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Axiom® 2.0 Assay Manual Target Prep Protocol Introduction and Stage 1: DNA Amplification

Introduction to Manual Target Preparation

Running the Axiom 2.0 Assay requires the following sets of steps:

1. Genomic DNA Prep, described in either the *Axiom® 2.0 gDNA Sample Prep QRC* (P/N 702987) or the *Axiom® gDNA Sample Prep for Genome-Wide BOS 1 Array Plate* (P/N 702975).
2. Target Prep of the samples, done using either:
   - Automated Target Prep, described in *Axiom® 2.0 Automated Target Prep Protocol QRC* (P/N 702962).
   - Manual Target Prep, described in this QRC.
3. Array Processing, described in *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC* (P/N 702988).

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 the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990) before performing manual target preparation.

NOTE: Array handling and processing protocols still require the use of a GeneTitan MC Instrument, as described in Chapter 5, *Array Processing with the GeneTitan® Multi-Channel Instrument* of the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990) and the QRC (P/N 702988) 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 master mixes 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 2.0 Reagent Kit (P/N 901758).
- See Chapter 4 of the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990) for a complete list of equipment and consumables required for each stage.

1. Preparation for Stage 1: DNA Amplification

Supplies Required

- Reagents from Axiom 2.0 Reagent Kit, Module 1, –20 °C, P/N 901711

Instrument Setup

- Set the oven temperature at 37 °C.
- Set the centrifuge temp at room temperature.

Reagent Preparation

1. Prepare reagents as shown in Table 1.1:

Table 1.1. Reagents Preparation

Reagent	Treatment
Axiom 2.0 Denat Soln 10X	Thaw, vortex, spin and keep at room temperature
Axiom 2.0 Neutral Soln	Thaw (see Note below) vortex and keep at room temperature
Axiom 2.0 Amp Soln	Thaw (see Note below) vortex and keep at room temperature
Axiom Water	Thaw (see Note below) vortex and keep at room temperature
Axiom 2.0 Amp Enzyme	Flick tube 3X, spin, and keep in –20 °C cooler until ready to use

NOTE: Allow ~ 1 hour for Axiom 2.0 Amp Soln to thaw on the benchtop at room temperature. If the solution is not completely thawed after 1 hour, vortex briefly and return to the benchtop to complete thawing. The bottles can also be thawed in a dish with Millipore water. The Axiom 2.0 Amp Soln must be thoroughly mixed before use.

2. 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: The gDNA samples must be at a volume of 20 µL for each sample. The DNA concentration is 5 ng/µL (for a total of 100 ng of gDNA in 20 µL) for gDNA that will be processed for the Axiom® Genome-Wide Pan-African Array. For gDNA that will be processed for all other Axiom genome-wide human arrays (CEU, ASI, EUR, and CHB), the concentration is 10 ng/µL (for a total of 200 ng gDNA in 20 µL).

NOTE: Carry out the master mix preparations and additions to the sample plate at room temperature.

2: Prepare Denaturation Mix

Prepare Denaturation Master Mix as shown in Table 1.2.

Table 1.2. Denaturation Master Mix

Reagent	per Sample	Master Mix 96+
Axiom 2.0 Denat Soln 10X	2 µL	400 µL
Axiom Water	18 µL	3.6 mL
Total Volume	20 µL	4 mL

3: Add Denaturation Master Mix to Samples

1. Add **20 µL of Denaturation Master Mix** to each sample.
2. Incubate the plate for **10 minutes** at room temperature. 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 Axiom 2.0 Neutral Soln to Samples

1. Add **130 µL of Axiom 2.0 Neutral Soln** to each sample.
2. Seal, vortex, and spin the Sample plate.
3. Proceed immediately to *5. Prepare and Add the Amplification Master Mix*.

5: Prepare and Add the Amplification Master Mix

1. Slowly add **230 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.
2. Seal tightly, vortex twice, and spin the plate for one minute at 1000 rpm.
3. Place the sealed plate in an oven set at **37 °C** and leave undisturbed for **23 ± 1 hr**.

Table 1.3. Amplification Master Mix

Reagent	per Sample (µL)	Master Mix 96+
To a 50 mL tube marked Amp MM, add:		
Axiom 2.0 Amp Soln	225 µL	26.0 mL
Axiom 2.0 Amp Enzyme	5 µL	578 mL
Total Volume	230 µL	26.58 mL
Vortex the Amplification master mix well, then invert the tube 2 times, and then vortex again.		

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® 2.0 Assay Manual Target Prep Protocol
Stage 2: Fragmentation and Precipitation

Preparation for Stage 2: Fragmentation and Precipitation

Supplies Required

- Selected reagents from Axiom 2.0 Reagent Kit (see Table 2.1):
  - Module 2-1, –20 °C, P/N 901528
  - Module 2-2, 2–8 °C, P/N 901529
- Isopropanol (supplied by user)

Instrument Setup

- Prepare the following instruments for this stage:
  - One oven at 65 °C
  - One oven at 37 °C
  - One centrifuge at room temperature

NOTE: If the plate of Amplifed DNA samples was frozen at the end of Stage 1, thaw the plate before beginning Stage 2. See instructions in Chapter 6 of the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990) for notes on thawing and spinning down prior to changing the seal to avoid cross contamination.

TIP: Keep a balance plate ready to avoid delays during the fragmentation steps.

1: Stop Amplification Reaction

1. Place the Sample plate in the **65 °C oven and incubate for 20 minutes**.
2. 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	Module	Treatment
Axiom 10X Frag Buffer	2-1	Thaw, vortex and keep on ice.
Axiom Frag Enzyme	2-1	Flick tube 3X, spin, and keep in –20 °C cooler until ready to use.
Precip Soln 2	2-1	Thaw, vortex, spin and keep at room temperature.
Axiom Frag Diluent	2-2	Thaw, vortex, spin, and keep on ice.
Axiom Frag Rxn Stop	2-2	Thaw, vortex and keep at room temperature.
Precip Soln 1	2-2	Thaw, vortex and keep at room temperature.
Isopropanol	N/A	Keep at room temperature.

3. Optional: Remove samples for quantifying amplification yield by the PicoGreen Assay at a later time.
   See Chapter 4, *Axiom 2.0 Assay: Manual Target Preparation* for more information.
4. 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 pour in a solution basin on the bench top at room temperature.

Table 2.2. Fragmentation Master Mix

Reagent	per Sample	Master Mix 96+
Axiom 10X Frag Buffer	45.7 µL	6.0 mL
Axiom Frag Diluent	10.3 µL	1.35 mL
Axiom Frag Enzyme	1.0 µL	131 µL
Total Volume	57 µL	7.48 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.

1. 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.
2. **Add 57 µL of Fragmentation Master Mix to each reaction.**
3. Seal and vortex twice.
4. Start the timer for 30 min.
5. Quick spin the Sample plate in the room temperature plate centrifuge.
6. 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 or shorter incubation times may lead to poor performance.

4: Aliquot the Stop Solution to the Plate

1. 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.
2. Remove the Sample plate from the oven and place on the bench top.
3. At the end of the 30 minute fragmentation incubation period, **add 19 µL of Stop Solution to each reaction**.
4. Seal, vortex, and spin.
5. Keep the Sample plate at room temperature while you prepare the Precipitation Master Mix.

5: Prepare and Add Precipitation Master Mix

Carry out the following steps at room temperature.

1. Prepare Precipitation Master Mix in a 50 mL tube. Add the reagents in the order and volumes shown in Table 2.3, vortex to mix and pour into a solution basin.

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

2. **Add 240 µL Precipitation Master Mix to each sample**. You do not need to mix up and down.
3. Seal the Sample plate, vortex, and spin.
4. **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. DO NOT vortex the plate after isopropanol addition to avoid cross contamination of the samples.
5. Blot the top of the plate with Kimwipe and seal tightly with a Microamp seal.
6. Carefully **transfer the Sample plate into the –20 °C freezer and incubate overnight** (16-24 hours).
7. After incubation, proceed to Stage 3.

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Axiom® 2.0 Assay Manual Target Prep Protocol Stage 3: Drying, Resuspension and QC

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Preparation for Stage 3. Drying, Resuspension, and QC

Supplies Required

- Selected Reagents from the Axiom 2.0 Reagent Kit (see Table 3.1):
  - Module 2-1, –20 °C, P/N 901528
  - Module 2-2, 2–8 °C, P/N 901529
- Other Reagents Required for QC steps (optional)
  - 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)
  - Invitrogen E-Gel® 48 4% agarose gels G8008-04

Instrument Setup

- Prepare the following instruments for this stage:
  - Oven preheated to 37 °C
  - Plate centrifuge set at 4 °C
  - Jitterbug or Titerplate shaker

Reagent Preparation

1. Prepare the 1000-fold Dilution of gel loading dye: 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.
2. Prepare reagents as shown in Table 3.1:

Table 3.1. Reagent Preparation

Reagent	Module	Treatment
Axiom Hyb Buffer	2-1	Vortex and keep at room temperature
Axiom Hyb Soln 1	2-1	Thaw, vortex, spin and keep at room temperature
Axiom Hyb Soln 2	2-1	Vortex, spin and keep at room temperature
Axiom Resusp Buffer	2-2	Warm to room temperature (1 hour)

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

1: Centrifuge and Dry Pellets and Thaw Reagents

1. Begin thawing/warming the reagents used in this stage as shown in Table 3.1.
2. Remove the Sample plate from the –20 °C freezer and **centrifuge the plate at 3200 xg at 4 °C for 40 min**.
3. During centrifugation prepare the resuspension and hybridization reagents as shown in Table 3.1.
4. 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**.

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

5. Turn the plate top side up and **place in an oven for 20 min at 37 °C to dry**. If using an GeneChip® Hybridization Oven 645, turn off the rotor during the 20 min drying time.

   NOTE: If you are proceeding directly to *2: Resuspension and Hybridization Master Mix Preparatio*n, you can prepare the Hybridization Master Mix at this time.
6. After 20 min remove the plate from the oven , even if some droplets of liquid remain, and either:
   - Proceed directly to *2: Resuspension and Hybridization Master Mix Preparation*. 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.
- Make sure the Axiom Resusp Buffer has equilibrated to room temperature before adding to dry pellets in Step 1, below.
- Carry out these steps at room temperature.

1. **Transfer 35 µL Axiom Resusp Buffer to each well** of the sample plate with a dry pellet. Avoid touching pellets with tip.
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 3.2. Vortex.
4. Inspect the Sample plate from the bottom. If the pellets are not dissolved, repeat
   Step 2. Quick spin.
5. Select a PCR plate appropriate to the type of approved thermal cycler you will use in Stage 4 and label as "Hyb Ready Plate [plate ID].".
6. Transfer the entire contents of each well of the resuspended Sample plate to the labeled Hyb Ready plate.
7. **Add 80 µL of the Hyb Master Mix to each well** of the Hyb Ready plate.
8. Seal tightly, vortex, and spin.

Table 3.2. Hybridization Master Mix

Reagent	per Sample	Master Mix 96+
Axiom Hyb Buffer	70.5 µL	7.8 mL
Axiom Hyb Soln 1	0.5 µL	55.6 µL
Axiom Hyb Soln 2	9 µL	1.0 mL
Total Volume	80 µL	8.86 mL

3: Recommended: Perform Quantitation and Fragmentation Quality Control Checks

Before proceeding to Stage 4: Denaturation and Hybridization, we recommend that you perform quantitation and fragmentation QC Checks.

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 2.0 Assay Manual Workflow User Guide* (P/N 702990) for more information.
3. Make and run Gel Samples:
   - **A.** Add 120 µL 1000-fold diluted gel loading dye to each well of the Gel Sample Plate.
   - **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 2.0 Assay Manual Workflow User Guide* (P/N 702990) 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® 2.0 Assay Manual Target Prep Protocol
Stage 4: Denaturation and Hybridization

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Preparation for Stage 4: Denaturation and Hybridization

Supplies Required

- Reagents from from the Axiom 2.0 Reagent Kit, Module 3, P/N 901472
- Following components from Axiom Array Plate Kit:
  - Axiom Genome-Wide Human or non-Human 96-array plate in a protective base
    or
  - Axiom myDesign™ Genotyping 96-array plate in a protective base
- Hyb tray from the Axiom GeneTitan® Consumables Kit (P/N 901606)

Instruments and Setup

- GeneTitan MC Instrument
- Approved Thermal Cycler
  - Must be programmed with the "Axiom 2.0 Denature" protocol of 95 °C for 10 min; 48 °C for 3 min; 48 °C for hold.
  - Use the heated lid option when setting up or running protocols.
- Hyb ready samples in plate appropriate to the thermal cycler model used
- 96-well metal chamber pre-heated in a 48 °C oven

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

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. Leave the Hyb Ready plate at room temperature.

2: Prepare Equipment and Perform Denaturation

1. Warm up the array plate on the bench top for a minimum of 25 minutes before setting up hybridization on the GeneTitan MC Instrument.
2. At the end of the array warm up time, open the pouch and scan the array plate barcode into the Batch Registration file.
3. Place Hyb Ready plate in thermal cycler block, secure lid, and **start the Axiom 2.0 Denature program**.
4. While the program is running:
   - **A.** Prepare the reagents from Module 3 by inverting the bottles 2 to 3 times to mix.
   - **B.** Upload the Batch Registration File.
   - **C.** Set up the GeneTitan MC Instrument. For more information, see:
     - *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC (P/N 702988).*
     - Chapter 5, *Array Processing with the GeneTitan® Multi-Channel Instrument* of the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990).

3: Prepare Hybridization Tray and Load into GeneTitan MC Instrument

1. Remove the hyb tray (from Axiom GeneTitan® Consumables Kit) from packaging.
2. Label the hyb tray; please refer to Figure 4.1 and the IMPORTANT note below the figure.
3. After the Axiom Denature program has completed, remove the Hyb Ready plate from the thermal cycler 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 µL, 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 Instrument**.

   The array plate is shipped with a clear top lid and a blue protective base (Figure 4.2). Before loading, the top lid must be removed.

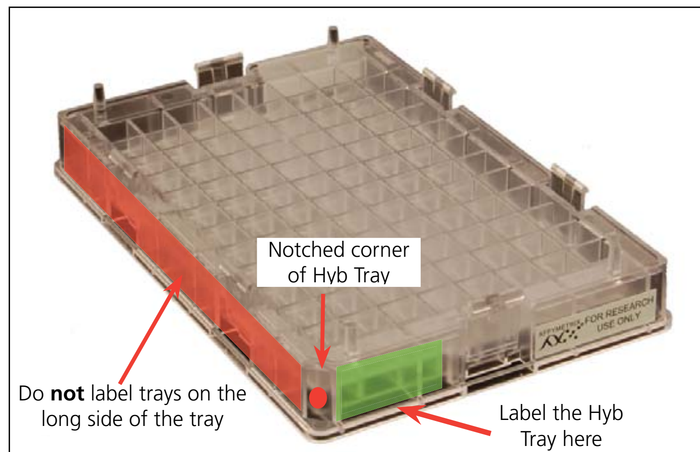
   The clear plastic lid on top of the array plate SHOULD NOT be loaded in the GeneTitan MC Instrument.

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

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

You must wait until the hybridization step on the GeneTitan MC Instrument 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



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

Figure 4.2. Array Plate as Shipped

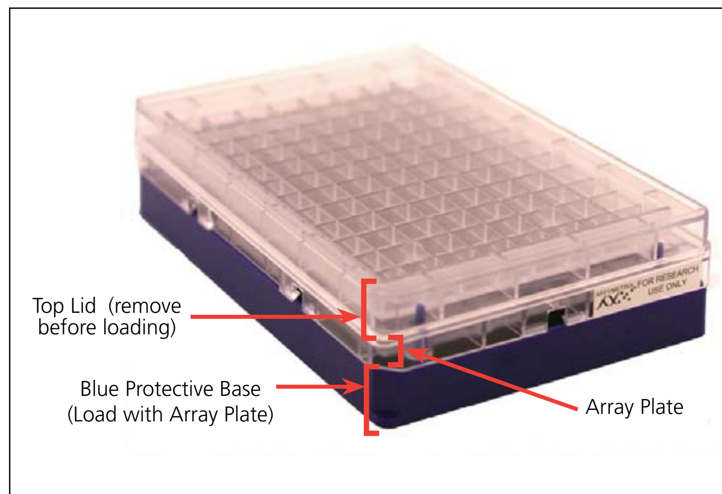
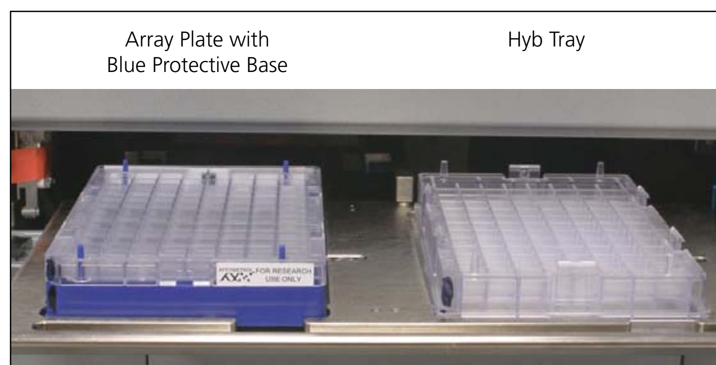


Figure 4.3. Array Plate and Hyb Tray Loaded in GeneTitan Drawer



IMPORTANT:

- The array plate must be loaded on its protective blue base, as shown above.
- After the GeneTitan MC Instrument 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.

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Axiom® 2.0 Assay Manual Target Prep Protocol

Stage 5: Manually Preparing Reagent Trays for the GeneTitan® MC Instrument

Preparation for Stage 5: Manually Preparing Reagent Trays for the GeneTitan MC Instrument

Reagents

- Reagents from Module 4-1, –20 °C, P/N 901278
- Reagents from Module 4-2, 2-8 °C, P/N 901276

Instrument

- GeneTitan MC Instrument

Consumables

- Aluminum foil (optional)
- Items from the Axiom GeneTitan® Consumables Kit (P/N 901606):
  - Scan Tray (1)
  - Stain Tray (5)
  - Covers for Trays (6)

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
Module 4-1 (P/N 901278)			
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
Module 4-2 (P/N 901276)			
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	Vortex and 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. 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 µL	22.2 mL
Axiom Stain Buffer	4.2 µL	463 µL
Axiom Stain 1-A	2.1 µL	231 µL
Axiom Stain 1-B	2.1 µL	231 µL
Total	210 µL (105 µL x 2)	23.13 mL

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. Place on ice and protect from direct light.

Table 5.3. Stain 2 Master Mix

Reagent	per Array	Master Mix 96+
Axiom Wash A	100.8 µL	11.1 mL
Axiom Stain Buffer	2.1 µL	231 µL
Axiom Stain 2-A	1.05 µL	115.6 µL
Axiom Stain 2-B	1.05 µL	115.6 µL
Total	105 µL	11.56 mL

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. Place on ice.

Table 5.4. Stabilization Master Mix

Reagent	per Array	Master Mix 96+
Axiom Water	93.19 µL	10.3 mL
Axiom Stabilize Diluent	10.50 µL	1.16 mL
Axiom Stabilize Soln	1.31 µL	144.8 µL
Total	105 µL	11.61 mL

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. Place back on ice.

Table 5.5. Ligation Master Mix: Stage 1

Reagent	per Array	Master Mix 96+
Axiom Ligate Buffer	66.15 µL	7.3 mL
Axiom Ligate Soln 1	13.12 µL	1.45 mL
Axiom Ligate Soln 2	3.15 µL	348 µL
Sub–Total	82.42 µL	9.10 mL

Ligation Master Mix: Stage 2

1. Remove the Axiom Ligation Enzyme from the –20 °C freezer and place in a cooler chilled to –20 °C.
2. Add reagents in the order shown in Table 5.6.
3. Gently flick the Axiom Ligate 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 5.6. Ligation Master Mix: Stage 2

Reagent	per Array	Master Mix 96+
Ligation Master Mix from Stage 1	82.42 µL	9.10 mL
Axiom Probe Mix 1	10.5 µL	1.16 mL
Axiom Probe Mix 2	10.5 µL	1.16 mL
Axiom Ligate Enzyme	1.58 µL	174.4 µL
Total	105 µL	11.59 mL

3: Aliquot Master Mixes and Axiom Hold Buffer into Trays

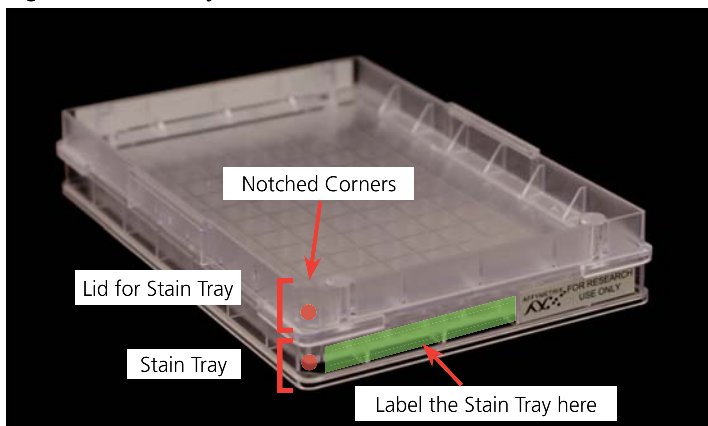
NOTE: It is not necessary to change pipette tips between additions of the same reagents to stain trays and scan trays.

Prepare Trays and Lids

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 Trays and Covers* of the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990) for the recommended technique.

Figure 5.1. Stain Tray with Lid



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

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:

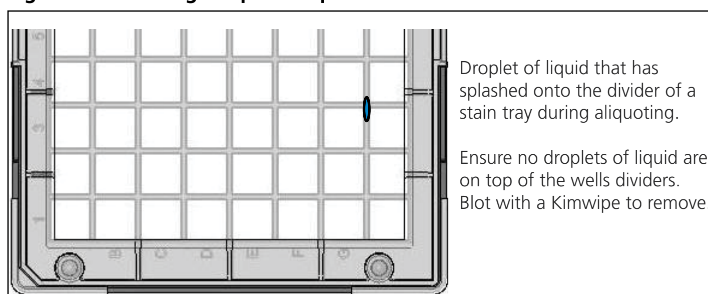
1. Aliquot **105 µL** per well of the appropriate Master Mix — dispense to the first stop only to avoid creating bubbles.
2. If:
   - Bubbles are present, puncture them with a pipette tip.
   - Droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove.
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 Instrument.

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 the GeneTitan MC Instrument.

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 base 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 protective base during this part of the process (Figure 5.4).

1. Pour the Axiom Hold Buffer into a solution basin, 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 base.
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.

Figure 5.3. Scan Tray with Top Lid and Black Protective Base

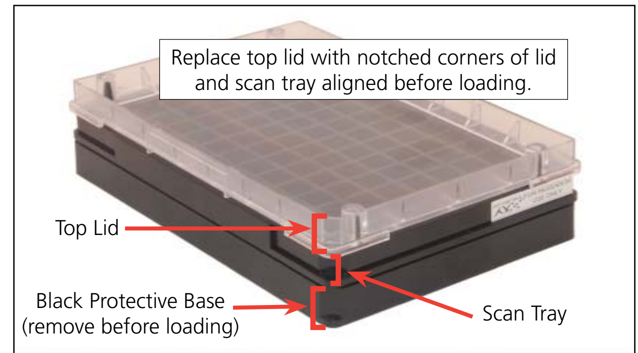
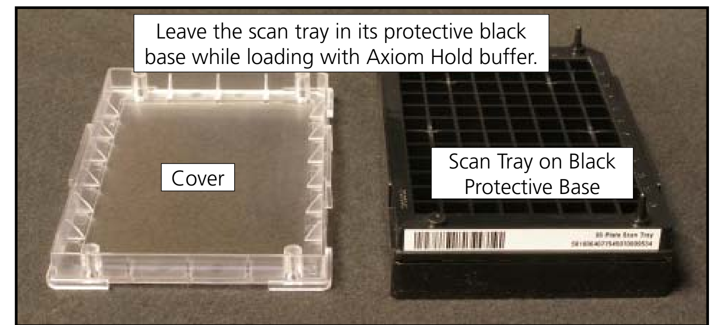


Figure 5.4. Scan Tray with Cover Removed



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

- *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC* (P/N 702988)
- *Chapter 5, Axiom Genotyping Assay: Array Processing* of the *Axiom® 2.0 Assay Manual Workflow User Guide* (P/N 702990)

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