

## Axiom™ Manual Target Prep Protocol

### Stage 1: DNA Amplification

#### Intro to Manual Target Preparation

Running the Axiom Assay requires the following sets of steps:

1. Genomic DNA Prep, described in *Axiom™ gDNA Sample Prep QRC* (P/N 702928).
2. Target Prep of the samples, done using either:
  - Automated Target Prep, described in *Axiom™ Automated Target Prep Protocol QRC* (P/N 702831).
  - Manual Target Prep, described in this QRC.
3. Array Processing, described in *GeneTitan™ MC Protocol for Axiom™ Array Plate Processing QRC* (P/N 702929).

**IMPORTANT:** This QRC contains an abbreviated set of instructions used to perform target preparation without the use of a liquid handling robot. You must carefully read all the instructions in *Before You Start* in *Chapter 4, Axiom Genotyping Assay: Manual Target Preparation* of the *Axiom Genotyping Assay User Manual* (P/N 702830) before performing manual target preparation.

**NOTE:** Array handling and processing protocols still require the use of a GeneTitan MC, as described in *Chapter 5, Axiom Genotyping Assay: Array Processing* of the *Axiom Genotyping Assay User Manual* (P/N 702830) and the QRC (P/N 702929) described above.

#### ADDITIONAL NOTES:

- We recommend that you prepare your genomic DNA sample plate in a clean room.
- Remove seals from plates carefully and discard used seals. Do not reuse seals.
- Use 12-channel pipettes for all sample transfers and additions of reagents and mastermixes to the samples and GeneTitan trays.
- Change pipette tips after each sample transfer or addition to the samples.
- Unless otherwise specified, all reagent Modules are from the Axiom Genome-Wide Human Reagent Kit (P/N 901281).

#### Supplies Required for Stage 1: DNA Amplification.

Reagents from Module 1, -20 °C, P/N 901275

See *Chapter 4, Axiom Genotyping Assay: Manual Target Preparation* of the *Axiom Genotyping Assay User Manual* (P/N 702830) for a full list of the equipment and consumables required for Stage 1.

#### 1: Perform Initial Preparation

1. Set the incubator/oven temperature at 30 °C.
2. Set the centrifuge temp at room temperature.
3. Prepare reagents as shown in Table 1.1:

**Table 1.1. Reagents Preparation**

Reagent	Temp Out of Module*	Treatment	Store before using in Master Mix
Axiom Amp Soln	Thaw at Room Temp (~1 hr)	Vortex twice	Place on ice
Axiom Water	Thaw at Room Temp	Vortex	Keep at Room Temp
Axiom Denat Soln	Thaw at Room Temp	Vortex and spin	Keep at Room Temp
Axiom Neutral Soln	Thaw at Room Temp	Vortex and spin	Keep at Room Temp
Axiom Amp Enzyme Soln	Keep at -20 °C	Just before use, flick tube 3X, spin, and place in -20 °C portable cooler	Keep in -20 °C cooler until ready to use

\*Temp Out of Module: temperature reagent is held at immediately after removal from module

4. Thaw Samples in gDNA Plate:
  - A. Bring your gDNA samples to room temperature on the bench top.
  - B. Vortex, spin, and leave at room temperature.

**NOTE:** gDNA samples must be 20 µL volume of each gDNA at a concentration of 10 ng/µL.

## 2: Prepare Denaturation and Neutralization Master Mixes

These steps should be carried out at room temperature.

**Table 1.2. Denaturation Master Mix**

Reagent	per sample	Master Mix 96+
Axiom Denat Soln 10X	2 µL	300 µL
Axiom Water	18 µL	2.7 mL
Total Volume	20µL	3 mL
Vortex and leave at room temperature.		

**Table 1.3. Neutralization Master Mix**

Reagent	per sample	Master Mix 96+
Axiom Neutral Soln 10X	4 µL	500 µL
Axiom Water	36 µL	4.5 mL
Total Volume	40 µL	5 mL
Vortex and leave at room temperature		

## 3: Add Denaturation Master Mix to Samples

1. Add 20 µL of Denaturation Master Mix to each sample.
2. Incubate the plate for 3 minutes at benchtop. Seal, vortex, and spin in a room temperature centrifuge during the incubation period.
3. After incubation immediately add the Neutralization Master Mix as described below.

## 4: Add Neutralization Master Mix to Samples

1. Add 40 µL of Neutralization Master Mix to each sample.
2. Seal, vortex, and spin the Sample plate.
3. Place the plate on ice.
4. Proceed immediately to 5. *Prepare and Add the Amplification Master Mix.*

## 5: Prepare and Add the Amplification Master Mix

**IMPORTANT:** Once the Amp Enzyme is added to the master mix, finish this step with no delays.

1. These steps should be carried out on ice.

**Table 1.4. Amplification Master Mix**

Reagent	Per Sample (µL)	Master Mix 96+
In a 50 mL tube add:		
Axiom Amp Soln	304 µL	34 mL
Axiom Amp Enzyme	16 µL	1.79 mL
Total Volume	320 µL	35.79 mL
<b>A.</b> Vortex the Amplification master mix well, then invert the tube 2 times, and then vortex again.		
<b>B.</b> Quick spin and return the tube to the ice bucket.		

2. Slowly add 320 µL Amplification Master Mix to each well of the Sample plate, pipetting down the wall of the well. Do not mix by pipetting up and down.
3. Seal, vortex twice, and spin the plate for one minute at 1000 rpm.
4. Place the sealed plate in an oven set at 30 °C and leave undisturbed for 23 ± 1 hr.

## 6: Freeze or Proceed

After the incubation finishes, you can either:

- Proceed to *Stage 2: Fragmentation and Precipitation.*
- Store the Sample plate at -20 °C.

**NOTE:** If freezing, do not perform the stop amplification reaction step before you store the Sample plate at -20 °C. The Stop Amplification Reaction step will be performed after thawing the frozen plate.

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## Axiom™ Genome-Wide Assay: Manual Target Prep

### Stage 2: Fragmentation and Precipitation

#### Supplies Required for Stage 2: Fragmentation and Precipitation

Selected reagents from:

- Module 2, Box 1, -20 °C, P/N 901528
- Module 2, Box 2, 2–8 °C, P/N 901529

Isopropanol (supplied by user)

See *Chapter 4, Axiom Genotyping Assay: Manual Target Preparation* of the *Axiom Genotyping Assay User Manual* (P/N 702830) for a full list of the equipment and consumables required for Stage 2.

#### 1: Stop Amplification Reaction

1. Prepare the following instruments for this stage:
  - One oven at 65 °C
  - One oven at 37 °C
  - One centrifuge at room temperature
2. Place the Sample plate in the 65 °C oven and incubate for 20 minutes.
3. Prepare reagents at the start of the 65 °C incubation of the amplification plate as shown in Table 2.1.

**Table 2.1. Reagent Preparation**

Reagent	Temp Out of Module*	Preparation Treatment	Store before using in Master Mix
<b>From Modules:</b>			
Axiom 10X Frag Buffer	Thaw at Room Temp	Vortex	Place on ice
Axiom Frag Diluent	Place on ice	Vortex and spin	Place on ice
Axiom Frag Enzyme	Keep at -20 °C	Just before use, flick tube 3X, spin, and place in -20 °C portable cooler	Keep in -20 °C cooler until ready to use.
Axiom Frag Rxn Stop	Room Temp	Vortex	Keep at Room Temp
Precip Soln 1	Place on ice	Vortex	Place on ice
Precip Soln 2	Thaw at Room Temp	Vortex and spin	Place on ice
<b>Supplied by user:</b>			
Isopropanol	Room Temp	Room Temp	Room Temp
Notes: *Temp Out of Module: temperature reagent is held at immediately after removal from module			

4. Optional: Remove samples for quantifying amplification yield by the PicoGreen Assay. See *Chapter 4, Axiom Genotyping Assay: Manual Target Preparation* for more information.
5. Transfer the Sample plate from the 65 °C oven to the 37 °C oven and incubate for 45 minutes.

#### 2: Prepare Fragmentation Master Mix

1. Start making the fragmentation master mix when there is still five minutes to the finish of the 37 °C incubation, using the values in Table 2.2.
  - A. Add the Axiom Frag Enzyme to the Fragmentation Master Mix at the end of the 45 minute 37 °C incubation.
2. Vortex twice and place on ice.

**Table 2.2. Fragmentation Master Mix**

Reagent	per sample	Master Mix 96+
Axiom 10X Frag Buffer	45.7 µL	5.3 mL
Axiom Frag Diluent	10.05 µL	1.17 mL
Axiom Frag Enzyme	1.25 µL	145.0 µL
Total Volume	57 µL	6.62 mL

### 3: Add Fragmentation Master Mix to Wells

**IMPORTANT:** Work quickly to perform this set of steps to minimize the time that the Fragmentation plate is out of the 37 °C oven.

- Carefully remove the Sample plate from the 37 °C oven and place on the bench top at room temperature.  
Do **not** place the Sample plate on ice.
- Add 57 µL of Fragmentation Master Mix to each reaction.**
- Seal and vortex.
- Start the timer for 30 min.
- Quick spin the Sample plate in the room temperature plate centrifuge.
- Quickly transfer plate to 37 °C oven and incubate for 30 min.

**CAUTION:** Be watchful for the end of the thirty minute incubation period. Fragmentation is an exact 30 minute incubation step. Longer and shorter incubation times may lead to poor performance of the assay.

### 4: Aliquot the Stop Solution to the Plate

- A few minutes before the end of the 30 minute incubation period, pour the Axiom Frag Rxn Stop solution in a solution basin.  
Leave the *Stop* solution basin at room temperature.
- Remove the Sample plate from the oven and place on the bench top.
- At the end of the 30 minute fragmentation incubation period, **add 19 µL of Stop Solution to each reaction.**
- Seal, vortex, and spin.
- Place the Sample plate on ice while you prepare the Precipitation Master Mix.

### 5: Prepare and Add Precipitation Master Mix

- Prepare Precipitation Master Mix in a 50 mL Falcon tube.
  - Add the reagents in the order and volumes shown in Table 2.3.
  - Vortex the *Precip Master Mix* tube and place on ice.
- Pour the Precipitation Master Mix into the solution basin.
- Add 240 µL Precipitation Master Mix to each sample. You do not need to mix up and down.
- Seal the Sample plate, vortex, and spin.
- Remove the Sample plate from the centrifuge and place on ice.
- Add 600 µL isopropanol to each sample and mix well by pipetting up and down within the solution to ensure mixing. The solution should look homogenous in the tips after pipetting 5-7 times.  
If not, repeat mixing a few more times until the solution looks mixed.
- Blot the top of the plate with Kimwipe and seal tightly with a Microamp seal.
- Carefully transfer the Sample plate into the -20 °C freezer and incubate overnight (16-24 hours).
- After incubation, proceed to Stage 3.

**Table 2.3. Precipitation Master Mix**

Reagent	per sample	Master Mix 96+
Axiom Precip Soln 1	238 µL	26 mL
Axiom Precip Soln 2	2 µL	218 µL
Total Volume	240 µL	26.22 mL

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#### Supplies Required for Stage 3. Drying, Resuspension, and QC

Reagents Required:

- Module 2, Box 1, -20 °C, P/N 901528
- Module 2, Box 2, 2–8 °C, P/N 901529

**Table 3.1. Other Reagents Required for QC steps (optional)**

Reagent
Invitrogen™ TrackIt Cyan/Orange Loading Buffer (Invitrogen P/N 10482-028)
25bp Invitrogen Ladder (Invitrogen P/N 10488-022)
Nuclease free water, ultrapure MB Grade (USB, P/N 71786); Module 2, Box 2, 2–8 °C, P/N 901529

See Chapter 4, *Axiom Genotyping Assay: Manual Target Preparation* of the *Axiom Genotyping Assay User Manual* (P/N 702830) for a full list of the equipment and consumables required for Stage 3.

#### 1: Centrifuge and Dry Pellets and Thaw Reagents

**CAUTION:** Some of the steps in this stage should be performed under a fume hood.

**CAUTION:** During this step, handle the Sample plate gently to avoid disturbing the pellets. Do not bump or bang the plate.

1. Preheat the oven to 37 °C.
2. Begin thawing/warming the reagents used in this stage as shown in Table 3.2.
3. Remove the Sample plate from the -20 °C freezer and centrifuge the plate at 3200 xg at 4 °C for 40 min.
4. During centrifugation prepare the resuspension and hybridization reagents as shown in Table 3.2.
5. Following centrifugation, empty the liquid from the Sample plate as follows:
  - A. Carefully remove the seal from the Sample plate and discard the seal.
  - B. Invert the plate over a waste container and allow the liquid to drain.
  - C. While still inverted, gently press the plate on a pile of Kimwipes on a bench and leave it for 5 min.
6. Turn the plate top side up and place in an oven for 20 min at 37 °C to dry.

**Table 3.2. Reagent Preparation**

Reagent	Temp Out of Module*	Treatment	Store before using
Axiom Hyb Buffer	Place on ice	Vortex	Place on ice
Axiom Hyb Soln 1	Thaw at Room Temp	Vortex and spin	Place on ice
Axiom Resusp Buffer	Warm to Room Temp (~ 1 hr)	Vortex	Keep at Room Temp
Axiom Hyb Soln 2	Place on ice	Vortex and spin	Place on ice

7. After 20 min remove the plate from the oven and either:
  - Proceed directly to 2: *Resuspension and Hybridization Master Mix Preparation*, even if some droplets of liquid remain. Leave the Sample plate at room temperature.
  - Tightly seal the plate and store at -20 °C.

#### 2: Resuspension and Hybridization Master Mix Preparation

**NOTE:** If a plate was stored at -20 °C after drying the pellets, allow the plate to sit at room temperature for 1.5 hour before carrying out resuspension.

**NOTE:** The Resuspension steps should be carried out at room temperature. Make sure the Axiom Resusp Buffer has equilibrated to room temperature before adding to dry pellets in Step 1, below.

1. **Transfer 35 µL Axiom Resusp Buffer to each well** of the sample plate with a dry pellet. Avoid touching pellets with tip. Change pipette tips after each addition.
2. Seal the Sample plate and put on one of the following shakers:
  - Titer Plate Shakers-4PL: at speed 9 for 10 min
  - Jitterbug: at speed 7 for 10 min

**CAUTION:** Perform the rest of the steps in this stage under a fume hood.

3. While the Sample plate is shaking, prepare the Hybridization Master Mix in a 15 mL tube as shown in Table 3.3. Vortex and place on ice.

**Table 3.3. Hybridization Master Mix**

Reagent	per sample	Master Mix 96+
Axiom Hyb Buffer	70.75 µL	7.9 mL
Axiom Hyb Soln 1	0.25 µL	27.8 µL
Axiom Hyb Soln 2	9 µL	1.0 mL
Total Volume	80 µL	8.89 mL

4. Inspect the Sample plate from the bottom. If the pellets are not dissolved, repeat Step 2.
5. Quick spin.
6. Select a PCR plate appropriate to the type of approved thermal cycler you will use in Stage 4.
7. Label the plate as "Hyb Ready Plate [plate ID]."
8. Transfer the entire contents of each well of the Resuspension plate to the labeled Hyb Ready plate.
9. **Add 80 µL of the Hyb Master Mix to each well** of the Hyb Ready plate.
10. Seal, vortex, and spin.

### 3: Recommended: Perform Quantitation and Fragmentation QC Checks

Before proceeding to Stage 4: Denaturation and Hybridization, we recommend that you perform quantitation and fragmentation quality control checks.

Reagents required:

- 14 mL of nuclease free water
- 1:1000 dilution of 6x Invitrogen TrackIt Cyan/Orange
  - Mix 49.95 mL of nuclease free water with 50 µL of 6x Invitrogen TrackIt Cyan/Orange Loading Buffer (Invitrogen P/N 10482-028)
- 15 fold dilution of 25bp Invitrogen Ladder (Invitrogen P/N 10488-022)

To perform the QC checks:

1. Make QC Dilution Plate:
  - A. Add 33 µL nuclease-free water to each well of a PCR plate labeled "QC Diln".
  - B. Transfer 3 µL of the Hyb Ready sample from each well of the Hyb Ready plate to the corresponding well of the QC Diln plate.
  - C. Seal, vortex, and spin.
2. Make and read OD Sample plate:
  - A. Transfer 10 µL of each QC Dilution Plate sample to the OD Plate (96-well UV Star plate, E&K Scientific P/N 25801).
  - B. Add 90 µL nuclease-free water to each well of the OD Plate and mix by pipetting up and down.
  - C. Read absorbance on a plate reader. See *Appendix B, Sample Quantitation after Resuspension of the Axiom Genotyping Assay User Manual* (P/N 702830) for more information.
3. Make and run Gel Samples:
  - A. Add 60 µL gel loading dye to each well of the Gel Sample Plate (Invitrogen P/N G8008-04).
  - B. Transfer 3 µL of each QC Dilution Plate sample to the Gel Sample Plate.
  - C. Seal, vortex, and spin.
  - D. Run Gel:

Consult *Appendix A, Fragmentation Quality Control Gel Protocol* of the *Axiom Genotyping Assay User Manual* (P/N 702830) for more information.

### 4: Freeze or Proceed to Stage 4

At this point you can:

- Proceed to *Stage 4: Denaturation and Hybridization*, or
- Store the Hyb Ready samples at -20 °C.

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## Axiom™ Manual Target Prep Protocol

### Stage 4: Denaturation and Hybridization

#### Required Supplies for Stage 4: Denaturation and Hybridization

Reagents from Module 3, P/N 901472

**Table 4.1. Components from Affymetrix Array Plate Kit required.**

Consumable	Vendor and Part Number	Quantity
One of the following Axiom Array plates:		
■ Axiom Genome-Wide Array Plate (96 arrays) on protective base	P/N 901608	1
■ Axiom Genome-Wide ASI Array (96 arrays) on protective base	P/N 901640	1
Hyb Tray		1

**Table 4.2. Other Required Equipment and Supplies**

√	Equipment	Quantity
	GeneTitan MC	1
	Approved Thermal Cycler programmed with the "Axiom Denature" protocol and appropriate plate	1
	Fume Hood	1

See Chapter 4, *Axiom Genotyping Assay: Manual Target Preparation* of the *Axiom Genotyping Assay User Manual* (P/N 702830) for a full list of the equipment and consumables required for Stage 4.

#### 1: Prepare Hyb Ready Samples Stored at -20 °C

Warm up the Hyb Ready plate at room temperature for 5 minutes.

1. Make sure the Hyb Ready plate is sealed well. If not, centrifuge the plate and change the seal.
2. Vortex the Hyb Ready plate briefly, then spin at 1000 rpm for 30 seconds.
3. Place the Hyb Ready plate at room temperature.

#### 2: Prepare Equipment and Perform Denaturation

1. Preheat the 96-well metal chamber in a 48 °C oven.
2. Warm up the array plate on the bench top for a minimum of 25 minutes before setting up hybridization on the GeneTitan MC.
3. At the end of the array warm up time, open the pouch and scan the array plate barcode into the Batch Registration file.
4. Make sure the thermocycler is powered on and the Axiom Denature program with the heated lid option has been selected.
  - Axiom Denature program: 95 °C for 20 min, 48 °C for 3 min, 48 °C hold
5. Start the Axiom Denature program.
6. While the program is running:
  - A. Prepare the reagents from Module 3 as described in Table 4.3.
  - B. Upload the Batch Registration File.
  - C. Set up the GeneTitan MC.

For more information, see:

*GeneTitan™ MC Protocol for Axiom™ Array Plate Processing QRC* (P/N 702929).

*Chapter 5, Axiom Genotyping Assay: Array Processing* of the *Axiom Genotyping Assay User Manual* (P/N 702830).

**Table 4.3. Reagent Preparation**

Reagent	Temp Out of Module*	Treatment
Axiom Wash Buffer A	Room Temp	Invert 2-3X for mixing before filling GT bottle
Axiom Wash Buffer B	Room Temp	Invert 2-3X for mixing before filling GT bottle
Axiom Water	Room Temp	N/A

Notes:

\*Temp Out of Module: temperature the reagent is held at immediately after removal from module

N/A: not applicable in this case

### 3: Prepare Hybridization Tray and Load into GeneTitan MC

1. Remove the Hyb Tray (from Axiom Array Plate kit) from packaging.
2. Label the Hyb Tray.

**IMPORTANT:** It is critical that you write only on the proper location of the Hyb tray, as shown in Figure 4.1. Do NOT write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure.

3. After the Axiom Denature program has completed, remove the Hyb Ready plate from the thermocycler and place into the preheated 96-well metal chamber.

**CAUTION:** Perform the next set of steps under a fume hood.

4. Using a pipette set at 105  $\mu$ L, slowly transfer the denatured samples from the Hyb Ready plate into the Hyb tray. Dispense to the first stop to avoid creating bubbles.
5. Load the array plate and Hyb tray into GeneTitan MC.

The Array plate is shipped with a clear top lid and a blue or black bottom cover (Figure 4.2). Before loading, the top lid must be removed.

**IMPORTANT:** The array plate must be loaded on its protective blue base, as shown in figure 4.3.

The clear plastic lid on top of the array plate SHOULD NOT be loaded in the GeneTitan MC.

The Hyb tray should not have any bubbles and there is no need to spread the liquid around the bottom of the wells.

**IMPORTANT:** After GeneTitan MC has stacked the array plate and hyb tray, manually check the stacking by gently pressing the six latching points to confirm that the two parts are clamped properly, and check underneath the arrays to make sure there are no bubbles. If bubbles are found, attempt to remove them by gently tapping the plate on top.

Hybridization continues on the GeneTitan for 23.5-24 hours before you will load the Ligation/Staining/Stabilization reagent trays into the GeneTitan.

You must wait until the hybridization step on the GeneTitan is approximately 1.5 hours from completion (22 hours after the start of hybridization) to begin Stage 5 of the Manual Target Prep.

Figure 4.1. Labeling Hyb Tray

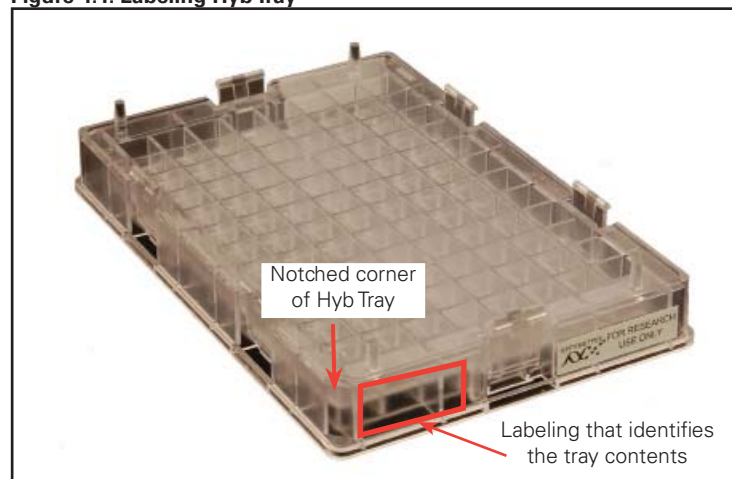


Figure 4.2. Array Plate as shipped

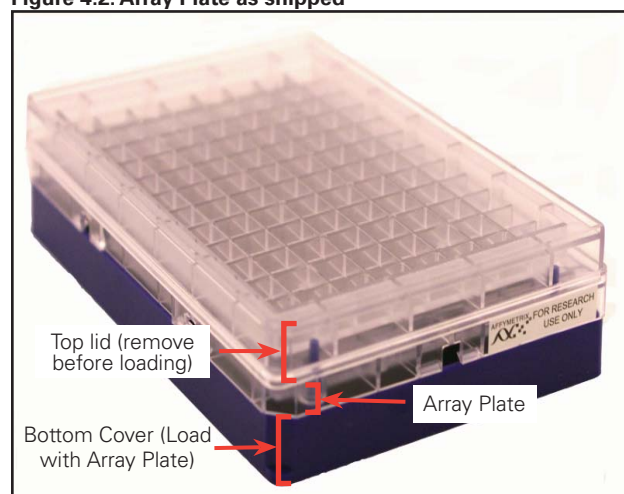
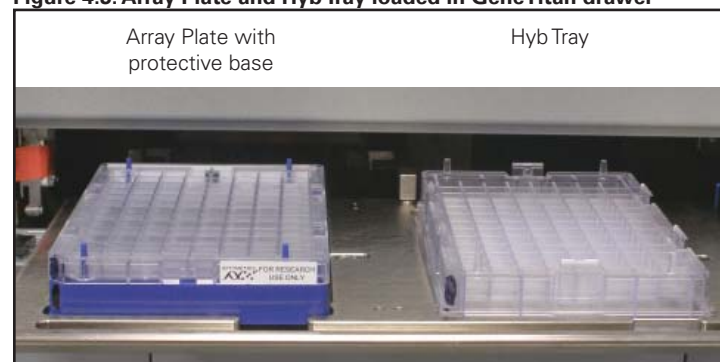


Figure 4.3. Array Plate and Hyb Tray loaded in GeneTitan drawer



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## Axiom™ Manual Target Prep Protocol

### Stage 5: Manually Preparing Reagent Trays for GeneTitan MC

#### Required supplies and Equipment

Reagents from Module 4, box 1, -20 °C, P/N 901278

Reagents from Module 4, box 2, 2-8 °C, P/N 901276

See *Chapter 4, Axiom Genotyping Assay: Manual Target Preparation* of the *Axiom Genotyping Assay User Manual* (P/N 702830) for a full list of the equipment and consumables required for Stage 5.

#### 1: Prepare Reagents

1. Prepare the reagents from Module 4 as described in the table below:

**Table 5.1. Reagents from Module 4**

Reagent	Temp Out of Module*	Treatment	Storage before Master Mix
<b>Module 4, box 1 of 2 (P/N 901278)</b>			
Axiom Ligate Buffer	Thaw at Room Temp	1. Place on bench top at room temp for 30 min 2. Examine for precipitate 3. Vortex twice 4. Examine for precipitate. If any: ▪ Warm bottle with your hands and vortex again for thirty seconds	Place on ice
Axiom Ligate Enzyme	Keep at -20 °C until ready to use	Just before use: 1. Flick 2 to 3 times to mix 2. Spin. 3. Place in -20 °C portable cooler until use.	Place in -20 °C portable cooler
Axiom Ligate Soln 1	Thaw at Room Temp	Vortex and Spin	Place on Ice
Axiom Probe Mix 1	Thaw at Room Temp	Vortex and Spin	Place on Ice
Axiom Stain Buffer	Thaw at Room Temp	Vortex and Spin	Place on Ice
Axiom Stabilize Soln	Thaw at Room Temp	Vortex and Spin	Place on Ice
<b>Module 4, box 2 of 2 (P/N 901276)</b>			
Axiom Ligate Soln 2	Thaw at Room Temp (do not place on ice!)	Vortex and Spin	Store at Room Temp.
Axiom Probe Mix 2#	Place on Ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Wash A	Leave on bench	1. Vortex twice 2. Place on Bench for 30 min. 3. Look for precipitate. 4. Vortex again if necessary.	Place on bench top at room temp
Axiom Stain 1-A#	Place on ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Stain 1-B#	Place on ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Stain 2-A#	Place on ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Stain 2-B#	Place on ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Stabilize Diluent	Place on ice	1. Vortex and Spin 2. Look for precipitate If any: ▪ Warm tube to room temperature and vortex again.	Place on ice
Axiom Water	Place on ice	N/A	Place on ice
Axiom Hold Buffer#	Room Temp	Vortex	Store at Room Temp away from light

Notes:

#: These solutions are light sensitive. Do not expose tubes to direct light for a prolonged period of time.

Temp Out of Module\*: temperature the reagent is held at immediately after removal from module

N/A: not applicable in this case

**NOTE:** The presence of some precipitate in Axiom Ligate Buffer will not adversely impact assay performance. Follow the instructions above to resuspend any precipitate before use.

**NOTE:** Occasionally, crystals are observed in Axiom Wash A and Axiom Stabilize Diluent upon removal from 2-8 °C storage. Before using these solutions, the crystals should be dissolved by warming the solutions to room temperature and then vortexing.

## 2: Prepare the Stain, Ligation and Stabilization Master Mixes

### Prepare Stain 1 Master Mix

1. Add reagents in the order shown in Table 5.2. This recipe will provide enough for both S1 reagent trays.
2. Gently invert the tube 10 times to mix.
3. Place the tube with the Stain 1 Master Mix on ice and protect from direct light.

### Prepare Stain 2 Master Mix

1. Add reagents in the order shown in Table 5.3.
2. Gently invert the tube 10 times to mix.
3. Place the tube with the Stain 2 Master Mix on ice and protect from direct light.

### Prepare Stabilization Master Mix

1. Add reagents in the order shown in Table 5.4.
2. Vortex the master mix at high speed for 3 sec.
3. Place the tube with the Stabilization Master Mix on ice.

### Prepare Ligation Master Mix

The Ligation Master Mix is prepared in two stages.

#### Ligation Master Mix: Stage 1

1. Place the Ligation Master Mix tube on ice.
2. Add reagents to the tube in the order shown in Table 5.5.
3. Mix well by vortexing the tube for 3 seconds.
4. Place the tube with the Ligation Master Mix back on ice.

#### Ligation Master Mix: Stage 2

1. Remove the Axiom Ligation Enzyme from the  $-20^{\circ}\text{C}$  freezer and place in a cooler chilled to  $-20^{\circ}\text{C}$ .
2. Add reagents in the order shown in Table 5.6.
3. Gently flick the Axiom Ligase Enzyme tube 2-3 times, then perform a quick spin immediately prior to adding the enzyme to the Master Mix.
4. Gently invert the Master Mix tube 10 times to mix (do not vortex).
5. Place on ice and protect from direct light.

**Table 5.2. Stain 1 Master Mix (for both S1 trays)**

Reagent	Per Array	Master Mix 96+
Axiom Wash A	201.6 $\mu\text{L}$	22.2 mL
Axiom Stain Buffer	4.2 $\mu\text{L}$	463 $\mu\text{L}$
Axiom Stain 1-A	2.1 $\mu\text{L}$	231 $\mu\text{L}$
Axiom Stain 1-B	2.1 $\mu\text{L}$	231 $\mu\text{L}$
Total	210 $\mu\text{L}$ (105 $\mu\text{L}$ x 2)	23.13 mL

**Table 5.3. Stain 2 Master Mix**

Reagent	Per Array	Master Mix 96+
Axiom Wash A	100.8 $\mu\text{L}$	11.1 mL
Axiom Stain Buffer	2.1 $\mu\text{L}$	231 $\mu\text{L}$
Axiom Stain 2-A	1.05 $\mu\text{L}$	115.6 $\mu\text{L}$
Axiom Stain 2-B	1.05 $\mu\text{L}$	115.6 $\mu\text{L}$
Total	105 $\mu\text{L}$	11.56 mL

**Table 5.4. Stabilization Master Mix**

Reagent	Per Array	Master Mix 96+
Axiom Water	93.19 $\mu\text{L}$	10.3 mL
Axiom Stabilize Diluent	10.50 $\mu\text{L}$	1.16 mL
Axiom Stabilize Soln	1.31 $\mu\text{L}$	144.8 $\mu\text{L}$
Total	105 $\mu\text{L}$	11.61 mL

**Table 5.5. Ligation Master Mix: Stage 1**

Reagent	Per Array	Master Mix 96+
Axiom Ligase Buffer	66.15 $\mu\text{L}$	7.3 mL
Axiom Ligase Soln 1	13.12 $\mu\text{L}$	1.45 mL
Axiom Ligase Soln 2	3.15 $\mu\text{L}$	348 $\mu\text{L}$
Sub-Total	82.42 $\mu\text{L}$	9.10 mL

**Table 5.6. Ligation Master Mix: Stage 2**

Reagent	Per Array	Master Mix 96+
Ligation Master Mix from Stage 1	82.42 $\mu\text{L}$	9.10 mL
Axiom Probe Mix 1	10.5 $\mu\text{L}$	1.16 mL
Axiom Probe Mix 2	10.5 $\mu\text{L}$	1.16 mL
Axiom Ligase Enzyme	1.58 $\mu\text{L}$	174.4 $\mu\text{L}$
Total	105 $\mu\text{L}$	11.58 mL

### 3: Aliquot Master Mixes and Axiom Hold Buffer into Trays

**NOTE:** It is not necessary to change pipette tips between additions of the same reagents to Stain trays and Scan trays.

#### Prepare Trays and Lids

**IMPORTANT:** It is critical that you write only on the proper location of the proper edge of the Stain trays, as shown in Figure 5.1. Do NOT write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure.

1. Label two Stain Trays S1 (for Stain 1 Master Mix)
2. Label the remaining stain trays:
  - S2 (for Stain 2 Master Mix)
  - Stbl (for Stabilization Master Mix)
  - Lig (for Ligation Master Mix)

3. Destatic the inside of each tray and cover.

See *Appendix E, Deionization Procedure for GeneTitan MC Instrument Trays and Covers of the Axiom Genotyping Assay User Manual (P/N 702830)* for the recommended technique.

#### About Aliquoting Reagents to Trays

**IMPORTANT:** Always aliquot reagents to the bottom of the tray. Avoid touching the sides or the top of the wells with the pipette tips. Droplets close to or on the top of the well dividers may cause the lid to stick to the tray during GeneTitan processing.

When aliquoting ligation, staining, and stabilization reagents to the trays, it is not necessary to spread the reagent to each corner of the well. The reagent will spread evenly when the array plate is inserted into the reagent tray during processing with GeneTitan MC.

#### Aliquoting Reagents to Stain Trays

You will need to aliquot the appropriate master mix into the S1, S2, Stbl, and Lig trays labeled in the previous step:

1. Aliquot **105 µL** per well of the appropriate Master Mix — dispense to the first stop only to avoid creating bubbles.
2. If:
  - Bubbles are present, puncture them with a pipette tip.
  - Droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove.
3. Place covers on the trays. Orient cover correctly on the tray with the notched corners together.
4. Protect the trays from light if not immediately loading onto the GeneTitan MC.

Figure 5.1. Stain Tray with lid

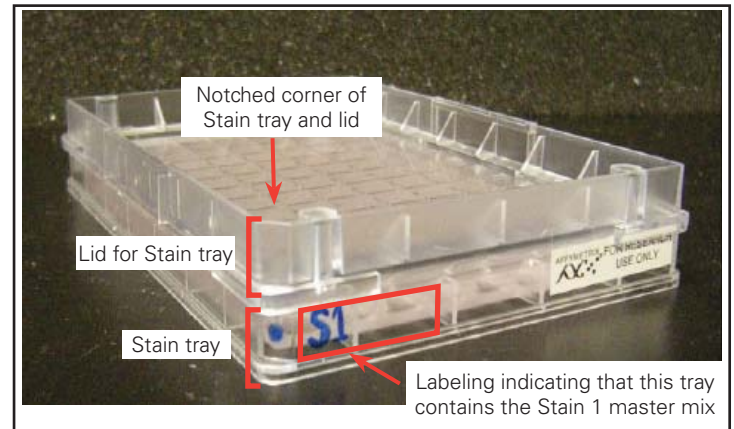
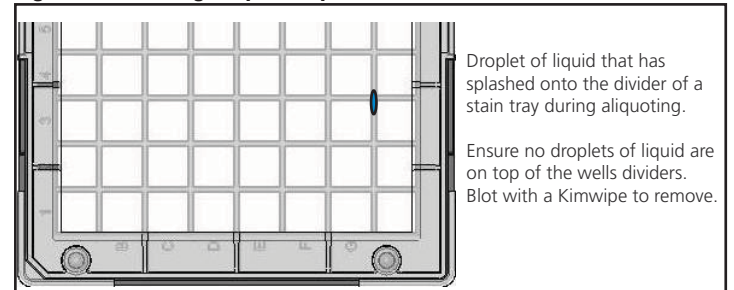


Figure 5.2. Blotting drops of liquid on dividers



### Aliquoting Hold Buffer to the Scan Tray

The Scan Tray is shipped with two covers, a bottom protective cover and a top lid (Figure 5.3).

The top cover is removed to fill the tray during the target prep process, while the Scan tray is left on the bottom cover during this part of the process (Figure 5.4).

1. Pour the Axiom Hold Buffer into the solution basin marked Hold, placed on the bench top at room temperature.
2. Remove the Scan tray from its pouch.
3. Remove the top scan tray lid, but leave the Scan Tray on its protective black cover.
4. Aliquot **150 µL** to each well of the Scan tray — dispense to the first stop and avoid touching the bottom of the tray.

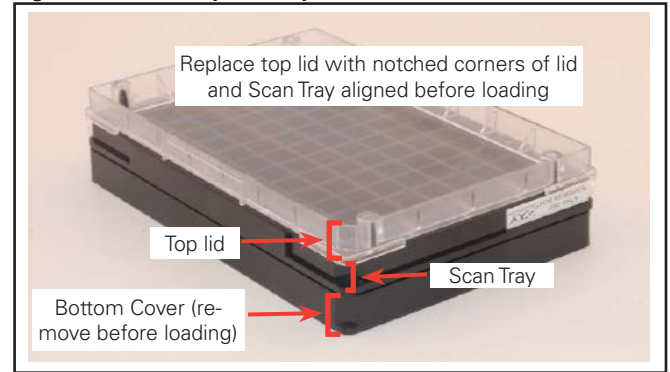
**IMPORTANT:** The Hold buffer requires 150 µL per well.

5. If droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove.
6. Cover the tray by orienting the notched corner of the lid over the notched edge of the tray, and leave on the bench top.

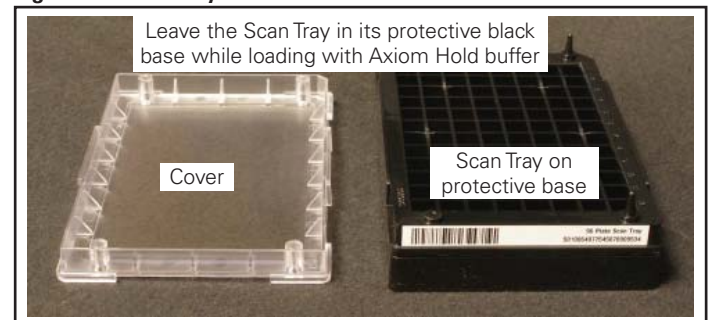
For more information on loading the reagent and scan trays, see:

- *GeneTitan™ MC Protocol for Axiom™ Array Plate Processing QRC* (P/N 702929)
- *Chapter 5, Axiom Genotyping Assay: Array Processing of the Axiom Genotyping Assay User Manual* (P/N 702830)

**Figure 5.3. Scan Tray with top and bottom covers**



**Figure 5.4. Scan Tray with cover removed**



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