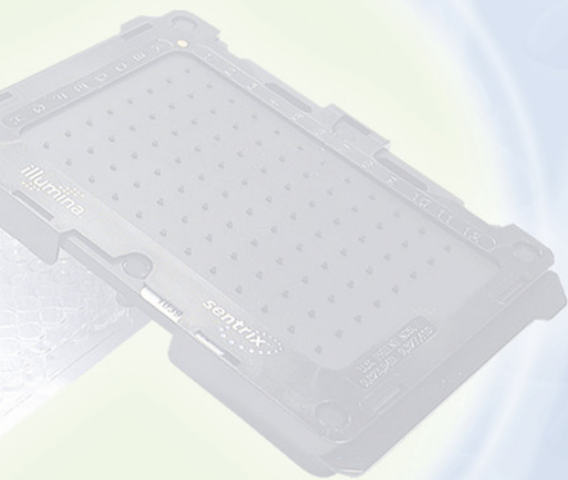


## Add MEL

| Process | Plates Used | Reagents Used    | Volume Required                         |
|---------|-------------|------------------|---|
| Add MEL | GS#-ASE     | GS#-AM1 (bottle) | 11 mL (10 mL for each additional plate) |
|         |             | GS#-UB1 (bottle) | 11 mL (10 mL for each additional plate) |
|         |             | GS#-MEL (tube)   | Entire tube contents                    |

- Remove **GS#-ASE** plate from heat block.
- Preheat heat block to 45°C and allow temperature to stabilize at 45°C.
- Thaw **GS#-MEL** to room temperature.
- Remove **GS#-AM1** and **GS#-UB1** from refrigerator.
- Pour 11 mL (+10 mL for each additional plate) **GS#-AM1** into sterile trough.
- Pour 11 mL **GS#-UB1** into another sterile trough.
- Centrifuge **GS#-ASE** plate to 250 Xg.
- Place **GS#-ASE** plate on magnetic plate for ~ 2 minutes, or until beads are completely captured.
- 
 If using the Illumina-provided raised bar magnetic plate, the beads in odd-numbered columns will be pulled to the right wall of the well, and the beads in even-numbered columns will be pulled to the left wall of the well.
- Remove heat seal from **GS#-ASE** plate; remove all liquid (~50 µL) from wells and discard liquid (leave beads in wells).
- With **GS#-ASE** plate on magnetic plate, use new pipet tips and add 50 µL **GS#-AM1** to each well of **GS#-ASE** plate.
- Seal **GS#-ASE** plate with adhesive film.
- Vortex **GS#-ASE** plate at **1600 rpm\*** for 20 seconds or until all beads are resuspended.
- Place **GS#-ASE** plate on magnetic plate for ~2 minutes, or until beads are completely captured.
- Using the same 8-channel pipet with the same tips, remove all **GS#-AM1** from each well.
- Repeat addition of 50 µL **GS#-AM1**, vortexing, and removal of buffer.
- Remove **GS#-ASE** plate from magnetic plate and, using new tips, add 50 µL **GS#-UB1** to each well.
- Place **GS#-ASE** plate onto magnetic plate for ~2 minutes, or until beads are completely captured.
- Remove all **GS#-UB1** from each well (using same tips).
- Repeat addition of 50 µL **GS#-UB1** and removal of buffer.
- Pour thawed **GS#-MEL** reagent into a new sterile trough.
- Using new pipet tips, add 37 µL **GS#-MEL** to each well of **GS#-ASE** plate.
- Seal plate with adhesive film, and vortex plate at **1600 rpm\*** for 1 minute.
- Incubate **GS#-ASE** plate on preheated 45°C heat block for 15 minutes.

\* Actual vortex speed



GENOME SCAN DNA ANALYSIS FINE-MAPPING GENOTYPING  
CORRELATION GENOME SCAN DNA ANALYSIS  
FINE-MAPPING CORRELATION GENOME SCAN DNA ANALYSIS  
GENOTYPING  
COMPLETE GENOTYPING

# GENOTYPING





## Make PCR

| Process  | Plates Used | Reagents Used                       | Volume Required      |
|----------|-------------|-------------------------------------|----------------------|
| Make PCR | GS#-PCR     | Illumina-recommended DNA Polymerase | 64 $\mu$ L           |
|          |             | UDG (1U/ $\mu$ L) (OPTIONAL)        | 50 $\mu$ L           |
|          |             | GS#-MMP (tube)                      | Entire tube contents |


- Thaw one tube **GS#-MMP** to room temperature.
- Add 64  $\mu$ L Illumina-recommended DNA Polymerase to **GS#-MMP** tube.
- Add 50  $\mu$ L UDG to **GS#-MMP** tube (OPTIONAL).
- Invert **GS#-MMP** tube several times to mix contents, and pour into a sterile trough.
- Apply a **GS#-PCR** barcode label to a new 96-well 0.2 mL microplate.
- Aliquot 30  $\mu$ L of mixture into each well of **GS#-PCR** plate.
- Seal **GS#-PCR** plate with clear adhesive film and pulse centrifuge to 250 Xg.

# PCR Preparation

## Inoc PCR

| Process  | Plates Used | Reagents Used    | Volume Required      |
|----------|-------------|------------------|----------------------|
| Inoc PCR | GS#-PCR     | GS#-UB1 (bottle) | 6 mL                 |
|          |             | GS#-IP1 (tube)   | Entire tube contents |

Remove **GS#-ASE** plate from heat block.

 **Do not transfer the plate to the 95°C heat block until all washes are completed!**

Immediately preheat the heat block to 95°C.

Pour 6 mL **GS#-UB1** into a sterile trough.

Pour entire contents of **GS#-IP1** tube into another sterile trough.

Place **GS#-ASE** plate on magnetic plate for ~2 minutes, or until beads are captured.

Remove clear adhesive film from **GS#-ASE** plate.

Remove and discard supernatant (~50 µL) from all wells of the **GS#-ASE** plate (leave beads in wells).

Leave **GS#-ASE** plate on magnetic plate.

With new pipet tips, add 50 µL **GS#-UB1** to each well of the **GS#-ASE** plate, and allow plate to rest on magnetic plate for at least 2 minutes.

Remove and discard all supernatant (~50 µL) from all wells of **GS#-ASE** plate (leave beads in wells).

Discard pipet tips.

Add 35 µL **GS#-IP1** to each column of **GS#-ASE** plate and seal with adhesive film.

Vortex at **1800 rpm\*** for 1 minute, or until all beads are resuspended.

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

Remove plate from heat block and place onto magnetic plate for 2 minutes, or until beads have settled completely.

Transfer 30 µL supernatant from first column of **GS#-ASE** plate into first column of **GS#-PCR** plate, and discard pipet tips.

Repeat transfer for each column of the **GS#-ASE** plate, using new pipet tips for each column.

Seal **GS#-PCR** plate with Microseal "A" PCR plate sealing film.

Immediately transfer **GS#-PCR** plate to the thermal cycler immediately.

**GS#-ASE** plate may be discarded.

\* Actual vortex speed

## Complete PCR Steps -- Cycle PCR

Place sealed plate into thermal cycler and run thermal cycler program.

| Temperature | Time at this Temperature |
|-------------|--------------------------|
| 37°C        | 10 minutes               |
| 95°C        | 3 minutes                |
| 95°C        | 35 seconds               |
| 56°C        | 35 seconds               |
| 72°C        | 2 minutes                |
| 72°C        | 10 minutes               |
| 4°C         | 5 minutes                |

X 34

Proceed immediately to *Bind PCR*, or seal and store GS#-PCR plate at -20°C.

# Prepare & Hybridize to Array

Post-PCR

## Bind PCR

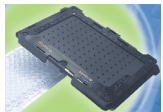
| Process  | Plates Used             | Reagents Used  | Volume Required      |
|----------|-------------------------|----------------|----------------------|
| Bind PCR | Filter Plate<br>GS#-PCR | GS#-MPB (tube) | Entire tube contents |

- Vortex **GS#-MPB** tube until beads are completely resuspended.
- Pour **GS#-MPB** into a sterile trough.
- Write the PCR plate barcode number in the space provided on a **Filter Plate: GS \_\_\_\_\_-PCR** label.
- Apply the **Filter Plate: GS \_\_\_\_\_-PCR** label to the top surface of the **Filter plate** adjacent to column 12.
- Pulse centrifuge **GS#-PCR** plate to 250 Xg.
- Using **new tips** on a multi-channel pipet (5-50  $\mu$ L), pipet 20  $\mu$ L resuspended **GS#-MPB** from trough into each column of **GS#-PCR** plate. It is not necessary to change pipet tips again until liquid has been transferred to all 12 columns.



*To avoid tip contamination, place the tips against the top edge of the wells.  
If you suspect the tips are contaminated with the contents of the well, discard the tips and use new tips.*

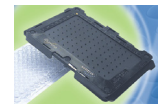
- Discard pipet tips.
- Using **new tips** on a multi-channel pipet (50-300  $\mu$ L) set to 85  $\mu$ L, pipet solution in the first column of the **GS#-PCR** plate up and down several times to mix the beads with PCR product, then transfer the mixed solution to the first column of the **Filter plate**.
- Using **new pipet tips** for each column, repeat the pipet mix and transfer step for the remaining columns of the **GS#-PCR** plate.
- Discard the empty **GS#-PCR** plate.
- Cover the **Filter plate** with its cover.
- Store at room temperature, protected from light, for 60 minutes.



*For SAM, proceed to Experienced User Cards #'s 6 and 7 (Make Hyb, Hyb SAM, & Image SAM).*



*For BeadChip, proceed to Experienced User Cards #'s 8 and 9 (Make INT, Hyb BeadChip, & Image BeadChip).*



## Make HYB

| Process  | Plates Used   | Reagents Used    | Volume Required      |
|----------|---|------------------|----------------------|
| Make HYB | Filter Plate<br>Filter Plate Adapter<br>Waste Plate<br>GS#-INT<br>GS#-HYB | 0.1N NaOH        | 5 mL                 |
|          |   | GS#-UB2 (bottle) | 10 mL                |
|          |   | GS#-MH1 (tube)   | Entire tube contents |



**User Cards 6 and 7 (Make HYB, HYB SAM, and Image SAM) apply to SAMs only. If you are using BeadChips, please skip these cards and start at User Card 8 (Make INT).**

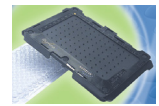
- Apply a **GS#-INT** barcode label to a new 96-well V-bottom plate.
- Apply a **GS#-HYB** barcode label to a Cliniplate 384-well microplate.
- Pour 10 mL **GS#-UB2** into a sterile trough.
- Pour 5 mL 0.1N NaOH into another sterile trough.
- Pour contents of **GS#-MH1** tube into another sterile trough.
- Place **Filter plate** adapter on a new 96-well V-bottom Waste plate.
- Place **Filter plate** containing the bound PCR products onto **Filter plate** adapter.
- Centrifuge at 1000 Xg for 5 minutes at 25°C.
- Remove **Filter plate** lid.
- Using new pipet tips, add 50 µL **GS#-UB2** from sterile trough to each column of **Filter plate**.
- Dispense slowly so as not to disturb the beads.*
- Re-lid the **Filter plate** and centrifuge at 1000 Xg for 5 minutes at 25°C.

### Prepare GS#-INT Plate

- Using new tips, dispense 30 µL **GS#-MH1** to all columns of **GS#-INT** plate.
- Replace the Waste plate with the **GS#-INT** plate.
- Orient the **GS#-INT** plate such that column A1 of the **Filter plate** matches column A1 of the **GS#-INT** plate.
- Using new tips, dispense 30 µL 0.1N NaOH to all wells of the **Filter plate**.
- Re-lid the **Filter plate** and centrifuge immediately at 1000 Xg for 5 minutes at 25°C.
- Discard the **Filter plate**; save the adapter.
- Gently mix the contents of the **GS#-INT** plate by moving it from side to side, without splashing.

### Transfer HYB Solution from GS#-INT to GS#-HYB Plate

- Using the Humidity Control Template as a guide under the **GS#-HYB** plate, dispense 30 µL **GS#-UB2** to the indicated **GS#-HYB** plate wells.
- Using the **GS#-HYB** Plate Template as a guide under the **GS#-HYB** plate, slowly pipet up and down several times to mix contents in the **GS#-INT** plate, then transfer 50 µL neutralized HYB solution from first column of **GS#-INT** plate into first marked column of **GS#-HYB** plate.
- Repeat for each column of the **GS#-INT** plate.
- Seal the plate with clear adhesive film and centrifuge at 3000 Xg for 4 minutes at 25°C.
- If the **GS#-HYB** plate is not used immediately, store it at -20°C.



## HYB SAM

| Process | Plates & Items Used                             | Reagents Used    | Volume Required |
|---------|---|------------------|-----------------|
| HYB SAM | GS#-HYB<br>2 OmniTrays<br>SAM Alignment Fixture | GS#-UB2 (bottle) | 70 mL           |
|         |   | 0.1N NaOH        | 60 mL           |

Enter the SAM ID number in the **Sentrix\_ID** column of the Sample Sheet.

Preheat oven to 60°C and allow oven to equilibrate.

If the **GS#-HYB** plate has been frozen, thaw it completely at room temperature in a light-protected drawer.

Centrifuge **GS#-HYB** plate at 3000 Xg for 4 minutes.

Dispense 70 mL **GS#-UB2** into first OmniTray, and label the tray "UB2".

Dispense 60 mL NaOH into the second OmniTray, and label the tray "NaOH".

Carefully place SAM, with the barcode facing up and the fiber bundles facing down, into the UB2 tray.

Agitate SAM gently (**10 seconds only**) to remove bubbles from bottoms of arrays. Set timer for 3 minutes.

After 3 minutes, move the SAM into NaOH tray and set timer for 30 seconds.

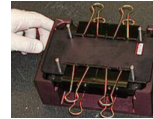
After 30 seconds, move the SAM back into the UB2 conditioning tray.

Allow SAM to sit in the UB2 conditioning tray for at least 30 seconds to neutralize the NaOH.

Gently remove the microplate clear adhesive film from the **GS#-HYB** plate.

**Film is bonded to the GS#-HYB plate very tightly. Remove this seal very carefully to avoid splashing.**

### If using SAM Alignment Fixture:



Insert **GS#-HYB** plate into the SAM alignment fixture and verify proper seating.

Apply a red perimeter gasket to the **GS#-HYB** plate top perimeter.

*Newer SAMs come with permanently affixed perimeter gaskets, and can be used with EITHER the old-style alignment fixture OR the Illumina® SAM HYB Cartridge (see below). When using these SAMs, do not attach the free-standing perimeter gasket.*

Align and seat the SAM into the SAM alignment fixture.

Place red proximal end gasket on top of SAM.

Place black metal top plate on top of proximal end gasket.

Place SAM alignment fixture clamps onto long sides of the fixture.

### If using SAM HYB Cartridge:



Slide **GS#-HYB** plate into Cartridge frame, using metal guide tabs and matching notched corners.

Place SAM, slowly lowering until firmly seated on **GS#-HYB** plate.

*Newer SAMs come with permanently affixed perimeter gaskets, and can be used with EITHER the old-style alignment fixture (see above) OR the Illumina® SAM HYB Cartridge. When using these SAMs, do not attach the free-standing perimeter gasket.*

Close SAM HYB Cartridge using gentle pressure on the lid when closing latch.

### Place in Oven

If necessary, remove BeadChip Hyb Wheel from inside the oven, using the tool provided.

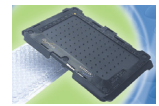
Place the **GS#-HYB** plate/SAM pair(s) into the hybridization oven (60°C).

Set timer for 30 minutes.

After 30 minutes, reset oven to 45°C.

Incubate overnight (at least 14 hours).

If experiment uses a single SAM, rename Sample Sheet using SAM ID. For multiple SAMs, Sample Sheet can be named at user discretion (e.g., experiment name). Enter the SAM ID(s) in the Sample Sheet "Sentrix\_ID" column.



## Image SAM

| Process   | Items Used  | Reagents Used    | Volume Required  |
|-----------|---|------------------|------------------|
| Image SAM | SAM<br>Illumina® BeadArray Reader<br>Four OmniTrays | 95% Ethanol      | See bottle label |
|           |   | 2-butanol        | See bottle label |
|           |   | GS#-IS1 (bottle) | 70 mL            |
|           |   | GS#-UB2 (bottle) | 140 mL           |

At first use of **GS#-IS1**, add an equal mixture of 95% ethanol and 2-butanol to the **GS#-IS1** bottle.

| Number of SAMs | GS#-IS1 Bottle Size | GS#-IS1 Volume per Bottle, as Shipped | Volume 1:1 Sec-Butanol/Ethanol to Add per GS#-IS1 Bottle |
|----------------|---------------------|---------------------------------------|--|
| One            | 125 mL              | 6 mL                                  | 94   |
| Six            | 1000 mL             | 60 mL                                 | 940  |

Replace cap on **GS#-IS1** bottle and shake to mix well. **GS#-IS1** bottle is now ready to be used.

Turn on BeadArray Reader and initialize, if not already on.

For each SAM to be imaged, label 2 OmniTrays "**GS#-UB2**" and fill with 70 mL **GS#-UB2**.

For each SAM to be imaged, label 1 OmniTray "**GS#-IS1**" and fill with 70 mL **GS#-IS1**.

Separate SAM from **GS#-HYB** plate.

*Take great care when separating the array from the HYB plate. Array bundles may sustain damage from rough handling.*

**UB2, 1 min.** Place the SAM into first **GS#-UB2** tray and agitate 1 minute at room temperature.

**UB2, 1 min.** Transfer SAM to the second **GS#-UB2** tray and agitate for 1 minute at room temperature.

*Ensure no bubbles on the bottom of array fiber bundles. These bubbles may prevent certain areas of the fiber bundle from contacting the liquid.*

**IS1, 5 min.** Dip the SAM into the **GS#-IS1** tray for 5 minutes.

Remove SAM from **GS#-IS1** tray and place on empty OmniTray to air dry 20 minutes, fiber bundles up.

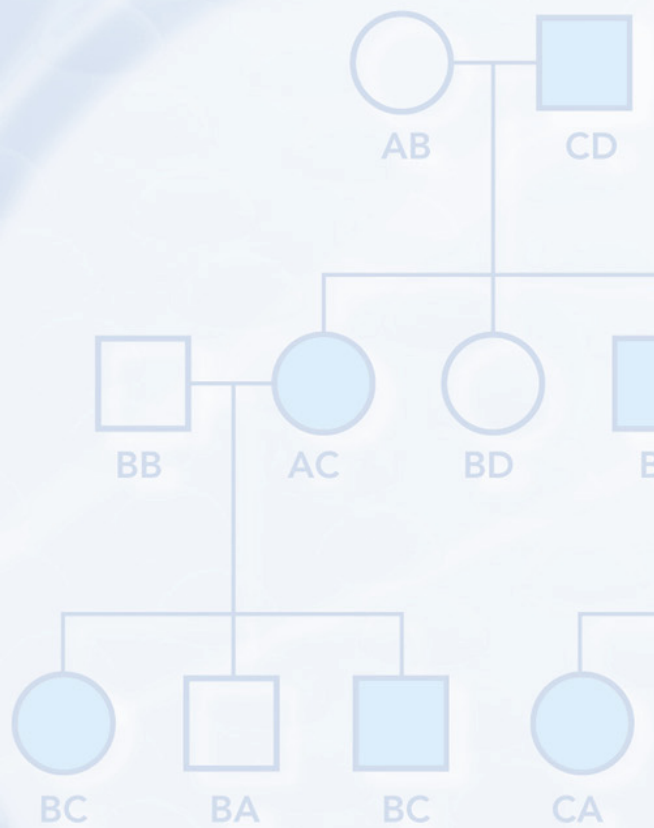
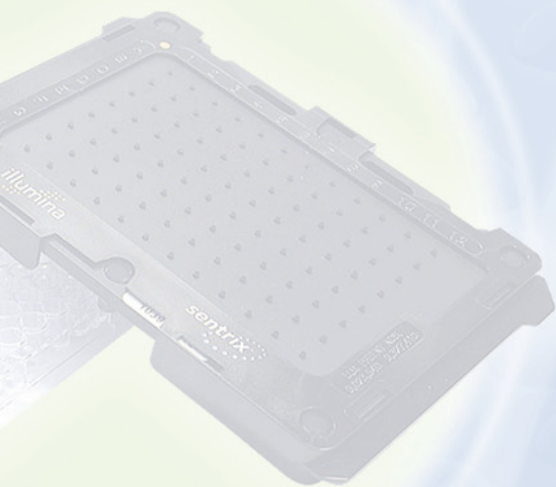
*Replace the **GS#-UB2** and **GS#-IS1** reagents for each subsequent array to be washed.*

Seal the **GS#-HYB** plate with clear adhesive sealing film and store at -20°C.

Clean proximal ends (barcode-side of SAM) of SAM bundles with canned air.

*DO NOT SHAKE the canned air before using. Shaking can cause damaging condensation.*

Place SAM in scanner tray and begin scan.

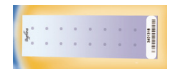


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# GENOTYPING







## Make INT

| Process  | Plates Used  | Reagents Used    | Volume Required (for one BeadChip) |
|----------|--|------------------|------------------------------------|
| Make INT | GS#-INT Plate<br>Filter Plate<br>Filter Plate Adapter<br>Waste Plate | 0.1N NaOH        | 2 mL                               |
|          |  | GS#-UB2 (bottle) | 2 mL                               |
|          |  | GS#-MH1 (tube)   | 480 µL                             |

- Apply a **GS#-INT** barcode label to a new 96-well V-bottom plate.
- Using a serological pipet, transfer 2 mL **GS#-UB2** into a sterile trough.
- Pour 2 mL 0.1N NaOH into another sterile trough.



### NOTE:

Scale up **GS#-UB2**, NaOH, and **GS#-MH1** volumes according to the number of slides being processed.

When processing 6 BeadChips (96 samples), pour the **GS#-MH1** tube's entire contents into a sterile trough. When any other combination of BeadChips is processed, aliquot 30 µL from the **GS#-MH1** tube into each well.

Place **Filter plate** adapter on a new 96-well V-bottom Waste plate.

Place **Filter plate** containing the bound PCR products onto **Filter plate** adapter.

Centrifuge at 1000 Xg for 5 minutes at 25°C.

Remove **Filter plate** lid.

Using **new pipet tips**, add 50 µL **GS#-UB2** from sterile trough to appropriate columns of the **Filter plate**.

Re-lid the **Filter plate** and centrifuge at 1000 Xg for 5 minutes at 25°C.

### Prepare GS#-INT Plate

Using **new tips**, dispense 30 µL **GS#-MH1** from stand-off tube to appropriate columns of **GS#-INT** plate.

Replace the Waste plate with the **GS#-INT** plate; discard the Waste plate.

Orient the **GS#-INT** plate such that column A1 of the **Filter plate** matches column A1 of the **GS#-INT** plate.

Using **new tips**, dispense 30 µL 0.1N NaOH to appropriate columns of the **Filter plate**.



To avoid tip contamination, place the tips against the top edge of the wells. If you suspect the tips are contaminated with the contents of the well, discard the tips and use new tips.

Re-lid the **Filter plate** and centrifuge immediately at 1000 Xg for 5 minutes at 25°C.

Discard the **Filter plate**; save the adapter.

Gently mix the contents of the **GS#-INT** plate by moving it from side to side, without splashing.

Seal **GS#-INT** plate with 96-well storage mat, then place plate in the dark until ready to dispense sample onto Sentrix BeadChip.



### NOTE:

If the **GS#-INT** plate is not used immediate in the **HYB BeadChip** protocol, store it at -20°C.



## HYB BeadChip

| Process      | Plates & Items Used  | Reagents Used  | Volume Required (for one BeadChip) |
|--------------|--|----------------|------------------------------------|
| HYB BeadChip | GS#-INT<br>Sentrrix BeadChip Hyb Cartridge<br>Hyb Oven w/Hyb Wheel | GS#-INT sample | 20 µL                              |

- Preheat oven to 60°C and allow oven to equilibrate.
- If **GS#-INT** plate is frozen, thaw it completely in a light-protected drawer, then pulse centrifuge **GS#-INT** plate to 250 Xg.
- Place **GS#-INT** plate into the 60°C oven for 10 minutes. Do not exceed 10 minutes.
- Enter the BeadChip ID number in the **Sentrrix\_ID** column of the Sample Sheet.
- To disassemble Hyb Cartridge, lift lever, remove coverseal alignment plate (CAP), then lower lever.
- Place BeadChip flat in Hyb Cartridge bottom plate, aligning barcode label with “Barcode This End” label.
- *To avoid BeadChip breakage: 1) Ensure no dirt, debris, or contamination exists beneath BeadChip when installed in Hyb Cartridge; and 2) Ensure Beadchip rests completely flat on Hyb Cartridge bottom plate.*
- Slide coverseal into slot on underside of CAP, aligning coverseal and CAP holes.
- After removal of release liner, verify that the slide has not been dislodged from its flat position in the bottom plate.
- Remove **GS#-INT** plate from oven and pulse centrifuge at 250 Xg.
- Carefully remove cap mat from **GS#-INT** plate; pipet sample up and down 5-10 times to thoroughly mix.
- Using the same tips, aliquot 20 µL assay sample and dispense to the first column of the Sentrrix BeadChip. **Do not allow the pipet tip to touch the array!**
- With fresh tips, repeat last three steps for second column of the **GS#-INT** plate.
- *To avoid contamination/evaporation, as soon as all arrays have received sample, proceed immediately to the next step.*
- With lever up, align CAP over the 3 bottom plate pins, with CAP pins are directly above lever slots.
- Using two hands, gently push CAP down to sit on the 3 Cartridge pins, then quickly lower lever, moving CAP down to seal each array on the BeadChip.
- Shake Hyb Cartridge up and down vigorously to move air bubbles in all directions.
- Observe all 16 sections for air bubbles moving upward; if necessary, shake Hyb Cartridge vigorously again until bubbles move freely in all directions.
- Record array(s) with inconsistent bubble size on BeadChip Tracking Worksheet.
- Place Hyb Cartridges on Oven Hyb Wheel such that their weight is balanced, and push gently to ensure secure placement.
- With Hyb Wheel speed set to 6, incubate for 30 minutes at 60°C; use timer.
- After 30 minutes, reset the oven temperature to 45°C. The oven should cool to 45°C (at least 14 hours).
- **Pre-Setup for Image BeadChip -- resuspend GS#-WC1 with 100% EtOH, and place on rocker overnight at room temperature.**
- If experiment uses a single SAM, rename Sample Sheet using SAM ID. For multiple SAMs, Sample Sheet can be named at user discretion (e.g., experiment name). Enter the SAM ID(s) in the Sample Sheet “Sentrrix\_ID” column.



## Image BeadChip

| Process        | Items Used  | Reagents Used    | Volume Required (for one BeadChip) |
|----------------|---|------------------|------------------------------------|
| Image BeadChip | Sentrix BeadChip & CD<br>50 mL centrifuge tubes<br>BeadArray Reader | GS#-UB2 (bottle) | 80 mL                              |
|                |   | GS#-WC1 (bottle) | 40 mL                              |
|                |   | GS#-AC1 (tube)   | 36 µL                              |

- Transfer 40 mL GS#-UB2 to each of two 50 mL centrifuge tubes labeled “UB2”.
- Transfer 40 mL GS#-WC1 to one 50 mL centrifuge tube labeled “WC1”. *(GS#-WC1 bottle must have been previously resuspended with 100% EtOH and placed on rocker at room temperature overnight.)*



### NOTE:

For multiple BeadChips, scale up number of centrifuge tubes.

- Turn the BeadArray Reader **ON**.
- Enter your username and click **Scan**.
- Allow the BeadArray Reader to warm up for 30 minutes, then double-click the SentrixScan icon on the BeadArray Reader PC desktop.

### Wash BeadChip

Do not remove Hyb Cartridge from the oven until wash solutions have been prepared.

Removal of Hyb Cartridge from oven and placing BeadChip into first wash should occur in less than 1 minute.

- Turn Hyb Wheel toggle switch to the OFF position.
- To remove BeadChip from Hyb Cartridge, lift lever; gently but firmly pull CAP and BeadChip off pins.
- Grasping barcode end of BeadChip, gently but firmly slide BeadChip out from under CAP.
- To remove Coverseal, insert the BeadChip into the Grip n’ Strip tool; use powder-free gloved hands and pull up on pointed release tab; remove Coverseal and discard.
- Immediately submerge BeadChip into the first 50 mL centrifuge tube containing GS#-UB2 solution, cap the tube and invert 5 times.
- Transfer BeadChip to second tube containing GS#-UB2 solution; incubate for 5 minutes.
- Using tweezers, transfer BeadChip to centrifuge tube containing GS#-WC1 solution, cap tube, invert 5 times, then incubate for 5 minutes.
- Using tweezers, remove BeadChip from the GS#-WC1 solution.
- Do not place tweezers into the GS#-WC1. To prevent wicking of GS#-WC1 onto the tweezers, grasp the top of the BeadChip (the part NOT in solution).
- Grasping BeadChip by the barcode end, stand it vertically on a lint-free absorbent cloth or paper towels for 1 minute.
- Transfer BeadChip to a non-absorbent surface, supported by the centrifuge tube cap, and allow to dry for 5 minutes or until completely dry.



## Apply CoverSlip

Using clean gloves, remove Coverslip from packaging; clean surfaces with a lint-free cloth and place on a new lint-free cloth until ready to use.

Place BeadChip on a flat surface, then place a gel-loading pipet tip on center of BeadChip, between rows 4 and 5.

Apply 18  $\mu$ L GS#-AC1 between rows 2 and 3, and again between rows 6 and 7.



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**NOTE:**

If bubbles appear near the surface of the GS#-AC1 spot on the BeadChip, remove them by touching lightly with a clean pipet tip.

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Place Coverslip slowly onto pipet tip, then place thumb and forefinger on opposite side of BeadChip to stabilize.

Slowly remove pipet tip until Coverslip contacts both spots of GS#-AC1.

Fully remove pipet tip; allow 5 minutes for the GS#-AC1 to spread evenly across BeadChip/Coverslip.



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**NOTE:**

If bubbles settle on an array, using a clean, firm pipet tip, press on the surface at the Coverslip above the bubble. Force the bubble away from the array.

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## Image BeadChip on the BeadArray Reader

Place BeadChip(s) in scanner tray and begin scan.