

A large, detailed 3D rendering of a DNA double helix structure, shown in a light gray color, running vertically along the left side of the page.

User Guide

Axiom[®] 2.0 Assay Manual Workflow

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About the Axiom® 2.0 Assay

The first Genome-Wide Association study (GWAS) was published in 2005 (1) when individuals carrying particular haplotypes of SNP rs380390 were found to have increased risk of developing age-related macular degeneration, a study performed with the Affymetrix GeneChip® Mapping 100K Array Set (2).

As of September, 2009, there have been over 400 peer-reviewed GWAS publications and over 1774 SNPs have been implicated in human disease (3). Initial GWAS studies focused on the “common disease, common variant” hypothesis (1) that held that haplotypes with a minor allele frequency (MAF) >5% would show measurable contribution to human disease research.

Current research is shifting towards “complex disease, complex/rare variant” studies. As such, these research projects require a broader catalog of human variation, such as is being generated by the 1000 Genomes Project (<http://www.1000genomes.org>). This project focuses on identifying alleles with a MAF <5% across a broader spectrum of human ethnicities. In order to allow our customers to take advantage of this novel and rare content for genome association and candidate gene studies in a cost effective and timely manner, Affymetrix is introducing a new genotyping product line: the Axiom® Genotyping Solution.

The Axiom Genotyping Solution introduces a new genotyping technology platform that includes novel assay biochemistry, array configuration and processing, and automated target preparation. This solution has applications in human disease research and basic and applied agriculture research.

For human disease research applications, Affymetrix conducted an empirical screen of genomic content from dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). The screen included markers from HapMap and the 1000 Genomes Project as well as other sources, using HapMap phase 3 samples and/or the original 270 HapMap samples. All of this information has gone into creating a proprietary Affymetrix database of validated markers that can be interrogated using the Axiom 2.0 Assay.

There are several arrays available for use with the Axiom 2.0 Assay which leverage the content of this proprietary Affymetrix database. For a complete list of supporting products please visit www.affymetrix.com.

The Axiom 2.0 Assay interrogates biallelic SNPs and simple indels (human only) in a single, fully automated assay workflow. Starting with genomic DNA, the samples are processed by performing either an automatic or manual target prep protocol followed by automated processing of the array plates in the GeneTitan MC Instrument.

- Target prep uses methods including DNA amplification, fragmentation, purification and resuspension of the target in hybridization cocktail.
- The hyb-ready targets are then transferred to the Affymetrix GeneTitan® Multi-Channel (MC) Instrument for automated, hands-free processing including hybridization, staining, washing and imaging.

Cel files generated by the GeneTitan Multi-Channel Instrument are processed using the Axiom® Genotyping Algorithm version 1 (Axiom GT1) available through Affymetrix Power Tools or Genotyping Console™ v4.1.

In summary, the Axiom Genotyping Solution is a product line that provides catalog arrays that:

- Are optimized for high genetic coverage of their population in question.
- Provide highly automated, reproducible results suitable for GWAS.

Related Documentation

- *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991
- *Axiom® 2.0 Manual Target Prep Protocol QRC*, P/N 702989
- *Axiom® Genotyping Solution Analysis Guide*, P/N 702961
- *Axiom® 2.0 gDNA Sample Prep Protocol QRC*, P/N 702987
- *Axiom® gDNA Sample Prep for Genome-Wide BOS 1 Array Plate QRC*, P/N 702975
- *GeneTitan® MC Protocol for Axiom 2.0 Array Plate Processing QRC*, P/N 702988
- *GeneTitan® Multichannel Instrument User's Manual*, P/N 08-0306
- *GeneTitan® Multichannel Instrument Site Preparation Guide*, P/N 08-0305
- *Affymetrix® GeneChip® Command Console® Software User Manual*, P/N 702569
- *Affymetrix® Genotyping Console™ 4.1 User Manual*, P/N 702982

Axiom 2.0 Assay Automated Workflow

- *Axiom® 2.0 Assay Automated Workflow User Guide*, P/N 702963
- *Axiom® 2.0 Assay Automated Workflow Site Prep Guide*, P/N 702984
- *Axiom® 2.0 Assay Automated Target Prep Protocol QRC*, P/N 702962
- *Biomek® Liquid Handler User's Manual*, Beckman Coulter P/N 987834
- *Biomek® Software User's Manual*, Beckman Coulter P/N 987835

References

1. Manolio T.A. and Collins F.S.: **The HapMap and Genome-Wide Association Studies in Diagnosis and Therapy**. *Annu Rev Medicine* 2009, **60**:443–56
2. Klein RJ, Zeiss C, Chew EY, et al.: **Complement factor H polymorphism in age-related macular degeneration**. *Science* 2005, **308**:385–89
3. Hindorff LA, Junkins HA, Mehta JP, and Manolio TA.: **A Catalog of Published Genome-Wide Association Studies**. Available at: www.genome.gov/gwastudies. Accessed 09/28/2009.

Overview of the Axiom 2.0 Manual Workflow Assay

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

1. Genomic DNA Prep--Resulting in samples that meet requirements spelled out in [Chapter 2, Genomic DNA Preparation and Requirements on page 11](#).
2. Target Prep of the samples (see [Chapter 4, Axiom® 2.0 Assay: Manual Target Preparation on page 32](#)).
3. Array Processing, done with
 - GeneTitan MC Instrument
 - GeneTitan Instrument Control software
 - AGCC Portal software

See [Chapter 5, Array Processing with the GeneTitan® Multi-Channel Instrument on page 67](#).

A list of the required equipment and supplies for running the Axiom 2.0 Assay manual target preparation can be found in the *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991.

Running Multiple Plate Workflows

Affymetrix provides workflows that allow you to run a set of samples and array plates through the protocol using a minimum of personnel and a forty-hour week. The timing of steps is critical, whether using automated target prep or manual target prep because of the following constraints:

- Incubation after DNA Amplification is 23 hours
- Hybridization in the GeneTitan Instrument is 23.5 hours.
- Reagent trays for wash/stain/imaging must be prepared as Hybridization finishes
- Limits to when a second hyb tray and array plate can be loaded into the GeneTitan Instrument.

These limitations require careful timing.

The details are covered in [Chapter 6, Manual Target Preparation for Processing Three Axiom® Array Plates per Week](#) on page 100

Safety Warnings and Precautions



CAUTION: All chemicals should be considered as potentially hazardous. We, therefore, recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as lab coat, safety glasses and gloves. Care should be taken to avoid contact with skin and eyes. In case of contact with skin or eyes, wash immediately with water. See MSDS (Material Safety Data Sheet) for specific advice.



WARNING: The following components contain harmful or toxic ingredients:

- Axiom Stabilize Soln: 8% Gluteraldehyde
- Axiom HybSoln 2: 100% Formamide
- Axiom Hyb Buffer: <55% Tetramethylammonium Chloride

As such we recommend the use of a fume hood when using these products during the Manual Target Preparation protocol. In all cases customers should use adequate local and general ventilation in order to minimize airborne concentrations.

For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Copies of the Material Safety Data Sheets for the kit components are available on the Affymetrix website at www.affymetrix.com.

Genomic DNA Preparation and Requirements

The general requirements for genomic DNA (gDNA) sources and extraction methods are described in this chapter. The success of this assay requires uniform amplification of the genome starting with relatively intact gDNA. To achieve this, the gDNA must be of high quality, and must be free of contaminants that may affect the enzymatic reactions to be performed.

For this protocol, you will use the Axiom® 2.0 Reagent Kit. The kit contains a tube labeled Genomic DNA. This DNA meets the requirements outlined below, and is included for use as a control. The size and purity of sample gDNA can be compared with those of the control DNA to assess sample quality. The control DNA should also be used routinely as an experimental positive control and for troubleshooting purposes.

Assay performance may vary for gDNA samples that do not meet the general requirements described below. However, the reliability of any given result should be assessed in the context of overall experimental design and goals.

The genomic DNA requirements and preparation are described in the following sections:

- *Sources of Genomic DNA*
- *General Requirements on page 12*
- *Genomic DNA Extraction/Purification Methods on page 13*
- *Genomic DNA Cleanup on page 13*
- *Genomic DNA Preparation on page 14*

Sources of Genomic DNA

The following sources of human gDNA have been successfully tested in the laboratories at Affymetrix for DNA that meets the above requirements.

- Blood
- Saliva
- Cell line
- WGA pre-amplified DNA: Genomic DNA amplified with the REPLI-g® Kit (a whole genome amplification kit; QIAGEN, P/N 150025) has been tested successfully with the Axiom 2.0 Genome-Wide Human Reagent Kit Assay. The REPLI-g Kit was used to amplify 20 ng genomic DNA, and the resulting yields were quantitated by a PicoGreen® assay. The amplified products (either 100 or 200 ng amplified DNA as required according to the Axiom array type) were used (without purification) as the input DNA sample in the subsequent Axiom 2.0 Assay steps. The stability of this amplified product to storage and repeated cycles of freeze/thaw have not been evaluated by Affymetrix.

Success with other types of samples will depend on quality (degree of degradation, level of purity, etc.) and quantity of gDNA extracted.

The following sources of bovine gDNA have been successfully tested in the laboratories at Affymetrix for DNA that meets the requirements below:

- Blood
- Semen
- Nasal swab
- Hair bulbs
- Ear punch tissue



NOTE: DNA derived from Formalin-Fixed Paraffin-Embedded (FFPE) blocks should not be used with this assay.

General Requirements

- Starting DNA must be double-stranded for the purpose of accurate concentration determination.
- DNA must be of high purity.
DNA should be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (i.e., EDTA). The gDNA extraction/purification method should render DNA that is generally salt-free because high concentrations of particular salts can also inhibit enzyme reactions. DNA purity is indicated by OD₂₆₀/OD₂₈₀ and OD₂₆₀/OD₂₃₀ ratios. The OD₂₆₀/OD₂₈₀ ratio should be between 1.8 and 2.0 and the OD₂₆₀/OD₂₃₀ ratio should be greater than 1.5. We recommend that DNA samples that do not meet these criteria be cleaned up as described under [Genomic DNA Cleanup](#) on page 13.
- DNA must not be degraded.
The approximate average size of gDNA may be assessed on a 1% agarose gel using an appropriate size standard control. Approximately 90% of the DNA must be greater than 10 Kb in size. Control DNA can be run on the same gel for side-by-side comparison.

Special Requirements

Pre-Amplification Area

Precautions are required when manipulating genomic DNA to avoid contamination with foreign DNA amplified in other reactions and procedures. It is recommended that genomic DNA manipulations are performed in a dedicated pre-amplification room or area separate from the main laboratory.

This pre-amplification area should have a dedicated set of pipettes and plasticware. If no dedicated area is available, use of a dedicated bench or a dedicated biosafety hood and dedicated pipettes is suggested. If no dedicated bench or biosafety hood is available, a set of dedicated pipettes is recommended.

Ideally, this pre-amplification area would be separate from the amplification staging area described in [Chapter 3, on page 19](#), however these areas may be combined due to space and equipment limitations.

Assessing the Quality of Genomic DNA Using 1% Agarose E-gels

We recommend this quality control step to assess the quality of the gDNA prior to starting the assay.

Equipment and Reagents Recommended

Table 2.1 E-Gel® and Reagents Required

Item	Supplier	Part Number
Mother E-Base Device	Life Technologies (formerly Invitrogen)	EB-M03
Daughter E-Base Device		EB-D03
E-Gel® 48 1% agarose gels		G8008-01
RediLoad™		750026
E-Gel® 96 High Range DNA Marker		12352-019

Guidelines for Preparing the Genomic DNA Plate for Gel Analysis

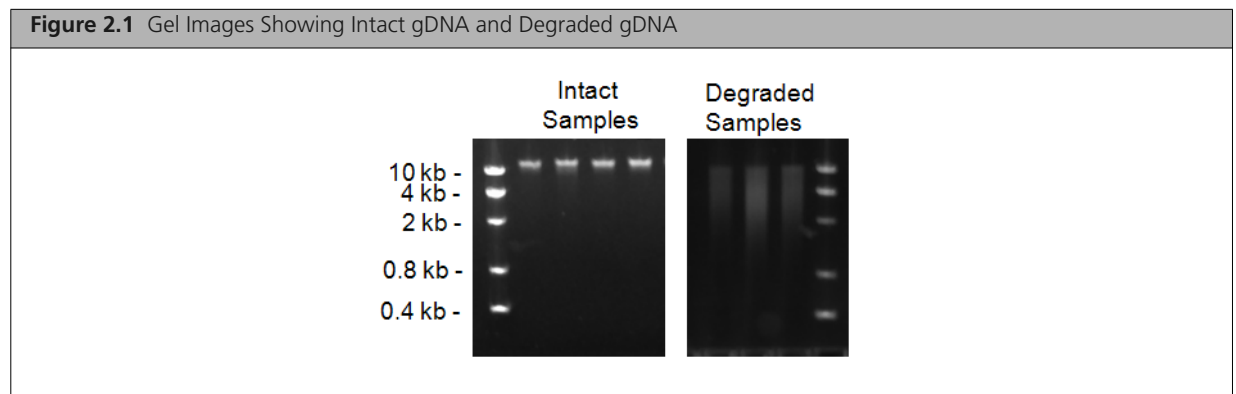
- Loading a DNA mass of 10 ng to 20 ng per well is recommended. If lower amounts are loaded, omission of the loading dye is recommended in order to improve visualization. Loading ≥ 25 ng gDNA per well can improve the image.
- Add 3 µL of 0.1X of RediLoad dye to each sample.
- Bring each sample to a total volume of 20 µL using H₂O (for example, if the volume of genomic DNA is 5 µL, add 3 µL of RediLoad, and bring to 20 µL total by adding 12 µL of H₂O).
- Seal, vortex and spin.

To Run the E-Gel:

1. Power on for E-Base (red light).
2. Push the Power/Prg button to make sure the program is at EG mode (not EP).
3. Insert the two 48 well 1% Agarose E-Gels into the slots.
4. Remove 2 combs.
5. Load 20 μ L from the above plate onto two 48 well 1% agarose E-Gels.
6. Load 15 μ L of diluted High Range DNA Marker (1:3 dilution or ~ 0.34 X from stock) into all marker wells (as needed).
7. Fill all empty wells with water.
8. Adjust the run time to ~ 27 min.
9. Push the Power/Prg button again (it will change from red to green).

When run time is reached (the ladder band reaches the end of the lane), the system will automatically shut off. The gel is then ready for imaging.

Figure 2.1 shows gel images of intact gDNA (that is suitable for use in the Axiom 2.0 Assay) and degraded gDNA samples. Customers whose gDNA is degraded (similar to the image in Figure 2.1) should perform a test experiment to investigate the performance of their samples in the Axiom Genotyping Assay prior to beginning any large scale genotyping projects.



Genomic DNA Extraction/Purification Methods

Genomic DNA extraction and purification methods that meet the general requirements outlined above should yield successful results. Methods that include boiling or strong denaturants are not acceptable because the DNA would be rendered single-stranded and can no longer be accurately quantitated using a PicoGreen-based assay.

Genomic DNA Cleanup

If a gDNA preparation is suspected to contain inhibitors, the following cleanup procedure can be used:

1. Add 0.5 volumes of 7.5 M NH_4OAc , 2.5 volumes of absolute ethanol (stored at -20°C), to gDNA.
2. Vortex and incubate at -20°C for 1 hr.
3. Centrifuge at 12,000 x g in a microcentrifuge at room temperature for 20 min.
4. Remove supernatant and wash pellet with 80% ethanol.
5. Centrifuge at 12,000 x g at room temperature for 5 min.
6. Remove the 80% ethanol and repeat the 80% ethanol wash one more time.
7. Resuspend the pellet in reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA).

Genomic DNA Preparation

This step needs to be done before proceeding with the DNA amplification stages.

The genomic DNA (gDNA) you will process using the Axiom 2.0 Assay should meet the general requirements listed earlier in this chapter. The amount of gDNA depends on which Axiom array will be used in the downstream protocol. All Axiom arrays (except the Axiom® Genome-Wide Pan-African Array Set) require a total of 200 ng. The Axiom Genome-Wide Pan-African Array Set requires a total of 300 ng, or 100 ng per array (there are three arrays in the Axiom Genome-Wide Pan-African Array Set).

To Prepare gDNA:

1. *Thaw Samples and Control*
2. *Quantitate and Dilute gDNA.*
3. *Aliquot the Diluted Samples and the Control*
4. *Freeze or Proceed*
5. *Create a Batch Registration File*

Duration

Thirty minutes to an hour for reagents to thaw and half an hour for setup.

Equipment, Consumables and Reagents Required

Equipment and Consumables

The equipment and consumables listed in [Table 2.2](#) are required for this stage.

Table 2.2 Equipment and Consumables Required for *Genomic DNA Preparation*

Quantity	Item
As required	Adhesive seals for plates
1	Ice bucket, filled with ice
1 each	Pipettes: ■ Single-channel P10 or P20 ■ Optional: multi-channel P10 or P20
As required	Pipette tips
1	Plate, deep well: ABGene 96 Square Well Storage; AB-0932*
1	Plate centrifuge
1	Plate spectrophotometer (required only if no OD measurements available for samples)
1	Vortexer

*A different deep well plate is used for the Automated Workflow version of the Axiom 2.0 Assay. See Chapter 2 of the Axiom 2.0 Assay Automated Workflow User Guide (PIN 702963).

Reagents

The reagents listed in [Table 2.3](#) are required for this stage.

Table 2.3 Reagents Required for *Genomic DNA Preparation*

Reagent	Supplier	Part Number
From the Axiom 2.0 Reagent Kit		
Axiom Reference Genomic DNA 103 (use as a positive control) Located in Module 1, -20 °C	—	—
User-supplied		
Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Affymetrix	75793

1. Thaw Samples and Control

Thaw the components listed below to room temperature:

- gDNA samples
- Axiom Reference Genomic DNA 103 (from the Axiom 2.0 Reagent Kit)

To Thaw, either:

- Place items on benchtop for one hour
- Thaw in a water bath:
 - Fill a small plastic dish with Millipore water. Do not overfill as the level of the water should not overflow when the sample tubes or plates are placed in the bath.
 - Thaw the sealed sample plate and Reference sample for a half-hour.
 - Wipe off the sample plate after removing and before removing the lid to minimize the chances that the water will enter the well and cause contamination or reaction failure.

2. Quantitate and Dilute gDNA

To Quantitate and Dilute the gDNA:

- Gently vortex (50% maximum) and spin the gDNA and Reference Genomic DNA 103.
- Recommendation:* quantitate each sample (*e.g.*, using the Quant-iT™ PicoGreen® dsDNA Kit).
- Dilute each sample to a concentration of either 10 ng (for all Axiom arrays except the Axiom Genome-Wide Pan-African Array Set) or 5 ng gDNA/μL (for the Axiom Genome-Wide Pan-African Array Set only) using reduced EDTA TE buffer.
- Seal, vortex and spin.



NOTE: Do NOT dilute the Reference Genomic DNA 103 control from the Axiom 2.0 Reagent Kit. It is already at a working concentration.

3. Aliquot the Diluted Samples and the Control

Next, the samples and control are placed in the following deep well plate for target preparation:

- For the **Manual Target Prep** protocol, use the ABgene 96 Square Well Storage; AB-0932. However, if you are running the Automated Target prep, see the *Axiom 2.0 Assay Automated Workflow User Guide* (P/N702963) for deep well plate recommendation.

Aliquot Diluted Samples and Reference Genomic DNA 103 to the Selected Deep Well Plate:

1. 20 μ L of each diluted gDNA sample (this should be the equivalent of 100 to 200 ng of gDNA, as required by the Axiom array).
2. 20 μ L of the Reference Genomic DNA 103 control.
We recommend including at least one positive control on each plate.
3. Seal and spin.



NOTE: For samples to be processed on the Axiom Genome-Wide Pan-African Array Set, three identical deep well plates of 100 ng gDNA per well should be made

4. Freeze or Proceed

At this point you can:

- Store the sample plate at -20°C , or
- Proceed to DNA Amplification for Manual Target Prep. See [Chapter 4, Axiom® 2.0 Assay: Manual Target Preparation](#) on page 32.



NOTE: You can leave the gDNA sample plate at room temperature if proceeding immediately to DNA Amplification.

5. Create a Batch Registration File



IMPORTANT: It is very important to create and upload a GeneTitan Array Plate Registration file with your sample information prior to loading the array plate and hyb tray in the GeneTitan Instrument. We recommend that you create (but not upload) this file at the same time you prepare your plate of genomic DNA. When your samples are ready for hybridization, you will scan the array plate barcode and upload the file to Affymetrix GeneChip Command Console (AGCC).

GeneTitan Array Plate Registration files contain information that is critical for:

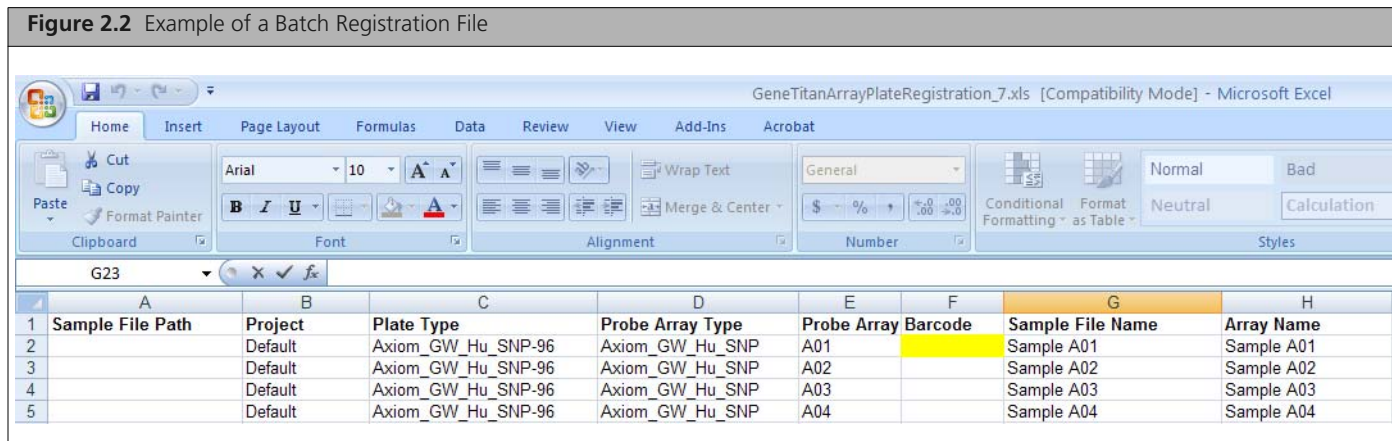
- Data file generation during imaging.
- Tracking the experimental results for each sample loaded onto an array plate.

Detailed instructions for creating this file are located in [Appendix D, Registering Samples in Affymetrix GeneChip® Command Console®](#) on page 127. See also [Figure 2.2](#) for a screen shot showing an example of a batch registration file.

1. Open AGCC Portal → Samples, and select:
 - A. GeneTitan Array Plate Registration.
 - B. The array plate format.
 - C. Click **Download**.
2. Enter a unique name for each sample and any additional information.
3. Save the file.

The array plate barcode will not be scanned until you are ready to load the array plate and samples onto the GeneTitan MC Instrument for processing.

Figure 2.2 Example of a Batch Registration File



The screenshot shows a Microsoft Excel spreadsheet titled "GeneTitanArrayPlateRegistration_7.xls [Compatibility Mode] - Microsoft Excel". The ribbon includes tabs for Home, Insert, Page Layout, Formulas, Data, Review, View, Add-Ins, and Acrobat. The "Home" tab is active, showing options for Clipboard, Font, Alignment, Number, and Styles. The spreadsheet data is as follows:

	A	B	C	D	E	F	G	H
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array	Barcode	Sample File Name	Array Name
2		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A01		Sample A01	Sample A01
3		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A02		Sample A02	Sample A02
4		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A03		Sample A03	Sample A03
5		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A04		Sample A04	Sample A04

Axiom® 2.0 Assay: Preparation Before You Start

This section provides information on procedures that are performed multiple times during manual target preparation and on steps that are critical to the success of the manual target preparation. It is essential that you familiarize yourself with the information in this section prior to running the manual target preparation for Axiom® 2.0 Assay.

A list of all equipment and resources required for the Axiom 2.0 Assay with manual target preparation is in the *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991.

Differences Between the Axiom Assays

The Axiom 2.0 Assay Manual Workflow contains important differences from the original Axiom Genotyping Assay. If you are experienced with running the original Axiom Assay, it is highly recommended that you familiarize yourself with these changes to prevent errors and ensure the success of the Axiom 2.0 Assay.

Reagent Kit Changes:

The new Axiom® 2.0 Reagent Kit contains a different Module 1 than the original Axiom® Reagent Kit; however, Modules 2, 3, and 4 are identical between both versions and may be used from either kit for this assay. Only use new Module 1 from the Axiom 2.0 Reagent Kit for the Axiom 2.0 Assay.

Amount of gDNA Required

The Axiom® Genome-Wide Pan-African Array Set requires 100 ng of gDNA per array. This array is packaged as a three array set so for each gDNA sample a total of 300 ng is required (100 ng/array x 3 arrays in the Axiom Genome-Wide Pan-African Array Set = 300 ng gDNA of each sample).

The Axiom Genome-Wide BOS, CEU, ASI, CHB, and EUR still require 200 ng gDNA per sample.

Manual Method and Equipment/Consumable Changes:

- New Vortex guideline: For deep well plates (such as ABgene 2.2 mL square well storage plates), the vortexing time per sector has been increased to 5 seconds. (Refer to [Seal, Vortex and Spin on page 23.](#))
- Only 3 incubator/oven temperatures are now required during the assay: 37 °C, 65 °C and 48 °C. (Refer to [Table 6.5 on page 102.](#))

Manual Assay Workflow Changes:

There are many changes in Axiom 2.0 manual assay workflow including how the reagents are handled out of modules, various master mixes preparation, incubation times and incubation temperature changes. The Amplification stage of the assay has changed most. These changes are indicated for all the five stages, but step-by-step instructions are detailed in each section of [Chapter 4, Axiom® 2.0 Assay: Manual Target Preparation on page 32.](#)

Amplification Stage Changes:

- All reagents of Module-1 (except Amp enzyme) are now thawed and kept at room temperature.
- There are new recipes for Denaturation Master Mix and Amplification Master Mix for Axiom 2.0 Assay.
- The Neutral Soln no longer requires dilution before use.
- Amplification master mix is now made and added to the plate at room temperature.
- The new incubation time for the Denaturation Plate is 10 minutes.
- The new oven temperature for Amplification Plate incubation is 37 °C.

Fragmentation and Precipitation Stage Changes:

- There is a new recipe for Fragmentation Master Mix preparation for Axiom 2.0 Assay.
- Regents for Precipitation Master Mix preparation (but not Fragmentation Master Mix preparation) are now handled at room temperature.

Drying, Resuspension and QC Stage Changes:

- Reagents for Hybridization Master Mix preparation are now handled at room temperature.
- There is a new recipe for Hybridization Master Mix for Axiom 2.0 Assay.
- Changes to the QC steps are:
 - The observed OD260 yields from in process QC are higher. It is recommended to consider troubleshooting if median yield of a plate is < 1000 µg.
 - QC gel samples are diluted more.

Denaturation and Hybridization Stage Changes:

- The new Axiom 2.0 Denature thermal cycler protocol for denaturation of the hyb ready samples is: 95 °C for 10min, 48 °C for 3 min and 48 °C hold.

Requirements and Recommendations

This section describes requirements and recommendations for facilities and equipment needed to perform the Axiom 2.0 Assay with manual target preparation.

Room Temperature

When referred to in the Axiom 2.0 Assay, room temperature is 18 to 25 °C.

Special Requirements

Amplification Staging Area

Precautions are required when setting up amplification reactions to avoid contamination with foreign DNA amplified in other reactions and procedures. It is recommended that amplification reaction set up is performed in a dedicated amplification staging area separate from the main laboratory.

This amplification staging area should have a dedicated set of pipettes and plasticware. If no dedicated amplification staging area is available, use of a dedicated bench or a dedicated biosafety hood and dedicated pipettes is suggested. If no dedicated bench or biosafety hood is available, a set of dedicated pipettes is recommended.

Fume Hood

At certain steps in the protocol we recommend the use of adequate local or general ventilation to keep airborne concentrations low.

A fume hood is suggested as a way to achieve the desired concentration. Thus, a fume hood is strongly recommended for several steps of this assay.

Safety Warnings and Precautions



CAUTION: All chemicals should be considered as potentially hazardous. We, therefore, recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as lab coat, safety glasses and gloves. Care should be taken to avoid contact with skin and eyes.



WARNING: The following components contain harmful or toxic ingredients:

- Axiom Stabilize Soln: 8% Gluteraldehyde
- Axiom HybSoln 2: 100% Formamide
- Axiom Hyb Buffer: <55% Tetramethylammonium Chloride

As such we recommend the use of a fume hood when using these products during the Manual Target Preparation protocol. In all cases customers should use adequate local and general ventilation in order to minimize airborne concentrations.

Copies of the Material Safety Data Sheets for the kit components are available on the Affymetrix website at www.affymetrix.com

Control Recommendations

A negative control is not required for this assay.

We recommend including one positive control with every set of samples processed. A positive control (Axiom Reference Genomic DNA 103) is included in the Affymetrix® Axiom 2.0 Reagent Kit.

Plate Requirements and Recommendations

The following types of plates are required for performing manual target preparation. Refer to the *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991, for vendor information.

- ABgene 96 Square Well Storage Plate, 2.2 mL
- Bio-Rad Hard Shell 96-well plate, P/N HSP-9631. Refer to the *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991, for vendor information.



NOTE: The ABI 9700 and the ABI 2720 use the half-skirted 96-well plates (P/N HSS-9601)

- 96-well UV Star Plates, 370 µL/well

Thermal Cycler Recommendations

The following thermal cyclers are recommended:

- BIO-RAD PTC-200, or
- Whatman Biometra TRobot 96, or
- BIO-RAD DNA Engine Tetrad 2 #PTC-0240, or
- ABI 9700 (with gold, silver, or aluminum block), or
- ABI 2720



IMPORTANT: Always use the heated lid option when programming protocols.

We have verified the performance of this assay using the following thermal cyclers: Bio-Rad PTC-200, Biometra TRobot 96, ABI 9700 (with a gold, silver or aluminum block), ABI 2720 and the Bio-Rad PTC-0240. The performance of this assay has not been verified with other thermal cyclers. Use of other thermal cyclers may result in assay failure and may violate the Axiom array and reagent replacement policy. The thermocycler needs to be programmed with the “Axiom 2.0 Denature” protocol:

1. 95 °C 10 min
2. 48 °C 3 min
3. 48 °C hold

Use the heated lid option when setting up or running the protocol.



WARNING: Evaporation during denaturation can negatively impact assay performance. Use the recommended thermal cycler consumables and sealing film to eliminate condensation and evaporation. For thermal cyclers with variable lid tension (such as the Bio-Rad PTC-200 or Tetrad 0240 or Biometra TRobot) please follow the manufacturer’s instructions for adjusting lid tension.

Thermal Cycler Consumables

Table 3.1 provides details into the consumables to be used with each thermal cycler.

Table 3.1 Thermal Cycler Consumables for the Axiom 2.0 Assay

Thermal Cycler Model	PCR Plate Type	Seal*
Bio-Rad PTC-200	Bio-Rad Hard-Shell Thin-Wall 96-Well Skirted PCR Plates, P/N HSP9631	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
TRobot	Bio-Rad Hard-Shell Thin-Wall 96-Well Skirted PCR Plates, P/N HSP9631	BioRad Arched Auto-Sealing Lids with Wide Tabs (P/N MSL-2032) with BioRad Micro seal 'P' Replacement Pads (MSP-1003)
ABI 9700	Bio-Rad P/N HSS-9601 (half skirted plate)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
ABI 2720	Bio-Rad P/N HSS-9601 (half skirted plate)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
Bio-Rad Tetrad® 2 PTC-0240	Bio-Rad Hard-Shell Thin-Wall 96-Well Skirted PCR Plates, P/N HSP9631	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)

* Microseal “B” film from BioRad (P/N MSB-1001) may be used in place of MicroAmp Clear Adhesive Film for the BioRad and ABI thermal cyclers.

Oven Recommendations

The following ovens are recommended:

- ED 53 drying oven by Binder
Refer to the *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991, for vendor information.
- Affymetrix GeneChip Hyb Oven 645



NOTE: The GeneChip® Hybridization Oven 640 is currently not supported with the Axiom 2.0 Assay; however, if you want to utilize it in the workflow please contact your Field Service Engineer (FSE) or Affymetrix Technical Support regarding the compatibility of this oven with the Axiom 2.0 Assay.

- If using an Affymetrix GeneChip Hyb Oven, set the rotation speed to 15 RPM to aid in even heat distribution.
- For either Affymetrix GeneChip Hyb Oven, plates are placed in the bottom of the oven. To avoid interfering with the rotation apparatus, do not stack plates in the oven.
- Up to 4 plates can fit into a Hyb Oven 645

Multiple ovens are required for manual target preparation. The exact number depends upon whether you are running only a single sample plate and array plate through the workflow, or if you are trying to run the three plate/week manual target preparation workflow.

- If you are running individual plates, you will need two ovens for the workflow.
- If you are running the three plate /week workflow, three ovens are recommended.

See *Changing Oven Temperatures for the Three Plate Workflow* on page 102 of Chapter 6, *Manual Target Preparation for Processing Three Axiom® Array Plates per Week* for more information.

Equipment Care and Calibration

Lab instrumentation plays an important role in the successful completion of this assay. To aid in maintaining consistency across samples and operators, all equipment must be regularly calibrated and well maintained, including:

- All pipettes, thermal cyclers, and ovens
- Plate spectrophotometer

Procedures

This section covers procedures you may need to do repeatedly during the workflow, or which are critical to the performance of the assay.

Seal, Vortex and Spin

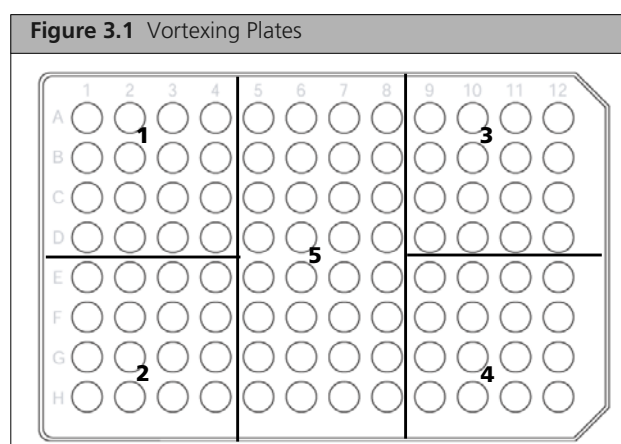
Unless otherwise noted, when the protocol instructs you to seal, vortex and spin:

- **Seal plates** — we recommend using MicroAmp Clear Adhesive Films to seal your plates.



IMPORTANT: Always ensure that your plates are tightly sealed. A tight seal will prevent sample loss and cross-well contamination, particularly when plates are being vortexed.

- **Spin** — when instructed to perform a brief spin down of plates or reagent vials, follow these guidelines unless otherwise instructed.
 - **Plates:**
 - Spin at room temperature.
 - Start the centrifuge, allow it to reach 1000 rpm and spin for 1 min.
 - **Reagent Vials:** 3 sec
- **Vortex reagents** 3 times, 1 sec each time.
- **Vortex plates**
 - For deep well plates (such as ABgene 2.2 mL square well storage plates), vortex 5 seconds in each sector for a total of 5 sectors ([Figure 3.1](#)).
 - For PCR plates (such as Bio-Rad Hard Shell or semi-skirted plates, vortex 2 seconds in each sector for a total of 5 sectors ([Figure 3.1](#)).



NOTE: In the procedures, “vortex twice” means to repeat the vortexing step.

Sample Quantitation

This protocol has been optimized using a PicoGreen assay to determine genomic DNA concentrations. Other quantitation methods such as UV Absorbance may give different readings. Therefore, you should correlate readings from other methods to the equivalent PicoGreen-determined concentration.

Please refer to [Chapter 2, Genomic DNA Preparation and Requirements](#) on page 11 for more information.

About the Reagents and Master Mix Preparation

Axiom 2.0 Reagent Kit Components

- Caps on the vials are color-coded by assay stage.
- Properly store all enzyme reagents, especially enzyme-containing vials. Improper storage methods can profoundly impact activity.



IMPORTANT: The Axiom® 2.0 Assay is compatible only with reagents from an Axiom Reagent Kit. These reagents are not interchangeable with reagents from other Affymetrix reagent kits, such as SNP 6.0, DMET Plus, etc. The new Axiom 2.0 Reagent Kit contains a different Module 1 than the original Axiom Reagent Kit; however, Modules 2, 3, and 4 are identical between both versions and may be used from either kit for this assay. Only use new Module 1 from the Axiom 2.0 Reagent Kit for the Axiom 2.0 Assay.

Reagents from Other Suppliers

- Use only fresh reagents from the recommended vendors to help eliminate changes in pH or the salt concentration of buffers.
- Consult the appropriate MSDS for reagent storage and handling requirements.

Master Mix Preparation

- Carefully follow each master mix recipe. Use pipettes that have been calibrated to $\pm 5\%$.
- If you run out of master mix during any of these procedures, a volume error has been made or the pipettes are not accurate. We recommend that you stop and repeat the experiment.



NOTE: The volumes of Master Mixes prepared are designed to provide consistent handling of reagents and consistent assay results. The percent overage of different master mixes may differ, depending upon the reagent volumes involved.

When Using Reagents at the Lab Bench

- Properly chill essential equipment such as reagent coolers before use.
- Ensure that enzymes are kept at -20°C until needed. When removed from the freezer, immediately place in a cooler that has been chilled to -20°C .

Pipettes and Pipetting

To efficiently process samples:

- Use a pipette of appropriate size for the volume of liquid being transferred ([Table 3.2](#)).

Table 3.2 Recommended Pipette Sizes

Pipette Size	Recommended Volume Range
Single channel P20 / 12-channel P20	1-20 µL
P50 (optional)	20-50 µL
Single channel P200 / 12-channel P200	20-200 µL
Single channel P1000 / 12-channel P1200	200-1000 µL

- We recommend the use of Rainin pipettes and tips. Affymetrix has only verified the use of Rainin 12-channel pipettes in this assay. The use of other pipettes (such as other brands or 8-channel pipettes) may impact the timing of the protocol and may adversely impact the assay. Pipette substitution may violate the terms of the Axiom 2.0 Assay and array replacement policy.
- Always use pipettes that have been calibrated.
- It is essential that you be proficient with the use of single- and multi-channel pipettes. To familiarize yourself with the use of multi-channel pipettes, we strongly recommend practicing several times before processing actual samples. Use water and solution basins to get a feel for aspirating and dispensing solutions to multiple wells simultaneously.

Single-channel Pipettes and Serological Pipettes

Use single-channel pipettes for preparing Master Mixes and for puncturing bubbles in GeneTitan trays. The single-channel pipettes will not be used for working with the plates or trays otherwise.

- Use single channel pipettes for volumes less than or equal to 2 mL. For volumes between 1 and 2 mL, add the reagent in two portions with a fresh tip for each portion.
- Use serological pipette for volumes > 2 mL.
- In most cases, 25 or 50 mL serological pipettes will not fit into the mouths of the reagents bottles. Multiple transfers using 5 or 10 mL serological pipettes will need to be performed.

Multi-Channel Pipettes

Use 12-channel pipettes when working to add Master Mix or to transfer samples to plates and GeneTitan trays.

- Use a pipette of appropriate size for the volume of liquid being transferred.
- Change pipette tips after each transfer or addition.

GeneTitan MC Instrument Consumables

All consumables for the GeneTitan MC Instrument are provided by Affymetrix. The following table provides guidance on the consumables that are shipped with the array plate.

! IMPORTANT: All GeneTitan trays and tray covers must have barcodes. Discard any consumable tray or tray cover without a barcode.

Table 3.3 Axiom GeneTitan Tray Consumables (from the Axiom® GeneTitan® Consumables Kit, P/N 901606)

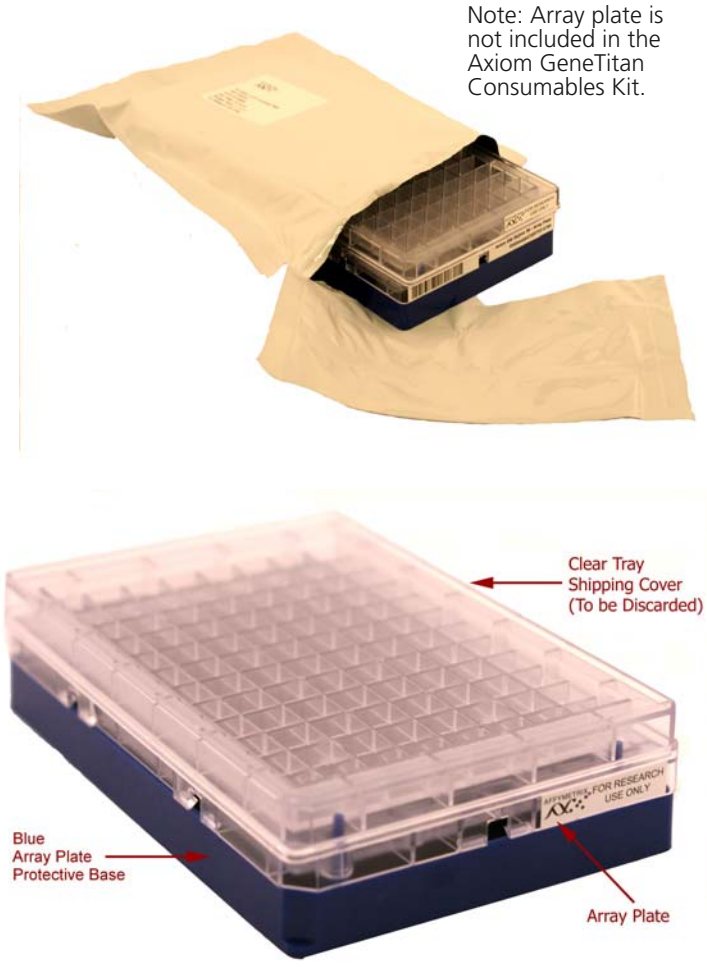
Item	Part Number	Labware Image	Information
Axiom Genome-Wide or Axiom myDesign Custom Array Plate Package	All array plates have the P/N 202091 etched on the plastic	 Note: Array plate is not included in the Axiom GeneTitan Consumables Kit.	The Axiom array plate shipping package includes the following: ■ The function of the clear plastic cover for the array plate is to protect the array plate during transport. You can discard this after removing the array plate. ■ The array plate must be protected at all times from damage or exposure to dust. The array plate must be in the blue array plate protective base at all times. ■ The blue array plate protective base in the package must be used to protect the array plate from damage.

Table 3.3 Axiom GeneTitan Tray Consumables (from the Axiom® GeneTitan® Consumables Kit, P/N 901606) (Continued)

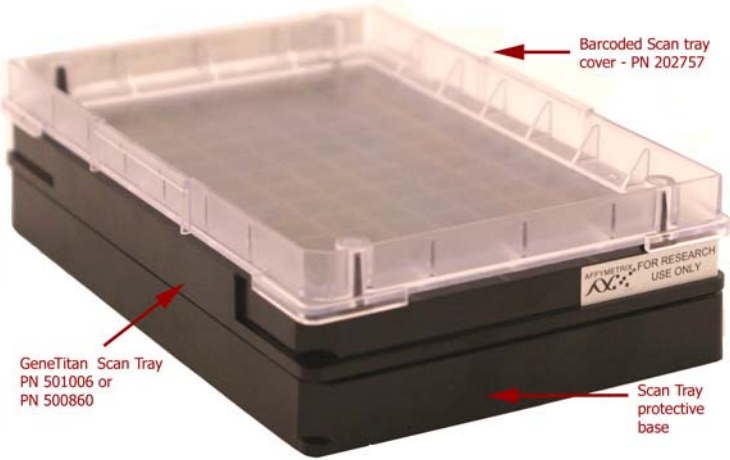
Item	Part Number	Labware Image	Information
			The HT scan tray shipping package includes the following: ■ The HT scan tray includes a scan tray cover. The tray cover should be used to cover the scan tray before placing the tray in the GeneTitan MC Instrument. The Tray cover should be de-ionized before use. See the section Appendix E, Deionization Procedure for GeneTitan Trays and Covers on page 130 for the anti-static procedure. ■ The scan tray must be protected at all times from damage or exposure to dust. The scan tray must be in the black plate cover at all times. ■ The black scan tray protective base in the package is used to protect the scan tray glass from damage. The black scan tray protective base is distinct from the blue array plate protective base and must not be used with the array plate. Remove the protective base from the scan tray before loading the scan tray with the scan tray cover.
Black Scan Tray protective base	P/N 202096		■ The black scan tray protective base in the package is used to protect the scan tray glass from damage. The black scan tray protective base is distinct from the blue array plate protective base and must not be used with the array plate. ■ Remove and discard the protective base from the scan tray before loading into the GeneTitan MultiChannel Instrument.

Table 3.3 Axiom GeneTitan Tray Consumables (from the Axiom® GeneTitan® Consumables Kit, P/N 901606) (Continued)

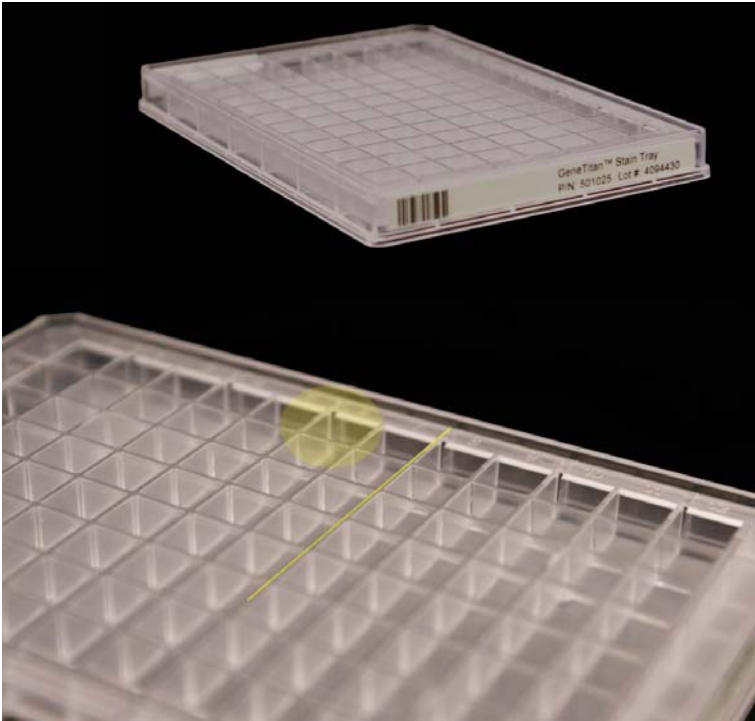
Item	Part Number	Labware Image	Information
Scan Tray with cover	Scan Tray 501006 Cover 202757		■ The GeneTitan scan tray must be loaded with the scan tray cover into the GeneTitan MC Instrument. ■ Do not load the scan tray with the protective base.
GeneTitan Stain Trays	501025		The GeneTitan stain trays are packaged in ziplock bags to keep them free of dust. The GeneTitan stain trays are barcoded and tray have separator walls that are flush with the frame of the stain tray as shown by the yellow line.

Table 3.3 Axiom GeneTitan Tray Consumables (from the Axiom® GeneTitan® Consumables Kit, P/N 901606) (Continued)

Item	Part Number	Labware Image	Information
GeneTitan Scan and Stain Tray cover	202757		■ The GeneTitan scan and stain tray covers are provided to prevent any evaporation of the stains in stain trays and the array holding buffer in the scan tray ■ All Stain and scan trays must be placed in the GeneTitan MC Instrument with the GeneTitan stain tray cover. ■ All tray covers must be de-ionized to remove static electricity prior to placing the cover on the tray. ■ See Appendix E, Deionization Procedure for GeneTitan Trays and Covers on page 130 for the anti-static procedure.
GeneTitan Stain Tray shown with the Stain Tray cover	Tray 501025 Cover 202757		■ The GeneTitan stain trays must be placed in the GeneTitan MC Instrument with the GeneTitan stain tray cover. ■ It is important to remove the static electricity on the cover and the stain tray prior to loading the tray into the GeneTitan MC Instrument. ■ See Appendix E, Deionization Procedure for GeneTitan Trays and Covers on page 130 for the anti-static procedure.
GeneTitan Hyb Tray	500867		■ The GeneTitan hybridization trays are packaged in white pouches with the label "HT Hybridization Tray." ■ After aliquoting the hyb cocktail into the hybridization tray, the tray should be loaded into the GeneTitan Instrument with the barcode facing away from the operator, i.e., barcode should be on the back side.

Labeling GeneTitan Hybridization and Reagent Trays

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan MC Instrument, you will need to mark each tray in a way that identifies its contents.

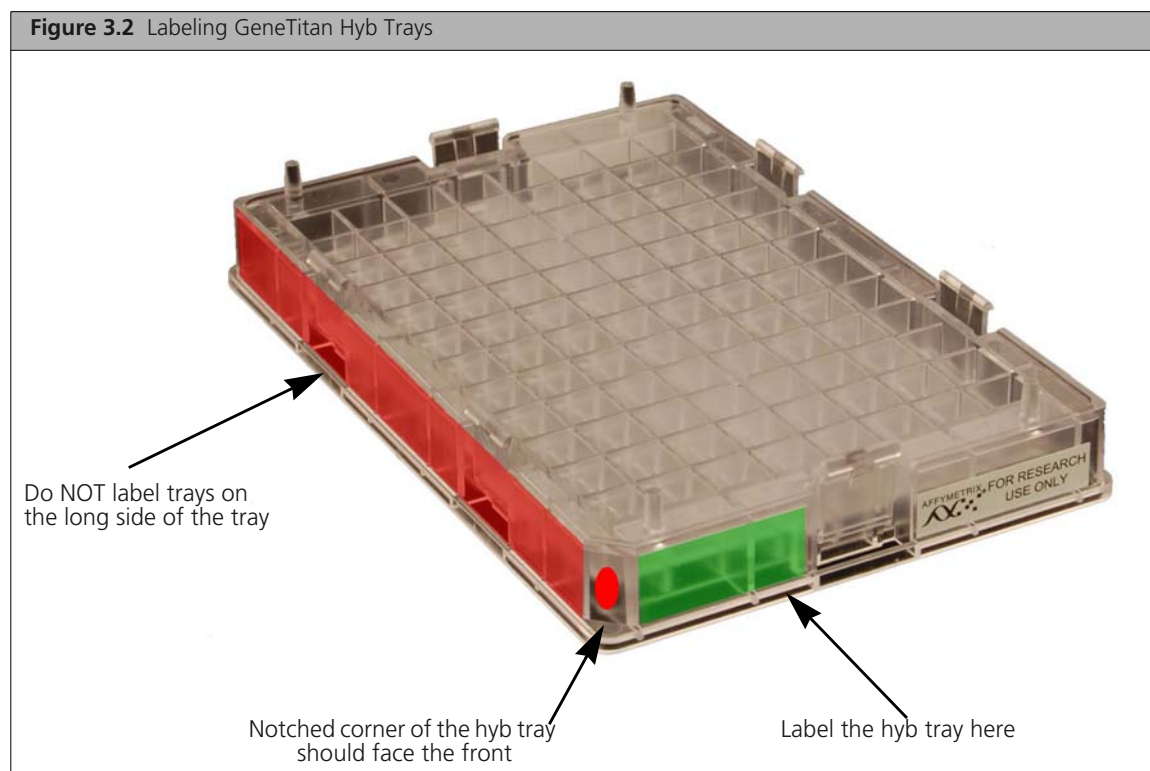
! IMPORTANT: It is critical that you write only on the proper locations of the proper sides of hyb and stain trays. Do **NOT** write in any other location, as this can interfere with sensors inside the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and lids.

Proper labeling for hyb trays and reagent trays is described in:

- *Labeling for Hyb Trays*, below
- *Labeling for Stain Trays* on page 31

Labeling for Hyb Trays

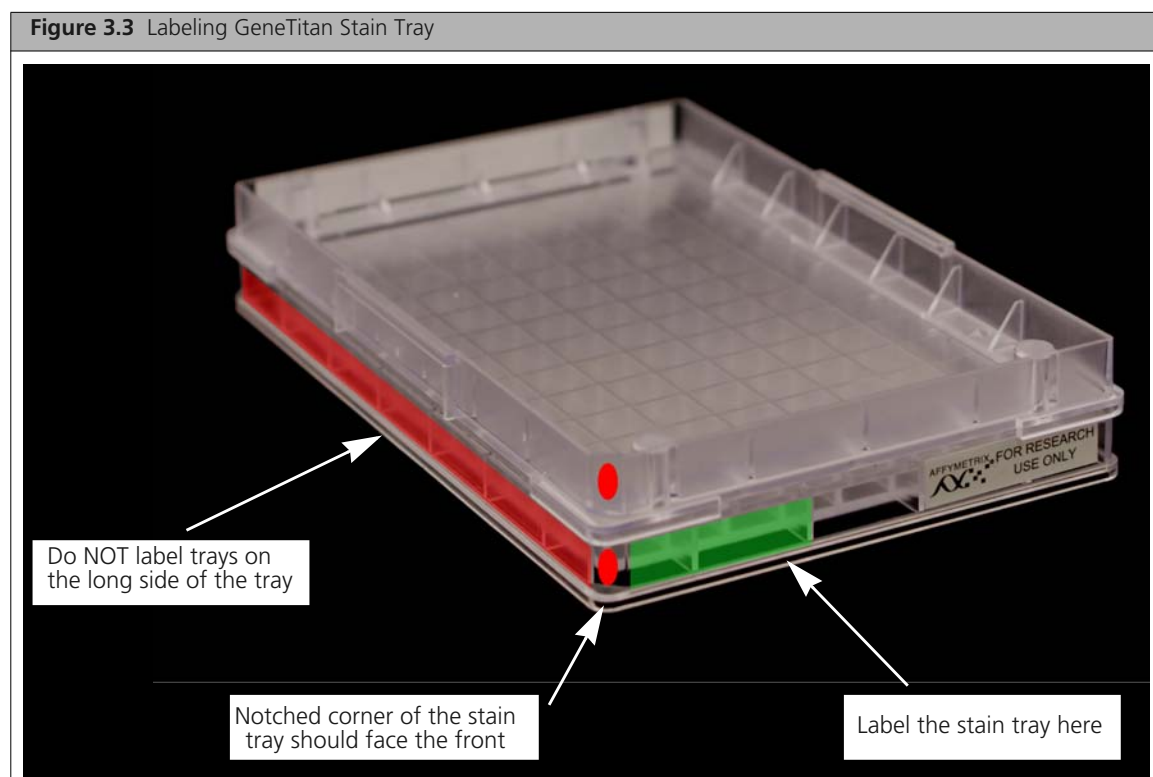
You may label the hyb tray on the front part of the **short side of the tray, next to the notch at the left**, as shown in [Figure 3.2](#). The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.



Writing on the wrong side of the hyb tray, or on the wrong part of the long side, may interfere with the operation of sensors in the GeneTitan MC Instrument.

Labeling for Stain Trays

You may label the stain trays on the **left side of the front of the tray** as shown in [Figure 3.3](#). The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.



(see *Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument* on page 55 for detailed information).

Axiom® 2.0 Assay: Manual Target Preparation

Manual target preparation for the Affymetrix Axiom Genome-Wide assay enables you to perform target preparation to process 96 samples at a time without the use of automation equipment.



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 on page 67](#).



IMPORTANT: Read all the instructions in [Chapter 3, Axiom® 2.0 Assay: Preparation Before You Start on page 18](#), before performing manual target preparation.

A list of all equipment and resources required for the Axiom 2.0 Assay with manual target preparation is in the *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991.

The protocol for manual target preparation is presented in the following sections:

- [Stage 1 — DNA Amplification on page 33](#)
- [Stage 2 — Fragmentation and Precipitation on page 38](#)
- [Stage 3 — Drying, Resuspension and QC on page 44](#)
- [Stage 4 — Denaturation and Hybridization on page 50](#)
- [Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument on page 55](#)



IMPORTANT: Before proceeding to DNA Amplification, perform the gDNA preparation described in [Chapter 2, Genomic DNA Preparation and Requirements on page 11](#).

Using the manual target preparation protocol, a single operator can process three gDNA and array plates a week during a forty-hour work week for a total of 288 arrays. See [Chapter 6, Manual Target Preparation for Processing Three Axiom® Array Plates per Week on page 100](#) for more information.

Stage 1 — DNA Amplification

! **IMPORTANT:** Before proceeding to DNA Amplification, perform the gDNA preparation described in [Chapter 2, Genomic DNA Preparation and Requirements on page 11](#).

📄 **NOTE:** For this protocol, the term *samples* includes the positive control.

The following sets of steps are necessary to perform DNA amplification:

- 1: *Initial Setup for DNA Amplification on page 35*
- 2: *Prepare the Denaturation Master Mix on page 36*
- 3: *Add Denaturation Master Mix to Samples on page 36*
- 4: *Add Neutralization Solution to Samples on page 37*
- 5: *Prepare and Add the Amplification Master Mix on page 37*
- 6: *Freeze or Proceed on page 38*

! **IMPORTANT:** Amplification preparation should take place in an a dedicated area such as a biosafety hood with dedicated pipettes, tips, vortex, etc. See [Amplification Staging Area on page 19](#) for more information.

Duration

For 96 samples:

- Time to thaw materials: 1 hr
- Hands-on time: approximately 0.5 hr
- Incubation at 37 °C: 23 ± 1 hr
- Total time required: approximately 24.5 hr

Input Required

gDNA Sample Plate, with 20 µL of each gDNA diluted to a concentration of 5 ng/µL or 10 ng/µL, as required according to the Axiom array that will be used, in an **ABgene 96 square well storage plate, 2.2 mL**.

See [Genomic DNA Preparation on page 14](#) for more information.

Equipment, Consumables and Reagents Required

Equipment and Consumables

The equipment and consumables listed in [Table 4.1](#) are required for this stage.

Table 4.1 Equipment and Consumables Required for Stage 1 — DNA Amplification

Quantity	Item
As required	Adhesive seals for 96-well plate - Applied Biosystems MicroAmp Clear adhesive film
1	Cooler, chilled to –20 °C
	
1	50 mL tube holder
1	15 mL tube holder
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1 each	Rainin Pipettes: ■ Single-channel P200 ■ Single-channel P1000 ■ Multi-channel P20 ■ Multi-channel P200 ■ Multi-channel P1200
As needed	Pipette tips
As needed	Pipette, serological ■ 5 x 1/10 mL (VWR P/N 53283-706) ■ 10 x 1/10 mL (VWR P/N 53283-708)
1	Pipet aid
1	Plate centrifuge, at room temperature
1	Oven, set at 37 °C
1	50 mL conical tube
1	15 mL conical tube
1	Vortexer
1	Timer
3	Solution basin, 100 mL sterile multichannel

Reagents Required

Table 4.2 Reagents Required for Stage 1 — DNA Amplification

Axiom 2.0 Reagent Kit	Module
Axiom 2.0 Denat Soln 10X	Module 1, –20 °C P/N 901711
Axiom 2.0 Neutral Soln	
Axiom 2.0 Amp Soln	
Axiom Water	
Axiom 2.0 Amp Enzyme	

1: Initial Setup for DNA Amplification

To Perform the Initial Setup

1. Set an incubator/oven temperature at 37 °C.
2. Set the centrifuge temp to room temperature.
3. Prepare reagents as shown in [Table 4.3](#):



NOTE: Leave the Axiom Amp Enzyme at –20 °C until ready to use.

Table 4.3 Initial Preparation of Reagents for Amplification

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.

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



IMPORTANT:

- gDNA samples must be brought to room temperature before proceeding with denaturation.
- gDNA samples must be **20 µL** volume of each gDNA at a concentration of **5 ng/µL or 10 ng/µL**, depending on the array type, in an ABgene 96 square well storage plate, 2.2 mL (see [Genomic DNA Preparation on page 14](#)).

5. Label the 15 mL and 50 mL conical tubes as indicated in the table below:

Label	Tube Size	Temperature	Contents
■ <i>D MM</i>	15 mL	leave tube at room temperature	Denaturation Master Mix
■ <i>Amp MM</i>	50 mL	leave tube at room temperature	Amplification Master Mix

6. Label three solution basins as indicated in the table below.

Label	Temperature	Contents
■ <i>D MM</i>	Leave basin at room temperature	Denaturation Master Mix
■ <i>N Soln</i>	Leave basin at room temperature	Neutralization Solution
■ <i>Amp MM</i>	Leave basin at room temperature	Amplification Master Mix

2. Prepare the Denaturation Master Mix

To Prepare the Denaturation Master Mix (carry out the following steps at room temperature):

1. Per [Table 4.4 on page 36](#), dilute the appropriate volume of Axiom 2.0 Denat Soln 10X using the Axiom Water.

Table 4.4 Preparing Denaturation Master Mix (*D MM*)

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked <i>D MM</i> , add:		
Axiom 2.0 Denat Soln 10X	2 µL	400 µL
Axiom Water	18 µL	3.6 mL
Total Volume	20µL	4 mL

2. Vortex and leave at room temperature.

3. Add Denaturation Master Mix to Samples

To Add the Denaturation Master Mix to Your Samples (carry out the following steps at room temperature):

1. Spin down the Sample plate.
Remember: Samples must be at room temperature for this step.
2. Pour the Denaturation Master Mix into the solution basin marked *D MM*.
3. Carefully remove the seal from the Sample plate and discard the seal.
4. Using a P20 12-channel pipette and pipetting directly into the liquid of each well, add **20 µL of Denaturation Master Mix** to each sample of the plate (total volume 40 µL/well).
Do not mix by pipetting up and down.
Change tips between each addition.
This plate is now known as the Denaturation plate.
5. Seal and vortex the Denaturation plate. Start the timer for **10 minute incubation**.
6. Do a quick spin on the Denaturation plate in a room temperature centrifuge by bringing centrifuge speed to 1000 rpm (takes ~ 1 minute).



NOTE: The quick spin time is included in the 10 minute incubation.

7. Visually examine the volume in each well (should be 40 μL /well) and:
 - A. Keep a record of any wells that visually appear to have a particularly low or high volume; these samples may need to be repeated.
 - B. Do **NOT** stop to measure volumes; proceed without delay.
8. Complete the **10 minute incubation** on the benchtop at room temperature.
While completing the incubation at room temperature, pour the Neutralization Soln into the solution basin as described in [Step 1 on page 37](#).
9. After incubation **immediately** add the Neutralization Soln as described in [4. Add Neutralization Solution to Samples on page 37](#).

4. Add Neutralization Solution to Samples

To Add the Neutralization Master Mix to Your Samples (carry out the following steps at room temperature):

1. Pour the Axiom 2.0 Neutral Soln into the solution basin marked *N Soln*.
2. Carefully remove the seal from the Denaturation plate and discard the seal.
3. Using a P200 12-channel pipette, pipetting down the wall of each well, add **130 μL of Axiom 2.0 Neutral Soln** to each sample (total volume 170 μL /well).
Change tips between each addition.
The plate is now known as the Neutralization Plate.
4. Seal, vortex, and spin the Neutralization plate.
5. Visually examine the volume in each well (should be ~ 170 μL /well) and:
 - A. Keep a record of any wells that visually appear to have a particularly low or high volume; these samples may need to be repeated.
 - B. Do **NOT** stop to measure volumes.
6. Proceed immediately to [5. Prepare and Add the Amplification Master Mix on page 37](#).

5. Prepare and Add the Amplification Master Mix

To Prepare and Add the Amplification Master Mix (carry out the following steps at room temperature):

1. Per [Table 4.5](#), pipette the appropriate amount of Axiom 2.0 Amp Soln into the 50 mL tube labeled *Amp MM* at room temperature.



NOTE: Use a 10 mL serological pipette to transfer Axiom 2.0 Amp Soln to the tube. The bottles have narrow openings, and a 25 mL pipette will not fit through the mouth of the bottle.

Table 4.5 Amplification Master Mix (*Amp MM*)

Reagent	Per Sample (μL)	Master Mix 96+
To the 50 mL tube marked <i>Amp MM</i> , add:		
Axiom 2.0 Amp Soln	225 μL	26.0 mL
Axiom 2.0 Amp Enzyme	5 μL	578 μL
Total Volume	230 μL	26.58 mL

2. Remove the Axiom 2.0 Amp Enzyme from the freezer and place in a portable cooler at -20 $^{\circ}\text{C}$.
 - A. Flick the Axiom 2.0 Amp Enzyme tube three times, then spin.
 - B. Per [Table 4.5 on page 37](#), add the appropriate amount of Axiom 2.0 Amp Enzyme to the tube labeled *Amp MM*.

- C. Vortex the Amplification Master Mix well, invert the tube 2 times, and then vortex again.
- D. **Slowly** pour the Amplification Master Mix to the solution basin labeled *Amp MM*.
- E. Carefully remove the seal from the Neutralization plate and discard the seal.
- F. Using a P1200 12-channel pipette, **slowly** add **230 µL Amplification Master Mix** to each well of the Neutralization plate, pipetting down the wall of the well (there will now be a total volume of 400 µL/well).
Do not mix by pipetting up and down.
Change tips between each addition.



NOTE: After adding the Amplification Master Mix, the plate is now known as the Amplification plate.

- G. Seal tightly, vortex twice, and spin the Amplification plate for one minute at 1000 rpm (as described in [Seal, Vortex and Spin on page 23](#)).
- H. Place the sealed amplification plate in an oven set at 37 °C and leave **undisturbed for 23 ± 1 hr**.



NOTE: If using a GeneChip® Hybridization Oven, place the plate on the bottom of the oven. Plates do not rotate. Set the rotor for 15 rpm speed. See [Oven Recommendations on page 22](#) for more information.

6. Freeze or Proceed

After the incubation finishes, you can either:

- Proceed to [Stage 2 — Fragmentation and Precipitation on page 38](#).
- Store the amplification plate at –20 °C.



NOTE: If freezing, do not perform the stop amplification reaction step before you store the Amplification plate at –20 °C. The Stop Amplification Reaction step will be performed after thawing the frozen plate, as described in [1: Stop Amplification Reaction on page 40](#).

Stage 2 — Fragmentation and Precipitation

The following sets of steps are necessary to perform fragmentation and precipitation:

- 1: [Stop Amplification Reaction on page 40](#)
- 2: [Prepare Fragmentation Master Mix on page 41](#)
- 3: [Add Fragmentation Master Mix to Wells on page 42](#)
- 4: [Aliquot the Stop Solution to the Fragmentation Plate on page 42](#)
- 5: [Prepare and Add Precipitation Master Mix on page 43](#)

Duration

Total time: approximately 2 hours.

Input Required

Amplification plate from [Stage 1 — DNA Amplification on page 33](#).

Equipment, Consumables and Reagents Required

Equipment and Consumables

The equipment and consumables listed in [Table 4.6](#) are required for this stage.

Table 4.6 Equipment and Consumables Required for Stage 2 — Fragmentation and Precipitation

Quantity	Item
As required	Adhesive seals for 96-well plates
1	Freezer set to -20°C (Designate a shelf where the precipitation plates can be left undisturbed)
1	Cooler, chilled to -20°C
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1 each	Rainin Pipettes: ■ Single channel P1000 ■ Single channel P200 ■ Multi-channel P20 ■ Multi-channel P200 ■ Multi-channel P1200
As needed	Pipette tips for pipettes listed above
1	Pipet-aid
1	Plate centrifuge set at room temp
1	Mini microcentrifuge (microfuge with microtube rotor)
2-3	Ovens (see Oven Recommendations on page 22): ■ One oven set at 37°C ■ One oven set to 65°C
1	15 mL conical tube
1	50 mL conical tube
1	50 mL conical tube holder
4	Solution basin, 100 mL sterile multichannel
1	Vortexer

Reagents Required

Table 4.7 Reagents Required for Stage 2 — Fragmentation and Precipitation

Reagent	Module
From the Axiom 2.0 Reagent Kit	
Axiom Frag Enzyme (leave at -20°C until ready to use)	Module 2-1, -20°C P/N 901528
Axiom 10X Frag Buffer	
Axiom Precip Soln 2	

Table 4.7 Reagents Required for Stage 2 — Fragmentation and Precipitation (Continued)

Reagent	Module
Axiom Frag Diluent	Module 2-2, 2–8 °C P/N 901529
Axiom Frag Rxn Stop	
Axiom Precip Soln 1	
User-supplied - Refer to the <i>Axiom® 2.0 Assay Manual Workflow Site Prep Guide</i> , P/N 702991	
Isopropanol (2-Propanol), 99.5%	96 samples: 65 mL per array plate

1: Stop Amplification Reaction

If you are running one plate per week, you will need two ovens to perform this step:

- One oven set at 37 °C.

Use an oven that can sustain a constant temperature of 37 °C and has a temperature accuracy of ± 1 °C.

- One oven set at 65 °C.

If you are running the three plate per week manual target preparation workflow, three ovens are recommended. See [Chapter 6, Manual Target Preparation for Processing Three Axiom® Array Plates per Week on page 100](#) for more information.



NOTE: If the plate has been frozen and stored, it must be thawed using the instructions in [Thawing Frozen Plates of Amplified DNA on page 103](#). Allow an hour to thaw.

To Stop the Amplification Reaction:

1. Place the Amplification plate in the 65 °C oven:

- If proceeding directly from the end of [Stage 1 — DNA Amplification on page 38](#), transfer the Amplification plate from the 37 °C oven to the **65 °C oven** and **incubate for 20 minutes**.
- If working with a thawed plate, place the thawed Amplification plate in the **65 °C oven** and **incubate for 20 minutes**.

2. Prepare reagents as shown in [Table 4.8](#) at the start of the **65 °C incubation** of the amplification plate.



NOTE: Leave the Axiom Frag Enzyme at –20 °C until ready to use.

Table 4.8 Reagent Preparation for Fragmentation and Precipitation

Reagent	Treatment
Axiom 10X Frag Buffer	Thaw, vortex and keep on ice.
Axiom Frag Diluent	Thaw, vortex, spin, and keep on ice.
Axiom Frag Enzyme	Flick tube 3X, spin, and keep in –20 °C cooler until ready to use.
Axiom Frag Rxn Stop	Thaw, vortex and keep at room temperature.
Axiom Precip Soln 1	Thaw, vortex and keep at room temperature.
Axiom Precip Soln 2	Thaw, vortex, spin and keep at room temperature.
Isopropanol	Keep at room temperature.

3. Optional: Remove samples for quantifying amplification yield by the PicoGreen Assay.
 - A. Carefully remove the seal from the Amplification plate and discard the seal.
 - B. Transfer 4 μL samples from each well to a 96 well PCR plate such as a Bio-Rad Hard Shell 96-well plate, HSP-9631 and set aside for later quantitation (e.g., using the Quant-iT™ PicoGreen® dsDNA Kit from Life Technologies).
 - C. Reseal the Amplification plate and continue with the Stop Amplification Step.
4. Transfer the Amplification plate from the 65 °C oven to the 37 °C oven and **incubate for 45 minutes**.
5. Set the plate centrifuge to room temperature.



TIP: Keep a labeled balance plate of equal weight ready to minimize any time delay before spinning the Fragmentation plate during later steps.

6. Label the 15 mL and 50 mL conical tubes as indicated in the table below:

Label	Tube Size	Temperature	Contents
■ <i>Frg MM</i>	15 mL	Place tube on ice	Fragmentation Master Mix
■ <i>Precip MM</i>	50 mL	Place tube at room temperature	Precipitation Master Mix

7. Label solution basins as indicated in the table below.

Label	Temperature	Contents
■ <i>Frg MM</i>	Leave basin at room temperature	Fragmentation Master Mix
■ <i>Stop</i>	Leave basin at room temperature	Frag Rxn Stop
■ <i>Precip MM</i>	Leave basin at room temperature	Precipitation Master Mix
■ <i>ISO</i>	Leave basin at room temperature	Isopropanol

2: Prepare Fragmentation Master Mix

To Prepare the 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 the table below.

Transfer the Axiom Frag Enzyme to a –20 °C portable cooler until ready to use.

Table 4.9 Axiom Fragmentation Master Mix

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked <i>Frg MM</i> , add:		
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	131.0 μL
Total Volume	57 μL	7.48 mL

Add the reagents from [Table 4.9](#) to the *Frg MM* tube in the order shown, using appropriate single channel and serological pipettes.

Just before the end of the **45 minute 37 °C incubation**, flick the Axiom Frag Enzyme tube 2 to 3 times, and spin.

Add the Axiom Frag Enzyme to the Fragmentation Master Mix at the end of the **45 minute 37 °C incubation**.



NOTE: Leave the Axiom Frag Enzyme at –20 °C until ready to use.

2. Vortex twice and place on ice.
3. Slowly pour the Fragmentation Master Mix in the solution basin labelled *Frg MM* placed at room temperature.

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 Amplification plate from the 37 °C oven and place on the bench top at room temperature.
Do not place the Amplification plate on ice.
2. Carefully remove the seal from the Amplification plate and discard the seal.
3. Pipetting directly into the liquid of each well, use a P200 12-channel pipette to add **57 µL of Fragmentation Master Mix** to each reaction.
Change tips after each addition.
After adding the Fragmentation Master Mix to the plate, the plate is now known as the Fragmentation plate.
4. Seal the Fragmentation plate and vortex twice.
5. Start the timer for **30 min**.
6. Spin the Fragmentation plate in the plate centrifuge at room temperature by bringing the centrifuge to 1000 rpm and stopping it.



IMPORTANT: Keep your timer in a safe place. It is helpful to note down the actual time when the incubation began in case the timer stops accidentally.

7. 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.

Prepare the Stop solution a few minutes before the end of the **30 minute incubation** period, as described in [4: Aliquot the Stop Solution to the Fragmentation Plate](#), below.

4: Aliquot the Stop Solution to the Fragmentation Plate

To Add the Stop Solution (carry out the following steps at room temperature):

1. A few minutes before the end of the 30 minute incubation period, pour the Axiom Frag Rxn Stop solution in the solution basin labelled *Stop*.
2. Remove the Fragmentation plate from the oven and place on the bench top at room temperature.
3. At the **end of the 30 minute fragmentation incubation period**, carefully remove the seal from the Fragmentation plate and discard the seal.

4. Using a P20 12-channel pipette, end the fragmentation reaction by adding **19 µL of Stop Solution** to each reaction.
Pipette directly into the liquid of each well.
Change tips after each addition.
Proceed immediately to the next step.
5. Seal and vortex and do a quick spin at 1000 rpm.
6. Leave the Fragmentation plate on the benchtop while you prepare the Precipitation Master Mix.

5: Prepare and Add Precipitation Master Mix

To Prepare and Add Precipitation Master Mix (carry out the following steps at room temperature):

1. Prepare Precipitation Master Mix in the 50 mL conical tube labelled *Precip MM*

Table 4.10 Precipitation Master Mix

Reagent	per sample	Master Mix 96+
To the 50 mL tube marked <i>Precip MM</i> , add:		
Axiom Precip Soln 1	238 µL	26 mL
Axiom Precip Soln 2	2 µL	218 µL
Total Volume	240 µL	26.22 mL



NOTE: Use a 5 or 10 mL serological pipette to pipette Axiom Precip Soln 1. The bottle has a narrow opening and a 25 mL serological pipette will not fit through the mouth of the bottle.

2. Vortex the *Precip MM* tube and place on benchtop at room temperature.
3. Pour the Precipitation Master Mix into the solution basin labeled *Precip MM*.
4. Carefully remove the seal from the Fragmentation plate and discard the seal.
5. Using a P1200 12-channel pipette, add **240 µL Precipitation Master Mix** to each sample. Rest each pipette tip against the wall of each well while delivering. You do not need to mix up and down. Change tips after each addition.



NOTE: After adding the Precipitation Master Mix, the plate is now known as the Precipitation plate.

6. Seal the Precipitation plate and vortex. Spin.
7. Remove the Precipitation plate from the centrifuge and place on benchtop at room temperature.
8. Pour isopropanol into the solution basin labeled *ISO*.
9. Carefully remove the seal from the Precipitation plate and discard the seal.
10. Using a P1200 12-channel pipette, 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.
Change the tips after each addition.
11. Blot the top of the plate with Kimwipe and seal tightly with a Microamp seal.
12. Carefully transfer the Precipitation plate into the -20°C freezer and **incubate overnight (16-24 hrs)**.



TIP: It is recommended to designate a shelf in a -20°C freezer where the plates can be left undisturbed.

Stage 3 — Drying, Resuspension and QC

This stage requires the following sets of steps:

- 1: *Centrifuge and Dry Pellets and Thaw Reagents* on page 46
- 2: *Prepare the Tubes, Basins, and Trays for Resuspension and Hyb Master Mix Preparation* on page 47
- 3: *Resuspension and Hybridization Master Mix Preparation* on page 47
- 4: *Recommended: Perform Quantitation and Fragmentation QC Checks* on page 49
5. *Freeze or Proceed* on page 50



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

Duration

- Centrifuge and dry plates: 1 hour 20 minutes
 - Resuspension and hyb mix preparation: 25 min
 - Gel QC and OD: 45 min
- total: 2.5 hr

Input Required

Precipitation plate from *Stage 2 — Fragmentation and Precipitation* on page 38.

Equipment, Consumables, and Reagents Required

The equipment and consumables listed in [Table 4.11](#) are required for this stage.

Table 4.11 Equipment and Consumables Required for Stage 3 — Drying, Resuspension and QC

Quantity	Item
As required	Adhesive seals for 96-well plates
1	Marker, fine point, permanent
1 each	Rainin Pipettes: ■ Single channel P20 ■ Single channel P-100 ■ Multi-channel P20 ■ Multi-channel P-200
As needed	Pipette tips for pipettes listed above
2	Bio-Rad Hard Shell 96-well plate, HSP-9631 or any 96-well PCR plate for making the dilutions: ■ QC Dilution Plate ■ Gel Samples Plate
1	Bio-Rad Hardshell 96 well plate, HSP-9631 (for Bio-Rad PTC-200, TRobot or Bio-Rad 0240G thermal cyclers) or Bio-Rad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate, P/N HSS-9601 (for ABI 9700 or ABI 2720 thermal cyclers) ■ Hyb Ready Plate
1	OD plate: 96-well UV Star, 370 µL/well
1	Oven set at 37 °C
1	Mini microcentrifuge (microfuge with microtube rotor)

Table 4.11 Equipment and Consumables Required for Stage 3 — Drying, Resuspension and QC (Continued)

Quantity	Item
1	Fume Hood
1	Plate centrifuge set at 4 °C
1	15 mL conical tube
1	10 mL Serological Pipette
1	Pipet aid
1	Shaker, either: ■ Titer Plate Shakers-4PL, 120V ■ Jitterbug
1	Vortexer
As needed	Solution basin, 100 mL sterile multichannel

Reagents Required

Table 4.12 Reagents Required for Stage 3 — Drying, Resuspension and QC

Reagent	Module
From the Axiom 2.0 Reagent Kit	
Axiom Hyb Buffer	Module 2-1, -20 °C P/N 901528
Axiom Hyb Soln 1	
Axiom Resusp Buffer	Module 2-2, 2–8 °C P/N 901529
Axiom Hyb Soln 2	
Other Reagents Required for QC steps (optional)	
TrackIt Gel Loading Buffer, 14 mL of 1000-fold dilution (see Appendix A, Fragmentation Quality Control Gel Protocol on page 114 for dilution instructions.)	
Gel Sample Plate	
15 fold dilution of 25bp Invitrogen Ladder (P/N 10488-022)	
Nuclease free water, ultrapure MB Grade, 14 mL (P/N 71786; for OD and Dilution Plate preparation)	

Gels and Related Materials Required

At the end of this stage, verifying the fragmentation reaction is highly recommended. See [Appendix A, Fragmentation Quality Control Gel Protocol on page 114](#) for the required gel and related materials.

1: Centrifuge and Dry Pellets and Thaw Reagents



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

To Centrifuge and Dry the Pellets:

1. Turn the oven on and preheat to 37 °C.
If using an Affymetrix GeneChip Hyb Oven, set the rotation speed to 15 rpm to distribute heat.
2. Begin thawing/warming the reagents used in this stage as shown in [Table 4.13 on page 46](#).
3. Remove the Precipitation plate from the –20 °C freezer and centrifuge the plate **for 40 min at 4 °C** at 3200 xg (4000 RPM for the Eppendorf 5810R centrifuge with the rotor configuration described in the *Axiom® 2.0 Assay Automated Workflow Site Prep Guide*).



NOTE: WARNING: Use rotor buckets with a soft rubber bottom to ensure that the deep well plates do not crack. Do not use buckets where the plates sit directly on a metal or hard plastic bottom, such as the A-4-62 rotor with a WO-15 plate carrier (hard bottom) for the Eppendorf 5810R centrifuge. Use of hard bottom plate carriers may result in cracked plates, loss of sample, unbalanced centrifugation, damage to the instrument and possible physical injury.



NOTE: If you are processing two plates at the same time, as in the three plate/week manual prep workflow, you can centrifuge both plates at the same time.

4. During the centrifugation time prepare the resuspension and hybridization reagents as shown in [Table 4.13](#):

Table 4.13 Reagent Preparation for Resuspension and Hybridization

Reagent	Treatment
Axiom Resuspension Buffer	Warm to room temperature (1 hour)
Axiom Hybridization Buffer	Vortex and keep at room temperature
Axiom Hybridization Solution 1	Thaw, vortex, spin and keep at room temperature
Axiom Hybridization Solution 2	Vortex, spin and keep at room temperature

5. Following centrifugation, empty the liquid from the Precipitation plate as follows:
 - A. Carefully remove the seal from the Precipitation 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.



NOTE: If using an Affymetrix 645 oven, turn off the rotor during the 20 min drying time.

7. If you are proceeding directly to [3: Resuspension and Hybridization Master Mix Preparation on page 47](#), you can prepare the Hybridization Master Mix at this time ([Step 6 on page 48](#)). You should also prepare the consumables detailed in [2: Prepare the Tubes, Basins, and Trays for Resuspension and Hyb Master Mix Preparation on page 47](#).

8. After 20 min remove the plate from the oven and either:
 - Proceed directly to [3: Resuspension and Hybridization Master Mix Preparation on page 47](#), even if some droplets of liquid remain. Leave the Precipitation plate at room temperature.
 - or
 - Tightly seal the plate and store at -20°C if not proceeding immediately to the next stage.

2: Prepare the Tubes, Basins, and Trays for Resuspension and Hyb Master Mix Preparation

To Prepare the Tubes, Basins, and Trays Used in the Procedure:

1. Label the 15 mL tube as indicated in the table below:

Label	Tube Size	Temperature	Contents
■ <i>Hyb MM</i>	15 mL	Room Temperature in Fume Hood	Hybridization Master Mix

2. Label solution basins as indicated in the table below.

Label	Temperature	Contents
■ <i>Resus</i>	Room Temperature	Axiom Resusp Buffer
■ <i>Hyb MM</i>	Room Temperature in Fume Hood	Hybridization Master Mix

3. If performing the recommended QC checks, label solution basins as indicated in the table below:

Label	Temperature	Contents
■ <i>NF H2O</i>	Leave basin at room temperature	Nuclease Free Water
■ <i>Loading Dye</i>	Leave basin at room temperature	Diluted Loading dye

3: Resuspension and Hybridization Master Mix Preparation



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

NOTE: Make sure the Axiom Resusp Buffer has equilibrated to room temperature before adding to dry pellets in [Step 3](#), below.

To Resuspend the Pellets (carry out the following steps at room temperature):

1. Pour Axiom Resusp Buffer in the solution basin labeled *Resus*.



NOTE: If you are processing two plates at the same time, as in the three plate/week manual prep workflow, you can add resuspension buffer to both plates at the same time and then place them both in the shaker.

2. If the Precipitation plate has a seal on it, carefully remove the seal from the Precipitation plate and discard the seal.
3. Using a P200 12-channel pipette, transfer **35 μL Axiom Resusp Buffer** to each well of the Precipitation plate with a dry pellet. Avoid touching pellets with tip. Change pipette tips after each addition. After adding Resuspension buffer, the plate is known as the Resuspension plate.
4. Seal the Resuspension plate.

5. Put the sealed Resuspension plate on one of the following shakers:

- Titer Plate Shakers-4PL: at **speed 9 for 10 min**
- Jitterbug: at **speed 7 for 10 min**



CAUTION: It is recommended that the remainder of the steps in this stage be preformed under a fume hood.

6. While the Resuspension plate is shaking, prepare the Hybridization Master Mix in the *Hyb MM* 15 mL tube.

- A. Add the reagents in [Table 4.14](#) to the *Hyb MM* tube in the order shown, using serological and single-channel pipettes as needed.

Table 4.14 Hybridization Master Mix

Reagent	per Sample	Master Mix 96+
To the 15 mL tube labeled <i>Hyb MM</i> , add:		
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

B. Vortex twice to mix.

7. Inspect the Resuspension plate from the bottom. If the pellets are not dissolved, repeat [Step 5](#).
8. Quickly spin at 1000 rpm.
9. Label a Bio-Rad Hard Shell 96-well plate, HSP-9631 as **Hyb Ready [Sample ID]** and keep covered. When using the ABI 9700 or ABI 2720 thermal cyclers, you should label a Bio-Rad **Full-Height 96-Well Semi-Skirted PCR Plate** (P/N HSS-9601) as **Hyb Ready [Sample ID]** and keep covered.
10. Set a P200 12-channel pipette to **45 µL** (this is slightly higher than the volume of sample in each well).
11. Using the P200 pipette, transfer the **entire contents of each well** of the Resuspension plate to the labeled Hyb Ready plate.
Change pipette tips after each transfer.
12. Pour the Hyb Master Mix to the solution basin labelled *Hyb MM* placed.
13. Using a P200 12-channel pipette, add **80 µL of the Hyb Master Mix** to each well of the Hyb Ready plate.
Change tips after each addition.
14. Seal, vortex twice, and spin.
15. Prepare the dilutions for the QC steps, as described in the next section.

4: Recommended: Perform Quantitation and Fragmentation QC Checks

Before proceeding to [Stage 4 — Denaturation and Hybridization](#), we highly recommend that you perform quantitation and fragmentation quality control checks.

The QC checks requires:

- 2 each 100 ml solution basin
 - Label one basin as *H2O*
 - Label the second basin as *Loading Dye*
- 2 each Bio-Rad Hard Shell 96-well plate, HSP-9631 or any 96-well PCR plate for making the dilutions:
 - Label one plate as *QC Diln*
 - Label the second plate as *Gel Sample*
- 1 each 96-well UV Star, 370 µL/well plate, labeled *OD*
- 14 mL Gel Loading dye:
 - 1000-fold dilution of 6x Invitrogen TrackIt Cyan/Orange as described in [Diluting the TrackIt Cyan/Orange Loading Buffer on page 115](#).
- 14 mL of nuclease free water, P/N 71786
- 15 fold dilution of 25bp Invitrogen Ladder, P/N 10488-022



NOTE: Change tips while transferring samples from the Hyb Ready plate and the QC Dilution plate to avoid cross-contamination.

To Perform the QC Checks (carry out the following steps at room temperature):

1. Pour nuclease free water into the solution basin labeled *H2O*. The water will be used to make the QC Dilution plate and the OD plate.
2. Make QC Dilution Plate:
 - A. Add **33 µL nuclease-free water** to each well of the *QC Diln plate*.
 - 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*. Change pipette tips after each transfer.
 - C. Seal, vortex, and spin.
3. Make OD Sample plate:
 - A. Carefully remove the seal from the QC Dilution plate and discard the seal.
 - B. Transfer **10 µL of each QC Dilution Plate sample** to the plate labeled *OD*.
This plate is known from here on as the *OD plate*.
Change pipette tips after each transfer.
 - C. Add **90 µL nuclease-free water** to each well of the OD Plate and mix by pipetting up and down.
Change pipette tips after each addition.
Final sample mass dilution is 120-fold.

See [Appendix B, Sample Quantitation after Resuspension on page 116](#) for more information on performing the Sample Quantitation.
4. Make Gel Samples:
 - A. Add **120 µL 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.
Change pipette tips after each transfer.
 - C. Seal, vortex, and spin the plate.
5. Run gel as described in [Appendix A, Fragmentation Quality Control Gel Protocol on page 114](#).
After the QC checks, the QC dilution plate, OD plate, and remaining gel samples can be discarded once satisfactory results from the gel and OD 260 readings have been obtained.

5. Freeze or Proceed

At this point you can:

- Proceed to [Stage 4 — Denaturation and Hybridization](#), below; or
- Store the Hyb Ready samples at -20°C .

Stage 4 — Denaturation and Hybridization

You will proceed to Stage 4 in one of two ways:

- Directly from Stage 3 without interruption.
- With Hyb Ready samples that were stored at -20°C after Stage 3.

To Perform Stage 4:

If the Hyb Ready plate was stored at -20°C , go to [1. Prepare Hyb Ready Samples Stored at \$-20^{\circ}\text{C}\$ on page 52](#)

If you are proceeding directly from the end of [Stage 4 — Denaturation and Hybridization on page 50](#), go to [2: Prepare Equipment and Perform Denaturation on page 52](#)



CAUTION: Parts of this stage should be performed under a fume hood.

Duration

- Hands-on: 45 minutes including denaturation time
- in GeneTitan MC Instrument: 23.5 to 24 hours Hyb Time

Required Input from Previous Stage

- Hyb Ready plate

Equipment, Consumables, and Reagents Required

The following thermal cyclers are recommended:

- BIO-RAD PTC-200, or
- Whatman Biometra TRobot, or
- BIO-RAD DNA Engine Tetrad 2 #PTC-0240, or
- ABI 9700, or
- ABI 2720



IMPORTANT: Always use the heated lid option when programming protocols.

The thermocycler needs to be programmed with the “Axiom 2.0 Denature” protocol (see [Thermal Cycler Recommendations on page 20](#)).

Table 4.15 Equipment Required for Stage 4 — Denaturation and Hybridization

Equipment		Quantity
GeneTitan MC Instrument		1
Rainin P200 12-channel Pipette		1
Pipette tips		As needed
Thermal Cycler	Appropriate thermal cycler, programmed with the "Axiom 2.0 Denature" protocol (see Thermal Cycler Recommendations on page 20).	1
96 well metal chamber warmed in a 48 °C oven*		1

* The metal chamber coming out of a 48 °C oven is warm to the touch. Gloves and mitts can be used if it feels too hot.

Table 4.16 Consumables Required for Stage 4 — Denaturation and Hybridization

Consumable	Vendor and Part Number	Quantity
■ One of the following Axiom array plates: □ One Axiom Genome-Wide human or non-human 96-array plate in a protective base, or □ One Axiom myDesign Genotyping 96-array plate in a protective base	Affymetrix various P/Ns	1
■ Hyb Tray*	P/N 500867	1

* The Consumables for the GeneTitan MC Instrument are packaged separately from the Axiom array plates. The consumables are available in the Axiom® GeneTitan Consumables Kit (P/N 901606). The hyb tray is available in the Axiom® GeneTitan® Consumables Kit.

Table 4.17 Reagents Required from the Axiom 2.0 Reagent Kit

Reagent	Module
Axiom Wash Buffer A (both bottles; 1L)	Module 3, Room Temperature P/N 901472
Axiom Wash Buffer B	
Axiom Water	

1. Prepare Hyb Ready Samples Stored at –20 °C

To Prepare Hyb Ready Samples That Were Stored at –20 °C:

1. Warm up the Hyb Ready plate at room temperature for 5 minutes. It is not necessary to equilibrate the plate for longer duration.
2. Make sure the Hyb Ready plate is sealed well.
If the plate is not sealed well:
 - A. Spin the plate and carefully remove the old seal.
 - B. If there is condensation on the top of the plate, blot dry gently with a Kimwipe.
 - C. Use a fresh seal and tightly reseal the plate.
3. Vortex the Hyb Ready plate briefly, then spin at 1000 rpm for 30 seconds.
4. 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 before setting up hybridization on the GeneTitan MC Instrument.
 - A. Leave the array plate in the pouch at room temperature, for a minimum of 25 minutes, before opening and loading on the GeneTitan MC Instrument to allow the plate to come to room temperature.
 - B. At the end of the array warm up time, open the pouch and scan the array plate barcode into the Batch Registration file (see [Stage 1 — Create and Upload Batch Registration File on page 75](#)).



WARNING: Do not remove the array plate from the protective base or touch the surface of any arrays.

3. Make sure the thermal cycler is powered on and the Axiom 2.0 Denature program with the heated lid option has been selected.
4. Open the lid of the thermal cycler and place the sealed Hyb Ready plate on the thermal cycler. Check the integrity of the seal as evaporation during denaturation can negatively impact assay performance.
5. Close the lid. For thermal cyclers with variable lid tension (such as the Bio-Rad PTC-200 or the BIO-RAD DNA Engine Tetrad 2 #PTC-0240) follow manufacturer's instructions for adjusting lid tension.
6. Start the **Axiom 2.0 Denature** program, described on [Thermal Cycler Recommendations on page 20](#)).
7. While the program is running:
 - A. Prepare the reagents from Module 3 as described in [Table 4.18](#):

Table 4.18 Reagents from Module 3 (P/N 901472)

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		

- B. Set up the GeneTitan MC Instrument (see [Setup the Instrument on page 76](#)).

- C. Upload the Batch Registration File (see [Stage 1 — Create and Upload Batch Registration File on page 75](#)).

3: Prepare Hybridization Tray and Load into GeneTitan MC Instrument



CAUTION: It is recommended to perform the next set of steps under a fume hood.

1. After the Axiom 2.0 Denature program has completed, remove the Hyb Ready plate from the thermocycler and place into a 96-well metal chamber that has been pre-warmed in an oven at 48 °C.
2. Move the metal chamber containing the denatured Hyb Ready plate to a fume hood.
3. Remove Microamp seal from Hyb Ready plate and discard.
4. Remove the hyb tray (from Axiom Array GeneTitan Consumables Kit) from packaging.
5. Label the hyb tray. See the note below and [Figure 3.2 on page 30](#) for more information.



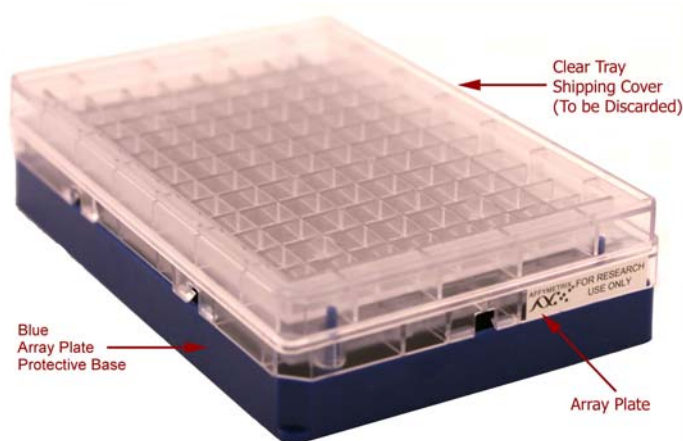
IMPORTANT: It is critical that you write only on the proper location of the hyb tray (on the edge in front of wells A1 and B1) as illustrated in [Figure 3.2 on page 30](#). Do **NOT** write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure.



IMPORTANT: Do not confuse hyb trays with stain trays.

6. Place the hyb tray under the fume hood.
7. Using a P200 12-channel 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. Change pipette tips after each transfer; discard the tip even if it shows some volume left. Ensure that there are no air bubbles present in the hyb tray. Puncture any air bubbles that you see using a clean pipette tip. There is no need to spread the sample around the bottom of the hyb tray wells. Sample distribution across the well will occur when the array plate is stacked together with the hyb tray by the GeneTitan MC Instrument.
8. Load the array plate and hyb tray into the GeneTitan MC Instrument (see [Load an Axiom Array Plate and Hyb Tray Onto the GeneTitan® MC Instrument on page 81](#)).

! **IMPORTANT:** The array plate must be loaded on the left side on its protective blue base, as shown in the figure below. The clear plastic cover on top of the array plate **SHOULD NOT** be loaded in the GeneTitan MC Instrument.



Load the hyb tray on the right side without any covering. The hyb tray should not have any bubbles.

! **IMPORTANT:** After the GeneTitan MC Instrument has stacked the array plate and hyb tray, the instrument will extend the drawer. 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. Do **NOT** tip/tilt the array plate/hyb tray sandwich while inspecting the bottom for bubbles. See [Step 3 on page 84](#) for detailed instructions.

Hybridization will continue on the GeneTitan MC Instrument for 23.5-24 hours before you can 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 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument](#), below.

Long delays between sample denaturation and loading into the GeneTitan MC Instrument for hybridization should be avoided. However, if denaturation has begun and the GeneTitan MC Instrument is found not to be ready for hybridization, then:

- If the Hyb Ready samples have not been transferred to the hyb tray (still in the Hyb Ready plate), the Hyb Ready plate should be held at **48 °C** in the thermocycler until the GeneTitan MC Instrument is ready, at which point you should begin at [Step 1 of 3: Prepare Hybridization Tray and Load into GeneTitan MC Instrument on page 53](#).
- If the samples have already been transferred to the hyb tray, the hyb tray should be sealed with plate sealing film and placed in an oven at **48 °C** until the GeneTitan MC Instrument is ready. Be sure to remove the plate sealing film before loading into the GeneTitan MC Instrument.

Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument

This stage needs to be done when hybridization in the GeneTitan MC Instrument is near completion (1.5 hours before completion), so the reagent trays can be loaded for the GeneTitan MC array processing steps.

Total time for this step: 1.5 hours, including reagent preparation, hands-on time and GeneTitan MC Instrument loading.

! **IMPORTANT:** The reagent trays prepared in this step, *Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument* are for the continued processing of an Axiom array plate that

- has completed the hybridization stage.
- is ready for transfer to the fluidics area.

The reagent trays for the fluidics stage on the GeneTitan MC Instrument should not be prepared in advance. Do not prepare these plates if there is no array plate ready for the fluidics stage. Once prepared, these plates must be loaded onto the instrument as soon as possible and should not be stored.

To prepare the reagent trays for the GeneTitan MC Instrument:

1: *Prepare the Reagents for Stage 5* on page 57

2: *Prepare the Stain, Ligation and Stabilization Master Mixes* on page 59

3: *Aliquot Master Mixes and Axiom Hold Buffer into Trays* on page 62

The following instructions are for manually preparing the reagents and trays required to process Axiom array plates on the GeneTitan MC Instrument. The reagents and trays required are as follows:

Table 4.19 Reagent Trays Required for the Axiom 2.0 Assay on the GeneTitan MC Instrument

Type of Tray	Number of Trays	Tray Designation	Master Mix/Reagent
Stain Tray with cover	2	<i>S1</i>	Stain 1 Master Mix
Stain Tray with cover	1	<i>S2</i>	Stain 2 Master Mix
Stain Tray with cover	1	<i>Stbl</i>	Stabilization Master Mix
Stain Tray with cover	1	<i>Lig</i>	Ligation Master Mix
Scan Tray	1	Scan Tray	Hold Buffer

Equipment, Consumables and Reagents Required

Table 4.20 Equipment Required for Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument

Equipment	Quantity
GeneTitan MC Instrument	1
Ice bucket with ice	1
Microcentrifuge	1
Pipetaid	1

Table 4.20 Equipment Required for Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument (Continued)

Equipment		Quantity
Rainin Pipettes — single channel ■ P200 ■ P1000	Rainin Pipettes — 12-channel: ■ P200	1 each
Vortexer		1

Table 4.21 Consumables Required for Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument

Consumable	Part Number	Quantity
Aluminum foil (optional)		As required
GeneTitan Consumables Kit	Affymetrix P/N 901606	1 kit includes:
□ Scan Tray	P/N 501006	1
□ Stain Tray	P/N 501025	5
□ Covers for trays	P/N 202757	6
Pipette, serological ■ 5 x 1/10 mL (VWR P/N 53283-706) ■ 10 x 1/10 mL (VWR P/N 53283-708)		1 2
Pipette tips		As required for pipettes listed in Table 4.20
Solution basin, 100 mL sterile multichannel		5
15 mL conical tube		3
50 mL conical tube		1

Reagents Required

Table 4.22 Axiom 2.0 Reagents Required for Stain and Ligation Stage (Continued)

Module	Reagent
Module 4-1, -20 °C P/N 901278	Axiom Ligase Buffer
	Axiom Ligase Enzyme
	Axiom Ligase Soln 1
	Axiom Probe Mix 1
	Axiom Stain Buffer
	Axiom Stabilize Soln
* These solutions are light sensitive. Keep tubes out of direct light for a prolonged period of time.	

Table 4.22 Axiom 2.0 Reagents Required for Stain and Ligation Stage (Continued)

Module	Reagent
Module 4-2, 2-8 °C P/N 901276	Axiom Ligate Soln 2
	Axiom Probe Mix 2*
	Axiom Wash A
	Axiom Stain 1-A*
	Axiom Stain 1-B*
	Axiom Stain 2-A*
	Axiom Stain 2-B*
	Axiom Stabilize Diluent
	Axiom Water
	Axiom Hold Buffer*
* These solutions are light sensitive. Keep tubes out of direct light for a prolonged period of time.	

1: Prepare the Reagents for Stage 5

To Prepare the Reagents:

1. Prepare the reagents from Module 4-1 as described in [Table 4.23](#):

Table 4.23 Reagents from Module 4-1, -20 °C (P/N 901278)

Reagent	Temp Out of Module*	Treatment	Storage before Master Mix
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
Notes: * Temperature the reagent is held at immediately after removal from module. # This bottle can also be thawed in a dish with room temperature Millipore water.			



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

2. Prepare the reagents from Module 4-2 as described in Table 4.24:

Table 4.24 Reagents from Module 4-2, 2-8 °C (P/N 901276)

Reagent	Temp Out of Module*	Treatment	Storage before Master Mix
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	Place at Room Temp away from light
Notes: * Temp Out of Module: temperature reagent is held at immediately after removal from module # These solutions are light sensitive. Keep tubes out of direct light for a prolonged period of time. N/A: not applicable in this case			



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

Label the Tubes and Solution Basins

To Label the Tubes:

1. Mark the side of each tube with one of designations shown in [Table 4.25](#).

Table 4.25 Labeling Master Mix Tubes for Stain, Ligation, and Stabilization Reagents

Conical Tube	Number of Tubes	Tube Designation	Contents	Place Tube:
50 mL	1	<i>S1</i>	■ Stain 1 Master Mix	On ice
15 mL	1	<i>S2</i>	■ Stain 2 Master Mix	On ice
15 mL	1	<i>Stbl</i>	■ Stabilization Master Mix	On ice
15 mL	1	<i>Lig</i>	■ Ligation Master Mix	On ice



NOTE: Use a 5 mL or 10 mL serological pipette to transfer Axiom Wash A, Axiom Water, and Axiom Ligate Buffer. These bottles have narrow openings and a 25 mL serological pipette will not fit.

2. Mark the side of each solution basin with one of the designations shown in [Table 4.26](#).

Table 4.26 Labeling Solution Basins

Basin Designation	Contents
<i>S1</i>	■ Stain 1 Master Mix
<i>S2</i>	■ Stain 2 Master Mix
<i>Stbl</i>	■ Stabilization Master Mix
<i>Lig</i>	■ Ligation Master Mix
<i>Hold</i>	■ Axiom Hold Buffer

Prepare Stain 1 Master Mix

To Prepare the Stain 1 Master Mix:

1. Use appropriate serological and single-channel pipettes to add reagents to the 50 mL tube labeled *S1* in the order shown in [Table 4.27](#). This recipe will provide enough for both *S1* reagent trays.

Table 4.27 Stain 1 Master Mix

Reagent	Per Array	Master Mix 96+
To the tube marked <i>S1</i> , add:		
■ 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

2. Gently invert the tube 10 times to mix.
3. Place on ice and protect from direct light (*e.g.*, cover with aluminum foil or ice bucket lid).

Prepare Stain 2 Master Mix

To Prepare the Stain 2 Master Mix:

1. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *S2* in the order shown in [Table 4.28](#).

Table 4.28 Stain 2 Master Mix

Reagent	Per Array	Master Mix 96+
To the tube marked <i>S2</i> , add:		
■ 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

2. Gently invert the *S2 MM* tube 10 times to mix.
3. Place on ice and protect from direct light (*e.g.*, cover with aluminum foil or ice bucket lid).

Prepare Stabilization Master Mix

To Prepare the Stabilization Master Mix:

1. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Stbl* in the order shown in [Table 4.29](#).

Table 4.29 Stabilization Master Mix

Reagent	Per Array	Master Mix 96+
To the tube marked <i>Stbl</i> , add:		
■ 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

2. Vortex the master mix at high speed for 3 sec.
3. Place on ice.

Prepare Ligation Master Mix

The Ligation Master Mix is prepared in two stages.

Ligation Master Mix: Stage 1

To Begin Preparing the Ligation Master Mix:

1. Place the 15 mL conical tube marked *Lig* on ice.
2. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Lig* in the order shown in [Table 4.30](#).

Table 4.30 Ligation Master Mix Preparation — **Stage 1**

Reagent	Per Array	Master Mix 96+
To the tube marked <i>Lig</i> , add:		
■ Axiom Ligase Buffer	66.15 µL	7.3 mL
■ Axiom Ligase Soln 1	13.12 µL	1.45 mL
■ Axiom Ligase Soln 2	3.15 µL	348 µL
Sub-Total	82.42 µL	9.10 mL

3. Mix well by vortexing the tube for 3 seconds.
4. Place the tube marked *Lig* back on ice.

Ligation Master Mix: Stage 2

To Finish Preparing the Ligation Master Mix:

1. Remove the Axiom Ligation Enzyme from the –20 °C freezer and place in a cooler chilled to –20 °C.
2. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Lig* in the order shown in [Table 4.31](#).

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.

Table 4.31 Ligation Master Mix Preparation — **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 Ligase Enzyme	1.58 µL	174.4 µL
Total	105 µL	11.59 mL

3. Gently invert 10 times to mix (do not vortex).
4. Place on ice and protect from direct light (e.g., cover with aluminum foil or ice bucket lid).

3: Aliquot Master Mixes and Axiom Hold Buffer into Trays

Label the Trays

1. Gather the scan tray and the stain trays and covers from the Axiom® GeneTitan® Consumables Kit.
2. Label two stain trays *S1*
3. Label the remaining stain trays:
 - *S2*
 - *Stbl*
 - *Lig*

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan MC Instrument, you will need to mark the front of each tray in a way that identifies its contents.

! **IMPORTANT:** It is critical that you write only on the proper side of the front edge of stain trays, as illustrated in [Figure 3.3 on page 31](#). The front edge of the tray is the short side with the lettering A through H. Do **NOT** write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and lids.

! **IMPORTANT:** Do not confuse hyb trays with stain trays.

Deionize Trays and Covers

Deionize the inside of each tray and cover now. Return the trays and covers to the bench top after deionizing

See [Appendix E, *Deionization Procedure for GeneTitan Trays and Covers* on page 130](#) for the recommended technique.

About Aliquotting 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 MC Instrument processing.

For all trays, pipette into trays on the bench top. If the trays are not being used immediately, protect them from light by covering with foil or placing in a cabinet.

! **IMPORTANT:** Remember to deionize the stain trays and the covers before aliquotting master-mixes

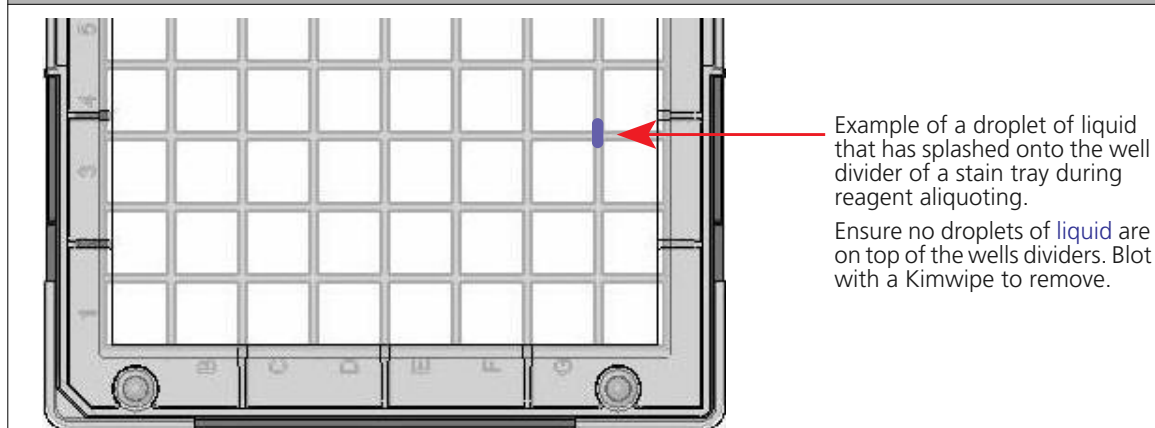
When aliquoting ligation, staining, and stabilization reagents to the trays, it is not necessary to spread the reagent to each corner of the well. The reagent will spread evenly when the array plate is inserted into the reagent tray during processing with the GeneTitan MC Instrument.

Stain 1 Master Mix

To Aliquot the Stain 1 Master Mix:

1. Pour the S1 Master Mix into the solution basin marked *S1*, placed on the bench top at room temperature.
2. Using a P200 12-channel pipette with new pipette tips, aliquot **105 μ L per well** to both *S1* trays — dispense to the first stop only to avoid creating bubbles.
You do not need to change pipette tips between additions of the Stain 1 Master Mix.
3. 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. (Figure 4.1).

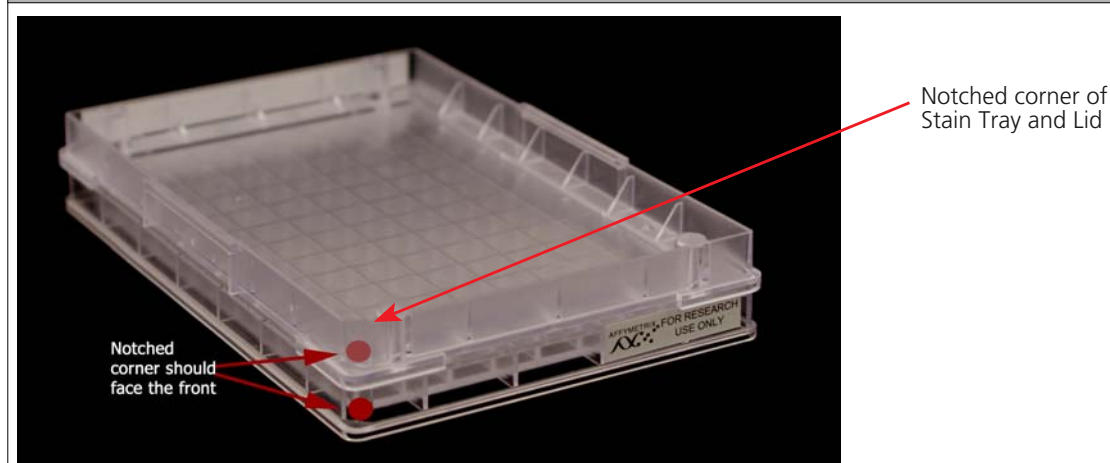
Figure 4.1 Well Dividers in Trays.



4. Place covers on the *S1* trays. Orient cover correctly on the tray with the notched corners together (Figure 4.2).

! IMPORTANT: Leaving liquid on the top of the dividers may cause excessive evaporation or may form a seal that will restrict the removal of the GeneTitan tray cover.

Figure 4.2 Placing Cover on Stain Tray



5. Protect the trays from light if not immediately loading onto the GeneTitan MC Instrument.

Stain 2 Master Mix

To Aliquot the Stain 2 Master Mix:

1. Pour the Stain 2 Master Mix into the solution basin marked *S2*, placed on the bench top at room temperature.
2. Using a P200 12-channel pipette with new pipette tips, aliquot **105 µL per well** to the *S2* tray — dispense to the first stop.
You do not need to change pipette tips between additions of the Stain 2 Master Mix.
3. 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.
4. Place a cover on the *S2* tray. Orient the cover correctly on the tray with the notched corners together (Figure 4.2).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Stabilization Master Mix

To Aliquot the Stabilization Master Mix:

1. Pour the Stabilization Master Mix into the solution basin marked *Stbl*, placed on the bench top at room temperature.
2. Using a 12-channel P200 pipette with new pipette tips, aliquot **105 µL per well** to the *Stbl* tray — dispense to the first stop.
You do not need to change pipette tips between additions of the Stabilization Master Mix.
3. If:
 - Bubbles are present, puncture them with a pipette tip.
 - Droplets of liquid splashed onto the well dividers, blot the top of the tray with a Kimwipe.
4. Place a cover on the tray. Orient cover correctly on the tray with the notched corners together (Figure 4.2).

Ligation Master Mix

To Aliquot the Ligation Master Mix:

1. Pour the Ligation Master Mix into the solution basin marked *Lig*, placed on the bench top at room temperature.
2. Using a 12-channel P200 pipette with new pipette tips, aliquot **105 µL per well** to the *Lig* tray — dispense to the first stop.
You do not need to change pipette tips between additions of the Ligation Master Mix.
3. 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.
4. Place a cover on the tray. Orient cover correctly on the tray with the notched corners together (Figure 4.2).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Axiom Hold Buffer

To Aliquot the Axiom Hold Buffer to the Scan Tray:

1. Ensure that the Axiom Hold Buffer has equilibrated to room temperature. Vortex and then 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 scan tray cover, but leave the scan tray on its protective black base.
4. Prepare the barcoded scan tray cover (P/N 202757) that came with the scan tray by completing the deionization procedure described in [Appendix E, Deionization Procedure for GeneTitan Trays and Covers on page 130](#). Place the cover as shown in [Figure 4.4 on page 66](#) to prevent dust or static from accumulating on the bottom of the cover.
5. Use a 12-channel P200 pipette with new pipette tips to aliquot **150 µL to each well** of a scan tray — dispense to the first stop and avoid touching the bottom of the tray.
You do not need to change pipette tips between additions of the Hold buffer.
6. If droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove.
7. Cover the tray by orienting the notched corner of the scan tray cover over the notched edge of the tray and the flat side of the cover against the scan tray ([Figure 4.3](#)).



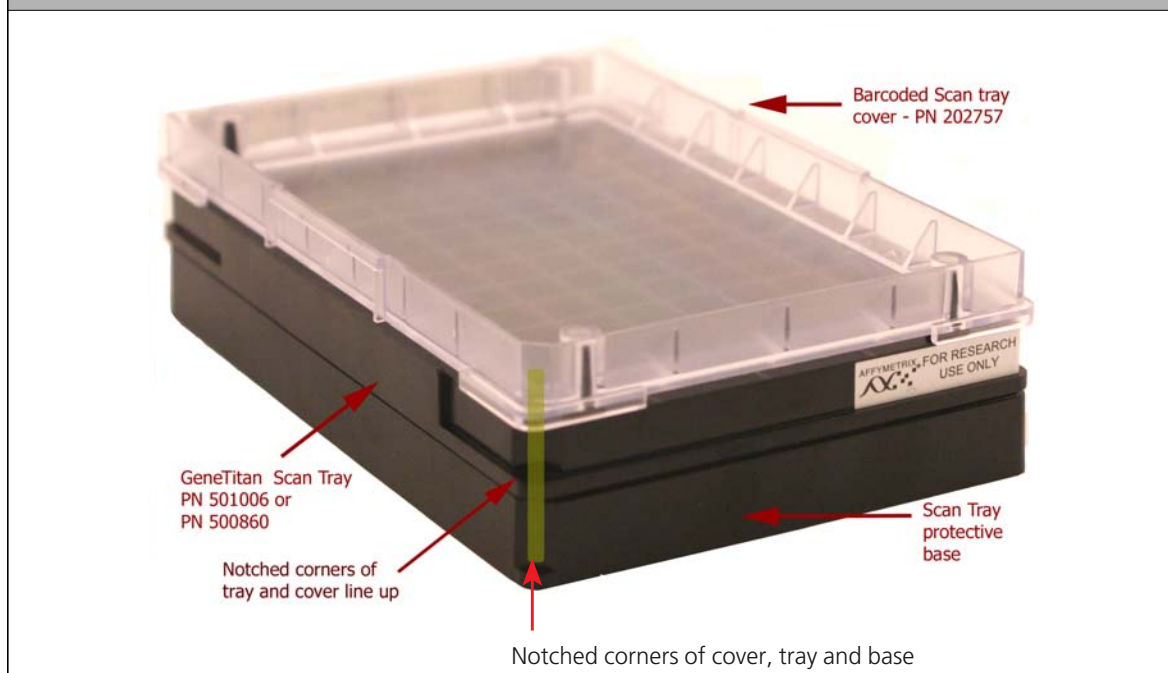
IMPORTANT: If your scan tray came with a cover that does not match the picture of the scan tray cover or if your scan tray cover does not have a barcode, then please contact your Field Application Specialist for a replacement scan tray cover. All tray covers must have a machine readable barcode.

IMPORTANT: The Hold buffer requires 150 µL per well

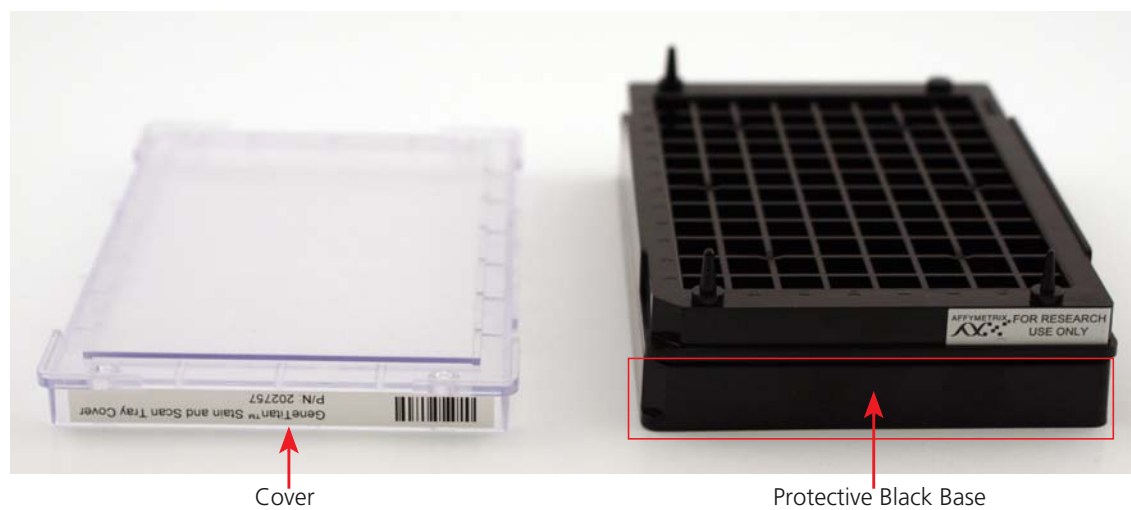


CAUTION: Do not remove the scan tray from its protective black base until loading onto the GeneTitan MC instrument. To avoid scratching, do not touch the bottom of the tray with pipette tips. Dispense hold buffer to the first stop only.

See [Stage 3 — Ligate, Wash, Stain and Scan on page 89](#) for instructions on loading the reagent trays.

Figure 4.3 Scan Tray with the Clear Cover and Protective Base**Figure 4.4** Loading the Scan Tray with Axiom Hold Buffer

Leave the scan tray in its protective black base while loading with Axiom Hold Buffer.



Array Processing with the GeneTitan® Multi-Channel Instrument

The Axiom® 2.0 Assay is designed for processing 96 samples at a time on Axiom® Genome-Wide and Custom myDesign™ Array Plates. The protocol is performed in two sets of steps:

- Target Preparation, performed on the lab bench without advanced automation. See [Chapter 4, Axiom® 2.0 Assay: Manual Target Preparation on page 32](#)
- Array processing, performed on the GeneTitan Multi-Channel (MC) Instrument

This chapter includes instructions for Part 2: Array Processing. These instructions are presented as follows:

- [Before Using the GeneTitan MC Instrument on page 67](#)
- [Stage 1 — Create and Upload Batch Registration File on page 75](#)
- [Stage 2 — Hybridization on page 76](#)
- [Stage 3 — Ligate, Wash, Stain and Scan on page 89](#)

Before Using the GeneTitan MC Instrument

Proper Tray Alignment and Loading

Proper alignment and loading of plates, covers and trays is critical when using the GeneTitan MC Instrument. Each plate, cover and tray has one notched corner. The notched corner of plates, trays, covers and bases must be in vertical alignment with each other, and placed in position A1 per the Tray Alignment guide inside each GeneTitan MC drawer ([Figure 5.1 on page 68](#) and [Figure 5.2 on page 69](#)).



IMPORTANT: When running a multi-plate workflow, you must pay careful attention to the software prompts that tell you which side of the drawer to place or remove a plate/tray.



TIP: Mark the notched corner of each plate, cover and tray with permanent marker to help ensure proper alignment and loading onto the GeneTitan MC Instrument.



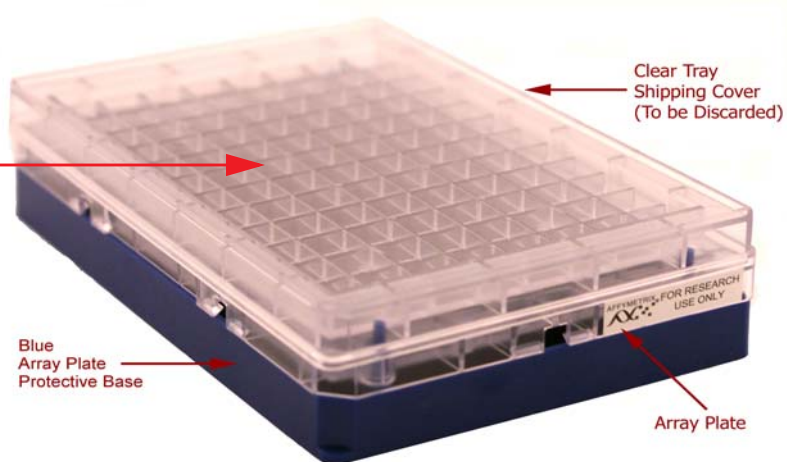
CAUTION: Take care not to damage the consumables or bend the blue cover posts or scan tray posts.



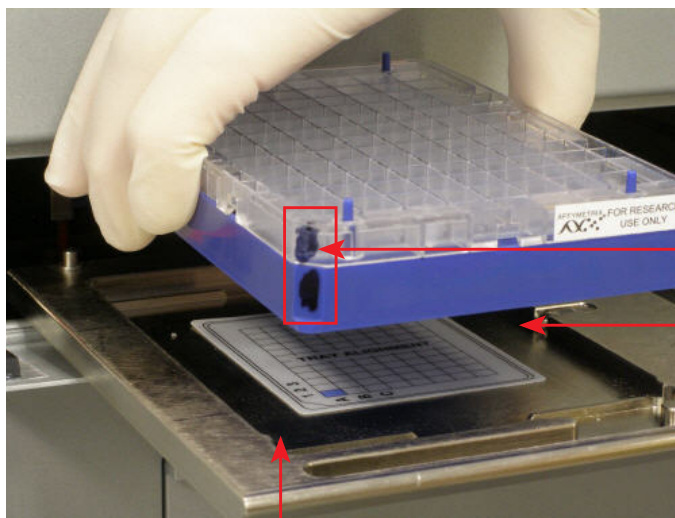
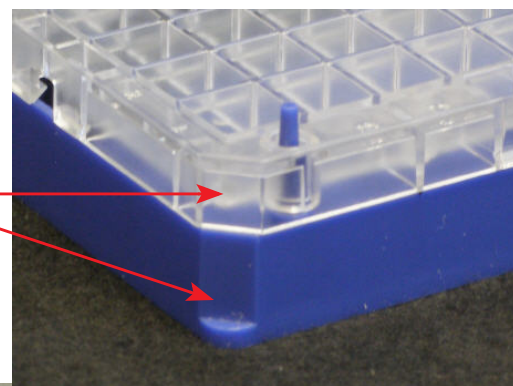
NOTE: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. Refer to [Appendix F, GeneTitan® Multi-Channel Instrument Care on page 133](#) for the message displayed to the user and the procedure for replacing the filters.

Figure 5.1 Proper Alignment and Loading of Plates, Covers and Trays in the GeneTitan MC Instrument

IMPORTANT: Remove the plastic protective shipping tray cover.



Notched corner of array plate aligned with notched corner of blue base.



The notched corner of all plates, bases, and covers and must be seated in this corner of the drawer per the Tray Alignment guide.

Tip

Mark the notched corner of each plate, cover and tray with permanent marker to help ensure proper alignment and loading.

Plates and trays must be seated in this groove.

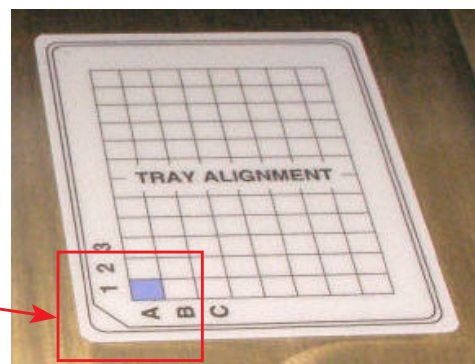
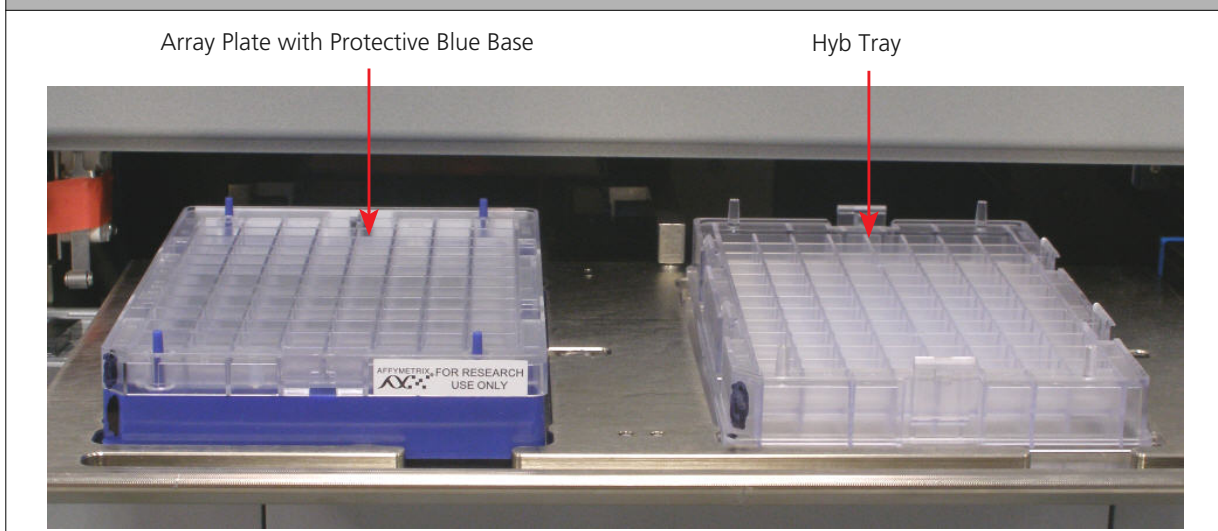


Figure 5.2 Array Plate with Protective Blue Base and the Hyb Tray Aligned and Properly Loaded into Drawer 6

! **IMPORTANT:** When you install the consumables, ensure that the fingers are retracted. Do not lay the consumables on top of the drawer fingers - this indicates that the instrument is not functioning correctly. Please notify your Field Service Engineer if the fingers do not retract automatically. You should place the trays into the instrument drawers when a drawer is fully extended by the instrument. The fingers are retracted when the drawer is open and are extended when the drawer is closed in order to restrain the consumable.

Stain Trays and Covers

! **IMPORTANT:** Always place the *flat* side of the cover against the stain tray.

Figure 5.3 Placement of Covers on Trays

Correct placement of cover on stain tray.



Incorrect placement of cover on stain tray.



Labeling GeneTitan Hybridization and Reagent Trays

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan MC Instrument, you will need to mark each tray in a way that identifies its contents.

! IMPORTANT: It is critical that you write only on the proper locations of the proper sides of hyb and stain trays. Do **NOT** write in any other location, as this can interfere with sensors inside the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and lids.

Proper labeling for hyb trays and reagent trays is described in:

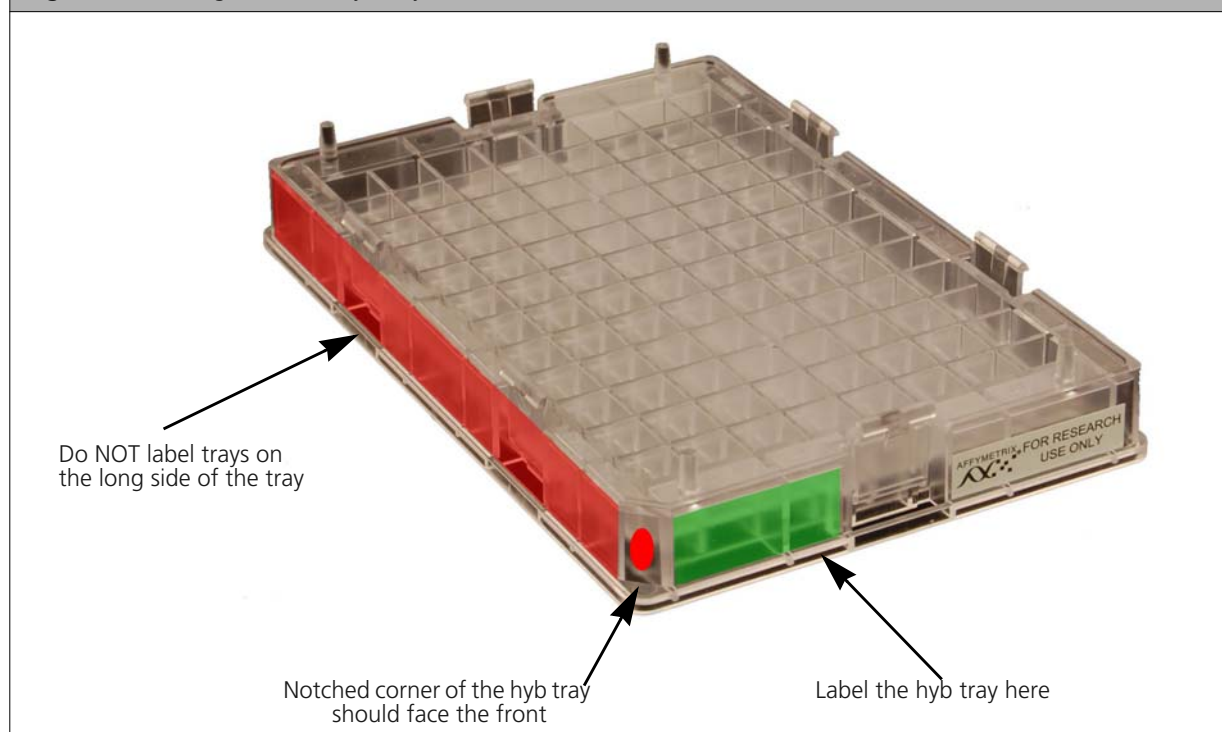
- *Labeling for Hyb Trays*, below
- *Labeling for Stain Trays* on page 71

! IMPORTANT: Do not confuse hyb trays with stain trays.

Labeling for Hyb Trays

You may label the hyb tray on the front part of the **short side of the tray, next to the notch at the left**, as shown in [Figure 5.4](#). The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.

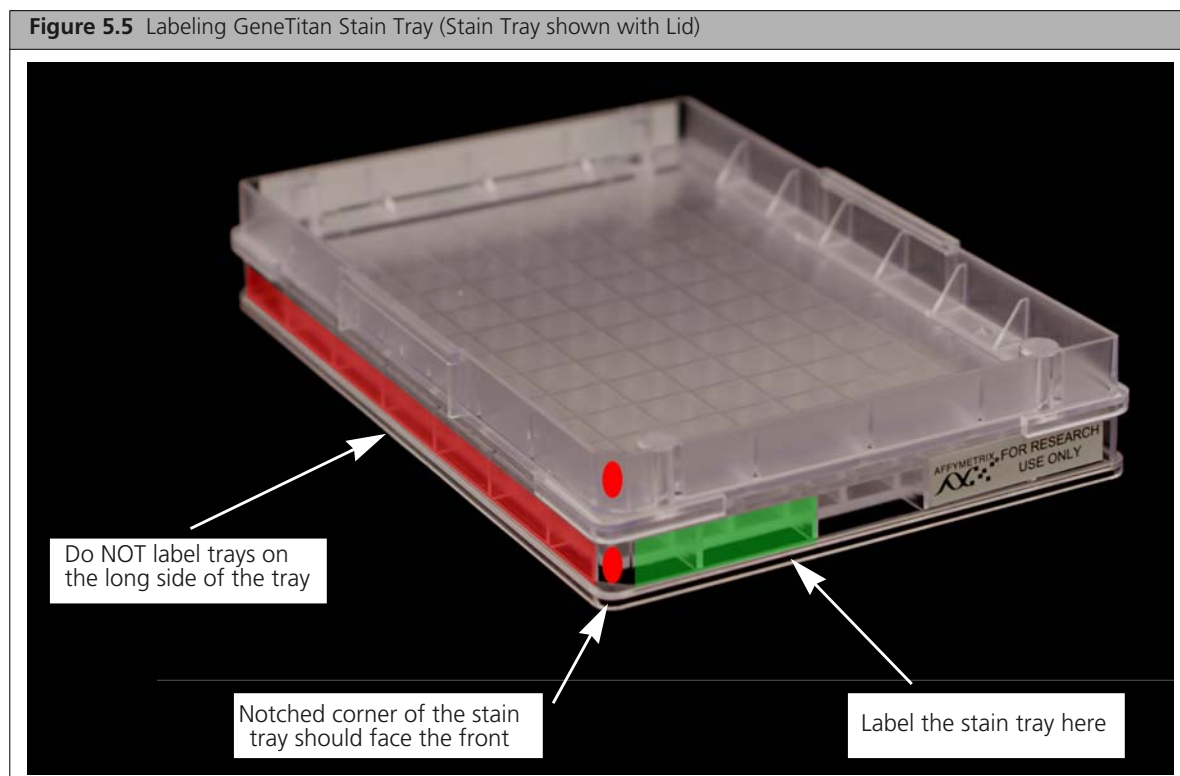
Figure 5.4 Labeling GeneTitan Hyb Trays



CAUTION: Writing on the wrong side of the hyb tray, or on the wrong part of the long side, may interfere with the operation of sensors in the GeneTitan MC Instrument.

Labeling for Stain Trays

You may label the stain trays on the **left side of the front of the tray** as shown in Figure 5.5. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.



E-mail and Telephone Notifications from the GeneTitan MC Instrument

We strongly recommend that you configure the Affymetrix GeneChip® Command Console (AGCC) software to send you GeneTitan MC notifications. It is critical that you know when the instrument requires your attention — either for sample handling or troubleshooting. Rapid notification can lessen the risk of sample loss.

Notifications can be sent to e-mail addresses and telephones. Refer to the AGCC user manual for instructions.

The types of notifications available will let you know when a process:

- Starts
- Completes
- Aborts
- Encounters an error

GeneTitan MC Instrument Lamp

The GeneTitan MC Instrument uses a xenon arc lamp system that is warranted for 500 hours to provide illumination for imaging the array at two wavelengths. The xenon lamp has a limited lifetime and needs to be replaced at regular intervals.

The GeneTitan Instrument Control software provides a timer that indicates the remaining useful life of the bulb and notifies you when it requires replacement. It is important to adhere to the warnings specified in the GeneTitan MC Instrument user guide.

Refer to the *GeneTitan MC Instrument User Guide*, P/N 08-0308, or [Appendix F, GeneTitan® Multi-Channel Instrument Care](#) on page 133 of this user guide for details on replacing the lamp.

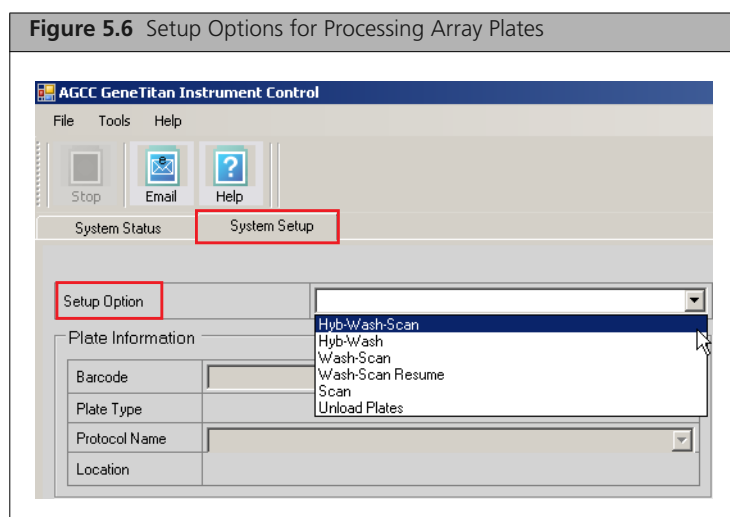
Refer to the *GeneTitan MC Instrument User Guide*, P/N 08-0308, for the Lambda LS and Smart controller system. The Lamp and the controller should NEVER be switched ON or OFF manually. The GeneTitan MC Instrument control software manages the lamp activity and will switch the lamp ON and OFF as required. It takes 10 minutes to warm-up the lamp. In idle mode the lamp will remain ON for 2 hours before it is automatically switched OFF and if there are no more plates being transferred from the fluidics to the imaging station. This is by design and intended behavior. Please do not try to save the lamp life by turning OFF the switch on the lamp.



NOTE: The power switch on the shutter box should be ON at all times. The OPEN/CLOSE switch on the shutter box should be at AUTO position at all times.

Setup Options for Array Plate Processing

The processes (setup options) available for processing array plates are shown in [Figure 5.6](#). A brief description of each option is given below.



Hyb-Wash-Scan

This setup option enables you to hybridize, wash-ligate-stain-fix, and scan an array plate on the GeneTitan MC Instrument.



IMPORTANT: When running a multi-plate workflow, you must pay careful attention to the software prompts that tell you which side of the drawer to place or remove a plate/tray.

- **Hyb:** the array plate is moved to the hybridization oven inside the instrument. Each denatured sample in the hyb tray is hybridized to an array on the array plate.
 - Duration for 96 samples = 23.5 hr
- **Wash:** samples on arrays are ligated, washed, stained and fixed.
 - Duration for 96 samples = ~ 5 hr



NOTE: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. Refer to [Appendix F, GeneTitan® Multi-Channel Instrument Care](#) on page 133 for the message displayed to the user and the procedure for replacing the filters

- **Scan:** The array plate is moved to the imaging device in the GeneTitan MC Instrument and each array is scanned.
 - Duration for 96 samples = ~ 7.5 hr

Hyb-Wash

If this setup option is selected, array plate processing will stop after the array has gone through fluidics processing. Use this option if an array plate cannot be scanned on the same GeneTitan MC Instrument as the one used for hybridization and fluidics processing.

If the Array Plate Cannot Be Scanned Immediately After the Hyb-Wash Process is Complete:

1. Wrap the array plate (in the scan tray with black protective base) in aluminum foil to protect from light.

No lid is required. Do not invert the plate stack. If inverted, the Hold Buffer will spill out of the tray. To prevent liquid spillage, try to keep the plate level when handling the plates. Do not touch the bottom optical surface of the scan tray.
2. Store at 4 °C.
3. Scan the array plate within 3 days or less.

When Ready to Scan the Array Plate:

1. Keeping the plate protected from light, bring the plate to room temperature for ~ 20 min.
2. Remove the aluminum foil and load onto the GeneTitan MC Instrument.

Wash-Scan

Use this option if:

- You wish to bypass the Hybridization step and perform only the Wash/Stain and Scan steps.

Wash-Scan-Resume

Use this option if:

- It was necessary to hybridize the array plate in an oven separate from the GeneTitan MC Instrument.
- Fluidics processing has been interrupted (*e.g.*, a power failure occurs at your facility).

Scan

Use this option:

- To rescan an entire array plate or specific arrays on a plate that failed to scan for reasons such as bubbles or gridding failure.
- If you have hybridized and performed the fluidics processes off the GeneTitan MC Instrument.

Unload Plates

Use this option to unload plates and trays from the instrument when:

- Array plate processing is complete.
- Array plate processing has been aborted.

Aborting a Process

If necessary, you can abort the processing of one or more array plates. Instructions and an example are shown below in [Figure 5.7](#).

If the instrument aborts a process, you can retrieve the array plate and related consumables as described in [Figure 5.7](#). An instrument-initiated abort may occur:

- Due to improper placement of plates
- If the UPS detects a long power interruption, draining the UPS to 75% power.

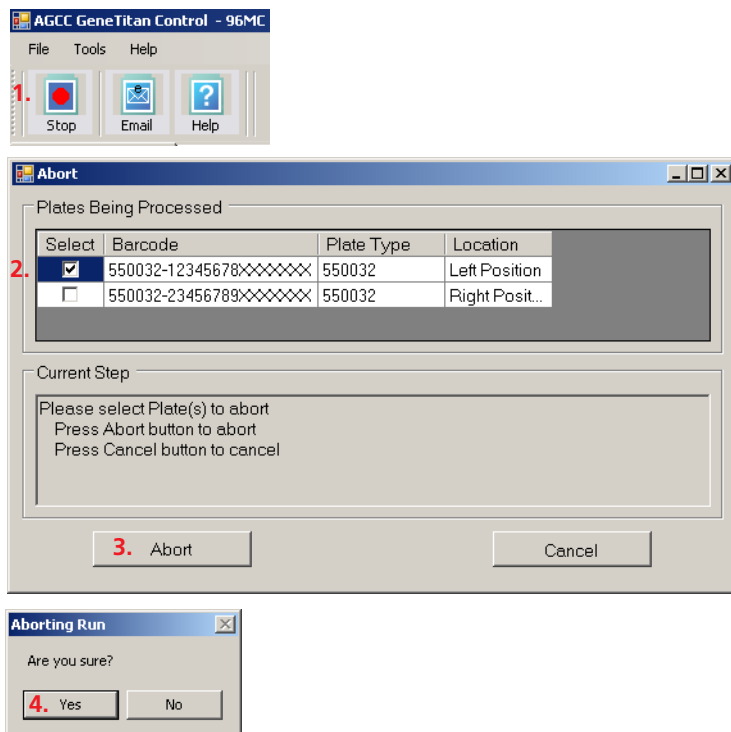
Figure 5.7 Manually Aborting an Array Plate

To abort array plate processing:

1. Click the **Stop** button.
2. Select the array plate that you want to abort.
3. Click **Abort**.
4. Click **Yes**.
5. Wait until the status of the array plate in the WorkFlow window changes from *AbortRequest...* to *Aborted* (5A and 5B).
6. Once aborted, retrieve the array plate and other related consumables by:
 - Using Setup Option: *Unload Plates*
 - Loading a new array plate.

Exception: If reagents are loading, abort the plate using the Cancel button displayed in the reagent load step.

Note: If the gripper is required to complete the Abort process, the plate will remain in the "AbortRequest" state until the gripper becomes available.



5A.					
Work Flow					
Barcode	Plate Type	Location	Hyb. Status	Fluidics Status	Scan Status
550032-12345678XXXXXX	550032	Left Position	AbortRequest...	Waiting	Waiting

5B.					
Work Flow					
Barcode	Plate Type	Location	Hyb. Status	Fluidics Status	Scan Status
550032-12345678XXXXXX	550032	Left Position	Aborted	Waiting	Waiting

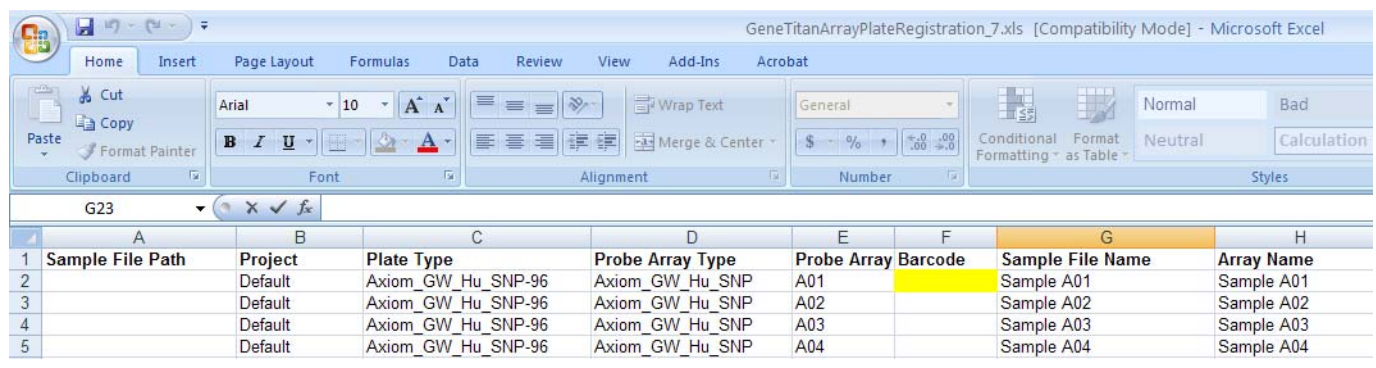
Stage 1 — Create and Upload Batch Registration File

In the AGCC software, you must create and upload a Batch Registration file before you begin [Stage 2 — Hybridization on page 76](#) (example shown in [Figure 5.8](#)). This file contains information critical for:

- Data file generation during scanning
 - Tracking the experimental results for each sample loaded onto an array plate
1. If you have not already created a batch registration file, create one now. (See [Appendix D, Registering Samples in Affymetrix GeneChip® Command Console®](#) on page 127 for detailed instructions.)
 2. In AGCC, select the array plate format (96 samples) and open a batch registration file template.
 3. Scan the array plate barcode into the yellow barcode field.
 4. Enter a unique name for each sample and any additional information.
 5. Save the file.
 6. Upload the file.

! IMPORTANT: It is very important to create and upload a batch registration file with your sample information prior to starting [Stage 2 — Hybridization on page 76](#).

Figure 5.8 Example of a Batch Registration File for an Array Plate



	A	B	C	D	E	F	G	H
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array	Barcode	Sample File Name	Array Name
2		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A01		Sample A01	Sample A01
3		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A02		Sample A02	Sample A02
4		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A03		Sample A03	Sample A03
5		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A04		Sample A04	Sample A04

Stage 2 — Hybridization

Reagents Required

Reagents Required

Table 5.1 Reagents Required from the Axiom 2.0 Reagent Kit

Reagent	Module
Axiom Wash Buffer A (both bottles; 1L)	Module 3, Room Temperature P/N 901472
Axiom Wash Buffer B	
Axiom Water	

- An Axiom Genome-Wide human or non-human array plate or an Axiom myDesign™ Genotyping 96-array plate is required for this step. Prior to inserting this plate into the GeneTitan MC Instrument for hybridization, the array plate should be brought to room temperature as described on [Step 2 on page 52](#).
- A hybridization tray containing denatured samples (from [Step 8 on page 53](#) in [Chapter 4](#)) is also required for this step. The denatured samples should be transferred to the hyb tray only after the GeneTitan MC Instrument is ready for loading the hyb tray in the [Load Trays onto the GeneTitan MC Instrument on page 91](#).

Setup the Instrument

To Setup the Instrument:

1. Launch AGCC Launcher and select **AGCC GeneTitan Control** ([Figure 5.9](#)).

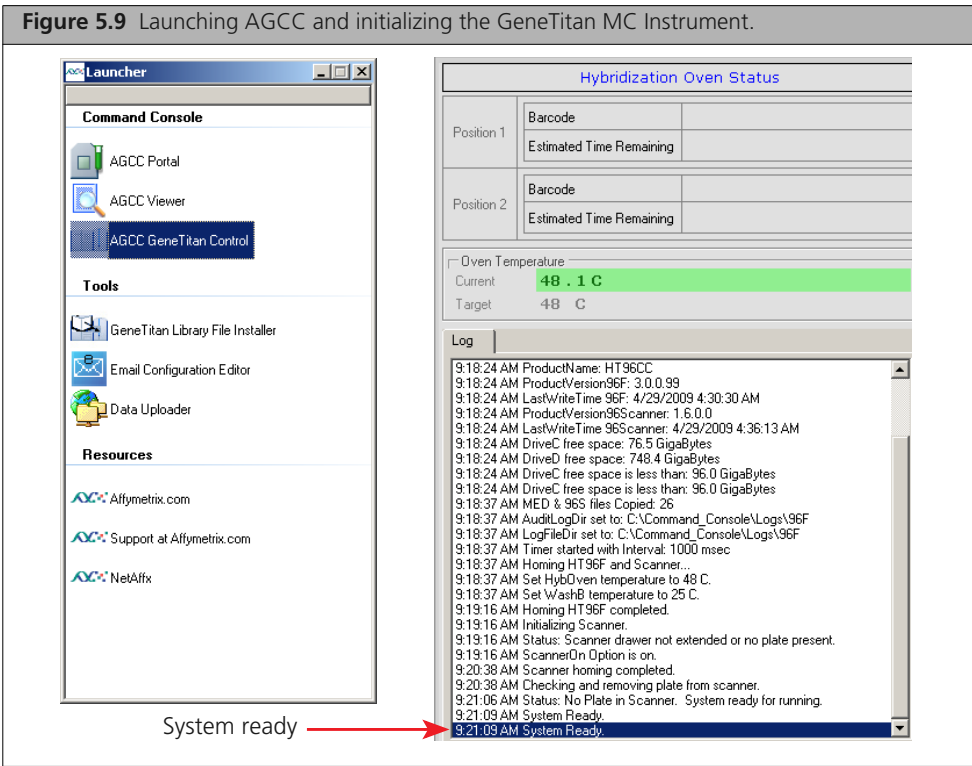
The system initializes. After initialization, the System Status tab is selected and the status of the Hybridization Oven is displayed at the bottom of the Log window. The status should read:
<Time of day> System Ready

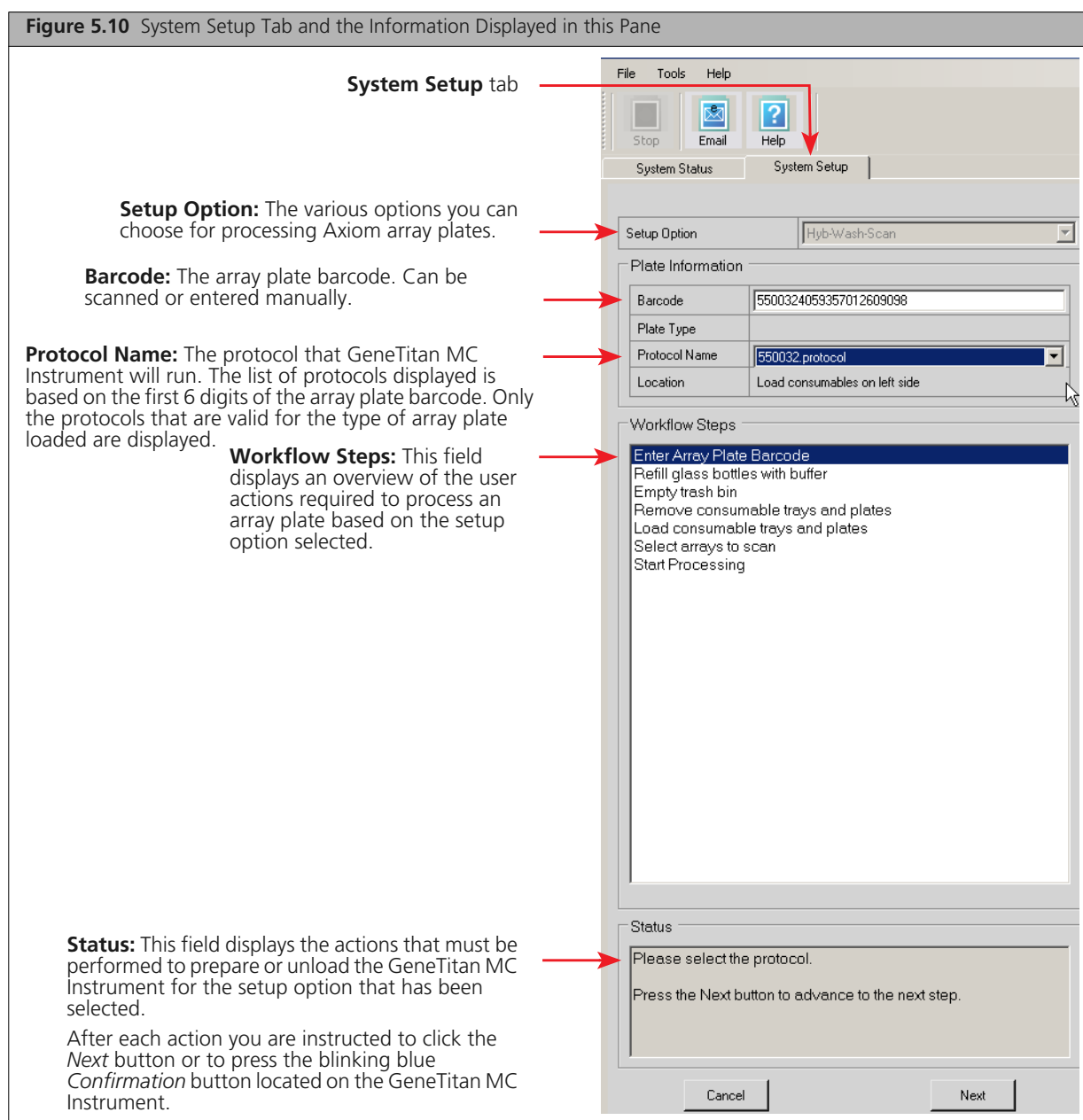


NOTE: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. Refer to [Appendix F, GeneTitan® Multi-Channel Instrument Care on page 133](#) for the message displayed to the user and the procedure for replacing the filters



IMPORTANT: Please do not close the scanner application by right-clicking on it and choosing the “Close” option. This will cause the scanner application to exit abnormally and cause undue delay in processing the next plate. The correct way to close the application is described in [Shutting Down the GeneTitan® MC Instrument on page 99](#).





2. Select the **System Setup** tab (Figure 5.10).
3. Configure the software as follows:
 - A. Setup Option: **Hyb-Wash-Scan**
Other options available are described under *Setup Options for Array Plate Processing* on page 72.
 - B. Click **Next**.



NOTE: If there is not enough disk space, a message is displayed.

Delete or move .dat files to another location to free up enough disk space for the data that will be generated by eight Axiom array plates.

- 96 Axiom array plate requires ~ 80 GB

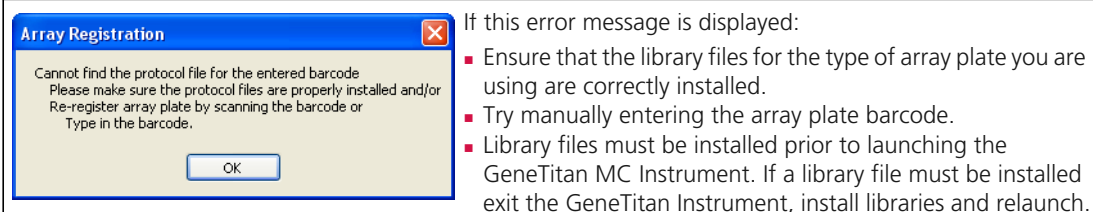
- C. Plate Information:

- **Barcode:** Scan or manually enter the Axiom array plate barcode and click **Next**.

The first six characters of the barcode identify the type of plate being loaded, the protocol GeneTitan MC Instrument will use to process the plate, and the imaging device parameters required for this type of plate.

550094 <barcode> = Affymetrix 96-array plate

Figure 5.11 Barcode Error Message



- **Protocol Name:** Select the protocol name and click **Next**.

The system reads the first 6 digits of the array plate barcode to determine which protocols can be run for the type of array plate that has been loaded. Only valid protocols are displayed.

550094.protocol = for Affymetrix 96-array plate barcodes

4. Complete the remaining workflow steps as follows:

A. Refill bottles with buffer (Figure 5.12 on page 80)

1) Fill these bottles:

- Wash A: fill with Axiom Wash Buffer A — keep at 2 L full
- Wash B: fill with Axiom Wash Buffer B — Use all 600 mL of Wash B 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 1 L full



IMPORTANT:

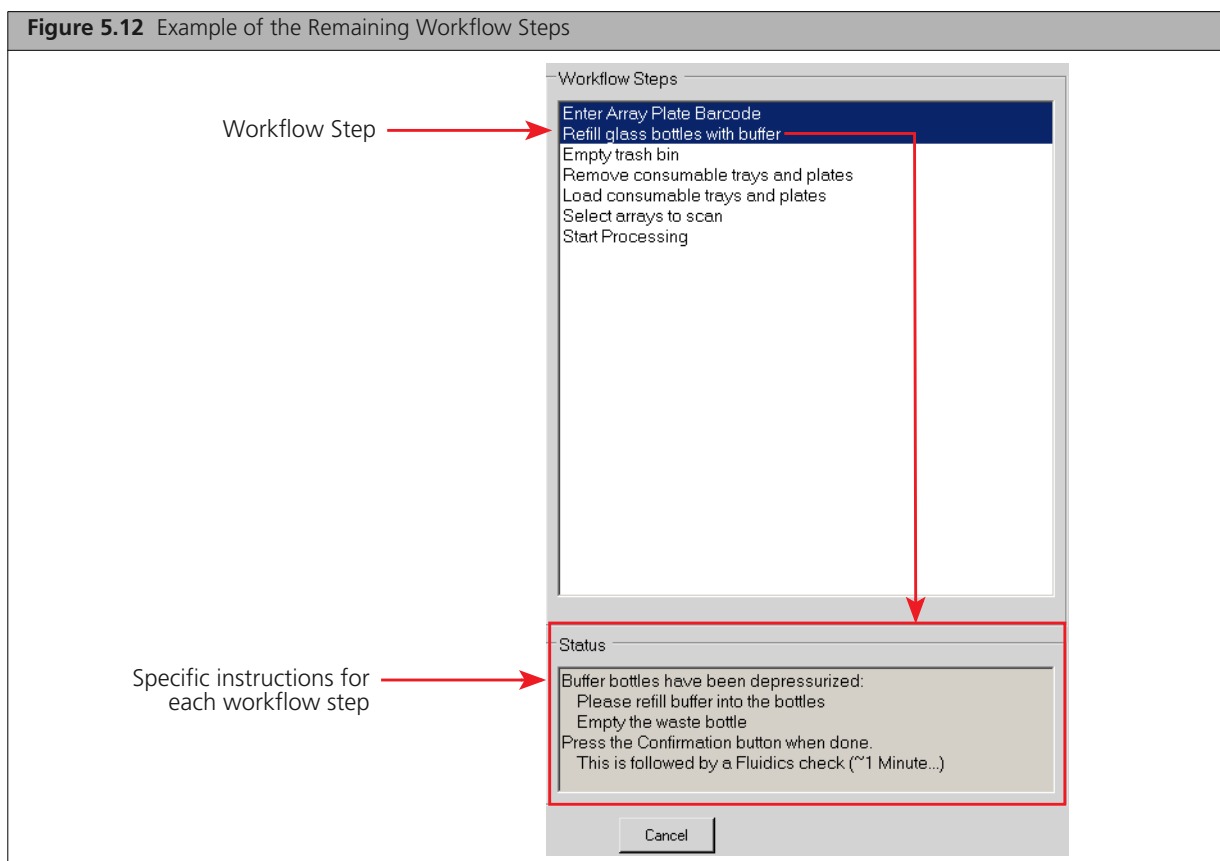
- Always ensure that the GeneTitan bottles containing Wash A and Rinse are above the 50% mark when setting up the system to process an Axiom HT array plate. All 600 mL of the Wash buffer B from the Axiom reagent kit should be emptied into the GeneTitan Wash B bottle when setting up the system to process a plate. This ensures that the GeneTitan Wash B bottle is filled to more than the requisite 35% of Wash B bottle volume. Also, do not overfill the bottles. Fill Wash Buffer B and Water bottles to the 1 L mark only. Wash A keep at 2 L. We strongly recommend refilling these bottles every time you are prompted to do so.

If the volume in any of these bottles becomes too low during a run, a message is displayed (see [Chapter 7, Troubleshooting on page 108](#)). However, even if you fill the bottle at this time, the instrument may not be able to successfully complete the step that was in progress.

- Wash B — if you intend to load two array plates on the same day, fill the Wash B bottle to the 1L mark (use both bottles from the Axiom 2.0 Reagent Kit).

2) Empty the waste bottle.

3) Press the Confirmation button on the GeneTitan MC Instrument to continue. A fluidics check is run (~ 1 min).



B. Empty trash bin

- 1) Open the trash bin and empty.

If already empty, the trash bin remains locked and the Status pane reads “Trash bin is empty.”

- 2) Press the **Confirmation** button to continue.

C. Remove consumable trays and plates

- 1) Remove used trays and plates when drawers open.

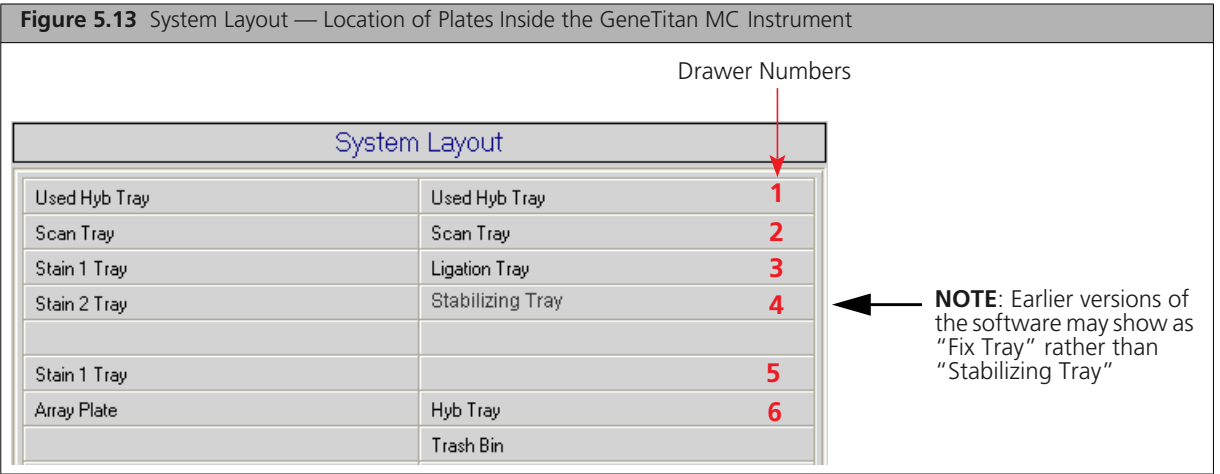
If no consumables to remove, the Status window reads “Drawers are empty.”

- 2) Press the **Confirmation** button to continue.

- D. Continue to [Load an Axiom Array Plate and Hyb Tray Onto the GeneTitan® MC Instrument](#) on page 81.

Load an Axiom Array Plate and Hyb Tray Onto the GeneTitan® MC Instrument

The System Layout pane indicates the position of the various trays in each drawer during a GeneTitan MC Instrument run at maximum throughput. This pane does not change as plates are loaded or removed.



To Load an Axiom Array Plate and Hyb Tray onto GeneTitan MC Instrument:

1. When drawer 6 opens, load the array plate and hyb tray as follows:
 - A. Examine the wells of the hyb tray for bubbles; puncture any bubbles with a pipette tip.

! IMPORTANT: Removing bubbles at this step greatly reduces the chance of bubbles under the arrays when the hyb tray and the Axiom array plates are clamped. Bubbles under an array can result in black spots on the array image.

- B. Load the hyb tray **without the cover** on the right side of the drawer (Figure 5.15 on page 82).

The array plate must be loaded on its protective blue base, as shown in Figure 5.15 on page 82 below. The clear plastic cover on top of the array plate **SHOULD NOT** be loaded in the GeneTitan MC Instrument. See Figure 5.1 on page 68 for more details on the correct way of loading the array plate.

- C. Remove the array plate and protective blue base from its package.

To avoid dust or other damage, leave the array plate packaged until ready to load onto the GeneTitan MC Instrument (Figure 5.14).

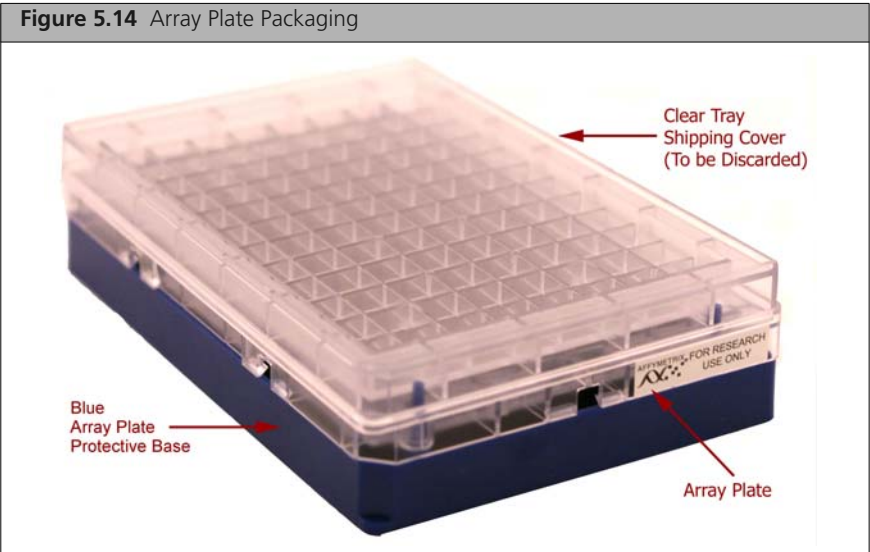
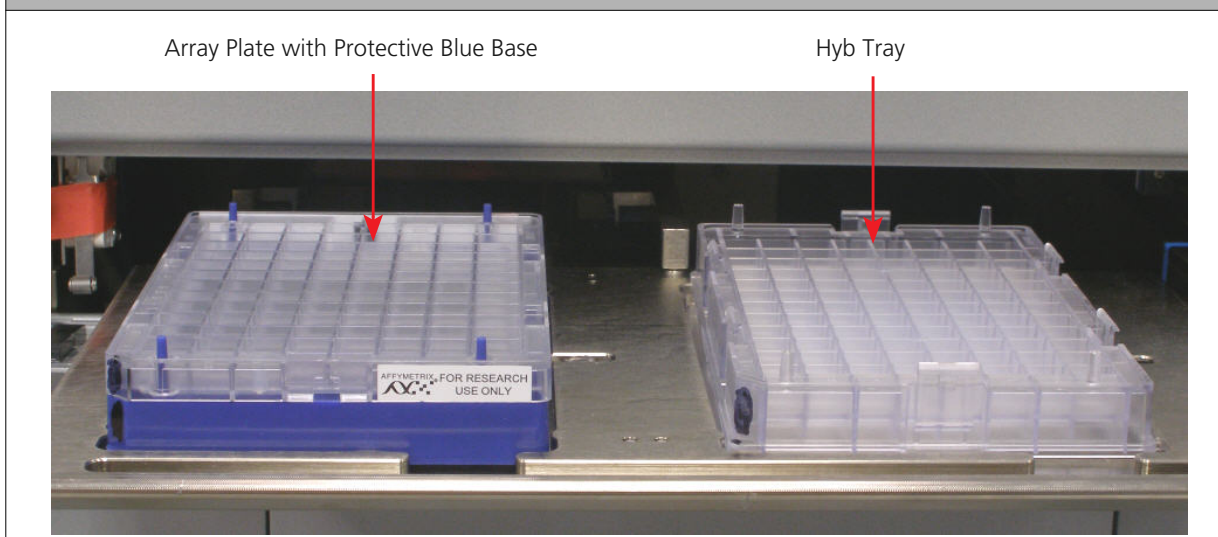


Figure 5.15 Array Plate with Protective Blue Base and the Hyb Tray Properly Loaded into Drawer 6.

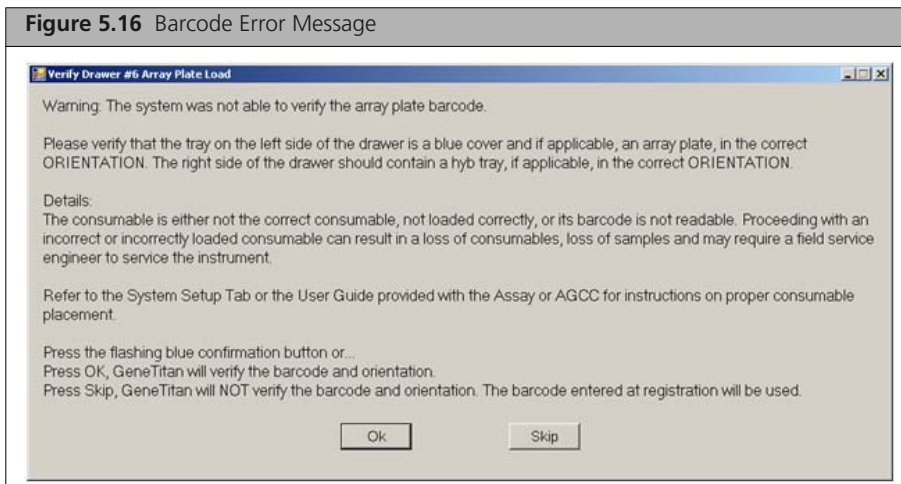
D. Load the array plate **with the protective blue base** on the left side of the drawer (Figure 5.15).



CAUTION: The notched corner of each plate, cover and tray must be aligned. When loading onto the GeneTitan MC Instrument, the notched edge plates, covers and trays must be aligned as indicated by the Tray Alignment guide in the drawer (Figure 5.15 on page 82).

The error message shown in may be displayed. Plate barcodes must face the internal barcode reader (back of the drawer). Improper tray positioning can cause the GeneTitan MC Instrument to crash, and can result in substantial damage to the instrument and loss of samples.

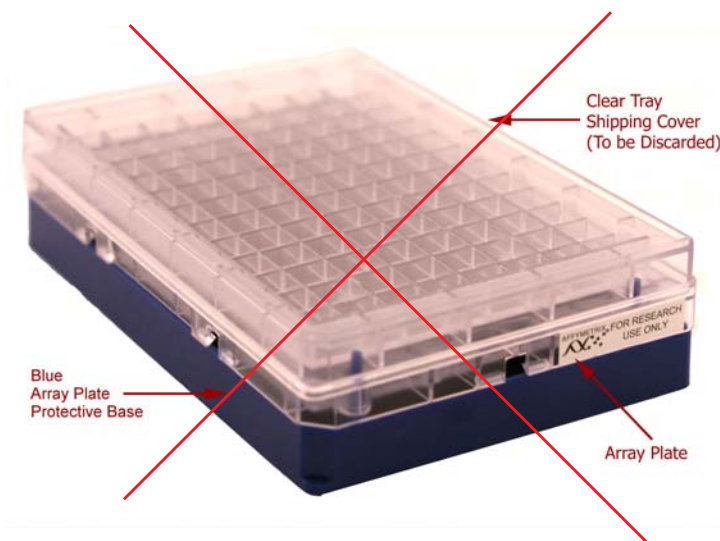
E. Press the **Confirmation** button.

Figure 5.16 Barcode Error Message

When you load the array plate left side of the drawer: The internal bar code reader reads the barcode of the array plate and compares it with the barcode and the plate type specified in the Barcode field and Plate Type field on the Setup page. If the information is correct, the application allows you to proceed to the next step. If the instrument is unable to read the barcode, it will push the tray out and will prompt (Figure 5.16) you to load the correct plate with the proper orientation into the instrument (Figure 5.15).

- Click OK to retry and check the loading of the array plate; or
- Click Skip if the instrument has problems reading the barcode and after verifying that the trays have been placed in the proper orientation.

! **IMPORTANT:** Do not install a 3 plate stack of trays. Confirm that you have removed the clear plastic shipping cover as shown in [Figure 5.1 on page 68](#).



- F. Select the arrays to scan (instructions in [Figure 5.17](#)).
By default, all arrays are selected.

Figure 5.17 Selecting Which Arrays to Scan an Array Plate

Status

Please select arrays to scan using array selector

Press the Next button to advance to the next step.

Message in Status Window.

Array Selection

☒ Manual Array Selection

	1	2	3	4	5	6	7	8	9	10	11	12
A	■	■	■	■	■	■	■	■	■	■	■	■
B	■	■	■	■	■	■	■	■	■	■	■	■
C	■	■	■	■	■	■	■	■	■	■	■	■
D	■	■	■	■	■	■	■	■	■	■	■	■
E	■	■	■	■	■	■	■	■	■	■	■	■
F	■	■	■	■	■	■	■	■	■	■	■	■
G	■	■	■	■	■	■	■	■	■	■	■	■
H	■	■	■	■	■	■	■	■	■	■	■	■

☒ Will Be Scanned
 ☐ Will Not Be Scanned
 ☐ Not Available

Default – all arrays are selected

Single array - click one box

Multiple arrays

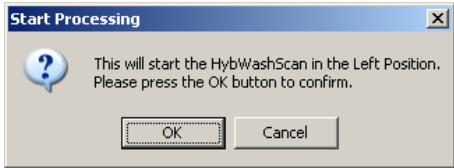
- Click one box
- Hold down the Ctrl key
- Click another box in the same column

Group of arrays:

- Click one box
- Hold down the Shift key
- Left click and drag the mouse

2. Click **Next**, then click **OK** to begin processing the samples ([Figure 5.18](#)).
The array plate is placed on top of the hyb tray and clamped (now referred to as the *plate stack*).

Figure 5.18 Click OK to Start Processing the First Array Plate and Hyb Tray



Click **OK** to confirm that you wish to proceed with hybridization. The plate stack is in the left position (the left side of the drawer).

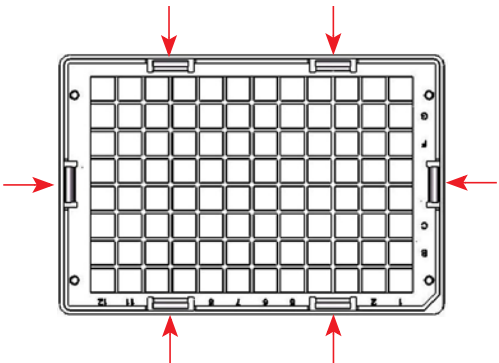
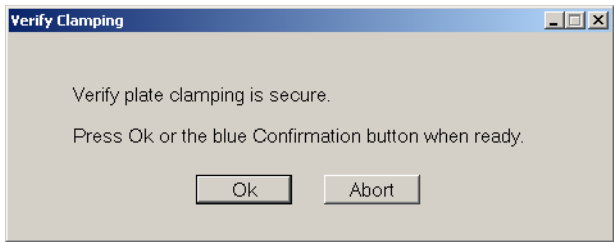
The software starts the process for clamping the array plate to the hybridization tray. Press **OK** on the dialog shown in [Figure 5.19](#) and wait for the drawer to open before retrieving the array plate and hybridization tray combo for inspection. The sandwich of the array plate and hybridization tray needs to be manually inspected before the array processing can begin. Once clamping is complete the dialog shown in [Figure 5.20](#) on page 84 will be displayed. If you do not press OK in [Figure 5.19](#) the dialog box will go away without intervention and [Figure 5.20](#) on page 84 will be displayed.

Figure 5.19

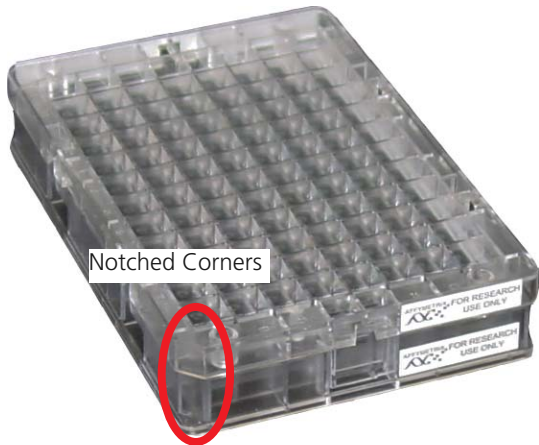


3. When drawer 6 opens and the prompt in [Figure 5.20](#) is displayed:

Figure 5.20 Location of Clamping Points on the Array Plate and Hyb Tray



Clamping Points on Axiom Array Plate and Hyb Tray



Array Plate

Hyb Tray

- A. Remove the plate stack and gently press the two plates together at each clamping point. Listen for a clicking sound which indicates that the plates are now clamped. No clicking sound indicates the plates are already clamped (See [Figure 5.21](#) for an example of a array plate hybridization tray sandwich).

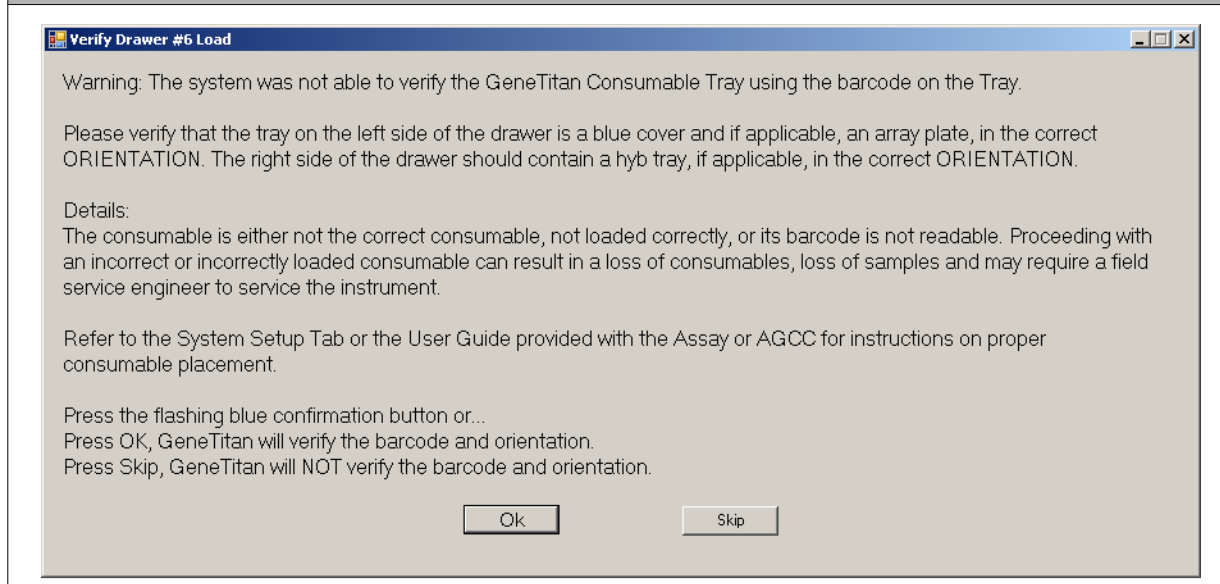
Figure 5.21 Array Plate Hyb Sandwich



- B. Inspect the bottom of the plate stack for bubbles under the arrays — **do NOT invert the plates.**
- C. If bubbles are present, gently tap the plate until the bubbles move out from under the arrays — **do NOT unclamp the plate stack.**
- D. Return the plate stack to the drawer, and press the **Confirmation** button to proceed.

The message in [Figure 5.22](#) may be displayed again if plate orientation is incorrect or if the hybridization tray barcode cannot be read. Click **OK** to proceed.

Figure 5.22 Verification Message



Load a Second Axiom Array Plate and Hyb Tray Onto the GeneTitan MC Instrument

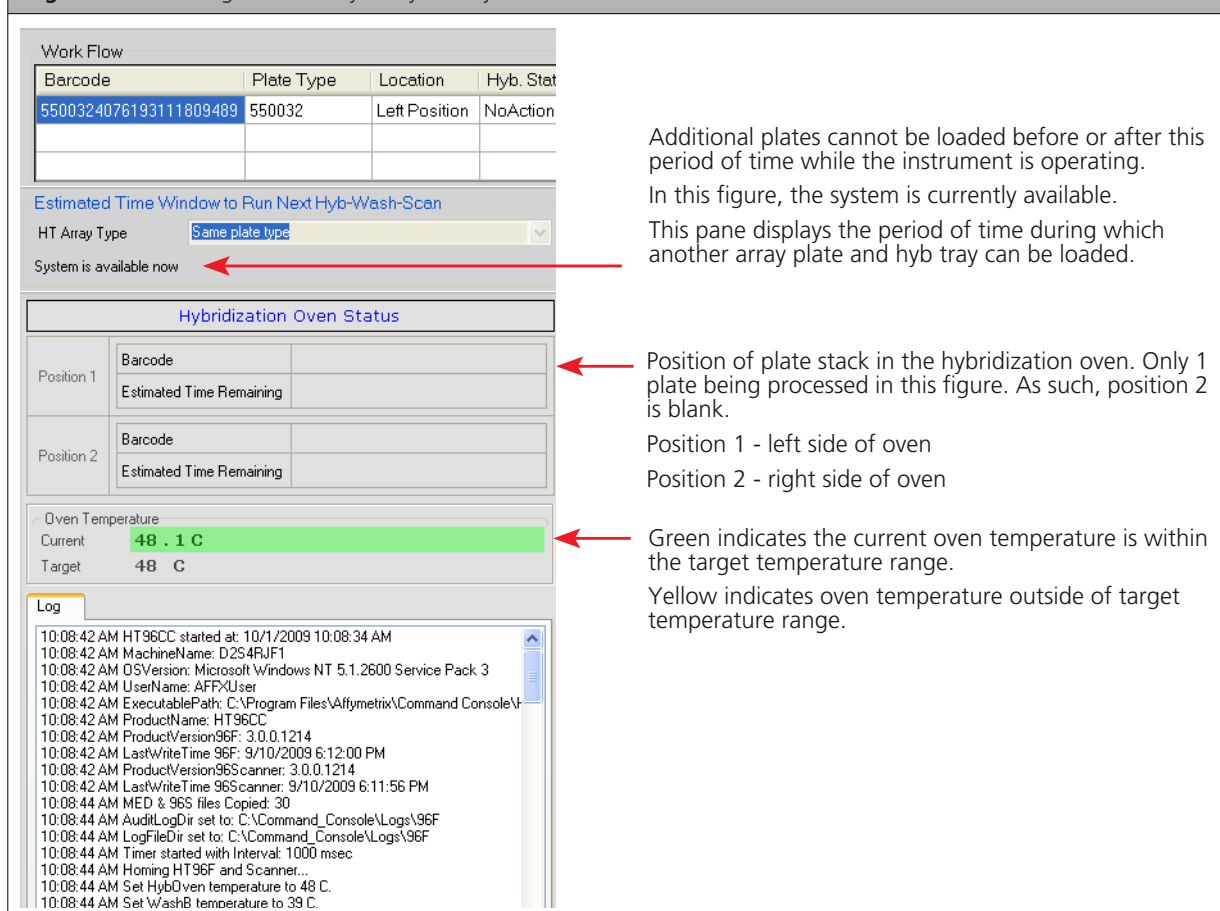
When You Can Load a Second Array Plate and Hyb Tray

Once processing begins, you have a specific period of time during which you can load another Axiom array plate and hyb tray. This period of time is displayed above the Hyb Oven Status pane (Figure 5.23). You cannot load another hyb tray before or after this period of time.

! IMPORTANT: You must load the next array plate and hyb tray during the period of time displayed above the Hyb Oven Status. You cannot load another hyb tray before or after this period of time. You will have to wait until the current process is finished.

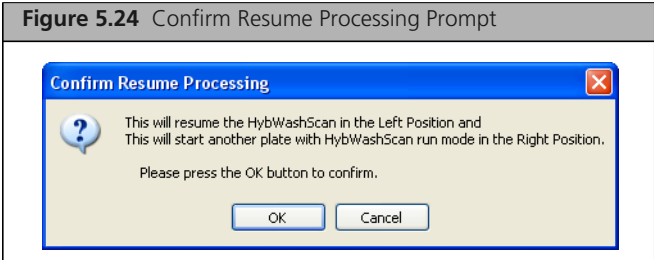
S NOTE: While the first plate is in the oven, you can load another plate if the time spacing requirement is met. This is to ensure that the second plate does not have to wait for system resources in its workflow. The time spacing is roughly equal to the longer of the wash-stain or scan time of the first plate.

Figure 5.23 Loading a Second Hyb Tray and Hybridization Oven Status Information

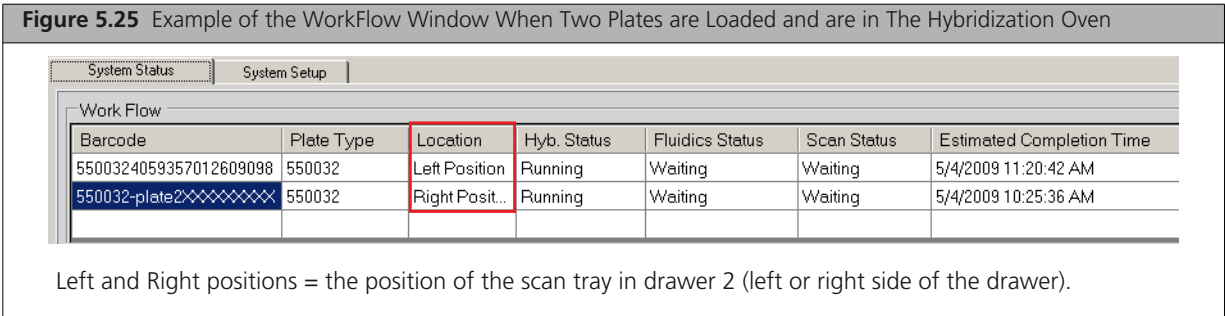


1. Select the System Setup tab.
2. Load an Axiom array plate and hyb tray in the same manner that you loaded the previous plate and tray.
 - A. Scan or manually enter the Axiom array plate barcode, then click **Next**.

- B. Load the Axiom array plates **with the blue base** and the hyb tray **without the cover**, then press the Confirmation button.
- C. Select the arrays to scan, then click **Next**.
- D. Ensure that the plates are clamped securely when prompted, then press the **Confirmation** button.
- E. Click **OK** when prompted to resume plate processing (Figure 5.24).



Select the System Status tab to view Axiom array plates status in the WorkFlow window (Figure 5.25).



Status Window Prompts and Actions Required

Table 5.2 Refilling Buffer Bottles and Emptying the Waste Bottle

Status Window Prompt	Action Required	Receptacle – Reagent
Buffer bottles have been depressurized. Please refill buffer into the bottles. Empty the waste bottle.	** Replenish the fluid in Wash Bottles A and B, and the Rinse bottle. - Empty the Waste Bottle. - Press the Confirmation button to continue.	- Wash Bottle A – fill with Axiom Wash Buffer A up to 2 L. - Wash Bottle B – fill with Axiom Wash Buffer B to the 1 L mark. - Rinse – fill with Axiom Water to the 1 L mark. Do not overfill these bottles.
** Every time you are prompted to refill the buffer bottles, the system runs a fluidics check (duration ~ 1 min).		

Table 5.3 Emptying the Trash Bin

Status Window Prompt	Action Required	Receptacle – Reagent
Empty trash bin	■ Open and empty the trash bin. ■ Press the <i>Confirmation</i> button to continue. NOTE: If the trash bin is empty, you will not be able to open it. Continue the process by pressing the blue confirmation button	—

Table 5.4 Loading the Array Plate and Hyb Tray; Barcode Error Messages

Status Window Prompt	Action Required	Reagent – Receptacle
Load Array Plate Tray on [Left/Right] side of Drawer. Load hyb tray without cover on [Left/Right] side of Drawer.	Load the array plate with the blue base and the hyb tray in drawer 6. ■ IMPORTANT: The blue base must remain in “left side HTA in” even when empty. ■ IMPORTANT: The trays must be positioned. If the trays are placed incorrectly, the software will display an error dialog box indicating the barcode could not be read. ■ Press the <i>Confirmation</i> button to continue.	■ Hyb tray loaded with denatured samples.
Text version of the error message Warning: The system was not able to verify the array plate barcode. Please verify that the tray on the left side of the drawer is a blue cover and if applicable, an array plate, in the correct ORIENTATION. The right side of the drawer should contain a hyb tray, if applicable, in the correct ORIENTATION. Details: The consumable is either not the correct consumable, not loaded correctly, or its barcode is not readable. Proceeding with an incorrect or incorrectly loaded consumable can result in a loss of consumables, loss of samples and may require a field service engineer to service the instrument. Refer to the System Setup Tab or the User Guide provided with the Assay or AGCC for instructions on proper consumable placement. Press the flashing blue confirmation button or... Press OK, The GeneTitan MC Instrument will verify the barcode and orientation. Press Skip, The GeneTitan MC Instrument will NOT verify the barcode and orientation. The barcode entered at registration will be used.		

Table 5.5 Selecting Which Arrays to Scan

Status Window Prompt	Action Required	Reagent and Receptacle
Select arrays to scan	■ Accept the default (all arrays selected) if appropriate. Otherwise, select the arrays to be scanned. ■ Click <i>Next</i>, then click <i>OK</i> to start processing.	—

Stage 3 — Ligate, Wash, Stain and Scan

Equipment, Consumables and Reagents Required

Scan Tray with Axiom Hold Buffer

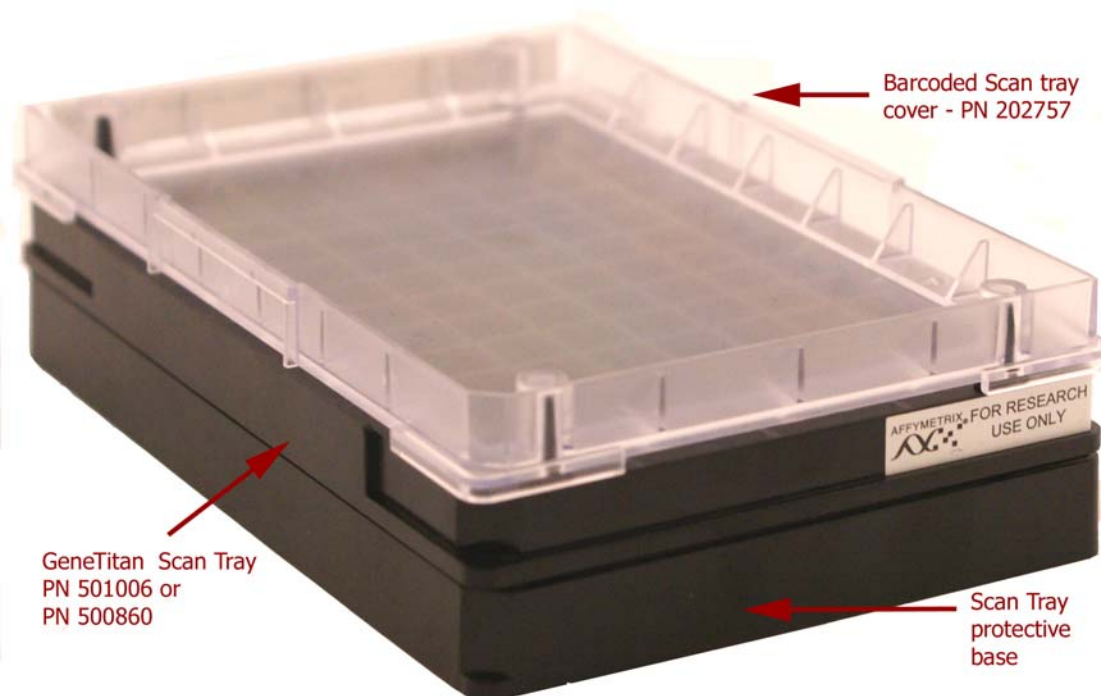
- Cover the tray by orienting the notched corner of the cover over the notched edge of the tray and leave on the bench top (no need to protect from light; [Figure 5.26](#)).



CAUTION: Do not remove the scan tray from its protective black base. Leave the scan tray in the base until loaded onto the GeneTitan MC Instrument. When handling the scan tray, the bottom glass surface of the tray should not be touched.

Figure 5.26 The Scan Tray with Cover on the Black Base.

Always leave the scan tray in its protective black base.



Proper Installation of the GeneTitan Tray Consumables.

It is very important that you install the GeneTitan tray consumables in the proper orientation. The barcode faces into the instrument.

Figure 5.27 You Must Rotate and install the Trays so that the Barcode Faces into the Instrument.

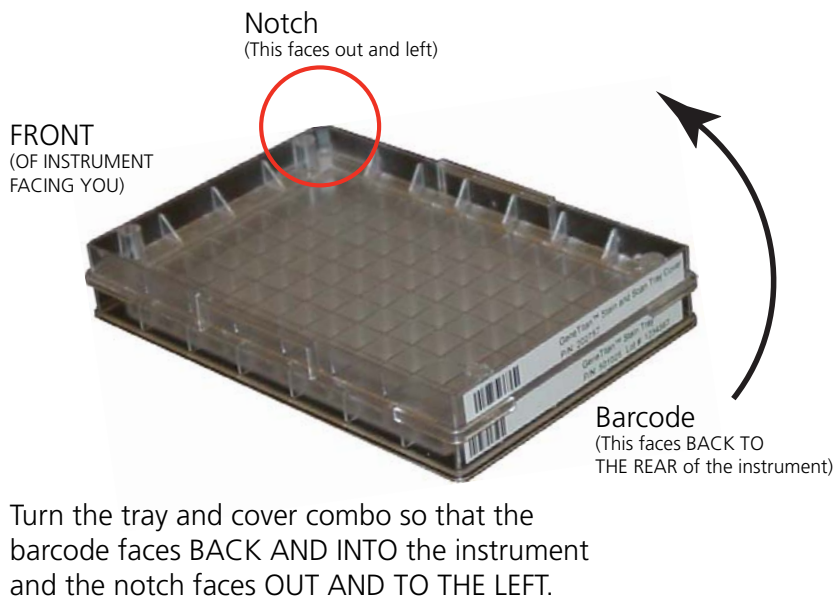
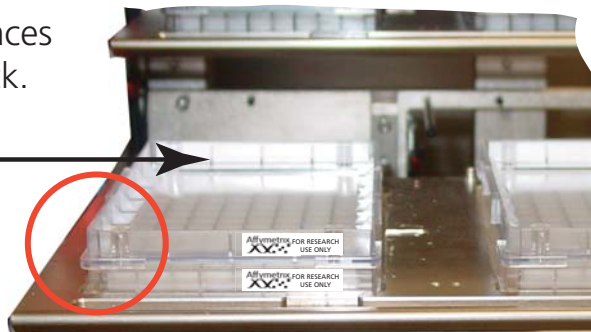


Figure 5.28 The Proper Installation of the GeneTitan Tray Consumables
(the image shows the Stain Tray and the Stain Tray cover as an example)

Barcode faces
in and back.



Notch faces out and left.

"Affymetrix For Research Use Only" faces out.

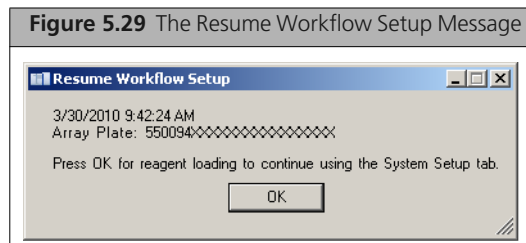


NOTE: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. Refer to [Appendix F, GeneTitan® Multi-Channel Instrument Care on page 133](#) for the message displayed to the user and the procedure for replacing the filters

Load Trays onto the GeneTitan MC Instrument

To Load Trays onto the GeneTitan MC Instrument:

When hybridization of an Axiom array plate has finished, a message (Figure 5.29) will alert you to resume the workflow setup. Press **OK** and the software takes you directly back to the System Setup tab.



This prompt to continue into reagent load (Figure 5.29) occurs when the hyb is complete. “Estimated Time Remaining” displayed under “Hybridization Oven Status” may display a time remaining of 0 to 30 minutes when the prompt occurs.

The GeneTitan MC Instrument will allow reagent load to take place after either:

- the estimated time counts down to zero or
- the actual real world hyb time (as indicated by the computer clock) indicates the hyb is complete.



NOTE: The time estimate displayed on some systems may lag due to high CPU utilization. The GeneTitan MC Instrument allows the workflow to synchronize with the system clock to compensate for this situation during the final half hour of the hyb time estimate. When this prompt to resume reagent loading is displayed to the user there is no need to wait for the estimated time to count down to zero.

Follow the prompts displayed to continue with staining, ligation, fixing and scanning.

1. Follow the prompts in the Status window.
 - A. Wash Bottles A and B, and the Rinse Bottle — refill as necessary (the system will prime itself again); Waste bottle — empty if necessary.
Wash bottle A — 2 L. Wash Bottle B and Rinse Bottle — fill to 1 L mark only.
 - B. Empty the trash bin.
 - C. Remove consumable trays and plates as instructed, except for the blue base.
Leave the blue array plate base in drawer 6 even though the base is empty.
2. Load consumable trays and plates as follows:
 - A. Follow the prompts in the Status window (load sequence and prompts in Table 5.6).
 - B. Once loaded, examine each cover for droplets of liquid.
 - C. If any liquid is present, remove the tray, clean the cover and top of the tray with Kimwipes, and reload the tray.



CAUTION:

- Orient trays as indicated by the guide inside the drawer. Improper orientation may cause the run to fail.
- Remove the protective black base from the scan tray immediately prior to loading (Figure 5.30 on page 93).
- Examine each cover for droplets of liquid after loading. Liquid on the cover can result in capillary phenomenon. As a result, the tray may stick to the cover and be lifted out of place inside the instrument.

Table 5.6 Sequence for Loading the Trays with Reagents

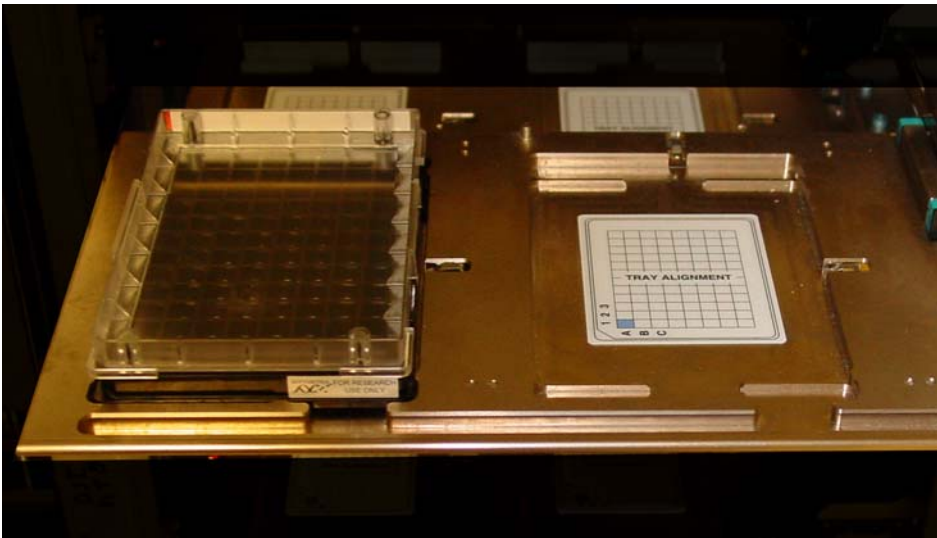
Loading Sequence by Drawer Number	Left	Right
	Note: If the software is unable to verify the barcode on the scan tray and the scan tray cover, the software will display the following error message Verify Drawer #2 Left Side Array Plate Load Warning: The system was not able to verify the array plate barcode. Please verify that the tray on the left side of the drawer is a SCAN tray with an ARRAY PLATE in the correct ORIENTATION. Details: The consumable is either not the correct consumable, not loaded correctly, or its barcode is not readable. Proceeding with an incorrect or incorrectly loaded consumable can result in a loss of consumables, loss of samples and may require a field service engineer to service the instrument. Refer to the System Setup Tab or the User Guide provided with the Assay or AGCC for instructions on proper consumable placement. Press the flashing blue confirmation button or... Press OK, GeneTitan will verify the barcode and orientation. Press Skip, GeneTitan will NOT verify the barcode and orientation. The barcode entered at registration will be used. Ok Skip	
2	Scan Tray with cover — do not load the protective <i>black base</i> (left side of drawer as indicated in Status window) Figure 5.30 on page 93 Status Load the Scan Tray with cover on Left side of Drawer. Press the Confirmation button when done.	
3	Stain Tray with Stain 1	Ligation Tray
	Figure 5.31 on page 94 Status Load the Stain 1 Tray with cover on Left side of Drawer. Load the Ligation Tray with cover on Right side of Drawer. Press the Confirmation button when done.	
4	Stain Tray with Stain 2	Stbl Tray
	Figure 5.32 on page 95 Status Load the Stain 2 Tray with cover on Left side of Drawer. Load the Fixing Tray with cover on Right side of Drawer. Press the Confirmation button when done.	

Table 5.6 Sequence for Loading the Trays with Reagents (Continued)

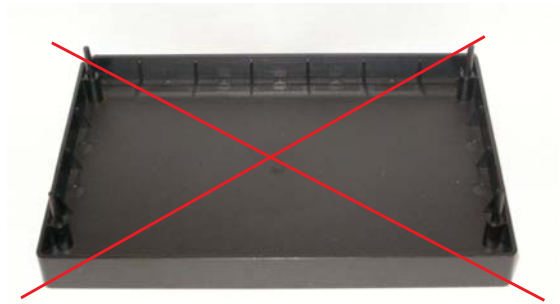
Loading Sequence by Drawer Number	Left	Right
5	Stain Tray with Stain 1	Empty
Status Load the Stain 1 Tray with cover on Left side of Drawer. Press the Confirmation button when done.		

Figure 5.30 Scan Tray loaded in Drawer 2.

Scan tray with cover loaded in drawer 2.



Do **NOT** load the protective black base packaged with the scan tray.



! **IMPORTANT:** When you load the plates, or trays, insert them under the tabs, or fingers, that may protrude into the stage. Confirm that the tray is not resting on these fingers.

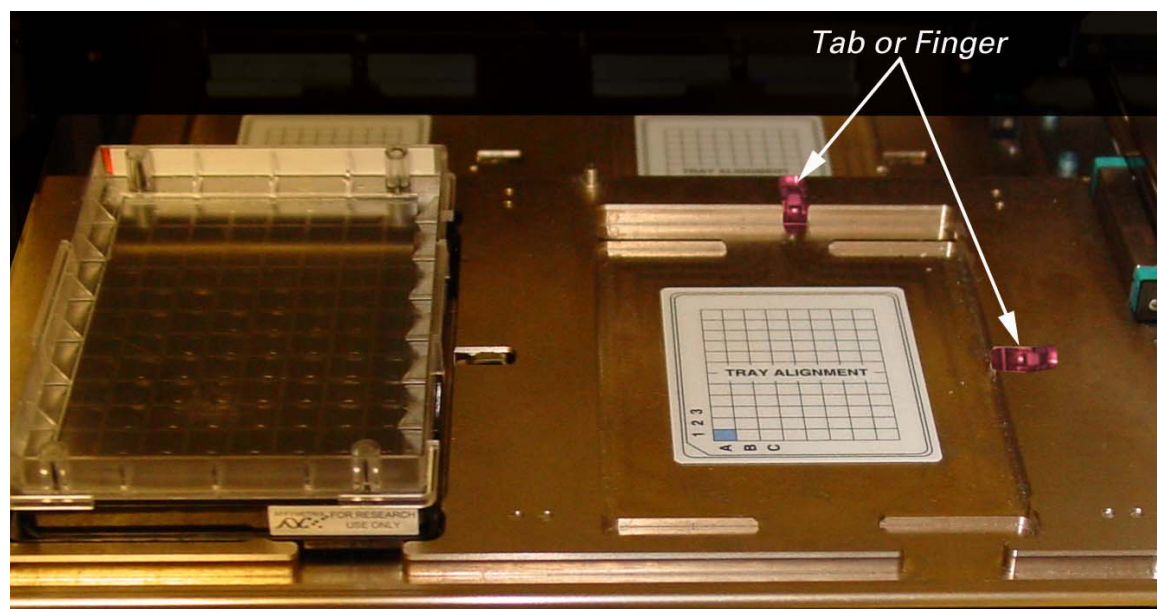


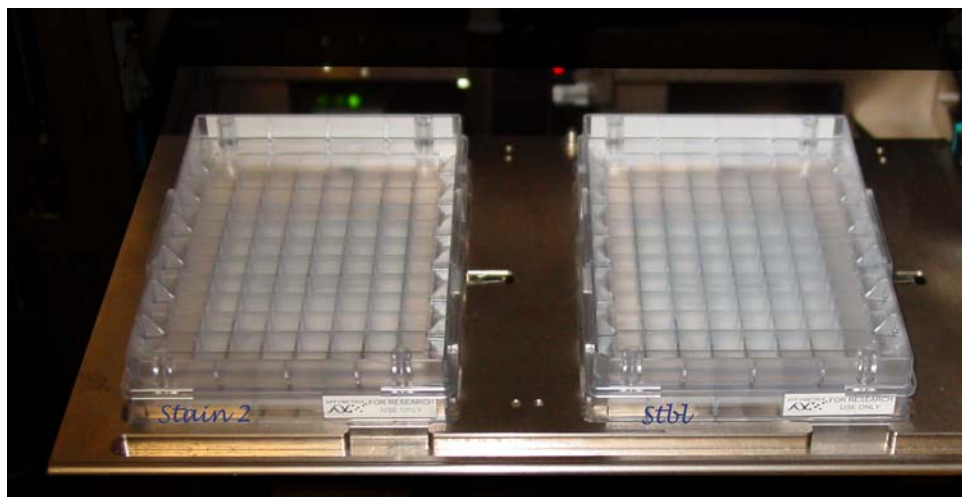
Figure 5.31 Stain 1 Tray and Ligation Tray Loaded in Drawer 3.

Stain 1 Tray and Ligation Tray loaded in drawer 3

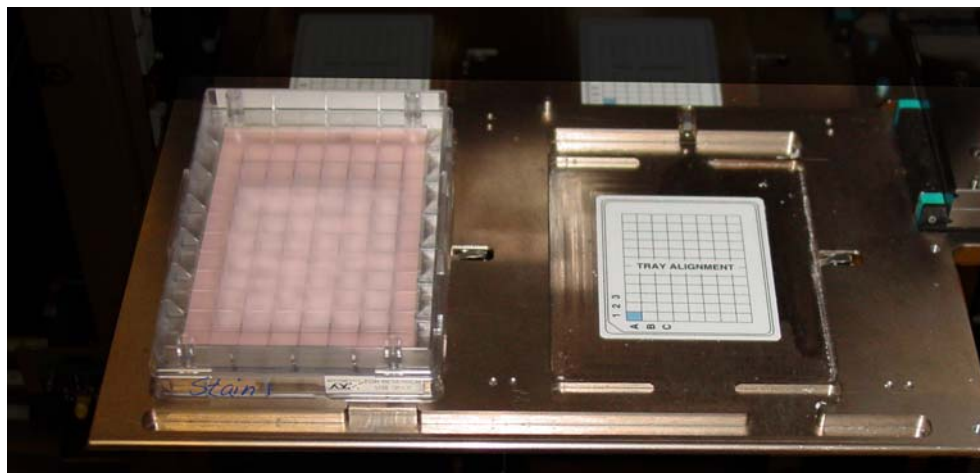


Figure 5.32 Stain 2 Tray and Stbl Tray Loaded in Drawer 4.

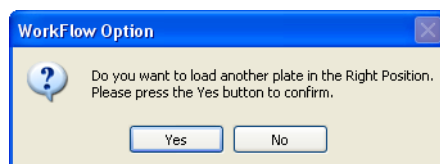
Stain 2 Tray and Stbl Tray loaded in drawer 4

**Figure 5.33** Stain 1 Tray Loaded in Drawer 5.

Stain 1 Tray loaded in drawer 5

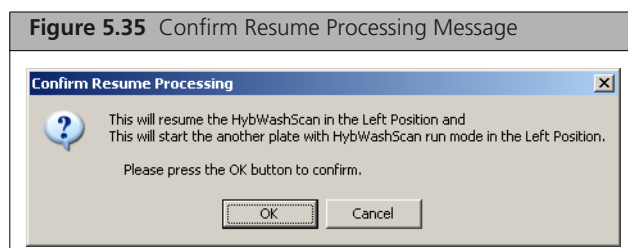


3. At the prompt shown in Figure 5.34, click **Yes** to load another Axiom array plate and hyb tray.

Figure 5.34 Prompt asking to load another plate. Right or left position determined by the position of Axiom array plates already in the GeneTitan MC Instrument.

4. Follow the prompts and:
 - A. Setup Option: select **Setup Another Run**, then click **Next**.
 - B. Scan or manually enter the Axiom array plate barcode, then click **Next**.
 - C. Select a protocol, then click **Next**.

- D. When drawer 6 opens:
 - 1) Remove the blue cover from the previous Axiom array plate.
 - 2) Load a new Axiom array plate and new blue base on the left; load a new hyb tray on the right.
 - 3) Press the **Confirmation** button.
- E. Click **OK** when prompted (Figure 5.35).



- F. When drawer 6 opens, confirm that the plate stack is securely clamped, then press the **Confirmation** button.

When processing resumes:

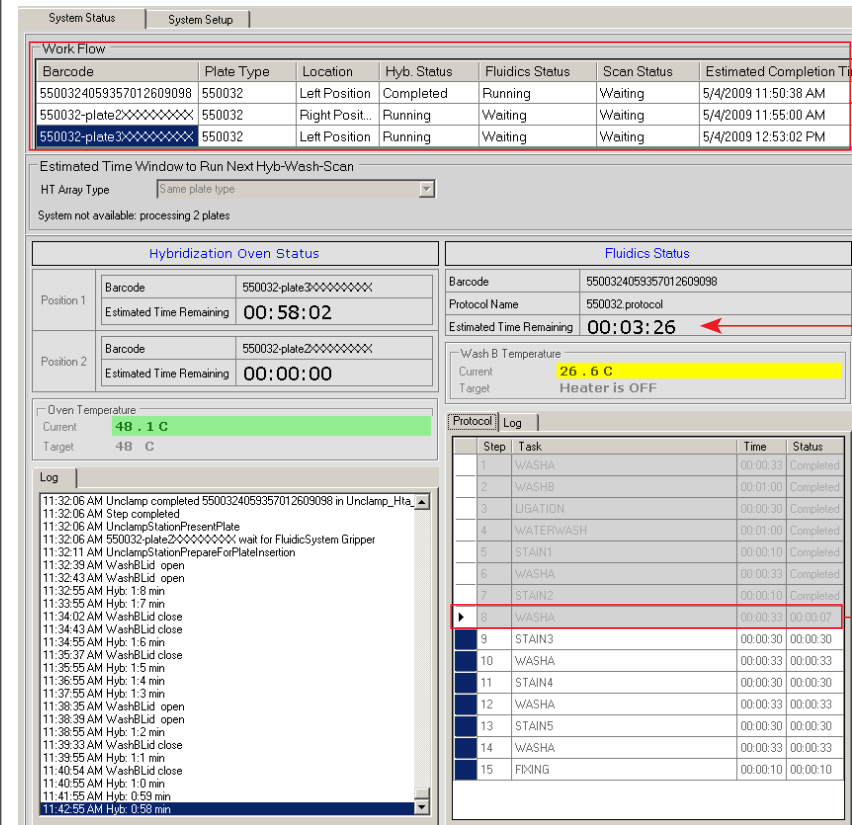
1. The plate stack which has finished hybridization is moved from the Hyb oven to drawer 1 temporarily and then moved to the unclamp station after step 2 (it remains clamped).
2. The plate stack in drawer 6 is moved to the Hyb oven.
3. The plate is moved to the unclamped station.
4. The plate stack in the unclamp area is unclamped and moved into the fluidics area.



NOTE: At the end of a Hyb-Wash-Scan run, all plate and tray covers and the fixing tray cover should be in the trash.

Figure 5.36 is an example of how the System Status Workflow window will appear when three Axiom array plates are being processed.

Figure 5.36 Example of the System Status window — three Axiom array plates are being processed.



Workflow indicates the number of plates being processed and where they are in the instrument. In this example, three Axiom array plates are being processed: 2 in the Hyb Oven and 1 in Fluidics.

Estimated Completion Time is for the current process.

Estimated Time Remaining for fluidics is adjusted as necessary. Adjustments can be due to process interruptions such as a drawer being opened.

Step currently executing in Fluidics.

Continuing the Workflow

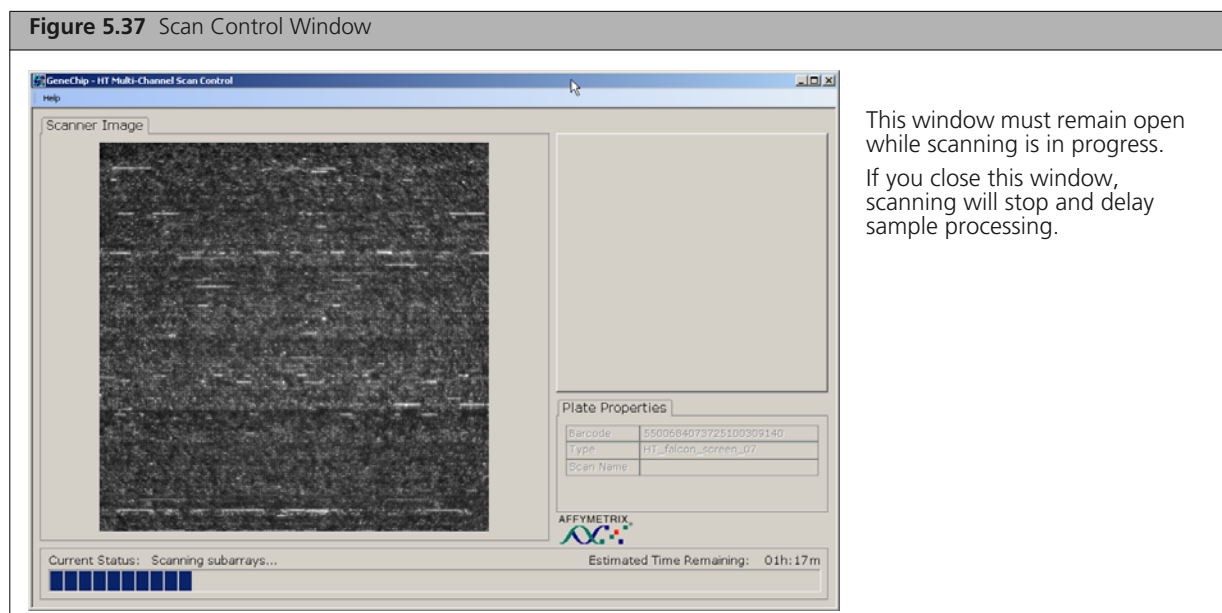
Once a plate has gone through the fluidics stage of the process, it is moved to the imaging device.

When the scanning process begins, the window shown in [Figure 5.37](#) is displayed. This window must remain open while Axiom array plates are being scanned.



CAUTION:

- The Scan Control window must remain open while Axiom array plates are being scanned. Closing this window will halt the scanning process. You can minimize this window if necessary without creating any interference to the imaging.
- Do not manually, or through the AGCC transfer utility, move any data associated with the current plate that is being processed/scanned. Transferring data will dramatically slow scanning and may cause the computer to freeze.



Storing Hyb Trays for Rehybridization

Hyb trays can be stored after processing for the purpose of rehybridization. Store hyb trays as directed below. See also [Appendix C, Rehybridization on page 125](#) for more information.

To Store a Hyb Tray with Processed Samples:

1. After completion of the GeneTitan MC Instrument run, unload the hyb tray and tightly seal it with an adhesive film.
The plate must be well-sealed to prevent cross-contamination between samples.
2. Press the four corners and sides of the tray to ensure that there is no space between the seal and plate.
3. Store the hyb tray at -20°C .

Shutting Down the GeneTitan® MC Instrument

This procedure assumes that all of the Axiom array plates loaded onto the GeneTitan MC Instrument have been processed.



WARNING: Do not attempt to shut down the GeneTitan MC Instrument while array plates are being processed.

To Shutdown the GeneTitan MC Instrument:

1. On the System Setup page, open the Setup Options drop-down menu and select **Unload Plates**.
2. Unload all of the consumables as prompted.
3. Power off the GeneTitan MC Instrument by opening **Tools** → **Shutdown** from the menu.
4. Exit the AGCC software if it does not close automatically.



NOTE: If the instrument is processing an array plate, the software will not allow you to shut down the system.

The three plates are referred to as Plates A, B, and C in the manual target prep and in the GeneTitan Array Processing.

In order to process three plates during a 40-hour week, the steps must be performed in the order and with the timing described in this chapter.

Table 6.1 Daily Steps for Manual Target Prep Workflow

Day	Activities	Plates
1	■ Amplify 3 plates of genomic DNA.	A, B, and C
2	■ Fragment and precipitate two plates amplified on Day 1. ■ Freeze one plate of amplified DNA for fragmentation later in the week.	■ A, B ■ C
3	■ Fragment and precipitate one plate. ■ Centrifuge, dry, resuspend and QC two plates precipitated on Day 2. ■ Denature and begin hybridization for one plate on the GeneTitan MC Instrument	■ C ■ A, B ■ A
4	■ Centrifuge, dry, resuspend and QC plates precipitated on Day 3 ■ Denature and begin Hybridization for two plates on the GeneTitan MC Instrument ■ GeneTitan reagent trays preparation and loading	■ C ■ B, C ■ A
5	■ GeneTitan reagent trays preparation and loading	■ B, C

The timing of these steps is critical because of constraints on both the target preparation, done on the lab bench, and the array processing, done using the GeneTitan MC Instrument.

These constraints are described in more detail in:

- [Timing Issues for Manual Target Preparation on page 101](#)
- [Timing Issues for GeneTitan MC Array Processing on page 102](#)

Timing Issues for Manual Target Preparation

The GeneTitan reagent trays for array processing cannot be loaded until the array plate has finished hybridization, and they should not be prepared more than 1.5 hours before hybridization will finish. The GeneTitan reagent trays cannot be prepared ahead of time and stored.

Table 6.2 Time Required for Manual Target Preparation

Manual Preparation	Hands-on Time Required	Total Prep Time*	Incubation/Hybridization/Processing
Amplification	0.5 hr	1.5 hr	23±1 hr
Fragmentation and Precipitation prep	2 hr	2 hr	Overnight Precipitation
Re-suspension and Hybridization Mix prep	25 min	25 min	N/A
Centrifugation/Drying	30 min	1 hr 20 min	N/A
QC gel and OD	45 min	45 min	N/A
Denaturation and hyb tray/array plate loading on the GeneTitan MC Instrument	25 min	45	23.5 - 24 hr hybridization
GeneTitan reagent tray preparation and loading on the GeneTitan MC Instrument	1 hr	1.5 hr	Additional time for processing: 96 arrays: 12.5 hr
* Total Prep Time includes reagent thawing time and hands-on time			

Timing Issues for GeneTitan MC Array Processing

! IMPORTANT: Maintaining consistent timing during the set up of the GeneTitan MC Instrument is critical to containing the user interventions of the three plate workflow within a work day. Once one process begins late, there is little opportunity to catch up until the end of the workflow.

The hybridization time for the Axiom® 2.0 Assay on the GeneTitan MC Instrument is 23.5 to 24 hr (Table 6.3). This provides a 30 min window during which you are prompted by the instrument control software to load the reagents required for washing and staining.

Table 6.3 Time Required for Array Plate Processing on the GeneTitan MC Instrument

Steps on the GeneTitan MC Instrument	Time Required
Hybridization of two plates in one day - First plate loaded at 9:30 a.m. - Second plate loaded at 5:00 p.m.	23.5 hr each plate
Loading reagent trays	15 min
Fluidics	5 hr each plate
Imaging	96 arrays: 7.5 hr

Changing Oven Temperatures for the Three Plate Workflow

Multiple ovens are required for manual target preparation. If you are running the three plate/week workflow, three ovens are recommended. Table 6.4 lists the different temperatures required for each step. Though only two ovens are strictly required, we recommend maintaining separate 37 °C ovens for the amplification and fragmentation stages to avoid confusion of plates and to minimize excess opening and closing of oven doors during incubation periods. Table 6.5 provides a list of suggested settings for three ovens when performing the three plate/week workflow.

Table 6.4 Oven Temperatures Needed for Each Step of the Workflow

Workflow step	Oven Temp
Amplification	37 °C
Stopping Amplification	65 °C
Pre-Fragmentation Incubation	37 °C
Fragmentation Incubation	37 °C
Drying	37 °C
Hybridization	48 °C

Table 6.5 Suggested Settings for Ovens When Performing Three Plate/Week Manual Target Prep Workflow

Day of Workflow	Oven 1	Oven 2	Oven 3
Day 1	37 °C	N/A	N/A
Day 2	37 °C	65 °C	37 °C
Day 3	48 °C	65 °C	37 °C
Day 4	48 °C	65 °C	37 °C
Day 5	N/A	N/A	N/A

Table 6.6 Manual Target Prep Workflow — Day 1 Activities

Activity	Plate ID	Approximate Start Times*
DNA Amplification	A	9:30 a.m.
DNA Amplification	C	10:30 a.m.
DNA Amplification	B	1:30 p.m.
*Approximate start time indicates start of thawing of reagents		

See [Stage 1 — DNA Amplification on page 33](#) for more information on the protocol.

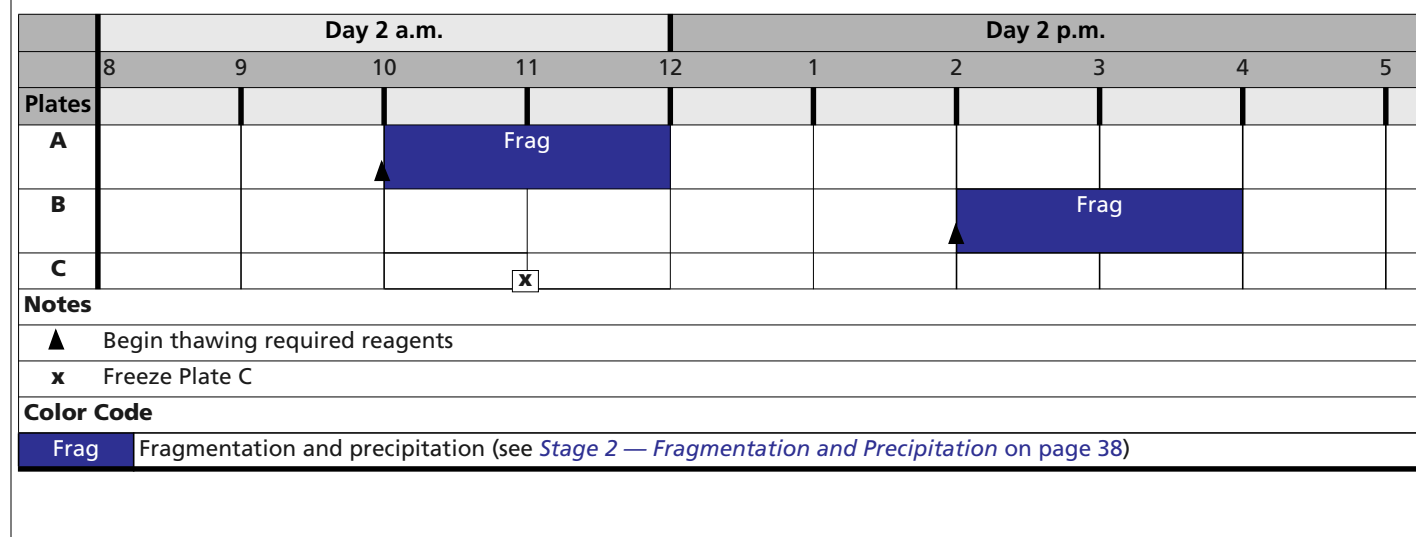
Manual Target Prep Workflow — Day 2

The tables below show the steps that need to be performed on the second day.

Plates A and B are fragmented and precipitated on Day 2 without freezing to preserve a 23 hr amplification incubation.

Precipitation is carried out at -20°C overnight.

! **IMPORTANT:** Store Plate C at -20°C immediately after the end of the 23 hr Amplification reaction (without performing the 65°C Stop Amplification Reaction step).

Figure 6.3 Manual Target Preparation Workflow — Day 2 Activities**Table 6.7** Manual Target Prep Workflow — Day 2 Activities

Activity	Plate ID	Approximate Start Times
Fragment and precipitate	A	10:00 a.m.
Freeze (-20°C)	C	11:00 a.m.
Fragment and precipitate	B	2:00 p.m.

Manual Target Prep Workflow — Day 3

- Centrifuge, dry, resuspend and QC Plates A and B.
- Thaw Plate C (see [Thawing Frozen Plates of Amplified DNA on page 103](#)).
- Fragment (including the 65 °C Stop Amplification Reaction step) and precipitate Plate C.
- Perform Denaturation on Plate A.
- Transfer Plate A samples to Hyb Tray A
- Load Hyb Tray A and array plate into GeneTitan MC Instrument and begin hybridization.



WARNING: The Hybridization Tray prep should take place under a running fume hood.



IMPORTANT: Amplified plates that are frozen must be thawed and thoroughly mixed by following the procedure under [Thawing Frozen Plates of Amplified DNA on page 103](#).

Figure 6.4 Manual Target Preparation Workflow — Day 3 Activities

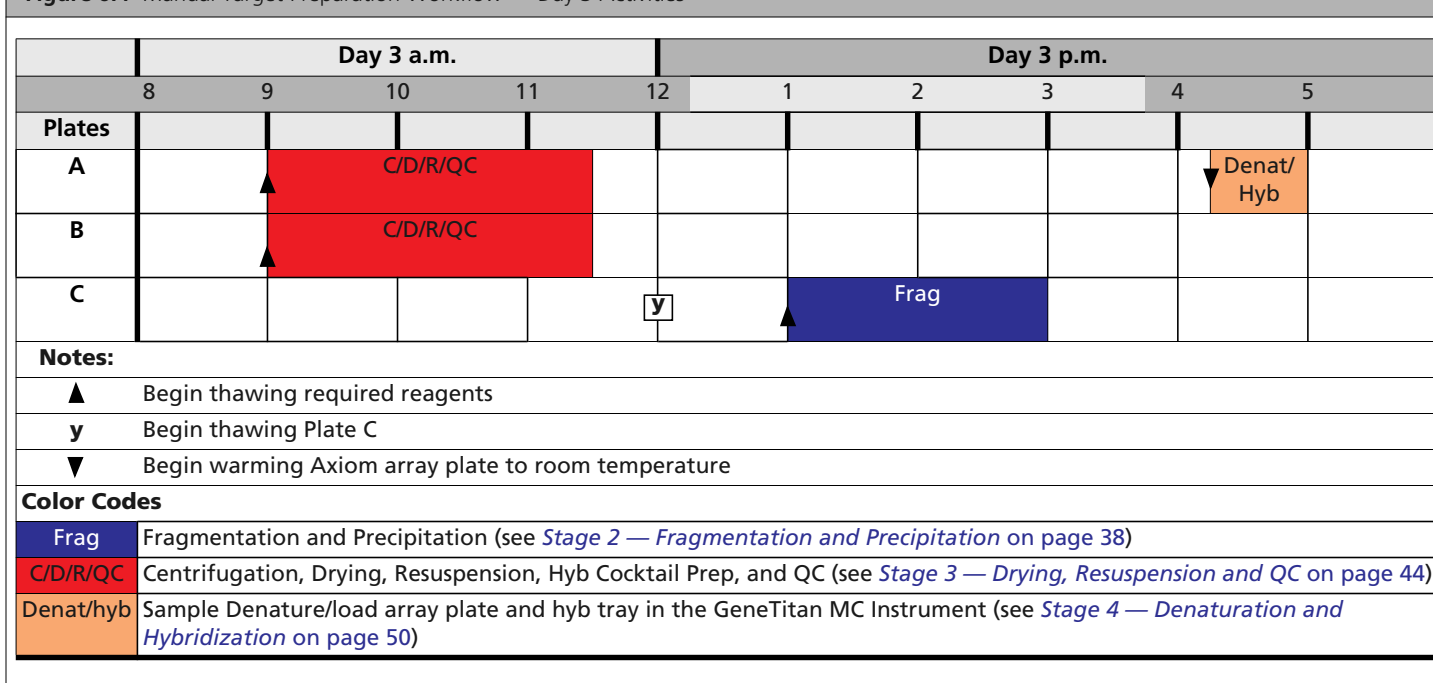


Table 6.8 Manual Target Prep Workflow — Day 3 Activities

Activity	Plate ID	Approximate Start Times
Centrifuge/Dry/Resuspend/QC	A, B	9:00 a.m.
Thaw Plate C	C	12:00 a.m.
Fragment and precipitate	C	1:00 p.m.
Denature and Hyb	A	4:00 p.m.

Manual Target Prep Workflow — Day 4

- Denaturation of Samples/Load array plate and hyb tray in the GeneTitan MC Instrument for Plates B and C
- Centrifuge, dry, resuspend, and QC Plate C
- GeneTitan reagent trays prep and loading for Plate A

▲ WARNING: The Hybridization Tray prep should take place under a running fume hood.

! IMPORTANT: The GeneTitan reagent trays for array processing cannot be loaded until the array plate has finished hybridization, and they should not be prepared more than 1.5 hours before hybridization will finish. The GeneTitan reagent trays cannot be prepared ahead of time and stored.

Figure 6.5 Manual Target Preparation Workflow — Day 4 Activities

Plates	Day 4 a.m.						Day 4 p.m.				
	8	9	10	11	12		1	2	3	4	5
A										▲ GT Reagent prep/load	z
B		▼ Denat/Hyb									
C			▲ C/D/R/QC							▼ Denat/Hyb	z
Notes											
▲ Begin thawing required reagents											
▼ Begin warming Axiom array plate to room temperature											
z Coupled operations on the GeneTitan MC Instrument: Load reagent trays for Plate A and hyb tray/array plate for Plate C											
Color Codes											
C/D/R/QC	Centrifugation, Drying, Resuspension, Hyb Cocktail Prep, and QC (see Stage 3 — Drying, Resuspension and QC on page 44)										
Denat/Hyb	Sample Denature/load array plate and hyb tray in the GeneTitan MC Instrument (see Stage 4 — Denaturation and Hybridization on page 50)										
GT Reagent prep/load	GeneTitan reagent trays prep and load (see Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument on page 55)										

Table 6.9 Manual Target Prep Workflow —Day 4 Activities

Activity	Plate ID	Approximate Start Times
Denature and Hyb	B	8:45 a.m.
Centrifugation/Drying/Resuspension/QC	C	9:30 a.m.
GeneTitan reagent prep and loading	A	3:30 p.m.
Denature and Hyb	C	4:15 p.m.

Manual Target Prep Workflow — Day 5

- GeneTitan reagents prep and loading for Plates B and C.

! **IMPORTANT:** The GeneTitan reagent trays for array processing cannot be loaded until the array plate has finished hybridization, and they should not be prepared more than 1.5 hours before hybridization will finish. The GeneTitan reagent trays cannot be prepared ahead of time and stored.

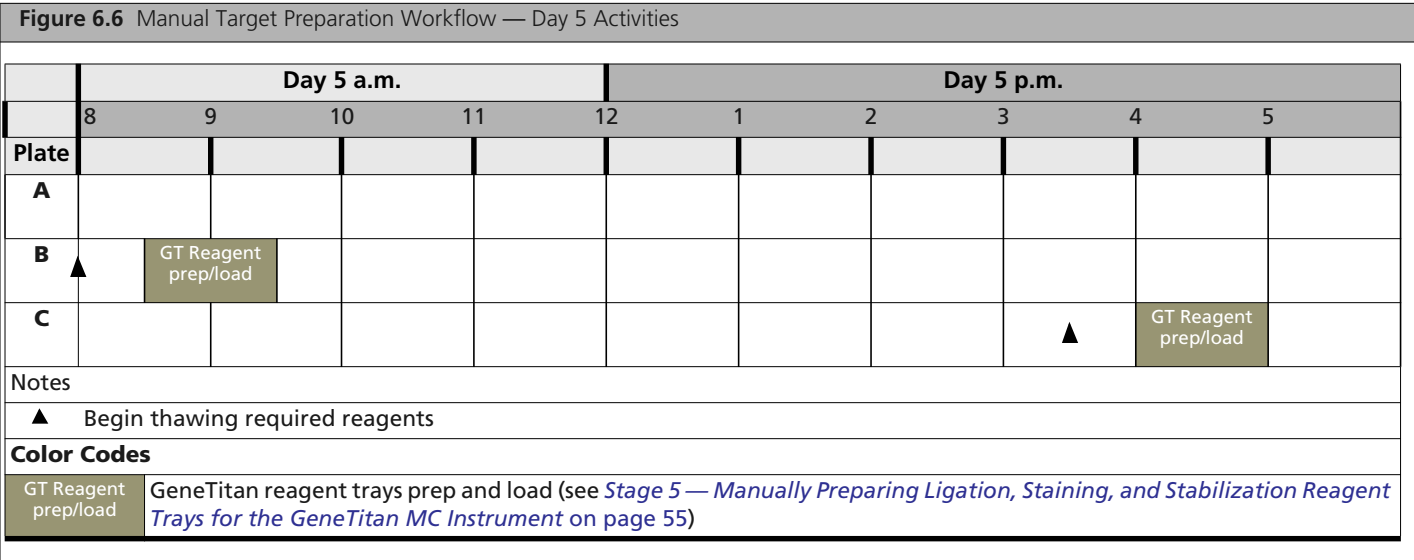


Table 6.10 Manual Target Prep Workflow — Day 5 Activities

Activity	Plate ID	Approximate Start Times
GeneTitan reagent tray prep and loading	B	8:00 a.m.
GeneTitan reagent tray prep and loading	C	3:30 p.m.

Troubleshooting

GeneTitan Multichannel Instrument

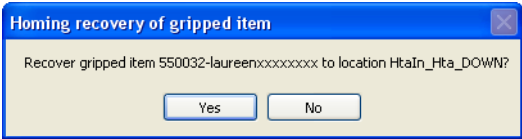
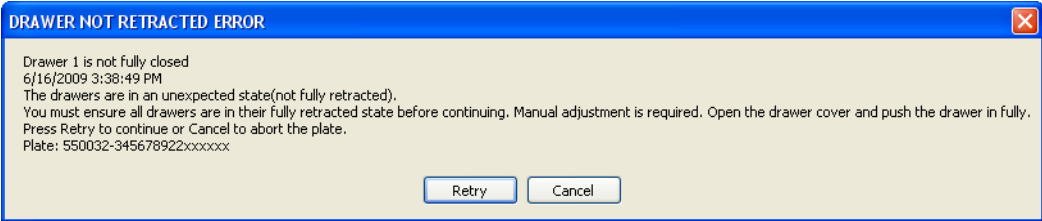
Refer to the *GeneTitan® Multi-Channel Instrument User's Manual*, P/N 08-0306 for further troubleshooting information.

Table 7.1 GeneTitan Multichannel Instrument troubleshooting guidelines for the Axiom® 2.0 Assay

Problem	Possible Causes	Possible Actions
Plate trapped in GeneTitan Multichannel Instrument.	■ Plate (or plate with lid) not properly loaded in drawer. ■ Cut edge of lid and plate not aligned. ■ Gripper failed to retrieve plate. ■ System requires adjustment.	1 Restart the GeneTitan Multichannel Instrument. 2 Run the setup option <i>Unload Plates</i> 3 If the plate remains trapped in the instrument, call Affymetrix support.
Computer frozen.	■ Too many processes running ■ Attempting to transfer data while an array plate is being scanned (imaged).	Restart the computer and unload all of the plates. ■ Plates in Hyb station: finish hybridization off-line. ■ Plate in Scanner: rescan using Scan Only function ■ Plate in Fluidics: use Wash/Scan Resume to resume the fluidics process Do not manually, or through the AGCC transfer utility, move any data associated with the current plate that is being processed/scanned.
Hybridization aborted: ■ System-initiated abort ■ User-initiated abort	System-initiated abort: ■ Power loss	Array plate and hyb tray are still clamped: ■ Contact your local field service engineer with information on the workstation model ■ The plate stack is moved to drawer 1. ■ Remove the plate stack and finish hybridization offline. ■ Return the hybridized array plate to the GeneTitan Multichannel Instrument and finish processing using the Wash/Scan process.
FAILED messages	See Failed Messages on page 110	
FLUIDIC DIAGNOSTIC messages	See Fluidic Diagnostic Messages on page 110 .	
Fluidics aborted: ■ System-initiated abort ■ User-initiated abort	System-initiated abort: ■ Power loss User-initiated abort: ■ Incorrect protocol selected	Follow the recommendations and instructions under Wash/Scan Resume on page 113 .

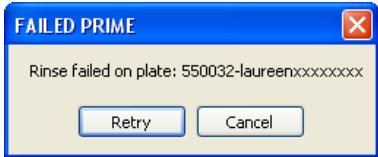
Miscellaneous Messages

Table 7.2 Miscellaneous messages and recommended actions

Message and Recommended Action	
 Indicates that an item is in the gripper, and normal startup of the GeneTitan Multichannel Instrument is not possible. The item must be removed from the instrument before you can begin processing array plates.	Recommendation: click Yes. If you click No, nothing will occur. Homing will not complete and you not be able to use the system. The item held by the gripper will be moved to either: ■ Drawer 2 — plates and trays ■ Trash Bin — covers The drawer names will reflect the location (left or Right) and the drawer number (1 through 6). Examples: Drawer2L_Hta_DOWN = Scan Tray on left side of drawer 2 HtaHyb = Clamped Hyb Tray and Array Plate Drawer(n)L/R_Hta_DOWN where n is the drawer number and L or R to indicate the left or right side. The _Hta_ (second term) indicates the item held. An example is drawer1R_HtaHyb_DOWN indicating it is an array plate with a Hyb Tray or Drawer2L_ScanHta_Pk_DOWN indicating it is an array plate with a scan tray
 The drawer listed in the message is not fully closed. Manually push the drawer back into the instrument until it is fully closed. There are two stop positions with audible clicks; push until you hear the second click and the drawer is fully seated.	
	■ Check that the array plate barcode has been entered correctly. ■ Ensure that the library files required for the type of array plate you are using have been installed, and are installed in the correct directory. ■ Restart the GeneTitan MC instrument control software after library files have been installed.

Failed Messages

Table 7.3

Problem and Possible Causes	
 Rinse bottle — fluid level too low or bottle empty.	If this message is displayed: - during a water wash step, array processing has been compromised. - during cleanup, array processing is OK, but cleanup will not be complete. Always ensure that the GeneTitan bottles containing Wash A and Rinse are above the 50% mark when setting up the system to process an Axiom HT array plate. All 600 mL of the Wash buffer B from the Axiom reagent kit should be emptied into the GeneTitan Wash B bottle when setting up the system to process a plate. This ensures that the GeneTitan Wash B bottle is filled to more than the requisite 35% of Wash B bottle volume.

Fluidic Diagnostic Messages

Table 7.4 Problem messages

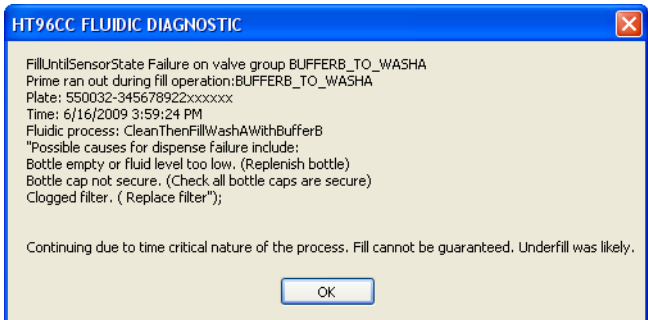
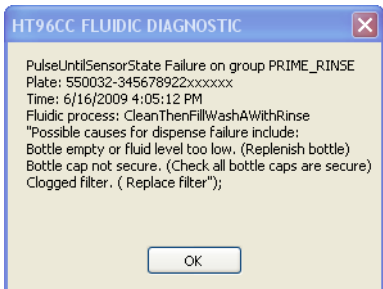
Problem and Possible Causes	
	About this message: - BUFFERX = Buffer bottle A, B or Rinse - WASHX = Wash A or B reservoir in the fluidics station. Recommended actions: - Replenish fluid level in the Rinse or Wash Bottle B to the 1L mark. Do not overfill. - Only replenish bottles when prompted by the UI. Replenishing during fluidic processing may cause system malfunction including overflowing inside the system and more problems. The only thing to do while a plate is running is to make sure bottle caps are secure. - Replenish fluid level in Wash Bottle A to 2 L. - Secure the bottle cap. - Replace the filter Instructions for filter replacement in the <i>GeneTitan® Multi-Channel Instrument User's Manual</i>, P/N 08-0306. If the problem persists, call Affymetrix support.
	The typical cause is an unsecure bottle cap. If the failure is detected during priming, the instrument will pause and wait for the problem to be corrected. If the failure is detected during another process, and if the cause is a clogged filter, wait until the end of the run to replace the filter. Instructions for filter replacement in the <i>GeneTitan® Multi-Channel Instrument User's Manual</i>, P/N 08-0306.

Table 7.4 Problem messages (Continued)

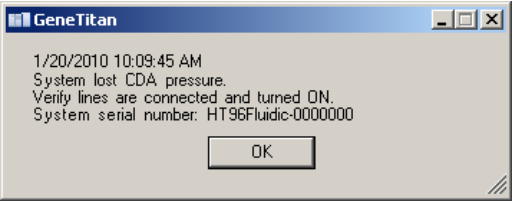
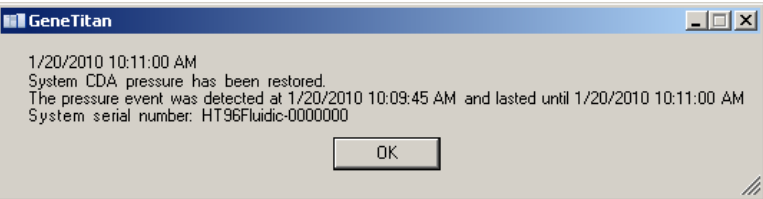
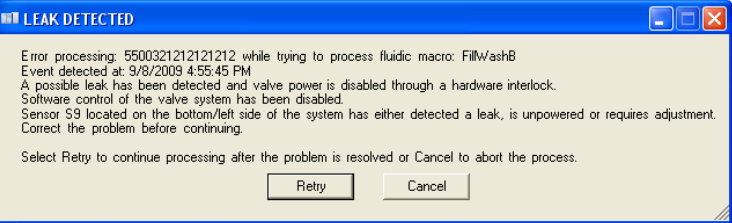
Problem and Possible Causes	
When the instrument experiences a loss in Clean Dry Air (CDA) pressure, the software will display the warning message.  1/20/2010 10:09:45 AM System lost CDA pressure. Verify lines are connected and turned ON. System serial number: HT96Fluidic-0000000 OK	Possible Causes Please verify that the facility CDA or the portable CDA compressor is in working condition. Refer to the GeneTitan MC Site Prep Guide for the portable compressor model that has been validated with the GeneTitan MC instrument. Contact your local field application specialist and notify the engineer about the error message.
When the pressure is detected again, a dialog message confirming the availability of CDA pressure is displayed.  1/20/2010 10:11:00 AM System CDA pressure has been restored. The pressure event was detected at 1/20/2010 10:09:45 AM and lasted until 1/20/2010 10:11:00 AM System serial number: HT96Fluidic-0000000 OK	
Leak Detected Leak checks are performed at application startup and any time a fluidic process (priming filling draining etc.) is performed. The leak detection is a hard-wired sensor which will shut off fluid flow without software control. Leaks are normally confined to the drip pan located inside the system.  Error processing: 5500321212121212 while trying to process fluidic macro: FillWashB Event detected at: 9/8/2009 4:55:45 PM A possible leak has been detected and valve power is disabled through a hardware interlock. Software control of the valve system has been disabled. Sensor S9 located on the bottom/left side of the system has either detected a leak, is unpowered or requires adjustment. Correct the problem before continuing. Select Retry to continue processing after the problem is resolved or Cancel to abort the process. Retry Cancel  3/1/2011 11:33:41 AM A possible leak has been detected and valve power is disabled through a hardware interlock. Software control of the valve system has been disabled. Sensor S9 located on the bottom/left side of the system has either detected a leak, is unpowered or requires adjustment. Correct the problem before continuing. No fluidic Process will be able to run until the problem is resolved. This message reflects the current system state. If the leak affects fluidic processing for a specific plate being processed that plate will display a similar message. Call Affymetrix Technical Support for Service. System serial number: HT96Fluidic-BETA002 OK	Causes: ■ System malfunction ■ User killing the application using task manager during a fill operation resulting in application exit without stopping flow. Solution: Contact Affymetrix field support. The system cannot be used for any fluidic processing until this is resolved.

Table 7.4 Problem messages (Continued)

Problem and Possible Causes	
Leak Resolved	This message is displayed when the leak is resolved (meaning the sensor LED is again lit up). If the original leak detected message was not acknowledged it will be automatically removed from the GUI and replaced by the following message. It will remain displayed until another leak is detected or the user acknowledges it by pressing OK. To resolve this issue complete the following tasks ■ Verify all internal and external tubing is connected and clean ■ Verify wash reservoirs are clean ■ Verify all bottle caps are secure and that no bottle cap is crimping a supply line. ■ Verify vacuum is working properly ■ Do not refill bottles or empty waste except when prompted to by the GeneTitan application. ■ Contact your facility group to ensure CDA is supplied to your GeneTitan system. Contact Affymetrix Field Service to have the sensor adjusted or replaced if the problem persists even after correcting for the usual causes outlined above.
Filter Error Messages	The filters in the GeneTitan fluidics bottles (Wash A, Wash B and DI Water) need to be replaced when the filters are worn out. The software displays warning message boxes for the filter in each reagent bottle when it detects a problem or shows a trend of increased fill times during fluid fill operations. If an error is detected as described above, then a message box titled “Filter Change Required” is displayed along with the information on the specific dispense operation. You should change all three filters when a warning is displayed for any one of the three filters. Refer to the section Replacing the Filter on page 136 in Appendix F.

Wash/Scan Resume

If a run is aborted during fluidics processing, the instrument will place the aborted array plate into the scan tray. To restart this process, remove the Axiom array plate from the scan tray and place it in its protective blue base.

The step at which the run was aborted can be identified by:

- Viewing the System Status window if you are aborting the last plate through the fluidics system.
 - Initiating the resume process.
1. System Setup tab: Select Wash/Scan Resume
 2. Follow the prompts to unload and reload all drawers.

The trays will be loaded. It is up to you to determine whether or not to load fresh reagents or reuse the trays already in the GeneTitan Multichannel Instrument. Base your decision upon the step where the problem occurred.

To Help Ensure that the Samples are Processed Correctly, We Recommend that You:

1. Load new stain trays with fresh reagents.
2. Load a new scan tray.

We do not recommend the use of trays without reagents or holding buffers for steps that appear to have already executed.

Resume Step

You must select the step at which you wish to resume plate processing. You can select any step that has not yet been started.

For certain steps, you can enter a duration in seconds (even if the step requires > 1 hr to run, you must enter the duration in seconds). You can set a step for less time than normal, but not for longer than the normal duration.

Aborting a Run

- Abort can take up to three minutes if a plate is in the Fluidics station. Status window Abort Requested changes to Abort Completed.
- Clamped Array-Plate -Hyb Tray sandwich aborted from the oven or from drawerIN are moved to drawer 1.
- Proceed as follows:
 - Use the Unload Plates option to remove the aborted plate(s).
 - Start another run which will force an unload of the aborted plate(s)

System-initiated

- Power interruption
- Plate loaded incorrectly
- Equipment malfunction

The system will abort the processing. Follow the instructions displayed in the user interface.

User-initiated

Can abort processing of individual array plates.

If multiple plates are being processed, the gripper may continue to process the remaining array plates.

Fragmentation Quality Control Gel Protocol

Protocol for Running a Fragmentation Quality Control Gel

Equipment Required

Table A.1 Equipment required

Item	Supplier	Part Number
Gel imager	Your choice	—
Pipette, multi- or single-channel P20	Your choice	—
Plate centrifuge	Your choice	—
Vortex	Your choice	—

E-Gels and Reagents

Table A.2 E-Gel and reagents required

Item	Supplier	Part Number
Mother E-Base Device	Life Technologies (formerly Invitrogen)	EB-M03
Daughter E-Base Device		EB-D03
E-Gel® 48 4% agarose gels		G8008-04
TrackIt 25 bp DNA Ladder		10488-022
TrackIt Cyan/Orange Loading Buffer		10482-028

Consumables

Table A.3 Gel and reagents required

Item	Supplier	Part Number
Adhesive film – use one of the following:		
■ MicroAmp Clear Adhesive Film	Applied Biosystems	4306311
■ Microseal 'B' Film	Bio-Rad	MSB1001
Pipette Tips	Same brand as pipette	—

Diluting the TrackIt Cyan/Orange Loading Buffer

The following recipe is for preparing a 1000-fold dilution of the TrackIt Cyan-Orange Loading Buffer.

To Dilute the TrackIt Cyan/Orange Loading Buffer:

1. Add 50 μ L of TrackIt Cyan/Orange Loading Buffer to 49.95 mL nuclease-free water.
Total volume 50 mL.
2. Mix well.
3. Store at room temperature.

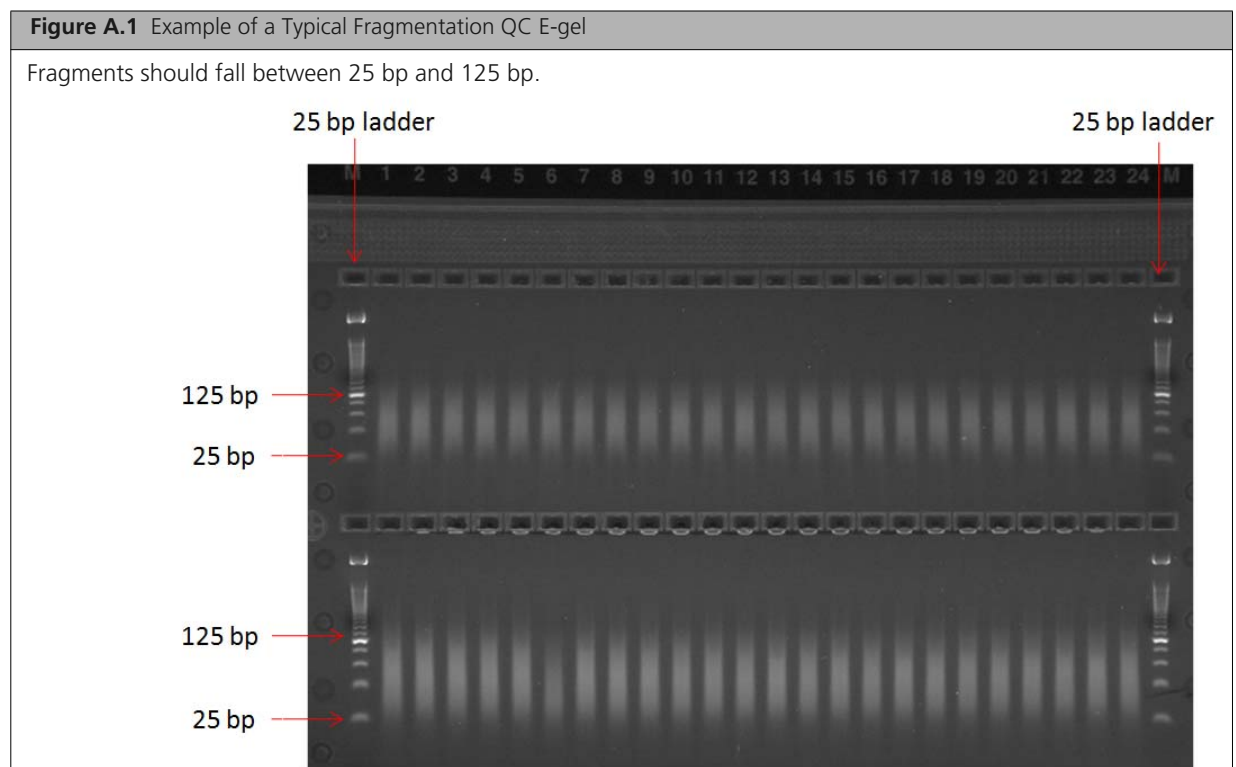
Fragmentation QC Gel Protocol

This protocol is based on running QC gels for 96 samples.

To Run a Fragmentation QC Gel:

1. Tightly seal the gel QC plate prepared during automated or manual target preparation.
2. Vortex the center of the plate for 3 sec, and spin at 2000 rpm for 30 sec.
3. Power on two E-Bases.
4. Push the Power/Prg button on each to ensure the program is in EG mode (not EP mode).
5. Place the E-Gels onto the base units.
6. Remove two combs from each gel.
7. Load 20 μ L from each well of the gel QC plate onto the gels.
8. Load 15 μ L of diluted TrackIt 25 bp ladder into the marker wells (M).
9. Load 20 μ L nuclease-free water into any unused wells.
10. Run the gels for 22 min.
11. Take a gel image.

Fragmentation QC gel images should look similar to the gel shown in [Figure A.1](#).



Sample Quantitation after Resuspension

Protocol for Sample Quantitation after Resuspension

Equipment Required

The following equipment is required for this protocol.

Table B.1 Equipment required for sample quantitation after resuspension

Quantity	Item
1	DTX 880 Multimode Detector with Genomic Filter Slide

Quantitate the Diluted Samples

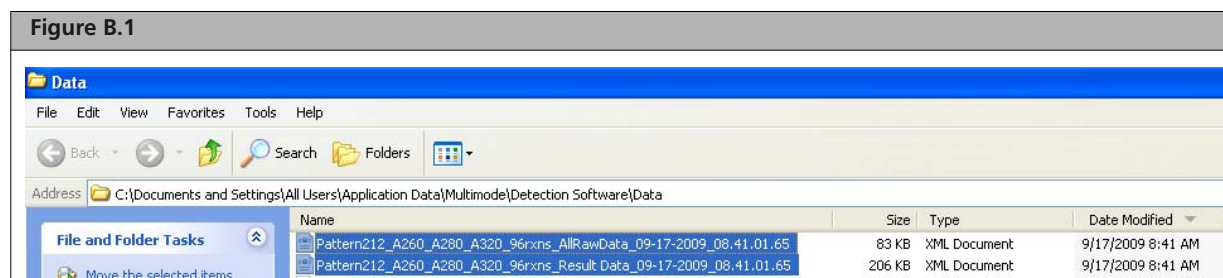
During target prep, two plates of diluted samples are prepared: one for OD quantitation and one for a QC gel to check the fragmentation reaction.

For OD quantitation, readings should be taken at wavelengths of 260, 280, and 320 nm. See [Suggested Protocol for OD Quantitation Using the DTX 880 on page 118](#) for more information.

To Quantitate the Diluted Samples Prepared for OD Quantitation:

1. Launch the *Multimode Analysis Software*.
2. When the **Protocol Selection List** is displayed, select the appropriate protocol.
3. Right click the protocol and select **Run the selected protocol**.
4. In the **Result Name** field, enter your experiment name.
5. Click the **Eject Plate Carrier** icon.
6. Load the OD plate onto the DTX 880.
7. Click the **Close Plate Carrier** icon.
8. Click the **Run the Selected Protocol** icon at the bottom of the window.

When the protocol is finished running, a list of results is displayed. If you used the formula provided in this appendix, two XML files are generated ([Figure B.1](#)). Open the ResultData file with Microsoft Excel to view and assess the OD readings. RawData file information is included in the ResultData file.



Assess the OD Readings

If using the formula provided in this appendix, the raw data is included in the final Result Data file. [Figure B.2](#) is an example of a Result Data file. Your OD readings should be similar to those displayed below.

Figure B.2 Example of Result Data file with acceptable OD readings

Well	Layout	REDUCTION_A1 - Abs260	REDUCTION_A2 - Abs280 ()	REDUCTION_A3 - Abs320 ()	REDUCTION_A4 - Purity ()	REDUCTION_A5 - Concentration	REDUCTION_A6 - Mass/rxn (ug)
A1	S1	0.5634	0.3138	0.0493	1.7954	10.6366	1223.2034
A2	S2	0.58	0.3195	0.0487	1.8153	10.9924	1264.1276
A3	S3	0.5757	0.3177	0.0494	1.8121	10.889	1252.231
A4	S4	0.5792	0.3204	0.0467	1.8077	11.0172	1266.9828
A5	S5	0.5693	0.3136	0.0496	1.8154	10.7524	1236.5276
A6	S6	0.5653	0.315	0.0534	1.7946	10.591	1217.969
A7	S7	0.6072	0.3394	0.0488	1.789	11.5531	1328.6069
A8	S8	0.595	0.329	0.0489	1.8085	11.2986	1299.3414
A9	S9	0.5921	0.3279	0.0498	1.8057	11.22	1290.3
A10	S10	0.6149	0.3413	0.0502	1.8016	11.6834	1343.5966
A11	S11	0.6103	0.3377	0.0497	1.8072	11.5986	1333.8414
A12	S12	0.5984	0.3309	0.0498	1.8084	11.3503	1305.2897
B1	S13	0.5786	0.3229	0.0522	1.7919	10.891	1252.469
B2	S14	0.5757	0.3208	0.0522	1.7946	10.831	1245.569
B3	S15	0.5501	0.305	0.0491	1.8036	10.3655	1192.0345
B4	S16	0.5415	0.2987	0.0505	1.8129	10.1586	1168.2414
B5	S17	0.5084	0.282	0.0485	1.8028	9.5152	1094.2448
B6	S18	0.5533	0.3061	0.0491	1.8076	10.4317	1199.6483
B7	S19	0.5502	0.304	0.052	1.8099	10.3076	1185.3724
B8	S20	0.5776	0.3187	0.0498	1.8124	10.92	1255.8
B9	S21	0.5673	0.3136	0.0535	1.809	10.6303	1222.4897
B10	S22	0.5602	0.3102	0.0493	1.8059	10.5703	1215.5897
B11	S23	0.5814	0.3206	0.0499	1.8135	10.9966	1264.6034
B12	S24	0.583	0.3235	0.0524	1.8022	10.9779	1262.4621
C1	S25	0.5424	0.3009	0.0475	1.8026	10.2393	1177.5207
C2	S26	0.5375	0.2973	0.0472	1.8079	10.1441	1166.5759
C3	S27	0.5196	0.2868	0.0473	1.8117	9.7717	1123.7483

OD Yield Assessment Guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the Axiom® 2.0 Assay. If the median yield for the plate is < 1000 µg DNA per sample:

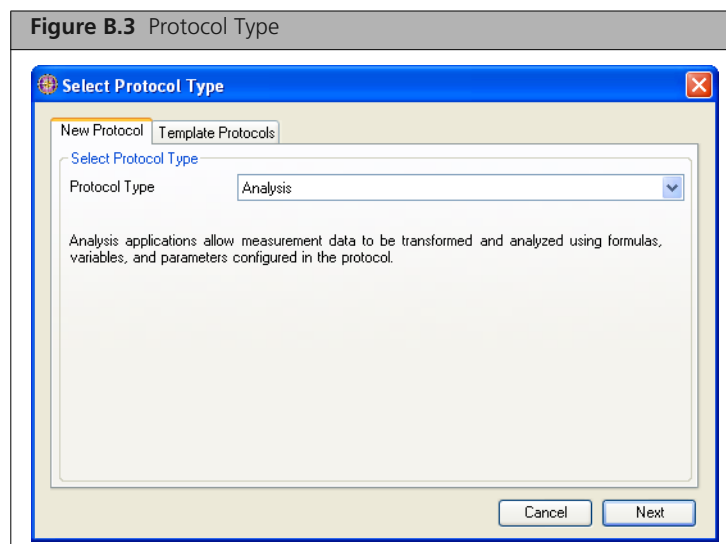
- Pause the protocol.
- Assess each of the steps performed to that point to determine the possible source of the low yields.

This DNA yield corresponds to an A260 value of approximately 0.49 and an A260-A320 value of approximately 0.42.

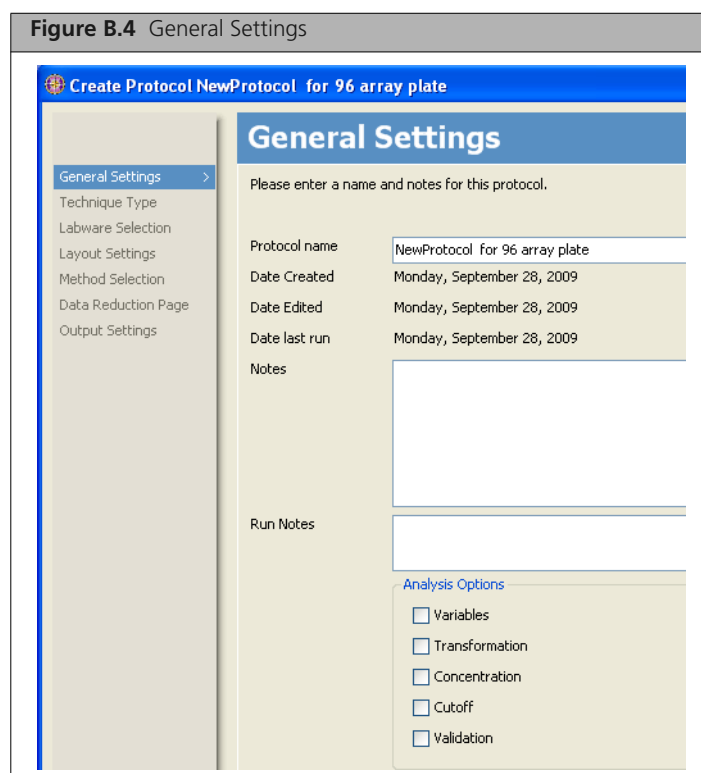
Suggested Protocol for OD Quantitation Using the DTX 880

The formula suggested below requires six passes. The settings and formula are shown below.

Protocol Type — Analysis



General Settings — enter a name for the protocol



Technique Type — select Absorbance.

Figure B.5 Technique Type

Technique Type

Select the desired technique type from the list below.

Technique Type

- ☒ Absorbance
- ☐ Luminescence
- ☐ FRET
- ☐ Fluorescence Intensity Top
- ☐ Fluorescence Intensity Bottom
- ☐ Fluorescence Polarization
- ☐ Time Resolved Fluorescence

Labware — x_Abs_Greiner 96 UV clear std (96 microplate format)

Figure B.6 Labware

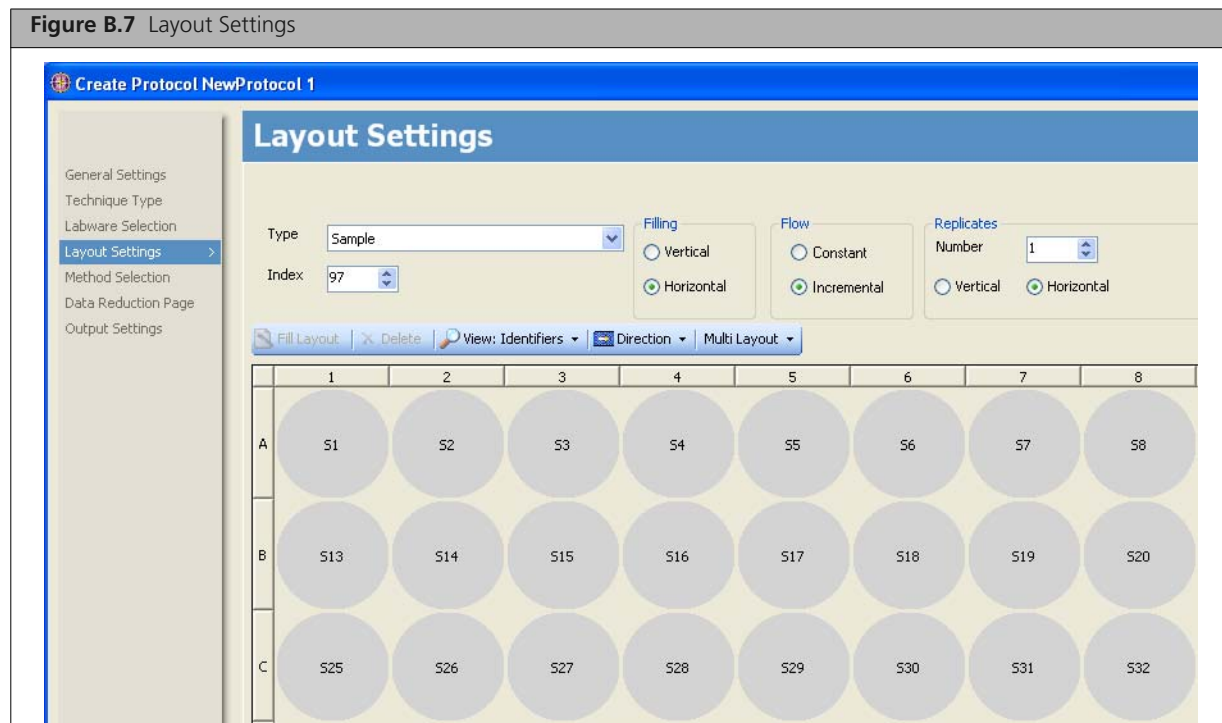
Labware Selection

Select the desired labware type from the list below.

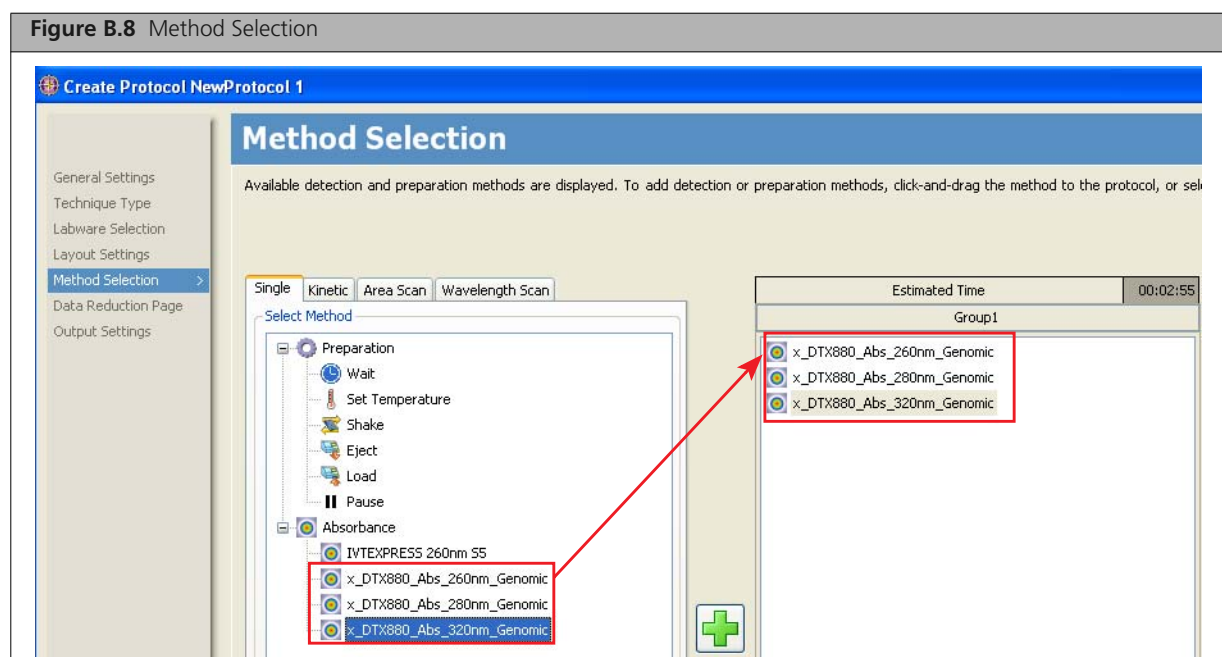
Type of Labware

Name	Microplate Format
Standard 96	96
Standard 384	384
Standard 1536	1536
x_DTX_Abs_Greiner 384 VIS clear std	384
x_Abs_Greiner 96 VIS clear std	96
x_Abs_Greiner 96 UV clear std	96

Layout Settings — as appropriate for 96-array plates



Method Selection — add (+) the three formulas created on the Data Reduction Page to the Group 1 box.



Data Reduction Page — create the formulas required for scans at 260, 280 and 320

This protocol consists of six passes. Click **Add new Pass** to create passes two through six, shown in these figures below.

Figure B.9 Data Reduction Page — First Pass

Create Protocol NewProtocol 1

Data Reduction Page

Press F1 for more information about data reduction functions and formulas.

General Settings
Technique Type
Labware Selection
Layout Settings
Method Selection
Data Reduction Page >
Output Settings

Group1

- A = x_DTX880_Abs_260nm_Genomic
- B = x_DTX880_Abs_280nm_Genomic
- C = x_DTX880_Abs_320nm_Genomic

First Pass Second Pass Third Pass Fourth Pass Fifth Pass Sixth Pass

REDUCTION_A1

Formula

A1

Formula

A

Name of Data Abs_260

Name of Units

Notes

Apply Formula for Wells with Category

☒ Sample

Figure B.10 Data Reduction Page — Second Pass

Create Protocol NewProtocol 1

Data Reduction Page

Press F1 for more information about data reduction functions and formulas.

General Settings
Technique Type
Labware Selection
Layout Settings
Method Selection
Data Reduction Page >
Output Settings

Group1

- A = x_DTX880_Abs_260nm_Genomic
- B = x_DTX880_Abs_280nm_Genomic
- C = x_DTX880_Abs_320nm_Genomic

First Pass Second Pass Third Pass Fourth Pass Fifth Pass Sixth Pass

REDUCTION_A2

Formula

A2

Formula

B

Name of Data Abs_280

Name of Units

Notes

Apply Formula for Wells with Category

☒ Sample

Figure B.11 Data Reduction Page — Third Pass

Create Protocol NewProtocol 1

Data Reduction Page

Press F1 for more information about data reduction functions and formulas.

General Settings
Technique Type
Labware Selection
Layout Settings
Method Selection
Data Reduction Page >
Output Settings

Group1

- A = x_DTX880_Abs_260nm_Genomic
- B = x_DTX880_Abs_280nm_Genomic
- C = x_DTX880_Abs_320nm_Genomic

First Pass Second Pass **Third Pass** Fourth Pass Fifth Pass Sixth Pass

REDUCTION_A3

Formula

Formula

C

Name of Data Abs_320

Name of Units

Notes

Apply Formula for Wells with Category

☒ Sample

Figure B.12 Data Reduction Page — Fourth Pass

Create Protocol NewProtocol 1

Data Reduction Page

Press F1 for more information about data reduction functions and formulas.

General Settings
Technique Type
Labware Selection
Layout Settings
Method Selection
Data Reduction Page >
Output Settings

Group1

- A = x_DTX880_Abs_260nm_Genomic
- B = x_DTX880_Abs_280nm_Genomic
- C = x_DTX880_Abs_320nm_Genomic

First Pass Second Pass Third Pass **Fourth Pass** Fifth Pass Sixth Pass

REDUCTION_A4

Formula

Formula

$((A-C)/0.29)*120*0.05$

Name of Data Concentration

Name of Units ug/L

Notes

0.29 = Pathlength
120 = Dilution Factor
0.05 = DNA extinction coefficient

Apply Formula for Wells with Category

☒ Sample

Figure B.13 Data Reduction Page — Fifth Pass

Create Protocol NewProtocol 1

Data Reduction Page

Press F1 for more information about data reduction functions and formulas.

General Settings
Technique Type
Labware Selection
Layout Settings
Method Selection
Data Reduction Page >
Output Settings

Group1

- A = x_DTX880_Abs_260nm_Genomic
- B = x_DTX880_Abs_280nm_Genomic
- C = x_DTX880_Abs_320nm_Genomic

First Pass Second Pass Third Pass Fourth Pass **Fifth Pass** Sixth Pass

REDUCTION_A5

Formula

A5

Formula
A/B

Name of Data Purity

Name of Units

Notes

Apply Formula for Wells with Category

☒ Sample

Figure B.14 Data Reduction Page — Sixth Pass

Create Protocol NewProtocol 1

Data Reduction Page

Press F1 for more information about data reduction functions and formulas.

General Settings
Technique Type
Labware Selection
Layout Settings
Method Selection
Data Reduction Page >
Output Settings

Group1

- A = x_DTX880_Abs_260nm_Genomic
- B = x_DTX880_Abs_280nm_Genomic
- C = x_DTX880_Abs_320nm_Genomic

First Pass Second Pass Third Pass Fourth Pass Fifth Pass **Sixth Pass**

REDUCTION_A6

Formula

A6

Formula
 $((A-C)/0.29)*690$

Name of Data Mass/rxn

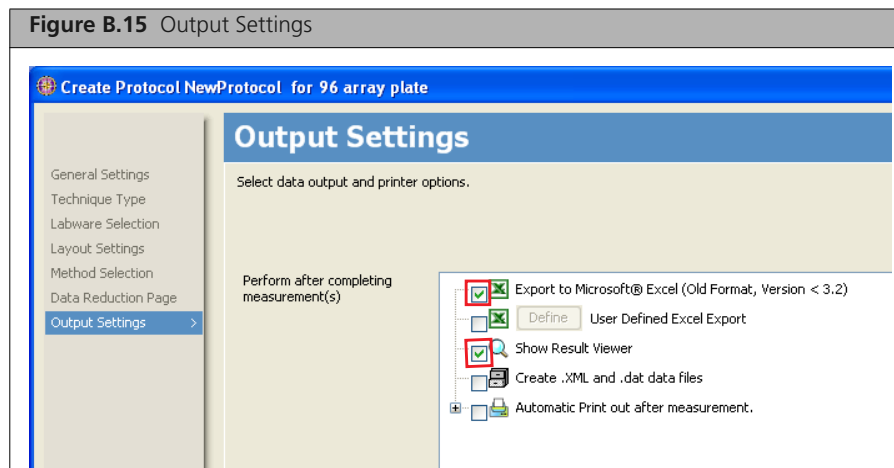
Name of Units ug

Notes
690 = 120 x 0.05 x 115
115 = volume per reaction of resuspended pellet and Hyb Mix

Apply Formula for Wells with Category

☒ Sample

Output Settings — Select Export to Microsoft® Excel and Show Result Viewer



Save the protocol.

If Performing Sample Quantitation on a Plate Reader Other than the DTX880

Your plate reader should be calibrated to ensure accurate readings.

The total yield in μg per well can be calculated as:

$$(A - C) * D * V * E / P$$

Where:

A = the observed OD260

C = the observed OD320 (an estimate of a blank reading)

D = 120 (the net dilution factor when preparing the OD Sample plate as described in the Automated or Manual protocol)

V = 115 (the volume of the sample in μL after the resuspension step)

E = 0.05 (the extinction coefficient of duplex DNA at 260 nm)

P = the optical path length for the plate type and plate reader used.

If your plate reader does not record the OD320, the OD260 of a blank solution of water only should be used for the parameter “B” above.

The optical path length is dependent on the type of plate and spectrophotometer used. Check your manufacturer's recommendations for the path length for your instrument and plate type or for recommendations on how to measure this quantity. The SpectraMax Plus384, described as an alternative spectrophotometer in the *Axiom® 2.0 Assay Manual Workflow Site Prep Guide*, P/N 702991, can employ an automated path length detection system. Consult this instrument's manual for more information.

The resulting yield calculations can be compared against the typical yields shown in column H of [Figure B.2 on page 117](#) and against *OD Yield Assessment Guidelines on page 117*.

Rehybridization

Protocol for Rehybridizing Samples

Rehybridization

The target prep rehybridization protocol is used to help identify potential sources of problem in sample failures.

You may occasionally encounter sub-optimal performance resulting in sample failures with the Axiom Genotyping System. Failures that cannot be attributed to instrumentation issues may be due to either sample quality and/or reagent and array issues.

To help isolate the potential cause of the problem, you may choose to perform a target prep re-hybridization protocol. This is a protocol by which customers can re-process previously used hybridization cocktails (that has been stored at -20°C) in combination with a new array. The results of this re-hybridization can indicate if the original sub-optimal performance was due to issues associated with the array, array processing, the DNA target prep, or reagent quality.

Table C.1 shows conclusions that can be drawn from the results of the re-hybridization of Axiom 2.0 Assay hybridization cocktail to the Axiom Genome-Wide Arrays.

Table C.1 Conclusions that can be drawn from the results of the re-hybridization of Axiom Genotyping Assay hybridization cocktail to the Axiom Genome-Wide Arrays.

Results of Re-hybridization	Details	Conclusion
Pass/Significant improvement over original hybridization	Re-hybridization of hyb cocktail results in data that passes performance metrics and is significantly better than original hybridization	Problems in original hyb were not due to target prep. Possibly due to the array, array processing, and/or reagents used for array processing.
Fail/No improvement over original hybridization	Re-hybridization of hyb cocktail results in data that is not significantly better than the original hybridization.	Inconclusive. Target Prep may be suspect however cannot exclude problems with array and reagents, or array processing issues.

Affymetrix has not established an upper limit for how long the hybridization tray can be stored at -20°C prior to re-hybridization. We have been successful in re-hybridizing plates that have been stored up to 11 days but individual results may vary as success depends on the quality of the original target prep and amount of target available for re-hybridization.

Equipment, Consumables and Reagents Required

Equipment and Consumables Required

- Multi-channel pipette and tips, P200
- PCR plate, BIO-RAD, P/N HSP9631
- or
- PCR Plate HSS-9601 plate for use with manual target prep
- Axiom® Genome-Wide or Custom myDesign™ Array Plate Kit (96 array)
- Axiom® GeneTitan® Consumables Kit

Reagents

- Modules 3 and 4 of the Axiom 2.0 Reagent Kit
- Water, nuclease-free ultrapure molecular-biology grade

Storing Hyb Trays for Rehybridization

To Store a Hyb Tray:

1. When processing on the GeneTitan MC Instrument is complete, unload the hyb tray and tightly seal it with an adhesive film.
2. Press the four corners and sides of the hyb tray to ensure that there is no open space between the seal and the plate.
The plate must be well-sealed to prevent cross-contamination between samples.
3. Store the hyb tray at -20°C .

Rehybridizing an Experiment



NOTE: It is recommended to perform these steps under a fume hood.

To Rehybridize an Experiment:

1. Remove the plate from the -20°C freezer, and spin briefly to collect all of the liquid to the bottom of the plate (bring up to 1000 rpm).
2. Slowly and carefully remove the seal to prevent cross-contamination.
3. Using a multichannel pipette, transfer the full volume from each well of the hyb tray to the corresponding wells of a new PCR plate.
Volume per well should be $\sim 45\ \mu\text{L}$.
4. To recover all of the remaining material from the hyb tray:
 - A. Add $50\ \mu\text{L}$ of molecular biology grade water to each well using a multichannel pipette.
 - B. Pipet up and down 10 times to mix, rinsing each corner of the well as you mix.
 - C. Transfer the full volume from each well to the corresponding wells of the PCR plate.
5. Tightly seal the PCR plate with an adhesive film.
6. Vortex each corner of the plate at maximum speed.
7. Spin briefly again to collect all of the liquid to the bottom of wells.
8. Perform denaturation and transfer samples to a new hybridization tray:
 - If using the Biomek FX^P Target Prep Express, run these methods:
 - *Denature samples*
 - *Transfer denatured samples to Hyb Tray*
 - If using the Manual Target Prep Workflow:
 - Perform Denaturation and Hybridization (see [Stage 4 — Denaturation and Hybridization on page 50](#))



IMPORTANT: Place a new hyb tray on the deck.

9. Transfer the hyb tray to the GeneTitan MC Instrument and:
 - A. Load the hyb tray and a new array plate onto the GeneTitan MC Instrument.
 - B. When hybridization is complete, prepare a new set of reagent trays using either:
 - The Biomek FX^P Target Prep Express.
 - Manual Reagent Tray Prep (see [Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument on page 55](#))
 - C. Transfer the reagent trays to the GeneTitan MC Instrument and finish processing the array plate.

Registering Samples in Affymetrix GeneChip® Command Console®

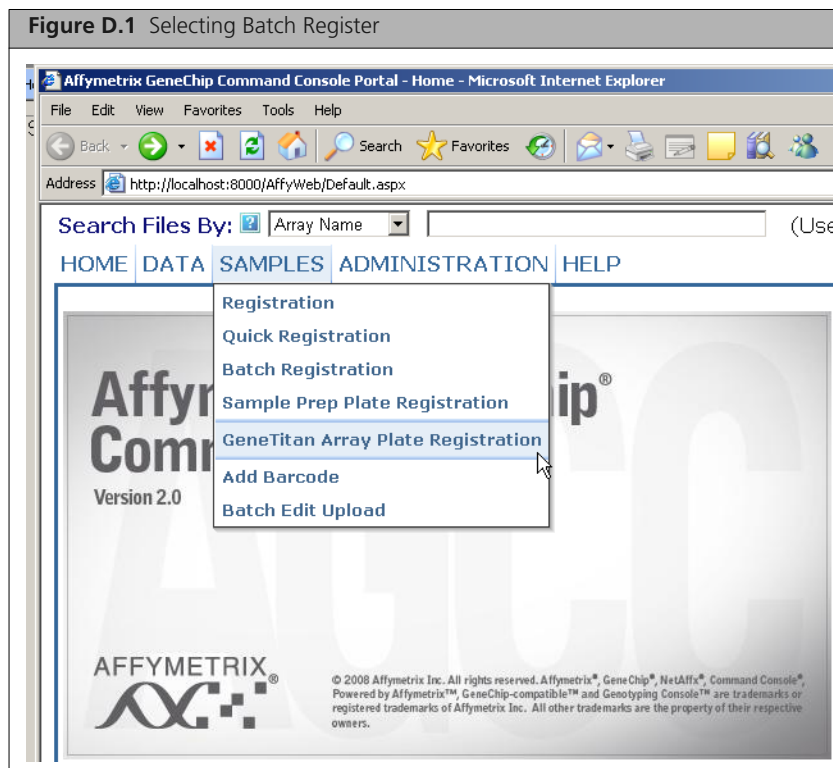
Creating a GeneTitan® Array Plate Registration File

A GeneTitan Array Plate Registration file is a Microsoft Excel spreadsheet that includes information on the samples you are processing on a single array plate. This information includes the array plate format, the array plate barcode, and sample file names so that you can track the samples that are loaded onto a particular array plate.

The version of Microsoft Excel must be 1997-2000 (file extension is .xls; not .xlsx).

To Create a GeneTitan Array Plate Registration File:

1. In AGCC Portal, open the **Samples** menu and select **GeneTitan Array Plate Registration**.



2. Step 1 — [Figure D.2 on page 128](#):

- A. Select the array plate type.
- B. Click **Download**.

Figure D.2 Selecting the type of array plate to be processed

HOME | **DATA** | **SAMPLES** | **ADMINISTRATION** | **HELP**

GeneTitan Array Plate Registration ⓘ ?

Step 1: Create a blank GeneTitan Array Plate registration file with the desired attributes

Select the templates with the attributes you wish to use for the sample files. ⓘ

- ☐ MIAME Sample Information
- ☐ Pedigree Template

GeneTitan Array Plate Type (Required): Axiom_GW_Hu_SNP-96

Project where to create samples: Default

Download

3. Step 2 — complete the registration file as follows:
 - A. Click the Microsoft Excel box on the bottom bar of the monitor to open the Excel spreadsheet.
 - B. Enter a unique name for each sample (Sample File Name) and any additional information you would like to include.
 - C. Do one of the following:
 - If you are ready to load the array plate onto the GeneTitan MC Instrument, scan the array plate barcode and proceed to the next step.
 - If you are not ready to load the array plate onto the GeneTitan MC Instrument, proceed directly to the next step.

Figure D.3 Entering sample information into a Batch Registration file.

	A	B	C	D	E	F	G	H
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array	Barcode	Sample File Name	Array Name
2		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A01		Sample A01	Sample A01
3		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A02		Sample A02	Sample A02
4		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A03		Sample A03	Sample A03
5		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A04		Sample A04	Sample A04

4. Save the file as follows:
 - A. Open **File** → **Save As**.
 - B. Enter a name for the array plate registration file.
 - C. Click **Save**.
By default, the file is saved in the Affymetrix_Downloads folder.
5. Step 3 — when ready to load the array plate onto the GeneTitan MC Instrument:
 - A. Click the **Browse** button, navigate to the file, and click **Open**.
 - B. Scan the array plate barcode if not already scanned.
 - C. Click the **Upload** button (Figure D.4), wait for the information to load, then click the **Save** button located at the *bottom* of the next page that is displayed.
If the samples are successfully registered, the message in Figure D.5 is displayed.

Figure D.4 Uploading the array plate registration file to AGCC.

Step 3: Upload the GeneTitan Array Plate registration file to create new sample (.ARR) files.

Enter the path, or click Browse to find the GeneTitan Array Plate registration file. If a plate barcode is not provided in the excel file being uploaded, one MUST be pr
barcode field below.

GeneTitan Array Plate registration file (Required):

C:\Documents and Settings\AFFXUser\Desktop\Affymetrix_Downloads\GeneTitanArrayPlateR

Browse...

GeneTitan Array Plate Barcode:

5500944077806010110488

Upload

Figure D.5 Array plate samples successfully registered.

HOME | DATA | SAMPLES | ADMINISTRATION | HELP

Confirm GeneTitan Arrays Plate Sample Registration -

Registered GeneTitan Array Plate Samples successfully.

Deionization Procedure for GeneTitan Trays and Covers

Use the following technique to destatic GeneTitan MC Instrument stain trays and lids.

! **IMPORTANT:** Except for the HT array tray and the Hybridization Tray, you must deionize all GeneTitan stain trays, stain tray covers and scan tray covers using an anti-static gun. You must do this before you fill the trays with reagents and before you place the covers on the trays. Deionization removes the static electricity. The presence of static electricity on the underside of the cover can cause the gripper to lift the tray along with the tray cover and can result in an aborted run. See [Figure E.1](#), [Figure E.2](#) and [Figure E.3](#).

Deionize the inner surface of each tray and lid:

- The surface of the tray with the wells that will hold reagents.
- The surface of the lid that will cover the reagents.

/// **CAUTION:** Do not deionize the scan tray or hybridization tray.

Figure E.1 Scan Tray with Cover. Deionize only the cover.



Figure E.2 Stain Tray with Cover. Deionize the cover and the tray.



Testing the Anti-Static Gun

Verify that the anti-static gun (P/N 74-0014, [Figure E.3.](#)) is in working condition. You can use the protective cap on the gun to determine if the anti-static gun is releasing ions. The procedure is as follows:

Keep the cap on the gun and press the trigger and release it. Observe the discharge through the viewing slot on the cap of the anti-static gun. There is a visible light observed in the viewing window on the cap when charged ions are discharged. If you cannot see the light, the gun may be un-usable and you should replace it.

Each anti-static gun is capable of 50,000 trigger operations which is sufficient for approximately 200-250 runs on the GeneTitan MC Instrument.

! **IMPORTANT:** Make sure you remove the cap from the gun when you deionize a tray or cover.

Deionization Procedure

The following process provides guidance on how to use the anti-static gun on the stain and scan tray covers only. See [Figure E.3.](#)

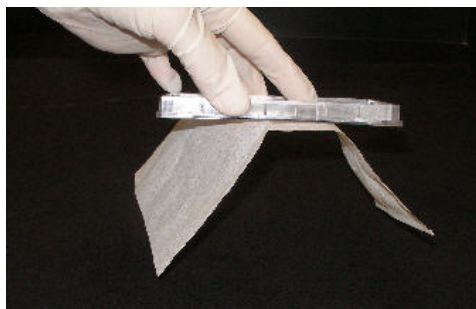
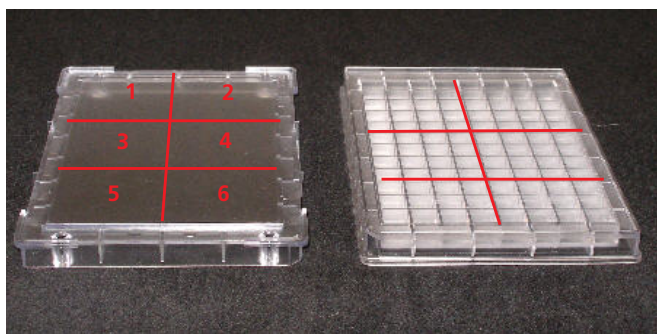
▲ **WARNING:** The deionization steps 4 and 5 will damage the HT arrays on the plate. Before using the anti-static gun, ensure that the HT array plates remain in their protective pouch and placed away from the deionization area. You must place the scan tray and hybridization tray away from the area where you are performing deionization.

1. Treat the plate or lid as if it were divided into 6 sections, and deionize as follows.
2. Place a Kimwipe on the benchtop.
3. Place the stain tray on a table top. Use the anti-static gun to aim at the center of each of the six sections on a 96-well cover or tray and pull the trigger. Ensure that a stream of ionized particles settles on all wells of the stain tray or cover to dissipate the static electricity. Squeeze and release the trigger slowly 3 times over each section (Squeeze for approximately two seconds and release for approximately two seconds).

4. Place the stain tray cover with the flat surface facing upward on the Kimwipe.
- Aim the anti-static gun (P/N 74-0014) approximately one-half inch away from the flat surface and pull the trigger. As you pull the trigger move the gun across the cover so that the stream of ionized particles settles on all areas of the cover and dissipates the static electricity. Squeeze and release the trigger slowly 3 times over each section (Squeeze for approximately two seconds and release for approximately two seconds).
5. Place the treated cover or tray on the Kimwipe and lift it up.
 6. Do one of the following:
 - If the Kimwipe does not adhere to the plastic, proceed with the step.
 - If the Kimwipe adheres to the plastic, then perform steps 3 and 4 again. If it continues to adhere to the plastic, then the gun is not working and you should replace it.

Figure E.3 Removing the Static Charge from Stain Trays and Lids

Treat the inside surface of stain trays (right) and cover (left).



IMPORTANT: Remove static gun cover before use.

If a Kimwipe sticks to treated surface, treat again following this procedure.
If the Kimwipe still adheres, replace the antistatic gun.

GeneTitan® Multi-Channel Instrument Care

This chapter provides instructions on caring for and maintaining the instrument and on troubleshooting if problems arise.

- Always run a Shutdown protocol when the instrument will be off or unused overnight or longer. This will prevent salt crystals from forming within the Fluidics system.
- Always use deionized water to prevent contamination of the lines. Change buffers with freshly prepared buffer at each system startup.

The GeneTitan® MC Instrument should be positioned on a sturdy level bench away from extremes in temperature and away from moving air.



IMPORTANT: Before performing maintenance turn off power to the instrument to avoid injury in case of an electrical malfunction.

Cleaning and Maintenance

The GeneTitan family of instruments require little in the way of customer maintenance. The instruments must be kept clean and free of dust. Dust buildup can degrade performance. Wipe the exterior surfaces clean using a mild dish detergent solution in water. Do not use ammonia based cleaners or organic solvents such as alcohol or acetone to clean the system because they may damage the exterior surfaces.

The following tasks should be performed regularly to ensure the Imaging Device remains in working order.

Monthly

Wipe down the outer surface of the Imaging Device with a dry cloth.

Every Six Months

Replace the cooling fan air filters at the rear of the instrument.

Replace the Micropore filters in the Wash A, Wash B, and Rinse bottles. If you run 4-8 plates/week then the micro-pore filters need to be replaced more frequently.

Servicing the Outer Enclosure Fan Filters

Cleaning Schedule

The GeneTitan fan filter cartridge ([Figure F.1](#)) should be cleaned at least every 90 days of service. Note that in some service locations, the presence of excessive dust or particulate matter may necessitate cleaning the cartridge more often than 90 days.

A plugged filter cartridge can cause excessive temperatures within the machine that can cause unwanted evaporation on test media.

Part details:

Affymetrix P/N: 01-0669

Number of filters required per GeneTitan instrument: 3

Figure F.1 The GeneTitan filter cartridge**Cleaning Procedure**

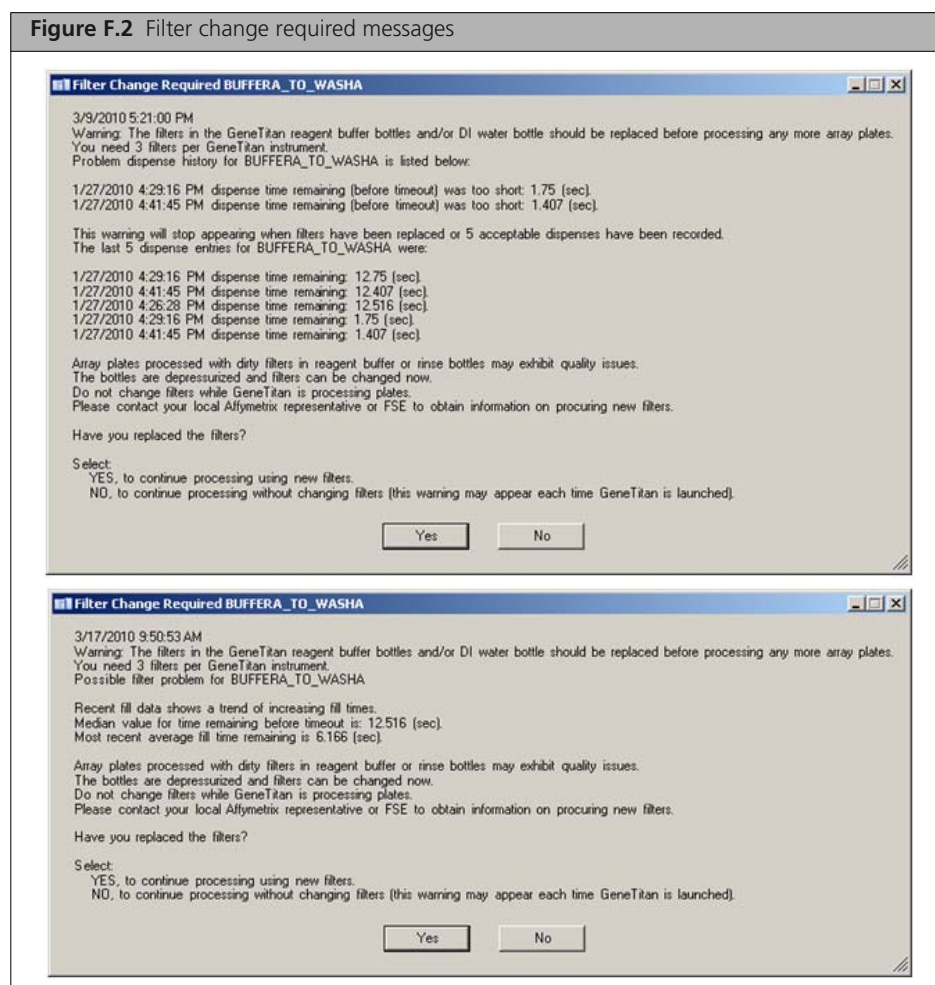
1. Slide the filter cartridge from the fan filter cartridge at the rear of the GeneTitan Instrument.
2. Submerge in clean DI water. Rinse and agitate gently to dislodge material.
3. Remove from water and dry with clean compressed air or towels.
4. When the filter cartridge is completely dry to the touch, re-install the cartridge.

Replacing the Bottle Filters

The bottles used in GeneTitan Instrument contain a filter to remove particulates that may exist in the buffers and DI water. The filters in the GeneTitan fluidics bottles (Wash A, Wash B and DI Water) need to be replaced when the filters are clogged.

The software displays warning message boxes for the filter in each reagent bottle when it detects a problem or shows a trend of increased fill times during fluid fill operations.

If an error is detected as described above, then a message box titled “Filter Change Required” is displayed (Figure F.2) along with the information on the specific dispense operation. You should change all three filters when a warning is displayed for any one of the three filters.



The message boxes displayed in Figure F.2 will provide information on fluid dispense errors that were detected by the instrument for any of the bottles or when the instrument detects an increase in the amount of time that is required to perform the fill operations.



NOTE: The reagent bottles are depressurized when this warning message is displayed. It is safe to change the filters in all three fluidic bottles when this message is displayed.

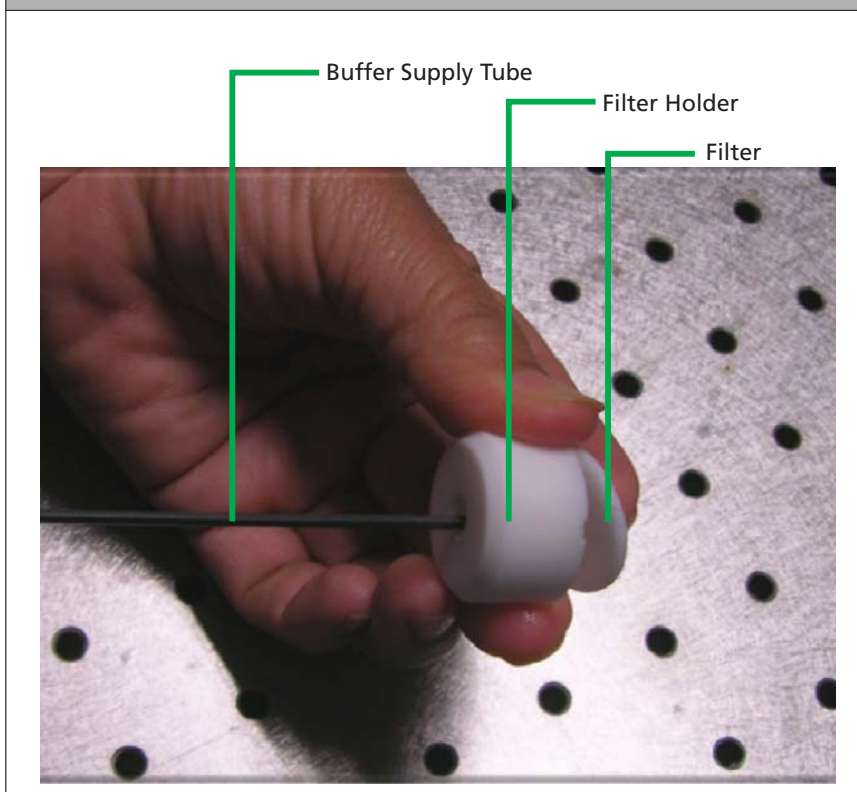
After changing the filters in all three bottles using the procedure described below, please press the **Yes** button to continue. If you choose to ignore the error message, press the **No** button. This warning message will be displayed each time AGCC instrument control software is launched. You may also experience data quality issues if particulate matter cannot be trapped by the filters if they are clogged.

We recommend that your site keep three spare filters in the event they need to be replaced. The procedure for replacing the filters is simple.

Part details:

Affymetrix P/N: 01-0671

Figure F.3 Replacing the Filter



Removing and inspecting the Filter

1. Loosen and remove the cap on the bottle.
2. Carefully remove the filter from the end of the filter body.
3. Visually inspect the filter. If one of the filters appears to have a concentration of dirt or contaminate in it, discard it and replace the filter with a new one.

Replacing the Filter

1. Insert the filter into the end of the filter body.
2. Replace the cap onto the bottle and tighten it.
3. Repeat for each bottle.



IMPORTANT: Replace one filter at a time to ensure the correct connection of the buffer supply tube to its respective bottle. The color of the buffer supply tubing matches the bottle color code.

Replacing the Xenon Lamp in the GeneTitan MC Instrument

This section applies to your site only if you have the GeneTitan Multi-Channel (MC) instrument. After the normal life expectancy of the lamp has expired, the software application will alert you to the requirement to replace the lamp. This procedure is simple but you must follow good health and safety precautions.

Affymetrix Part Number: 01-0740

! IMPORTANT: Please **DO NOT** try to replace the lamp when a plate is being processed either in the fluidics or scanner system.

Lamp Life/Imaging Device Status Notices

The Imaging Status pane displays lamp life and Imaging Device status notices for the GeneTitan MC. In normal operation, the pane displays the hours of life left in the lamp:

Figure F.4 Lamp Life above tolerance

Imaging Device Status	
Barcode	
Estimated Time Remaining	
Lamp Life Remaining	166 hours

It displays a red or yellow notice when the lamp life is getting short:

Figure F.5 Lamp Life above tolerance

Imaging Device Status	
Barcode	
Estimated Time Remaining	
Lamp Life Remaining	1 hours -- Replace lamp as soon as possible

It also displays a red notice when the Imaging Device is offline:

Figure F.6 Imaging Device Offline

Imaging Device Status	
Barcode	
Estimated Time Remaining	
Scanner Status	Offline: scanning is not available.

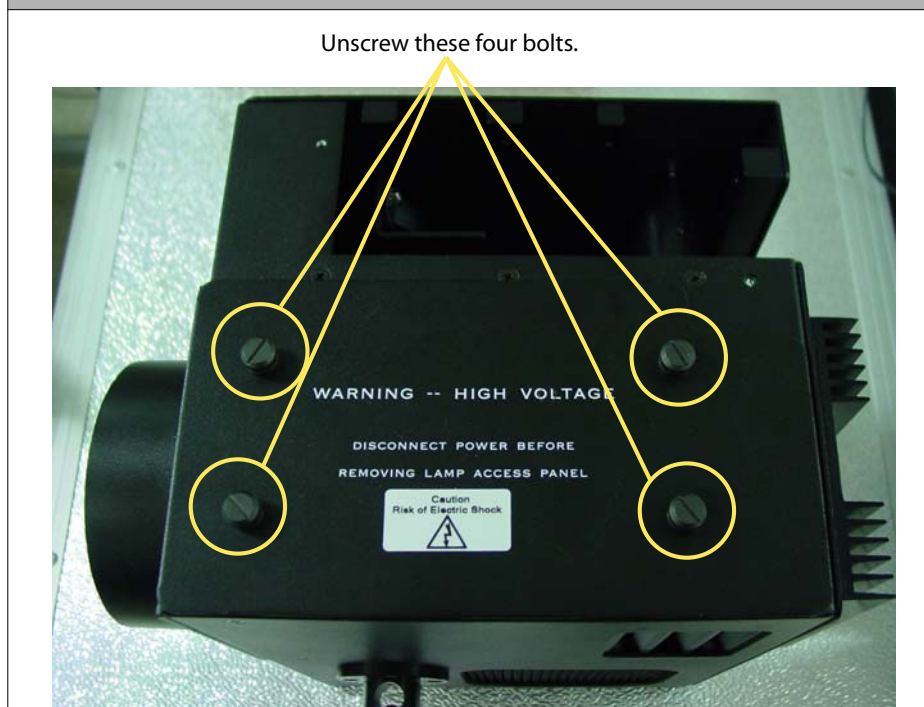
NOTE: The 300 Watt Xenon lamp in the GeneTitan MC instrument is warranted for 500 hours. The instructions to replace the lamp are available on the following page. After changing the lamp, it is necessary to reset the lamp life clock manually.

▲ WARNING: You must turn off the lamp using the power switch in the rear of the unit and remove the power cord. Allow the lamp to cool before attempting to replace the lamp

Removing the Xenon Lamp

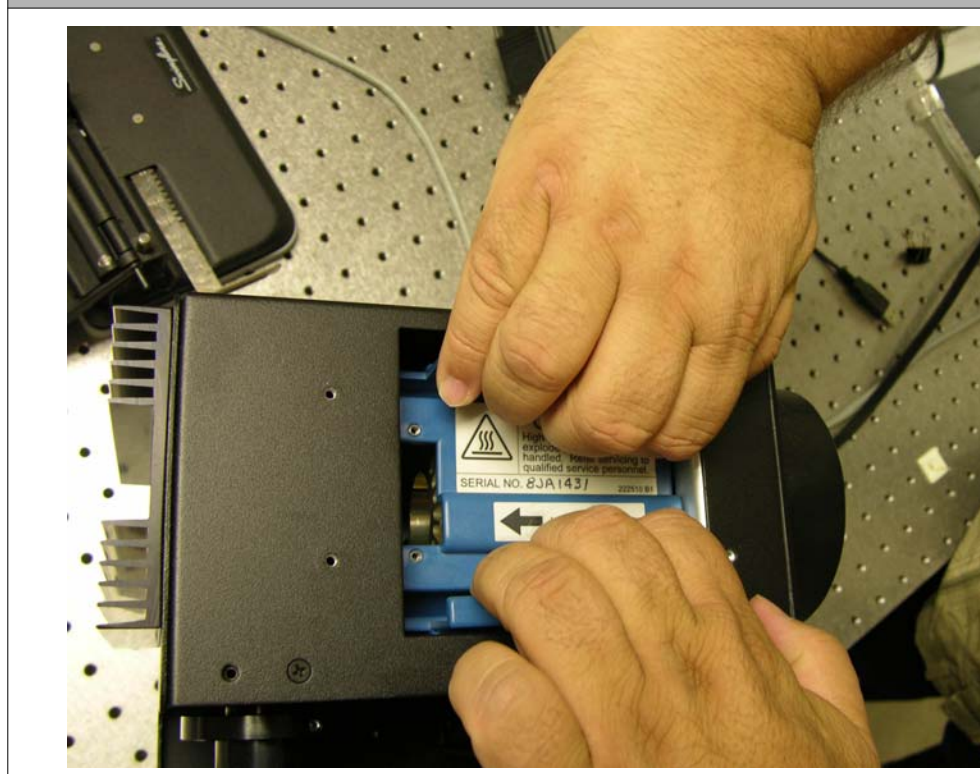
1. Unscrew the four retaining bolts. They should be finger tight ([Figure F.7](#)).

Figure F.7 Unscrewing the Bolts



2. Place each hand on each side of the blue plastic flange and lift out the lamp in a vertical motion ([Figure F.8](#)). You must use both hands to remove the lamp successfully. Apply equal pressure on each side of the lamp and gently lift.

Figure F.8 Lifting out the lamp



Replacing the Lamp



CAUTION: Ensure that you install the lamp in the correct orientation.

1. Hold the lamp by the blue plastic flanges. Ensure that the lamp bulb faces inward toward the reflecting mirror (Figure F.9) and vertically insert the lamp (Figure F.10).
2. Replace the warning cover and hand tighten the bolts (Figure F.7).

Figure F.9 The Reflecting mirror

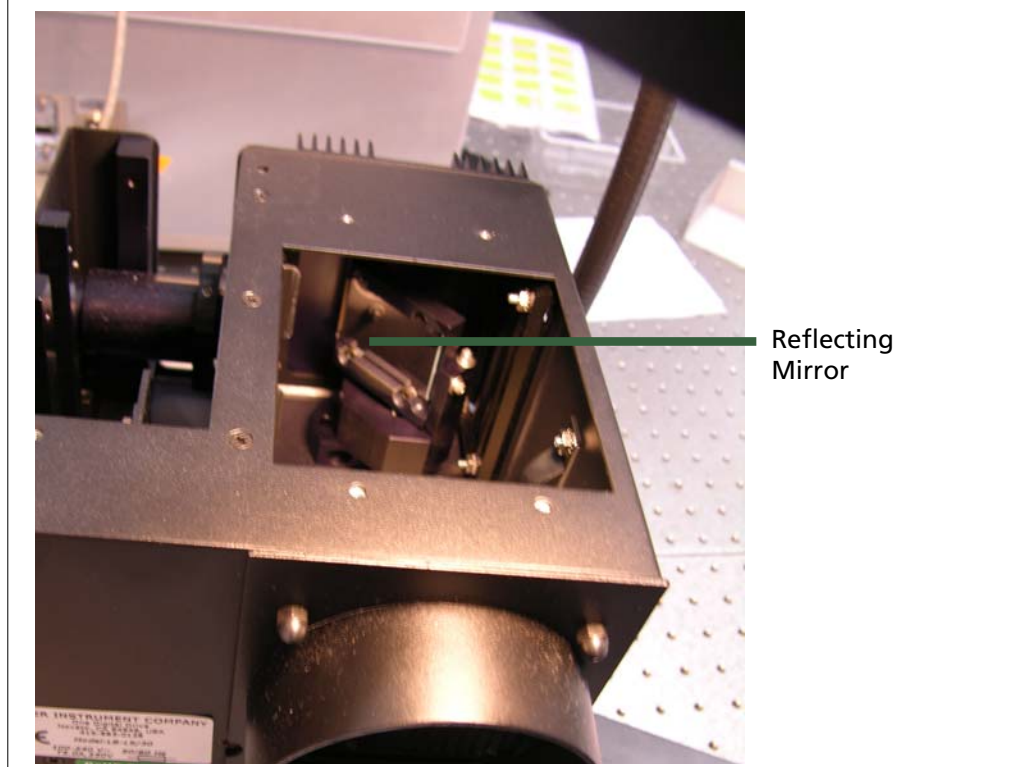
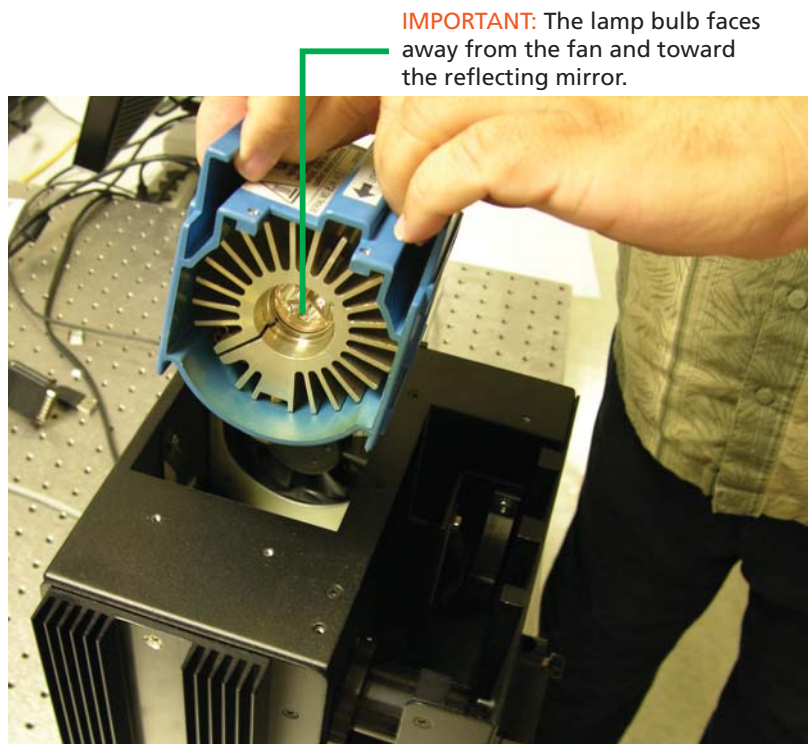


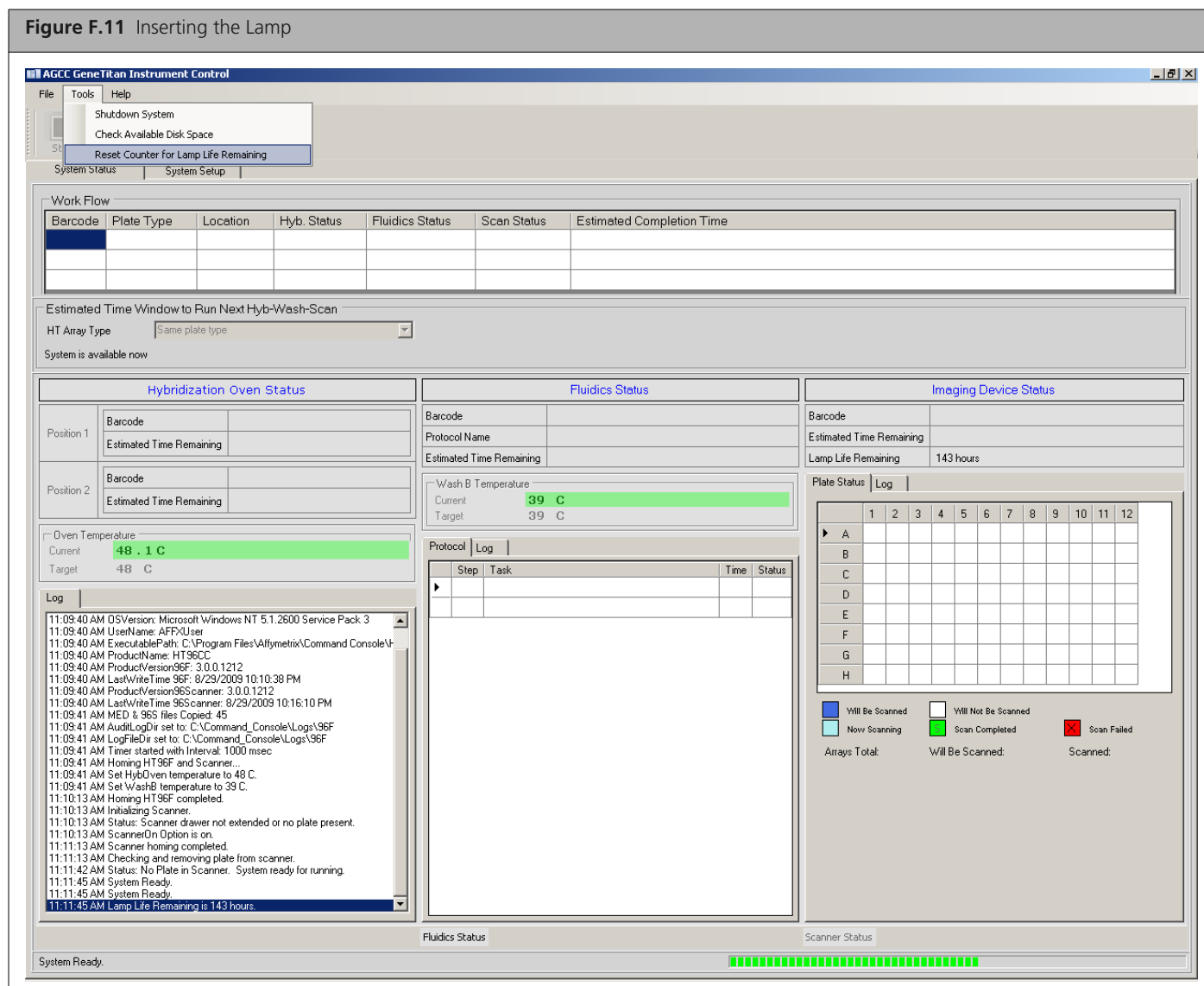
Figure F.10 Inserting the Lamp

Resetting the Lamp Counter

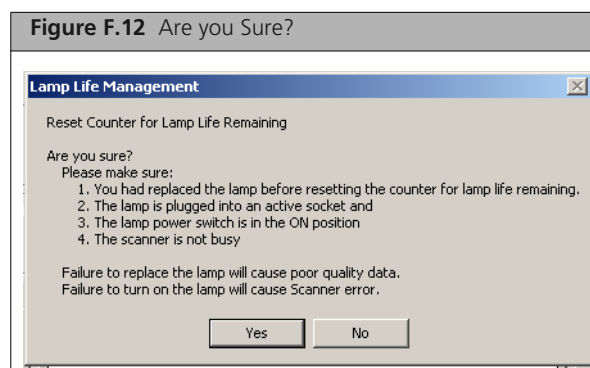
You must alert the software application that you have replaced the lamp so that the hours of the lamp counter are reset to zero. This menu option is only available when the system is not processing any plates.

1. On the software application click **Tools** → **Reset Counter for Life Remaining** (Figure F.11).

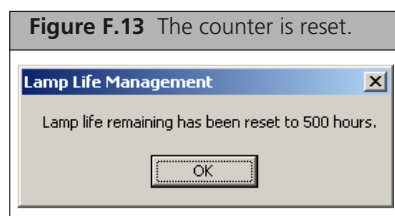
Figure F.11 Inserting the Lamp



2. The software will display a message that allows you to change your mind.



3. Click **Yes** if you want to reset the counter. The software will display a message that confirms that the software has reset the counter (Figure F.13).



Troubleshooting

This section provides instructions on how to identify and solve simple problems with the GeneTitan MC Instrument. If a problem or error occurs that is not listed in this chapter contact a Affymetrix technical support for assistance.

For software errors that do not involve hardware crashes the most common solution is to shut down the application and then restart it. If the same error occurs shut down both the application and the computer and then restart. If it still occurs shut down the GeneTitan MC Instrument and then restart.

Log Files

The log files are produced by different AGCC components. The logs provide a record of the tasks performed by different components, such as the migration tools and installer. These log files provide useful information for troubleshooting problems. These files may be requested by your field application specialist (FAS), field service engineer (FSE), or the Affymetrix call center.

AGCC Log Files

The following files apply to the GeneTitan Instruments. All the AGCC log files from C:\Command_Console\Logs The different log files include:

Systemlog.XML	XML file with system information.
DEC.log	Text file with information on the use of the Data Exchange Console.
DECError.log	Text file with information on errors created while using DEC.
AGCC_LibFileImporter. log (with date and time code)	Text file with info on use of the Library File Importer.

Other AGCC Files

Your FAS and/or FSE may request you to send the following files for troubleshooting:

1. Library files (*.PARAMS, *.MASTER, *.WORKFLOW, *.SMD, *.MEDIA) located in C:\Command_Console\Library, excluding the large analysis library files (CDF, PSI, GRC).
2. Provide a list of all sub folders and their contents under the library files folder located in C:\Command_Console\Library. Please ensure there are no duplicate library files, as these can cause problems.
3. AGCC system configuration file located at C:\Command_Console\Configuration\Calvin.System.config
4. Pending job order files located in C:\Command_Console\Jobs
5. Other AGCC related information, such as:
 - A. The number of files under C:\Command_Console\Data, including sub directory.
 - B. If the system is a networked system or a standalone system.
 - C. Other applications installed on the system, such as antivirus application, MS Office, Internet Explorer versions.

AGCC Log Files for GeneTitan MC Systems

Log files for the GeneTitan MC Instrument control processes are placed in subdirectories of the Command Console\Logs\ folder. Affymetrix may need the following files for troubleshooting:

GeneTitan MC Fluidics

1. C:\Command_Console\Logs\96F\
 - A. subdirectories named by date (e.g., Log7-29-2009)
 - 1) Collect all dated directories and contents since the GeneTitan application was started, not just the date of the event (some logging goes into files from the date the application started so this can be critical for us).
 - 2) Absolutely required are all the log directories from the date the run was started to the date of the event.
2. C:\Command_Console\Logs\96F\FluidicErrorLog - all files in this directory

GeneTitan MC Imaging Device

1. C:\Affymetrix\GeneChipHTScanControlMC\Log - collect all dated directories and contents since the GeneTitan application was started
2. C:\Affymetrix\GeneChipHTScanControlMC\RunLog - collect all dated directories and contents since the GeneTitan application was started

Problems and Solutions

This section provides instructions on how to identify and solve problems with the unit.

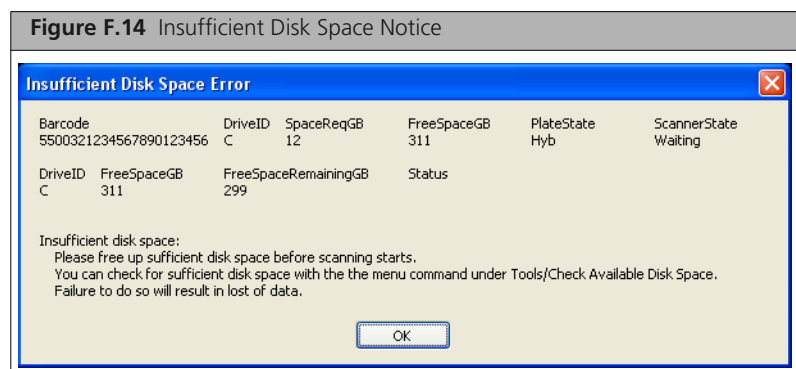
If problems arise with the instruments use the following tables to locate the description that matches the problem. If you cannot find a solution call Affymetrix technical support for assistance.

For software errors that do not involve hardware crashes the most common solution is to shut down the application and then restart it. If the same error occurs shut down both the application and the computer and then restart. If it still occurs shut down the entire unit and then restart.

Insufficient Disk Space Notice

If there is not enough memory on the computer's drives to save the data from an array plate, a notice appears when:

- You first initialize the software and instrument
- You select arrays for imaging.



If you see this notice, you will need to free up sufficient disk space before imaging starts.

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