

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 QRC* (P/N 702975).
2. Target Prep of the samples, done using either:
 - Automated Target Prep, described in the *Axiom[™] 2.0 Automated Target Prep Protocol QRC* (P/N 702962).
 - Manual Target Prep, described in the *Axiom[™] 2.0 Manual Target Prep Protocol QRC* (P/N 702989).
3. Array Processing, described in this QRC. The array processing is performed using:
 - GeneTitan[®] MC Instrument
 - Affymetrix[®] GeneChip[®] Command Console[®] (AGCC) Portal Software
 - AGCC GeneTitan[®] Instrument Control Software

IMPORTANT: This Quick Reference Card (QRC) contains an abbreviated set of instructions. Before running the array processing workflow, read the following documentation carefully:

- *Chapter 7, Controlling the GeneTitan[®] Instruments* of the *Affymetrix[®] GeneChip[®] Command Console[®] User Manual* (P/N 702569)
- The Array Processing chapter in either the *Axiom 2.0 Assay Automated Workflow User Guide* (P/N 702963) or the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990).

GeneTitan MC processing for Axiom Array Plates takes place in two sets of steps:

- Hybridization: loading Hybridization (Hyb) trays and Array plates
After loading the Hyb Tray and Array plate, hybridization continues for ~ 24 hours on the GeneTitan Instrument.
- Wash/Stain/Scan: Loading Reagent and Scan Trays for the Array plate when hybridization is nearly complete
After loading the reagent and Scan trays, the Wash/Stain/Scan steps continue for approximately ~ 5 hours on the GeneTitan Instrument.

IMPORTANT: If you are processing one array plate at a time, you cannot prepare and load reagent and scan trays for the Array Plate until hybridization is nearly complete.

You can process multiple array plates in a staggered sequence using the high-throughput workflow, described in *High Throughput Workflow Guidelines* on page 3 of this QRC. See either the *Axiom 2.0 Assay Automated Workflow User Guide* (P/N 702963) or the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990) for more information about high-throughput workflows for both Automated and Manual Target Prep.

Labeling Hyb Trays and Stain Trays for Hybridization

Labeled and filled trays are required for processing Axiom Array plates. The Hyb tray and Stain trays are labeled and filled during the target prep process. See the QRCs for the automated and manual target prep for more information.

IMPORTANT: It is critical that you write only on the proper location of the proper edge of the Hyb tray and Stain trays, as shown in Figures 1 and 2. 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 1. Labeled Hyb Tray

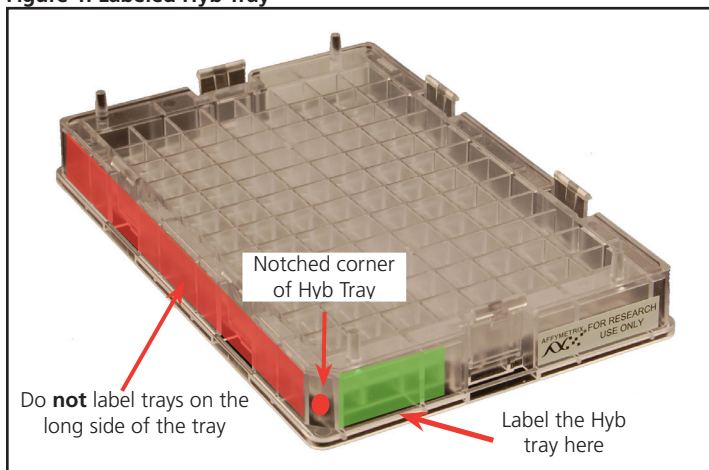
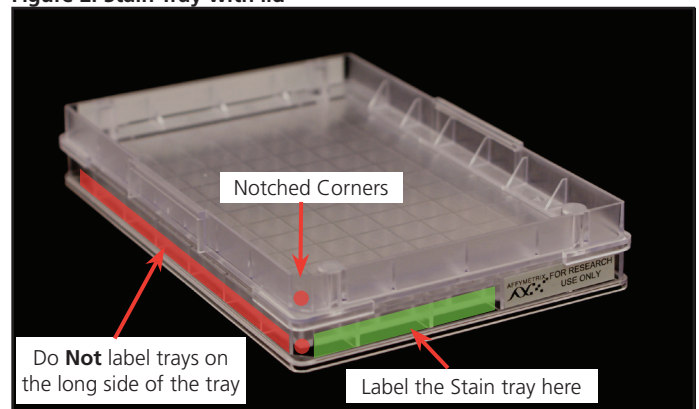


Figure 2. Stain Tray with lid



Loading Array Plates and Hyb Trays for Hybridization

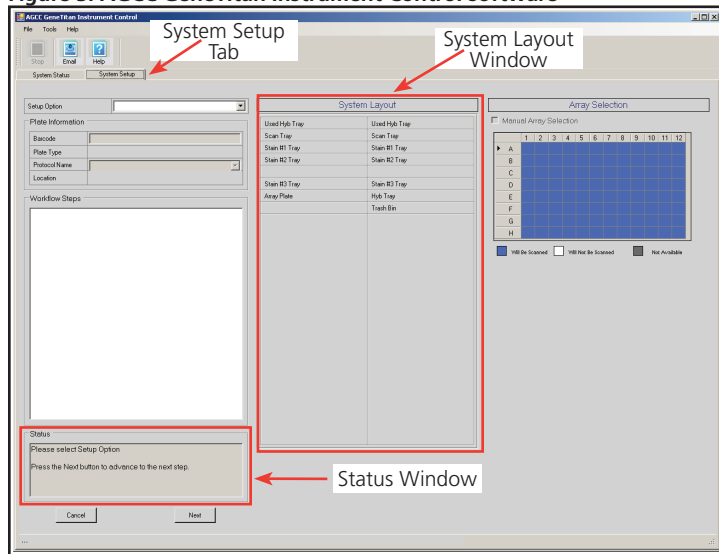
To load the Array Plate and Hyb Tray:

- Open the AGCC software Launcher and select:
 - AGCC Portal
 - GeneTitan Instrument Control software
- Upload your sample (batch) registration file using AGCC Portal now.

IMPORTANT Upload this file prior to starting Hybridization.
- In the GeneTitan Instrument Control software (Figure 3), select the System Setup tab; then select the Hyb-Wash-Scan Setup Option.

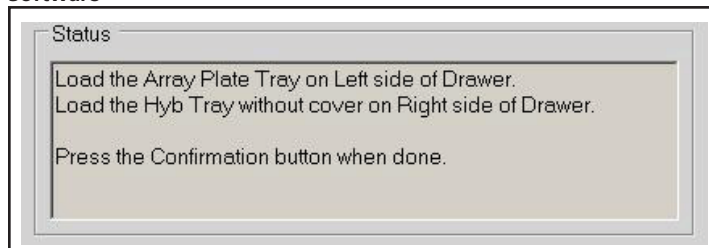
NOTE: See the AGCC User Manual and the Axiom 2.0 Assay (Automated or Manual Workflow) User Guide for information on using other setup options.

Figure 3. AGCC GeneTitan Instrument Control software



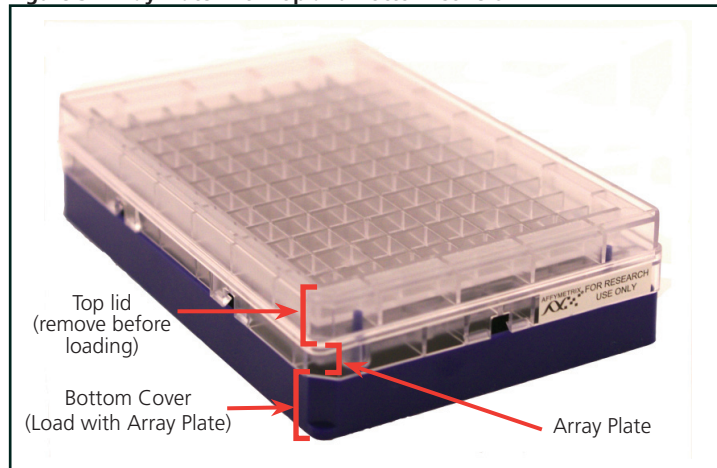
- Scan the Axiom array plate barcode. The correct protocol is automatically selected.
- Press the Next button and follow the prompts displayed in the Status window (Figure 4).
 - Fill the Wash A, Wash B and Rinse bottles.
 - Wash A: Fill with Axiom Wash buffer A-- keep at 2L full.
 - Wash B: Fill with Axiom Wash Buffer B -- use all 600 mL from the reagent kit per Axiom plate. Fill to 1L mark when processing two plates on the same day.
 - Rinse: Fill with Axiom Water -- keep at 1L full.
 - Empty the waste bottle.
 - Unload previously loaded trays and plates.
 - Load the Array plate and Hyb tray.

Figure 4. Status window in AGCC GeneTitan Instrument Control Software



The array plate is shipped with two covers: a bottom protective cover and a top lid (Figure 5).

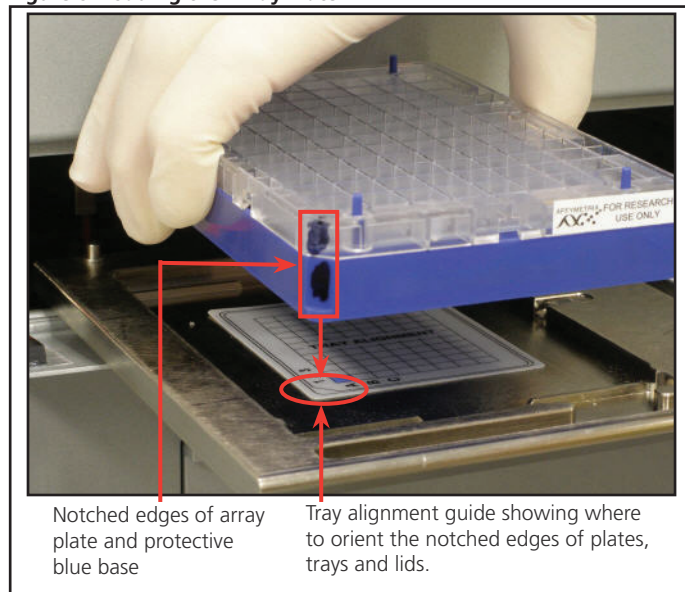
Figure 5. Array Plate with Top and Bottom Covers



The top lid must be removed before loading the Array plate and bottom cover in GeneTitan MC Instrument.

NOTE: Align notched corners of the Array plate and Hyb tray as shown on the drawer alignment guide (Figure 6).

Figure 6. Loading the Array Plate.



- Press the OK button to begin hybridization.

IMPORTANT: 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, gently tap the plate on top and the bubbles should disappear.

Hybridization will continue on the GeneTitan Instrument for ~ 24 hours before you can load the Ligation/Staining/Stabilization reagent trays for this Array Plate.

Loading Trays for Wash/Stain/Scan

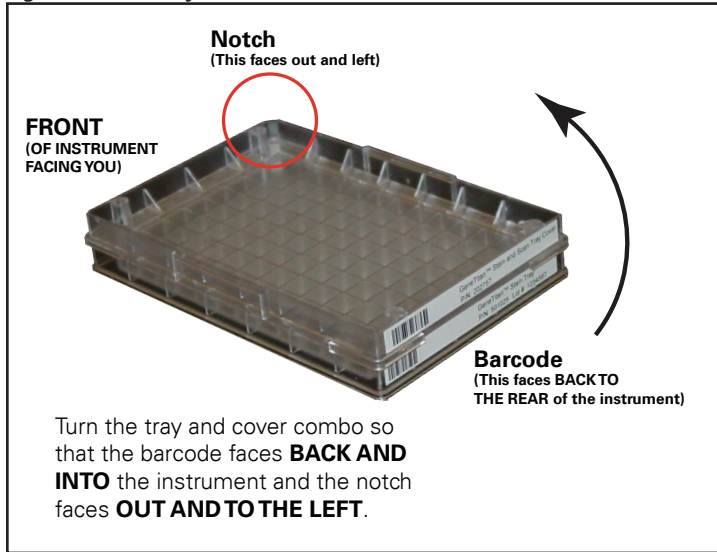
The trays for the wash/stain/scan process are labeled and filled during the Target Prep. They must be prepared as the Array plate is finishing the hybridization step for immediate loading.

NOTE: The Stain and Scan trays and lids should be treated with the antistatic gun before filling and loading.

The wash/stain/scan step requires:

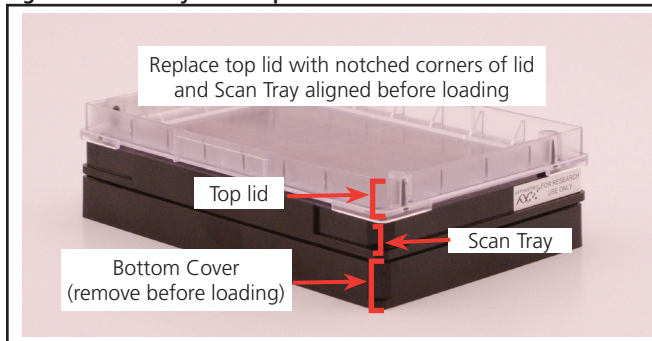
- Two trays containing Stain 1 with lids
 - One tray containing Stain 2 with lid
 - One tray containing Ligation mix with lid
 - One tray containing Stabilization solution with lid
 - One Scan tray containing Holding Buffer, with lid
- The lids must be placed on the stain trays with the notched corners aligned (Figure 6).

Figure 6. Stain Tray with Lid



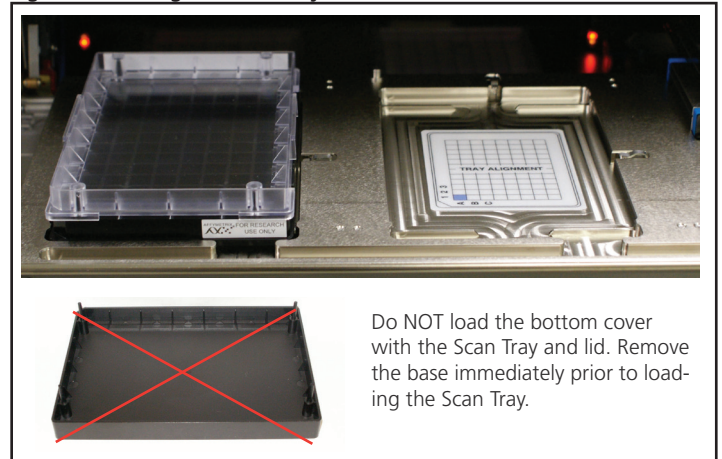
The Scan tray is shipped with two covers, a bottom protective cover and a barcoded top lid (Figure 7).

Figure 7. Scan Tray with Top and Bottom Covers



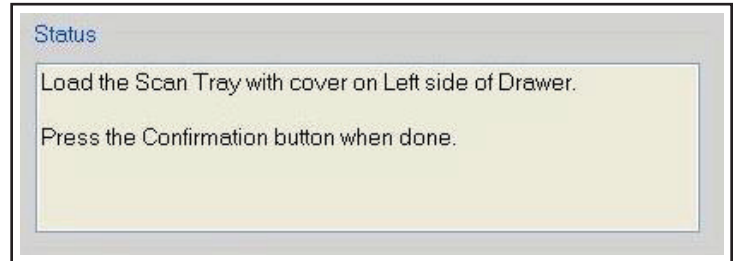
The Scan tray must be covered with the barcoded Scan tray cover with the notched corners aligned correctly. The bottom cover must be removed before loading (Figure 8).

Figure 8. Loading the Scan Tray and Lid.



Carefully follow the prompts in the Status window of the GeneTitan Instrument Control software to ensure that the correct tray is loaded on the proper side of each drawer (Figure 9).

Figure 9. Status window in AGCC GeneTitan Instrument Control Software



High-Throughput Workflow Guidelines

The high-throughput workflow allows you to process multiple array plates in a staggered sequence that requires loading:

- The reagents and scan tray for an Array Plate finishing hybridization
- An Array plate and Hyb tray to begin hybridization.

When running a multi-plate workflow:

- Pay careful attention to the software prompts that indicate which side of the drawer to place or remove a plate/tray.
- If you are loading reagents and a Scan tray for the fluidics process as well as another Array plate for hybridization, you will always load the trays for fluidics first.

See either the *Axiom 2.0 Assay Automated Workflow User Guide* (P/N 702963) or the *Axiom 2.0 Assay Manual Workflow User Guide* (P/N 702990) for more information about running high-throughput workflows.

Shutting Down the GeneTitan MC Instrument

1. Ensure that all consumables and plates have been unloaded (Unload Plates function).
2. In the GeneTitan Control software, open **Tools > Shutdown** and exit.

For Research Use Only

Not for use in Diagnostic Procedures.

P/N 702988 Rev. 1

© 2011 Affymetrix, Inc. All rights reserved. Affymetrix®, Axiom™, myDesign™, Command Console®, DMET™, GeneAtlas™, GeneChip®, GeneChip-compatible™, GeneTitan®, Genotyping Console™, NetAffx®, and Powered by Affymetrix™ are trademarks or registered trademarks of Affymetrix Inc. All other trademarks are the property of their respective owners.