Affymetrix®

User Manual

Axiom™ Genotyping Assay

For Axiom™ Genome-Wide and
Custom myDesign™ Array Plates

P/N 702830 Rev 4
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About the Axiom™ Genotyping 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.

Affymetrix conducted an empirical screen of genomic content from dbSNP (http://www.ncbi.nlm.nih.gove/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 Genotyping Assay.

Currently two arrays are available for use with the Assay:

- Axiom Genome-Wide CEU 1 Array: optimized to provide high genetic coverage (r2 >= 0.8) in Northern European populations (often referred to as Caucasian or CEU), with lower coverage in ASI and YRI populations.
- Axiom Genome-Wide ASI 1Array: optimized to provide high genetic coverage (r2 > 0.8) in East Asian populations (specifically the HapMap populations of Han Chinese and Tokyo Japanese), with lower coverage in CEU and YRI populations.

These arrays are the first in a suite of population specific peg arrays that leverages the content of this proprietary Affymetrix database.

The Axiom Genotyping Assay interrogates biallelic SNPs and simple indels 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.
  - Automated target prep uses the Beckman Coulter *Biomek FX^P Target Prep Express* liquid handler.
  - Manual target prep enables lab workers to perform the steps without the use of automation equipment.
- 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 MC Instrument are processed using the Axiom™ Genotyping Algorithm version 1 (Axiom GT1) available through Affymetrix Power Tools or Genotyping Console™ v4.0.

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.
- Utilize the latest content from dbSNP.
- Provide highly automated, reproducible results suitable for GWAS.

Related Documentation

- *Affymetrix GeneTitan® Multi-Channel Instrument User's Guide*, P/N 08-0308
- *Affymetrix GeneTitan® Multi-Channel Instrument Site Preparation Guide*, P/N 08-0305
- *Affymetrix GeneChip® Command Console™ 3.1 User Manual,* P/N 702569
- *Genotyping Console User Guide*
- Axiom gDNA Sample Prep QRC P/N 792928
- Axiom Automated Target Prep QRC Setup P/N 702831
- GeneTitan MC Protocol for Axiom Array Plate Processing QRC P/N 702929

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, 3**08**: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 Assay

Running the Axiom 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 9*.
2. Target Prep of the samples:

   The target prep can be done using either:

   - Biomek Automation (see Chapter 3, *Axiom™ Genotyping Assay: Target Preparation with Biomek FXP Target Prep Instrument on page 15*)
   - Manual Target Prep (see Chapter 4, *Axiom Genotyping Assay: Manual Target Preparation on page 95*)
3. Array Processing, done with
   - GeneTitan MC Instrument
   - GeneTitan Instrument Control software
   - AGCC Portal software

   See Chapter 5, *Axiom™ Genotyping Assay: Array Processing with the GeneTitan® MC Instrument on page 149*.

A list of the required equipment and supplies for running the Axiom Assay using automated target preparation and Manual target preparation can be found in the *Axiom Site Prep Guide*, P/N 702858.

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 GeneTitan 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 GeneTitan.

These limitations require careful timing.

The details are covered in:

- Chapter 6, *Automated Target Preparation for Processing Eight Axiom Array Plates Per Week on page 183*
- Chapter 7, *Automated Target Preparation for Processing Two Axiom Array Plates per Week on page 203*
- Chapter 8, *Manual Target Preparation for Processing Three Axiom Array Plates per Week on page 209*

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.

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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 Reagent Kit (96 reaction, P/N 901281). These kits contain a tube labeled Axiom Reference Genomic DNA 103. 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 Human 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

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

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 OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios. The OD_{260}/OD_{280} ratio should be between 1.8 and 2.0 and the OD_{260}/OD_{230} 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 11*.
- 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.

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

We recommend this quality control step to asses 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
*Redi*Load™		750026
E-Gel® 96 High Range DNA Marker		12352-019

Guidelines for Preparing the Genomic DNA Plate

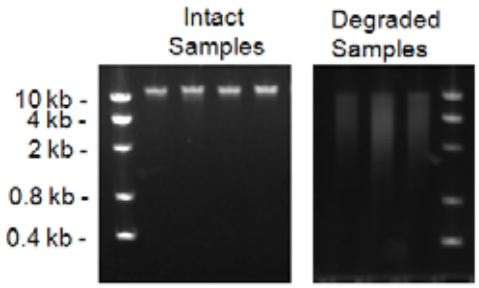
- Loading a DNA mass of 10 ng to 20 ng per well is recommended. If lower amounts are loaded, omission of the loading dye is recommended in order to improve visualization. Loading ≥ 25 ng gDNA per well can improve the image.
- Add 3 µL of 0.1X of *Redi*Load dye to each sample.
- Bring each sample to a total volume of 20 µL using H_2O (for example, if the volume of genomic DNA is 5 µL, add 3 µL of *Redi*Load, and bring to 20 µL total by adding 12 µL of H_2O).
- 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 2*48 well 1% Agarose E-Gel into slot.
4. Remove 2 combs.
5. Load 20 µL from the above plate onto 2*48 well 1% agarose E-Gel.
6. Load 15 µL 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 Gel images showing intact gDNA and degraded gDNA.



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).

   (See the *Axiom Site Preparation Guide* P/N 702858 for reagents, equipment, labware and consumables for Axiom Assay).

Genomic DNA Preparation

This step needs to be done before proceeding with the DNA amplification stages for either automated or manual target prep.

The human genomic DNA (gDNA) you will process using the Axiom Assay should meet the general requirements listed earlier in this chapter. A total of 200 ng gDNA is required.

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: ▪ For Automated Target Prep: Beckman Deep Well Titer, polypropylene; P/N 267007 ▪ For Manual Target Prep: ABGene 96 Square Well Storage; AB-0932
1	Plate centrifuge
1	Plate spectrophotometer (required only if no OD measurements available for samples)
1	Vortexer

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 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)	USB TEKnova	75793 T0223

1. Thaw Samples and Control

Thaw the components listed below to room temperature:

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

To thaw, either:

- Place items on benchtop for one hour
- Thaw in a water bath:

A. 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.

B. Thaw the sealed sample plate and Reference sample for a half-hour.

C. 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:

1. Gently vortex (50% maximum) and spin the gDNA and Reference Genomic DNA 103.
2. *Recommendation*: quantitate each sample (*e.g.,* using the Quant-iT™ PicoGreen® dsDNA Kit).
3. Dilute each sample to a concentration of 10 ng gDNA/µL using reduced EDTA TE buffer.

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

4. Seal, vortex and spin.

3. Aliquot the Diluted Samples and the Control

Next, the samples and control are place in one of the following deep well plates for target preparation:

- For **Automated Target Prep**: Beckman Deep Well Titer, polypropylene; P/N 267007
- For **Manual Target Prep**: ABgene 96 Square Well Storage; AB-0932

Aliquot diluted samples and Reference Genomic DNA 103 to the selected deep well plate as follows:

1. 20 µL of each diluted gDNA sample (this should be the equivalent of 200 ng of gDNA).
2. 20 µL of the Reference Genomic DNA 103 control.
   We recommend including at least one positive control on each plate.
3. Seal and spin.

4. Freeze or Proceed

At this point you can:

- Store the sample plate at –20 °C, or
- Proceed to DNA Amplification for:
  - Automated Target Prep (see Chapter 3, *Axiom™ Genotyping Assay: Target Preparation with Biomek FXP Target Prep Instrument on page 15*)
  - Manual Target Prep (see Chapter 4, *Axiom Genotyping Assay: Manual Target Preparation on page 95*)

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 GeneTitan. 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:

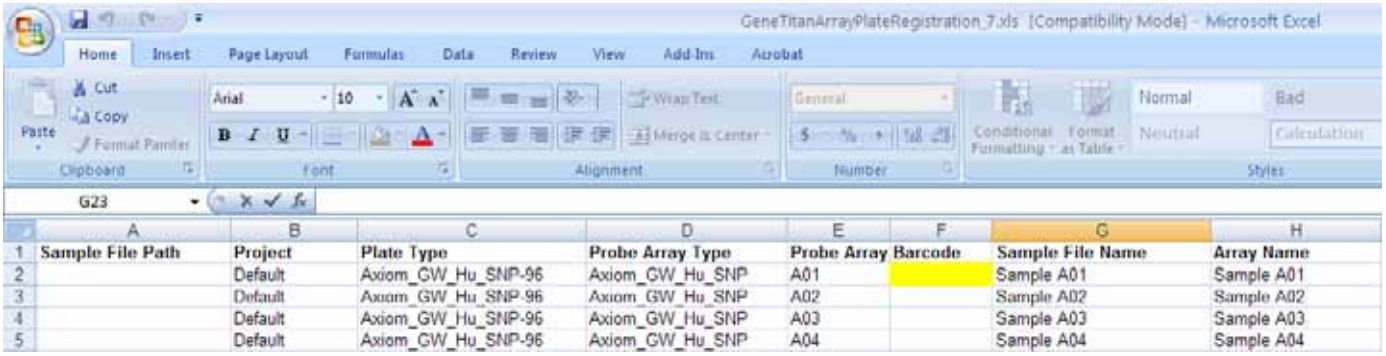
- Data file generation during imaging.
- Tracking the experimental results for each sample loaded onto an array plate.

Detailed instructions for creating this file are located in Appendix D, *Registering Samples in Affymetrix GeneChip® Command Console on page 237*. 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



	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™ Genotyping Assay: Target Preparation with Biomek FX^P Target Prep Instrument

The Axiom™ Genotyping Assay with automated target preparation is designed for processing 96 samples at a time on Axiom™ Genome-Wide and Custom myDesign™ Array Plates. The protocol is performed on two instruments:

- Part 1: Target preparation is performed on the Biomek FX^P Target Prep Express
- Part 2: Array processing is performed on the GeneTitan® Multi-Channel (MC) Instrument

A list of all equipment and resources for the Axiom Genotyping Assay with Automated Target Prep is in the *Axiom Site Preparation Guide* P/N 702858.

This chapter includes instructions for Part 1: target preparation. These instructions are presented as follows:

IMPORTANT: Before proceeding to DNA Amplification, do the gDNA preparation described in **Chapter 2, *Genomic DNA Preparation and Requirements on page 9***.

Before Using the Biomek Workstation

Seal, Vortex and Spin

Unless otherwise stated in the protocol, follow the guidelines below when instructed to seal, vortex and spin plates or reagent tubes for the Biomek FX^P Target Prep Express portion of this assay.

- **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.

- **Vortex:**
  - Reagents 3 times, 1 sec each time at the maximum setting.
  - Plates 1 sec each corner, and 1 sec in the center at the maximum setting.

- **Spin** — when instructed to spin plates or reagent vials, follow these guidelines *unless otherwise instructed* (for example, when centrifuging and drying pellets, *5. Centrifuge and Dry Pellets on page 61*).
  - **Plates**:
    - Spin at 1000 rpm for 1 min at 4 °C.
    - Do not spin for more than 1 min.
  - **Reagent Vials**: 3 sec

Breaking the Light Curtain

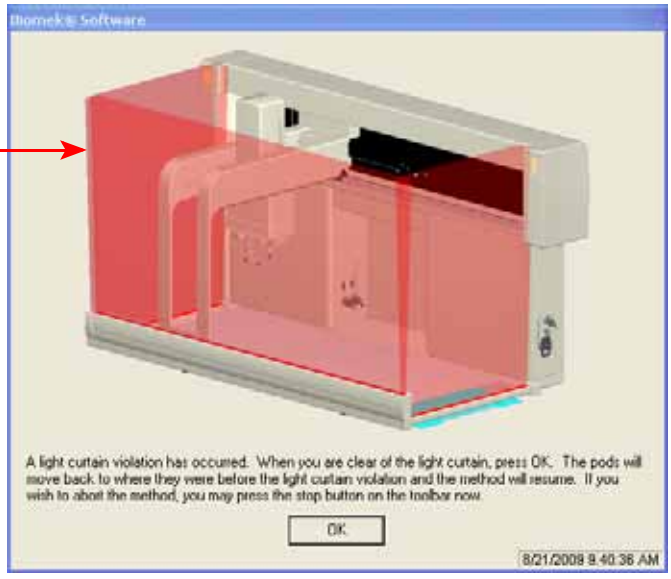
For your safety, the Biomek FX^P Target Prep Express is designed to immediately halt all movement when the light curtain is broken.

Light Curtain Broken While Running the Assay

For your safety, the Biomek FX^P Target Prep Express is equipped with a light curtain (Figure 3.1). The light curtain senses when an object (such as a hand or an arm) enters the space surrounding the deck. When this curtain is broken, all movement on the deck halts until the user either clicks OK to resume the activity that was taking place, or aborts the activity. Incubation timers are not interrupted.

Figure 3.1 Prompt displayed when the light curtain is broken during the process referred to as *Homing All Axes*.

The light curtain covers the front of the instrument. The sides are protected by Plexiglas. Both areas are shown as red in this figure. Any penetration of the light curtain from outside the deck halts all movement of the workstation. To resume activity on the deck, click OK. To abort the step or other activity, click Stop on the toolbar.



Pipette Tip Usage

Table 3.1 Pipette tip usage for one full run of the Axiom™ Genotyping Assay on the Biomek Workstation

Step	Multi-Channel P50, Pink 96 tips, P/N A21586	Multi-Channel AP96 P250, Aqua 96 tips, P/N 717253	Span-8 Span P250, Green 96 tips, P/N 379503	Span-8 Span P1000, Yellow 96 tips, P/N 987925
	96 rxns	**96 rxns**	**96 rxns**	**96 rxns**
DNA Amplification	—	96 tips	—	34 tips
Fragmentation	—	96 tips	24 tips	20 tips
Resuspension and Hybridization Preparation	96 tips	96 tips	17 tips	15 tips
Preparation for GeneTitan™ ▪ Denature samples ▪ Transfer denatured samples to Hyb Tray ▪ Prepare GeneTitan reagent plates	—	96 tips	26 tips	96 tips
TOTAL	**96 tips**	**384 tips**	**67 tips**	**165 tips**

Plate Centrifuge

One plate centrifuge is required for the Axiom™ Genotyping Assay. Refer to the *Axiom Site Preparation Guide,* P/N 702858, for an appropriate plate centrifuge that can be used with the Axiom Genotyping Solution When centrifuging and drying pellets as instructed under *5. Centrifuge and Dry Pellets on page 61*, the centrifuge must be able to spin down plates at:

- rpm: 4000
- rcf: 3200
- temperature: 4 °C

In addition, the bottom of the rotor buckets should be soft rubber to ensure that the deep well plates do not crack. Do not spin plates in metal or hard plastic buckets.

NOTE: Refer to the *Axiom Site Preparation Guide*, P/N 702858, for an appropriate plate centrifuge that can be used with the Axiom Genotyping Solution.

Set the Biomek Software Default Settings

Typically you will select the default settings for the Biomek software once. The settings you select will:

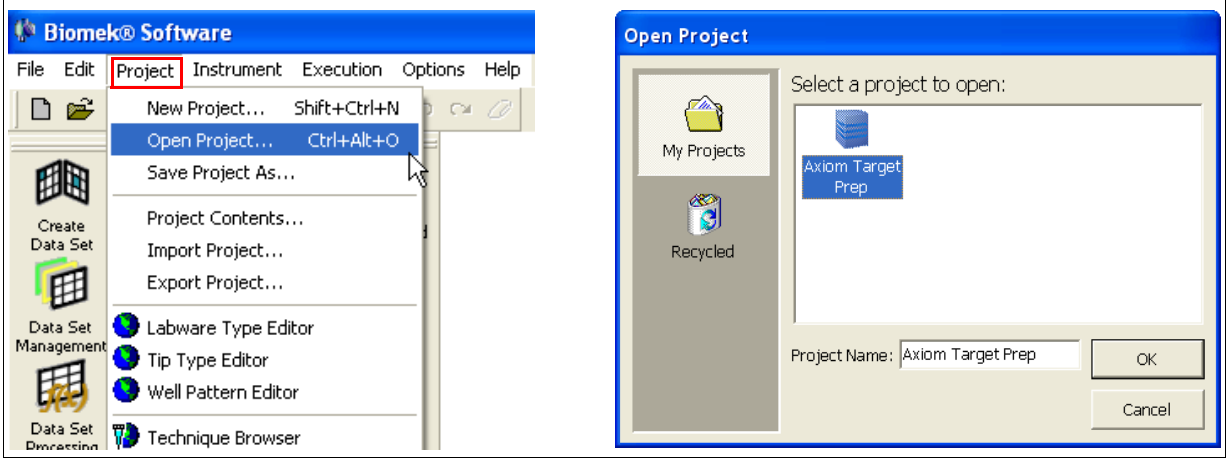
- Determine the default step selected when the user prompt is displayed at the start of each run.
- Determine which process controls (if any) that will be run during stages 2 and 3 (Fragmentation and Resuspension). The process controls include:
  - Preparation of sample dilution plates for OD and gel analysis during Resuspension and Hyb Prep. The dilution plates are taken off-deck. One is used for OD quantitation to evaluate DNA mass; the other is used to check fragment size.
  - Prompt you to manually take samples for DNA quantitation prior to fragmentation.

To select Biomek Software default settings:

1. Launch the Biomek Software.

2. Open **Project > Open Project > Axiom Target Prep** and click **OK** (Figure 3.2).

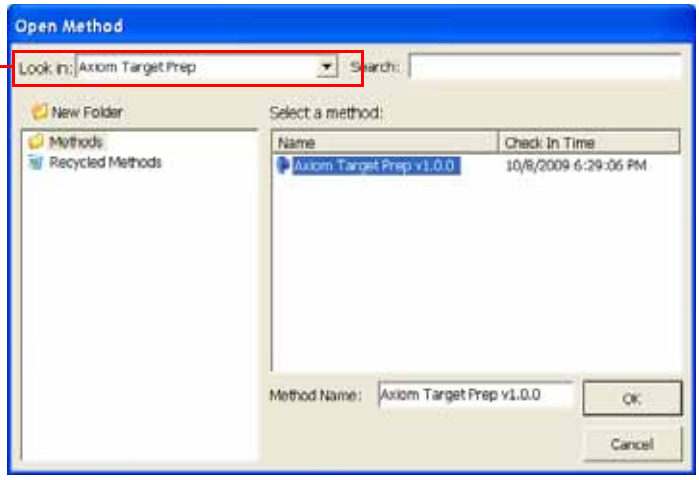
Figure 3.2 Opening the Axiom Target Prep project



3. Open **File > Open** to display the *Open Method* window (Figure 3.3).
   Or click the Open Method icon 
4. Select **Axiom Target Prep** and click **OK**.

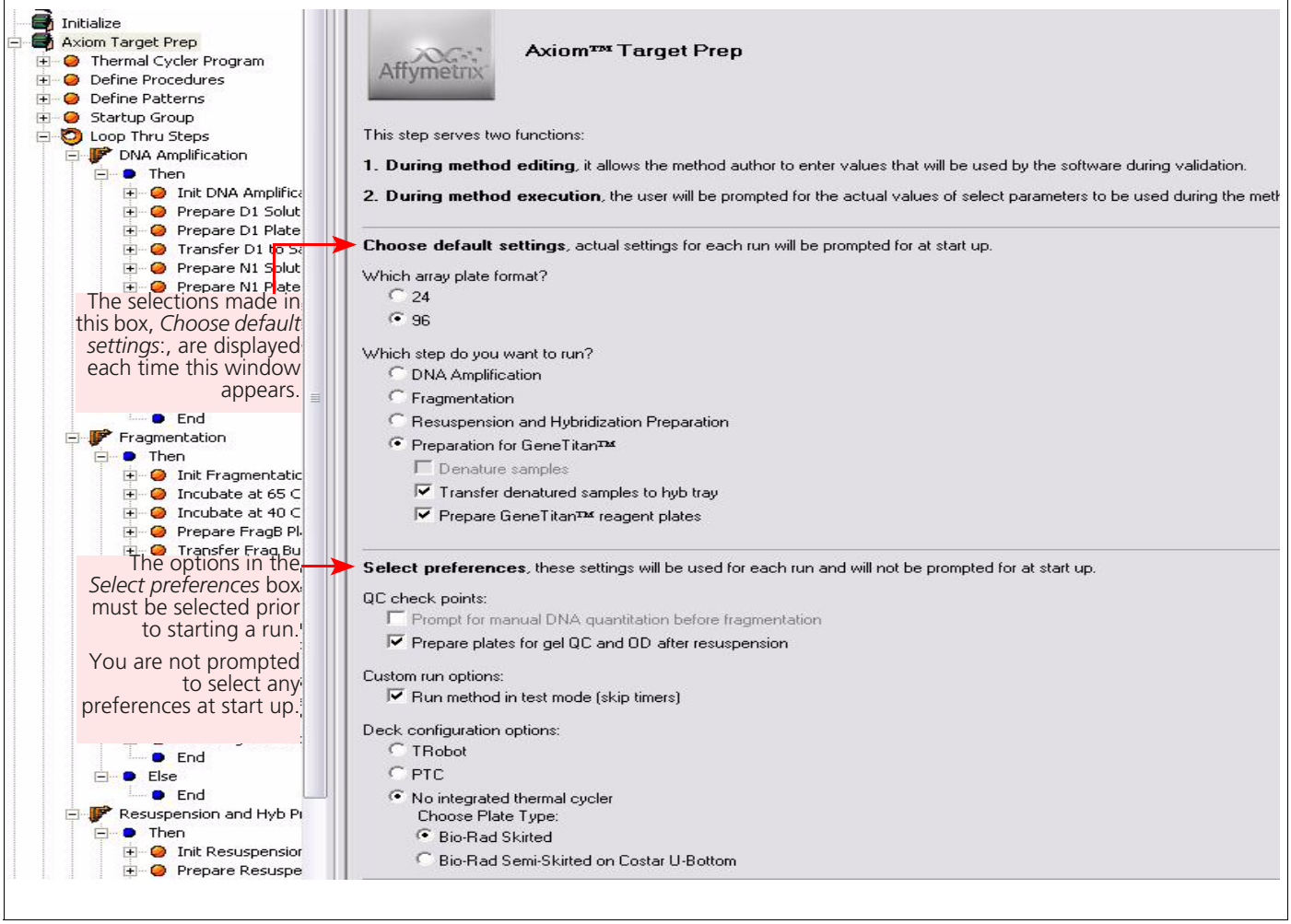
Figure 3.3 Opening the Axiom Target Preparation method

The Axiom Target Prep project folder must be displayed in this menu.



5. In the left pane of the window, select **Axiom Target Prep** (Figure 3.4).
   The default settings window is displayed on the right.

Figure 3.4 Click Startup Dialog to open the default settings window



6. Select your default settings.

   Choose defaults settings — these settings can be changed at the start of each step

   - Which array plate format?

> **NOTE:** At present, the Axiom Genome-Wide Assay supports only the 96-array plate format.

   Select 96.

   - Which step do you want to run?

     The step that you choose will be the default setting for the runtime prompt. The actual step selected when the runtime prompt is shown reflects the state of the last completed run of the Axiom Target Preparation method. For example, if DNA Amplification was the step completed in the last run, then the step chosen in the subsequent run would be Fragmentation (the next step in the process). If there is no record of the previous run, then the default choice specified is selected.

   Select preferences — These settings are displayed in this window only. You must select/deselect here.

   - QC check points

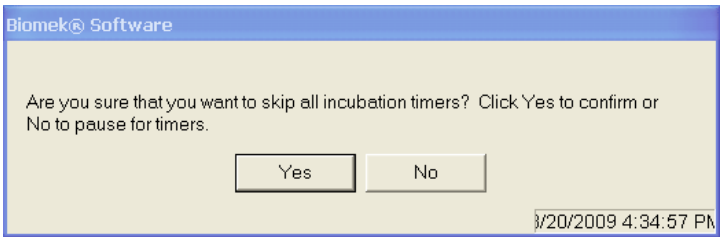
- *Prompt for manual DNA quantitation before fragmentation* — the workstation will pause following inactivation of the DNA amplification reaction to allow you to manually remove an aliquot of each sample for off-line (manual) DNA quantitation. This extra quality control step is available for troubleshooting the DNA amplification reaction.
- *Prepare plates for gel QC and OD after resuspension* — the workstation will prepare two plates of resuspended samples properly diluted for the fragmentation gel QC and OD quantitation process control checks. See Appendix A, *Fragmentation Quality Control Gel Protocol on page 223* and Appendix B, *Sample Quantitation after Resuspension on page 225* for instructions and result assessment guidelines.

IMPORTANT: **For troubleshooting and support purposes, we strongly recommend that you perform the gel QC and OD quantitation process controls after resuspension.**

- Custom run options
  - *Run method in test mode* — select this option to skip all of the incubation timers in a step. If selected, a prompt is displayed asking you to confirm that you want to run a step in test mode (Figure 3.5). Use this option to perform runs using water only, not actual reagents or samples.

CAUTION: **Never process samples in test mode. The assay will fail; all of your samples and reagents will be lost.**

Figure 3.5 Prompt displayed when the custom run option, *Run method in test mode*, is selected.



- Deck configuration options
  - TRobot- select this option to perform the denaturation of the Axiom Hyb Ready Plate on the integrated Biometra TRobot 96 thermal cycler
  - PTC - select this option to perform the denaturation of the Axiom Hyb Ready Plate on the integrated Bio-Rad PTC-200 thermal cycler
  - No integrated thermal cycler - select this option to perform the denaturation of the Axiom Hyb Ready Plate on an off-deck thermal cycler or if your Biomek does not have an integrated thermal cycler. A list of thermal cyclers that have been verified with the assay can be found below. When selecting this option, select the appropriate plate type that should be used for the Hyb Ready Plate.
    - Select the Bio-Rad Skirted option when using the HSP 9631 plate for the PTC-200 or the Bio-Rad Tetrad 0240G thermal cycler.
    - Select the Bio-Rad Semi-Skirted on Costar Round Bottom option for the ABI 9700 or the ABI 2720 thermal cycler. The Bio-Rad HSS 9601 plate must be stacked onto the Costar Round Bottom plate from Corning (VWR International P/N 29442-392, E&K Scientific: EK 680568, Corning Mfg PN 3795) for the robot to prepare the Hyb Ready Plate.

We have verified the performance of this assay using the Bio-Rad PTC-200/PTC-200G and Biometra TRobot 96 on the Beckman Biomek Target Prep Express liquid handler. We have also verified the performance of this assay using the following off-deck thermal cyclers:

- Bio-Rad PTC-200G
- Biometra TRobot 96

- ABI 9700 with a gold, silver or aluminum block
- ABI 2720
- Bio-Rad MJ 0240G

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.

IMPORTANT: **The default settings you select here will be displayed each time the Axiom Target Prep window is displayed.**

The options displayed in the Startup Dialog box as shown in Figure 3.4 on page 19 must be selected prior to starting a run. These settings are not prompted for at runtime.

The thermocycler needs to be programmed with the "Axiom Denature" protocol:

A. 95 °C 20 min

B. 48 °C 3 min

C. 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. The arched, auto-sealing metal plate with P pads as shown in Table 3.2 on page 22 should be replaced after use as per the manufacturers recommendation.**

7. Click **Start** at the top of the left pane to close the default settings window.

Equipment, Consumables, Labware and Reagents Required

Labware used on the Biomek Workstation deck

Table 3.2 Labware used on the Biomek Workstation deck

Labware	Supplier and Part Number	Labware Image
Biomek AP96 – P250 Pipette Tips (aqua box; pre-sterile, barrier)	Beckman Coulter P/N 717253	
Biomek Span P1000 Pipette Tips (yellow box; pre-sterile, barrier, conductive)	Beckman Coulter P/N 987925	
Biomek Span P250 Pipette Tips (green box; pre-sterile, barrier)	Beckman Coulter P/N 379503	

Table 3.2 Labware used on the Biomek Workstation deck

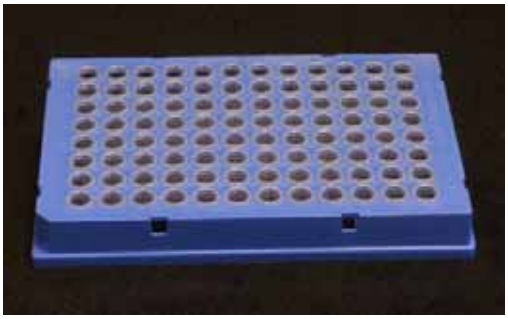
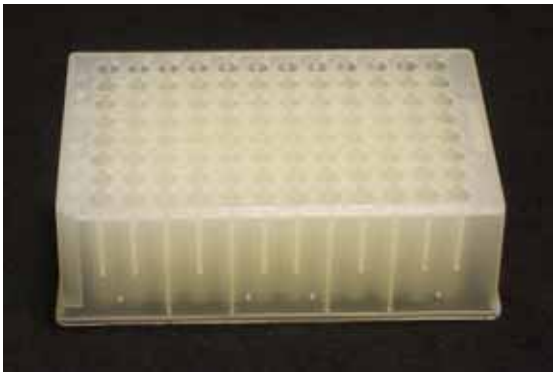
Labware	Supplier and Part Number	Labware Image
Biomek Span P50 Pipette Tips (pink box; pre-sterile, barrier)	Beckman Coulter P/N A21586	
BIO-RAD Hard Shell 96-well plate (available in multiple colors)	BIO-RAD P/N HSP9631	
Lid, metal (arched, auto-sealing with P pads)	BIO-RAD P/N MSL-2032 and P Pad P/N: MSP-1003	Front view  Side view 
Beckman Deep Well Titer Plate (polypropylene)	Beckman Coulter P/N 267007	

Table 3.2 Labware used on the Biomek Workstation deck

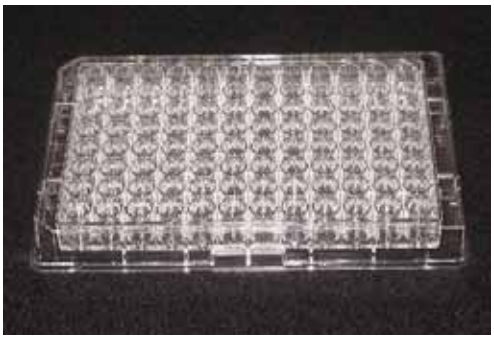
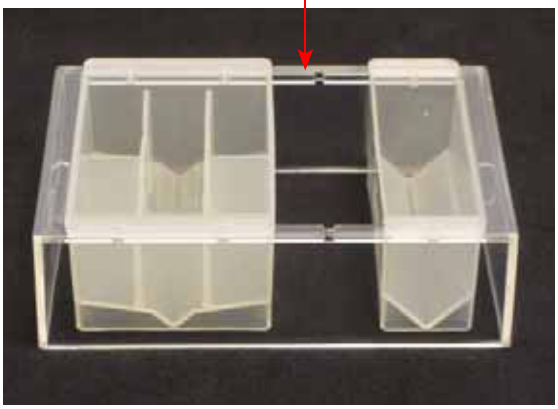
Labware	Supplier and Part Number	Labware Image
ABGene 96 Square Well Storage Plate (2.2 mL)	Thermo Scientific P/N AB-0932	
OD Plate, UV	E & K Scientific EK-25801	
Frame for Reservoirs	Beckman Coulter P/N 372795	

Table 3.2 Labware used on the Biomek Workstation deck

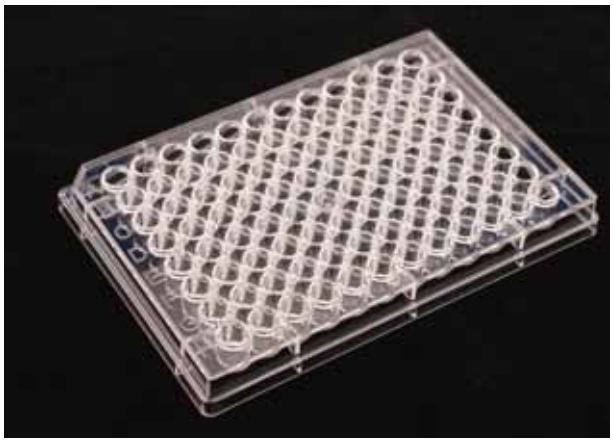
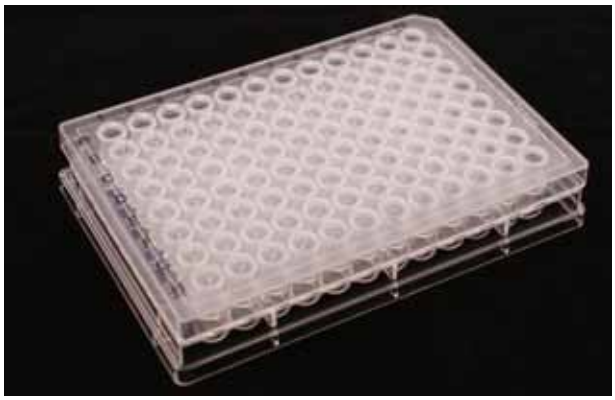
Labware	Supplier and Part Number	Labware Image
Plate, Costar Brand Serocluster round bottom plate from Corning ▫ Note: this consumable is required if using an off-deck ABI 9700 or ABI 2720 thermal cycle	VWR International P/N 29442-392 E&K Scientific EK 680568 Corning Mfg PN 3795	
Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate required only if using off-deck ABI 2720 or ABI 9700 Thermal cyclers	Bio-Rad HSS-9601	
The ABI 9700 and the ABI 2720 use the semi-skirted 96-well plates (PN HSS-9601) stacked on a Costar brand Serocluster 96-well Round Bottom Microtitration plate as shown in the figure below A 96-well semi skirted PCR plate stacked on a Costar Branded Round Bottom 96 well plate.		
Half Reservoir Half module, 75 mL capacity	Beckman Coulter P/N 372786	

Table 3.2 Labware used on the Biomek Workstation deck

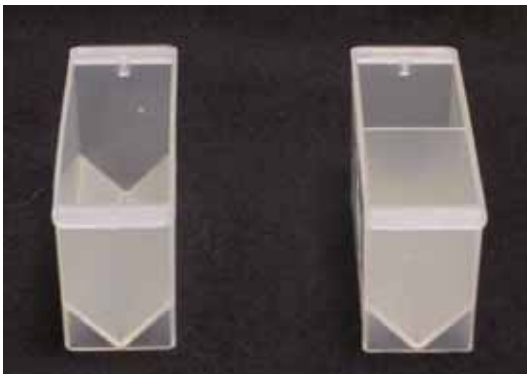
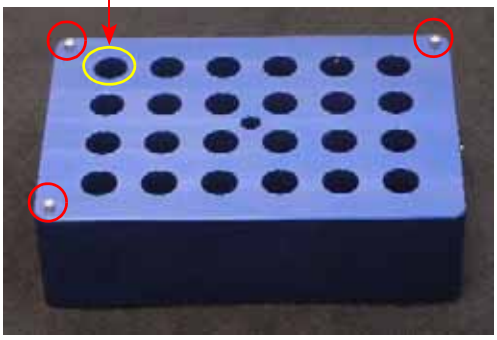
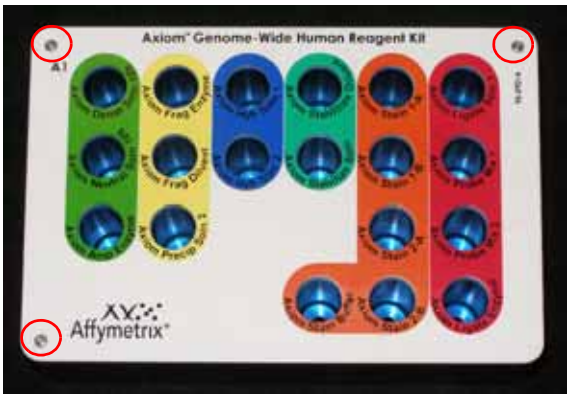
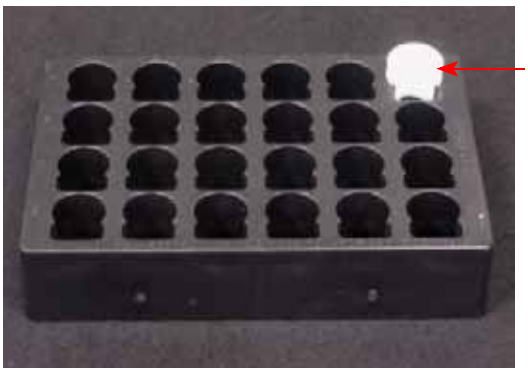
Labware	Supplier and Part Number	Labware Image
Quarter Reservoirs ▪ Quarter module, 40 mL capacity ▪ Quarter module divided by width, 19 mL capacity each receptacle	Beckman Coulter P/N 372790 (40 mL) P/N 372792 (19 mL)	Undivided 40 mL capacity Divided by width 19 mL capacity 
Reagent block, chilled to 4 °C	Beckman Coulter P/N A83054	**A1** Metal posts on block circled in red. 
Reagent block template (designed specifically for use with the Axiom Reagent Kit)	Contact Affymetrix	Template on reagent block. Metal posts on block circled in red. 

Table 3.2 Labware used on the Biomek Workstation deck

Labware	Supplier and Part Number	Labware Image
24-Position Tube Rack with one 11 mm tube insert in position A6.	Beckman Coulter P/N 373661 (rack) P/N 373696 (insert)	
Adaptor, Deep Well Plate (installed on the Shaking Peltier) This adaptor is typically installed by a Beckman Coulter field service technician during new system installation or a system upgrade. Ensure that you have one of these adaptors on the deck prior to running this assay.	Beckman Coulter P/N A83050	

GeneTitan MC 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 covers must have barcodes. Discard any cover without a barcode.**

Table 3.3 Axiom GeneTitan Tray consumables

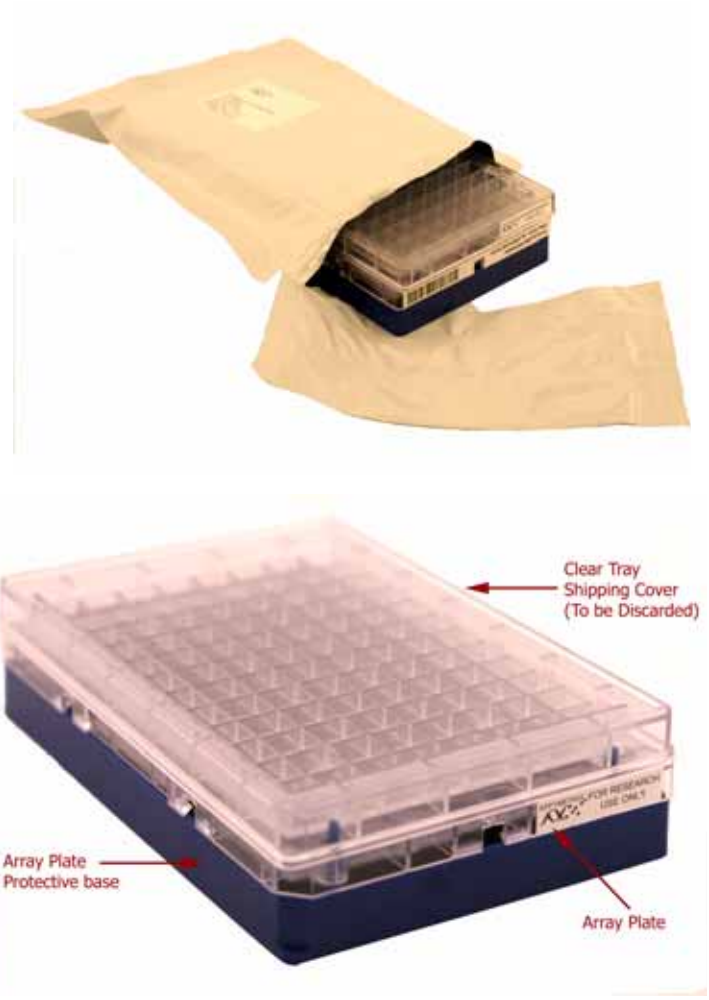
Item	Part Number	Labware Image	Information
HT Array Plate Package	All array plates have the PN 202091 etched on the on the plastic		The HT Array Plate shipping package includes the following: ▪ The function of the clear plastic cover for the Array Plate is to protect the Array Plate during transport. 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 (Continued)

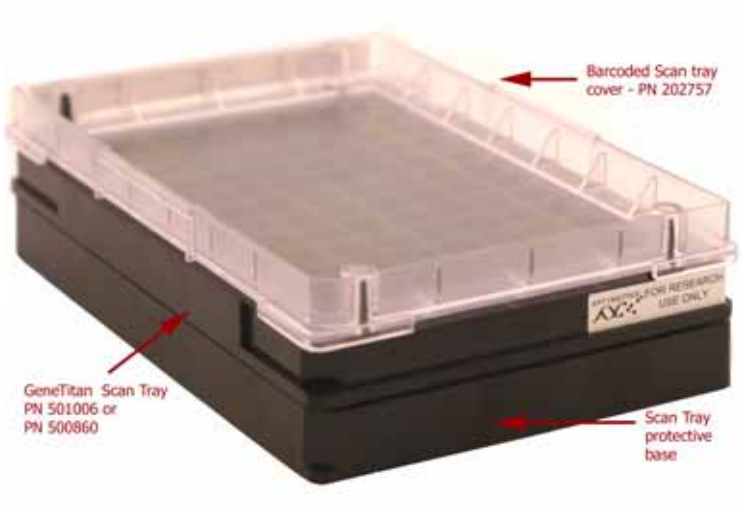
Item	Part Number	Labware Image	Information
			The HT Scan Tray shipping package includes the following: ▪ The HT Scan tray includes a Scan Tray cover. The tray cover should be used to cover the scan tray before placing the tray in the GeneTitan MC Instrument. The Tray cover should be de-ionized before use. See the section Appendix E, *Deionization Procedure for GeneTitan Trays and Covers on page 241* 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 cover in the package is used to protect the scan tray glass from damage. The black scan tray 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	PN 202096		▪ The Black Scan tray cover in the package is used to protect the scan tray glass from damage. The black scan tray 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

Table 3.3 Axiom GeneTitan Tray consumables (Continued)

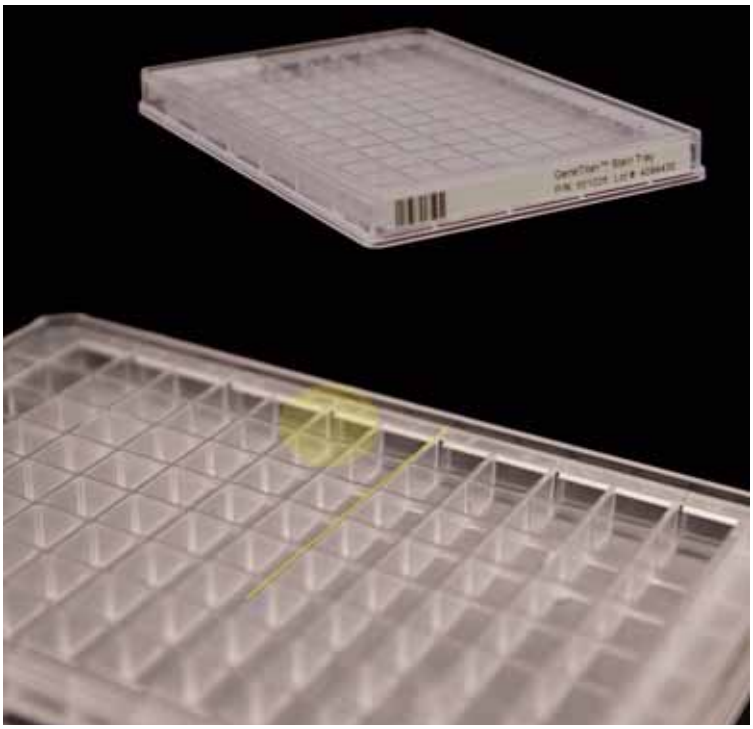
Item	Part Number	Labware Image	Information
Scan Tray with cover	Scan Tray 501006 Cover 202757		▪ The GeneTitan Scan tray must be loaded with the scan tray cover into the GeneTitan instrument ▪ Do not load the scan tray with the protective base
GeneTitan Stain Trays	501025		The GeneTitan stain trays are 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 (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 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 the section *Deionization Procedure for GeneTitan Trays and Covers* on page 241 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 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 instrument. ▪ See the section *Deionization Procedure for GeneTitan Trays and Covers* on page 241 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.

Proper Tray Alignment and Loading

Proper alignment and loading of plates, covers and trays is critical when using the GeneTitan MC. 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 3.6 and Figure 3.7 on page 34).

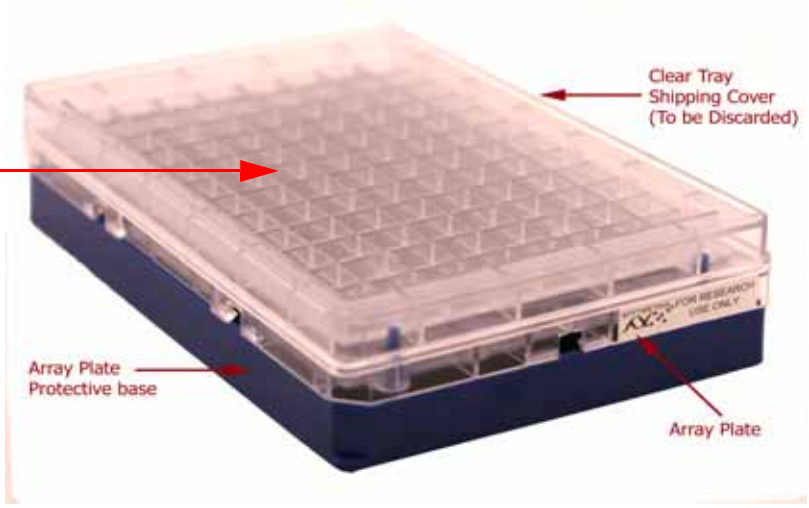
IMPORTANT: When running a multi-plate workflow, you must pay careful attention to the software prompts that tell you which side of the drawer to place or remove a plate/tray.

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.

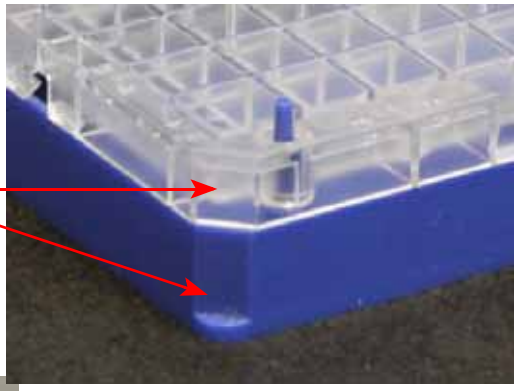
CAUTION: Take care not to damage the consumables or bend the blue base posts or scan tray posts.

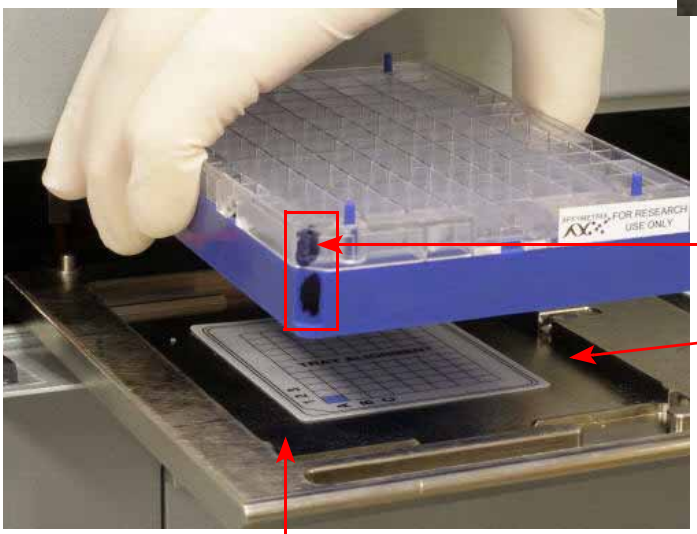
Figure 3.6 Proper alignment and loading of plates, covers and trays in the GeneTitan MC.

IMPORTANT: Remove the plastic protective shipping tray cover.



Notched corner of Array Plate aligned with notched corner of blue base.

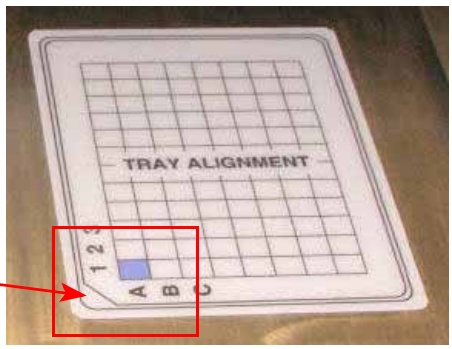




Tip

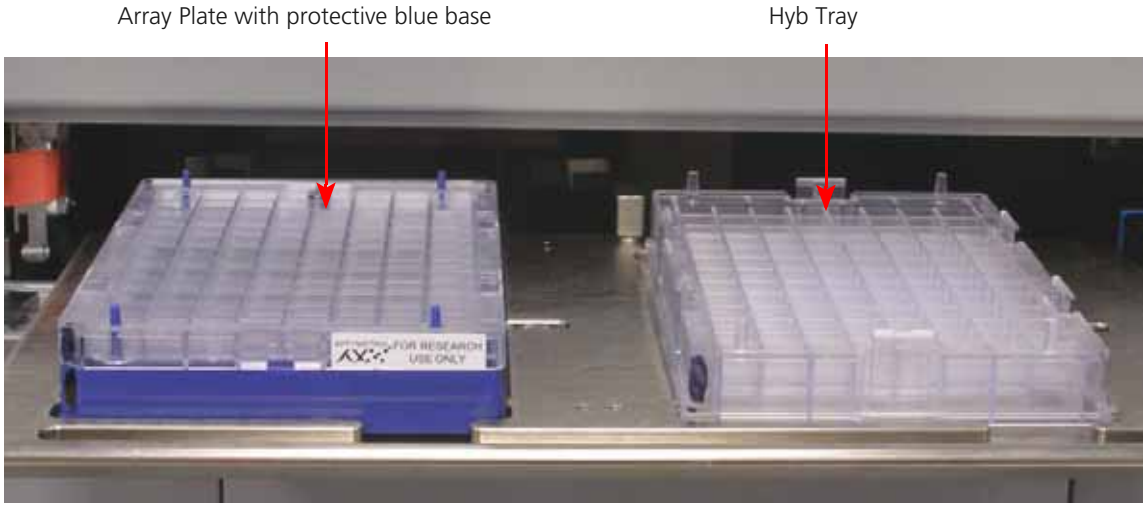
Mark the notched corner of each plate, cover and tray with permanent marker to help ensure proper alignment and loading.

Plates and trays must be seated in this rectangular recess.



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

Figure 3.7 Array Plate with protective blue base and the Hyb Tray aligned and properly loaded into drawer 6.



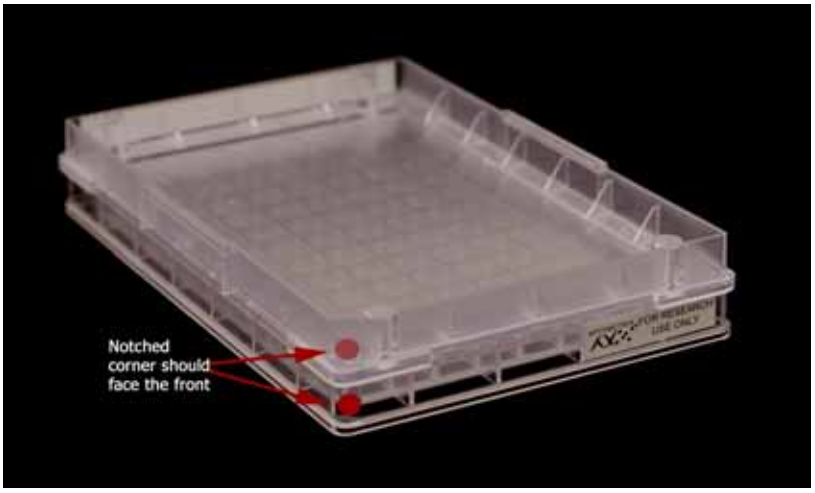
IMPORTANT: When you install the consumables, ensure that the fingers are retracted (do not put the consumables onto the drawer tab, or fingers - this indicates that the instrument is not functioning correctly. See the Important note on on page 37 for an image of the tabs.

Stain Trays and Covers

IMPORTANT: Always place the *flat* side of the cover against the Stain Tray.

Figure 3.8 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.

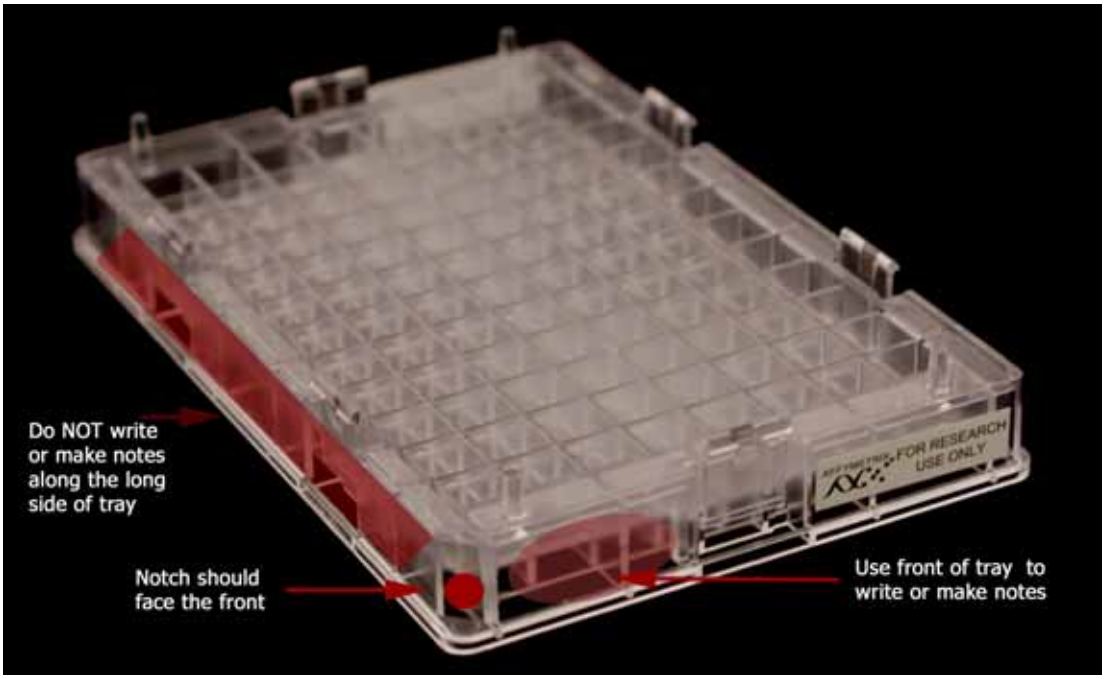
Proper labeling for Hyb Trays and reagent trays is described in:

- *Labeling for Hyb Trays*, below
- *Labeling for Stain Trays* *on page 36*

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 3.9. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.

Figure 3.9 Labeling GeneTitan hyb trays



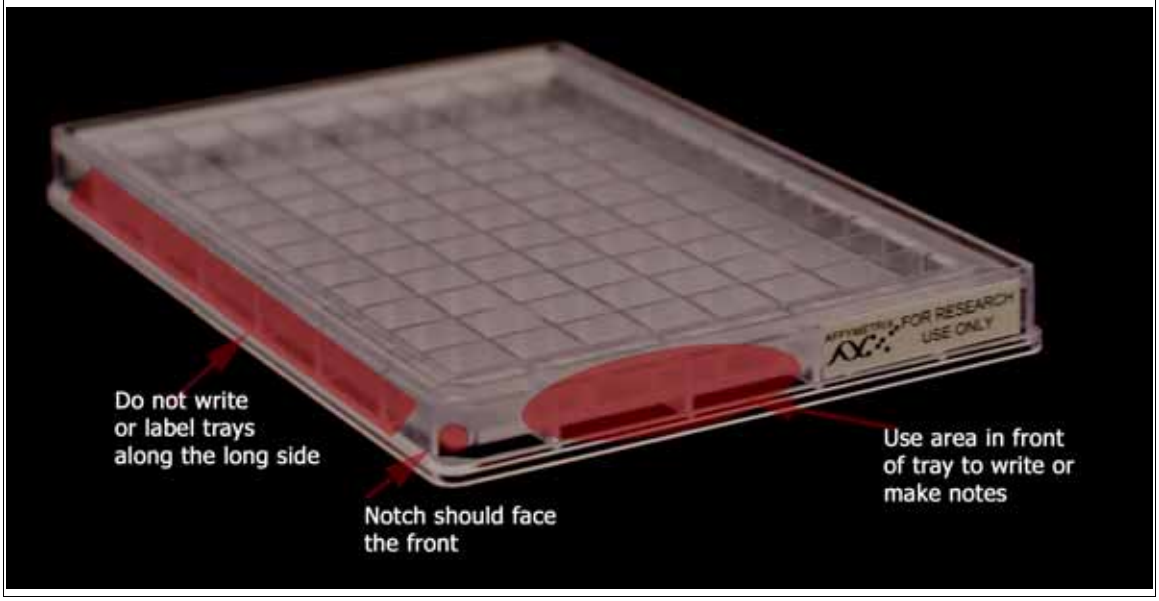
CAUTION: Writing on the wrong side of the Hyb tray may interfere with the operation of sensors in the GeneTitan MC Instrument.

Labeling for Stain Trays

You may label the stain trays on the **left side of the front of the tray** as shown in Figure 3.10. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.

IMPORTANT: Do not confuse hyb trays with stain trays.

Figure 3.10 Labeling GeneTitan stain tray

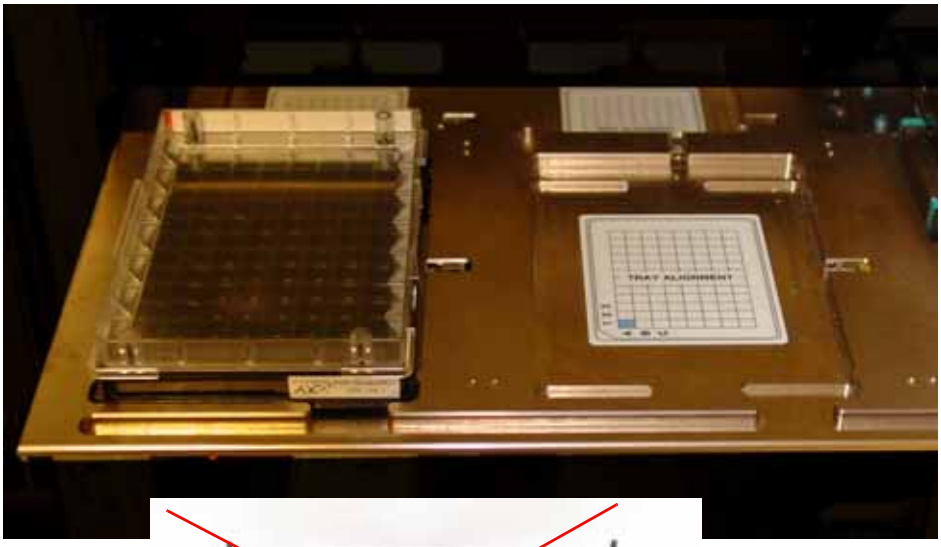


Loading Tray Consumables onto the GeneTitan MC

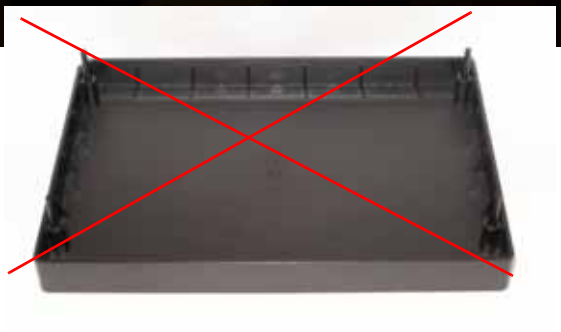
Loading, or installing, the trays and plates onto the GeneTitan MC Instrument is a simple procedure, but there are certain precautions that you must take in order to ensure an error-free procedure. The following figures show you how to do it.

Figure 3.11 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.**

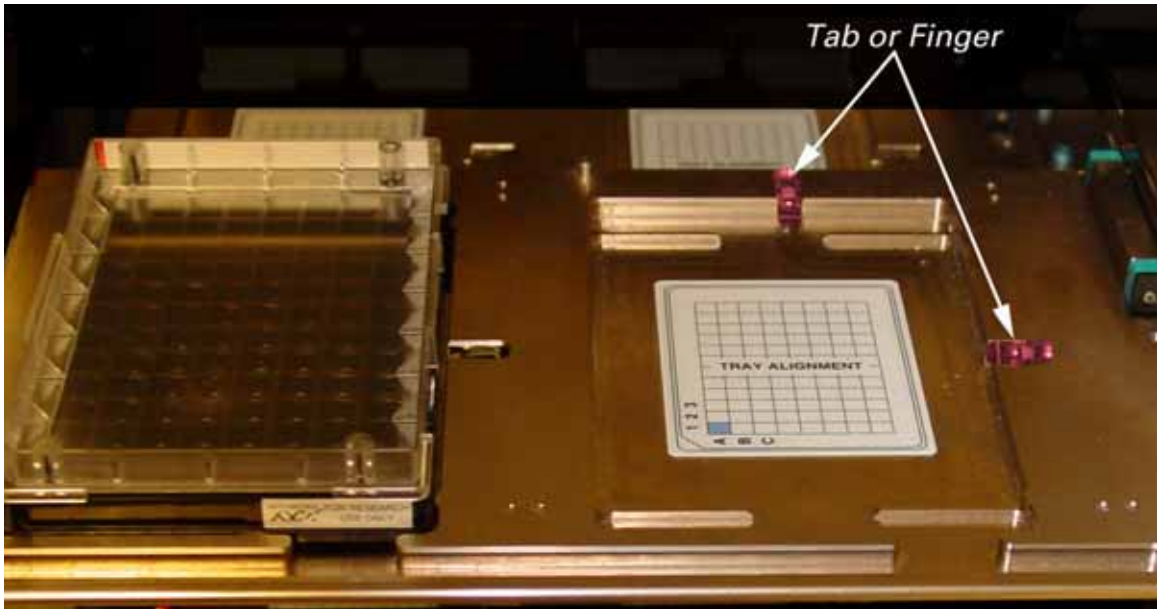


Figure 3.12 Stain 1 Tray and Ligation Tray loaded in drawer 3.

Stain 1 Tray and Ligation Tray loaded in drawer 3

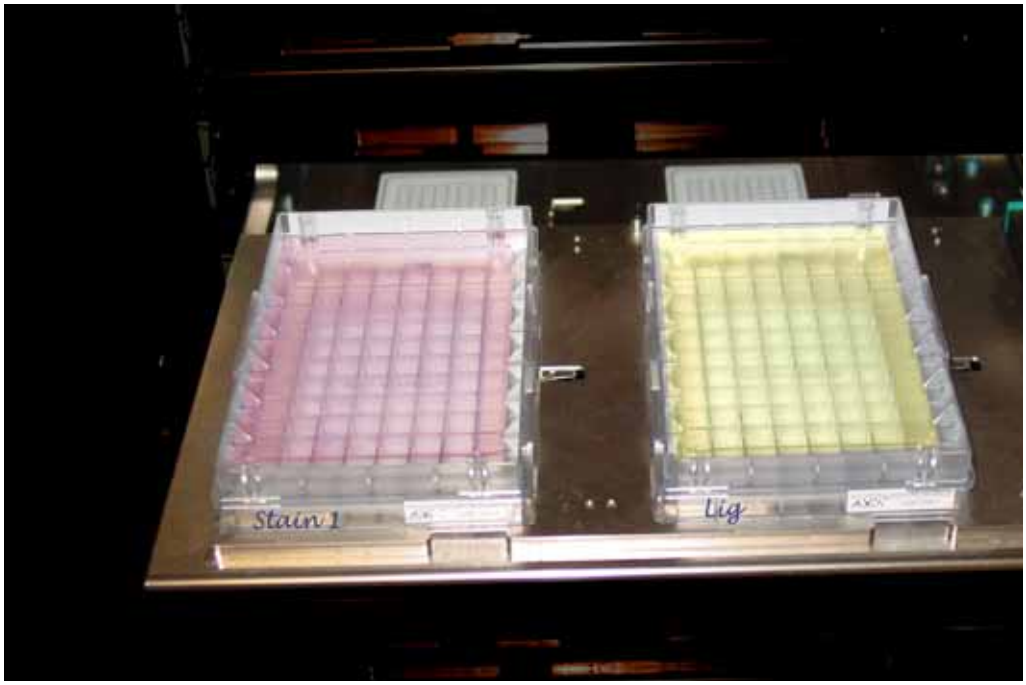


Figure 3.13 Stain 2 Tray and Fix Tray loaded in drawer 4.

Stain 2 Stain Tray and Stabilize Tray loaded in drawer 4

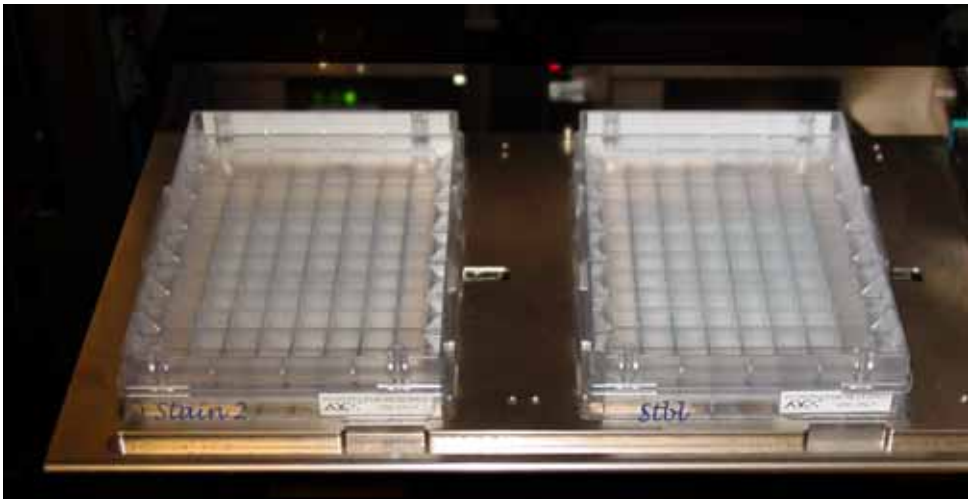
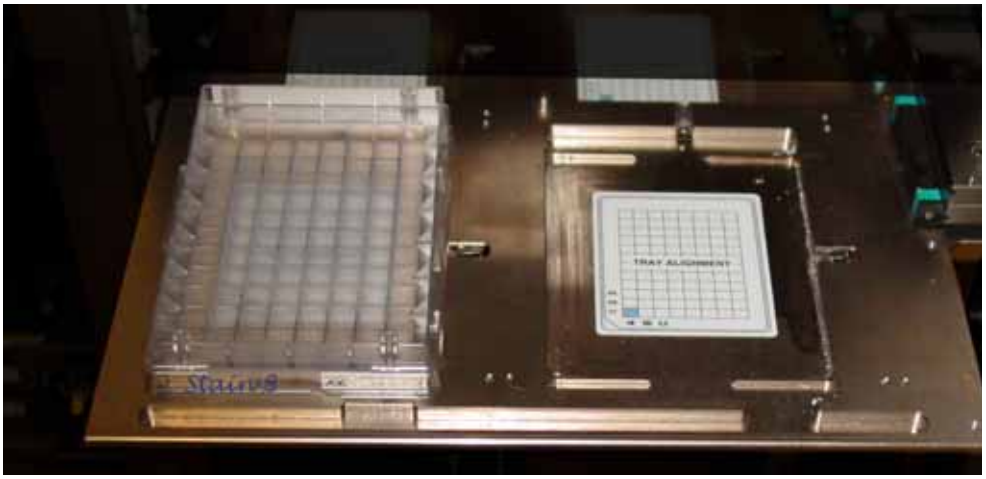


Figure 3.14 Stain 1 Tray loaded in drawer 5.

Stain 1 Tray loaded in drawer 5.



Reagent Block Template

The Axiom Reagent Kit template fits precisely onto the top of the chilled reagent block, and is held in place by the metal posts on the block (Figure 3.15). Using this template will help ensure the proper placement of reagent tubes onto the block for each method.

Figure 3.15 Axiom reagent template for the reagent block

Related Biomek FX^P Target Prep Express Documentation

The following user manuals are installed at the same time as the Biomek FX^P Target Prep Express software (**Start > All Programs > Beckman Coulter > Manuals**). Refer to these for troubleshooting the Biomek workstation.

- *Biomek® Liquid Handler User's Manual*, Beckman Coulter P/N 987834
- *Biomek® Software User's Manual*, Beckman Coulter P/N 987835

Stage 1 — DNA Amplification

IMPORTANT: Before proceeding to DNA Amplification, do the gDNA preparation described in Chapter 2, *Genomic DNA Preparation and Requirements on page 9*.

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

Duration

NOTE: A 22-24 hr incubation is required at the end of this stage.

Table 3.4 Time required for *Stage 1 — DNA Amplification*

Hands-on time	~ 30 min
Biomek FX^P Target Prep Express	~ 19 min
Incubation	23 hr
Total time	~ 24 hr

Equipment, Consumables, Labware and Reagents Required

Equipment and Labware Required

Table 3.5 Equipment and labware required

Quantity	Item
As required	Adhesive seals for plates
1	Bench top cooler, chilled to –20 °C
1	Ice bucket, filled with ice
As required	Kimwipes®
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Oven (must maintain a constant temperature of 30 °C for at least 23.5 hr with a temperature accuracy of +/– 1 °C) ▪ > 3 array plates per week — we recommend using the Binder ED 53 ▪ ≤ 3 Array plates per week — OK to use the GeneChip Hybridization Oven or the Binder ED 53
1	Plate centrifuge
1	Vortex
Biomek Workstation Labware	
1 box of each	Barrier pipette tips: ▪ Biomek Span P1000 (yellow) ▪ Biomek AP96, P250 (aqua)
2	Plate, BIO-RAD hard shell PCR 96-well
2	Plate, deep well titer
1	Reagent block, chilled to 4 °C
2	Reservoir, quarter module (40 mL)
3	Reservoir, half module (75 mL)

Reagents Required

Table 3.6 Reagents required for DNA amplification

Axiom Reagent Kit	Module
Axiom Denat Soln 10X	Module 1, –20 °C
Axiom Neutral Soln 10X	
Axiom Amp Soln	
Axiom Amp Enzyme	
Axiom Water	

1. Perform the Pre-Run Checklist

Check the Water and Waste Containers

To check the system water and waste containers:

1. If necessary, fill the system water container using distilled, deionized water (no need for ultra-pure water).
2. If necessary, empty the system waste bottle.

Turn on the Biomek FX^P Target Prep Express

To turn on the workstation:

1. Power on the workstation.
2. Ensure that all of the peripherals are powered on.
   - Watlow temperature controllers

     Control the Static Peltier (Pelt_1) and the Shaking Peltier (SPelt_96); no additional power supply.
   - Thermal cycler:

     BIO-RAD PTC-200 or Whatman Biometra TRobot 96 if present on the Biomek workstation deck. Otherwise, a stand-alone thermal cycler can be used.
3. Launch the Biomek Software by double-clicking the Biomek Software icon on the desktop 

. You can also open **Start > All Programs > Beckman Coulter > Biomek Software**.

Close the thermal cycler (on deck thermal cycler)

If your Biomek Workstation includes a PTC200 or a Biometra TRobot 96, the lid may remain open upon startup. You must close the thermal cycler lid prior to homing the axes or starting a method.

The lid of the PTC-200 opens automatically when the device is powered up. While the lid of the TRobot does not power up automatically, it is possible that the lid may be open from a previous run or at the beginning of the next run.

CAUTION: Close the thermal cycler lid:

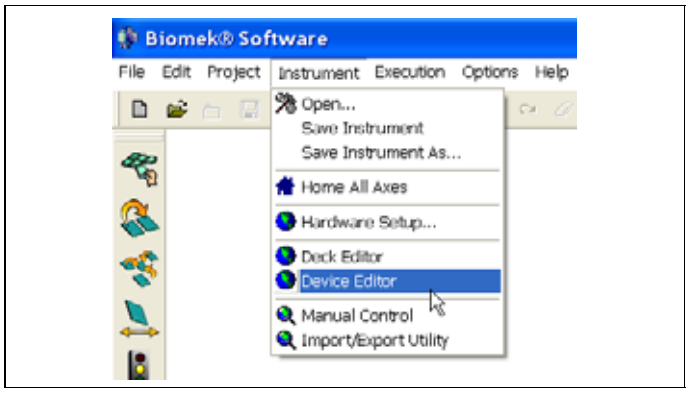
- If it remains open after you have powered on the workstation.
- If the lid is up before you home the axes or before you begin a method.

If not closed, the MC Pod may collide with the thermal cycler lid and damage the instrument.

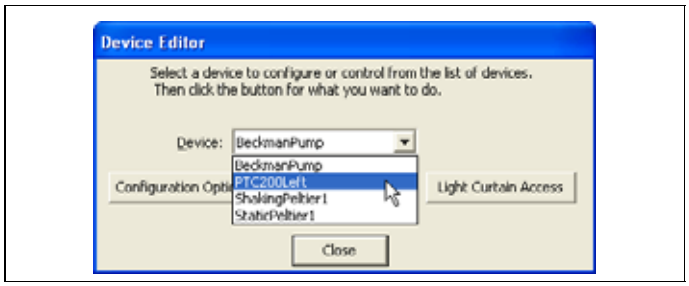
NOTE: Refer to page 12 of the Setup Guide and User's manual for Biometra TRobot on the Biomek FX^P. After following the instructions to close the thermal cycler lid, proceed to *Home All Axes* *on page 44*.

Closing the thermal cycler lid on the PTC-200

1. Open **Instrument > Device Editor.**



2. Open the Device drop-down menu and select **PTC200Left**.



3. Click **Action Commands**.
4. Select the following (Figure 3.16 on page 44):
   A. In the *Actions* box, select **Close**.
   B. In the *Open/Close* box, select **Without plate**.

> **IMPORTANT:** It is critical that you select ***Without plate*** in the Open/Close box. If a plate is present, remove it now.

5. Click **Close Lid**.
   A Status window is displayed while the lid is being closed (Figure 3.17).
6. Click **OK** when the *Command executed* prompt is displayed (Figure 3.17).
7. Click **Cancel**; then click **Close**.

Figure 3.16 Closing the thermal cycler lid

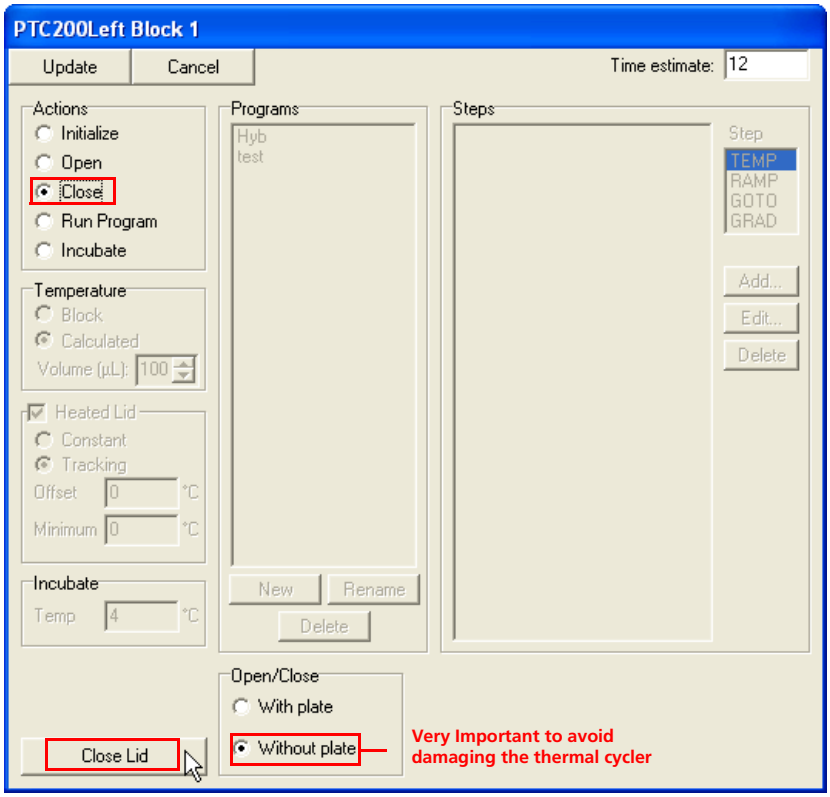
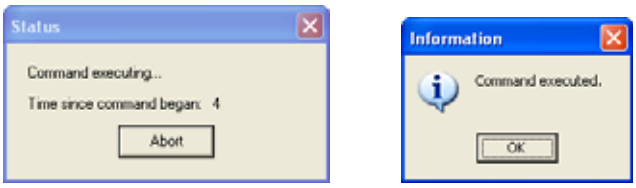


Figure 3.17 Closing PTC Lid prompts.



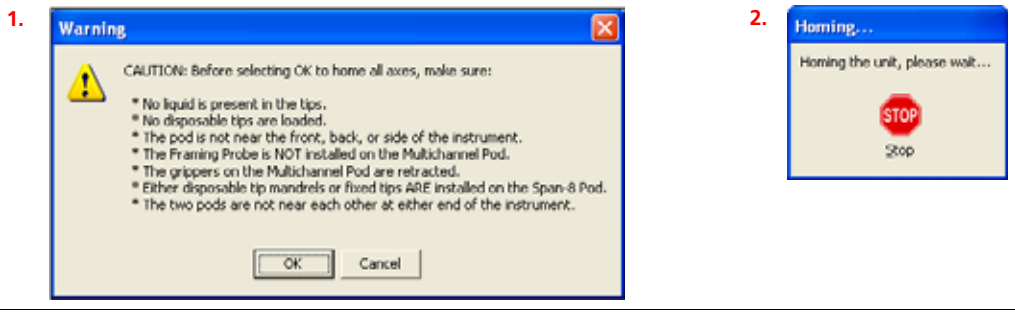
Home All Axes

This procedure will home all axes and prime the fluidics lines.

To home all axes:

1. Open **Instrument > Home All Axes**.
2. Ensure all conditions in the Warning prompt Figure 3.18 (1.) are met, then click **OK**. An icon instructing you to Stop and wait while the instrument homes is displayed (2.).

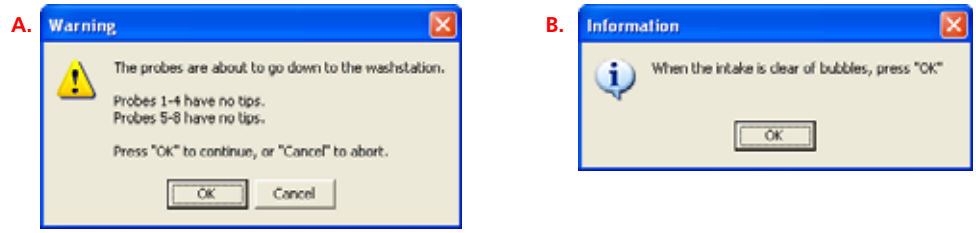
Figure 3.18 Homing all axes



3. When:
   - **A.** The Warning prompt in Figure 3.19 (A.) is displayed, confirm that no tips are loaded in the Span-8 Pod, and click **OK**.

     The lines for the Span-8 tips are primed and the next prompt shown in Figure 3.19 (B.) is displayed.
   - **B.** When the intake (syringes and tubing) for the Span-8 tips is clear of bubbles, click **OK**.

Figure 3.19 Prompts displayed for priming the Span-8 Pod fluidic lines.



2. Thaw and Prepare the Reagents and Sample Plate

Thaw and Prepare the Reagents and Sample Plate

To thaw and prepare the reagents:

1. Thaw the sample plate on the bench top at room temperature and spin.

   NOTE: Do not place a frozen sample plate directly on the workstation deck.

2. Thaw the following reagents on the bench top at room temperature.
   - Axiom Denat Soln 10X
   - Axiom Neutral Soln 10X
   - Axiom Amp Soln
   - Axiom Water

   Leave the Axiom Amp Enzyme in the freezer until ready to use.
3. Vortex and spin all reagents (except Axiom Amp Enzyme), then place on ice.
   - Vortex the Axiom Amp Soln for 30 sec to thoroughly mix.
   - For the Axiom Amp Enzyme, just before placing on the deck gently flick the tube 3 times to mix and spin.
4. Preheat the Oven to 30 °C.

We recommend using one of these ovens:

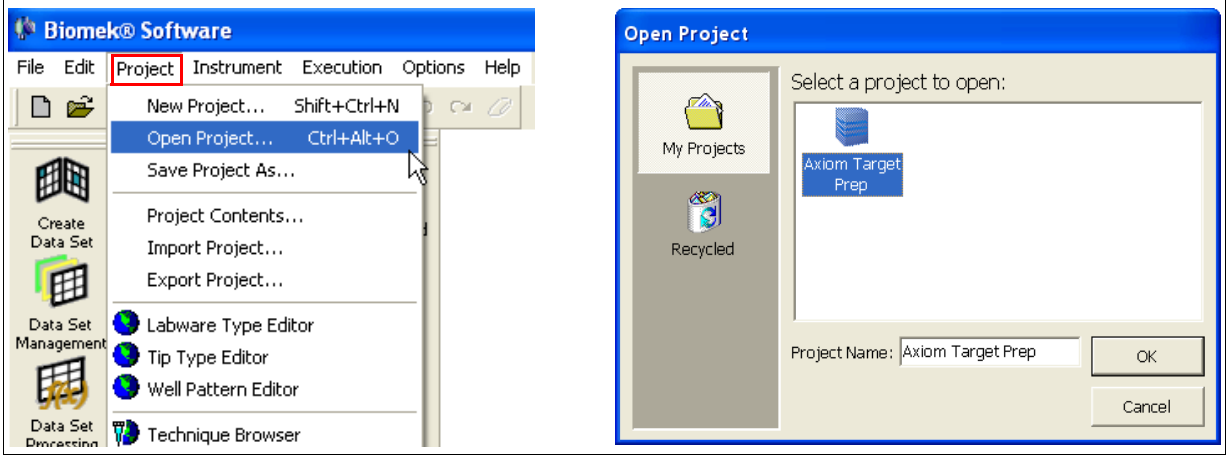
- Binder FD53
- Affymetrix GeneChip® Hybridization Oven (turn rotation on to 15 rpm)

4. Run the DNA Amplification Step

To run the DNA Amplification step:

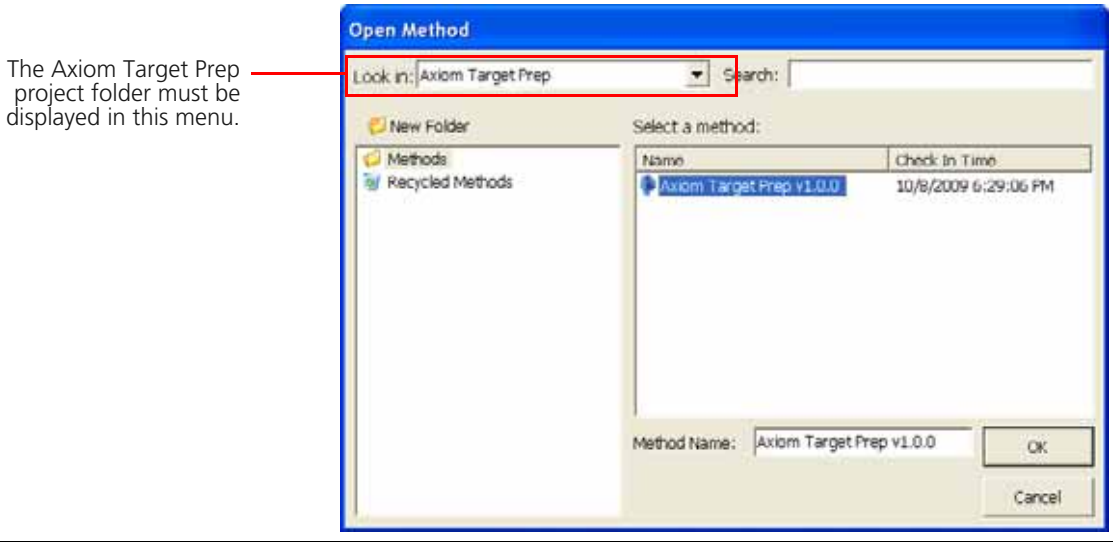
1. Open **Project > Open Project > Axiom Target Prep** (Figure 3.20) and click **OK**.

Figure 3.20 Opening a project



2. Open **File > Open** to display the *Open Method* window (Figure 3.21).
   Or click the Open Method icon 
3. Select **Axiom Target Prep** and click **OK**.

Figure 3.21 Opening the Axiom Target Preparation method

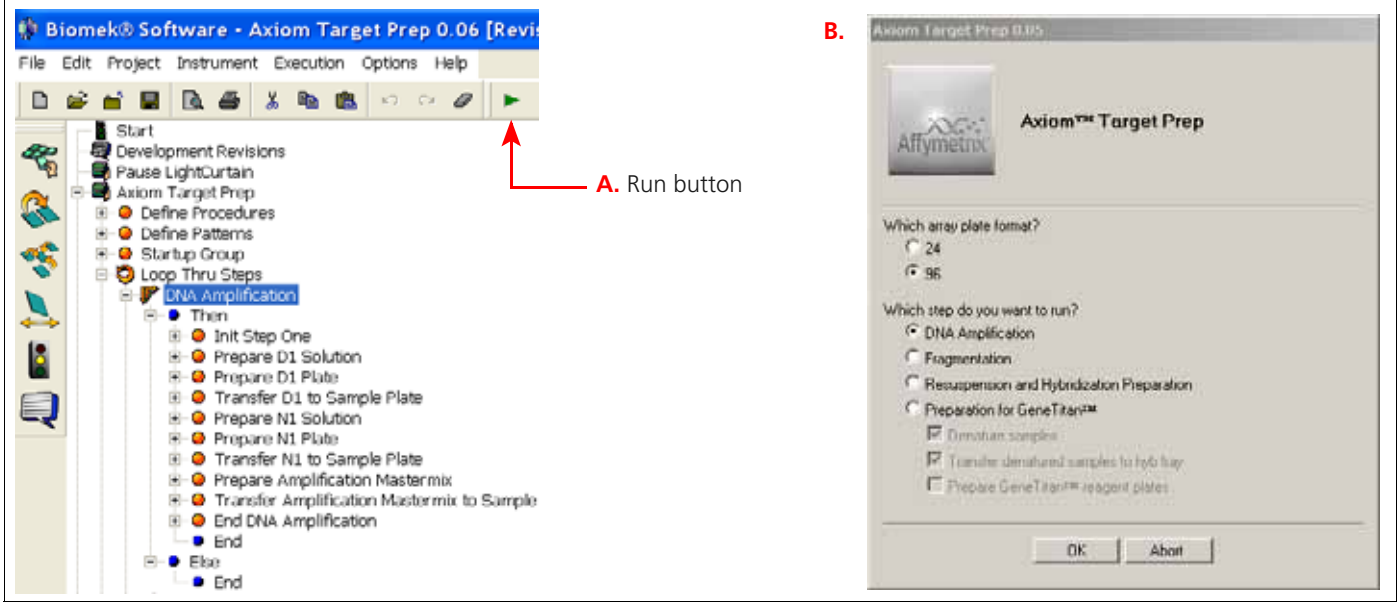


4. At the top of the main window, click the **Run** button to open the *Axiom™ Target Prep* window (Figure 3.22 – **A.**).
5. In the *Axiom Target Preparation* window (Figure 3.22 – **B.**):
   - **A.** Select your Array Plate format.
   - **B.** Select **DNA Amplification.**

C. Click **OK**.

The Deck Layout for DNA Amplification is displayed (Figure 3.23 on page 48).

Figure 3.22 Opening the Axiom Target Preparation methods window



6. Place the labware and reagents on the deck as directed in the following figures and table:
   - Figure 3.23 on page 48 — deck layout
   - Table 3.7 on page 48 — labware and reagents
   - Figure 3.24 on page 49 — reagent block

IMPORTANT: **Axiom Amp Enzyme — Immediately prior to placing on the reagent block, gently flick the tube with your finger two to three times to mix; then spin. Do NOT vortex.**

Figure 3.23 Deck layout window for the DNA Amplification method

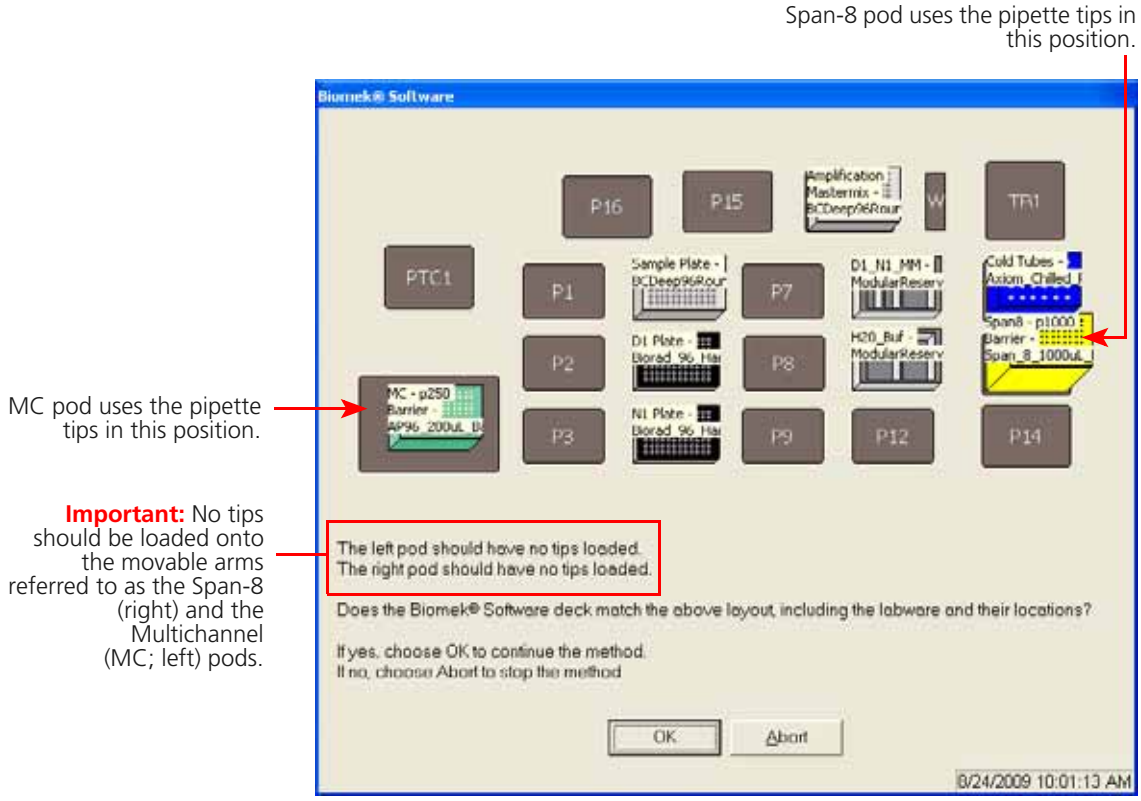


Table 3.7 Labware and reagent locations on the deck for the DNA Amplification method

Position on Deck	Labware	Reagent or Samples
TL1	Biomek AP96 – P250 Pipette Tips (aqua)	—
P4	Beckman Deep Well Titer Plate	gDNA samples
P5	BIO-RAD Hard Shell 96-well plate (any color)	—
P6	BIO-RAD Hard Shell 96-well plate (any color)	
SPelt96_1	Beckman Deep Well Titer Plate	—
P10	Reservoirs in frame: ▪ Quarter module (1) ▪ Quarter module (2) ▪ Half module (3) Leave all reservoirs empty	1 Empty Reservoir 2 Empty Reservoir 3 Empty Reservoir

Table 3.7 Labware and reagent locations on the deck for the DNA Amplification method

Position on Deck	Labware	Reagent or Samples
P11	Reservoirs in frame: ▪ Half module (1) ▪ Half module (2) ▪ Pour Axiom Water into reservoir 1 ▪ Pour Axiom Amp Soln into reservoir 2	1: Axiom Water 2: Axiom Amp Soln
Pelt_1	Reagent block, chilled to 4 °C	See Figure 3.24.
P13	Biomek Span P1000 Pipette Tips (yellow)	—

Figure 3.24 Placement of reagents on chilled reagent block for GeneTitan reagent tray preparation

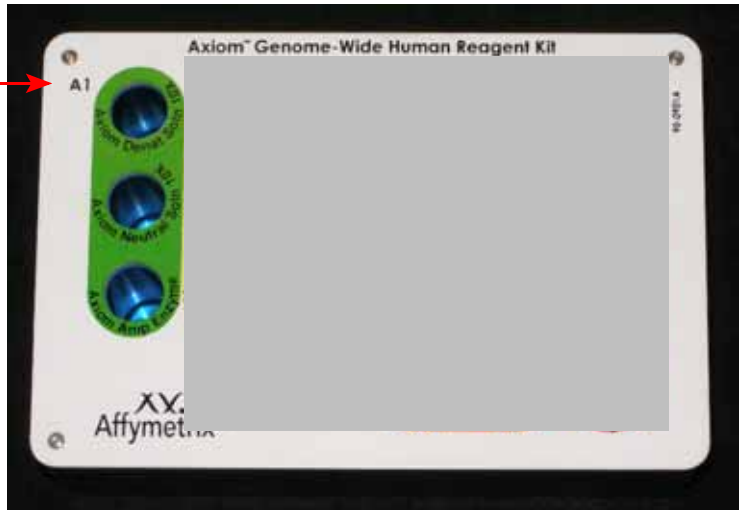
IMPORTANT:

- Position all plates and the chilled reagent block with A1 in the upper left corner of the frame.
- Press reagent tubes into the block to ensure that they are fully seated.

A1

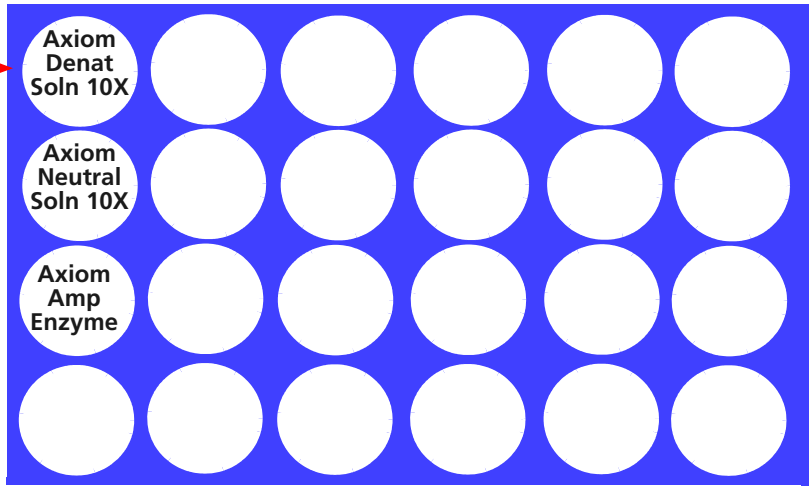
Reagent block with template.
Only the positions used for this method are shown.

Flick and spin the Amp Enzyme before placing in block.



A1

Diagram of reagent block without template



7. Check the deck layout to ensure that all labware and reagents are in the proper locations.

> **NOTE: If the physical deck does not match the Deck Layout and Confirmation window exactly (Figure 3.23 on page 48), either modify the physical deck to match exactly or choose Abort in the Deck Layout and Confirmation window.**

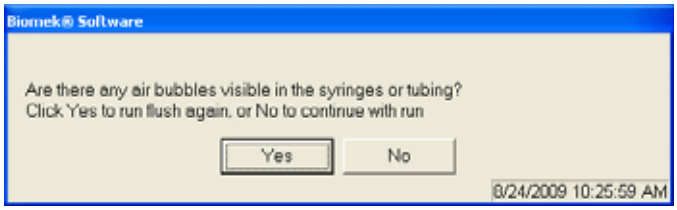
8. Click **OK**.

   The system flushes the Span-8 fluidics system. Observe the lines and syringes for air bubbles.

9. At the prompt to repeat the Span-8 fluidics system flush:
   - Click **No** if no air bubbles are present.
   - Click **Yes** if air bubbles are present. Repeat the flush until no air bubbles are present.

Figure 3.25 Flushing the Span-8 fluidics system to purge air bubbles.

Prompt for Span-8 flush



The DNA Amplification step runs until the Amplification Master Mix has been added to the sample plate. Once complete, the instructions and prompt shown in Figure 3.26 are displayed.

- Follow the instructions for the sample plate (see also *User Intervention* below).
- Follow the instructions for clearing the workstation deck.

Figure 3.26 Instructions for clearing the workstation deck

IMPORTANT:
- Seal the sample plate before placing in the oven.
- Always discard the used multi-channel pipette tips in position P3.
- Always store the reagent block at 4 °C.

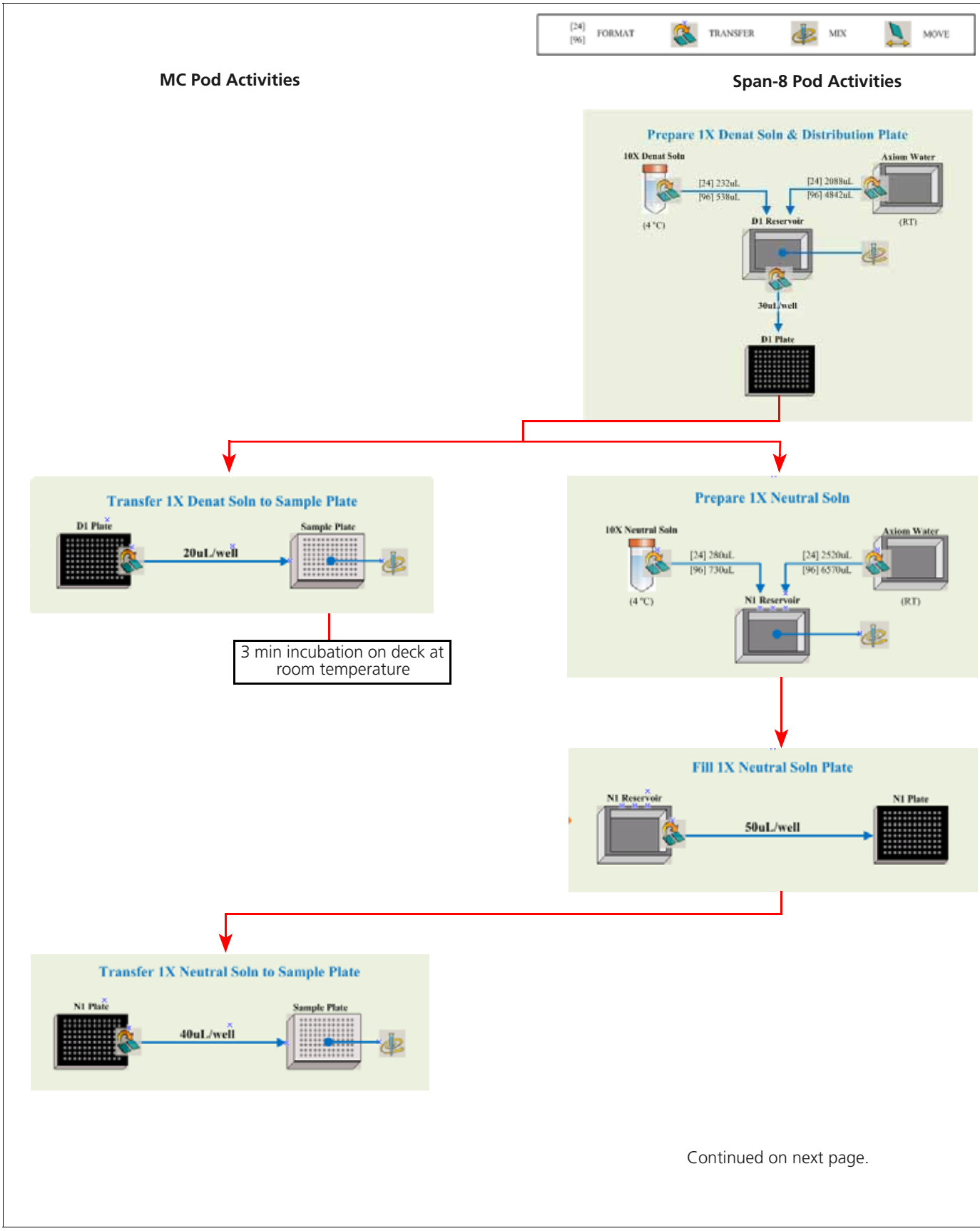
User Intervention

1. Remove the sample plate.
2. Blot the top of the plate with a Kimwipe to remove any droplets that may be present.
3. Tightly seal the plate.
4. Place in a preheated oven and incubate at 30 °C for 22 to 24 hr.

> **NOTE: If using a GeneChip® Hybridization Oven, place the plate on the bottom of the oven. Plates do not rotate.**

5. After 22 to 24 hr of incubation, do one of the following:
   - Proceed directly to *Stage 2 — Fragmentation and Purification* *on page 53*
   - Tightly seal and store the amplified samples at –20 °C.

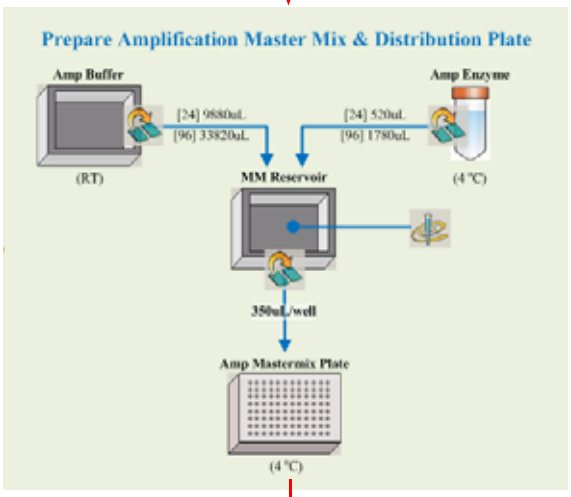
Summary of DNA Amplification



Continued on next page.

MC Pod Activities

Span-8 Pod Activities



Stage 2 — Fragmentation and Purification

NOTE: Purification is done by precipitation (*4. Precipitation on page 61*).

Duration

Table 3.8 Time required for *Stage 2 — Fragmentation and Purification*

Hands-on time	~ 25 min ~ 50 min if frozen DNA from Step 1
Biomek FX^P Target Prep Express ▫ Deactivation incubation — 40 min to deactivate the amplification reaction ▫ Fragmentation incubation — 30 min	~ 1:35 hr — 96 samples
Total time	2:00 to 2:25 hr

Equipment, Consumables, Labware and Reagents Required

Equipment and Labware Required

Table 3.9 Equipment, consumables and labware required

Quantity	Item
As required	Adhesive seals for plates
1	Bench top cooler, chilled to –20 °C
1	Freezer, –20 °C
1	Ice bucket, filled with ice
As required	Kimwipes
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Oven, preheated to 48 °C, with a temperature accuracy of +/– 1 °C
1	Plate centrifuge, 4 °C
1	Vortex (for plates and microtubes)
Beckman Labware	
1 box of each	Barrier pipette tips: ▪ Biomek AP96, P250 (aqua) ▪ Biomek Span P250 (green) ▪ Biomek Span P1000 (yellow)
3	Plate, BIO-RAD hard shell PCR 96-well
1	Plate, ABgene 96 Square Well Storage
1	Reagent block, chilled to 4 °C
3	Reservoir, quarter module
1	Reservoir, half module

Reagents Required

Table 3.10 Reagents required for the Fragmentation method

Reagent	Module
From the Axiom Reagent Kit	
Axiom Frag Enzyme (leave at –20 °C until ready to use)	Module 2 Box 1, –20 °C
Axiom 10X Frag Buffer	
Axiom Precip Soln 2	
Axiom Frag Diluent	Module 2 Box 2, 2–8 °C
Axiom Frag Rxn Stop	
Axiom Precip Soln 1	
User-supplied	
Isopropanol, 99.5%	96 samples: 65 mL

1. Perform the Pre-Run Checklist

The following actions are the same as described under *1. Perform the Pre-Run Checklist* *on page 42*. Refer back to this step for details. Some or all of these steps may not be required depending upon the current state of the Biomek workstation.

To perform the pre-run checklist:

1. Power on the Biomek FX^P Target Prep Express and all peripherals.
2. Check the water and waste containers; replenish or empty as required.
3. Launch the Biomek Software.
4. If applicable, close the thermal cycler lid (on page 42).
5. Home all axes (on page 44).

2. Thaw and Prepare the Samples and Reagents

Thaw and Prepare the Sample Plate

If the plate of amplified samples is frozen:

1. Place the deep well plate in a small water bath.
   For example, pour Millipore water into a small tray. Place the frozen plate on the water in the tray.
2. Leave the plate in the water bath for ~ 50 min until all wells have thawed.
3. Spin down at 1000 rpm for 30 sec.
4. To avoid cross-contamination of wells during vortexing:
   A. Remove the seal and blot the top of the plate with a Kimwipe.
   B. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 sec to thoroughly mix.
6. Spin at 1000 rpm for 30 sec.

Thaw and Prepare the Reagents

To thaw and prepare the reagents:

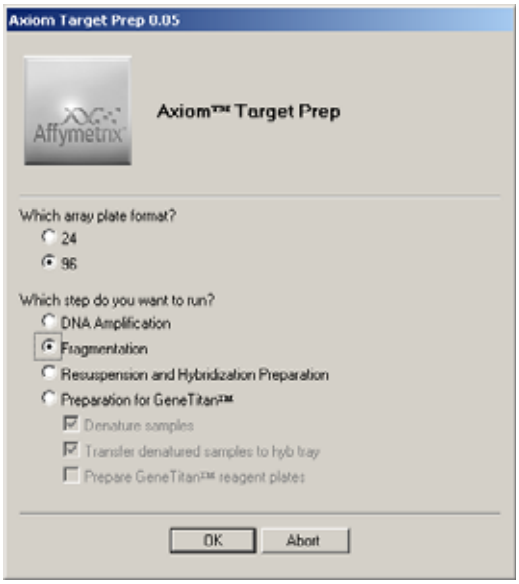
1. Thaw the following reagents on the bench top at room temperature.
   - Axiom 10X Frag Buffer
   - Axiom Precip Soln 2
2. Vortex and spin all reagents (except Axiom Frag Enzyme), then place on ice.
   - For the Axiom Frag Enzyme: Leave at -20 degrees C until ready to use. Just before placing on the deck gently flick the tube 3 times to mix and spin

3. Run the Fragmentation Step

To open the Target Preparation Methods window:

1. Open **Project > Open Project > Axiom Target Prep** and click **OK**.
2. Click the **Open Method** icon, select **Axiom Target Prep**, and click **OK**.
3. Click the **Run** button.
4. Select the array plate format and **Fragmentation**, then click **OK** (Figure 3.27).
   The deck layout for Fragmentation is displayed (Figure 3.28).

Figure 3.27



5. Setup the deck with the labware and reagents as shown in Figure 3.28 on page 56, Table 3.11 on page 56, and Figure 3.29 on page 57.

IMPORTANT: Remove the seal from the sample plate before placing on the deck.

Figure 3.28 Deck layout for the Fragmentation method

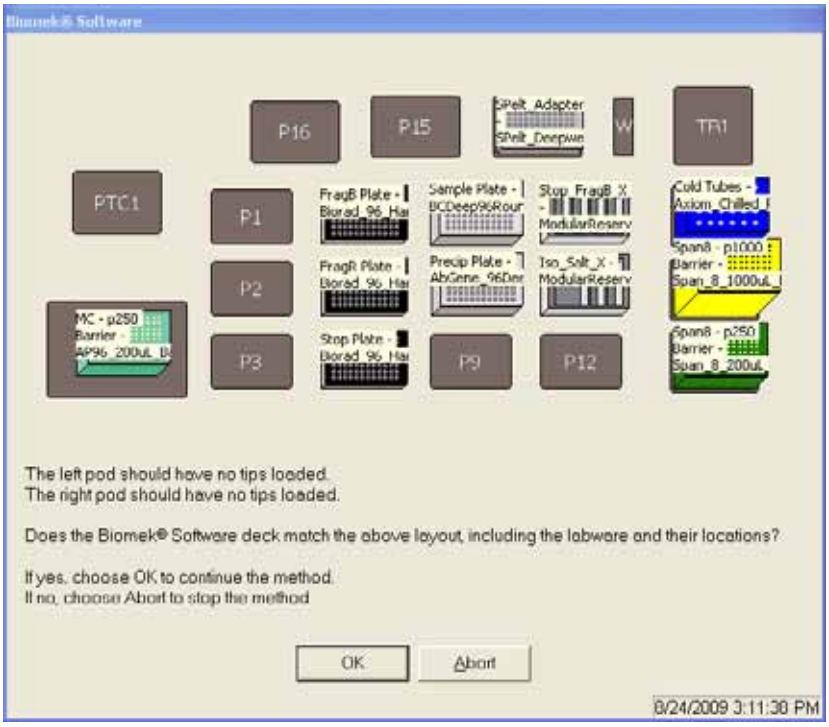


Table 3.11 Labware and reagent locations on the deck for the Fragmentation

Position on Deck	Labware	Reagent or Samples
TL1	Biomek AP96 – P250 Pipette Tips (aqua)	—
P4	BIO-RAD Hard Shell 96-well plate	—
P5	BIO-RAD Hard Shell 96-well plate	—
P6	BIO-RAD Hard Shell 96-well plate	—
P7	Beckman Deep Well Titer Plate	Amplified gDNA
P8	ABgene 96 Square Well Storage Plate	—
P10	Reservoirs in frame: ▪ Quarter module (1) ▪ Quarter module (2) ▪ Pour Axiom Frag Rxn Stop into reservoir 1 ▪ Pour Axiom 10X Frag Buffer into reservoir 2	1 Axiom Frag Rxn Stop 2 Axiom 10X Frag Buffer X
P11	Reservoirs in frame: ▪ Half module (1) ▪ Quarter module (2) ▪ Pour Isopropanol into reservoir 1 ▪ Pour Axiom Precip Soln 1 into reservoir 2	1 Isopropanol 2 Axiom Precip Sol 1 X

Table 3.11 Labware and reagent locations on the deck for the Fragmentation

Position on Deck	Labware	Reagent or Samples
Pelt_1	Reagent block, chilled to 4 °C	See Figure 3.29 on page 57.
P13	Biomek Span P1000 Pipette Tips (yellow)	—
P14	Biomek Span P250 Pipette Tips (green)	—

Figure 3.29 Placement of reagents on chilled reagent block for GeneTitan reagent tray preparation

IMPORTANT:

- Always position the chilled reagent block with A1 in the upper left corner of the frame.
- Press reagent tubes into the block to ensure that they are fully seated.

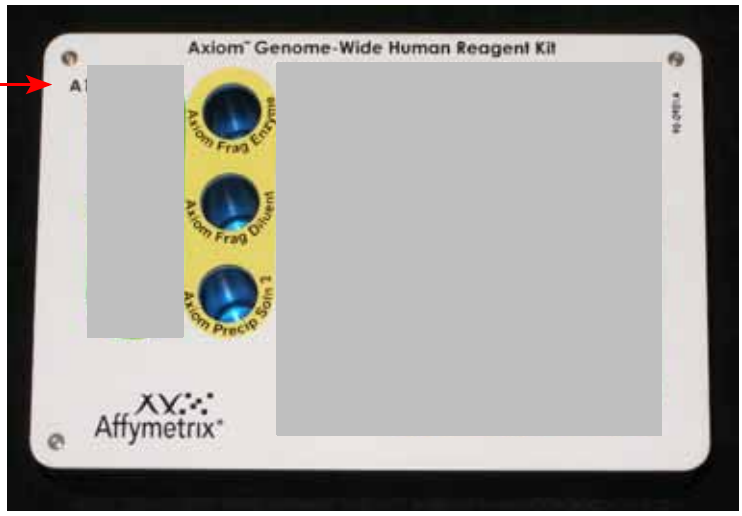
A1

Reagent block with template.

Only the positions used for this method are shown.

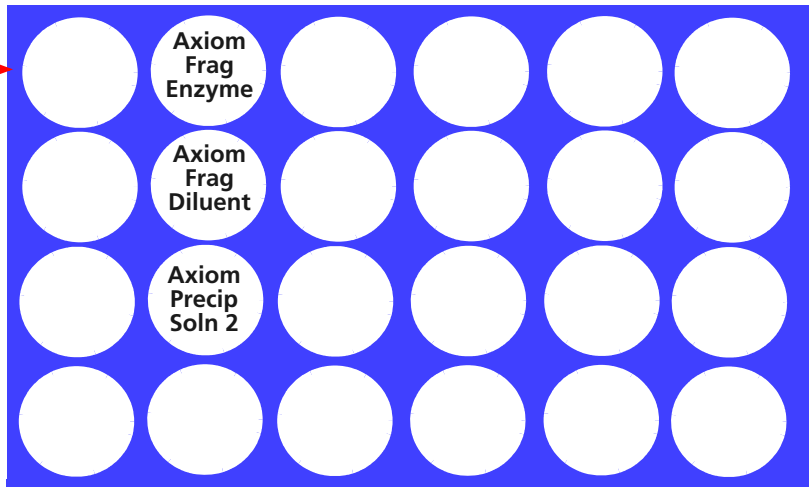
Axiom Frag Enzyme:

Flick to mix 3X and spin immediately prior to placing on the chilled reagent block.



A1

Diagram of reagent block without template



6. Check the deck layout to ensure that the labware, reagents and samples are in the proper locations.

NOTE: If the physical deck does not match the Deck Layout and Confirmation window exactly (Figure 3.28 on page 56), either modify the physical deck to match exactly or choose Abort in the Deck Layout and Confirmation window.

7. Click **OK** to continue.

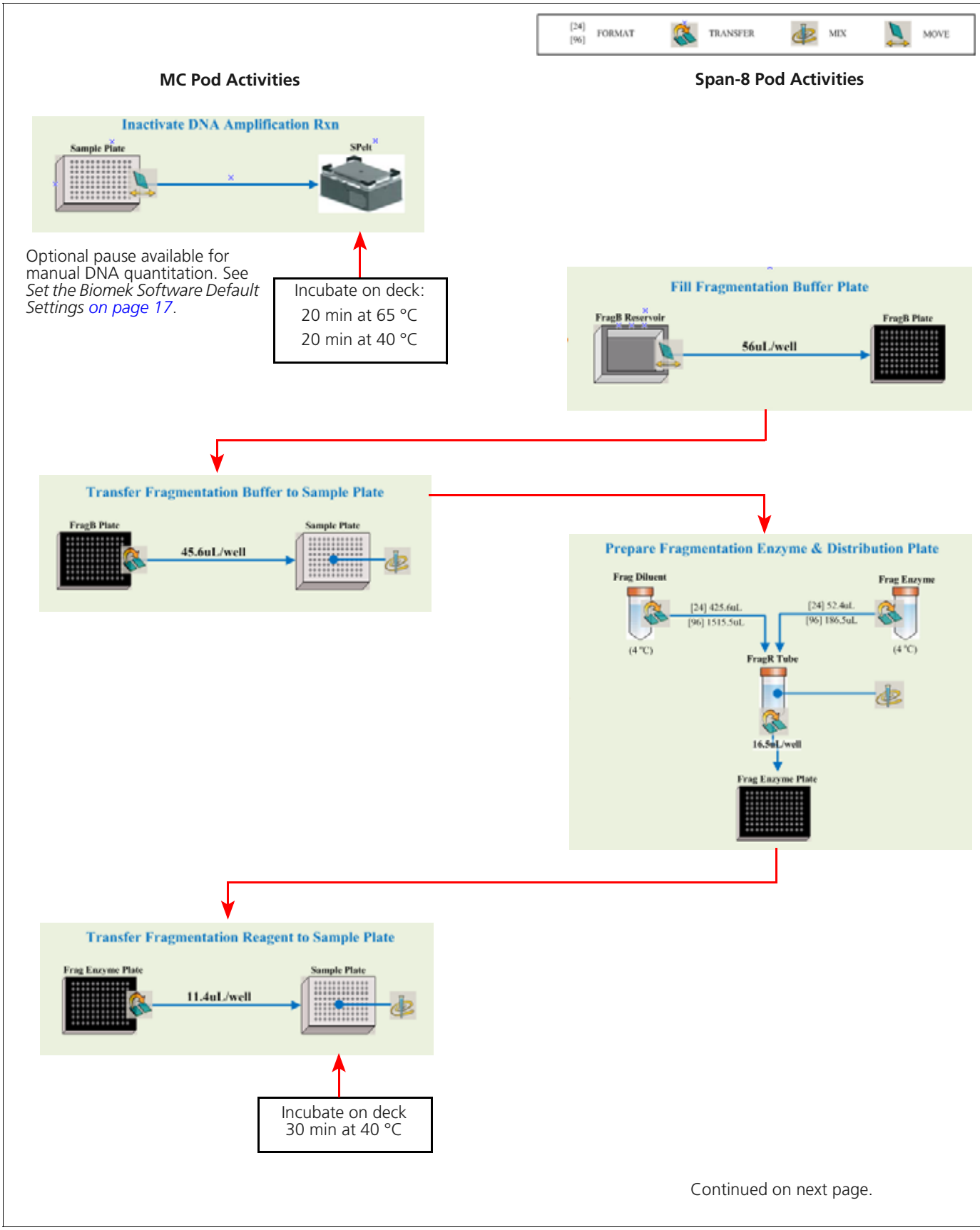
The system will automatically flush the Span-8 fluidics system. Observe the lines and syringes for air bubbles.

8. At the prompt to repeat the Span-8 fluidics system flush:
   - Click **No** if no air bubbles are present.
   - Click **Yes** if air bubbles are present. Repeat the flush until no air bubbles are present.

The Fragmentation step begins. The sample plate is incubated at 65 °C to inactivate amplification. If you selected *Prompt for manual DNA quantitation* in the default software settings, you will then be prompted to remove an aliquot of each sample for a quantitation process control. The plate will remain at 40 °C until the aliquots have been collected.

NOTE: Remain near the Biomek FX^P Target Prep Express if you are going to remove aliquots for quantitation. Avoid leaving the samples at 40 °C for a long period of time.

Summary of Fragmentation



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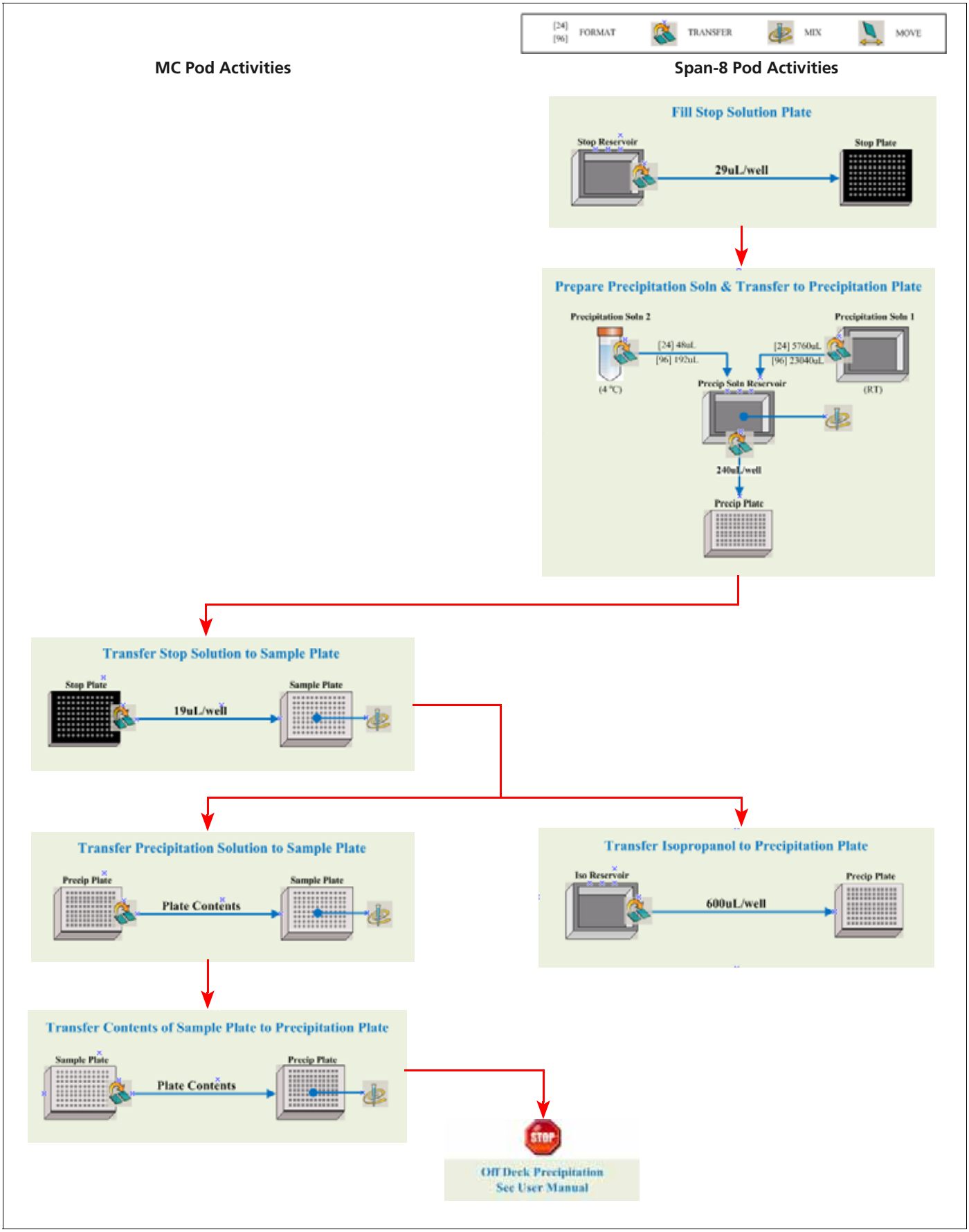

[24]
[96]
FORMAT
TRANSFER
MIX
MOVE
MC Pod Activities
Span-8 Pod Activities
Fill Stop Solution Plate
Stop Reservoir
29uL/well
Stop Plate
Prepare Precipitation Soln & Transfer to Precipitation Plate
Precipitation Soln 2
[24] 48uL
[96] 192uL
(4 °C)
Precipitation Soln 1
[24] 5760uL
[96] 23040uL
(RT)
Precip Soln Reservoir
240uL/well
Precip Plate
Transfer Stop Solution to Sample Plate
Stop Plate
19uL/well
Sample Plate
Transfer Precipitation Solution to Sample Plate
Precip Plate
Plate Contents
Sample Plate
Transfer Isopropanol to Precipitation Plate
Iso Reservoir
600uL/well
Precip Plate
Transfer Contents of Sample Plate to Precipitation Plate
Sample Plate
Plate Contents
Precip Plate
STOP
Off Deck Precipitation
See User Manual

4. Precipitation

To freeze the samples:

1. Remove the Precipitation Plate from the deck.
2. Blot the top of the plate with a Kimwipe and seal tightly.
3. Place the plate in a –20 °C freezer overnight to precipitate.
4. Return to the Biomek workstation and clear the deck.

5. Centrifuge and Dry Pellets

CAUTION: During this step, handle the 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 48 °C.

   Use an oven that can sustain a constant temperature of 48 °C and has a temperature accuracy of +/– 1 °C (we recommend the Binder FD53). If processing three or fewer array plates, you can use a GeneChip Hybridization Oven.
2. Centrifuge the plate at 3200 xg (or rcf) at 4 °C for 40 min.

   WARNING: We strongly recommend that you use the Eppendorf 5810R at 3200 xg (4000 rpm/) with:

   - **Rotor A-4-81**
   - **Plate carrier DL 050 (rubber bottom)**

   If not using the Eppendorf 5810R with recommended rotor and plate carrier, centrifuge the samples at 3200 xg (or rcf) at 4 °C.

   Do not use the 5810R with the A-4-62 rotor and WO-15 plate carrier (hard bottom). Use of this rotor and plate carrier may result in cracked plates, loss of sample, unbalanced centrifugation, damage to the instrument and possible physical injury.
3. Immediately after the 40 min centrifugation period, empty the liquid from the plate as follows:
   A. Remove 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.
4. Turn the plate right side up and place in an oven for 20 min at 48 °C to dry.

   NOTE: If using a GeneChip® Hybridization Oven, place the plate on the bottom of the oven. Plates do not rotate.
5. Do one of the following:
   - Proceed directly to *Stage 3 — Resuspension and Hybridization Preparation* *on page 62*, even if some droplets of liquid remain. Leave the sample plate at room temperature.
   - Tightly seal the plate and store at –20 °C.

Stage 3 — Resuspension and Hybridization Preparation

Duration

Resuspension and Hybridization Preparation

Table 3.12 Time required for resuspension

Hands-on time	15 min
Frozen pellet equilibration to room temperature	1.5 hr
Biomek FX^P Target Prep Express	40 min
Total time	55 min to 2.6 hr

Equipment, Consumables, Labware and Reagents Required

Equipment and Labware Required

Table 3.13 Equipment, consumables and labware required

Quantity	Item
As required	Adhesive seals for plates
1	Bench top cooler, chilled to –20 °C
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Plate centrifuge, 4 °C
As required	Adhesive seals for plates
1	Vortex
Biomek FX^P Target Prep Express Labware	
1 box of each	Barrier pipette tips: ▪ Biomek Span P50 (pink) ▪ Biomek AP96, P250 (aqua) ▪ Biomek Span P250 (green) ▪ Biomek Span P1000 (yellow)
5 plates for on-deck cycling OR: 6 plates for off-deck cycling	BIO-RAD hard shell PCR 96-well For on-deck thermal cycling or when using the PTC-0240/PTC-200 off-deck thermal cycler 4 BIO-RAD (HSP-9631) Hard-Shell PCR 96-well Plate and 1 HSS-9601 Hard-Shell Full-Height 96-Well Semi- Skirted PCR Plate 1 Plate, Costar Brand Serocluster round bottom plate For off-deck thermal cycling using the ABI 9700 or ABI 2720 thermal cycler ▫ Note: The HSS-9601 plate stacked on the Costar brand serocluster round-bottom plate should only be used on position P2 on the Biomek FX^P deck. See Figure 3.35 on page 75.
1	Plate, OD

Table 3.13 Equipment, consumables and labware required

Quantity	Item
1	Reagent block, chilled to 4 °C
1	Reservoir, 19 mL (quarter module, divided)
3	Reservoir, 40 mL (quarter module)

Reagents Required

Table 3.14 Reagents required for resuspension and hybridization

Reagent	Module
From the Axiom Reagent Kit	
Axiom Hyb Buffer	Module 2 Box 1, –20 °C
Axiom Hyb Soln 1	
Axiom Resusp Buffer	Module 2 Box 2, 2–8 °C
Axiom Hyb Soln 2	
Other Reagents Required	
Nuclease-Free Water, ultrapure MB grade (USB, P/N 71786; for OD and gel plate preparation)	To fill line of divided reservoir
TrackIt Gel Loading Buffer, diluted (see Appendix A, *Fragmentation Quality Control Gel Protocol on page 223* for dilution instructions.)	96-array plate: 8 mL

1. Preparing Frozen Pellets and Axiom Resusp Buffer

IMPORTANT: The pellets and the resuspension buffer must be at room temperature before proceeding with this step.

Allow the pellets and the Axiom Resusp Buffer to equilibrate to room temperature for 1.5 hr prior to starting this step.

2. Perform the Pre-Run Checklist

The following actions are the same as described under *1. Perform the Pre-Run Checklist on page 42*. Refer back to this step for details. Some or all of these steps may not be required depending upon the current state of the Biomek workstation.

To perform the pre-run checklist:

1. Power on the Biomek FX^P Target Prep Express and all peripherals.
2. Check the water and waste containers; replenish or empty as required.
3. Launch the Biomek Software.
4. If applicable, close the thermal cycler lid (on page 42).
5. Home all axes (on page 44).

3. Thaw and Prepare the Reagents

Thaw and Prepare the Reagents

To thaw and prepare the reagents:

1. Thaw Axiom Hyb Soln 1 on the bench top at room temperature.
2. Vortex the Axiom Resusp Buffer and the Axiom Hyb Buffer.
3. Vortex and spin Axiom Hyb Soln 1 and Axiom Hyb Soln 2.

4. Run the Resuspension and Hybridization Preparation Step

NOTE: We strongly recommend that you run two quality process controls during this step:

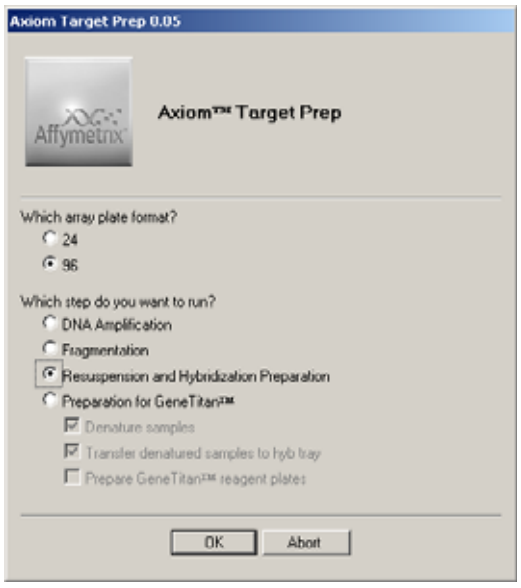
- **A gel to verify successful fragmentation**
- **An OD quantitation of each resuspended sample**

The Biomek FX^P Target Prep Express can be set to prepare fragmentation and OD plates that are ready for processing. These process controls must be selected as a run preference prior to starting a run. See *Set the Biomek Software Default Settings* *on page 17* for instructions.

To run the Resuspension and Hybridization Preparation step:

1. Open **Project > Open Project > Axiom Target Prep** and click **OK** (Figure 3.30).
2. Click the **Open Method** icon, select **Axiom Target Prep**, and click **OK**.
3. Click the **Run** button.
4. Select your array plate format and **Resuspension and Hybridization Preparation**.
   The deck layout for this method is displayed (Figure 3.31 on page 65).

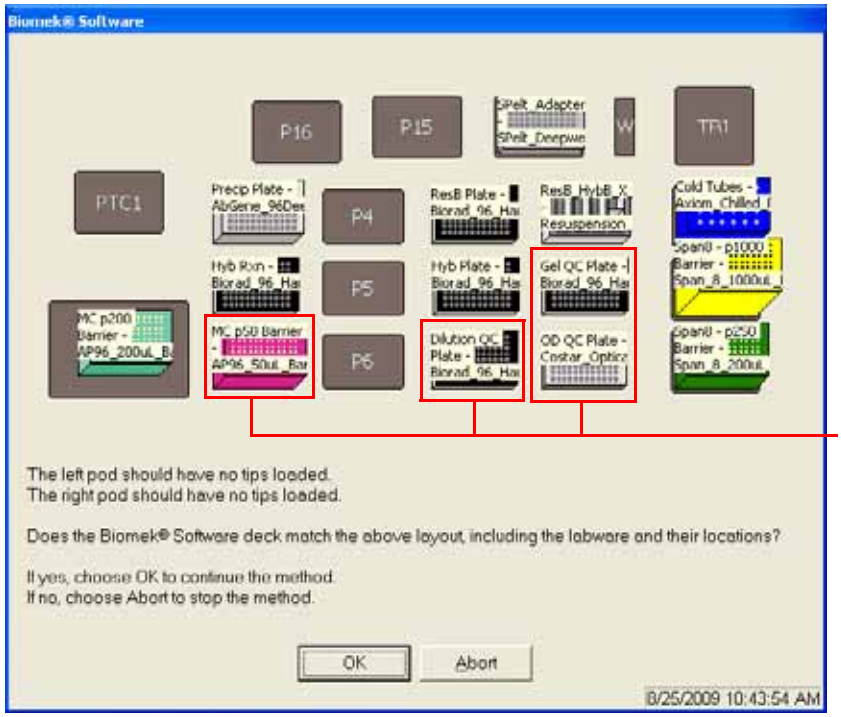
Figure 3.30 Selecting the Resuspension and Hybridization Preparation method



5. Prepare the deck as shown in Figure 3.31, Table 3.15 and Figure 3.32 on page 67.
   Label the BIO-RAD plates placed on the deck in positions P2 and P11. For example:
   - P2 — Hyb Ready + <sample description>
   - P11 — Gel QC

NOTE: Verify the appropriate plastic consumables are being used on the deck for the Hyb Reaction Plate when using the ABI 9700 or ABI 2720 thermal cycle.

Figure 3.31 Deck layout for the Resuspension and Hybridization Preparation method



If *Prepare plates for gel QC and OD after resuspension* is selected in your run preferences, the following labware is required on the deck:

- Multichannel P50 pipette tips
- Dilution QC plate
- Gel QC plate
- OD QC plate (do NOT touch bottom of plate)

If not selected, these plates will not appear in the deck layout.

See *Set the Biomek Software Default Settings on page 17* for more information.

Table 3.15 Labware and reagent locations on the deck for Resuspension and Hybridization Preparation

Position on Deck	Labware	Reagent or Samples
TL1	Biomek AP96 – P250 Pipette Tips (aqua)	—
P1	ABgene 96 Square Well Storage plate	Pelleted samples
P2	BIO-RAD Hard Shell 96-well plate (Hyb Reaction Plate). ▫ Bio-Rad Hard Shell 96-well plate (HSP 9631) for on-deck or off-deck thermal cycling using the TRobot 96, PTC-200 or PTC 0240. ▫ Bio-Rad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (HSS 9601) stacked on a Costar Round Bottom plate for off-deck thermal cycling with the ABI 9700 or 2720 thermal cycler	

Table 3.15 Labware and reagent locations on the deck for Resuspension and Hybridization Preparation (Continued)

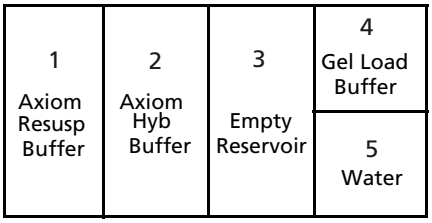
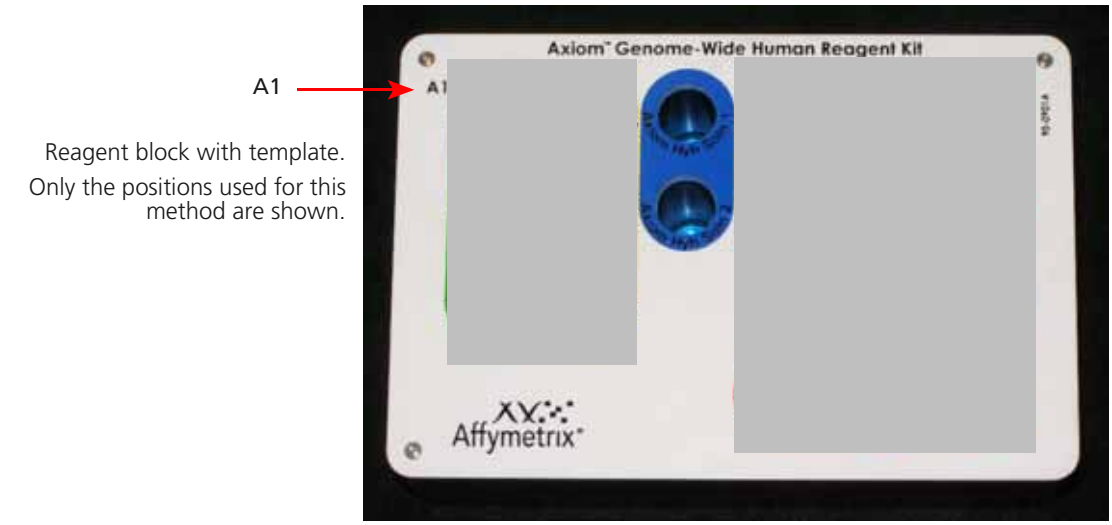
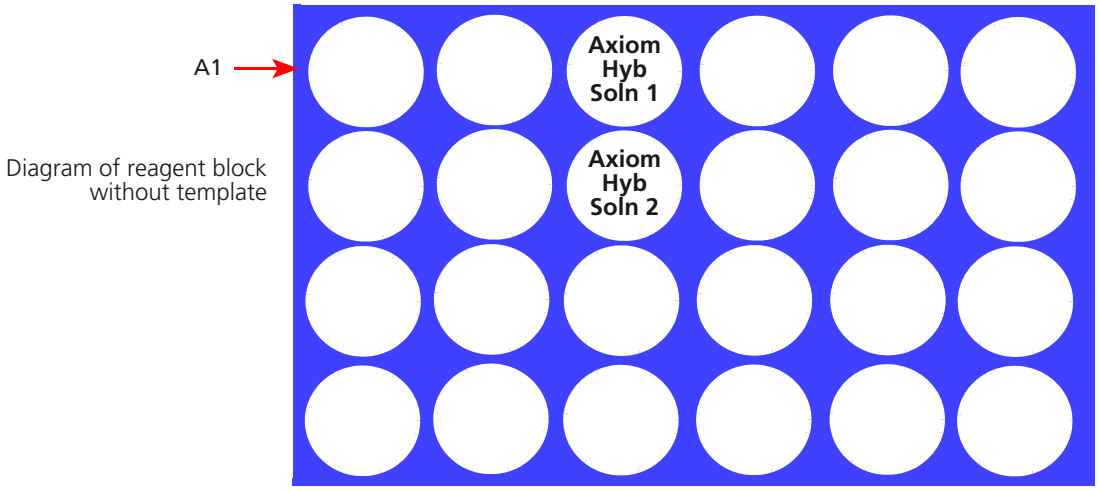
Position on Deck	Labware	Reagent or Samples
Warning: When using the ABI 9700 or the ABI 2720 off-deck thermal cycler for denaturing the hyb-ready plate, the Bio-Rad HSS 9601 Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate must be stacked on a CoStar Brand Serocluster Round Bottom plate (29442-392 from VWR International or EK-680568 from E&K Scientific) as shown in Table 3.2 on page 22. **The HSS9601 and HSP9631 PCR plates are not interchangeable on the Biomek FXp deck.**		
P3	Biomek Span P50 Pipette Tip (pink)	—
P7	BIO-RAD Hard Shell 96-well plate	—
P8	BIO-RAD Hard Shell 96-well plate	
P9	BIO-RAD Hard Shell 96-well plate	
P10	Reservoirs in frame: ▪ Quarter module (1) ▪ Quarter module (2) ▪ Quarter module (3) ▪ Quarter module, divided (4 and 5) ▪ Pour Axiom Resus Buffer into reservoir 1 ▪ Pour Axiom Hyb Buffer into reservoir 2 ▪ Leave reservoir 3 empty ▪ Pour diluted gel loading buffer into reservoir 4: – 8 mL for 96 samples ▪ Pour nuclease-free water into reservoir 5 to fill line	
P11	BIO-RAD Hard Shell 96-well plate (for Gel QC)	—
P12	OD plate, 96-well UV	—
Pelt_1	Reagent block, chilled to 4 °C	See Figure 3.32 on page 67.
P13	Biomek Span P1000 Pipette Tips (yellow)	—
P14	Biomek Span P250 Pipette Tips (green)	—

Figure 3.32 Placement of reagents on chilled reagent block for GeneTitan reagent tray preparation

IMPORTANT:

- Position all plates and the chilled reagent block with A1 in the upper left corner of the frame.
- Press reagent tubes into the block to ensure that they are fully seated.





6. Check the deck layout to ensure that the labware, reagents and samples are in the proper locations.

NOTE: If the physical deck does not match the Deck Layout window exactly (Figure 3.31 on page 65), either modify the physical deck to match exactly or choose Abort in the Deck Layout window.

7. Click **OK** to continue the method.

   The system will automatically flush the Span-8 fluidics system. Observe the lines and syringes for air bubbles.

8. At the prompt to repeat the Span-8 fluidics system flush:
   - Click **No** if no air bubbles are present.
   - Click **Yes** if air bubbles are present. Repeat the flush until no air bubbles are present.

9. Run the fragmentation gel and OD quantitation process controls.

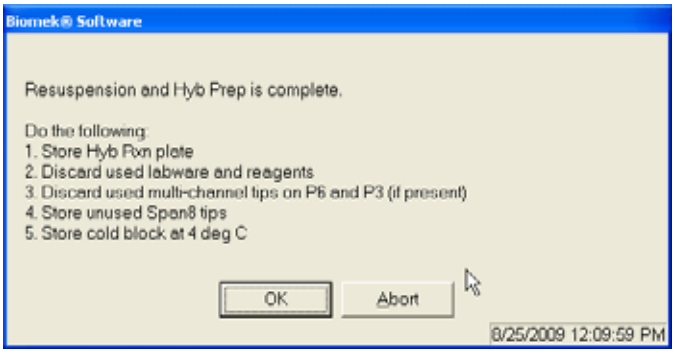
   For instructions and guidelines on assessing gel and OD results, see:
   - Appendix A, *Fragmentation Quality Control Gel Protocol on page 223*

- Appendix B, *Sample Quantitation after Resuspension on page 225*

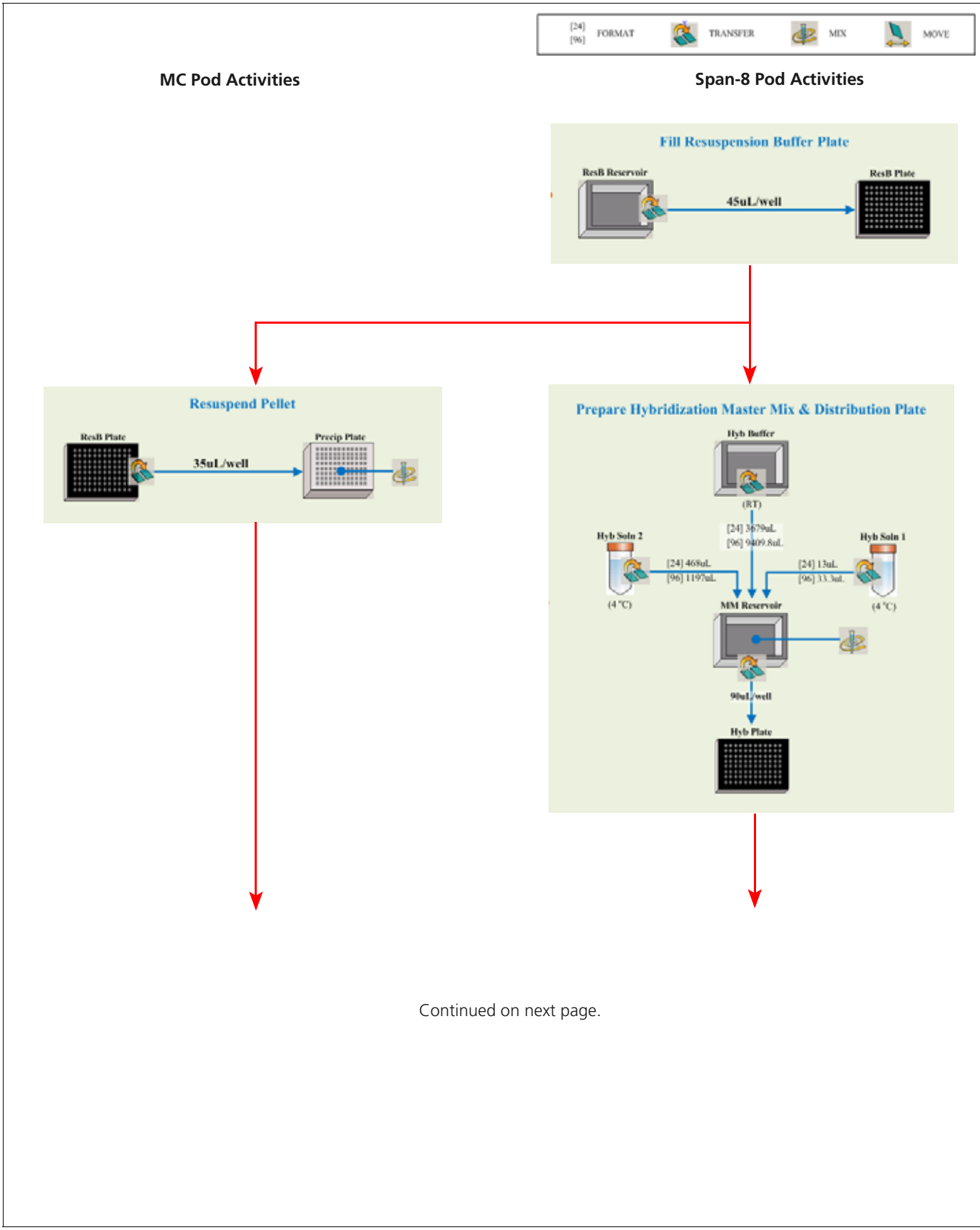
10. Do one of the following:

- If the GeneTitan MC Instrument is free, and if the gel and OD quantitation results are good, you can proceed directly to *Stage 4 — Preparation for the GeneTitan MC Instrument on page 72*.
- If the GeneTitan MC Instrument is not free, then follow the instructions shown in Figure 3.33. Tightly seal the Hyb Rxn plate and store at –20 °C.

Figure 3.33 Instructions to follow if the GeneTitan MC Instrument is not free



Summary of Resuspension and Hybridization Preparation



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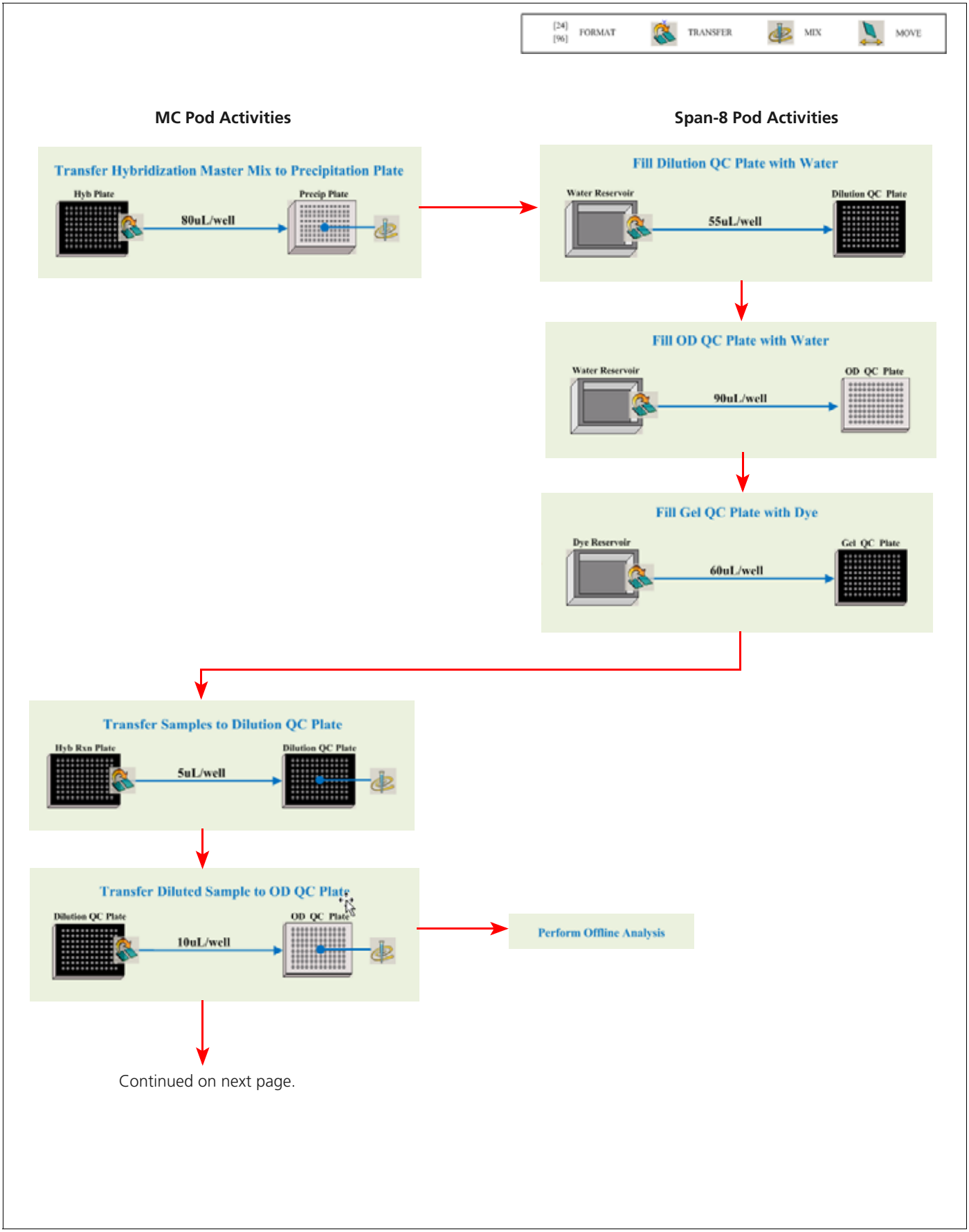
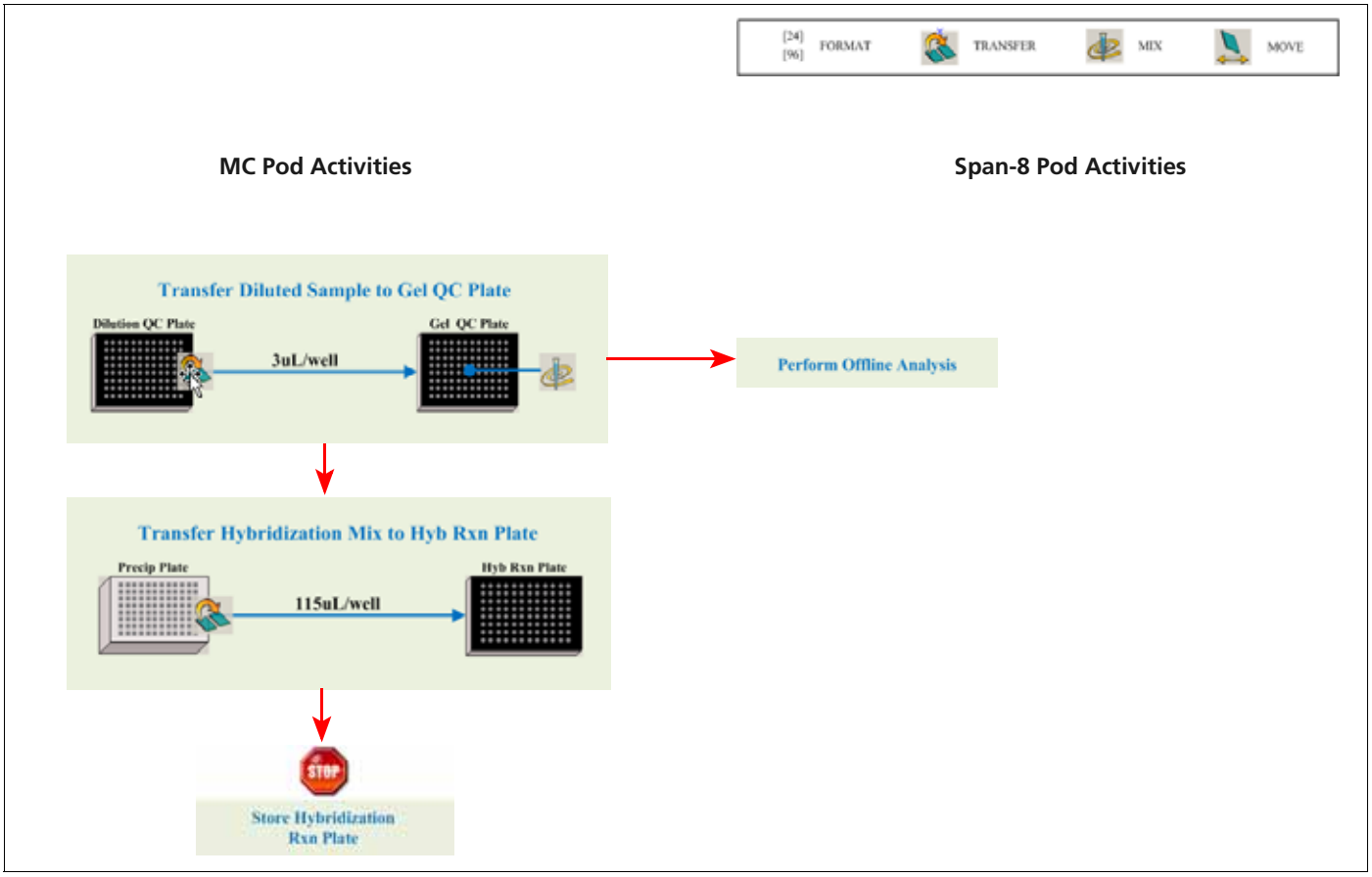

[24]
[96]
FORMAT
TRANSFER
MIX
MOVE
MC Pod Activities
Span-8 Pod Activities
Transfer Hybridization Master Mix to Precipitation Plate
Hyb Plate
80uL/well
Precip Plate
Fill Dilution QC Plate with Water
Water Reservoir
55uL/well
Dilution QC Plate
Fill OD QC Plate with Water
Water Reservoir
90uL/well
OD QC Plate
Fill Gel QC Plate with Dye
Dye Reservoir
60uL/well
Gel QC Plate
Transfer Samples to Dilution QC Plate
Hyb Rxn Plate
5uL/well
Dilution QC Plate
Transfer Diluted Sample to OD QC Plate
Dilution QC Plate
10uL/well
OD QC Plate
Perform Offline Analysis
Continued on next page.


[24]
[96]
FORMAT
TRANSFER
MIX
MOVE
MC Pod Activities
Span-8 Pod Activities
Transfer Diluted Sample to Gel QC Plate
Dilution QC Plate
3uL/well
Gel QC Plate
Perform Offline Analysis
Transfer Hybridization Mix to Hyb Rxn Plate
Precip Plate
115uL/well
Hyb Rxn Plate
STOP
Store Hybridization
Rxn Plate

Stage 4 — Preparation for the GeneTitan MC Instrument

About Stage 4

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

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

This stage, *Preparation for GeneTitan*, can include any combination of the options shown in Figure 3.36 on page 76 and Figure 3.37 on page 82. The first two options complete target preparation on the Biomek FX^P Target Prep Express.

The options are:

Option 1

- **Denature samples** - the Hyb Rxn plate is placed on the thermal cycler and the samples are denatured. At this step, you must also select Transfer denatured samples to Hyb Tray. After the denature program has completed, the block will hold temperature until the user has dismissed the prompt, indicating that they are ready to continue the method to transfer the samples to the hyb tray and then carry it to the GeneTitan. Do not leave samples on the thermal cycler for a long period of time.

Option 2

- **Transfer denatured samples to Hyb Tray** - the denatured samples are transferred from the Hyb Rxn plate to the Hyb Tray, and are ready to load onto the GeneTitan MC Instrument.

NOTE: When using an ABI 9700 or the ABI 2720 thermal cycler for off-deck denaturation, the Bio-Rad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (hyb reaction plate) must be stacked on a Costar brand Serocluster Round Bottom plate from Corning (Corning Mfg P/N 3795)

Option 3

- **Prepare GeneTitan® reagent plates** (option 3) - the solutions required for the fluidics stage of array processing on the GeneTitan MC Instrument are prepared and aliquoted to the appropriate trays (three stain trays, one ligation tray, one fix tray and one scan tray with Holding Buffer).

When performing a 1 plate workflow, select **two options** (option 1 and option 2).

- **Denature samples** and **Transfer denatured samples to hyb tray** when you are preparing the samples to begin hybridization in the GeneTitan.

- or select **one option** (option 3)**-**

- **Prepare GeneTitan reagent plates** to prepare reagents for loading onto the GeneTitan the following day when the plate is finished with the hybridization period and about to begin GeneTitan fluidics processing.

Options for performing a multi-plate workflow

When performing a multi-plate workflow (see Chapter 6, *Automated Target Preparation for Processing Eight Axiom Array Plates Per Week on page 183* for a description of the 8 plate workflow), the need to carry out preparation of reagents for one plate while simultaneously hybridizing a second plate will arise. In this case, all three options in the Biomek FXᴾ method can be carried out concurrently. Select **all three options** when using an on-deck thermal cycler.

- **Denature samples** and **Transfer denatured samples to hyb tray** will prepare samples for the new plate going into the GeneTitan to begin hybridization.

-and-

- **Prepare GeneTitan reagent plates** will prepare reagents for the plate that is already in the GeneTitan hyb oven and is ready to go into the Wash, Ligation, Stain, and Scan phases of GeneTitan array processing.

NOTE: In the 8 plate workflow, all three options will only be selected for middle days of the workflow. On the first day of the workflow, only the Denature samples and Transfer denatured samples to hyb tray options will be used. On the last day of the workflow, only the Prepare GeneTitan reagent plates option will be used.

NOTE: Note: When using an off-deck thermal cycler, avoid letting the samples sit a room temperature for an extended period of time after denaturation. Do not begin denaturation at the same time as the GeneTitan reagent preparation.

Off-deck thermal cycler option

The steps for performing a simultaneous preparation of GeneTitan reagent plates and hybridization of a second plate required in the course of a multi-plate workflow are modified slightly when the off-deck thermal cycler option is selected. The reagent trays to be loaded into the GeneTitan MC instrument are prepared in an initial run of the method.

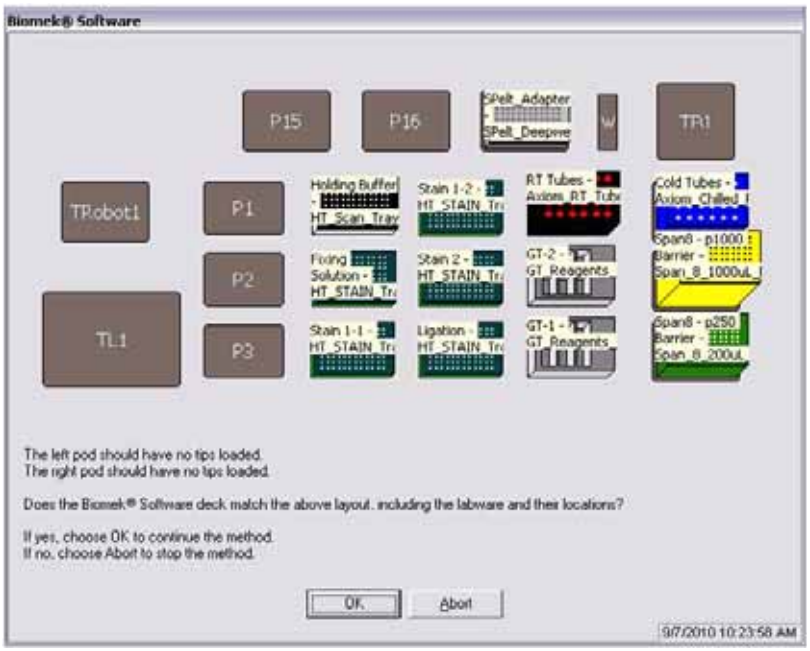
Denaturation of the hyb ready samples in the off-deck thermal cycler begins **mid way** through the GeneTitan reagent prep on the Biomek deck.

After loading the reagent trays into the GeneTitan MC instrument, you must perform a second run of the Biomek method to transfer the denatured samples to the hyb tray for loading into the GeneTitan MC instrument.

The modified steps are:

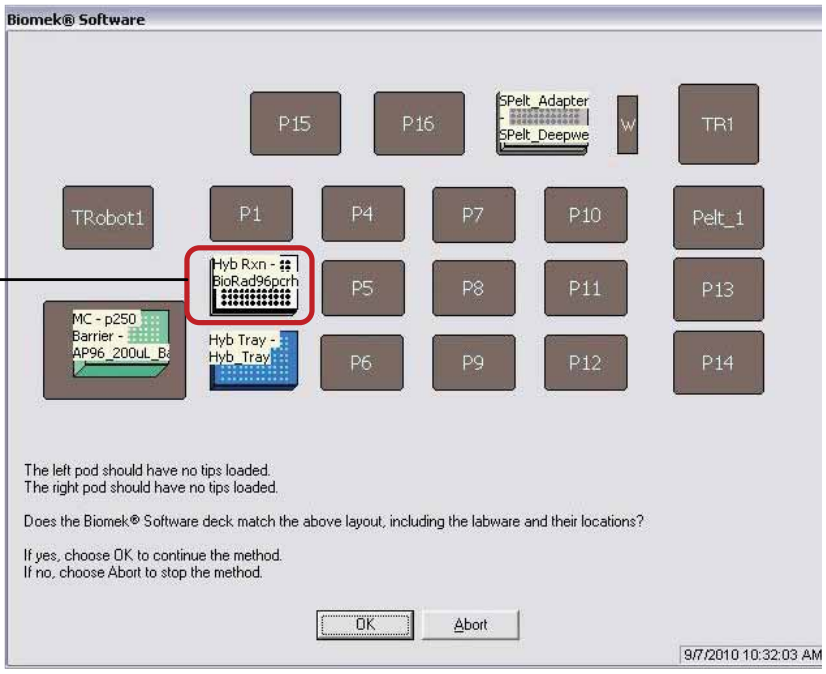
1. Select **Preparation for GeneTitan** step with only the **Prepare GeneTitan reagent plates** box checked, click **OK**.
2. Prepare deck as shown in Figure 3.34.

Figure 3.34 Deck Layout for Off-deck thermal cycler option



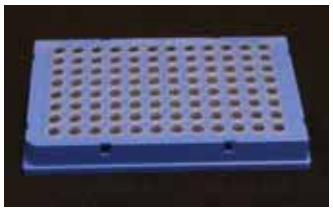
3. Click **OK**.
4. Wait 18 minutes, then begin denaturation of the hyb ready samples using the off-deck thermal cycler.
5. Upon completion of the GeneTitan reagent plate preparation, load reagent plates and scan tray into the GeneTitan MC instrument
6. Once the reagents are loaded into the GeneTitan MC instrument and the denaturation method on the thermal cycler is complete, retrieve the denatured hyb ready samples from the thermal cycler
7. Return to Biomek FXP and begin a new method, select **Preparation for GeneTitan** step with only the **Transfer denatured samples to hyb tray** box checked, click **OK**.
8. Prepare deck as shown in Figure 3.35 (note that a spacer must be used with HSS-9601 plates for ABI thermal cyclers).

Figure 3.35 Deck Layout for Off-deck thermal cycler option and position P2



The Hyb Reaction Plate in deck position P2 must be one of the following:

Bio-Rad Hard Shell 96-well plate (HSP 9631) for on-deck or off-deck thermal cycling using the TRobot 96, PTC-200 or PTC 0240



OR

Bio-Rad Hard-Shell Full-Height 96-Well Semi- Skirted PCR Plate(HSS 9601) stacked on a Costar Round Bottom plate for off-deck thermal cycling with the ABI 9700 or 2720 thermal cycler

9. Click **OK**.
10. After the denatured samples have been transferred to the GeneTitan hyb tray, load hyb tray into GeneTitan

If a plate is already in the hybridization oven

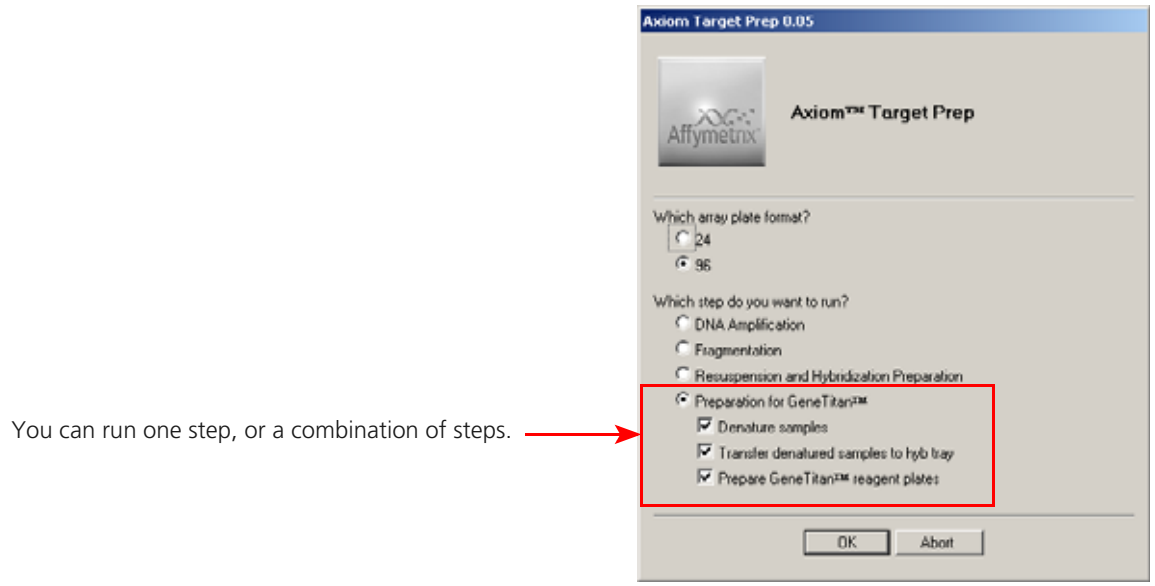
For the plate that is already in the GeneTitan hyb oven and that is ready to go into Ligation, Wash-Stain and Scan, select **option 3**, **Prepare GeneTitan reagent plates.** The solutions required for the fluidics stage of array processing on the GeneTitan MC Instrument are prepared and aliquoted to the appropriate trays (three stain trays, one ligation tray, one fix tray and one scan tray with Holding Buffer).

IMPORTANT: **The reagent plates prepared in the third sub-step, *Prepare GeneTitan® reagent plates*:**

- **Are NOT for use with the Hyb Tray currently being prepared on the Biomek workstation.**
- **Are for the continued processing of an Axiom Array Plate that is:**
  - **already on the GeneTitan MC Instrument.**
  - **has completed the hybridization stage.**
  - **is ready for transfer to the fluidics area.**

The reagent plates for the fluidics stage on the GeneTitan MC Instrument CANNOT 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 cannot be stored.

Figure 3.36 Software setup for *Preparation for GeneTitan*



Duration

Denaturation

Table 3.16 Time required to denature samples

Hands-on time:	~ 3 min
Biomek FX^P Target Prep Express	23 min
Total time:	~ 26 min

Denatured Sample Transfer to Hyb Tray

Table 3.17 Time required to transfer denatured samples to the Hyb Tray

Hands-on time	2 min
Biomek FX^P Target Prep Express	~ 2 min
Total time	~ 4 min

Preparation of GeneTitan Reagent Trays

Table 3.18 Time required to prepare reagent trays for the GeneTitan MC Instrument

Hands-on time	~15 min
Prepare reagents	~ 30 min
Biomek FX^P Target Prep Express	~ 30 min
Total time	~ 75 min

NOTE: When you select Denaturation and Preparation of the GeneTitan Reagent Trays, the on-deck processes run concurrently.

Equipment and Consumables Required

Denature Samples

Table 3.19 Equipment required for denaturing sample.

If denaturing samples:	Item	Quantity
▪ On the Biomek workstation	Lid, arched metal	1

Transfer to Hyb Tray

Table 3.20 Consumables required for transferring denatured samples to a Hyb Tray

Item	Quantity
Hyb Tray (from an Axiom Genome-Wide CEU 1 Array Plate Kit or the GeneTitan Consumable Kit, P/N 901606)	1

Table 3.20 Consumables required for transferring denatured samples to a Hyb Tray

Item	Quantity
Pipette tips, Biomek AP96 – P250 (aqua)	1 full box
Costar brand Serocluster Round Bottom plate from Corning Corning Mfg PN 3795) to be used for stacking under the HSS-9601 semi-skirted (hyb reaction plate) plate used with the ABI 9700 and ABI 2720 thermal cyclers	1

Prepare GeneTitan Reagent Plates

Table 3.21 Consumables and other equipment required

Item	Quantity
Axiom Genome-Wide CEU 1 Array Plate Kit or the GeneTitan Consumable Kit: ▪ Scan Tray with cover ▪ Stain Tray ▪ Cover for Stain Tray	▪ 1 Scan Tray with cover and protective base ▪ 5 Stain Trays ▪ 5 Covers
Pipette tips, Biomek AP96 – P250 (aqua)	1 full box
Pipette tips, Biomek Span P1000 (yellow)	1 full box
Pipette tips, Biomek Span P250 (green)	1 full box
Reagent block, chilled to 4 °C	1
Reservoirs, quarter module divided	3
Reservoirs, quarter module	3
Tube rack, 24 position with insert (room temperature rack)	1
Zerostat AntiStatic Gun	1

NOTE: See Table 3.3 on page 28 for GeneTitan MC Consumable part numbers.

1. Perform the Pre-Run Checklist

The following actions are the same as described under *1. Perform the Pre-Run Checklist* *on page 42*. Refer back to this step for details. Some or all of these steps may not be required depending upon the current state of the Biomek workstation.

To perform the pre-run checklist:

1. Power on the Biomek FX^P Target Prep Express and all peripherals.
2. Check the water and waste containers; replenish or empty as required.
3. Launch the Biomek Software.
4. If open, close the thermal cycler lid (on page 42).
5. Home all axes (on page 44).

2. Prepare the Reagents for GeneTitan Reagent Plate Preparation

IMPORTANT: Ligation Buffer and Ligation Solution 2 require approximately 30 to 40 min to thaw on the bench top at room temperature.

Reagents Required

Table 3.22 Reagents required for GeneTitan MC Instrument reagent plate preparation.

Module	Reagent	Thaw on Bench Top, Then Place on Ice	Place on Ice	Place on Bench Top at Room Temperature
Module 3 Room Temperature	Axiom Wash Buffer A			✓
	Axiom Wash Buffer B			✓
	Axiom Water			✓
Module 4 Box 1 of 2 –20 °C	Axiom Ligate Buffer			✓ for 30 min
	Axiom Ligate Enzyme	Keep at –20 °C until ready to use		
	Axiom Ligate Soln 1	✓		
	Axiom Probe Mix 1	✓		
	Axiom Stain Buffer	✓		
	Axiom Stabilize Soln	✓		
Module 4 Box 2 of 2 2 to 8 °C	Axiom Ligate Soln 2			✓ for 30 to 40 min
	Axiom Probe Mix 2*		✓	
	Axiom Wash A			✓ for 30 min
	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.**

Preparing Axiom Wash A

During storage of the Axiom Wash A from Module 4 at 4 °C, precipitation in the form of clear crystals can sometimes occur. Therefore, follow the procedure below to ensure that any precipitate is returned to solution prior to use.

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

To prepare the Axiom Wash A:

1. Vortex the bottle for 30 sec.
2. Place on the bench top at room temperature for 30 min.
3. Examine the reagent for precipitate (look into the top of the bottle).
4. If precipitate is still present, vortex again for 30 sec.
5. Pour Axiom Wash A into the appropriate reagent reservoir and leave on the bench top.

Preparing Axiom Ligate Buffer

White precipitate is sometimes observed when the Axiom Ligate Buffer is thawed. Follow this procedure to ensure that any precipitate is returned to solution prior to use.

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

To prepare the Axiom Ligate Buffer:

1. Place on the bench top at room temperature for 30 min.
2. Examine the buffer for precipitate (look into the top of the bottle).
3. If precipitate is present, vortex the bottle for 30 sec.
4. Re-examine the buffer for precipitate.
5. If precipitate is still present, warm the bottle with your hands and vortex again for 30 sec.
6. Pour the Axiom Ligate Buffer into the appropriate reagent reservoir and leave on the bench top.

Prepare the Remaining Reagents

To prepare the remaining reagents for GeneTitan MC Instrument plate preparation:

1. Leave the Axiom Ligate Enzyme at –20 °C until ready to use.
2. Thaw the following reagents from Module 4, box 1 of 2 on the bench top at room temperature, then vortex, spin and place on ice:
   - Axiom Ligate Soln 1
   - Axiom Probe Mix 1
   - Axiom Stabilize Soln
   - Axiom Stain Buffer
3. Prepare the remaining reagents from Module 4, box 2 of 2 as follows:
   A. Gently flick each tube 2 to 3 times to mix, then spin.
   B. Place reagents on ice, *except for the Axiom Ligate Soln 2 and Axiom Water*— leave these reagents on the bench top at room temperature.

3. Prepare the Sample Plate if Stored at –20 °C and the Array Plate

To prepare samples that were stored at –20 °C:

1. Vortex briefly; then spin at 1000 rpm for 30 sec.
2. Place on ice.

To prepare the Array Plate:

1. Remove the array plate box from the 4 °C refrigerator where it is stored.
2. Open the box and remove the pouch containing the array plate and protective base.

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

3. Leave the array plate in the pouch, unopened but placed on the bench for a minimum of 25 minutes before opening and loading on the GeneTitan MC to allow the plate to come to room temperature.

4. Run the Preparation for GeneTitan® Step

To run the Preparation for GeneTitan step:

1. Open **Project > Open Project > Axiom Target Prep** and click **OK**.
2. Click the **Open Method** icon, select **Axiom Target Prep**, and click **OK**.
3. Click the **Run** button.
4. Select your array plate format.
5. Select **Preparation for GeneTitan®** and the sub-steps that you wish to run; then click **OK**.
   Based upon your choices, the appropriate deck layout is displayed (Figure 3.38).
6. Deionize the GeneTitan stain trays. Refer to the section *Deionization Procedure for GeneTitan Trays and Covers* *on page 241* for the deionization procedure.

IMPORTANT: It is important to deionize the GeneTitan Instrument trays to remove any static electricity on the trays. Static attraction by the trays may prevent the tray cover from being lifted up by the instrument.

7. Prepare the deck as shown in:
   - Figure 3.38
   - Table 3.23 and Figure 3.41 on page 86
   - Figure 3.42 on page 86

NOTE: Prior to placing the arched metal lid on the deck, be sure to clean the attached Microseal P pad with 70% ethanol and dry it. Refer to the package insert for this product for further information on cleaning and replacement.

Figure 3.37 Software setup for *Preparation for GeneTitan*

- Select **Denature Samples** and **Transfer denatured samples to hyb tray**
  - if you are preparing to load the array plate into the GeneTitan Instrument for Hybridization.
- Select **Prepare GeneTitan Reagent Plates**
  - if you are preparing to load the GeneTitan Instrument for Wash-Stain and Imaging.
- Select **Denature Samples** and **Transfer denatured samples to hyb tray** and **Prepare GeneTitan Reagent Plates**
  - if you are running an 8 plate workflow then you will need to select all three check boxes in some instances in preparation for the plate that will go into the GeneTitan Instrument for Hybridization.
- Select **Prepare GeneTitan Reagent Plates** for the array plate that has completed hybridization and is ready to be processed in the GeneTitan Instrument for Ligation- Wash-Stain and Imaging.

You can run each step individually, or any combination of steps. If you do not select Prepare GeneTitan reagent plates, the deck layout will be different than the one shown in Figure 3.38.

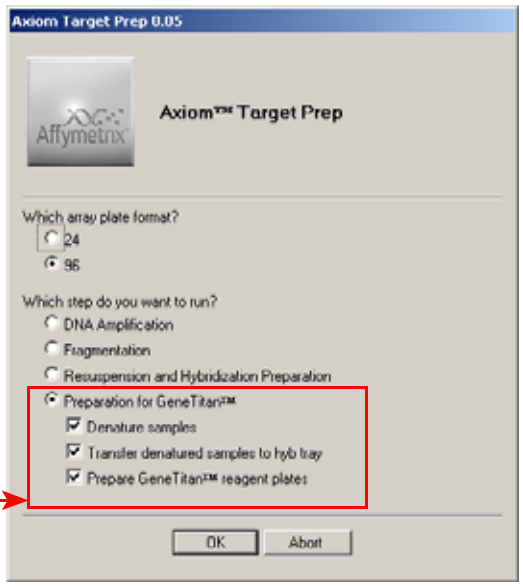


Figure 3.38 Deck layout for *Preparation for GeneTitan* — denature, transfer to Hyb Tray, and GeneTitan plate preparation

IMPORTANT: Destatic the bottom of the Hyb Tray, Stain Trays, and the protective base of the Scan Tray prior to placing on the deck. See the section *Deionization Procedure for GeneTitan Trays and Covers* on page 241.

Included in deck layout if *Prepare GeneTitan® reagent plates* is selected.

The deck layout displayed is based upon the selections you make for this step.

Included in deck layout if:
- *Denature samples*
- *Transfer denatured samples to Hyb Tray* are selected.

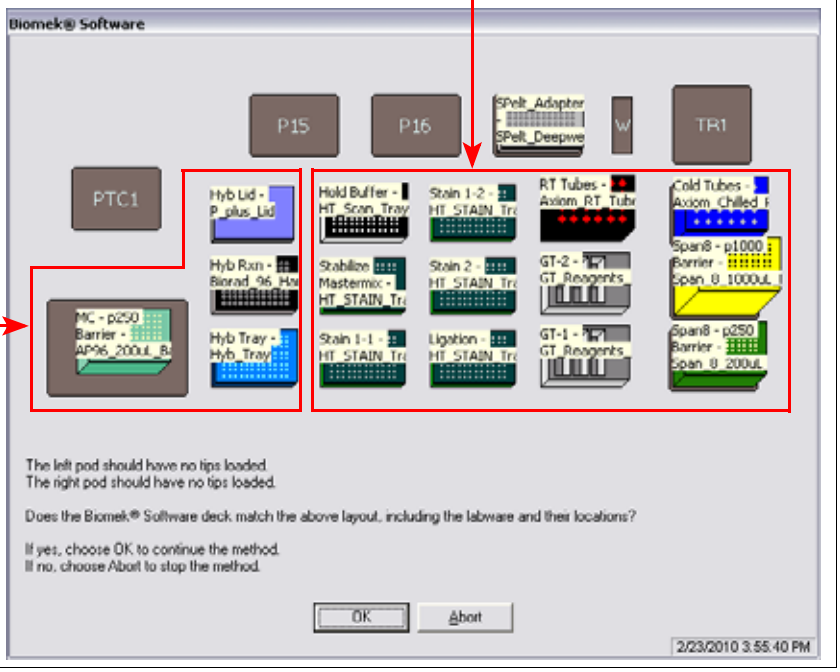
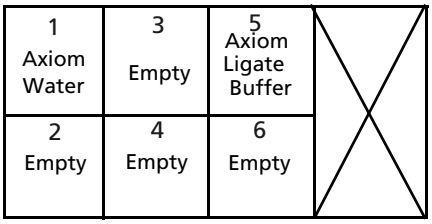
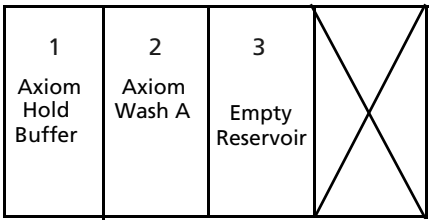


Table 3.23 Labware and reagent locations on the deck for GeneTitan reagent preparation

Position on Deck	Labware	Reagent or Samples
If *Denature samples* and *Transfer denatured samples to Hyb Tray is selected,* (no reagent tray preparation), only the labware listed below is required:		
TL1	Biomek AP96 – P250 Pipette Tips (aqua)	—
P1	Lid, arched metal Clean before use (70% ethanol).	—
P2	Hyb Reaction Plate++	Hyb-ready samples
P3	Hyb Tray*	—
If *Prepare GeneTitan® reagent plates*, the labware listed below is required:		
P4	Scan Tray on protective base*	—
P5	*Fix**✂	—
P6	*Stain 1**✂ (first of two trays with Stain 1)	—
P7	*Stain 1**✂ (second of two trays with Stain 1)	—
P8	*Stain 2**✂	—
P9	*Lig**✂	—
P10	Tube block with one insert, room temperature	See Figure 3.42 on page 86.
P11	Reservoirs in frame: ▪ Quarter module, divided (1 and 2) ▪ Quarter module, divided (3 and 4) ▪ Quarter module, divided (5 and 6) ▪ Pour Axiom Water into reservoir 1 ▪ Pour Axiom Ligation Buffer into reservoir 5	
P12	Reservoirs in frame: ▪ Quarter module (1) ▪ Quarter module (2) ▪ Quarter module (3) ▪ Pour Axiom Hold Buffer into reservoir 1 ▪ Pour Axiom Wash A into reservoir 2 ▪ Leave reservoir 3 empty	
Pelt_1	Reagent block, chilled to 4 °C	See Figure 3.41 on page 86
P13	Biomek Span P1000 Pipette Tips (yellow)	—
P14	Biomek Span P250 Pipette Tips (green)	—

++Bio-Rad Hard Shell 96-well plate (HSP 9631) for on-deck or off-deck thermal cycling using the TRobot 96, PTC-200 or PTC 0240. Bio-Rad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (HSS 9601) stacked on a Costar Round Bottom plate for off-deck thermal cycling with the ABI 9700 or 2720 thermal cycler

*These trays are included in Axiom Genome-Wide and Custom myDesign™ Array Plate Kits.

✂ Label each of these stain trays as described above as described in *Labeling GeneTitan Hybridization and Reagent Trays*. For example, label the Stain tray to be placed in P9 with the word Lig. This tray will contain the Ligation master mix.

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:

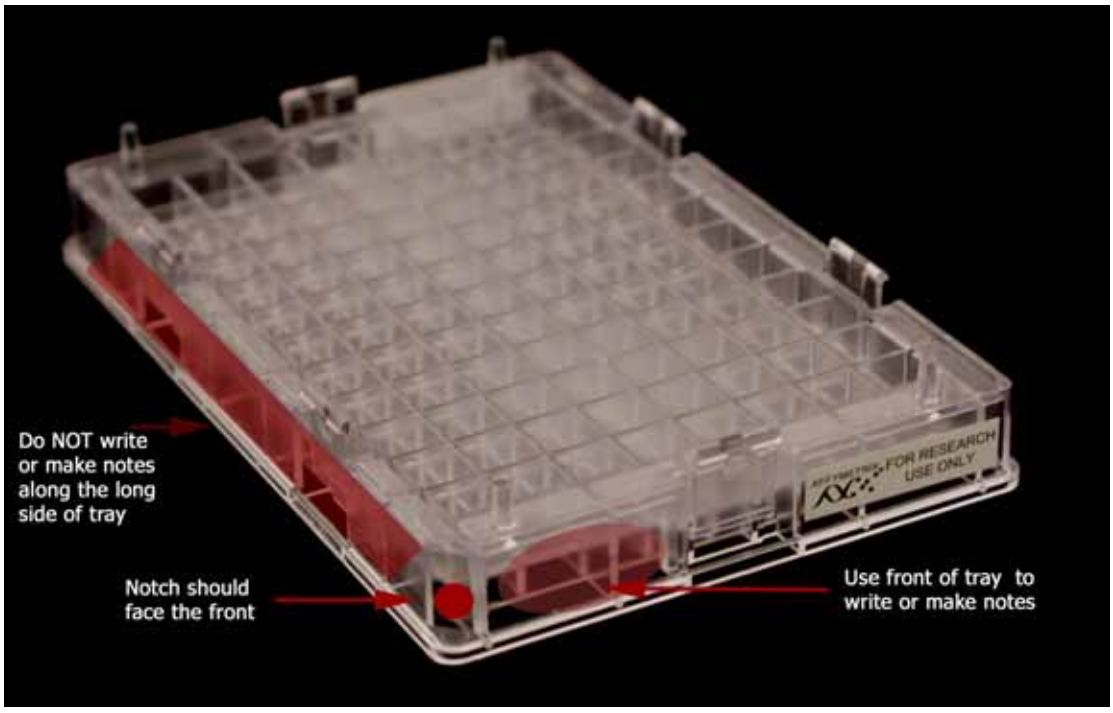
- *Labeling for Hyb Trays*, below
- *Labeling for Stain Trays* *on page 36*

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 3.39. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.

Figure 3.39 Labeling GeneTitan Hyb Trays



Writing on the wrong area of the Hyb tray may interfere with the operation of sensors in the GeneTitan MC.

Labeling for Stain Trays

You may label the stain trays on the **left side of the front of the tray** as shown in Figure 3.40. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.

Figure 3.40 Labeling GeneTitan stain trays

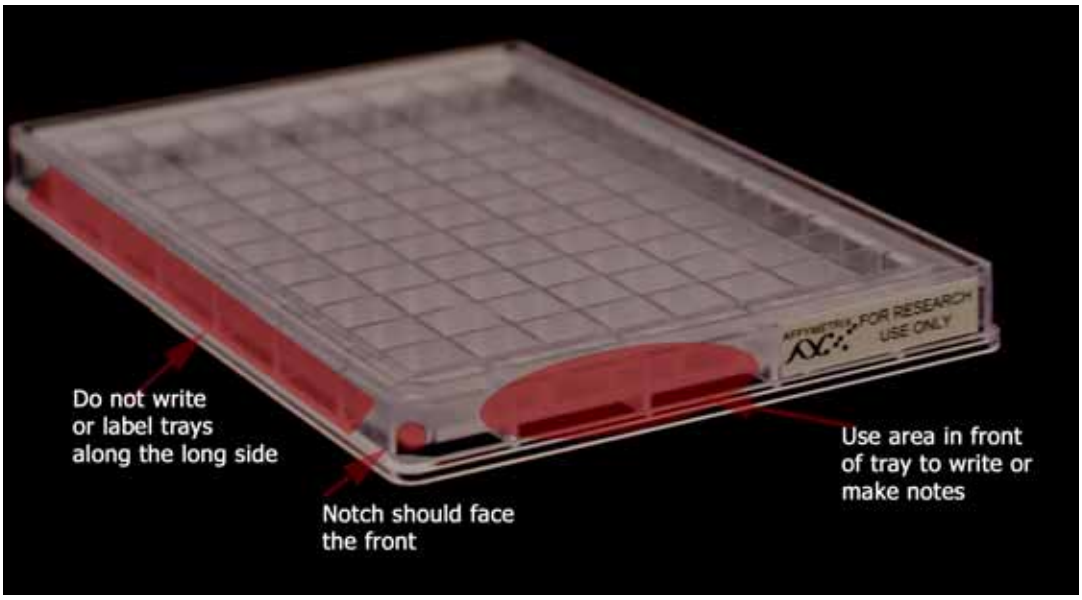


Figure 3.41 Placement of reagents on chilled reagent block for GeneTitan reagent tray preparation

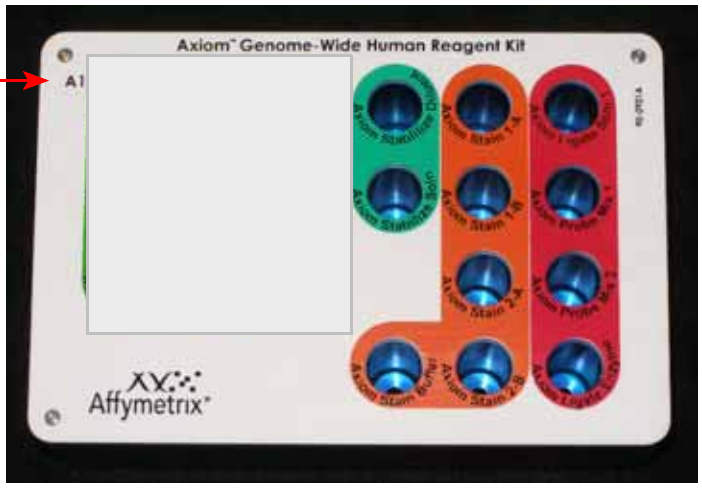
IMPORTANT:

- Position all plates and the chilled reagent block with A1 in the upper left corner of the frame.
- Press reagent tubes into the block to ensure that they are fully seated.

A1

Reagent block with template.

Only the positions used for this method are shown.



A1

Diagram of reagent block without template

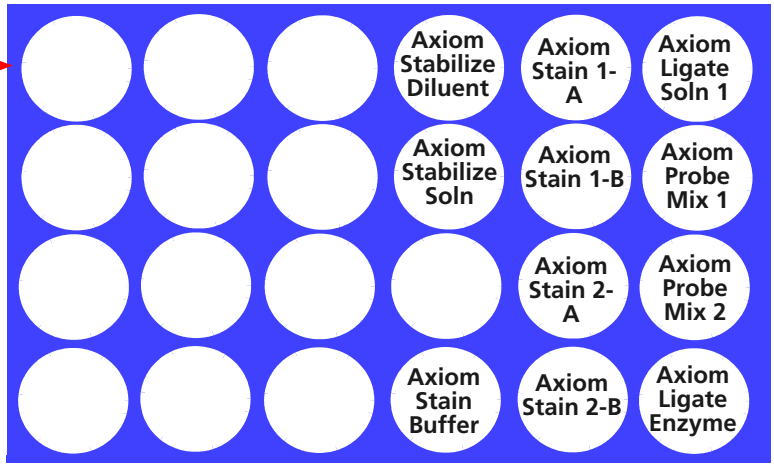
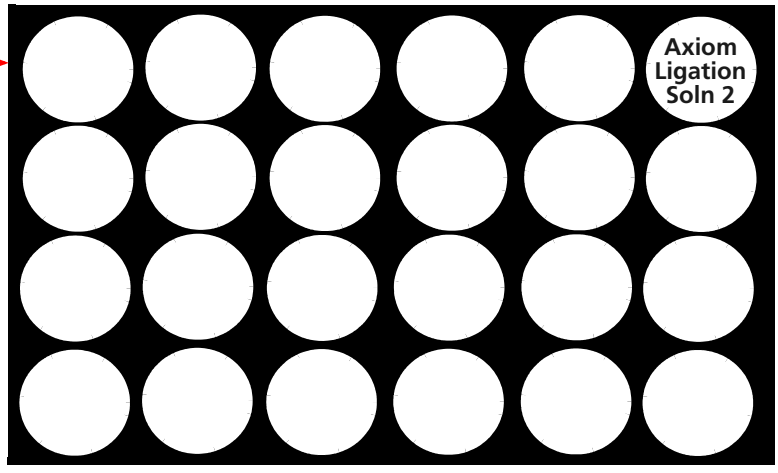


Figure 3.42 Placement of reagents on room temperature tube block

A1



8. Check the deck layout to ensure that the labware, reagents and samples are in the proper locations.

   NOTE: If the physical deck does not match the Deck Layout window exactly (Figure 3.38 on page 82), either modify the physical deck to match exactly or choose Abort in the Deck Layout window.

9. Click **OK** to continue the step.

   The system will automatically flush the Span-8 fluidics system. Observe the lines and syringes for air bubbles.

10. At the prompt to repeat the Span-8 fluidics system flush:

    - Click **No** if no air bubbles are present.

    Click **Yes** if air bubbles are present. Repeat the flush until no air bubbles are present.

If the "Denature Samples" option is selected, the Biomek FX^P Target Prep Express places the samples onto the thermal cycler and runs the denaturation program. If *Prepare GeneTitan reagent plates* is also selected, reagent plate preparation for the GeneTitan MC Instrument starts while the samples are being denatured.

6. Prepare the GeneTitan MC Instrument

While your samples are being denatured and/or the Biomek FX^P Target Prep Express is preparing reagent plates, ensure that the GeneTitan MC Instrument is ready for use.

One or more of the following steps may need to be performed:

1. Launch AGCC and select **AGCC GeneTitan Control.**
2. Upload your sample registration file now.

   If you do not upload your samples before scanning the array plate barcode, the software will assign names to your samples.
3. Select the **System Setup** tab (Figure 3.43).
4. 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 154*.

   B. Click **Next**.

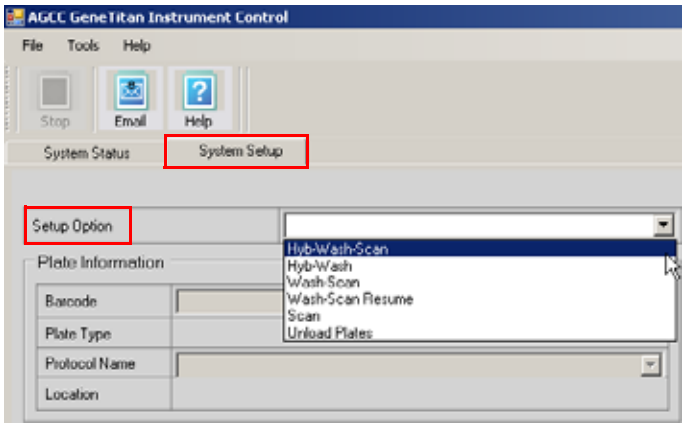
   C. Plate Information:

      Barcode: Scan or manually enter the Axiom Array Plate barcode and click **Next**.

   D. **Protocol Name:** Select the protocol name and click **Next**.
5. Fill the Wash A, Wash B and Rinse bottles.
6. Empty the Waste bottle.

- After preparing the GeneTitan MC Instrument, perform steps 7a, 7b or 7c as appropriate for your workflow:
  - Go to Step 7a: if you are denaturing the samples and transferring them to the hyb tray.
  - Go to Step 7b: if you are preparing reagents for the Ligation-Wash-Stain
  - Go To Step 7c: if you are performing a 2-8 plate workflow, and you are denaturing samples for the 2nd plate and are preparing ligation reagents for the 1st plate. The samples for the 2nd HT array plate will be denatured and transferred into the Hyb tray. The Hyb tray will be placed in the GeneTitan with the array plate in preparation for hybridization. The robot will also prepare reagents for Ligation-Wash-Stain for the 1st array plate that was placed in the GeneTitan hyb oven 24 hours ago.

Figure 3.43 Setup options for processing array plates



Step 7a. Complete Stage 4 — Preparation for GeneTitan - Hybridization Trays

If you selected **Denature samples and Transfer denatured samples to Hyb tray** in Step 4 (See Step *4. Run the Preparation for GeneTitan® Step* *on page 81* and Figure 3.37 on page 82), use the following instructions:

Figure 3.44 Prompt indicating denaturation is complete.

-

IMPORTANT: Immediately load the Array Plate and Hyb Tray into the GeneTitan MC Instrument.

1. Once the GeneTitan MC Instrument is ready, return to the Biomek FX^P Target Prep Express click **OK** (prompt shown in Figure 3.44 above).

   The denatured samples are then taken off the thermal cycler and are transferred to the Hyb Tray.

   - Ensure that there are no air bubbles present in the hyb tray. Puncture any air bubbles that you see using a pipette tip.
2. Transfer the Hyb Tray to the GeneTitan MC Instrument and load. Refer to the section *Axiom™ Genotyping Assay: Array Processing with the GeneTitan® MC Instrument* *on page 149* for the proper way of loading the array plate and the hyb tray.
3. Return to the Biomek FX^P Target Prep Express and clear the deck (Figure 3.45).
   - Always discard the used multi-channel pipette tips in position P9.
   - Always store the reagent block at 4 °C.
   - Clean the Microseal P Pad by wiping with 70% EtOH and dry.

     Refer to the package insert for this product for further information on cleaning and replacement.

Figure 3.45 Instructions displayed at the end of Step 4.

Step 7b. Complete Stage 4 - Preparation for GeneTitan - Reagent Trays

If you selected **Prepare GeneTitan Reagent Plates** in Step 4 (See Step *4. Run the Preparation for GeneTitan® Step on page 81* and Figure 3.37 on page 82), use the following instructions:

1. Once the prompt shown in Figure 3.45 appears, remove the reagent plates and Scan Tray with Hold Buffer from the deck and cover with the appropriate lids.
2. Examine each tray to ensure that:
   - All of the wells as appropriate (96) have been filled. If any wells do not contain reagents, then manually add reagents to these wells.
   - There are no air bubbles present. Puncture any air bubbles that you see using a pipette tip.

IMPORTANT: **Immediately load the reagent plates onto the GeneTitan MC Instrument. Do not leave denatured samples or reagent plates at room temperature for any length of time.**

3. Transfer the reagent plates, Scan Tray to the GeneTitan MC Instrument and load. Refer to the section *Stage 3 — Ligate, Wash, Stain and Scan on page 172* to continue the process on the GeneTitan MC instrument
4. Return to the Biomek FXP Target Prep Express and clear the deck (See Figure 3.43 on page 88).
   - Always discard the used multi-channel pipette tips in position P9.
   - Always store the reagent block at 4 °C.
   - Clean the Microseal P Pad by wiping with 70% EtOH and dry.

Refer to the package insert for this product for further information on cleaning and replacement.

7c. Complete Stage 4 — Preparation for GeneTitan - Multiple plate workflow

If you selected all three options in Step 4 (See Step *4. Run the Preparation for GeneTitan® Step on page 81* and Figure 3.37 on page 82)) and are preparing hyb tray and reagent trays in a 2+ plate workflow, use he following instructions:

The Hyb tray is prepared for a new HT array plate that will be loaded into the GeneTitan. The reagent trays are prepared for the HT array plate that is in the hybridization oven in the GeneTitan MC and is ready to move to the next stage of the process - Ligation, Wash-Stain and Scan.

NOTE: **Note: When using an of-deck thermal cycler please refer to the instructions for the *Off-deck thermal cycler option on page 73*.**

1. Once the reagent plates are prepared and sample denaturation is complete, the prompt in Figure 3.44 on page 88 is displayed (**do not click OK yet**). Remove the reagent plates and Scan Tray with Hold Buffer from the deck and cover with the appropriate lids.
2. Examine each tray to ensure that:
   - All of the wells as appropriate (96) have been filled. If any wells do not contain reagents, then manually add reagents to these wells.
   - There are no air bubbles present. Puncture any air bubbles that you see using a pipette tip.
3. Transfer the reagent plates, Scan Tray and Array Plate to the GeneTitan MC Instrument and load.

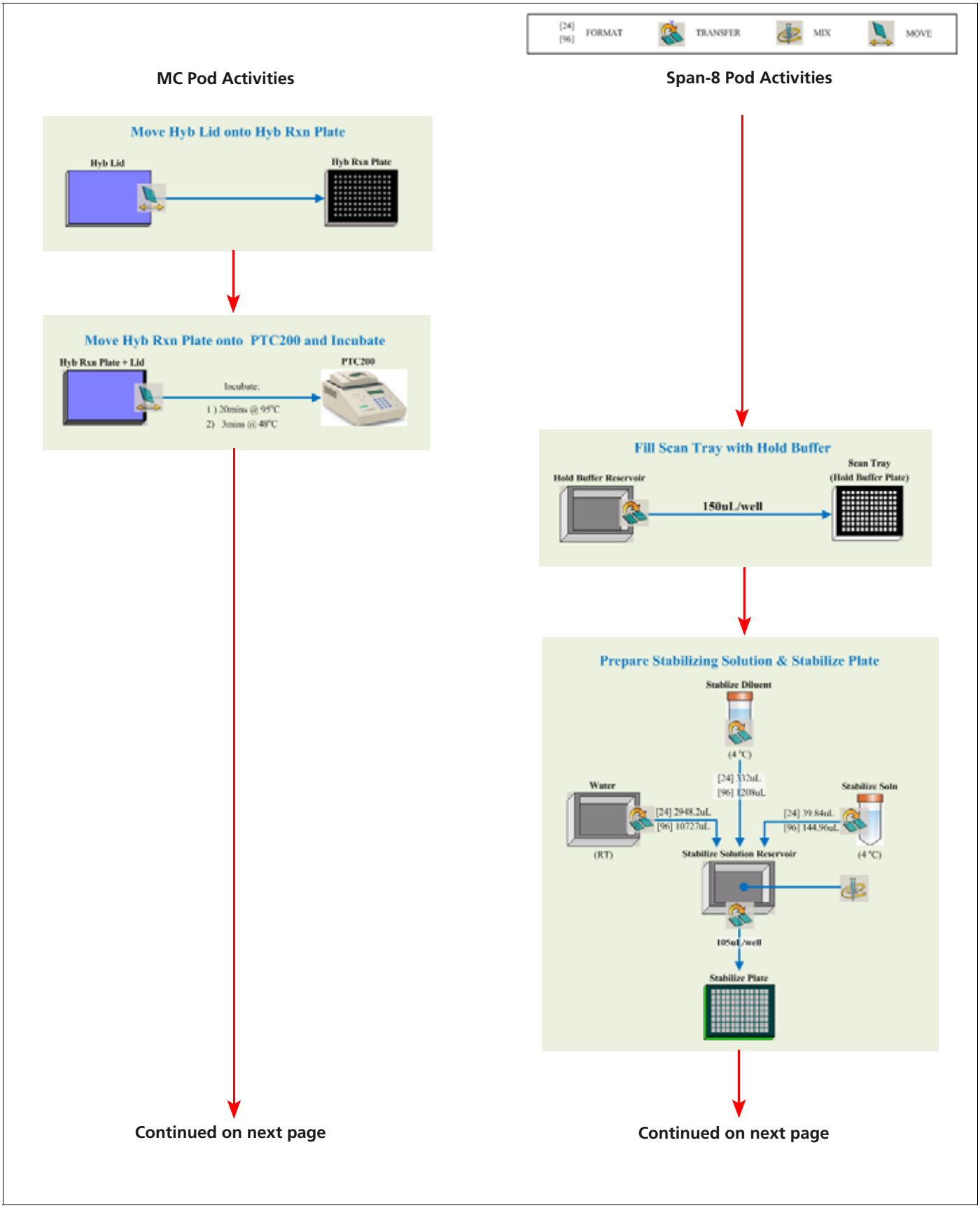
   IMPORTANT: Immediately load the reagent plates and the Hyb Tray onto the GeneTitan MC Instrument. Then load the Array Plate and Hyb Tray. Do not leave denatured samples or reagent plates at room temperature for any length of time.

4. Return to the Biomek FX^P Target Prep Express click **OK** at the prompt shown in Figure 3.44 on page 88.

   The denatured samples are then taken off the thermal cycler and are transferred to the Hyb Tray.
5. Transfer the Hyb Tray to the GeneTitan MC Instrument and load. Refer to the section *Axiom™ Genotyping Assay: Array Processing with the GeneTitan® MC Instrument on page 149* for the proper way of loading.
6. Transfer the reagent plates, Scan Tray to the GeneTitan MC Instrument and load. Refer to the section *Axiom™ Genotyping Assay: Array Processing with the GeneTitan® MC Instrument on page 149* to continue the process on the GeneTitan MC Instrument
7. Return to the Biomek FX^P Target Prep Express and clear the deck (Figure 3.45 on page 89).
   - Always discard the used multi-channel pipette tips in position P9.
   - Always store the reagent block at 4 °C.
   - Clean the Microseal P Pad by wiping with 70% EtOH and dry.

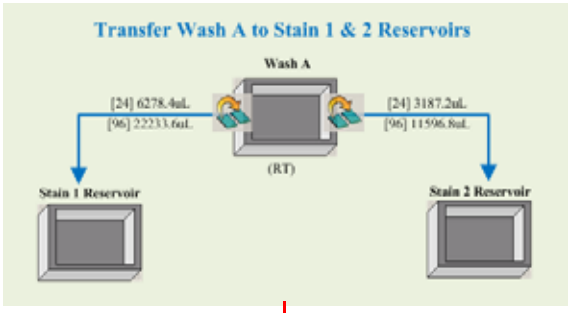
     Refer to the package insert for this product for further information on cleaning and replacement.

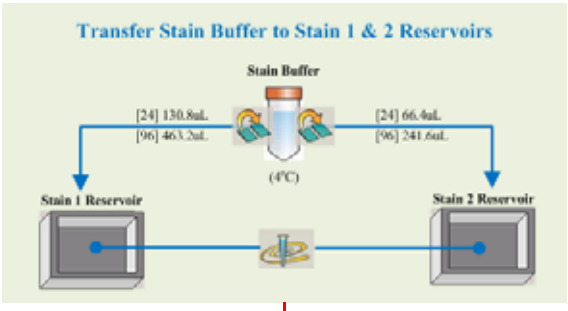
8.Summary of Preparation for GeneTitan MC Instrument

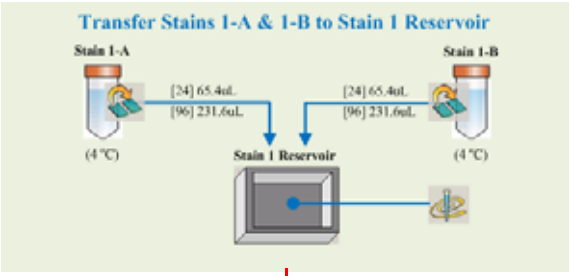


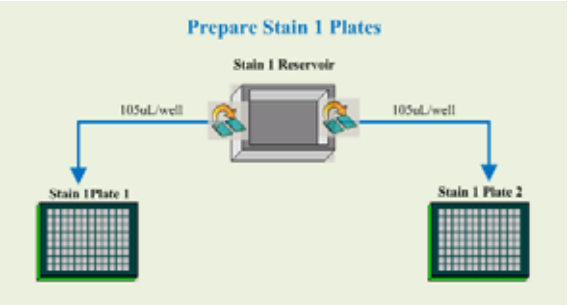
MC Pod Activities

Span-8 Pod Activities









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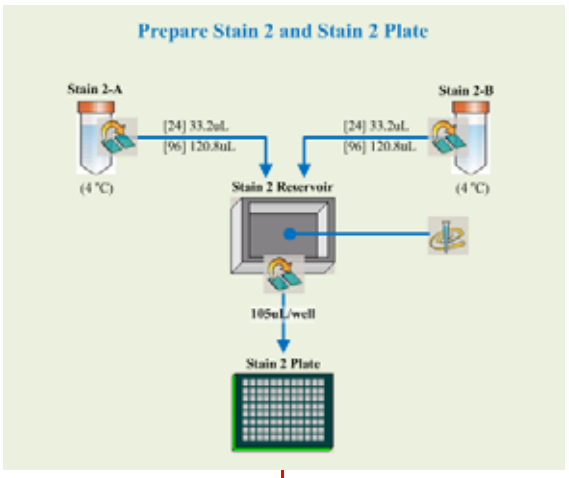
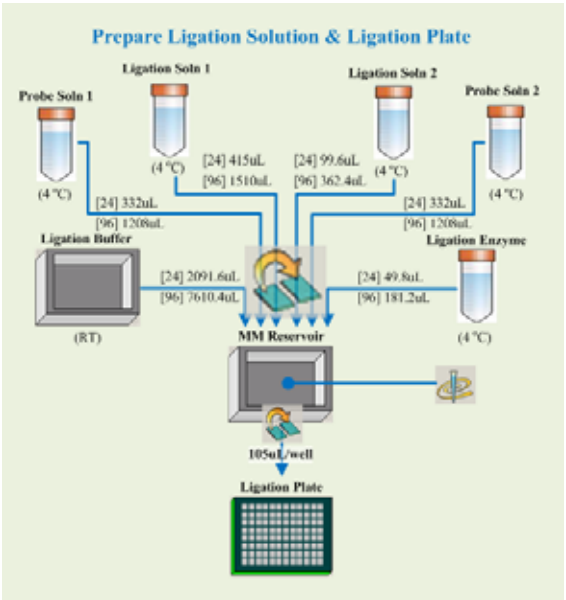
MC Pod Activities

Span-8 Pod Activities





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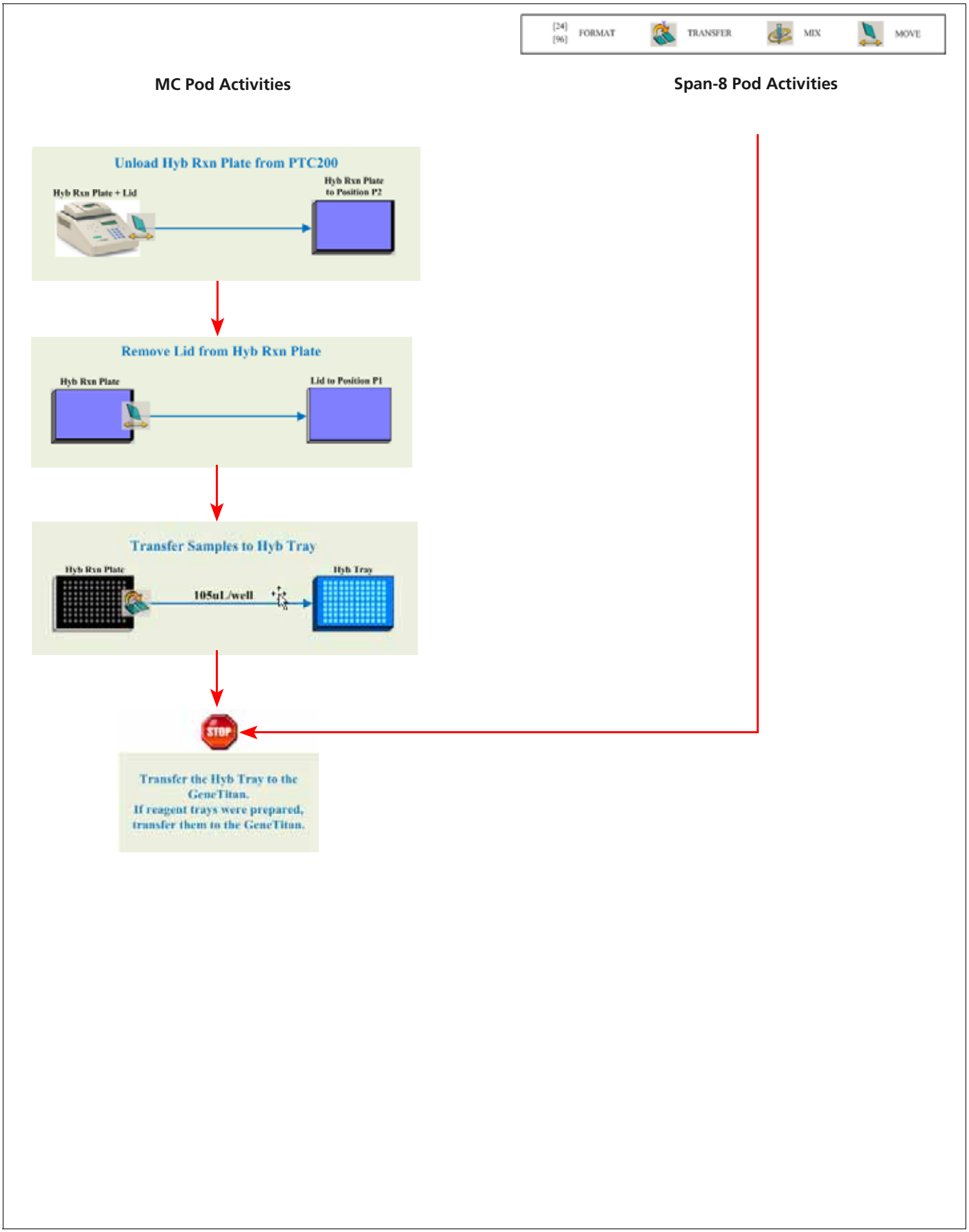

[24]
[96]
FORMAT
TRANSFER
MIX
MOVE
MC Pod Activities
Span-8 Pod Activities
Unload Hyb Rxn Plate from PTC200
Hyb Rxn Plate + Lid
Hyb Rxn Plate to Position P2
Remove Lid from Hyb Rxn Plate
Hyb Rxn Plate
Lid to Position P1
Transfer Samples to Hyb Tray
Hyb Rxn Plate
105uL/well
Hyb Tray
STOP
Transfer the Hyb Tray to the GeneTitan.
If reagent trays were prepared, transfer them to the GeneTitan.

Axiom Genotyping 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, as described in Chapter 5, *Axiom™ Genotyping Assay: Array Processing with the GeneTitan® MC Instrument on page 149*.

IMPORTANT: Read all the instructions in *Before You Start*, below, before performing manual target preparation.

A list of all equipment and resources required for the Axiom Assay with manual target preparation is in the *Axiom Site Prep Guide* P/N 702858.

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

IMPORTANT: Before proceeding to DNA Amplification, perform the gDNA preparation described in Chapter 2, *Genomic DNA Preparation and Requirements on page 9*.

Using the manual target preparation protocol, a single operator can process three sample and array plates a week during a forty-hour work week for a total of 288 samples. See Chapter 8, *Manual Target Preparation for Processing Three Axiom Array Plates per Week on page 209* for more information.

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™ Genotyping Assay.

Requirements and Recommendations

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

Room Temperature

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

Special Requirements

Amplification Staging Room

Precautions are required when manipulating genomic DNA or setting up amplification reactions to avoid contamination with foreign DNA amplified in other reactions and procedures.

It is highly recommended that genomic DNA manipulations and amplification reaction set up are performed in a dedicated amplification staging room separate from the main laboratory. This amplification staging room should have a dedicated set of pipettes and plasticware.

If no dedicated amplification staging room is available, use of a dedicated bench or a dedicated biosafety hood and dedicated pipettes is suggested.

If no dedicated bench 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 Reagent Kit.

Plate Requirements and Recommendations

The following types of plates are required for performing manual target preparation:

- ABgene 96 Square Well Storage Plate, 2.2 mL

- Bio-Rad Hard Shell 96-well plate, p/n HSP9631\Refer to the *Axiom Site Prep Guide,* P/N 702858, for vendor information.
- 96-well UV Star Plates, 370 µL/well

Thermal Cycler Recommendations

The following thermal cyclers are recommended:

- BIO-RAD PTC200 or
- Whatman Biometra TRobot
- BIO-RAD MJ 0240G
- ABI 9700
- ABI 2720

NOTE: **The ABI 9700 and the ABI 2720 use the half-skirted 96-well plates (PN HSS-9601)**

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 MJ 0240G). 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 Denature” protocol:

1. 95 °C 20 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. The arched, auto-sealing metal plate with P pads (used with the TRobot thermal cycler) as shown in Table 3.2 on page 22 should be replaced after use as per the manufacturers recommendation.**

Thermal cycler Consumables

Table 4.1 provides details into the consumables to be used with each thermal cycler.

Table 4.1 Thermal Cycler Consumables for Axiom

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 withWide 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 (supplier VWR)
  - Specifications:
    for the 115 V version:
    50/60 Hz, 115 V, 1200W
    VWR P/N 47746-744
    Mfg P/N 9010-0131
    for the 230 V version:
    50/60 Hz, 230 V, 1200 W
    VWR P/N 47746-690
    Mfg P/N 9010-0078
- Affymetrix GeneChip Hyb Oven 640 or 645
  - If using either 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 2 plates can fit in a Hyb Oven 640
  - Up to 4 plates can fit into a Hyb Oven 645
  - For the Hyb Oven 640, place the plate away from the heat vent.

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, you will need three ovens.

See *Changing Oven Temperatures for the Three Plate Workflow on page 211* of Chapter 8, *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

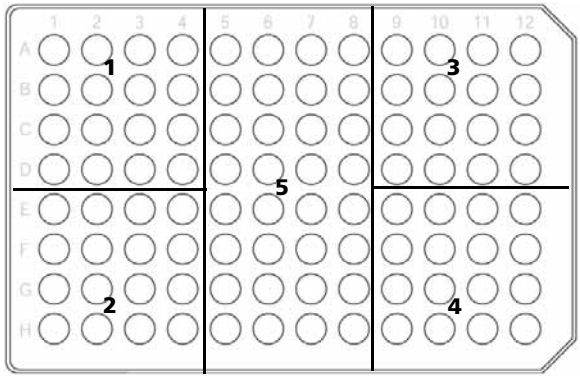
Unless otherwise noted, when the protocol instructs you to seal, vortex and spin:

- **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** 1 to 2 sec each sector for a total of 5 sectors (Figure 4.1).

Figure 4.1 Vortexing plates



> **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 9* for more information.

About the Reagents and Master Mix Preparation

Axiom 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: **Use only the reagents from the Axiom Reagent Kit for this assay. These reagents are not interchangeable with reagents from other Affymetrix reagent kits.**

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 ± 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 12-channel pipettes to transfer samples between plates and to add Master Mix to plates or GeneTitan Trays
- 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 a pipette of appropriate size for the volume of liquid being transferred (Table 4.2).

Table 4.2 Recommended pipette sizes

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

- We recommend the use of Rainin pipettes and tips. Affymetrix has only verified the use of Rainin multi 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 Assay and Array Replacement policy.
- Always use pipettes that have been calibrated to ± 5%.
- 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 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 4.3 Axiom GeneTitan Tray consumables

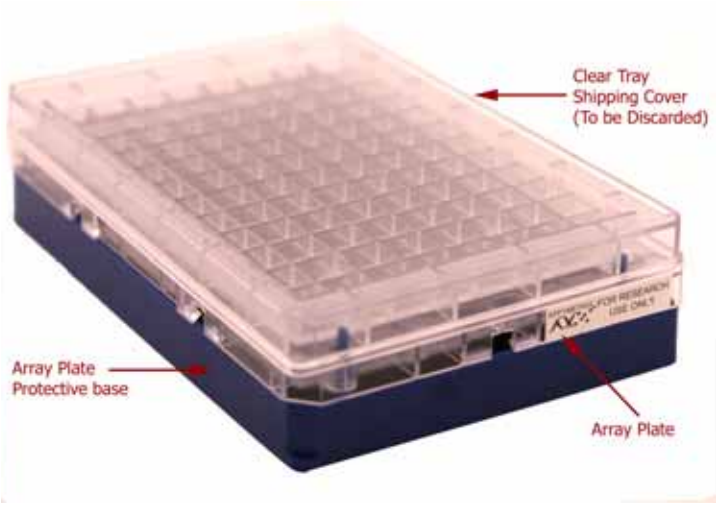
Item	Part Number	Labware Image	Information
HT Array Plate Package	All array plates have the PN 202091 etched on the on the plastic	 	The HT Array Plate shipping package includes the following: ▪ The function of the clear plastic cover for the Array Plate is to protect the Array Plate during transport. 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 4.3 Axiom GeneTitan Tray consumables (Continued)

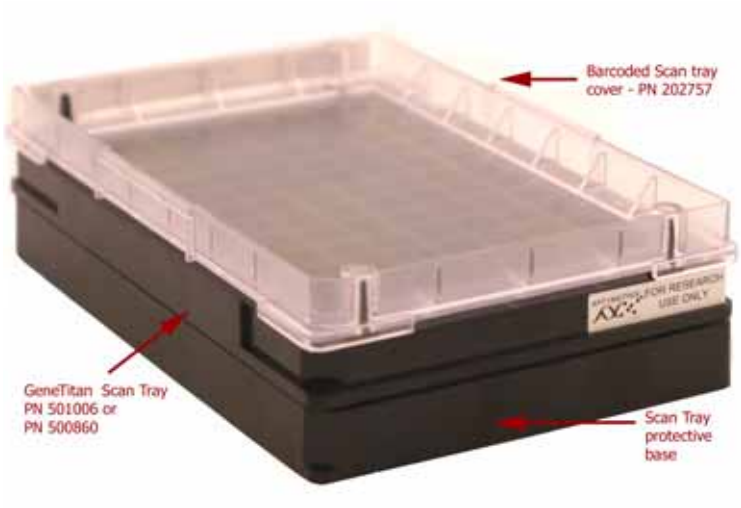
Item	Part Number	Labware Image	Information
			The HT Scan Tray shipping package includes the following: ▪ The HT Scan tray includes a Scan Tray cover. The tray cover should be used to cover the scan tray before placing the tray in the GeneTitan MC Instrument. The Tray cover should be de-ionized before use. See the section Appendix E, *Deionization Procedure for GeneTitan Trays and Covers on page 241* 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 cover in the package is used to protect the scan tray glass from damage. The black scan tray 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	PN 202096		▪ The Black Scan tray cover in the package is used to protect the scan tray glass from damage. The black scan tray 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

Table 4.3 Axiom GeneTitan Tray consumables (Continued)

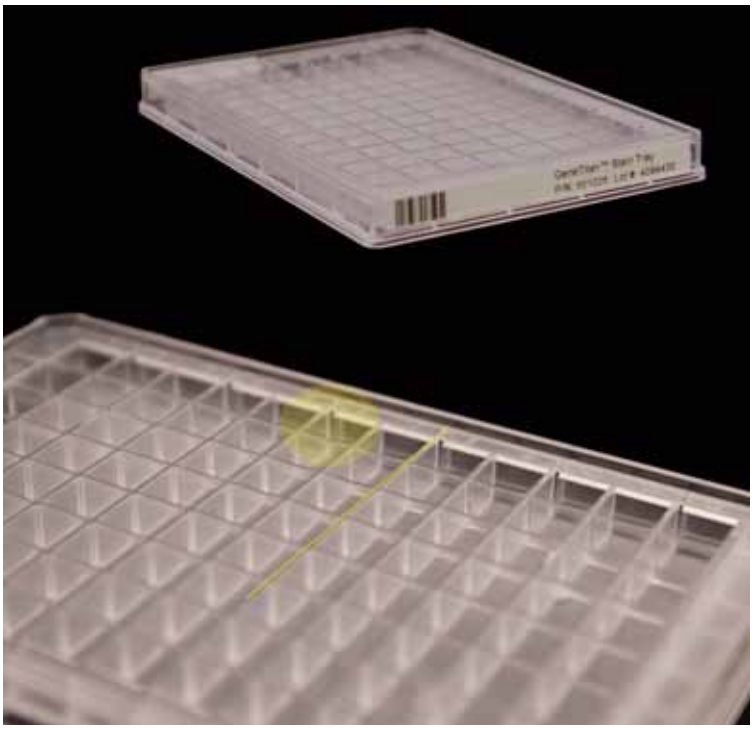
Item	Part Number	Labware Image	Information
Scan Tray with cover	Scan Tray 501006 Cover 202757		▪ The GeneTitan Scan tray must be loaded with the scan tray cover into the GeneTitan instrument ▪ Do not load the scan tray with the protective base
GeneTitan Stain Trays	501025		The GeneTitan stain trays are 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 4.3 Axiom GeneTitan Tray consumables (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 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 the section *Deionization Procedure for GeneTitan Trays and Covers* *on page 241* 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 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 instrument. ▪ See the section *Deionization Procedure for GeneTitan Trays and Covers* *on page 241* 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.

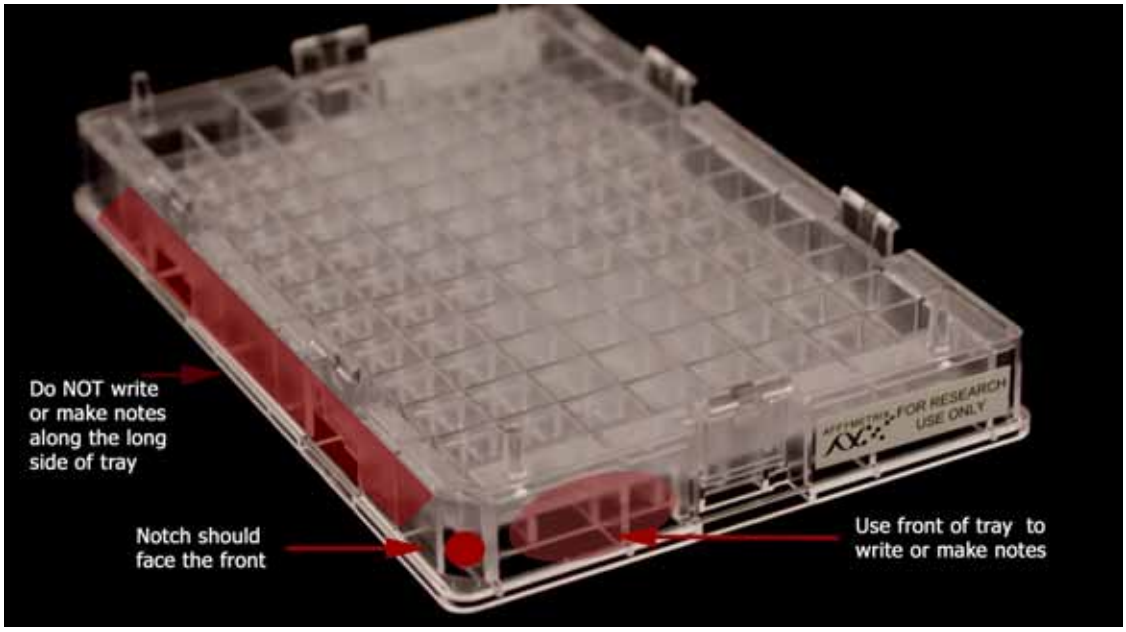
Proper labeling for Hyb Trays and reagent trays is described in:

- *Labeling for Hyb Trays*, below
- *Labeling for Stain Trays* *on page 106*

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 4.2. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.

Figure 4.2 Labeling GeneTitan Hyb Trays

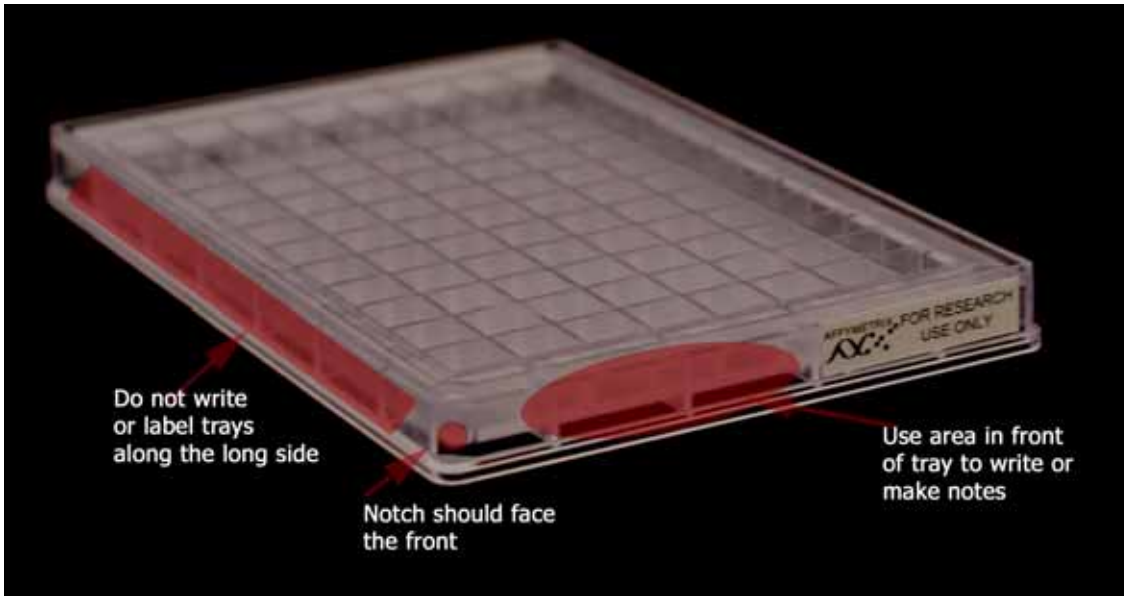


Writing on the wrong side of the Hyb tray, or on the wrong part of the long side, may interfere with the operation of sensors in the GeneTitan MC.

Labeling for Stain Trays

You may label the Stain trays on the **left side of the front of the tray** as shown in Figure 4.3. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.

Figure 4.3 Labeling GeneTitan Stain Tray



(see *Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument* *on page 134* for detailed 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 9**.

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 109*

2. Prepare the Denaturation Master Mix and the Neutralization Master Mix *on page 111*

3. Add Denaturation Master Mix to Samples *on page 112*

4. Add Neutralization Master Mix to Samples *on page 113*

5. Prepare and Add the Amplification Master Mix *on page 113*

6. Freeze or Proceed *on page 115*

IMPORTANT: Amplification preparation should take place in an amplification staging room or dedicated area such as a biosafety hood with dedicated pipettes, tips, vortex, etc. See ***Amplification Staging Room* on page 96** for more information.

Duration

For 96 samples:

- Time to thaw materials: 1 hr
- Hands-on time: approximately 0.5 hr
- Incubation at 30 °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 10 ng/µL.

See *Genomic DNA Preparation on page 11* for more information.

Equipment, Consumables and Reagents Required

Equipment and Consumables

The equipment and consumables listed in Table 4.4 are required for this stage.

Table 4.4 Equipment and consumables required for *Stage 1 — DNA Amplification*.

Quantity	Item
As required	Adhesive seals for 96-well plate
1	Metal chamber for 50 mL tubes, chilled to 4 °C on ice (do not freeze) 
1	Cooler, chilled to –20 °C 
1	15 mL tube holder
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)

Table 4.4 Equipment and consumables required for *Stage 1 — DNA Amplification*.

Quantity	Item
1 each	Pipettes: ▪ Single-channel P100 ▪ Single-channel P200 ▪ Single-channel P1000 ▪ Multi-channel P20 ▪ Multi-channel P50 or P200 ▪ Multi-channel P1200
As needed	Pipette tips (Use tips manufactured specifically for your brand of pipette.)
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 30 °C
1	50 mL Falcon tube
2	15 mL Falcon tube
1	Vortexer
1	Timer
As needed	Solution basin, 100 mL sterile multichannel

Reagents Required

Table 4.5 Reagents required for *Stage 1 — DNA Amplification* *on page 107*

Axiom Reagent Kit	Module
Axiom Denat Soln 10X	Module 1, –20 °C P/N 901275
Axiom Neutral Soln 10X	
Axiom Amp Soln	
Axiom Amp Enzyme	
Axiom Water	

1: Initial Setup for DNA Amplification

In this set of steps you will:

- Set temperatures for the oven and centrifuge
- Label the Master Mix tubes and solution basins
- Thaw the reagents and sample plate.

To perform the initial setup

1. Set an incubator/oven temperature at 30 °C.
2. Set the centrifuge temp to room temperature.

3. Prepare reagents as shown in Table 4.6:

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

Table 4.6 Initial Preparation of reagents for Amplification

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

Notes:

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

NOTE: Allow ~ 1 hour for Axiom 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.

IMPORTANT: The Axiom Amp Soln must be thoroughly mixed before use. Place on ice immediately after thawing.

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 **10 ng/µL** (see *Genomic DNA Preparation* *on page 11*).

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

Label	Tube Size	Temperature	Contents
▪ *D MM*	15 mL	leave tube at room temperature	Axiom Denaturation Master Mix
▪ *N MM*	15 mL	leave tube at room temperature	Axiom Neutralization Master Mix
▪ *Amp MM*	50 mL	place tube on ice	Amplification Master Mix

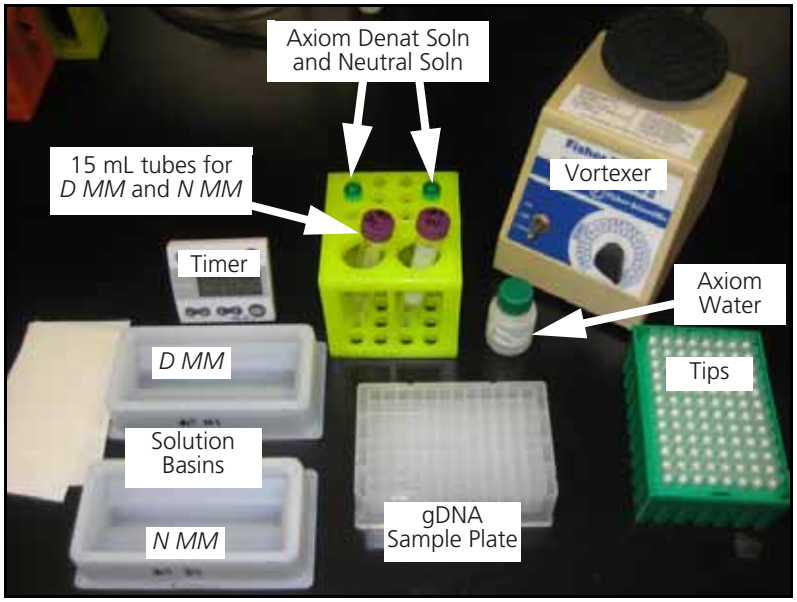
NOTE: It is recommended to use a chilled metal chamber for 50 mL tubes to stabilize the *Amp MM* tube in the ice bucket.

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

Label	Temperature	Contents
▪ *D MM*	Leave basin at room temperature	Axiom Denaturation Master Mix
▪ *N MM*	Leave basin at room temperature	Axiom Neutralization Master Mix
▪ *Amp MM*	Place basin on ice	Amplification Master Mix

A suggested bench setup for denaturation is shown in the figure below.

Figure 4.4 Work area prepared for denaturation



2. Prepare the Denaturation Master Mix and the Neutralization Master Mix

Preparing the Denaturation Master Mix

To prepare the Denaturation Master Mix:

1. Per Table 4.7 on page 112, dilute the appropriate volume of Axiom Denat Soln 10X using the Axiom Water.

Table 4.7 Preparing Axiom Denaturation Master Mix (*D MM*)

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked *D MM*, add:		
Axiom Denat Soln 10X	2 µL	300 µL
Axiom Water	18 µL	2.7 mL
Total Volume	**20µL**	**3 mL**

2. Vortex and leave at room temperature.

Preparing the Neutralization Master Mix

To prepare the Neutralization Master Mix:

1. Per Table 4.8, dilute the appropriate volume of Neutral Soln 10X using the Axiom Water.

Table 4.8 Preparing Axiom Neutralization Master Mix (*N MM*)

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked *N MM*, add:		
Axiom Neutral Soln 10X	4 µL	500 µL
Axiom Water	36 µL	4.5 mL
Total Volume	**40 µL**	**5 mL**

2. Vortex and leave at room temperature.

3. Add Denaturation Master Mix to Samples

To add the Denaturation Master Mix to your samples:

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*. Leave the solution basin on the benchtop.
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).
   Change tips between each addition.

NOTE: Pipette directly into the liquid of the well; do not mix by pipetting up and down.
NOTE: This plate is now known as the Denaturation plate.

5. Seal and vortex the Denaturation plate. Start the timer for **3 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 3 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 **3 minute incubation** on the benchtop at room temperature.

   While completing the incubation at room temperature, pour the Neutralization Master Mix into the solution basin as described in Step 1 on page 113.

9. After incubation **immediately** add the Neutralization Master Mix as described in *4. Add Neutralization Master Mix to Samples* *on page 113*.

4. Add Neutralization Master Mix to Samples

To add the Neutralization Master Mix to your samples:

1. Pour the Neutralization Master Mix into the solution basin marked *N MM*. Leave the solution basin on the benchtop.
2. Carefully remove the seal from the Denaturation plate and discard the seal.
3. Using a P50 or P200 12 channel pipette, pipetting directly into the liquid of each well, add **40 µL of Neutralization Master Mix** to each sample (total volume 80 µL/well).
   Change tips between each addition.

   NOTE: Pipette directly into the liquid of the well; do not mix by pipetting up and down.
   NOTE: This 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 ~80 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.

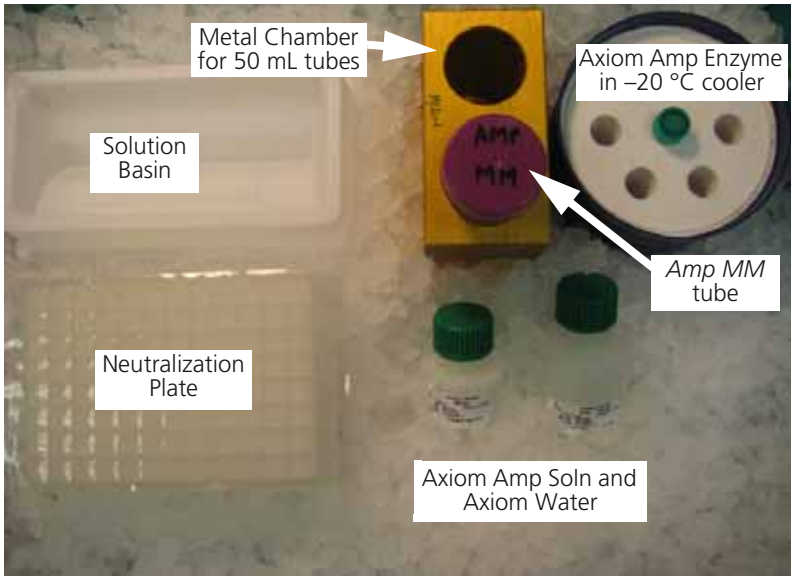
   B. Do **NOT** stop to measure volumes.

6. Place the Neutralization plate on ice.
7. Proceed immediately to *5. Prepare and Add the Amplification Master Mix* *on page 113*.

5. Prepare and Add the Amplification Master Mix

A suggested ice bucket setup for Amplification is shown in the figure below.

Figure 4.5 Ice bucket set up for Amplification



IMPORTANT: Complete this step as efficiently as possible and with minimal delays. Once the Amp Enzyme is added to the master mix, finish this step with no delays.

To prepare and add the Amplification Master Mix:

1. Place the 50 mL tube labeled *Amp MM* on ice.

NOTE: It is recommended to use a chilled metal chamber for 50 mL tubes to stabilize the *Amp MM* tube in the ice bucket.

2. Per Table 4.9, pipette the appropriate amount of Axiom Amp Soln into the 50 mL tube labeled *Amp MM* in the chilled metal chamber.

NOTE: Use a 10 mL serological pipette to transfer Axiom 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 4.9 Amplification Master Mix (*Amp MM*)

Reagent	Per Sample (µL)	Master Mix 96+
To the 50 mL tube marked *Amp MM*, add:		
Axiom Amp Soln	304 µL	34 mL
Axiom Amp Enzyme	16 µL	1.79 mL
Total Volume	**320 µL**	**35.79 mL**

3. Remove the Axiom Amp Enzyme from the freezer and place in a portable cooler at –20 °C.
   A. Flick the Axiom Amp Enzyme tube three times, then spin.
   B. Per Table 4.9 on page 114, add the appropriate amount of Axiom Amp Enzyme to the tube labeled *Amp MM* and place on ice.

4. **Moving through the remaining steps without delay**:
   - **A.** Vortex the Amplification master mix well, then invert the tube 2 times, and then vortex again.
   - **B.** Quick spin and return the *Amp MM* tube to the ice bucket.
   - **C.** Place the solution basin labeled *Amp MM* on ice.
   - **D.** S***lowly*** pour the Amplification Master Mix to the solution basin labeled *Amp MM*.

> **IMPORTANT:** Keep the Amplification master mix solution basin and Neutralization plate on ice throughout this procedure.

   - **E.** Carefully remove the seal from the Neutralization plate and discard the seal.
   - **F.** Using a P1200 12 channel pipette, ***slowly*** add **320 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, vortex twice, and spin the Amplification plate for one minute at 1000 rpm.
   - **H.** Place the sealed amplification plate in an oven set at **30** °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 98* for more information.

6. Freeze or Proceed

After the incubation finishes, you can either:

- Proceed to *Stage 2 — Fragmentation and Precipitation* *on page 115*.
- 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 117*.

Stage 2 — Fragmentation and Precipitation

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

Duration

Total time: approximately 2 hours.

Input Required

Amplification plate from *Stage 1 — DNA Amplification* *on page 107*.

Equipment, Consumables and Reagents Required

Equipment and Consumables

The equipment and consumables listed in Table 4.10 are required for this stage.

Table 4.10 Equipment and consumables required for *Stage 2 — Fragmentation and Precipitation*

Quantity	Item
As required	Adhesive seals for 96-well plates
1	Metal chamber for 50 mL tubes, chilled to 4 °C (do not freeze) 
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	Pipettes: ▪ Single channel P1000 ▪ Single channel P200 ▪ Multi-channel P200 ▪ Multi-channel P1200
As needed	Pipette tips for pipettes listed above (Use tips manufactured specifically for your brand of pipette.)

Table 4.10 Equipment and consumables required for *Stage 2 — Fragmentation and Precipitation*

Quantity	Item
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 98): ▪ One oven set at 37 °C ▪ One oven set to 65 °C
1	15 mL Falcon Tube
1	50 mL Falcon Tube
4	Solution basin, 100 mL sterile multichannel
1	Vortexer

Reagents Required

Table 4.11 Reagents required for *Stage 2 — Fragmentation and Precipitation*

Reagent	Module
From the Axiom Reagent Kit	96 reactions: 901281
Axiom Frag Enzyme (leave at –20 °C until ready to use)	Module 2 Box 1, –20 °C P/N 901528
Axiom 10X Frag Buffer	
Axiom Precip Soln 2	
Axiom Frag Diluent	Module 2 Box 2, 2–8 °C P/N 901529
Axiom Frag Rxn Stop	
Axiom Precip Soln 1	
User-supplied	
Isopropanol, 99.5%	96 samples: 65 mL

1: Stop Amplification Reaction

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

- One oven which will have the temperature changed between 30 °C and 37 °C, depending upon the step being performed.

  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, you will need three ovens. See Chapter 8, *Manual Target Preparation for Processing Three Axiom Array Plates per Week on page 209* 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 212*. 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 115, transfer the Amplification plate from the 30 °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. If using two ovens, set the 30 °C oven previously used for amplification to 37 °C.
3. Prepare reagents as shown in Table 4.12 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 4.12 Reagent Preparation for Fragmentation and Precipitation

Reagent	Temp Out of Module*	Treatment	Store before using in Master Mix
Axiom 10X Frag Buffer	Thaw at Room Temp	Vortex	Place on ice
Axiom Frag Diluent	Place on ice	Vortex and spin	Place on ice
Axiom Frag Enzyme	Keep at -20 °C	Just before use, flick tube 3X, spin, and place in -20 °C portable cooler	Keep in -20 °C cooler until ready to use.
Axiom Frag Rxn Stop	Room Temp	Vortex	Keep at Room Temp
Precip Soln 1	Place on ice	Vortex	Place on ice
Precip Soln 2	Thaw at Room Temp	Vortex and spin	Place on ice
Isopropanol	Room Temp	Room Temp	Room Temp

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

4. Optional: Remove samples for quantifying amplification yield by the PicoGreen Assay.
   A. Carefully remove the seal from the Amplification plate and discard the seal.
   B. Transfer 4 µL samples from each well to a Bio-Rad Hard Shell 96-well plate, HSP-9631.
   C. Quantitate each sample (e.g., using the Quant-iT™ PicoGreen® dsDNA Kit).
   D. Reseal the Amplification plate.
5. Transfer the Amplification plate from the **65** °C oven to the **37** °C oven and **incubate for 45 minutes**.
6. 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.

7. Label the 15 mL and 50 mL Falcon tubes as indicated in the table below:

Label	Tube Size	Temperature	Contents
▪ *Frg MM*	15 mL	Place tube on ice	Axiom Fragmentation Master Mix
▪ *Precip MM*	50 mL	Place tube on ice	Axiom Precipitation Master Mix

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

Label	Temperature	Contents
▪ *Frg MM*	Leave basin at room temperature	Axiom Fragmentation Master Mix
▪ *Stop*	Leave basin at room temperature	Axiom Frag Rxn Stop
▪ *Precip MM*	Place basin on ice	Axiom Precipitation Master Mix
▪ *ISO*	Place basin on ice	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 4.13 Axiom Fragmentation Master Mix

Reagent	per sample	Master Mix 96+
To the 15 ml tube marked *Frg MM*, add:		
Axiom 10X Frag Buffer	45.7 µL	5.3 mL
Axiom Frag Diluent	10.05 µL	1.17 mL
Axiom Frag Enzyme	1.25 µL	145.0 µL
Total Volume	**57 µL**	**6.62 mL**

   Add the reagents from Table 4.13 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.

NOTE: 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:

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*.
   Leave the *Stop* solution basin at room temperature.
2. Remove the Fragmentation plate from the oven and place on the bench top.
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 twice and do a quick spin at 1000 rpm.
6. Place the Fragmentation plate on ice while you prepare the Precipitation Master Mix.

5: Prepare and Add Precipitation Master Mix

To prepare and add Precipitation Master Mix:

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

   A. Add the reagents in the order and volumes shown in Table 4.14, using the appropriate serological pipette and single-channel pipette. The tip of a 25 or 50 mL serological pipette will not fit through the narrow opening of the bottles.

Table 4.14 Axiom Precipitation Master Mix

Reagent	per sample	Master Mix 96+
To the 50 mL tube marked *Precip MM*, 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.

   B. Vortex the *Precip MM* tube and place on ice.

TIP: It is recommended to place the *Precip MM* tube in the chilled 50 mL metal chamber and put the chamber on ice.

2. Place the *Precip MM* solution basin on ice.
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 twice. Spin.
7. Remove the Precipitation plate from the centrifuge and place on ice.
8. Place the solution basin labeled *ISO* on ice and pour the isopropanol into it.
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.

    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 hours)**.

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:

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

Duration

- Centrifuge and dry plates: 1 hour 15 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 115.

Equipment, Consumables, and Reagents Required

The equipment and consumables listed in Table 4.15 are required for this stage.

Table 4.15 Equipment and consumables required for *Stage 3 — Drying, Resuspension and QC*

Quantity	Item
As required	Adhesive seals for 96-well plates
1	96-well cooling chamber, chilled to 4 °C (do not freeze) 
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1 each	Pipettes: ▪ Single channel P20 ▪ Single channel P-100 ▪ Multi-channel P20 ▪ Multi-channel P-200

Table 4.15 Equipment and consumables required for *Stage 3 — Drying, Resuspension and QC*

Quantity	Item
As needed	Pipette tips for pipettes listed above (Use tips manufactured specifically for your brand of pipette.)
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)
1	Fume Hood
1	Plate centrifuge set at 4 °C
1	Tube, Falcon 15 mL
1	10 mL Serological Pipette
1	Pipetaid
1	Shaker, either: ▪ Titer Plate Shakers-4PL, 120V ▪ Jitterbug
1	Vortexer
As needed	Solution basin, 100 mL sterile multichannel

Reagents Required

Table 4.16 Reagents required for *Stage 3 — Drying, Resuspension and QC*

Reagent	Module
From the Axiom Reagent Kit	
Axiom Hyb Buffer	Module 2 Box 1, –20 °C P/N 901528
Axiom Hyb Soln 1	
Axiom Resusp Buffer	Module 2 Box 2, 2–8 °C P/N 901529
Axiom Hyb Soln 2	

Table 4.16 Reagents required for *Stage 3 — Drying, Resuspension and QC*

Reagent	Module
Other Reagents Required for QC steps (optional)	
TrackIt Gel Loading Buffer, 1000-fold dilution (see Appendix A, *Fragmentation Quality Control Gel Protocol on page 223* for dilution instructions.)	8 mL
Gel Sample Plate	
15 fold dilution of 25bp Invitrogen Ladder (P/N 10488-022)	
Nuclease free water, ultrapure MB Grade (P/N 71786; for OD and Dilution Plate preparation)	14 mL

Gels and Related Materials Required

At the end of this stage, verifying the fragmentation reaction is recommended. See Appendix A, *Fragmentation Quality Control Gel Protocol on page 223* 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.

   If using the Affymetrix GeneChip Hyb Oven 640, keep the plate on the side away from the bottom heat vent.
2. Begin thawing/warming the reagents used in this stage as shown in Table 4.17 on page 125.
3. Remove the Precipitation plate from the -20 °C freezer and centrifuge the plate at 3200 xg (or rcf) at 4 °C for 40 min.

   WARNING: We strongly recommend that you use the Eppendorf 5810R at 3200 xg (4000 rpm/) with:

   - **Rotor A-4-81**
   - **Plate carrier DL 050 (rubber bottom)**

   If not using the Eppendorf 5810R with recommended rotor and plate carrier, centrifuge the samples at 3200 xg (or rcf) at 4 °C.

   Do not use the 5810R with the A-4-62 rotor and WO-15 plate carrier (hard bottom). Use of this rotor and plate carrier 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 4.17:

Table 4.17 Reagent Preparation for Resuspension and Hybridization

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

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

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.
7. If you are proceeding directly to *3: Resuspension and Hybridization Master Mix Preparation on page 126*, you can prepare the Hybridization Master Mix at this time (Step 6 on page 126).
8. After 20 min remove the plate from the oven and either:
   - Proceed directly to *3: Resuspension and Hybridization Master Mix Preparation on page 126*, even if some droplets of liquid remain. Leave the Precipitation plate at room temperature. Seal if not proceeding immediately to the next set of steps.
   - Tightly seal the plate and store at –20 °C.

2: Prepare the Tubes, Basins, and Trays

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
▪ *Hyb MM*	15 mL	On ice in Fume Hood	Axiom Hybridization Master Mix

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

Label	Temperature	Contents
▪ *Resus*	Room Temperature	Axiom Resusp Buffer
▪ *Hyb MM*	On Ice in Fume Hood	Axiom Hybridization Master Mix

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

Label	Temperature	Contents
▪ *NF H2O*	Leave basin at room temperature	Nuclease Free Water
▪ *Loading Dye*	Leave basin at room temperature	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:

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

   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.

   NOTE: 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 to perform the rest of the steps in this stage under a fume hood.

6. While the Resuspension plate is shaking, prepare the Hybridization Master Mix in the *Hyb MM* 15 mL tube.
   A. Keep the *Hyb MM* tube on ice.
   B. Add the reagents in Table 4.18 to the *Hyb MM* tube in the order shown, using serological and single-channel pipettes as needed.

Table 4.18 Axiom Hybridization Master Mix

Reagent	per sample	Master Mix 96+
To the 15 mL tube labeled *Hyb MM*, add:		
Axiom Hyb Buffer	70.75 µL	7.9 mL
Axiom Hyb Soln 1	0.25 µL	27.8 µL
Axiom Hyb Soln 2	9 µL	1.0 mL
Total Volume	**80 µL**	**8.89 mL**

   C. Vortex twice and place on ice
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 on ice.

   When using the ABI 9700 or ABI 2720, you should label a Bio-Rad **Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate** (P/N HSS 9601) as **Hyb Ready [Sample ID]** and keep covered on ice.

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 on ice.

    Change pipette tips after each transfer.
12. Pour the Hyb Master Mix to the solution basin labelled *Hyb MM* placed on ice.
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. Keep the Hyb Ready plate on ice while preparing the dilutions for the QC steps, as described in the next section. We recommend placing the Hyb Ready plate on a 96-well metal chamber sitting on ice to stabilize it.

4: Recommended: Perform Quantitation and Fragmentation QC Checks

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

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*
- 8ml Gel Loading dye:
  - 1:1000 dilution of 6x Invitrogen TrackIt Cyan/Orange as described in *Diluting the TrackIt Cyan/Orange Loading Buffer* *on page 224*.
- 14 mL of nuclease free water, P/N 71786
- 15 fold dilution of 25bp Invitrogen Ladder, P/N 10488-022

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

To perform the QC checks:

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 twice, 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 1:120.

   See Appendix B, *Sample Quantitation after Resuspension on page 225* for more information on performing the Sample Quantitation.
4. Make Gel Samples:
   A. Add **60 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 223*.
   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 °C on page 130*

If you are proceeding directly from the end of *Stage 4 — Denaturation and Hybridization* on page 128, go to *2: Prepare Equipment and Perform Denaturation on page 130*

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

Duration

- Hands-on: 45 minutes including denaturation time
- in GeneTitan MC: 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/PTC-200G Thermal Cycler or
- Whatman Biometra TRobot 96
- BIO-RAD MJ 0240G
- ABI 9700
- ABI 2720

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

The thermocycler needs to be programmed with the "Axiom Denature" protocol (see *Thermal Cycler Recommendations* on page 97).

Table 4.19 Equipment Required for *Stage 4 — Denaturation and Hybridization*

Equipment		Quantity
GeneTitan MC		1
Pipetaid		1
P200 12-channel Pipette		1
Pipette tips		As needed
Thermal Cycler	Appropriate thermal cycler, programmed with the "Axiom Denature" protocol (see *Thermal Cycler Recommendations* on page 97).	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 4.20 Consumables Required for *Stage 4 — Denaturation and Hybridization*

Consumable	Vendor and Part Number	Quantity
Following components from Axiom Array Plate Kit: ▪ One of the following Axiom Array plates: ▫ Axiom Genome-Wide CEU 1 Array Plate (96 arrays) on protective base	Affymetrix P/N 901608	1 kit includes: 1
▫ Axiom Genome-Wide ASI 1 Array (96 arrays) on protective base	P/N 901640	1

Table 4.20 Consumables Required for *Stage 4 — Denaturation and Hybridization*

Consumable	Vendor and Part Number	Quantity
▪ Axiom™ myDesign™ Genotyping Array Plates, 1x96 format one 96-array plate of one array type	P/N 000780**	one 96-array plate of one array type
▪ Axiom™ myDesign™ Genotyping Array Plates, 2x48 format one 96-array plate of two array types	P/N 000786**	one 96-array plate of one array type
▪ Axiom™ myDesign™ Genotyping Array Plates, 4x24 format one 96-array plate of four array type	P/N 000787**	one 96-array plate of one array type
**The Consumables for the GeneTitan MC instrument are packaged separately from the Axiom myDesign™ Genotyping Array Plates. The consumables are available in the GeneTitan Consumable kit (P/N 901606)		
▪ Hyb Tray	P/N 500867	1

Table 4.21 Reagents required from the Axiom Reagent Kit

Module	Reagent	Thaw on Bench Top, Then Place on Ice	Place on Ice	Place on Bench Top at Room Temperature
Module 3, Room Temperature 96-Sample: P/N 901472	Axiom Wash Buffer A			✓
	Axiom Wash Buffer B (both bottles; 1L)			✓
	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.
   A. Remove the array plate box from the 4 °C refrigerator where it is stored.
   B. Open the box and remove the pouch containing the array plate and protective base.

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

C. Leave the array plate in the pouch, unopened but placed on the bench for a minimum of 25 minutes before opening and loading on the GeneTitan MC to allow the plate to come to room temperature.

D. 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 157*).

3. Make sure the thermocycler is powered on and the Axiom 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.
5. Close the lid.
6. Start the **Axiom Denature program**, described on *Thermal Cycler Recommendations* *on page 97*).
7. While the program is running:

A. Prepare the reagents from Module 3 as described in Table 4.22:

Table 4.22 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 (see *Setup the Instrument* *on page 158*).

C. Upload the Batch Registration File (see *Stage 1 — Create and Upload Batch Registration File* *on page 157*).

3: Prepare Hybridization Tray and Load into GeneTitan MC

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

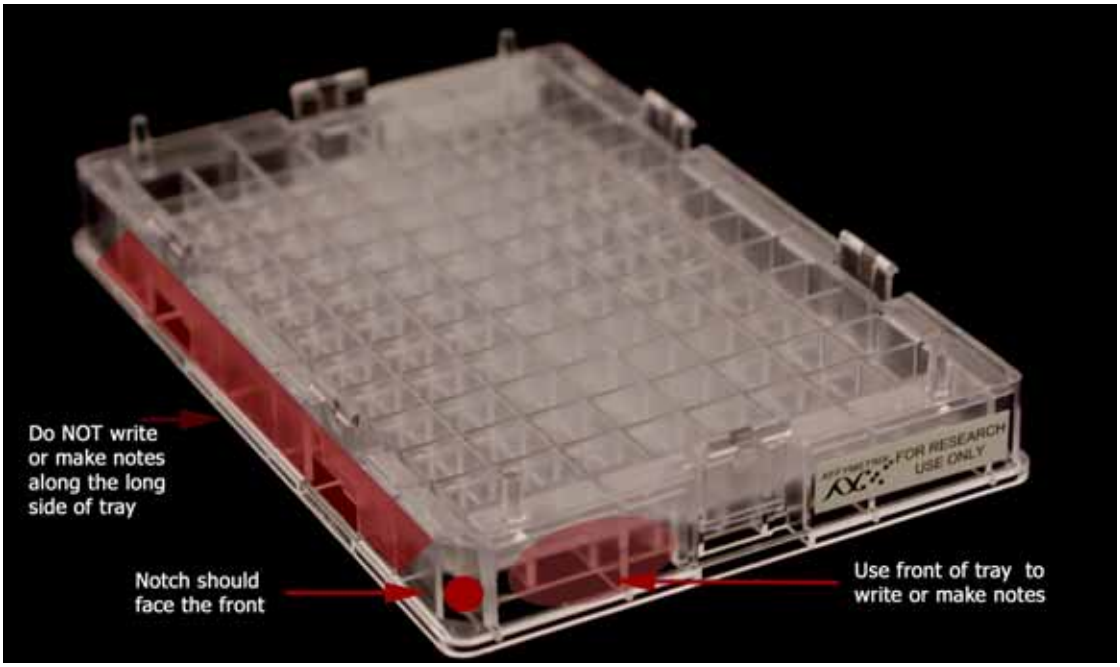
1. After the Axiom Denature program has completed, remove the Hyb Ready plate from the thermocycler and place into a 96-well metal chamber that has been pre-warmed in an oven at 48 °C.
2. Move the metal chamber containing the denatured Hyb Ready plate to a fume hood.
3. Remove Microamp seal from Hyb Ready plate and discard.
4. Remove the Hyb Tray (from Axiom Array Plate kit) from packaging.
5. Label the Hyb Tray. See the note below and Figure 4.6 on page 132 for more information.

IMPORTANT: It is critical that you write only on the proper location of the proper edge of the Hyb tray. 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.

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 4.6. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.

IMPORTANT: Do not confuse hyb trays with stain trays.

Figure 4.6 Labeling GeneTitan hyb trays

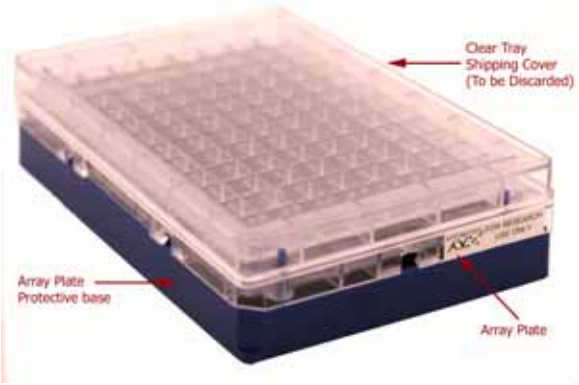


Writing on the wrong side of the Hyb tray, or on the wrong part of the long side, may interfere with the operation of sensors in the GeneTitan MC.

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.

   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.
8. Load the array plate and Hyb tray into GeneTitan MC (see *Load an Axiom Array Plates and Hyb Tray Onto the GeneTitan MC* *on page 163*).

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.



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

IMPORTANT: After GeneTitan MC 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 166** for detailed instructions.

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

You must wait until the hybridization step on the GeneTitan is approximately 1.5 hours from completion (22 hours after the start of hybridization) to begin *Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument*, below.

Long delays between sample denaturation and loading into the GeneTitan for hybridization should be avoided. However, if denaturation has begun and the GeneTitan 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 when the GeneTitan is ready, at which point you should begin at Step 1 of *3: Prepare Hybridization Tray and Load into GeneTitan MC on page 131*.
- 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 is ready. Be sure to remove the plate sealing film before loading into the GeneTitan.

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 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 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 plates 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 GeneTitan MC:

1: Prepare the Reagents for Stage 5 on page 136

2: Prepare the Stain, Ligation and Stabilization Master Mixes on page 138

3: Aliquot Master Mixes and Axiom Hold Buffer into Trays on page 140

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 4.23 Reagent trays required for the Axiom assay on the GeneTitan MC

Type of Tray	Number of Trays	Tray Designation	Master Mix/Reagent
Stain tray with cover	2	*S1*	Stain 1 master mix
Stain tray with cover	1	*S2*	Stain 2 master mix
Stain tray with cover	1	*Stbl*	Stabilization master mix
Stain tray with cover	1	*Lig*	Ligation master mix
Scan tray	1	Scan Tray	Hold Buffer

Equipment, Consumables and Reagents Required

Table 4.24 Equipment Required for *Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument*

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

Table 4.24 Equipment Required for *Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument*

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

Table 4.25 Consumables Required for *Stage 5 — Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument*

Consumable	Vendor and 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
MicroAmp Clear Adhesive Film		As required
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 4.24
Solution basin, 100 mL sterile multichannel		5
15 mL Falcon tube	Becton Dickinson P/N 352097	3
50 mL Falcon tube		1

Reagents Required

Table 4.26 Axiom Reagents required for Stain and Ligation Stage

Module	Reagent
Module 4 Box 1 of 2 96-Sample: P/N 901278	Axiom Ligate Buffer
	Axiom Ligate Enzyme
	Axiom Ligate 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 4.26 Axiom Reagents required for Stain and Ligation Stage

Module	Reagent
Module 4 Box 2 of 2 96-Sample: 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, box 1 of 2 as described in Table 4.27:

Table 4.27 Reagents from Module 4, box 1 of 2 (96-sample: 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:
Temp Out of Module*: temperature the reagent is held at immediately after removal from module
N/A: not applicable in this case

NOTE: The presence of some precipitate in Axiom Ligate Buffer 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, box 2 of 2 as described in Table 4.28:

Table 4.28 Reagents from Module 4, box 2 of 2 (96-sample: 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	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Wash A	Leave on bench	1.Vortex twice 2.Place on Bench for 30 min. 3.Look for precipitate. 4.Vortex again if necessary.	Place on bench top at room temp
Axiom Stain 1-A#	Place on ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Stain 1-B#	Place on ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Stain 2-A#	Place on ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Stain 2-B#	Place on ice	Flick 2 to 3 times to mix, then spin	Place on ice
Axiom Stabilize Diluent	Place on ice	1.Vortex and Spin 2.Look for precipitate If any: ▫ Warm tube to room temperature and vortex again.	Place on ice
Axiom Water	Place on ice	N/A	Place on ice
Axiom Hold Buffer#	Room Temp	Vortex	Store at Room Temp away from light

Notes:

These solutions are light sensitive. Keep tubes out of direct light for a prolonged period of time.

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

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 4.29.

Table 4.29 Labeling master mix tubes for stain, ligation, and stabilization reagents

Conical Tube	Number of Tubes	Tube Designation	Contents	Place Tube:
50 mL	1	*S1*	▪ Stain 1 Master Mix	On ice
15 mL	1	*S2*	▪ Stain 2 Master Mix	On ice
15 mL	1	*Stbl*	▪ Stabilization Master Mix	On ice
15 mL	1	*Lig*	▪ Ligation Master Mix	On ice

NOTE: Use a 5mL 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 4.30.

Table 4.30 Labeling master mix tubes for stain, ligation, and stabilization reagents

Basin Designation	Contents
S1	▪ Stain 1 Master Mix
S2	▪ Stain 2 Master Mix
Stbl	▪ Stabilization Master Mix
Lig	▪ Ligation Master Mix
Hold	▪ 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 4.31. This recipe will provide enough for both *S1* reagent trays.

Table 4.31 Stain 1 Master Mix

Reagent	Per Array	Master Mix 96+
To the tube marked *S1*, 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 4.32.

Table 4.32 Stain 2 Master Mix

Reagent	Per Array	Master Mix 96+
To the tube marked *S2*, 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 4.33.

Table 4.33 Stabilization Master Mix

Reagent	Per Array	Master Mix 96+
To the tube marked *Stbl*, 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 4.34.

Table 4.34 Ligation Master Mix Preparation — **Stage 1**

Reagent	Per Array	Master Mix 96+
To the tube marked *Lig*, add:		
▪ Axiom Ligate Buffer	66.15 µL	7.3 mL
▪ Axiom Ligate Soln 1	13.12 µL	1.45 mL
▪ Axiom Ligate Soln 2	3.15 µL	348 µL
Sub–Total	**82.42 µL**	**9.10 mL**

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 4.35.

   Gently flick the Axiom Ligate Enzyme tube 2-3 times, then perform a quick spin immediately prior to adding the enzyme to the Master Mix.

Table 4.35 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 Ligate Enzyme	1.58 µL	174.4 µL
Total	**105 µL**	**11.58 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 Array Plate kit.
2. Label two Stain Trays *S1*

3. Label the remaining stain trays:
   - *S2*
   - *Stbl*
   - *Lig*

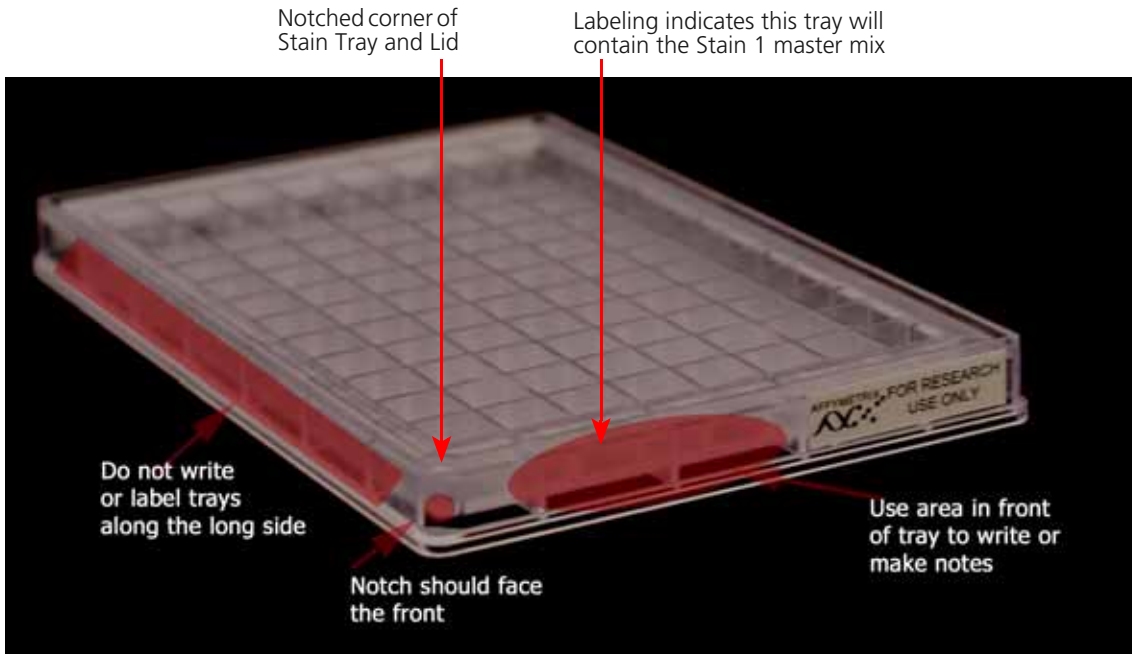
   When preparing the hybridization and reagent trays to be loaded onto the GeneTitan MC Instrument, you will need to mark the front of each tray in a way that identifies its contents.

> **IMPORTANT:** It is critical that you write only on the proper side of the front edge of Stain Trays. 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.

   You may label the Stain trays on the **left side of the front of the tray** as shown in Figure 4.3. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.

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

Figure 4.7 Labeling GeneTitan 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 241* for the recommended technique.

About Aliquoting Reagents to Trays

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

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 preparing the stain master-mixes

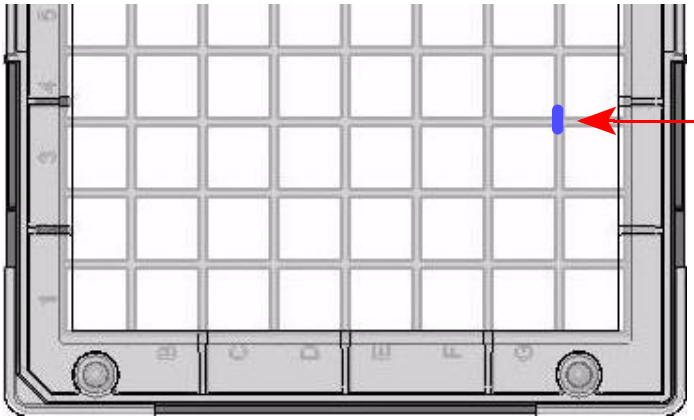
When aliquoting ligation, staining, and stabilization reagents to the trays, it is not necessary to spread the reagent to each corner of the well. The reagent will spread evenly when the array plate is inserted into the reagent tray during processing with GeneTitan MC.

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 µL 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 4.8).

Figure 4.8 Well dividers in trays.



4. Place covers on the *S1* trays. Orient cover correctly on the tray with the notched corners together (Figure 4.9).

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 4.9 Placing cover on stain tray

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

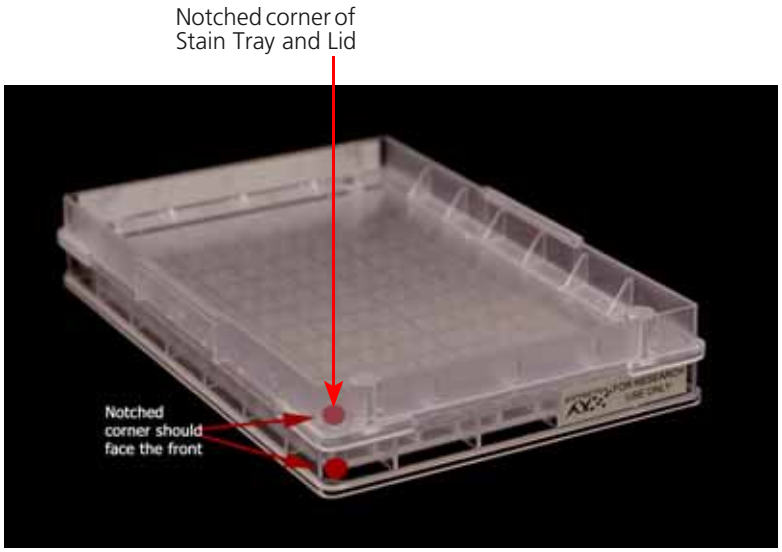
Stain 2 Master Mix

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 4.10).

Figure 4.10 Placing cover on stain tray



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.

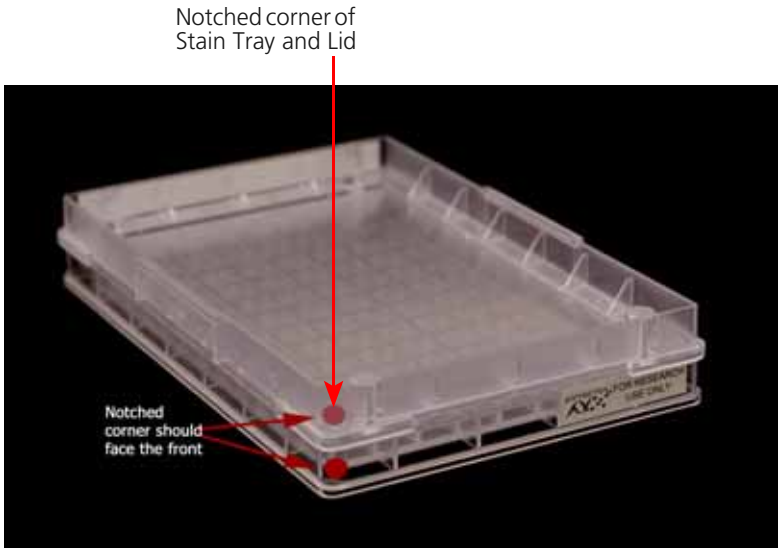
Ligation Master Mix

To aliquot the Ligation Master Mix:

1. Pour the ligation master mix into the solution basin marked *Lig*. Keep the solution basin on ice.
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 4.11).

Figure 4.11 Placing cover on stain tray



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. 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.

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.

4. Prepare the barcoded Scan tray cover (PN 202757) that came with the scan tray by completing the deionization procedure described in *Deionization Procedure for GeneTitan Trays and Covers on page 241*. Place the cover as shown in Figure 4.13 on page 146 to prevent dust or static from accumulating on the bottom of the cover.

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.

Figure 4.12 Scan tray with the clear cover and protective base

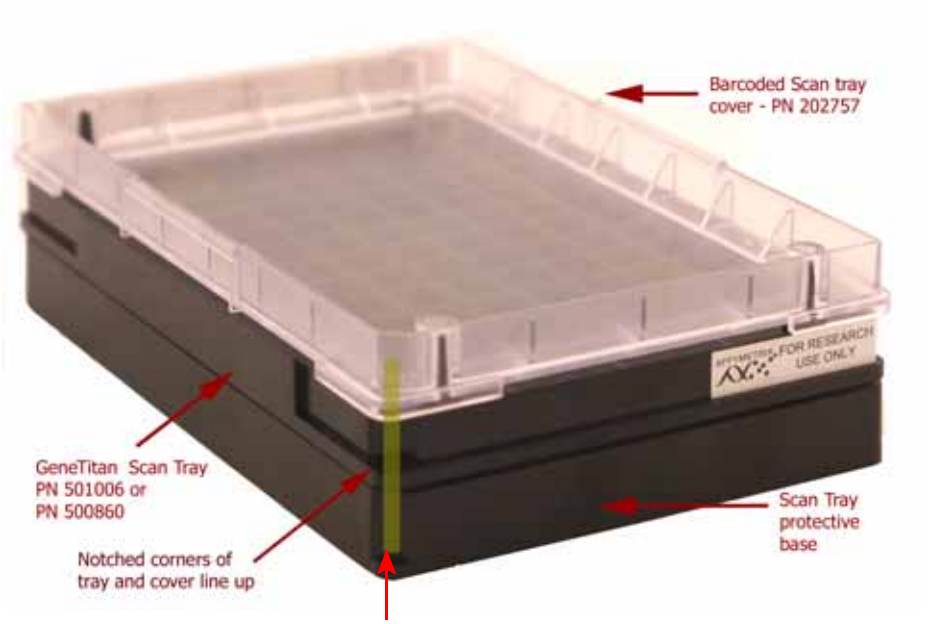


Figure 4.13 Loading the Scan Tray with Axiom Hold Buffer

Leave the Scan Tray in its protective black base while loading with Axiom Hold Buffer.

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.

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

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 and leave on the bench top (no need to protect from light (Figure 4.13)).

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 172* for instructions on loading the reagent trays.

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Axiom™ Genotyping Assay: Array Processing with the GeneTitan® MC Instrument

The Axiom™ Genotyping 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 with either:
  - Automated target prep, performed with the Biomek FX^P Target Prep Express

    See Chapter 3, *Axiom™ Genotyping Assay: Target Preparation with Biomek FXP Target Prep Instrument on page 15*
  - Manual target prep, performed on the lab bench without advanced automation

    See Chapter 4, *Axiom Genotyping Assay: Manual Target Preparation on page 95*
- 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 on page 149*
- *Stage 1 — Create and Upload Batch Registration File on page 157*
- *Stage 2 — Hybridization on page 158*
- *Stage 3 — Ligate, Wash, Stain and Scan on page 172*

Before Using the GeneTitan MC

Proper Tray Alignment and Loading

Proper alignment and loading of plates, covers and trays is critical when using the GeneTitan MC. 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 and Figure 5.2 on page 151).

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.

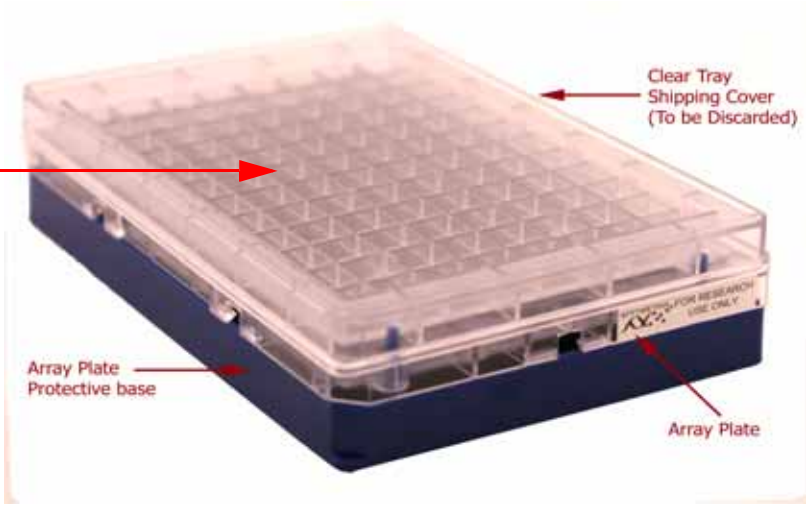
TIP: Mark the notched corner of each plate, cover and tray with permanent marker to help ensure proper alignment and loading onto the GeneTitan MC.

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

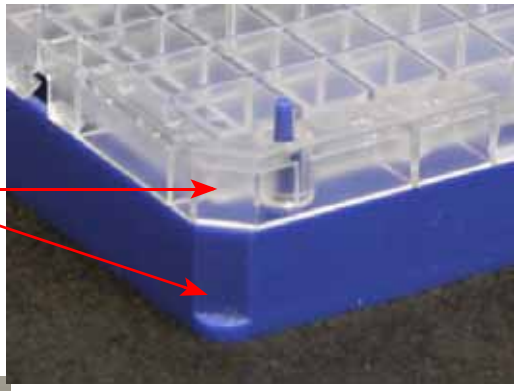
NOTE: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. Refer to **Appendix F, *GeneTitan® MC Instrument Care on page 247*** for the message displayed to the user and the procedure for replacing the filters.

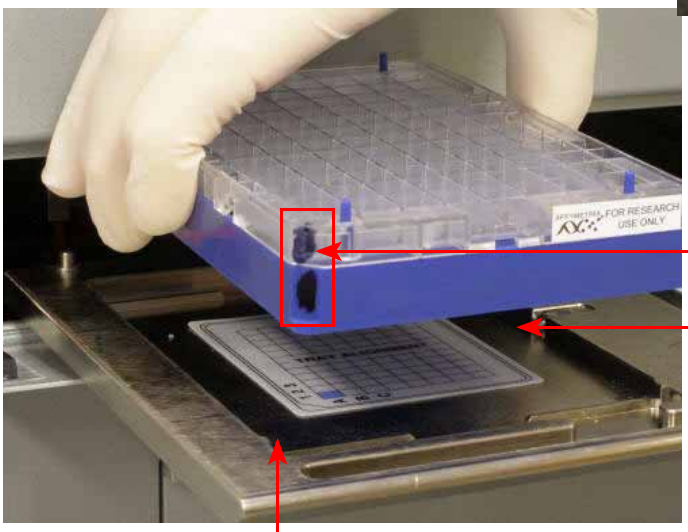
Figure 5.1 Proper alignment and loading of plates, covers and trays in the GeneTitan MC.

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.

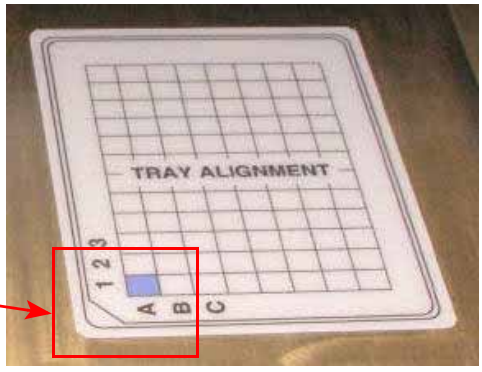
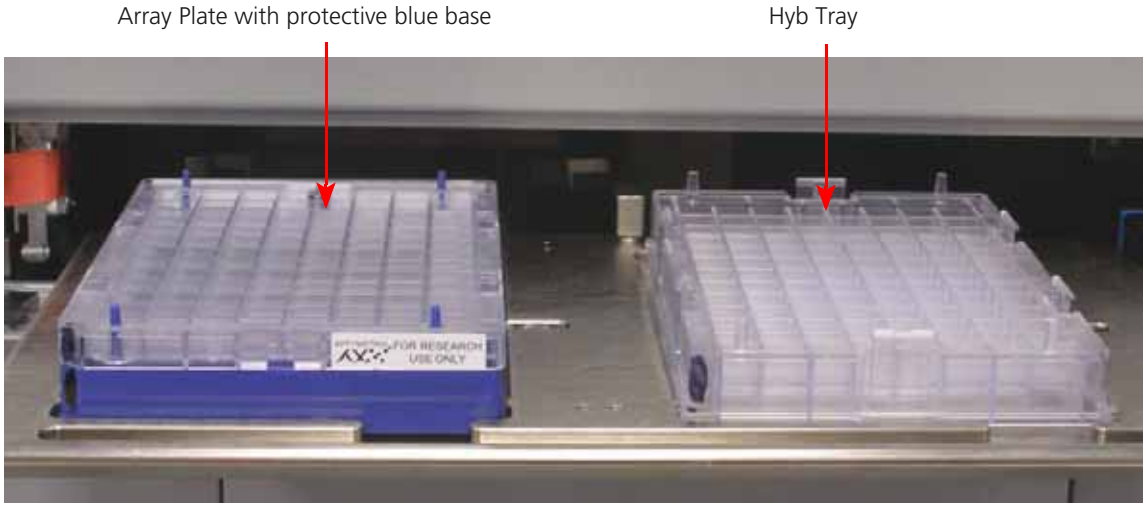


Figure 5.2 Array Plate with protective blue base and the Hyb Tray aligned and properly loaded into drawer 6.



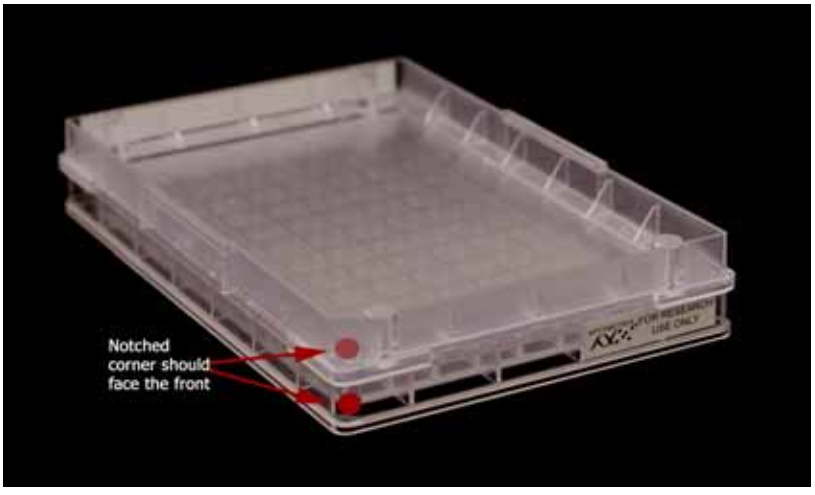
IMPORTANT: When you install the consumables, ensure that the fingers are retracted. Do not lay the consumables on top of the drawer fingers - this indicates that the instrument is not functioning correctly. Please notify your Field Service Engineer if the fingers do not retract automatically. You should 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:

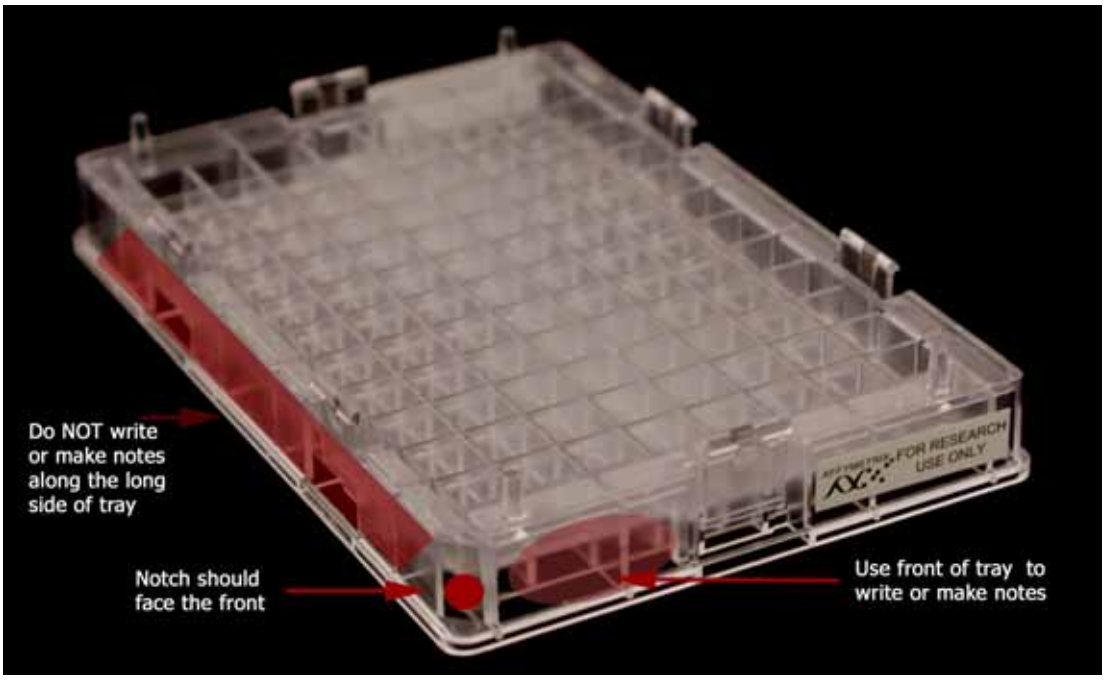
- *Labeling for Hyb Trays*, below
- *Labeling for Stain Trays* *on page 153*

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

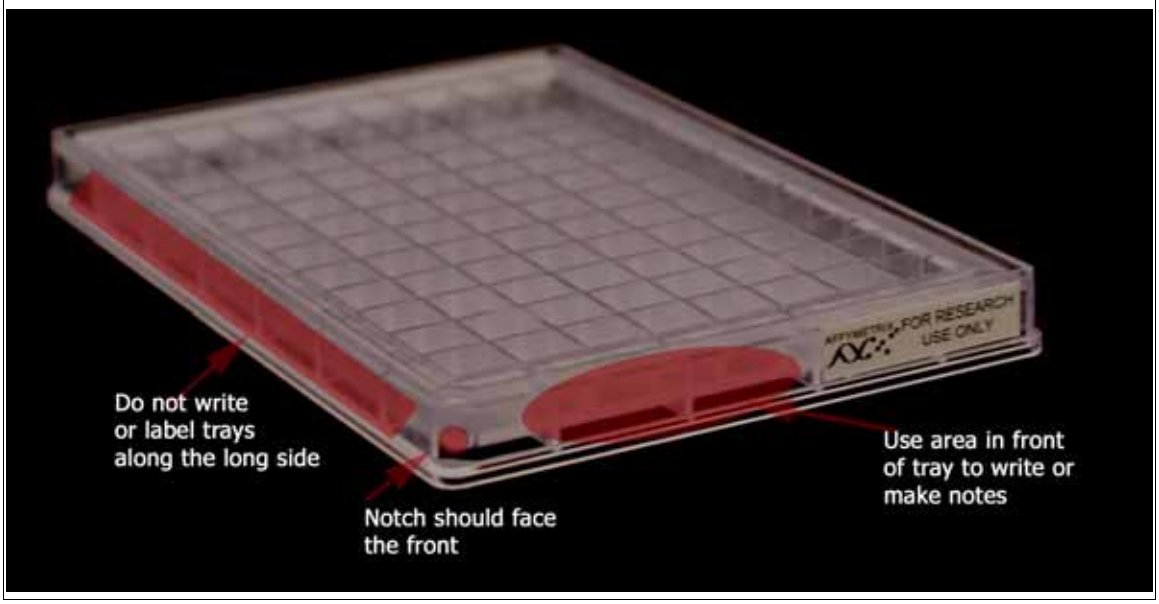


CAUTION: Writing on the wrong side of the Hyb tray, or on the wrong part of the long side, may interfere with the operation of sensors in the GeneTitan MC.

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.

Figure 5.5 Labeling GeneTitan Stain Tray



E-mail and Telephone Notifications from the GeneTitan MC

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 Lamp

The GeneTitan MC 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 user guide.

Refer to the *GeneTitan MC Instrument User Guide*, P/N 08-0308, or Appendix F, *GeneTitan® MC Instrument Care on page 249* 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

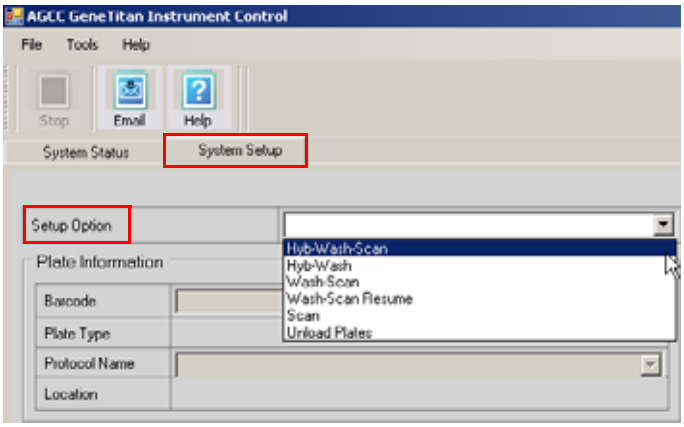
required. It takes 10 minutes to warm-up the lamp. In idle mode the lamp will remain ON for 2 hours before it is automatically switched OFF and if there are no more plates being transferred from the fluidics to the imaging station. This is by design and intended behavior. Please do not try to save the 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.

Figure 5.6 Setup options for processing array plates



Hyb-Wash-Scan

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

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® MC Instrument Care on page 247* 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 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 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

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.
- 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.

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.

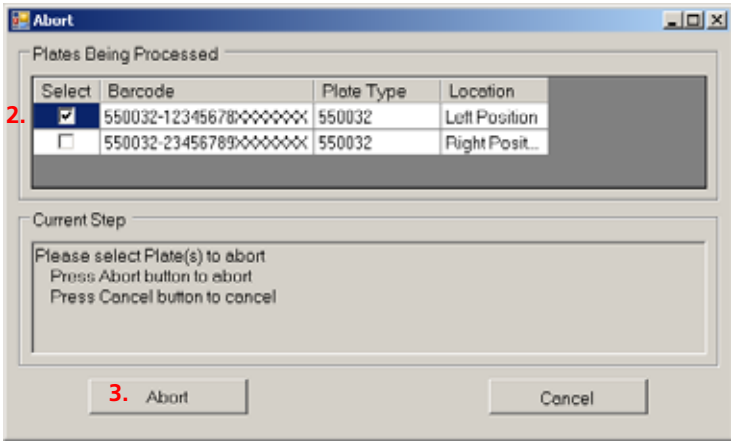
Figure 5.7 Manually aborting an array plate.

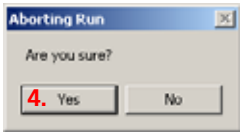
To abort array plate processing:

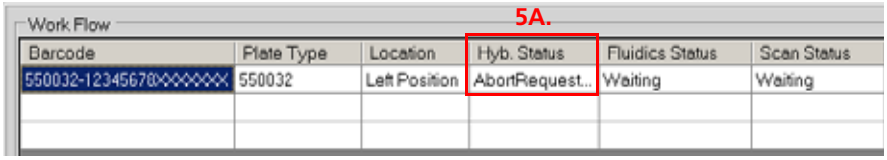
1. Click the **Stop** button.
2. Select the array plate that you want to abort.
3. Click **Abort**.
4. Click **Yes**.
5. Wait until the status of the array plate in the WorkFlow window changes from *AbortRequest…* to *Aborted* (5A and 5B).
6. Once aborted, retrieve the array plate and other related consumables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

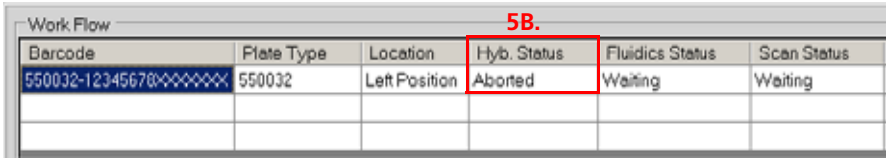
Exception: If reagents are loading, abort the plate using the Cancel button displayed in the reagent load step.

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









Stage 1 — Create and Upload Batch Registration File

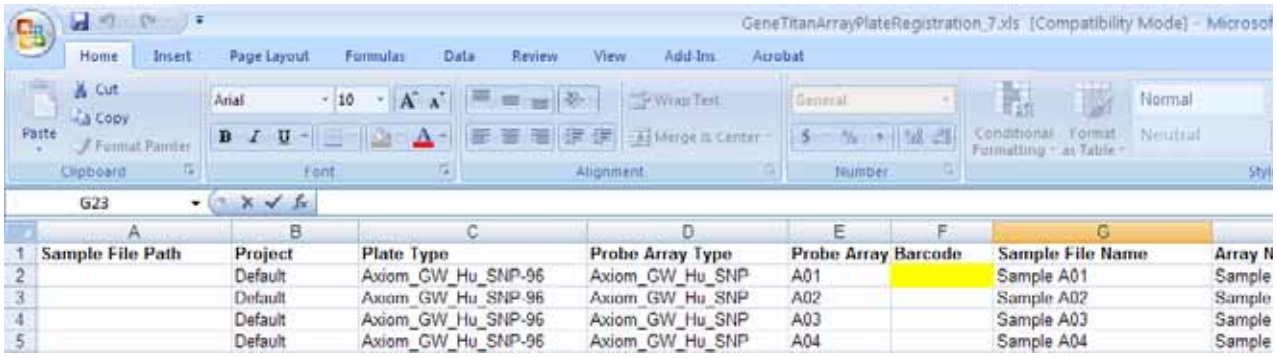
In the AGCC software, you must create and upload a Batch Registration file before you begin *Stage 2 — Hybridization* *on page 158* (example shown in Figure 5.8). This file contains information critical for:

- 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 237* 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 158*.**

Figure 5.8 Example of a batch registration file for an array plate



Stage 2 — Hybridization

Reagents Required

Reagents Required

Table 5.1 Reagents required from the Axiom Reagent Kit

Module	Reagent	Thaw on Bench Top, Then Place on Ice	Place on Ice	Place on Bench Top at Room Temperature
Module 3, Room Temperature	Axiom Wash Buffer A			✓
	Axiom Wash Buffer B (both bottles; 1L)			✓
	Axiom Water			✓

- An Axiom Genome-Wide CEU 1 array plate or an Axiom Genome-Wide and custom myDesign™ Array Plate is required for this step. Prior to inserting this plate into the GeneTitan for hybridization, the array plate should be brought to room temperature as described on Step 2 on page 130.
- A hybridization tray containing denatured samples (from Step 2 on page 88 in Chapter 3 or Step 8 on page 132 in Chapter 4) is also required for this step. The denatured samples should be transferred to the hyb tray only after the GeneTitan is ready for loading the hyb tray in the "Load an Array Plate and Hyb Tray Onto the GeneTitan MC Instrument" step on page 121.

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® MC Instrument Care on page 247*** 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 on page 182***.

Figure 5.9 Launching AGCC and initializing the GeneTitan MC instrument.

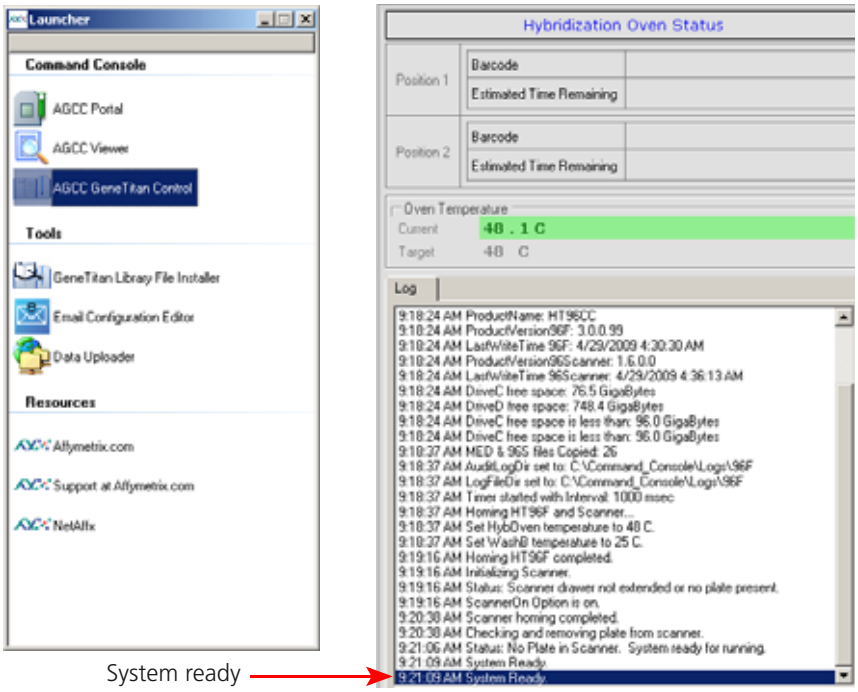
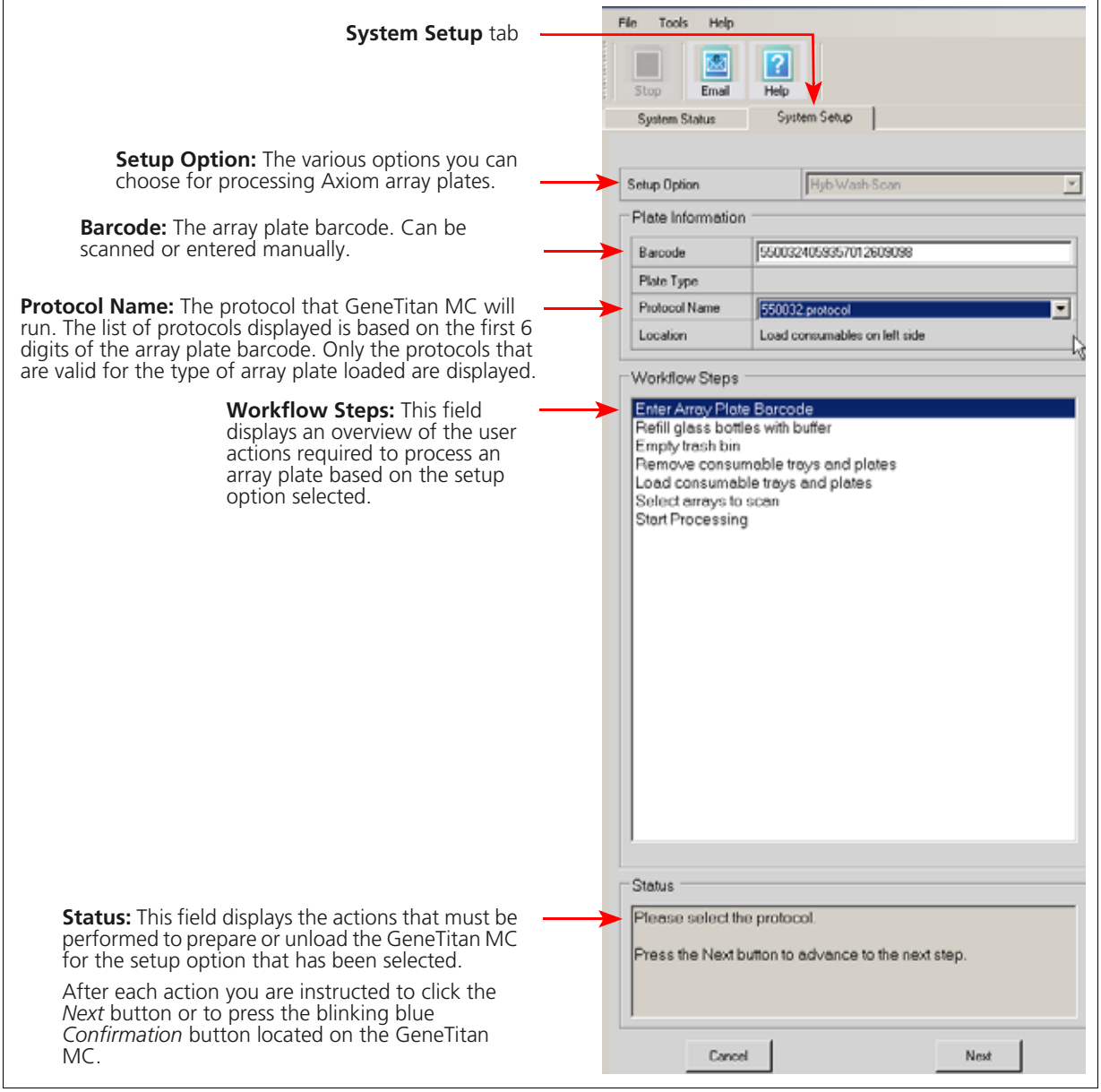


Figure 5.10 System setup tab and the information displayed in this pane.



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 154*.
   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 Platess.**
>
> - **96 Axiom Array Plate requires ~ 80 GB**

   C. Plate Information:

- **Barcode:** Scan or manually enter the Axiom Array Plates barcode and click **Next**.

  The first six characters of the barcode identify the type of plate being loaded, the protocol GeneTitan MC 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

If this error message is displayed:

- Ensure that the library files for the type of array plate you are using are correctly installed.
- Try manually entering the array plate barcode.
- Library files must be installed prior to launching GeneTitan. If a library file must be installed exit GeneTitan, install libraries and relaunch GeneTitan.

- **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 162)**

      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 l L full

! IMPORTANT:

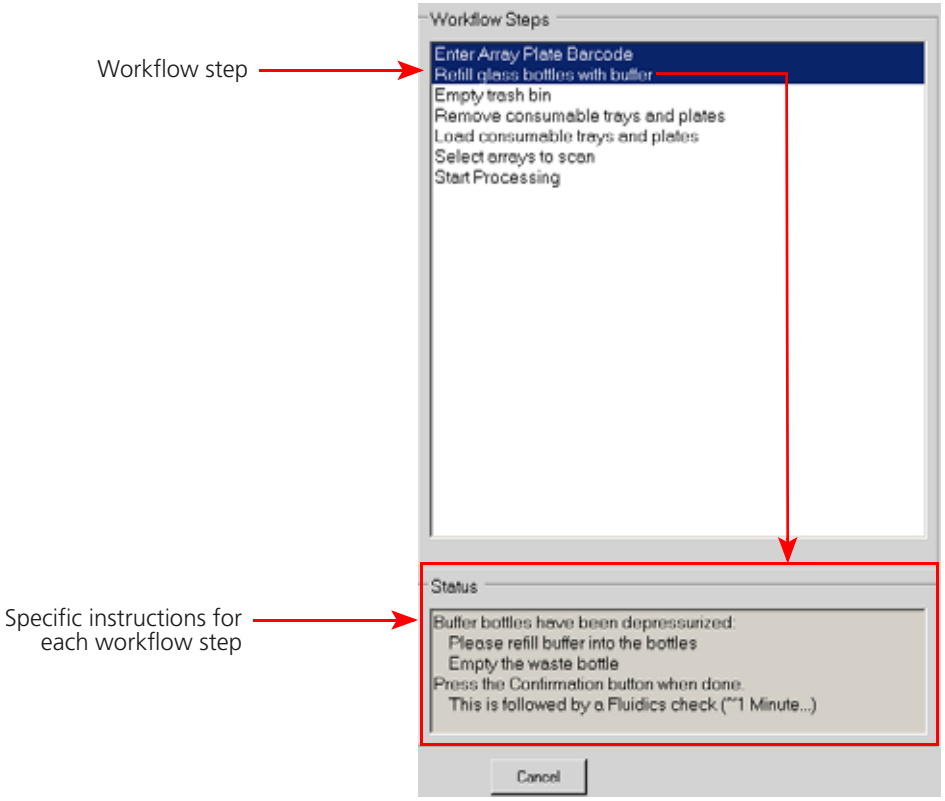
- **Always ensure that the GeneTitan bottles containing Wash A and Rinse are above the 50% mark when setting up the system to process an Axiom HT array plate. All 600 mL of the Wash buffer B from the Axiom reagent kit should be emptied into the GeneTitan Wash B bottle when setting up the system to process 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 9, *Troubleshooting on page 217*). 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 Reagent Kit).**

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

Figure 5.12 Example of the remaining workflow steps



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."

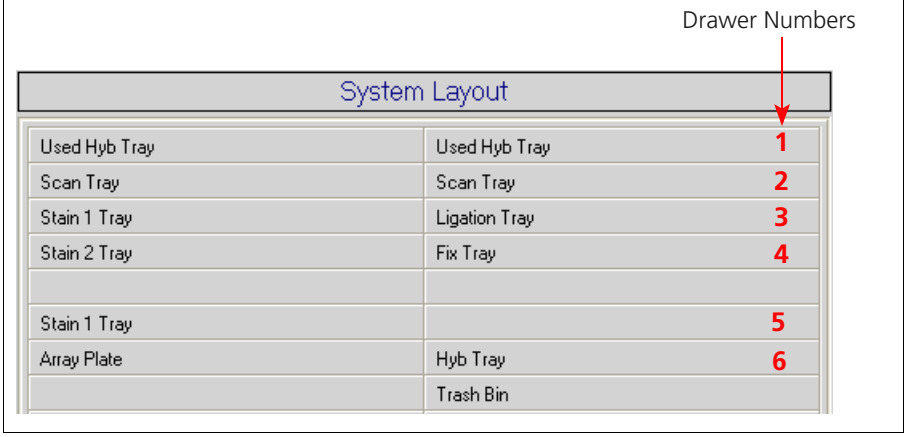
2) Press the **Confirmation** button to continue.

D. Continue to *Load an Axiom Array Plates and Hyb Tray Onto the GeneTitan MC* *on page 163*.

Load an Axiom Array Plates and Hyb Tray Onto the GeneTitan MC

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

Figure 5.13 System layout — location of plates inside the GeneTitan MC



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

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 164).

The array plate must be loaded on its protective blue base, as shown in Figure 5.15 on page 164 below. The clear plastic cover on top of the array plate SHOULD NOT be loaded in the GeneTitan MC. See Figure 5.1 on page 150 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 (Figure 5.14).

Figure 5.14 Array Plate packaging

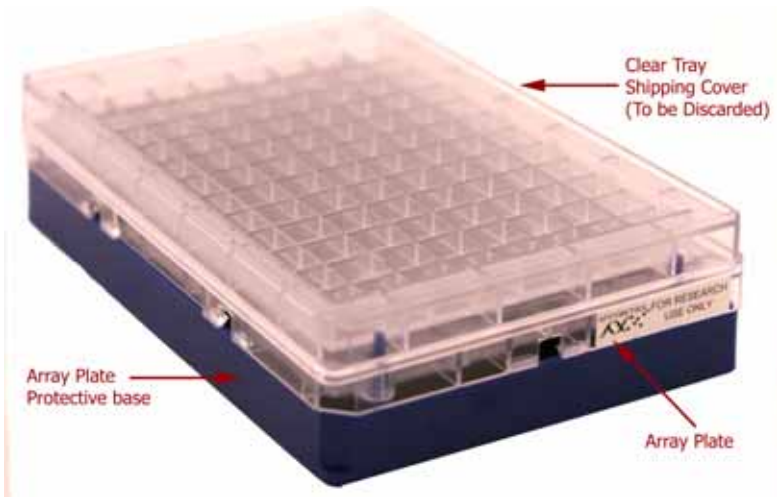
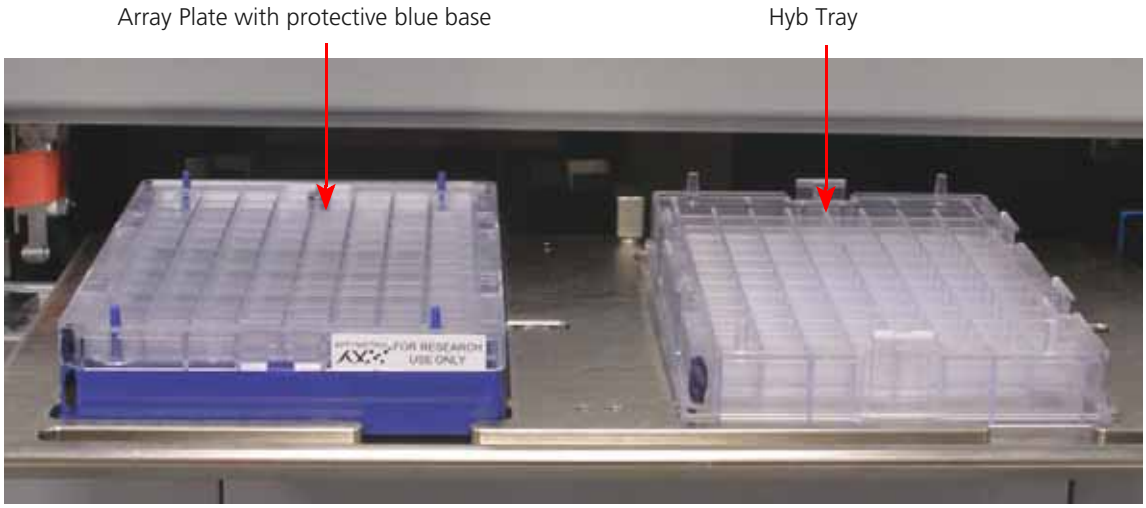


Figure 5.15 Array Plate with protective blue base and the Hyb Tray properly loaded into drawer 6.



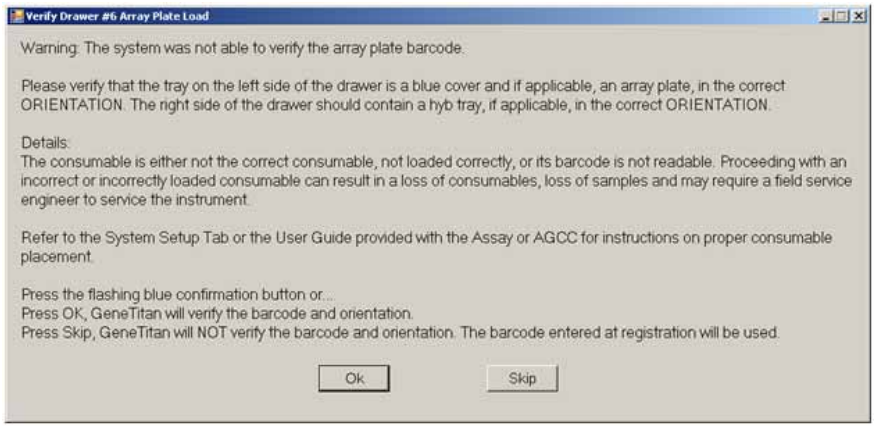
D. Load the Array Plate **with the protective blue base** on the left side of the drawer (Figure 5.15).

! CAUTION: The notched corner of each plate, cover and tray must be aligned. When loading onto the GeneTitan MC, 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 164).

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 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

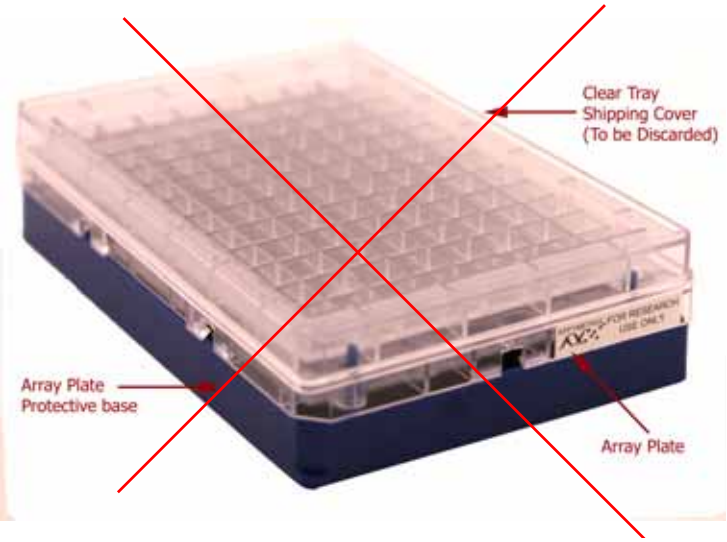


When you load the array plate left side of the drawer: The internal bar code reader reads the barcode of the array plate and compares it with the barcode and the plate type specified in the Barcode field and Plate Type field on the Setup page. If the information is correct, the application allows you 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

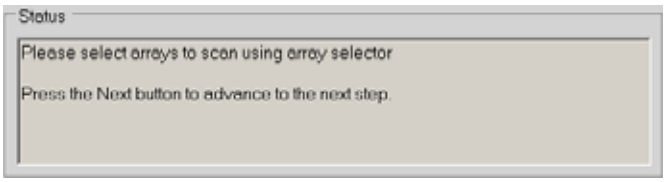
- Click Skip if the instrument has problems reading the barcode and after verifying that the trays have been placed in the proper orientation.

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 150.

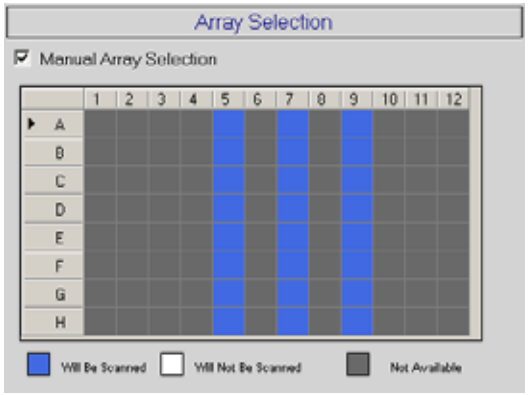


F. Select the arrays to scan (instructions in Figure 5.17).
By default, all arrays are selected.

Figure 5.17 Selecting which arrays to scan an array plate



Message in Status Window.



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 System Status window is automatically selected and brought to the front of the computer monitor.

3. When drawer 6 opens and the prompt in Figure 5.20 on page 167 is displayed:

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 5.19 for an example of a array plate hybridization plate sandwich).

Figure 5.19 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 5.21 may be displayed again if plate orientation is incorrect or if the Hyb Tray barcode cannot be read. Click **OK** to proceed.

Figure 5.20 Location of camping points on the Array Plate and Hyb Tray

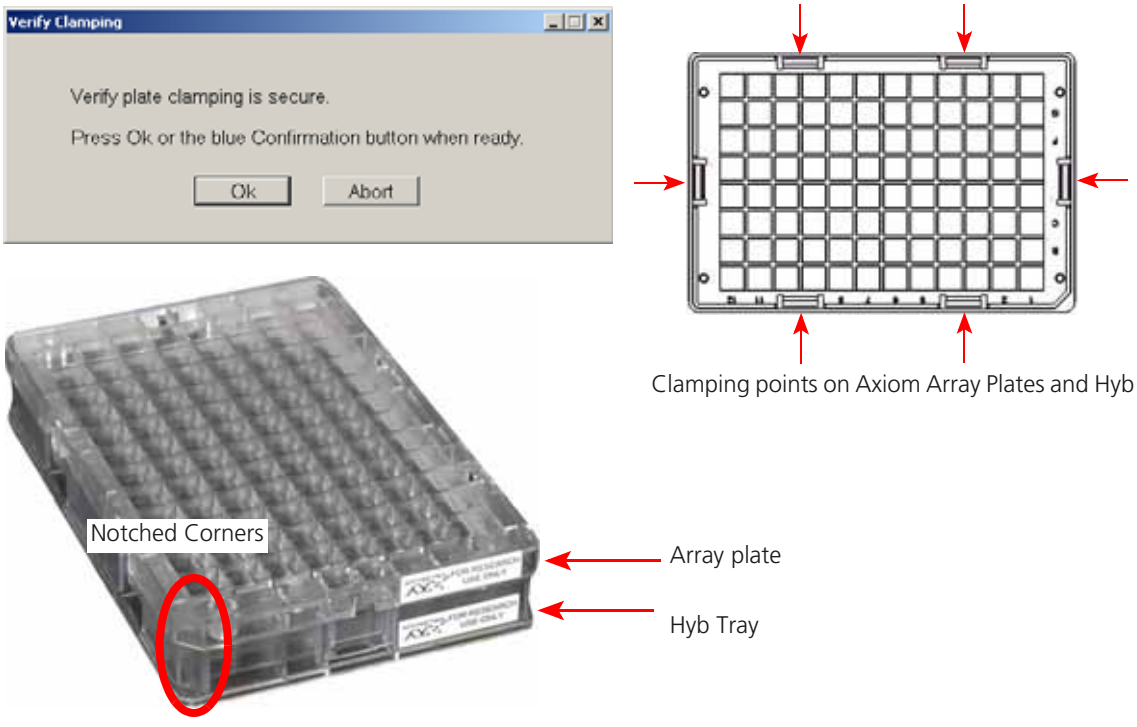
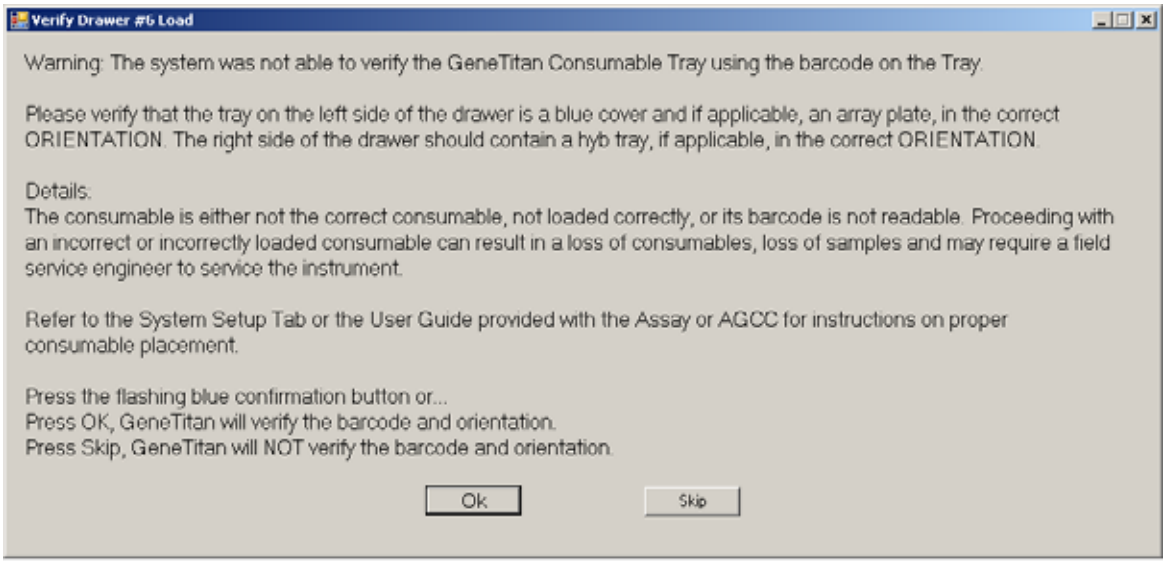


Figure 5.21 Verification message



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

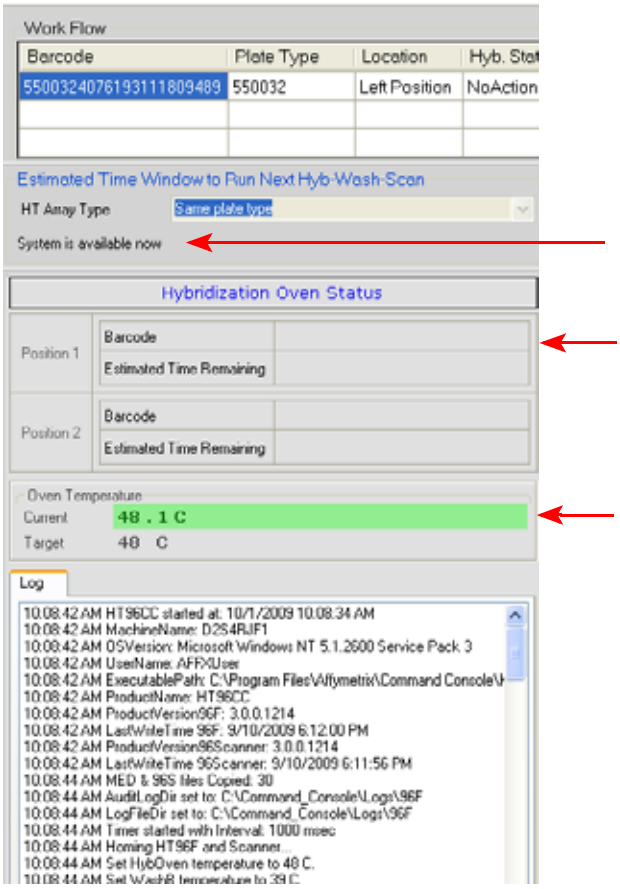
When You Can Load a Second Array Plate and Hyb Tray

Once processing begins, you have a specific period of time during which you can load another Axiom Array Plates and Hyb Tray. This period of time is displayed above the Hyb Oven Status pane (Figure 5.22). 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.**

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 5.22 Loading a second Hyb Tray and hybridization oven status information



Additional plates cannot be loaded before or after this period of time while the instrument is operating.

In this figure, the system is currently available.

This pane displays the period of time during which another Array Plate and Hyb Tray can be loaded.

Position of plate stack in the hybridization oven. Only 1 plate being processed in this figure. As such, position 2 is blank.

Position 1 - left side of oven

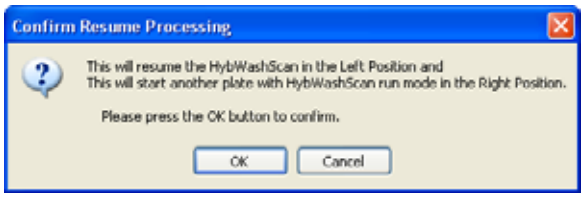
Position 2 - right side of oven

Green indicates the current oven temperature is within the target temperature range.

Yellow indicates oven temperature outside of target temperature range.

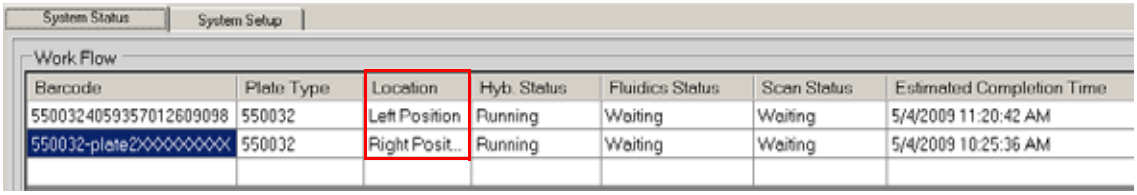
1. Select the System Setup tab.
2. Load an Axiom Array Plates and Hyb Tray in the same manner that you loaded the previous plate and tray.
   - **A.** Scan or manually enter the Axiom Array Plates barcode, then click **Next**.
   - **B.** Load the Axiom Array Plates **with the blue base** and the Hyb 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 5.23).

Figure 5.23 Confirm resume processing prompt



Select the System Status tab to view Axiom Array Plates status in the WorkFlow window (Figure 5.24).

Figure 5.24 Example of the WorkFlow window when two plates are loaded and are in the hybridization oven



Left and Right positions = the position of the Scan Tray in drawer 2 (left or right side of the drawer).

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 *Confirmation* 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 *Confirmation* 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, 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.		These messages are displayed if: ▪ A plate has been loaded improperly. ▪ The bar code is missing or obscured

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 *Next*, then click *OK* to start processing.	—

Stage 3 — Ligate, Wash, Stain and Scan

Equipment, Consumables and Reagents Required

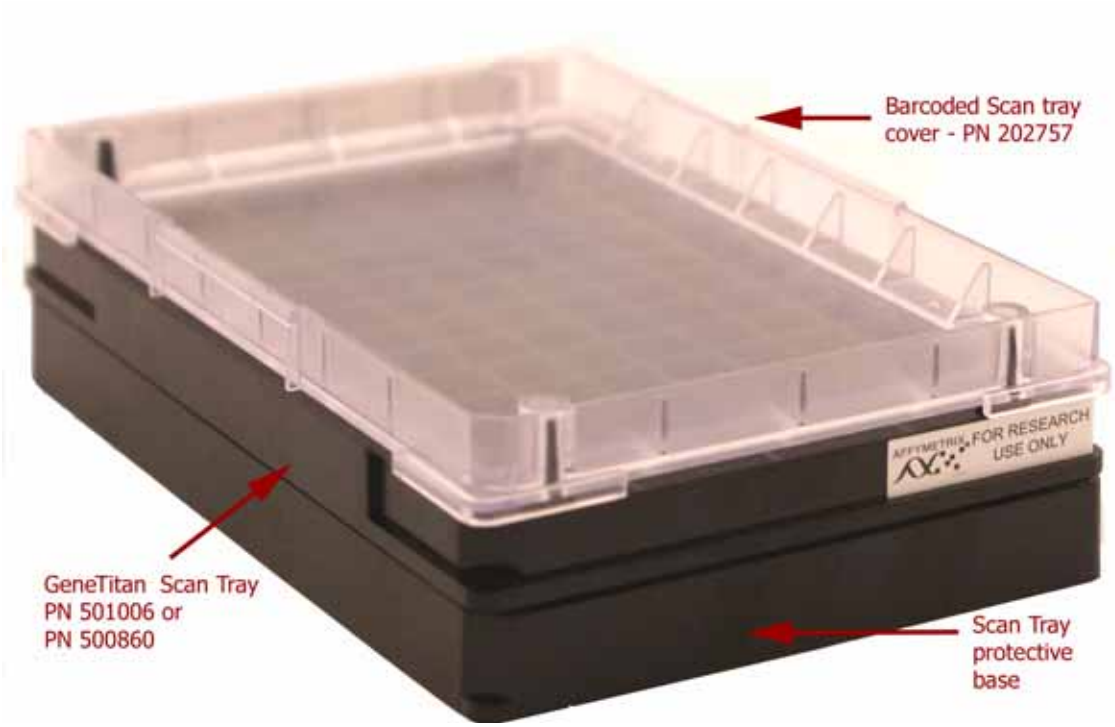
Scan Tray with Axiom Hold Buffer

- 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 5.25).

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. When handling the Scan Tray, the bottom glass surface of the tray should not be touched.

Figure 5.25 The Scan Tray with cover on the black base.

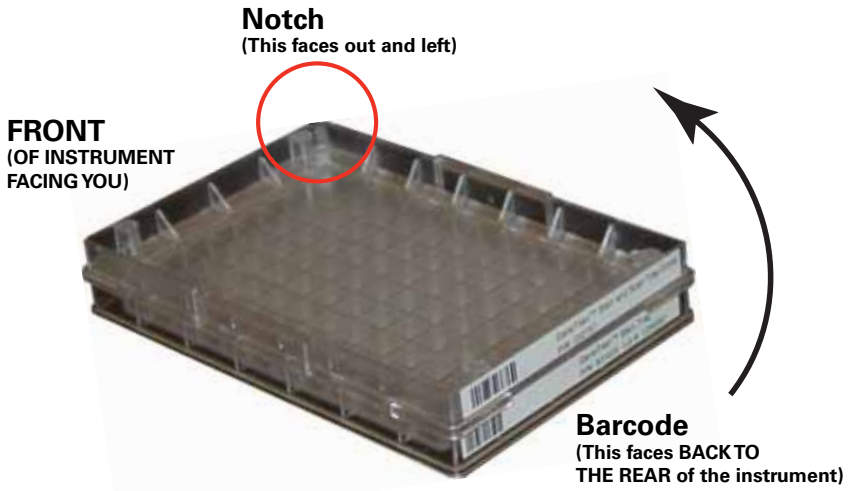
Always leave the Scan Tray in its protective black base.



Proper Installation of the GeneTitan Tray Consumables.

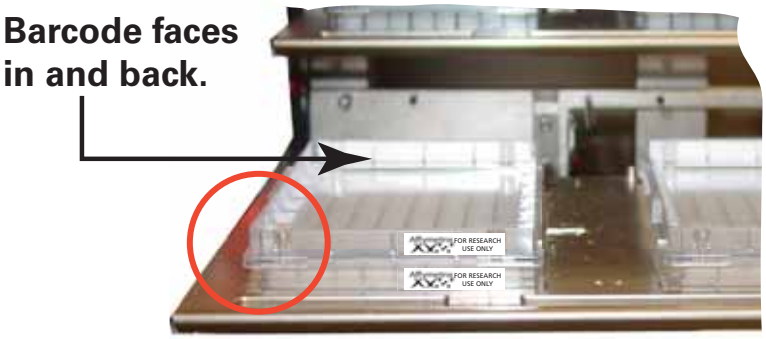
It is very important that you install the GeneTitan tray consumables in the proper orientation. The barcode faces into the instrument.

Figure 5.26 You must rotate and install the trays so that the barcode faces into the instrument.



Turn the tray and cover combo so that the barcode faces **BACK AND INTO** the instrument and the notch faces **OUT AND TO THE LEFT**.

Figure 5.27 The proper installation of the GeneTitan tray consumables (the image shows the Stain Tray and the Stain Tray cover as an example)



Notch faces out and left.
"Affymetrix For Research Use Only" faces out .

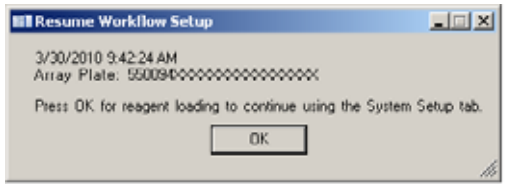
NOTE: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. Refer to *Appendix F, GeneTitan® MC Instrument Care on page 247* for the message displayed to the user and the procedure for replacing the filters

Load Trays onto the GeneTitan MC

To load trays onto the GeneTitan MC:

When hybridization of an Axiom Array Plates has finished, a message (Figure 5.28) will alert you to resume the workflow setup. Press **OK** and the software takes you directly back to the System Setup tab.

Figure 5.28 The Resume Workflow Setup Message



This prompt to continue into reagent load (Figure 5.28) 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.

GeneTitan 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. GeneTitan 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 5.29 on page 176).**
- **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

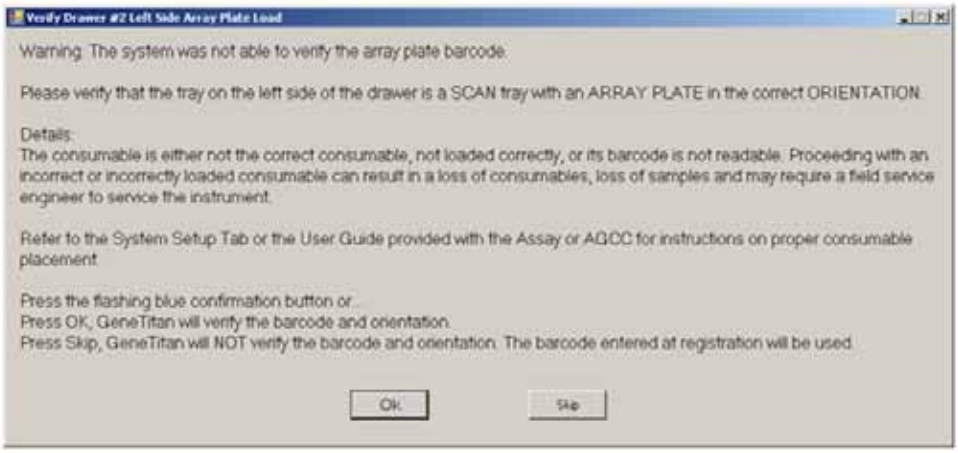
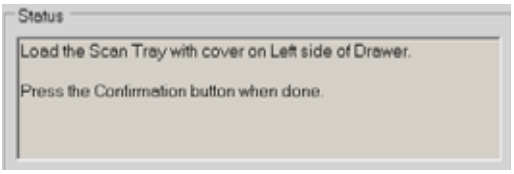
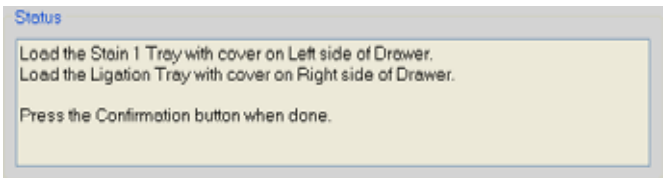
Loading Sequence by Drawer Number	Left	Right
	Note: If the software is unable to verify the barcode on the scan tray and the scan tray cover, the software will display the following error message 	
2	**Scan Tray with cover — do not load the protective *black base*** (left side of drawer as indicated in Status window) Figure 5.29 on page 176 	
3	Stain Tray with Stain 1	Ligation Tray
	Figure 5.30 on page 177 	

Table 5.6 Sequence for loading the trays with reagents

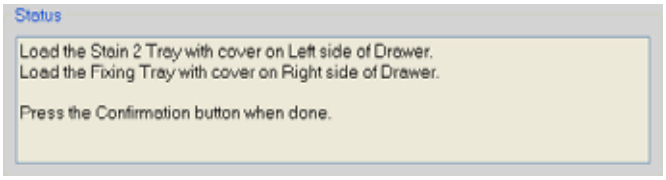
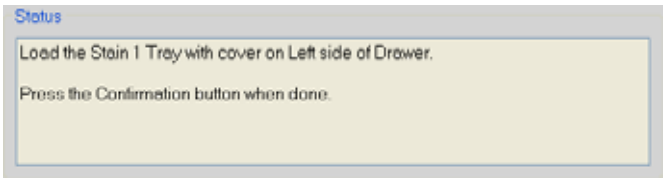
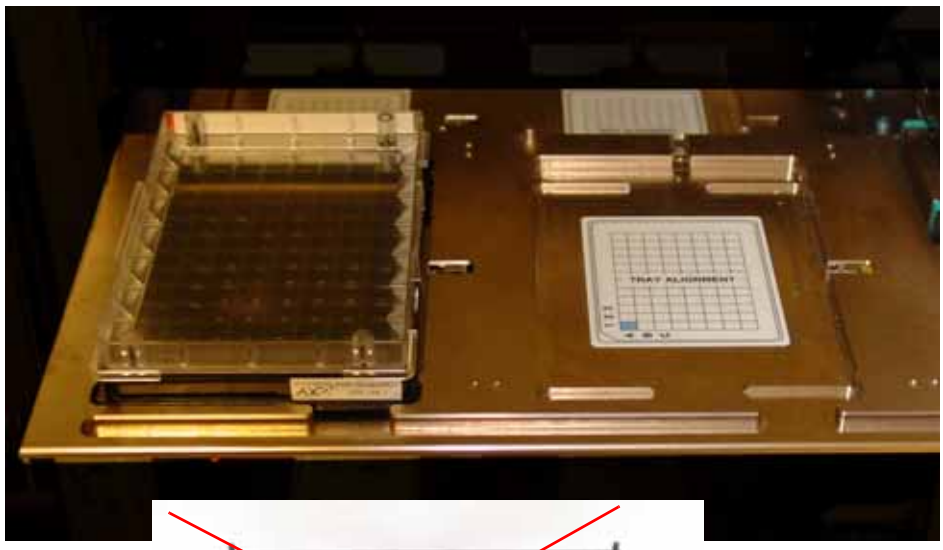
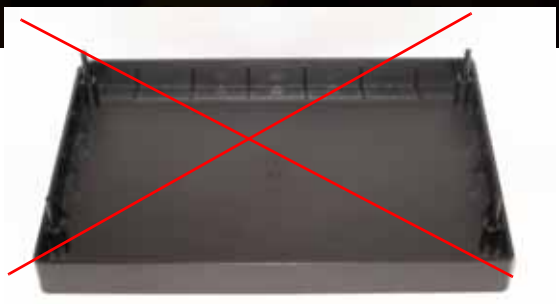
Loading Sequence by Drawer Number	Left	Right
4	Stain Tray with Stain 2 Figure 5.31 on page 178 	Fix Tray
5	Stain Tray with Stain 1 	Empty

Figure 5.29 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.**

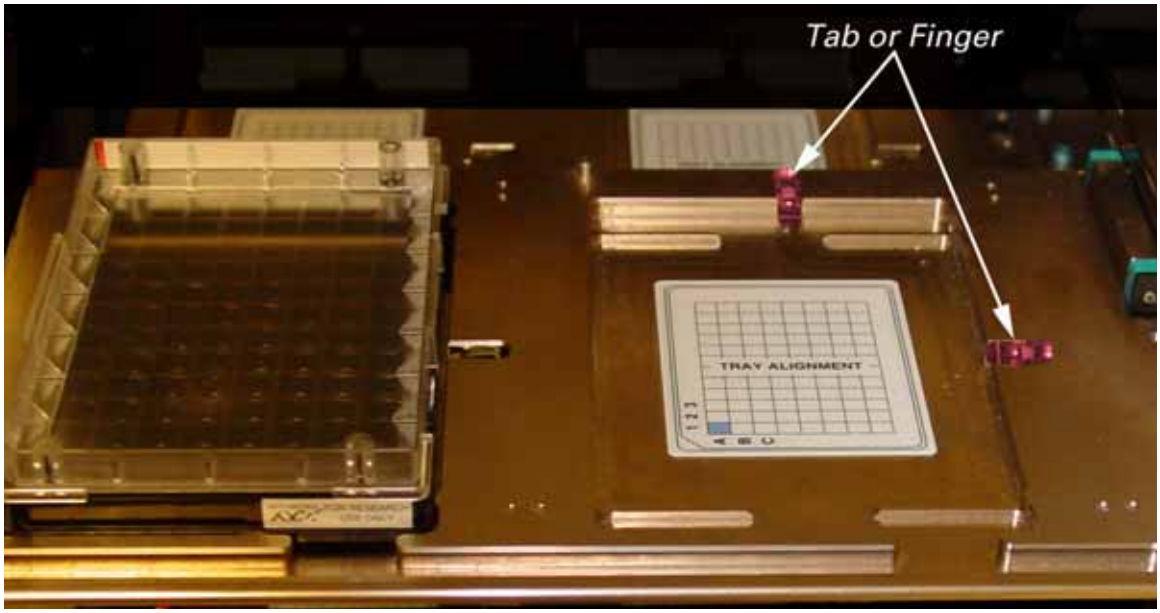


Figure 5.30 Stain 1 Tray and Ligation Tray loaded in drawer 3.

Stain 1 Tray and Ligation Tray loaded in drawer 3

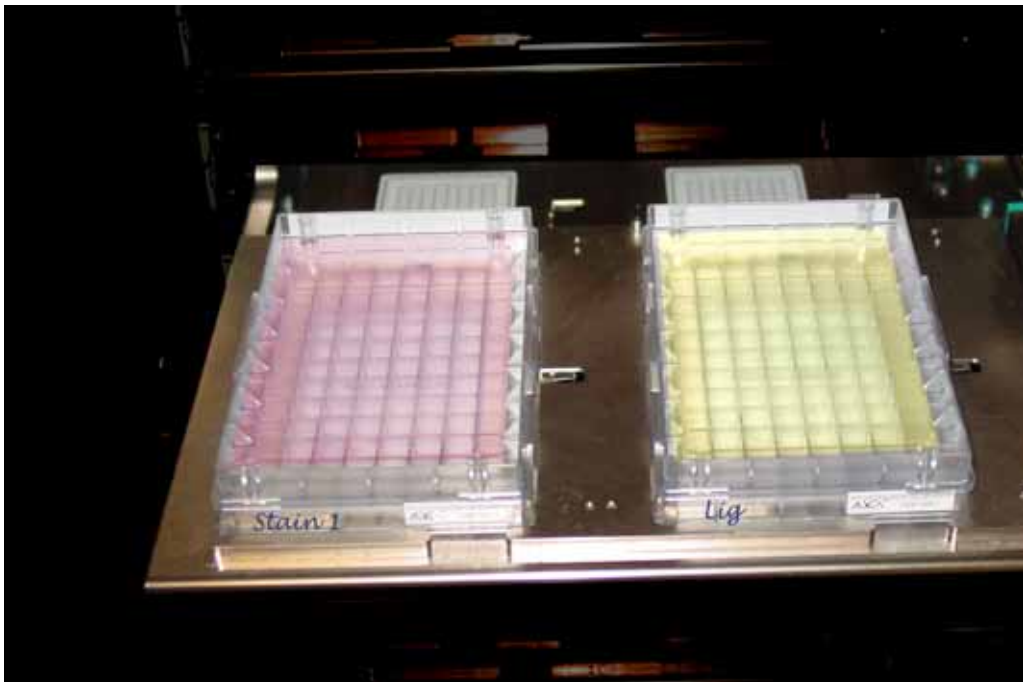


Figure 5.31 Stain 2 Tray and Fix Tray loaded in drawer 4.

Stain 2 Tray and Fix Tray loaded in drawer 4

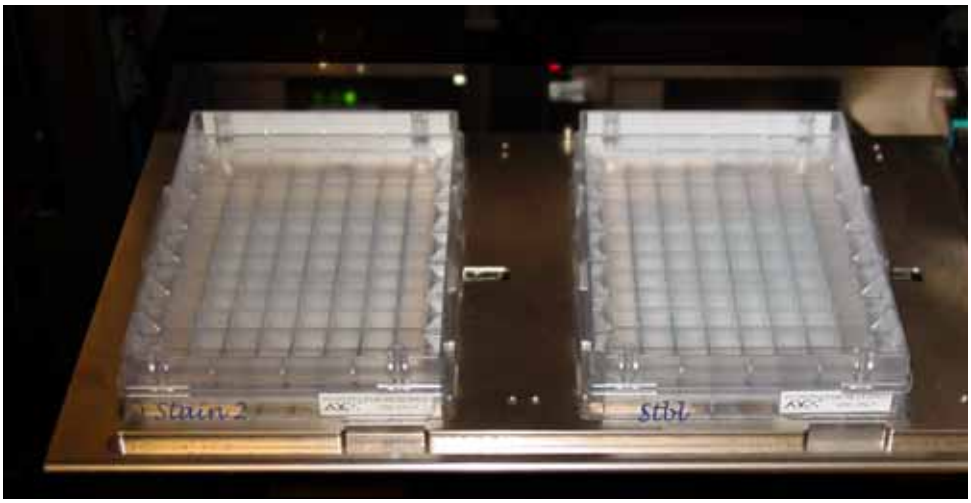
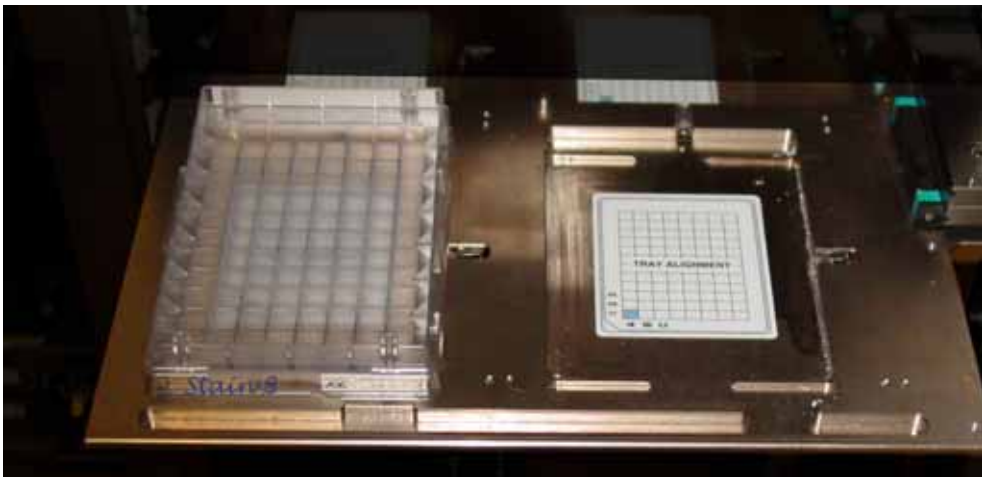


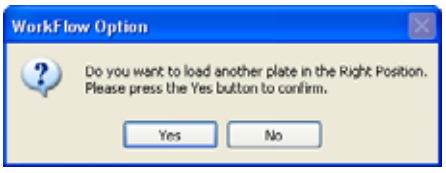
Figure 5.32 Stain 1 Tray loaded in drawer 5.

Stain 1 Tray loaded in drawer 5.



3. At the prompt shown in Figure 5.33, click *Yes* to load another Axiom Array Plates and Hyb Tray.

Figure 5.33 Prompt asking to load another plate. Right or left position determined by the position of Axiom Array Platess already in the GeneTitan MC.



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

D. When drawer 6 opens:

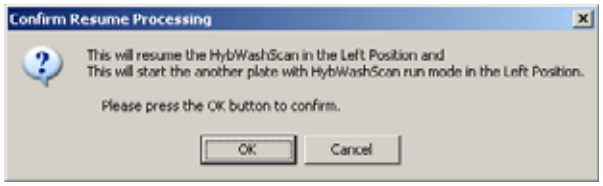
   1) Remove the blue cover from the previous Axiom Array Plates.

   2) Load a new Axiom Array Plates 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 5.34).

Figure 5.34 Confirm Resume Processing message



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

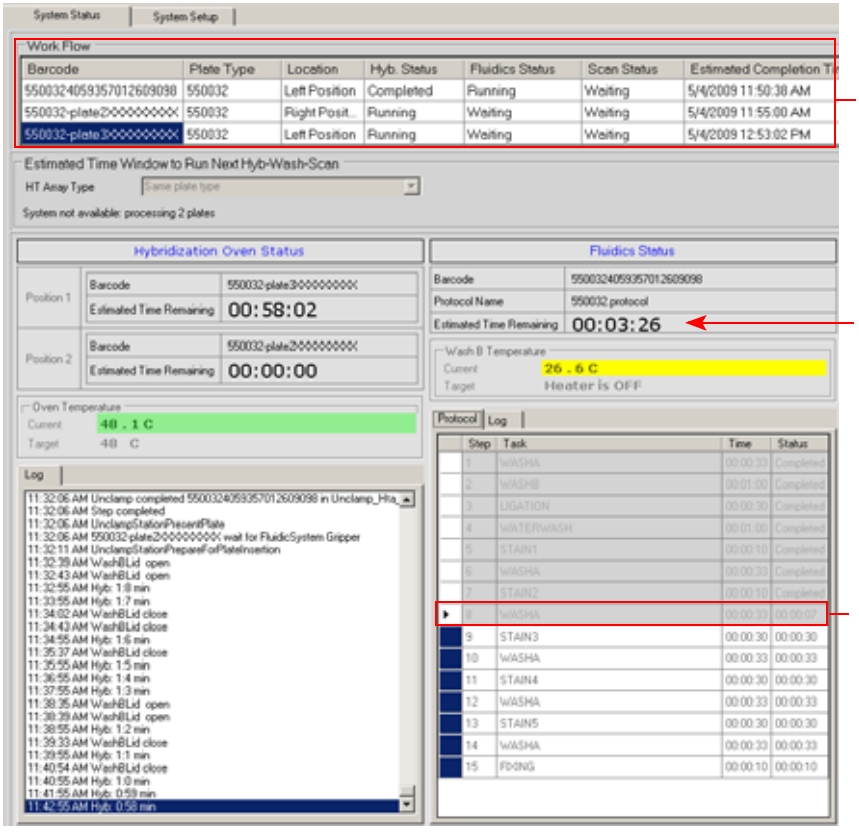
When processing resumes:

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 5.35 is an example of how the System Status Workflow window will appear when three Axiom Array Platess are being processed.

Figure 5.35 Example of the System Status window — three Axiom Array Platess are being processed.



Workflow indicates the number of plates being processed and where they are in the instrument. In this example, three Axiom Array Platess 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

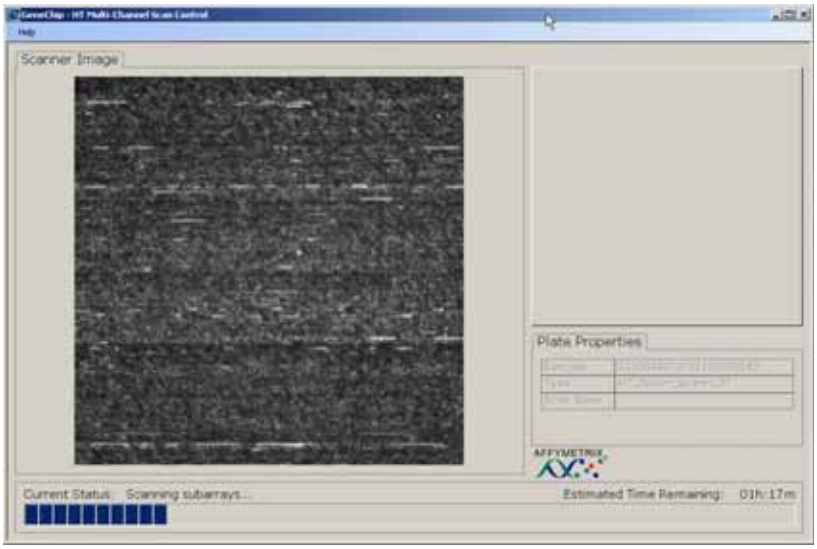
Once a plate has gone through the fluidics stage of the process, it is moved to the imaging device.

When the scanning process begins, the window shown in Figure 5.36 is displayed. This window must remain open while Axiom Array Platess are being scanned.

CAUTION:

- **The Scan Control window must remain open while Axiom Array Platess 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.**

Figure 5.36 Scan control window



This window must remain open while scanning is in progress.

If you close this window, scanning will stop and delay sample processing.

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 235* for more information.

To store a Hyb Tray with processed samples:

1. After completion of the GeneTitan 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

This procedure assumes that all of the Axiom Array Platess loaded onto the GeneTitan MC have been processed.

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

To shutdown the GeneTitan MC:

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 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.

Automated Target Preparation for Processing Eight Axiom Array Plates Per Week

Using one Biomek FX^P Target Prep Express (Biomek workstation) and one GeneTitan MC Instrument, the Axiom™ assay (using the 96-array plate) can be run at a throughput of eight Axiom™ Array Plates per five-day work week. This chapter includes tables that present the timing of the steps required to perform this workflow per 5-day work week, eight hours per day.

IMPORTANT: **Experienced users and careful timing are critical for the successful execution of this workflow.**

During the initial week of startup, all work is done on the Biomek FX^P Target Prep Express. You will process eight plates of genomic DNA samples. At the end of this week, you will have what are now referred to as eight plates of *hyb-ready target* (target).

Subsequent weeks of the workflow involve the simultaneous processing of plates on the Biomek FX^P Target Prep Express and on the GeneTitan MC Instrument. Each week:

- Eight plates of target from the previous week are denatured and transferred to the GeneTitan MC Instrument for hybridization and array plate processing.
- Eight new plates of target are prepared on the Biomek FX^P Target Prep Express.

Plate Numbering Scheme

- Target preparation on the Biomek FX^P Target Prep Express: sample plates are numbered 1 through 8.
- Target prepared the previous week is processed on the GeneTitan MC Instrument. The plates denatured and loaded onto the GeneTitan are now referred to as plates A through H. Target prepared on the Biomek FX^P Target Prep Express can be run on the GeneTitan MC Instrument in random order.

Overview of the 8-plate Workflow on the Biomek FX^P Target Prep Express

Table 6.1 Overview of the Biomek FX^P Target Prep Express workflow

Day	Activities	Plates
1	▪ Amplify eight plates of genomic DNA.	1 through 8
2	▪ Fragment and precipitate three plates amplified on Day 1. ▪ Freeze five plates of amplified gDNA for fragmentation later in the week.	▪ 1, 5, 8 ▪ 2, 3, 4, 6, 7
3	▪ Fragment and precipitate two more amplified plates. ▪ Centrifuge, dry, resuspend and QC the three plates precipitated on Day 2.	▪ 2, 3 ▪ 1, 5, 8
4	▪ Fragment and precipitate the three remaining amplified plates. ▪ Centrifuge and dry the two plates precipitated on Day 3.	▪ 4, 6, 7 ▪ 2, 3
5	▪ Resuspend and QC the two plates dried on Day 4. ▪ Centrifuge, dry, resuspend and QC the three plates precipitated on Day 4.	▪ 2, 3 ▪ 4, 6, 7

Table 6.2 Time required for target preparation on the Biomek FX^P Target Prep Express

Steps on the Biomek FX^P Target Prep Express	Time Required
Amplification	30 min
Fragmentation	2 hr
Resuspension	45 min
Off-deck centrifugation and drying	75 min
Off-deck QC gel and OD	45 min
Denaturation only	30 min
Denaturation and GeneTitan reagent plate preparation	45 min
Transfer denatured samples to GeneTitan	15 min
Transfer reagent plates to the GeneTitan	15 min
Time Required includes setup.	

Overview of the 8-plate Workflow on the GeneTitan MC Instrument

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

Table 6.3 Overview of the GeneTitan MC Instrument 8 plate workflow

Day	Activities	Plates
1	▪ Hybridize two plates of denatured target.	▪ A and B
2	▪ Hybridize two plates of denatured target. ▪ Load reagent plates for fluidics and imaging of plates loaded on day 1	▪ C and D ▪ A and B
3	▪ Hybridize two plates of denatured target. ▪ Load reagent plates for fluidics and imaging of plates loaded on day 2	▪ E and F ▪ C and D
4	▪ Hybridize two plates of denatured target. ▪ Load reagent plates for fluidics and imaging of plates loaded on day 3	▪ G and H ▪ E and F
5	▪ Load reagent plates for fluidics and imaging of plates loaded on day 4	▪ G and H

The hybridization time for the Axiom assay on the GeneTitan MC Instrument is 23.5 to 24 hr (Table 6.4). 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. We recommend that you begin loading the reagent plates onto the GeneTitan at the mid-point of this 30 min window. As such, the wash procedures will begin 24 hr after the start of hybridization. If catch-up is required in the framework of the 8-plate workflow, begin loading reagents at the beginning of this 30 min window (*i.e.,* immediately after prompted by the software).

Table 6.4 Time required for array plate processing on the GeneTitan MC Instrument

Steps on the GeneTitan MC Instrument	Time Required
Hybridization of two plates First plate loaded at 9:30 a.m. Second plate loaded at 5:00 p.m.	23.5 hr each plate
Loading reagent plates that were prepared on the Biomek FX^P Target Prep Express	15 min
Fluidics	5 hr each plate
Imaging	96 arrays: 7.5 hr

Thawing Frozen Plates of Amplified DNA

To thaw frozen plates of amplified DNA:

1. Place the deep well plate in a small water bath.
   For example, pour Millipore water into a small tray. Place the frozen plate on the water in the tray.
2. Leave the plate in the water bath for ~ 50 min until all wells have thawed.
3. Spin down at 1000 rpm for 30 sec.
4. To avoid cross-contamination of wells during vortexing:
   A. Remove the seal and blot the top of the plate with a Kimwipe.
   B. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 sec to thoroughly mix.
6. Spin at 1000 rpm for 30 sec.

Initial Target Prep Week — Biomek FX^P Target Prep Express

Initial Target Prep Week — Day 1

- Amplify eight plates of gDNA samples.

IMPORTANT:

- **All amplifications should be set up on Day 1 to allow for a 22 to 24 hr amplification incubation for each plate.**
- **Begin thawing the amplification reagents, particularly the Axiom Amp Soln, 60 min prior to the start of each reaction.**

Table 6.5 Initial Target Prep Week — Day 1 Activities

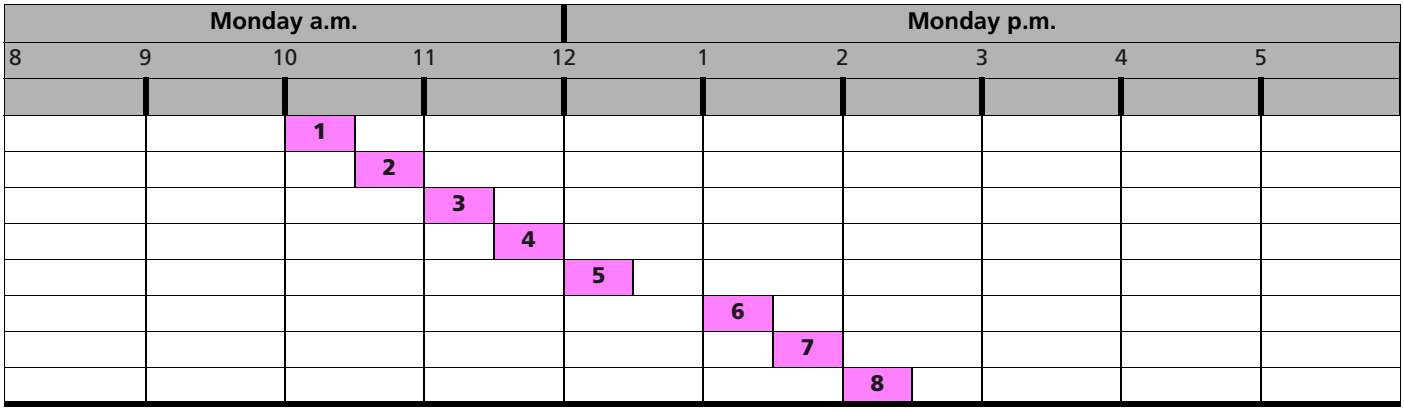


Table 6.6 Initial Target Prep Week — Day 1 Activities

Activity	Plate Number	Instrument	Approximate Start Times
DNA Amplification	1	Biomek FX^P Target Prep Express	10:00 a.m.
DNA Amplification	2		10:30 a.m.
DNA Amplification	3		11:00 a.m.
DNA Amplification	4		11:30 a.m.
DNA Amplification	5		12:00 p.m.
DNA Amplification	6		1:00 p.m.
DNA Amplification	7		1:30 p.m.
DNA Amplification	8		2:00 p.m.

Initial Target Prep Week — Day 2

- Fragment and precipitate plates 1, 5 and 8.
- Freeze amplified plates 2, 3, 4, 6, and 7 after each plate has incubated for 23 hr.

IMPORTANT:

- **Plates 1, 5 and 8 are fragmented and precipitated on Day 2 without freezing to preserve a 23 hr amplification incubation.**
- **Store all plates not fragmented and precipitated on Day 2 at –20 °C following 23 hr of amplification reaction incubation.**
- **Precipitation is carried out at –20 °C overnight.**

Table 6.7 Initial Target Prep Week — Day 2 Activities

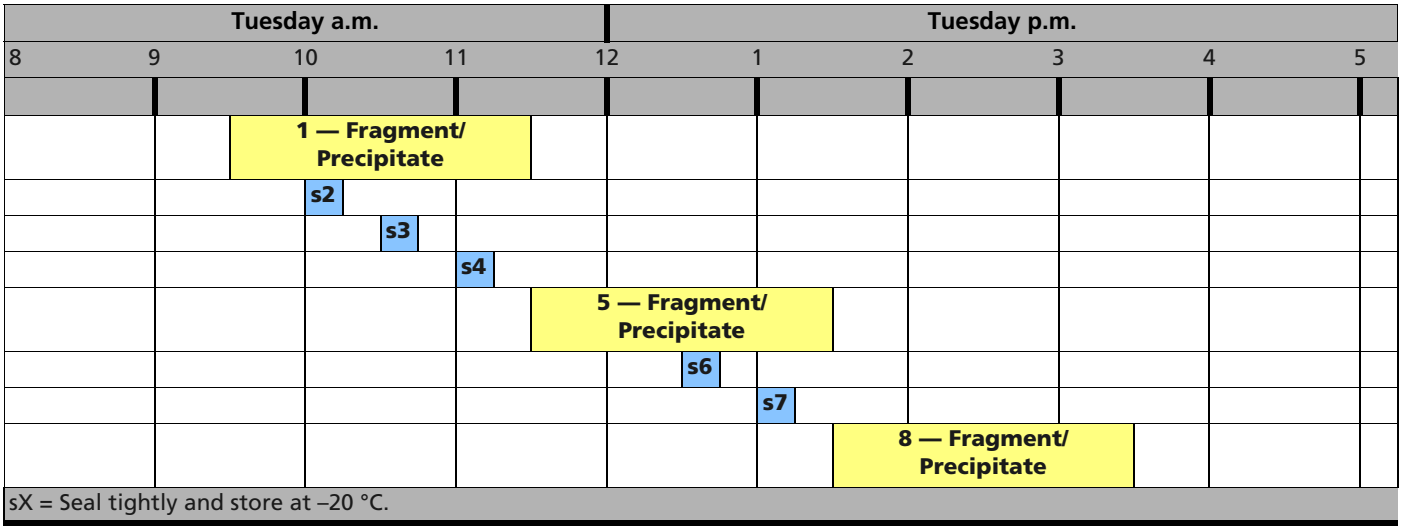


Table 6.8 Initial Target Prep Week — Day 2 Activities

Activity	Plate Number	Instrument	Approximate Start Times
Fragment and precipitate	1	Biomek FX^P Target Prep Express	9:30 a.m.
Freeze (–20 °C)	2	—	10:00 a.m.
Freeze (–20 °C)	3	—	10:30 a.m.
Freeze (–20 °C)	4	—	11:00 a.m.
Fragment and precipitate	5	Biomek FX^P Target Prep Express	11:30 a.m.
Freeze (–20 °C)	6	—	12:30 p.m.
Freeze (–20 °C)	7	—	1:00 p.m.
Fragment and precipitate	8	Biomek FX^P Target Prep Express	1:30 p.m.

Initial Target Prep Week — Day 3

- Thaw plates 2 and 3 (see *Thawing Frozen Plates of Amplified DNA* *on page 185*).
- Fragment and precipitate plates 2 and 3.
- Centrifuge, dry, resuspend and QC plates 1, 5 and 8.

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 185*.**
- **After being centrifuged and dried, plates 1, 5 and 8 are sealed and placed in a 4 °C refrigerator until further processing later the same day.**
- **Precipitation is carried out at –20 °C overnight.**
- **Prior to resuspension and QC, plates 1, 5 and 8 must be brought to room temperature (place on bench top for 30 min).**

Table 6.9 Initial Target Prep Week — Day 3 Activities

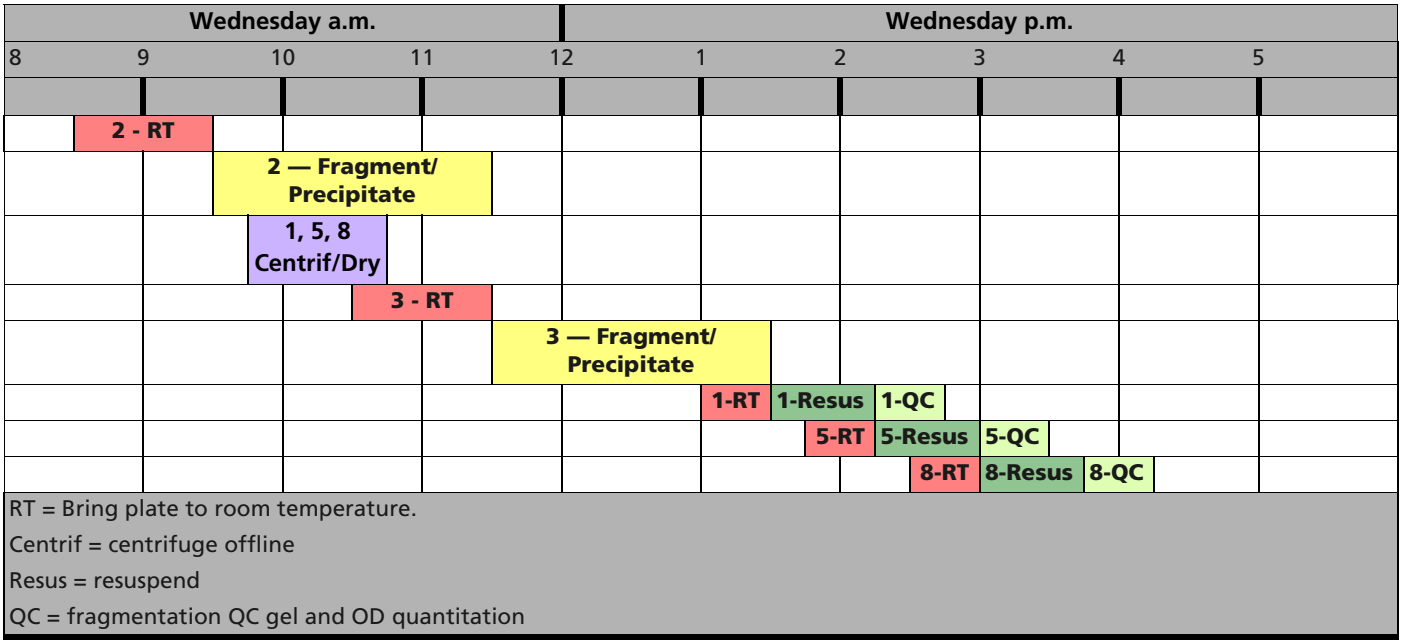


Table 6.10 Initial Target Prep Week — Day 3 Activities

Activity	Plate Number	Instrument	Approximate Start Times
Bring plate to room temperature (RT)	2	—	8:30 a.m.
Fragment and precipitate	2	Biomek FX^P Target Prep Express	9:30 a.m.
Spin and dry	1, 5, 8	Plate centrifuge and oven	9:45 a.m.
Bring plate to room temperature (RT)	3	—	10:30 a.m.
Fragment and precipitate	3	Biomek FX^P Target Prep Express	11:30 a.m.
Resuspension	1	Biomek FX^P Target Prep Express,	1:30 p.m.

Table 6.10 Initial Target Prep Week — Day 3 Activities

Activity	Plate Number	Instrument	Approximate Start Times
Off-deck QC	1	Plate spectrophotometer, e-gel system	2:15 p.m.
Resuspension	5	Biomek FX^P Target Prep Express	2:15 p.m.
Off-deck QC	5	Plate spectrophotometer, e-gel system	3:00 p.m.
Resuspension	8	Biomek FX^P Target Prep Express	3:00 p.m.
Off-deck QC	8	Plate spectrophotometer, e-gel system	3:45 p.m.

Initial Target Prep Week — Day 4

- Fragment and precipitate plates 4, 6 and 7.
- Centrifuge and dry plates 2 and 3.

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 185*.**
- **Precipitation is carried out at –20 °C overnight.**
- **After being centrifuged and dried, plates 2 and 3 are sealed and stored at –20 °C.**

Table 6.11 Initial Target Prep Week — Day 4 Activities

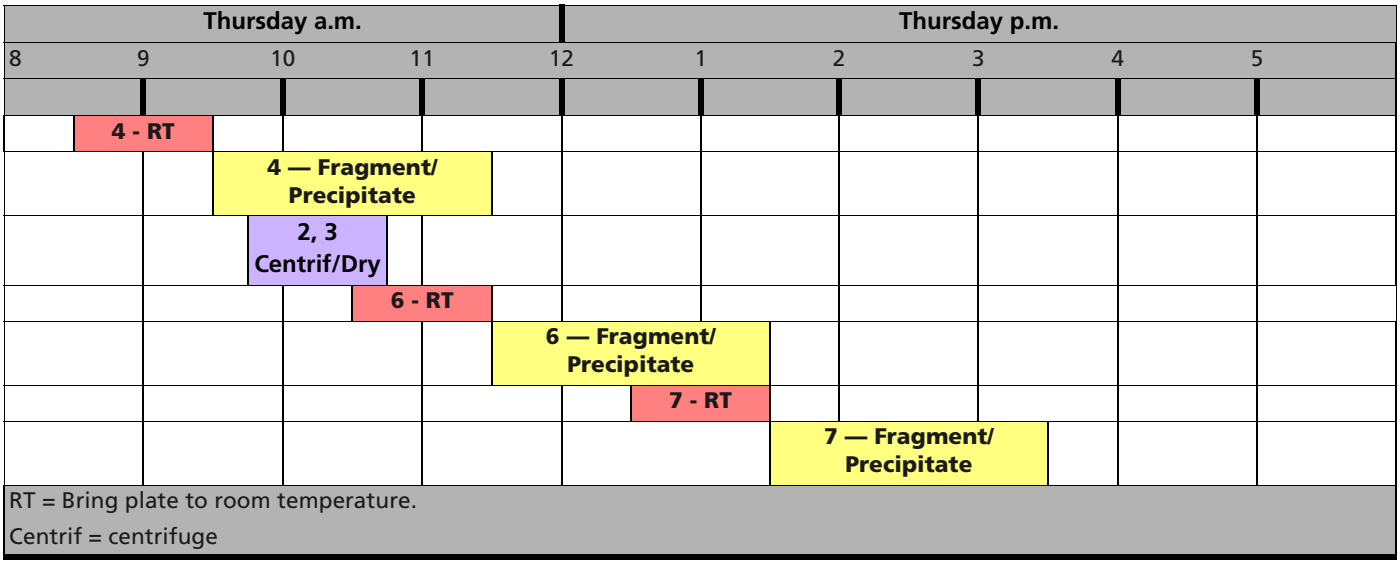


Table 6.12 Initial Target Prep Week — Day 4 Activities

Activity	Plate Number	Instrument	Approximate Start Times
Thaw	4	—	8:30 a.m.
Fragment and precipitate	4	Biomek FXᴾ Target Prep Express	9:30 a.m.
Centrifuge and dry	2, 3	Plate centrifuge and oven	9:45 a.m.
Thaw	6	—	10:30 a.m.
Fragment and precipitate	6	Biomek FXᴾ Target Prep Express	11:30 a.m.
Thaw	7	—	12:30 p.m.
Fragment and precipitate	7	Biomek FXᴾ Target Prep Express	1:30 p.m.

Initial Target Prep Week — Day 5

- Centrifuge and dry plates 4, 6 and 7.
- Resuspend and QC plates 2, 3, 4, 6 and 7.

IMPORTANT:

- **Plates 2 and 3 must be brought to room temperature for 90 min prior to resuspension.**
- **After being centrifuged and dried, plates 4, 6 and 7 are sealed. Place plates 6 and 7 in a 4 °C refrigerator until further processing later the same day. Plate 4 can be left on the bench top.**
- **Prior to resuspension and QC, plates 6 and 7 must be brought to room temperature (place on bench top for 30 min).**

Table 6.13 Initial Target Prep Week — Day 5 Activities

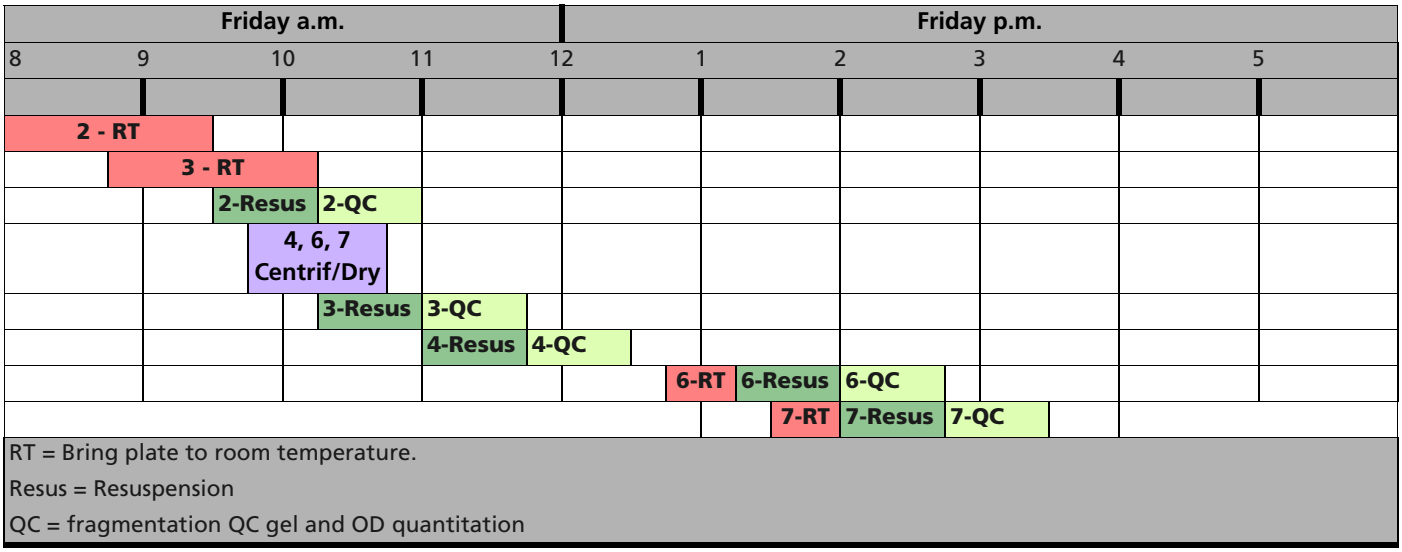


Table 6.14 Initial Target Prep Week — Day 5 Activities

Activity	Plate Number	Instrument	Approximate Start Times
Bring to room temperature	2	—	8:00 a.m.
Bring to room temperature	3	—	8:45 a.m.
Resuspension and QC	2	Biomek FX^P Target Prep Express	9:30 a.m.
Spin and dry	4, 6, 7	Plate centrifuge and oven	9:45 a.m.
Resuspension and QC	3	Biomek FX^P Target Prep Express	10:15 a.m.
Resuspension and QC	4		11:00 a.m.
Resuspension and QC	6		1:15 p.m.
Resuspension and QC	7		2:00 p.m.

Simultaneous Eight-Plate Workflow

The tables on the following pages provide a breakdown of the timing involved to simultaneously:

- **GeneTitan MC Instrument**: Process eight array plates per week with target prepared the previous week on the Biomek FX^P Target Prep Express.
- Biomek FX^P Target Prep Express: Prepare eight new plates of target for processing the following week on the GeneTitan MC Instrument.

! **IMPORTANT: Timing is critical.**

- **The Biomek FX^P Target Prep Express will be in operation for a large percentage of each work day.**
- **Plates for target preparation on the Biomek FX^P Target Prep Express are numbered 1 through 8.**
- **Plates with target that are now ready for denaturation and transfer to the GeneTitan MC Instrument for hybridization, fluidics processing, and imaging are lettered A through H.**
- **Plates of target from the previous week (1–8) can be processed on the GeneTitan MC Instrument in any order.**

Eight Plate Workflow — Day 1

Biomek FX^P Target Prep Express Activities

- Denature two plates of target prepared the previous week (plates A and B).
- Amplify eight new plates of genomic DNA (1 through 8)

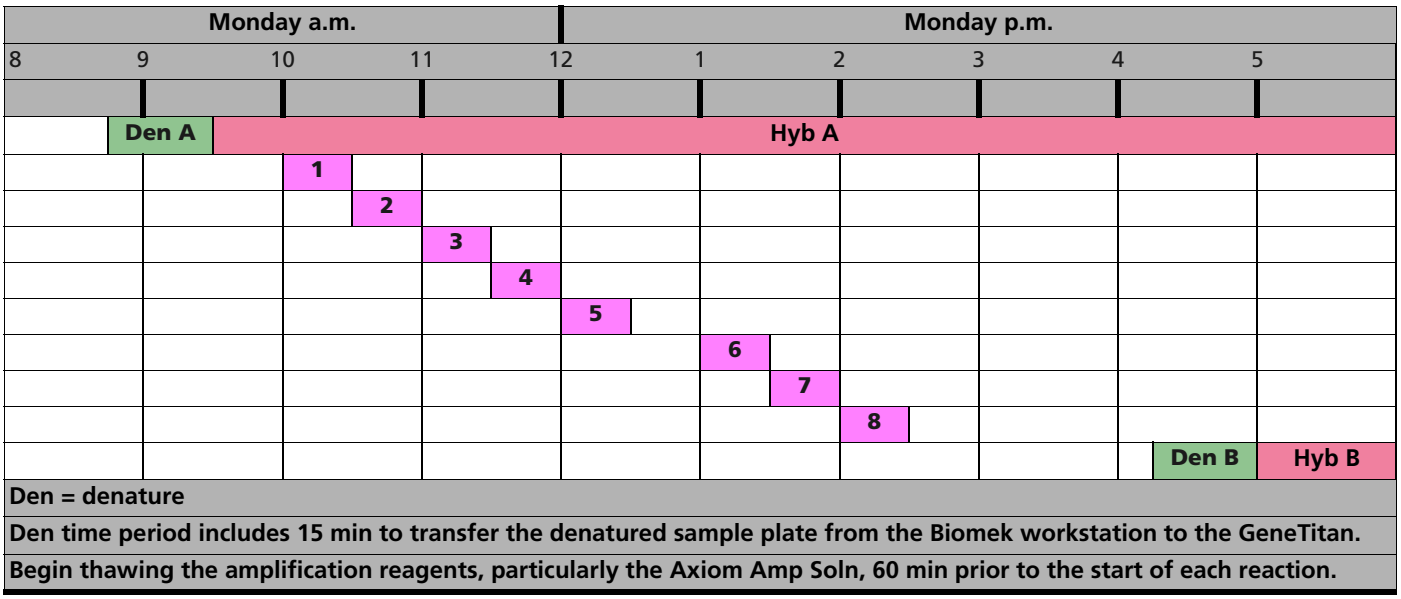
GeneTitan MC Instrument Activities

- Transfer denatured plates to the GeneTitan MC Instrument and begin hybridization (plates A and B).

! **IMPORTANT:**

- **Plates 1 through 8 are referred to as A through H when denatured and loaded onto the GeneTitan MC Instrument. Plates 1 through 8 can go onto the GeneTitan in any order.**
- **All amplifications are set up on Day 1 to maintain a 22 to 24 hr amplification incubation for each plate.**
- **Begin thawing the amplification reagents, particularly the Axiom Amp Soln, 60 min prior to the start of each reaction.**

Table 6.15 Eight Plate Workflow — Day 1 Activities



Den = denature

Den time period includes 15 min to transfer the denatured sample plate from the Biomek workstation to the GeneTitan.

Begin thawing the amplification reagents, particularly the Axiom Amp Soln, 60 min prior to the start of each reaction.

Table 6.16 Eight Plate Workflow — Day 1 Activities

Activity	Plate Number	Instrument	Approximate Start Times
Denature on the FX	A	Biomek FX^P Target Prep Express	8:45 a.m.
Load onto the GT and hybridize	A	GeneTitan MC Instrument	9:30 a.m.
DNA Amplification	1	Biomek FX^P Target Prep Express	10:00 a.m.
DNA Amplification	2		10:30 a.m.
DNA Amplification	3		11:00 a.m.
DNA Amplification	4		11:30 a.m.
DNA Amplification	5		12:00 p.m.
DNA Amplification	6		1:00 p.m.
DNA Amplification	7		1:30 p.m.
DNA Amplification	8		2:00 p.m.
Denature on the FX	B	Biomek FX^P Target Prep Express	4:15 p.m.
Load onto the GT and hybridize	B	GeneTitan MC Instrument	5:00 p.m.

Eight Plate Workflow — Day 2

Biomek FX^P Target Prep Express Activities

- Denature two plates of target from the previous week (plates C and D).
- Prepare reagent plates for the GeneTitan MC Instrument (for plates A and B already on the GeneTitan).
- Transfer the denatured samples and reagent plates to the GeneTitan MC Instrument.
- Fragment and precipitate plates 1, 5 and 8.
- Freeze amplified plates 2, 3, 4, 6, and 7 after each plate has incubated for 23 hr

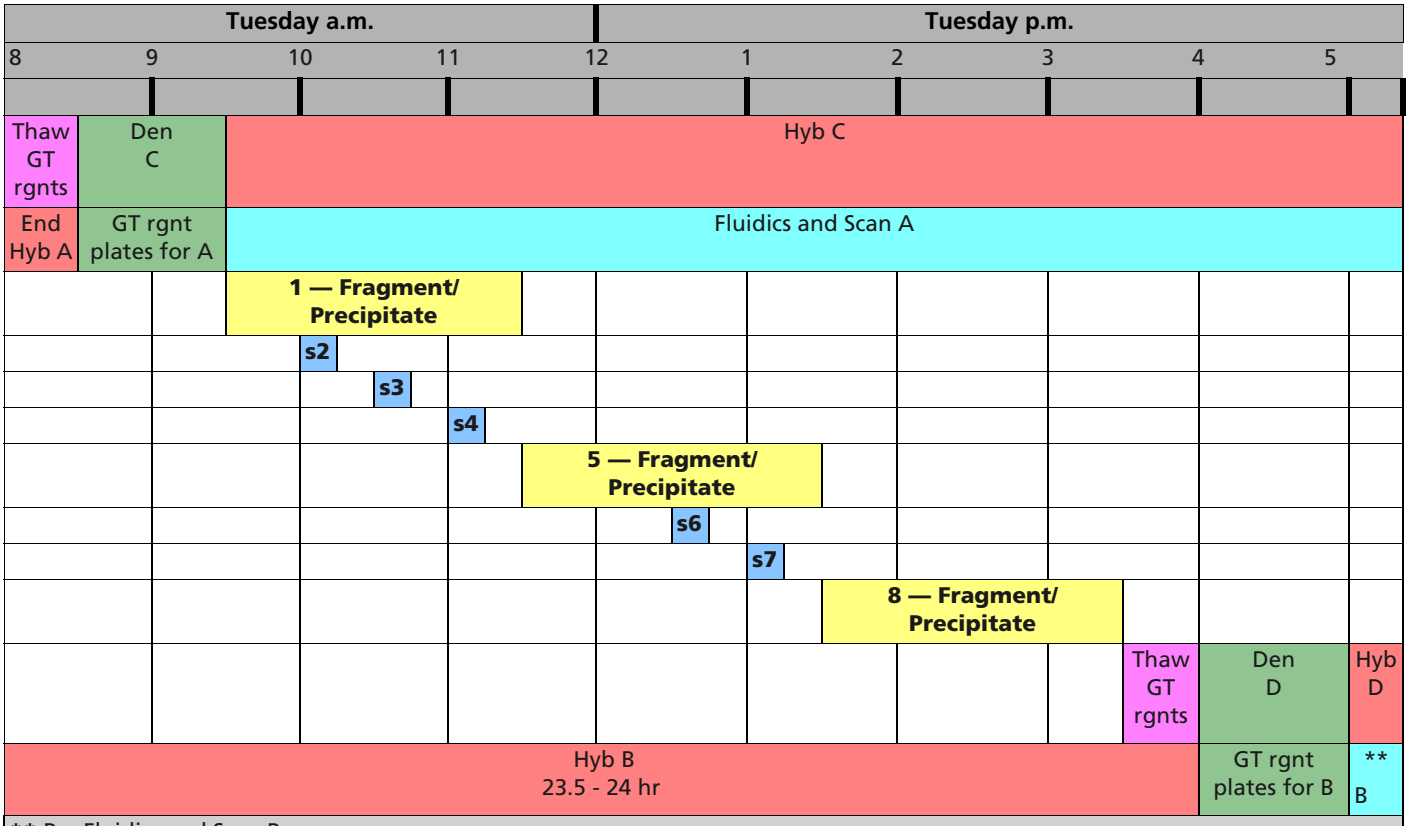
GeneTitan MC Instrument Activities

- Load reagent plates for A and B. These plates are moved from the hyb oven to the fluidics area. After fluidics, the plates move to the imaging area of the instrument. Load plates C and D for hybridization.

! **IMPORTANT:**

- **Plates 1, 5 and 8 are fragmented and precipitated without freezing to preserve a 23 hr amplification incubation.**
- **All plates not fragmented and precipitated on Day 2 should be stored at –20 °C following 23 hr of amplification reaction incubation.**
- **Precipitation is carried out at –20 °C overnight.**

Table 6.17 Eight Plate Workflow — Day 2 Activities



** B = Fluidics and Scan B

Den = denature

Denature and GT rgnt plate time periods include 15 min to transfer the denatured plate and reagent plates from the Biomek to the GeneTitan.

sX = Seal tightly and store at –20 °C.

GT rgnt plates = reagent plates for GeneTitan array plate processing.

Table 6.18 Eight Plate Workflow — Day 2 Activities

Activity	Plate Designation	Instrument	Approximate Start Times
Thaw reagent for GT rgnt plate preparation	A	—	8:00 a.m.
Concurrently: ▪ Denature C ▪ Prepare GT rgnt plates for A ▪ Transfer to GeneTitan	Denature C Reagents for A	Biomek FX^P Target Prep Express	8:30 a.m.
▪ Load GT rgnt plates and begin fluidics processing for A ▪ Load C and begin hybridization	A to fluidics C to hyb oven	GeneTitan MC Instrument	9:30 a.m.
Fragment and precipitate	1	Biomek FX^P Target Prep Express	9:30 a.m.
Freeze (–20 °C)	2	—	10:00 a.m.
Freeze (–20 °C)	3	—	10:30 a.m.
Freeze (–20 °C)	4	—	11:00 a.m.
Fragment and precipitate	5	Biomek FX^P Target Prep Express	11:30 a.m.
Freeze (–20 °C)	6	—	12:30 p.m.
Freeze (–20 °C)	7	—	1:00 p.m.
Fragment and precipitate	8	Biomek FX^P Target Prep Express	1:30 p.m.
Thaw reagent for GT rgnt plate preparation	B	—	3:30 p.m.
Concurrently: ▪ Denature D ▪ Prepare GT rgnt plates for B ▪ Transfer to GeneTitan	Denature D Reagents for B	Biomek FX^P Target Prep Express	4:00 p.m.
▪ Load GT rgnt plates and begin fluidics processing for B ▪ Load D and begin hybridization	B to fluidics D to hyb oven	GeneTitan MC Instrument	5:00 p.m.
Denature and GT rgnt plate preparation time periods include 15 min to transfer the denatured plate and reagent plates from the Biomek to the GeneTitan.			

Eight Plate Workflow — Day 3

Off-Deck Activities

- Thaw plates 2 and 3.
- QC — OD quantitation and fragmentation gel for plates 1, 5, 8.

Biomek FX^P Target Prep Express Activities

- Denature two plates of target from the previous week (plates E and F).
- Prepare reagent plates for the GeneTitan MC Instrument (for plates C and D already on the GeneTitan).
- Transfer the denatured samples and reagent plates to the GeneTitan MC Instrument.
- Fragment and precipitate plates 2 and 3.
- Centrifuge, dry, resuspend and QC plates 1, 5, 8.

GeneTitan MC Instrument Activities

- Load reagent plates for plates C and D. These plates are moved from the hyb oven to the fluidics area. After fluidics, the plates will move to the imaging area of the instrument. Also load plates E and F for hybridization.

Table 6.19 Eight Plate Workflow — Day 3 Activities

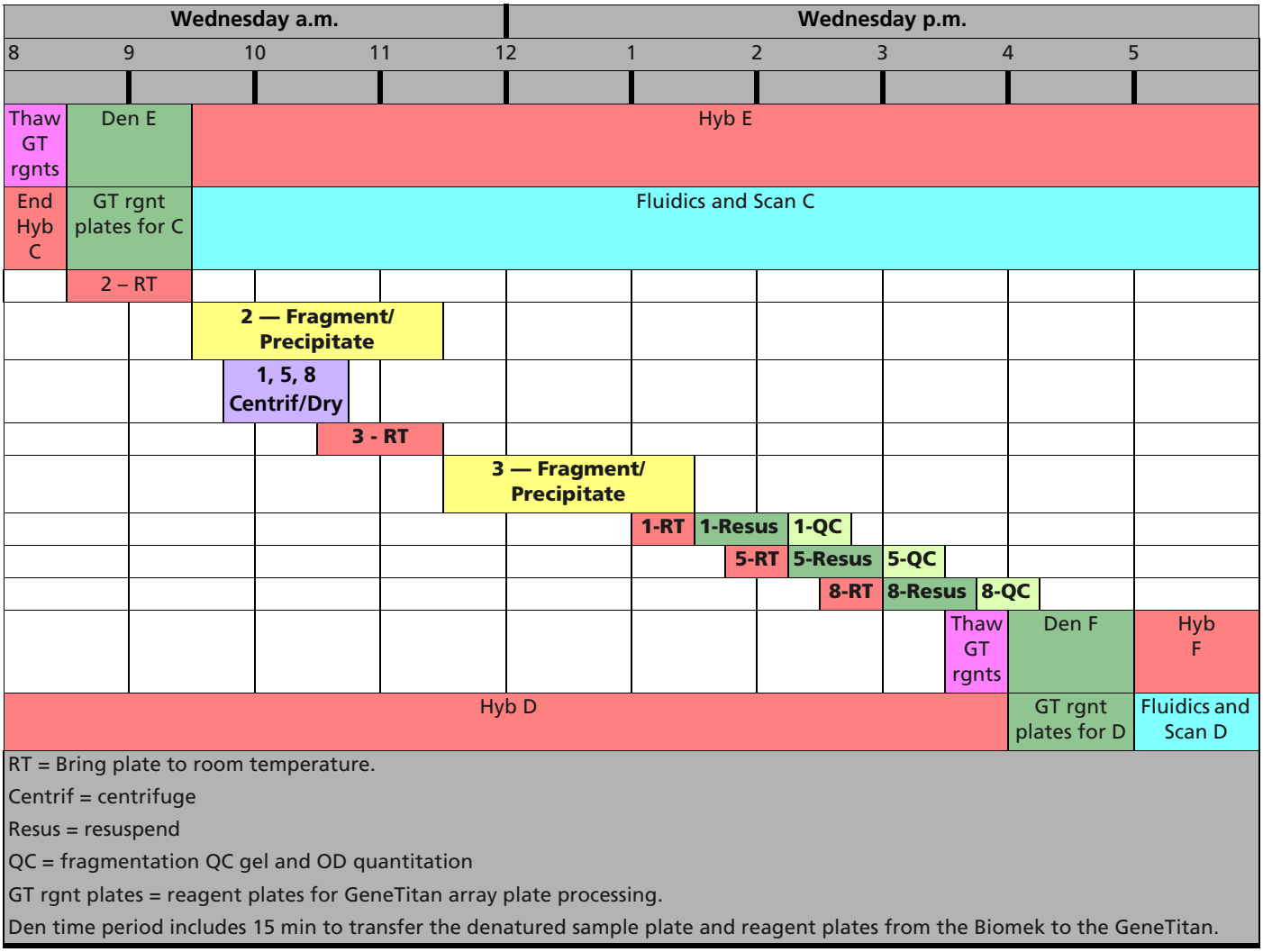


Table 6.20 Eight Plate Workflow — Day 3 Activities

Activity	Plate Number	Instrument	Approximate Start Times
Thaw reagents for GT rgnt plate preparation	C	—	8:00 a.m.
Bring plate to room temperature (RT)	2	—	8:30 a.m.
Concurrently: ▪ Denature E ▪ Prepare GT rgnt plates for C ▪ Transfer to GeneTitan	Denature E Reagents for C	Biomek FX^P Target Prep Express	8:30 a.m.
▪ Load GT rgnt plates and begin fluidics processing for C ▪ Load E and begin hybridization	C to fluidics E to hyb oven	GeneTitan MC Instrument	9:30 a.m.
Fragment and precipitate	2	Biomek FX^P Target Prep Express	9:30 a.m.
Spin and dry	1, 5, 8	Plate centrifuge and oven	9:45 a.m.
Bring plate to room temperature (RT)	3	—	10:30 a.m.
Fragment and precipitate	3	Biomek FX^P Target Prep Express	11:30 a.m.
Resuspension	1	Biomek FX^P Target Prep Express,	1:30 p.m.
Off-deck QC	1	Plate spectrophotometer, e-gel system	2:15 p.m.
Resuspension	5	Biomek FX^P Target Prep Express	2:15 p.m.
Off-deck QC	5	Plate spectrophotometer, e-gel system	3:00 p.m.
Resuspension	8	Biomek FX^P Target Prep Express	3:00 p.m.
Thaw reagents for GT rgnt plate preparation	F	—	3:30 p.m.
Off-deck QC	8	Plate spectrophotometer, e-gel system	3:45 p.m.
Concurrently: ▪ Denature F ▪ Prepare GT rgnt plates for D ▪ Transfer to GeneTitan	Denature F Reagents for D	Biomek FX^P Target Prep Express	4:00 p.m.
▪ Load GT rgnt plates and begin fluidics processing for D ▪ Load F and begin hybridization	D to fluidics F to hyb oven	GeneTitan MC Instrument	5:00 p.m.

Eight Plate Workflow — Day 4

Off-Deck Activities

Bring plates 4, 6 and 7 to room temperature.

Biomek FX^P Target Prep Express Activities

- Denature two plates of target from the previous week (plates G and H).
- Prepare reagent plates for the GeneTitan MC Instrument (for plates E and F already on the GeneTitan).
- Transfer the denatured samples and reagent plates to the GeneTitan MC Instrument.
- Fragment and precipitate plates 4, 6, 7.
- Centrifuge and dry plates 2 and 3.

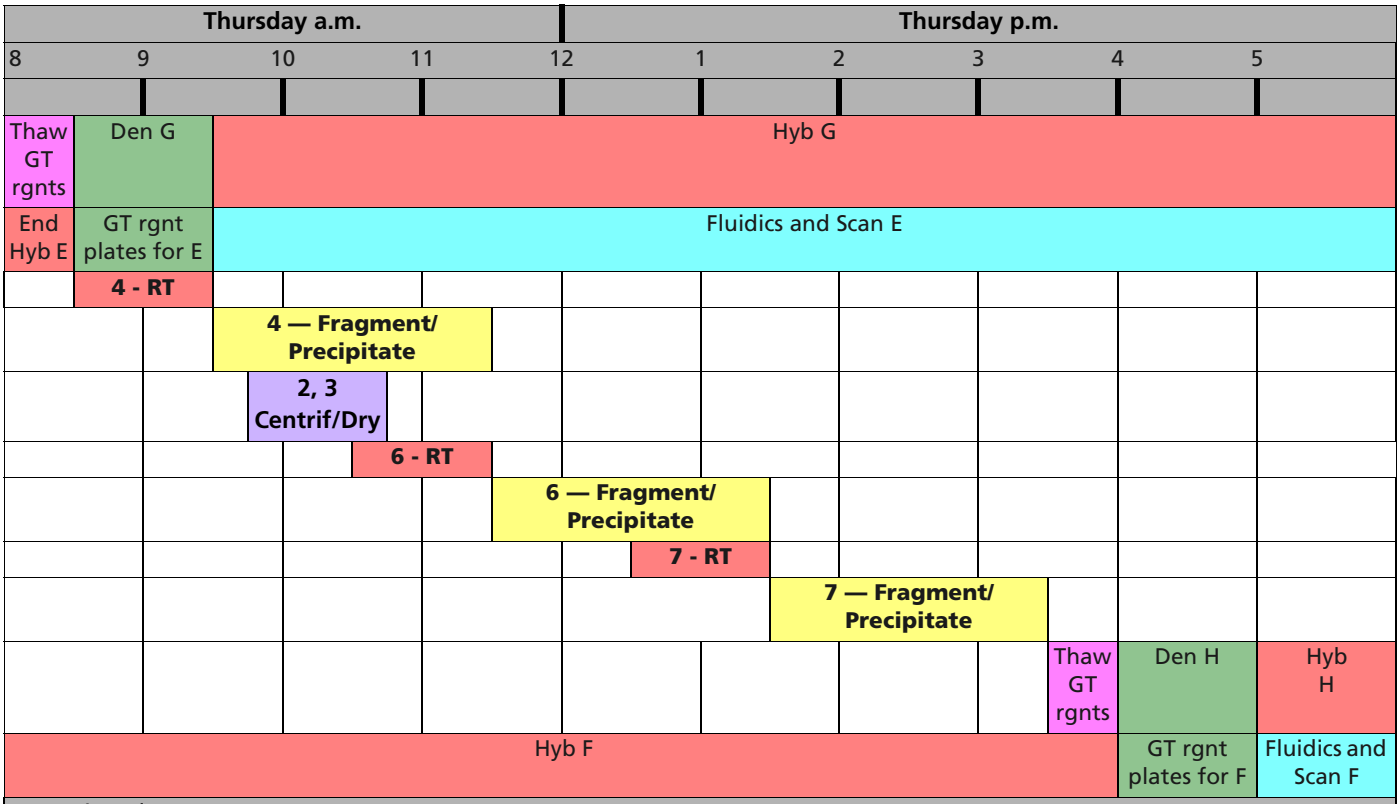
GeneTitan MC Instrument Activities

- Load reagent plates for E and F. These plates are moved from the hyb oven to the fluidics area. After fluidics, the plates move to the imaging area of the instrument. Load plates G and H for hybridization.

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 185*.**
- **Precipitation is carried out at –20 °C overnight.**
- **After being centrifuged and dried, plates 2 and 3 are sealed and stored at –20 °C.**

Table 6.21 Eight Plate Workflow— Day 4 Activities



RT = Bring plate to room temperature.

Centrif = centrifuge

Denature and GT rgnt plate preparation time periods include 15 min to transfer the denatured plate and reagent plates from the Biomek to the GeneTitan.

Table 6.22 Eight Plate Workflow— Day 4 Activities

Activity	Plate Designation	Instrument	Approximate Start Times
Thaw reagents for GT rgnt plate preparation	E	—	8:00 a.m.
Bring plate to room temperature (RT)	4	—	8:30 a.m.
Concurrently: ▪ Denature G ▪ Prepare GT rgnt plates for E ▪ Transfer to GeneTitan	Denature D Reagent for E	Biomek FX^P Target Prep Express	8:30 a.m.
▪ Load GT rgnt plates and begin fluidics processing for E ▪ Load G and begin hybridization	E to fluidics G to hyb oven	GeneTitan MC Instrument	9:30 a.m.
Fragment and precipitate	4	Biomek FX^P Target Prep Express	9:30 a.m.
Spin and dry	2, 3	Plate centrifuge and oven	9:45 a.m.
Bring plate to room temperature (RT)	6	—	10:30 a.m.
Fragment and precipitate	6	Biomek FX^P Target Prep Express	11:30 a.m.
Bring plate to room temperature (RT)	7	—	12:30 a.m.
Fragment and precipitate	7	Biomek FX^P Target Prep Express	1:30 a.m.
Thaw reagents for GT rgnt plate preparation	F	—	3:30 p.m.
Concurrently: ▪ Denature H ▪ Prepare GT rgnt plates for F ▪ Transfer to GeneTitan	Denature H Reagents for F	Biomek FX^P Target Prep Express	4:00 p.m.
▪ Load GT rgnt plates and begin fluidics processing for F ▪ Load H and begin hybridization	F to fluidics H to hyb oven	GeneTitan MC Instrument	5:00 p.m.

Eight Plate Workflow — Day 5

Off-Deck Activities

Bring plates 2 and 3 to room temperature.

Biomek FX^P Target Prep Express Activities

- Centrifuge and dry plates 4, 6, 7.
- Resuspend and QC plates 2, 3, 4, 6, 7.

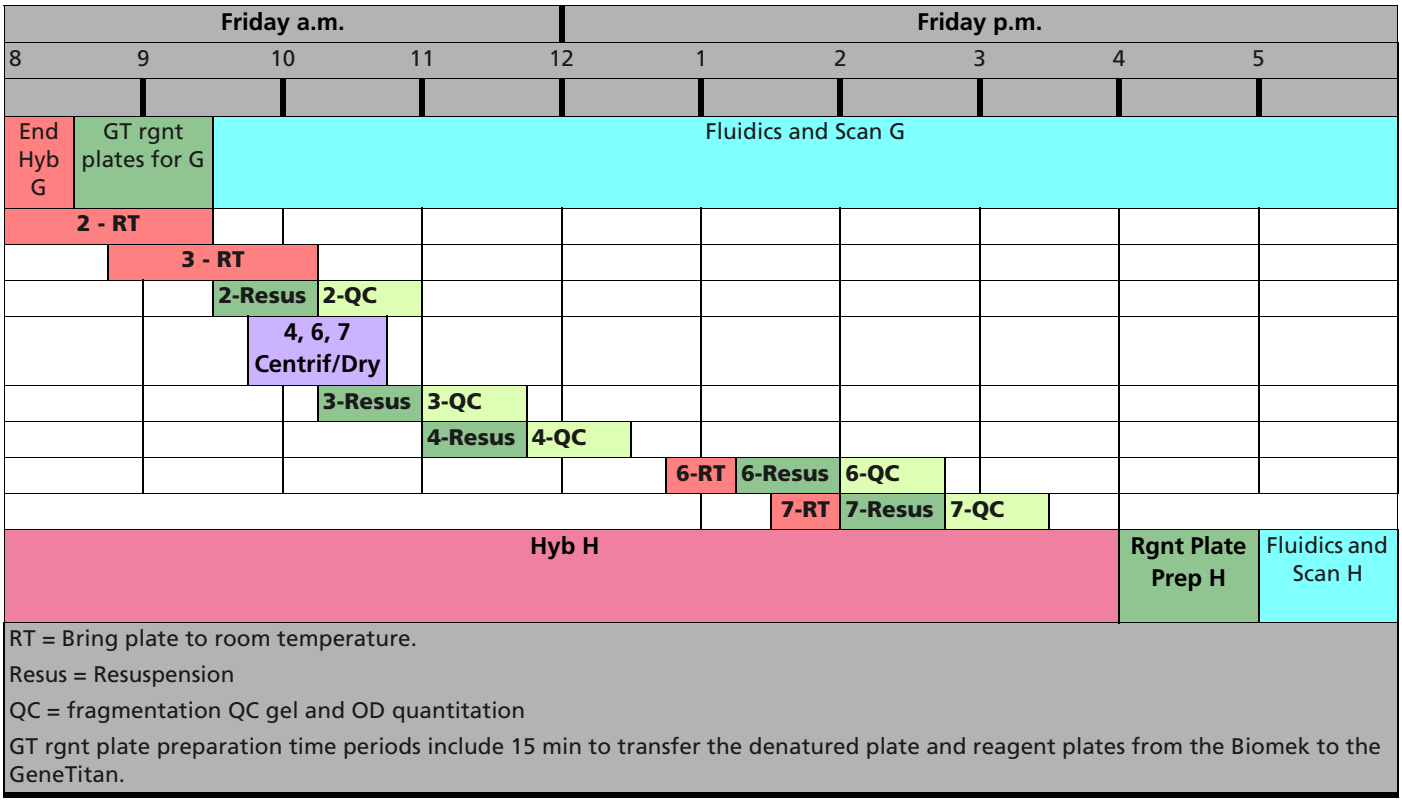
GeneTitan MC Instrument Activities

Load reagent plates for plates E and F. These plates are moved from the hyb oven to the fluidics area. After fluidics, the plates will move to the imaging area of the instrument. Also load plates G and H for hybridization.

IMPORTANT:

- **Plates 2 and 3 must be brought to room temperature for 90 min prior to resuspension.**
- **After being centrifuged and dried, plates 4, 6 and 7 are sealed. Place plates 6 and 7 in a 4 °C refrigerator until further processing later the same day. Plate 4 can be left on the bench top.**
- **Prior to resuspension and QC, plates 6 and 7 must be brought to room temperature (place on bench top for 30 min).**

Table 6.23 Eight Plate Workflow — Day 5 Activities



RT = Bring plate to room temperature.

Resus = Resuspension

QC = fragmentation QC gel and OD quantitation

GT rgnt plate preparation time periods include 15 min to transfer the denatured plate and reagent plates from the Biomek to the GeneTitan.

Table 6.24 Eight Plate Workflow — Day 5 Activities

Activity	Plate Number	Instrument	Approximate Start Times
Bring to room temperature	2	—	8:00 a.m.
Prepare GeneTitan reagent plates	G	Biomek FX^P Target Prep Express	8:30 a.m.
Load reagent plates and begin fluidics processing for G	G	GeneTitan MC Instrument	9:30 a.m.
Bring to room temperature	3	—	8:45 a.m.
Resuspension and QC	2	Biomek FX^P Target Prep Express	9:30 a.m.
Spin and dry	4, 6, 7	Plate centrifuge and oven	9:45 a.m.
Resuspension and QC	3	Biomek FX^P Target Prep Express	10:15 a.m.
Resuspension and QC	4		11:00 a.m.
Resuspension and QC	6		1:15 p.m.
Resuspension and QC	7		2:00 p.m.
Prepare GeneTitan reagent plates	H	Biomek FX^P Target Prep Express	4:00 p.m.
Load reagent plates and begin fluidics processing for H	H	GeneTitan MC Instrument	5:00 p.m.

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Automated Target Preparation for Processing Two Axiom Array Plates per Week

The two array plate/week automated target prep workflow enables you to do the target preparation and array processing for the Axiom Genome -Wide 96 Array in the same week.

This workflow is performed using one Biomek FX^P Target Prep Express (Biomek workstation) (see Chapter 3, *Axiom™ Genotyping Assay: Target Preparation with Biomek FXP Target Prep Instrument on page 15*) and one GeneTitan MC Instrument (see Chapter 5, *Axiom™ Genotyping Assay: Array Processing with the GeneTitan® MC Instrument on page 149*). This chapter includes tables that present the timing of the steps required to perform this workflow per 5-day work week, eight hours per day.

IMPORTANT: **Experienced users and careful timing are critical for the successful execution of this workflow.**

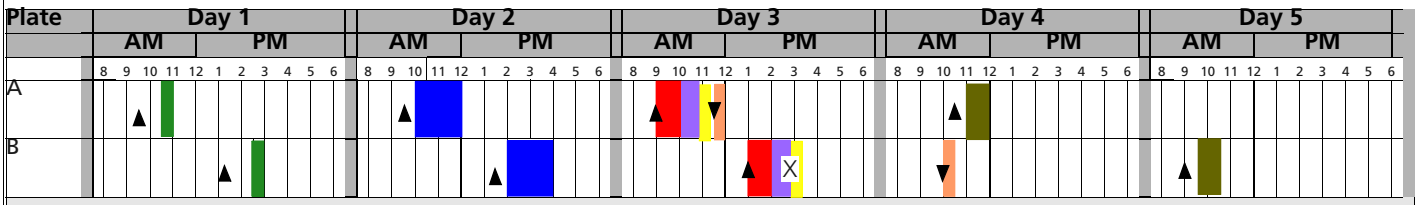
The two plate per week automated workflow is described in the following sections:

- *Overview of the 2-plate Workflow for Automated Target Preparation*
- *Thawing Frozen Plates of Amplified DNA* *on page 204*
- *Target Prep and Array Processing* *on page 204*

Overview of the 2-plate Workflow for Automated Target Preparation

The table below displays the timing and duration of the hands-on processing necessary for performing the two-plate workflow by one person.

Figure 7.1 Two plate per week automated target prep workflow



Notes

▲ Begin Thawing required reagents for the process.

▼ Begin warming Axiom array plate to room temperature

x Day 3 2:45 PM: Store Hyb Ready Plate B at –20 °C

Color Code

- Amplification
- Fragmentation and Precipitation
- Centrifugation and Drying
- Resuspension and Hybridization Mix Prep
- QC
- Sample Denature/load array plate and Hyb Plate in GeneTitan MC
- GeneTitan MC reagent plates prep and loading

The two plates are referred to as plates A and B in the target prep and in the GeneTitan Array Processing.

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

Table 7.1 Daily steps for Automated Target Prep workflow for two plates/week

Day	Activities
1	▪ Amplify 2 plates of genomic DNA.
2	▪ Fragment and precipitate two plates amplified on Day 1.
3	▪ Centrifuge, dry, resuspend and QC Plate A. ▪ Denature Plate A and load samples and Array Plate into GeneTitan MC ▪ Centrifuge, dry, resuspend and QC Plate B. ▪ Store Plate B at –20 °C
4	▪ Denature Plate B and load samples and Array Plate into GeneTitan MC ▪ Prepare GeneTitan Reagent plates for Plate A and load into GeneTitan MC
5	▪ Prepare GeneTitan Reagent plates for Plate B and load into GeneTitan MC

Table 7.2 Time required for target preparation on the Biomek FX^P Target Prep Express

Steps on the Biomek FX^P Target Prep Express	Time Required
Amplification	30 min
Fragmentation	2 hr
Resuspension	45 min
Off-deck centrifugation and drying	75 min
Off-deck QC gel and OD	45 min
Denaturation only	30 min
Transfer denatured samples to GeneTitan MC	15 min
Prepare and Load reagent plates to the GeneTitan MC	60 min
Time Required includes deck setup.	

Thawing Frozen Plates of Amplified DNA

The automated two plate workflow described in this chapter does not require freezing the plates of DNA following amplification. If plates of amplified DNA are frozen because of an interruption of this workflow, you should thaw them prior to the fragmentation step on Day 2 as described in section *2. Thaw and Prepare the Reagents and Sample Plate* *on page 45*.

Target Prep and Array Processing

The tables in the sections below show the steps that need to be performed on each day of the workflow.

Two Plate/Week Workflow - Day 1

NOTE: Genomic DNA sample for amplification should be prepared as described in *Genomic DNA Preparation and Requirements* *on page 9*. Affymetrix recommends that you carry out genomic DNA preparation in a dedicated pre-amplification room, or area, with dedicated consumables such as pipettes, tips, vortexes, etc.

IMPORTANT: **Amplification preparation should take place in an Amplification Staging Room or dedicated area such as biosafety hood with dedicated pipettes, tips, vortex, etc.**

Figure 7.2 Two Plate/Week Automated Target Prep Workflow — Day 1 Activities

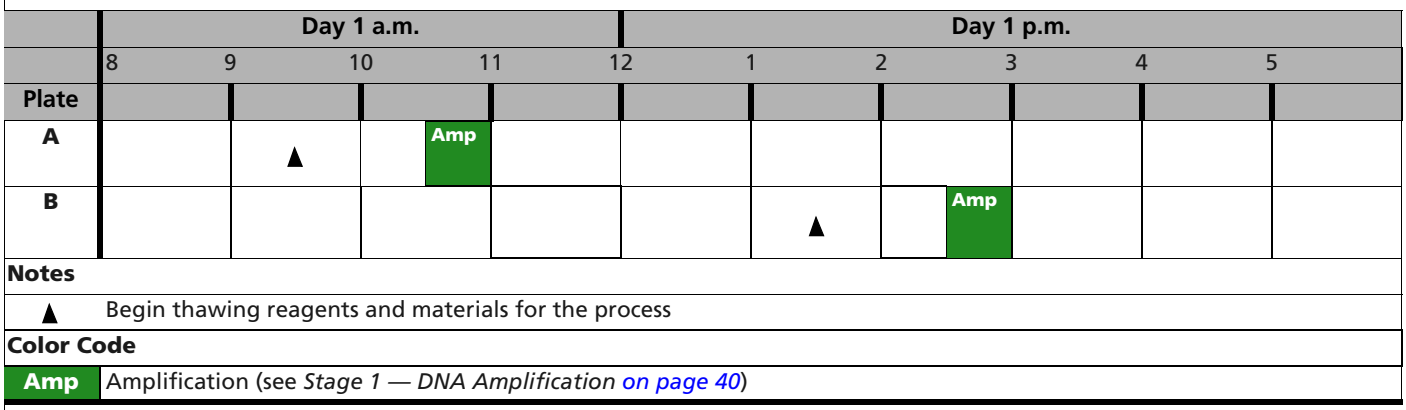


Table 7.3 Two plate/week Auto Target Prep Workflow--Day 1

Time	Topic
9:30	Prepare DNA amplification reagents for Plate A
10:30	Start DNA amplification Plate A
1:30	Prepare DNA amplification reagents for Plate B
2:30	Start DNA amplification Plate B

See *Stage 1 — DNA Amplification* on page 40 for more information on the protocol.

Two Plate/Week Workflow — Day 2

Figure 7.3 Two Plate/Week Workflow — Day 2 Activities

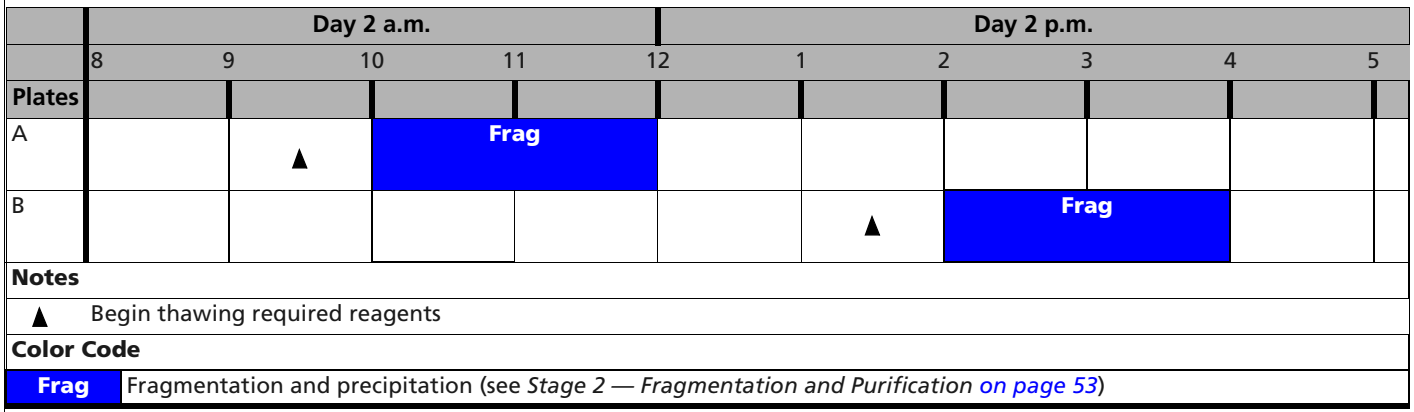
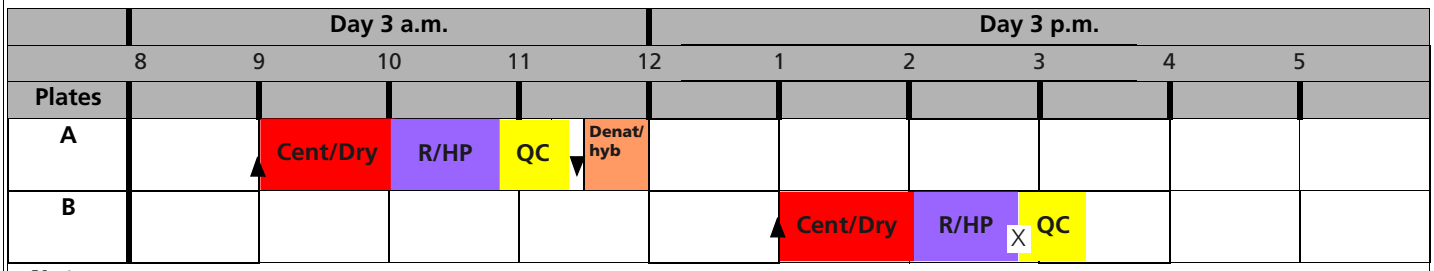


Table 7.4 Two plate/week Auto Target Prep Workflow--Day 2

Time	Topic
9:30	Prepare Frag reagents for Plate A
10:00	Start Fragmentation Plate A
1:30	Prepare Frag reagents for Plate B
2:00	Start Fragmentation Plate B

Two Plate/Week Workflow — Day 3

Figure 7.4 Two Plate/Week Automated Target Prep Workflow — Day 3 Activities



Notes:	
▲	Begin thawing required reagents
▼	Begin warming Axiom array plate to room temperature
X	Store Hyb Ready Plate B at –20 °C
Color Codes	
Cent/Dry	Centrifugation and Drying (see *5. Centrifuge and Dry Pellets on page 61*)
R/HP	Resuspension and Hyb Mix Prep (see *Stage 3 — Resuspension and Hybridization Preparation on page 62*)
QC	Quality control checks
Denat/hyb	Sample Denature/load array plate and Hyb Plate in GeneTitan MC (see *Stage 4 — Preparation for the GeneTitan MC Instrument on page 72*)

Table 7.5 Two plate/week Automated Target Prep Workflow--Day 3

Time	Topic
9:00	Start Centrifugation of Plate A
9:00	Prepare Hyb reagents for Plate A
9:40	Start Drying of Plate A
10:05	Start Resuspension and Hybridization Preparation Plate A
10:50	Run QC
11:30	Start Denature Sample for Plate A
12:00	Start Hyb-Wash-Scan run Plate A

Table 7.5 Two plate/week Automated Target Prep Workflow--Day 3

Time	Topic
1:00	Start centrifugation of Plate B
1:00	Prepare Hyb reagents for Plate B
1:40	Start Drying of Plate B
2:05	Start Resuspension and Hybridization Preparation Plate B
2:50	Run QC and Put Plate B in Freezer (-20C)

Two Plate/Week Workflow — Day 4

IMPORTANT: The GeneTitan reagent plates 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 plates cannot be prepared ahead of time and stored.

Figure 7.5 Two plate/week Automated Target Prep Workflow--Day 4

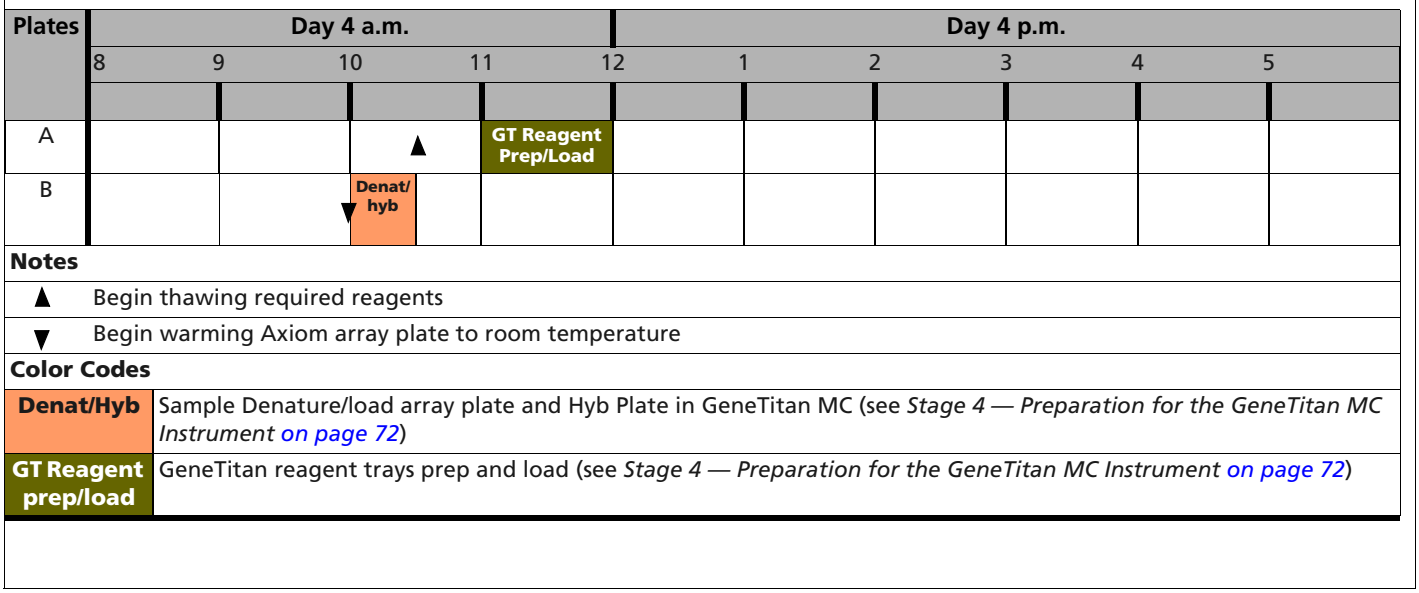


Table 7.6 Two plate/week Auto Target Prep Workflow--Day 4

Time	Topic
10:00	Start Denature Sample for Plate B (See *Stage 4 — Preparation for the GeneTitan MC Instrument on page 72*)
10:30	Start Hyb-Wash-Scan run for Plate B
10:30	Prepare Wash-Stain reagents for Plate A
11:00	Prepare GeneTitan reagent trays for Plate A (See *Stage 4 — Preparation for the GeneTitan MC Instrument on page 72*)
12:00	Load GeneTitan reagent trays for Plate A

Two Plate/Week Workflow — Day 5

IMPORTANT: **The GeneTitan reagent plates 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 plates cannot be prepared ahead of time and stored.**

Figure 7.6 Two plate/week Automated Target Prep Workflow--Day 5

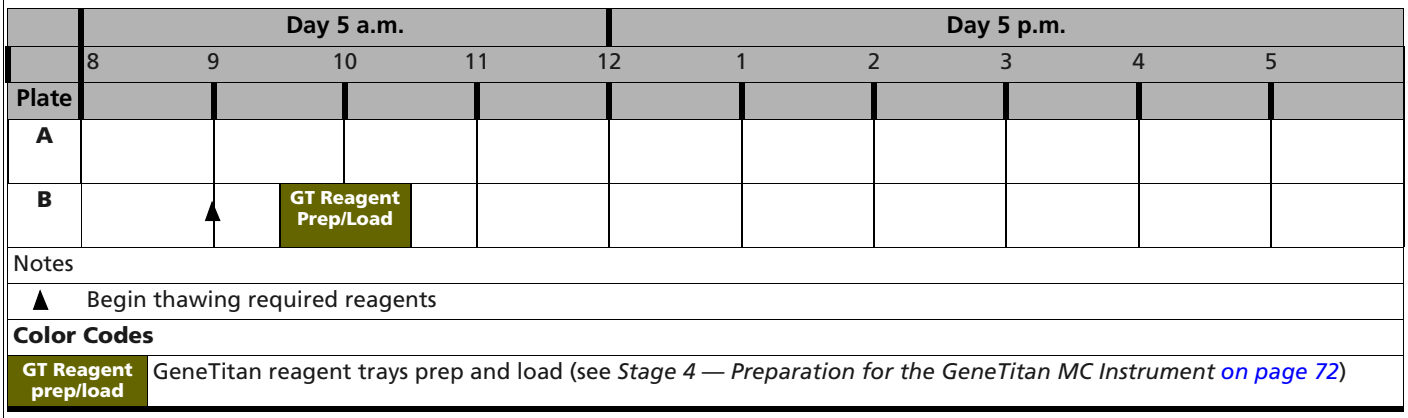


Table 7.7 Two plate/week Auto Target Prep Workflow--Day 5

Time	Topic
9:00	Prepare Wash-Stain reagents for Plate B
9:30	Prepare GeneTitan reagent trays for Plate B (See *Stage 4 — Preparation for the GeneTitan MC Instrument* on page 72)
10:30	Load GeneTitan reagent trays for Plate B

Manual Target Preparation for Processing Three Axiom Array Plates per Week

When using the manual target prep protocol, one person can process up to three Axiom Genome-Wide 96-Array Plates in one forty-hour work week.

This chapter describes the timing of the steps for each sample and array plate that are required to perform this workflow.

IMPORTANT: Experienced users and careful timing are critical for the successful execution of this workflow.

The three plate per week workflow is described in the following sections:

- *Overview of the 3-plate Workflow for Manual Target Preparation*
- *Thawing Frozen Plates of Amplified DNA* *on page 212*
- *Manual Target Prep and Array Processing* *on page 212*

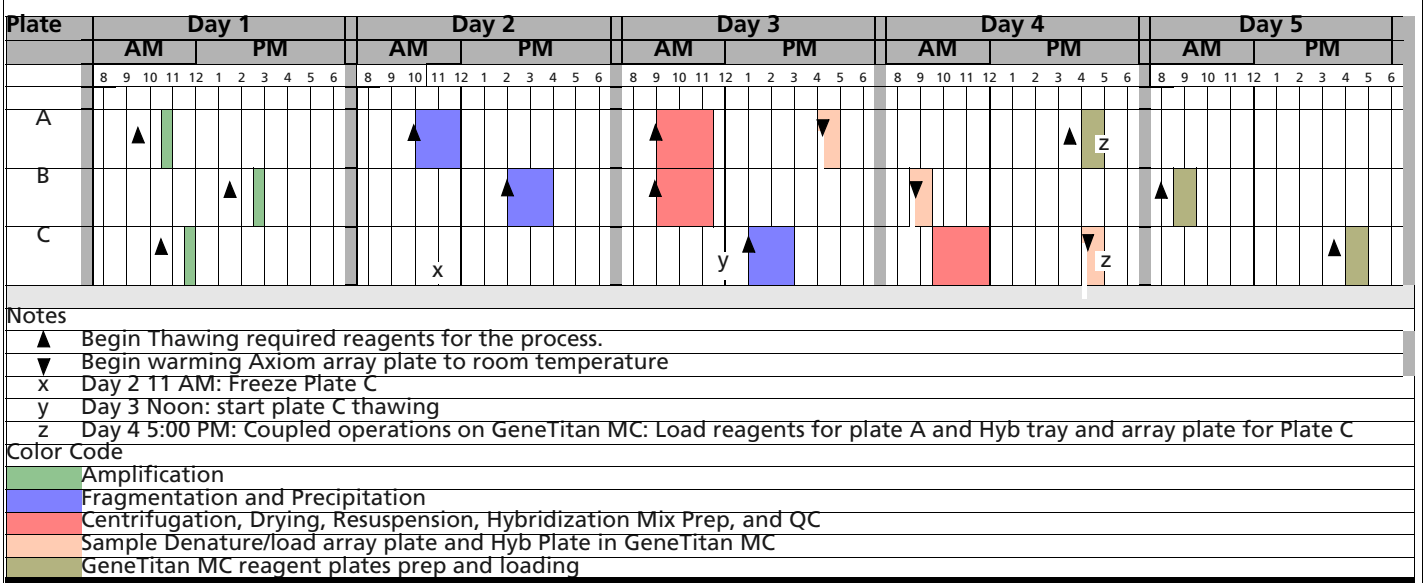
Detailed instructions for the manual target prep protocol and the array plate processing are given in:

- Chapter 4, *Axiom Genotyping Assay: Manual Target Preparation* *on page 95*
- Chapter 5, *Axiom™ Genotyping Assay: Array Processing with the GeneTitan® MC Instrument* *on page 149*

Overview of the 3-plate Workflow for Manual Target Preparation

The table below displays the timing and duration of the hands-on processing necessary for performing the three plate workflow by one person.

Figure 8.1 Three plate per week manual target prep workflow



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 8.1 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 GeneTitan	▪ C ▪ A, B ▪ A
4	▪ Centrifuge, dry, resuspend and QC plates precipitated on Day 3 ▪ Denature and begin Hybridization for two plates on GeneTitan ▪ GeneTitan reagent plates preparation and loading	▪ C ▪ B, C ▪ A
5	▪ GeneTitan reagent plates 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 GeneTitan MC.

These constraints are described in more detail in:

- *Timing Issues for Manual Target Preparation* *on page 210*
- *Timing Issues for GeneTitan MC Array Processing* *on page 211*

Timing Issues for Manual Target Preparation

The GeneTitan reagent plates 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 plates cannot be prepared ahead of time and stored.

Table 8.2 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
Centrifugation/Drying/Resuspension	1 hr 20 min	2 hr	N/A
QC gel and OD	30 min	30 min	N/A
Denaturation and hyb tray/array plate loading on GeneTitan	25 min	45	23.5-24 hr hybridization
GeneTitan reagent plate preparation and loading on GeneTitan	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 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 assay on the GeneTitan MC Instrument is 23.5 to 24 hr (Table 8.3). 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 8.3 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 plates	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, you will need three ovens. Table 8.4 lists the different temperatures required for each step. Table 8.5 provides a list of suggested settings for three ovens when performing the three plate/week workflow.

Table 8.4 Oven temperatures needed for each step of the workflow

Workflow step	Oven Temp
Amplification	30 °C
Stopping Amplification	65 °C
Pre-Fragmentation Incubation	37 °C
Fragmentation Incubation	37 °C
Drying	37 °C
Hybridization	48 °C

Table 8.5 Suggested settings for ovens when performing three plate/week manual target prep workflow

Day of Workflow	Oven 1	Oven 2	Oven 3
Day 1	30 °C	N/A	N/A
Day 2	30 °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

Thawing Frozen Plates of Amplified DNA

To thaw frozen plates of amplified DNA:

1. Place the deep well plate in a small water bath.
   For example, pour Millipore water into a small tray. Place the frozen plate in the water in the tray.
2. Leave the plate in the water bath for ~ 50 min until all wells have thawed.
3. Spin down at 1000 rpm for 30 sec.
4. To avoid cross-contamination of wells during vortexing:
   A. Remove the seal and blot the top of the plate with a Kimwipe.
   B. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 sec to thoroughly mix.
6. Spin at 1000 rpm for 30 sec.

Manual Target Prep and Array Processing

Manual Target Prep Workflow — Day 1

On this day you start amplification of the three plates: each plate must incubate 23±1 hours after amplification begins.

All amplifications should be set up on Day 1 to allow for a 23±1 hr amplification incubation for each plate and to minimize movement between pre-amplification and post-amplification areas.

Begin thawing the amplification reagents, particularly the Axiom Amp Soln, 60 min prior to the start of each reaction.

IMPORTANT: Amplification preparation should take place in an Amplification Staging Room or dedicated area such as biosafety hood with dedicated pipettes, tips, vortex, etc. See *Amplification Staging Room* on page 96 for more information.

Figure 8.2 Manual Target Preparation Workflow — Day 1 Activities

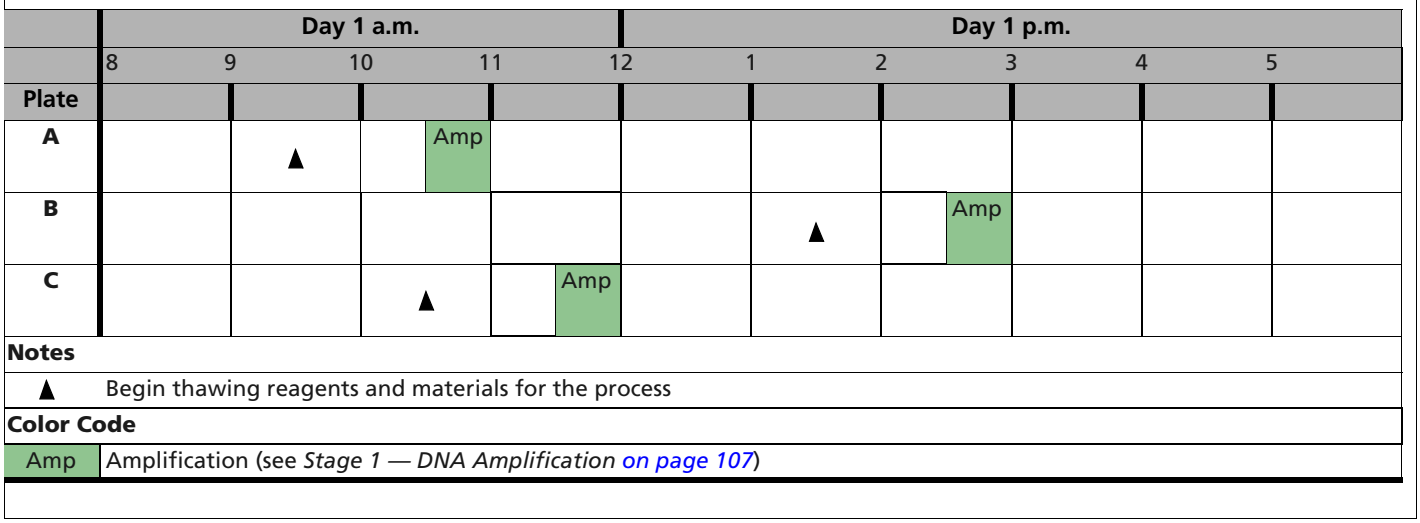


Table 8.6 Manual Target Prep Workflow — Day 1 Activities

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 107 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 immediately after the end of the Amplification reaction without stopping the Amplification reaction, at –20 °C following 23 hr of amplification reaction incubation.

Figure 8.3 Manual Target Preparation Workflow — Day 2 Activities

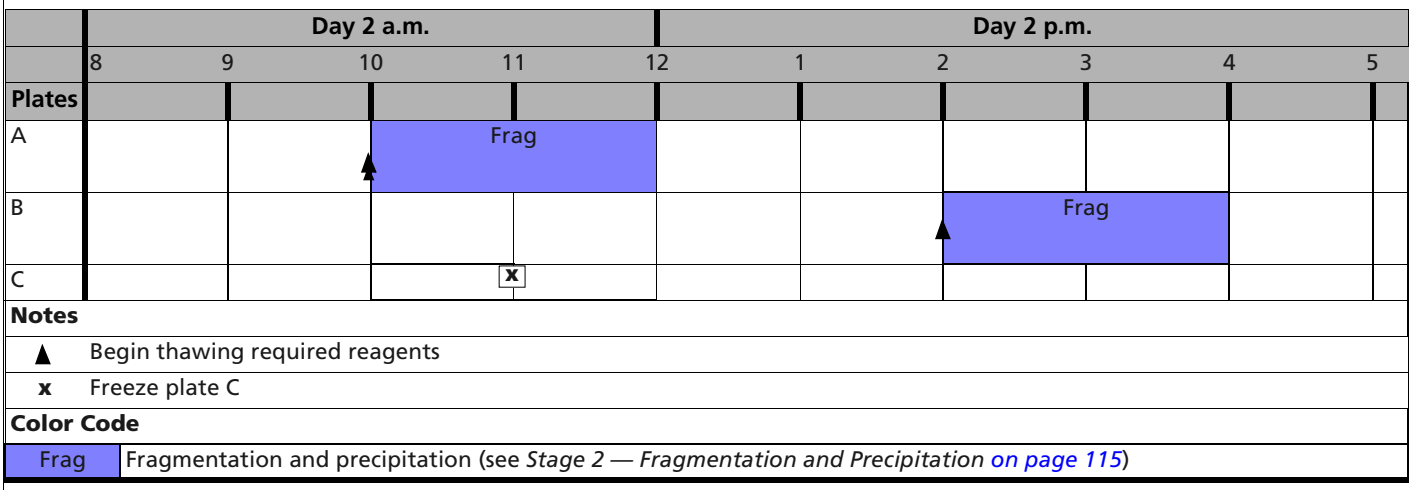


Table 8.7 Manual Target Prep Workflow — Day 2 Activities

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.

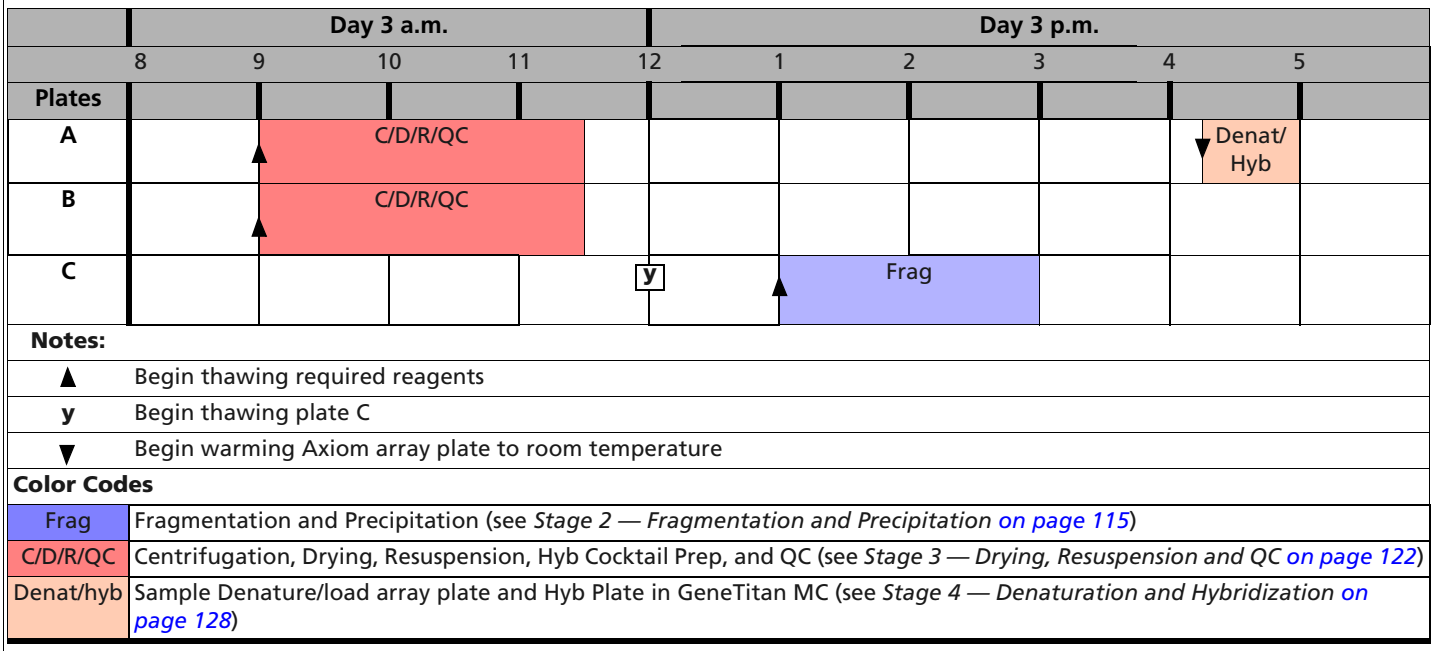
Manual Target Prep Workflow — Day 3

- Centrifuge, dry, resuspend and QC plates A and B.
- Thaw plate C (see *Thawing Frozen Plates of Amplified DNA* *on page 212*).
- Fragment 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 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 212*.**

Figure 8.4 Manual Target Preparation Workflow — Day 3 Activities



Notes:	
▲	Begin thawing required reagents
y	Begin thawing plate C
▼	Begin warming Axiom array plate to room temperature

Color Codes	
Frag	Fragmentation and Precipitation (see *Stage 2 — Fragmentation and Precipitation* *on page 115*)
C/D/R/QC	Centrifugation, Drying, Resuspension, Hyb Cocktail Prep, and QC (see *Stage 3 — Drying, Resuspension and QC* *on page 122*)
Denat/hyb	Sample Denature/load array plate and Hyb Plate in GeneTitan MC (see *Stage 4 — Denaturation and Hybridization* *on page 128*)

Table 8.8 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.

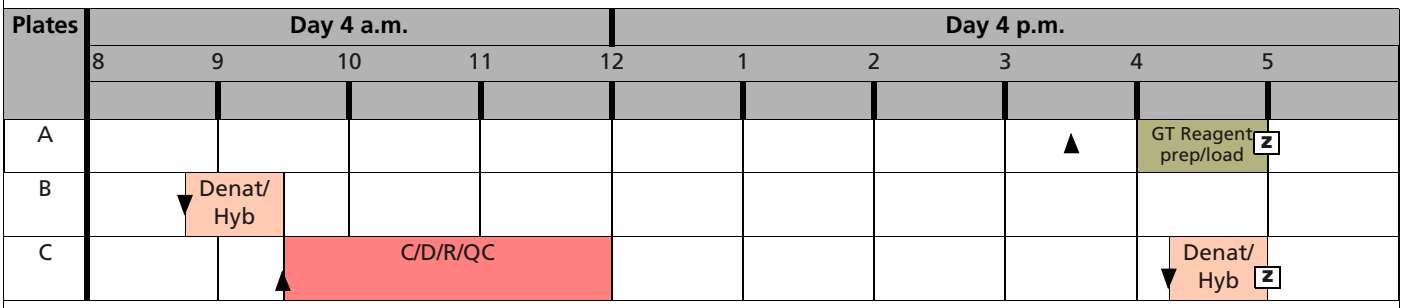
Manual Target Prep Workflow — Day 4

- Denaturation of Samples/Load array plate and Hyb Plate in GeneTitan MC for plates B and C
- Centrifuge, dry, resuspend, and QC plate C
- GeneTitan reagent plates prep and loading for plate A

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

IMPORTANT: The GeneTitan reagent plates 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 plates cannot be prepared ahead of time and stored.

Figure 8.5 Manual Target Preparation Workflow — Day 4 Activities



Notes	
▲	Begin thawing required reagents
▼	Begin warming Axiom array plate to room temperature
z	Coupled operations on GeneTitan MC: 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 122)
Denat/Hyb	Sample Denature/load array plate and Hyb Plate in GeneTitan MC (see *Stage 4 — Denaturation and Hybridization* on page 128)
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 134)

Table 8.9 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 plates 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 plates cannot be prepared ahead of time and stored.**

Figure 8.6 Manual Target Preparation Workflow — Day 5 Activities

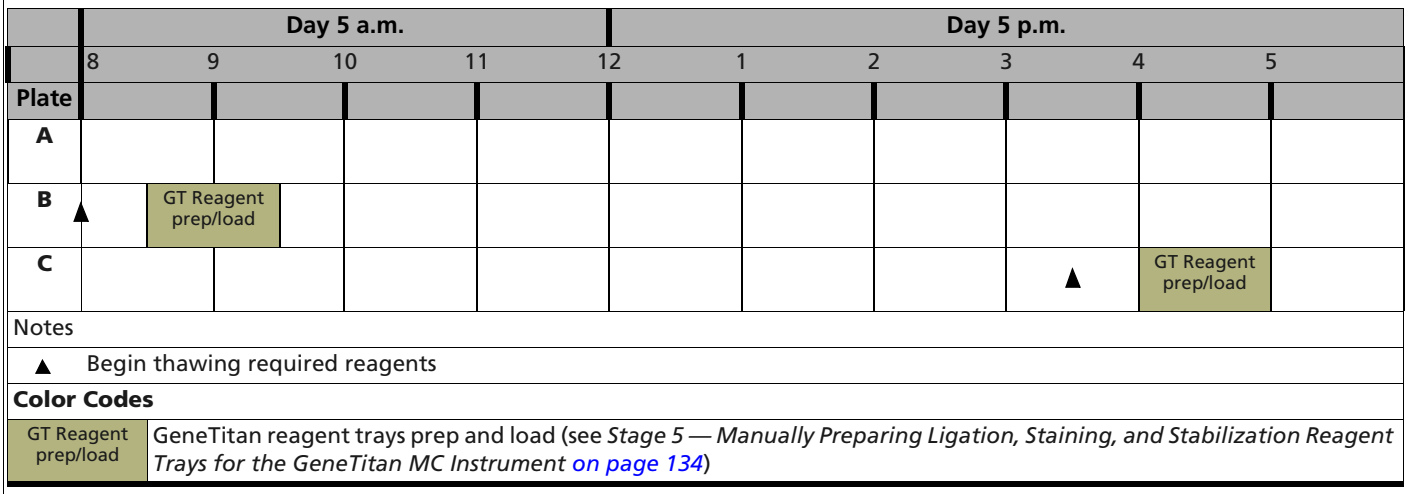


Table 8.10 Manual Target Prep Workflow — Day 5 Activities

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

Biomek FX^P Target Prep Express

If a hardware problem is encountered while running the Axiom target preparation methods on the Biomek FX^P Target Prep Express, you can do the following:

- Refer to these documents:
  - *Biomek® Liquid Handler User's Manual*, Beckman Coulter P/N 987834
  - *Biomek® Software User's Manual*, Beckman Coulter P/N 987835
- For information on recovering a run, contact your Affymetrix Field Application Specialist.
- For additional information on Biomek FX^P Target Prep Express hardware, error messages, or to request service, contact Beckman Coulter. Be sure to have the serial number of your workstation available.

GeneTitan Multichannel Instrument

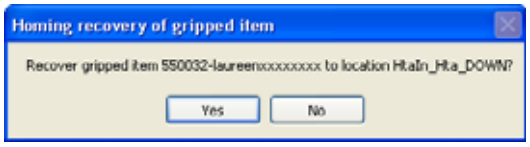
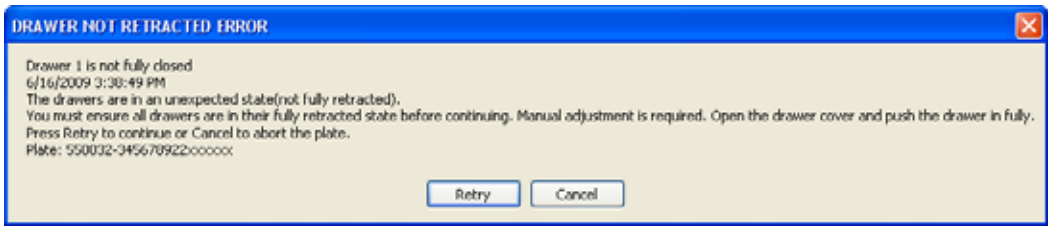
Refer to the *GeneTitan® Multi-Channel Instrument User's Manual*, P/N 08-0306 for further troubleshooting information.

Table 9.1 GeneTitan Multichannel Instrument troubleshooting guidelines for the Axiom 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 *Unload Plates* 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 219	
FLUIDIC DIAGNOSTIC messages	See *Fluidic Diagnostic Messages* on page 219.	
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 221.

Miscellaneous Messages

Table 9.2 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 instrument control software after library files have been installed.

Failed Messages

Table 9.3

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 9.4 Problem messages

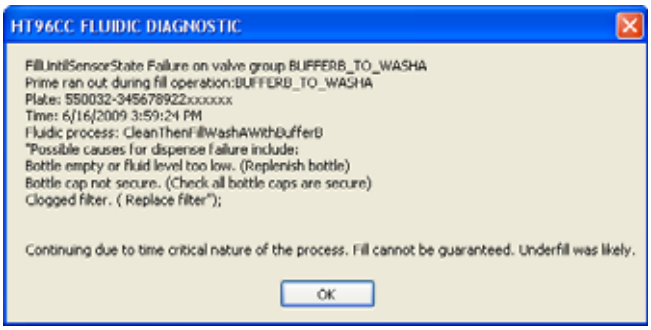
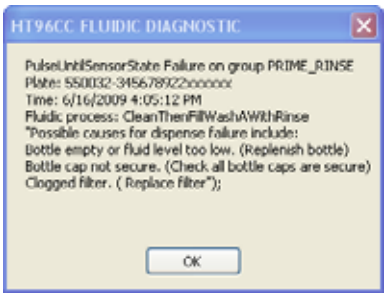
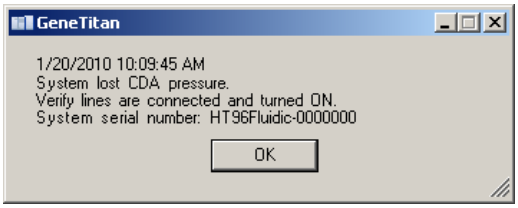
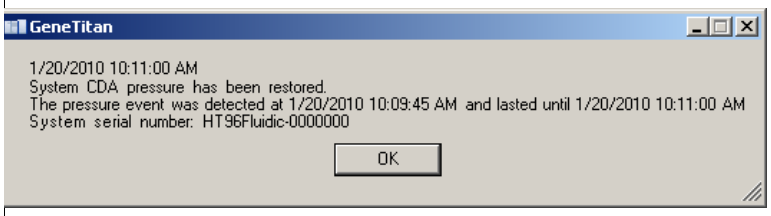
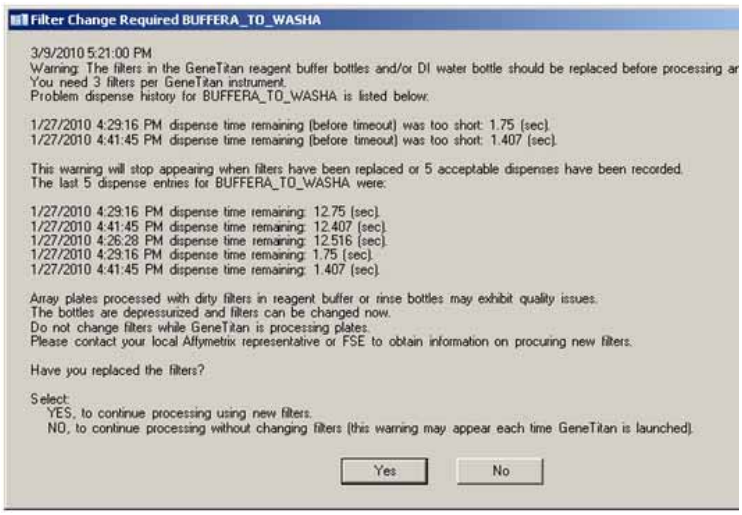
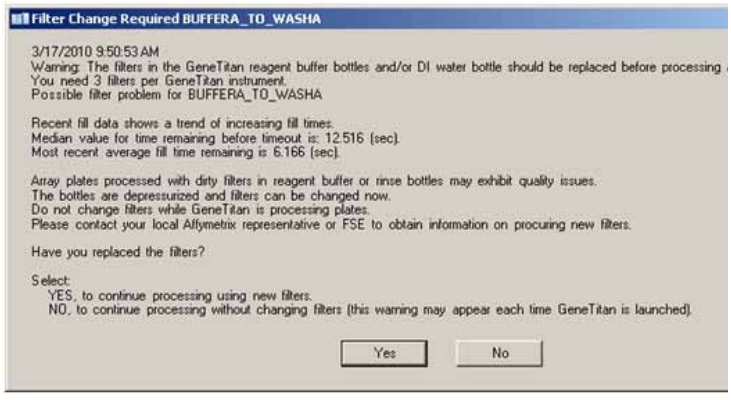
Problem and Possible Causes	
	About this message: ▪ BUFFERX = Buffer bottle A, B or Rinse ▪ WASHX = Wash A or B reservoir in the fluidics station. Recommended actions: ▪ Replenish fluid level in the Rinse or Wash Bottle B to the 1L mark. Do not overfill. ▫ Only replenish bottles when prompted by the 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 *GeneTitan® Multi-Channel Instrument User's Manual*, 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 *GeneTitan® Multi-Channel Instrument User's Manual*, P/N 08-0306.

Table 9.4 Problem messages

Problem and Possible Causes	
When the instrument experiences a loss in Clean Dry Air (CDA) pressure, the software will display the warning message.  When the pressure is detected again, a dialog message confirming the availability of CDA pressure is displayed. 	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.
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 248* 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.

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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, 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 ▪ Microseal 'B' Film	 Applied Biosystems Bio-Rad	 4306311 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 µL 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

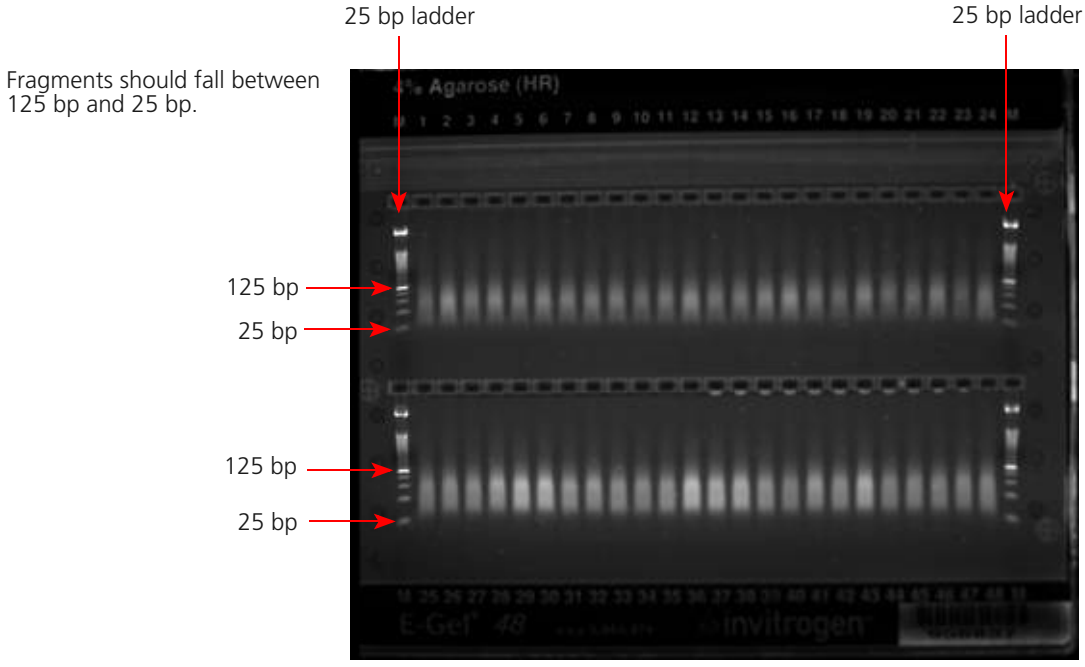
This protocol is based on running QC gels for 96 samples.

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 µL from each well of the gel QC plate onto the gels.
8. Load 15 µL of diluted TrackIt 25 bp ladder into the marker wells (M).
9. Load 20 µL 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.

Figure A.1 Example of a typical fragmentation QC E-gel



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 260, 280 and 320. See *Suggested Protocol for OD Quantitation Using the DTX 880* *on page 227* 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.x
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.

Figure B.1

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

	A	B	C	D	E	F	G	H
1								
2	Well	Layout	REDUCTION_A1 - Abs_260 ()	REDUCTION_A2 - Abs_280 ()	REDUCTION_A3 - Abs_320 ()	REDUCTION_A4 - Concentration	REDUCTION_A5 - Purity ()	REDUCTION_A6 - Mass/rxn (ug)
3	A1	S1	0.4164	0.244	0.0389	7.8103	1.7066	898.1897
4	A2	S2	0.3671	0.2116	0.0339	6.8938	1.7349	792.7862
5	A3	S3	0.4303	0.239	0.035	8.1786	1.8004	940.5414
6	A4	S4	0.4019	0.2137	0.0392	7.5041	1.8807	862.9759
7	A5	S5	0.3844	0.2177	0.036	7.2083	1.7657	828.9517
8	A6	S6	0.3745	0.2101	0.0386	6.9497	1.7825	799.2103
9	A7	S7	0.4401	0.2432	0.0358	8.3648	1.8096	961.9552
10	A8	S8	0.373	0.2047	0.0342	7.0097	1.8222	806.1103
11	A9	S9	0.4134	0.2346	0.0378	7.771	1.7621	893.669
12	A10	S10	0.4218	0.2379	0.0366	7.9697	1.773	916.5103
13	A11	S11	0.4015	0.226	0.0396	7.4876	1.7765	861.0724
14	A12	S12	0.4022	0.2345	0.0368	7.56	1.7151	869.4
15	B1	S13	0.4457	0.2414	0.036	8.4766	1.8463	974.8034
16	B2	S14	0.4302	0.2306	0.0387	8.1	1.8656	931.5
17	B3	S15	0.4442	0.2521	0.0376	8.4124	1.762	967.4276
18	B4	S16	0.3773	0.213	0.0372	7.0366	1.7714	809.2034
19	B5	S17	0.3496	0.195	0.0332	6.5462	1.7928	752.8138
20	B6	S18	0.3349	0.1929	0.0366	6.1717	1.7361	709.7483
21	B7	S19	0.3941	0.2209	0.0365	7.3986	1.7841	850.8414
22	B8	S20	0.4095	0.2295	0.0354	7.74	1.7843	890.1
23	B9	S21	0.4384	0.252	0.0405	8.2324	1.7397	946.7276
24	B10	S22	0.3944	0.219	0.0347	7.4421	1.8009	855.8379
25	B11	S23	0.4042	0.2285	0.0371	7.5952	1.7689	873.4448
26	B12	S24	0.3467	0.1998	0.0356	6.4366	1.7352	740.2034

OD Yield Assessment Guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the Axiom assay. If the median yield for the plate is < 500 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.

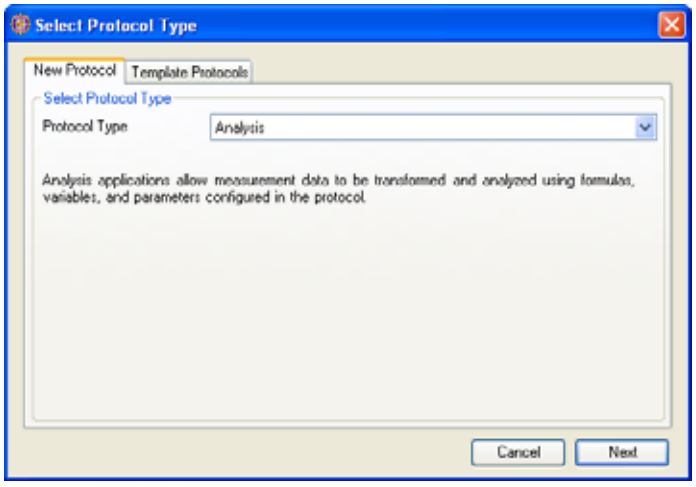
This DNA yield corresponds to an A260 value of 0.245 and an A260-A320 value of 0.210.

Suggested Protocol for OD Quantitation Using the DTX 880

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

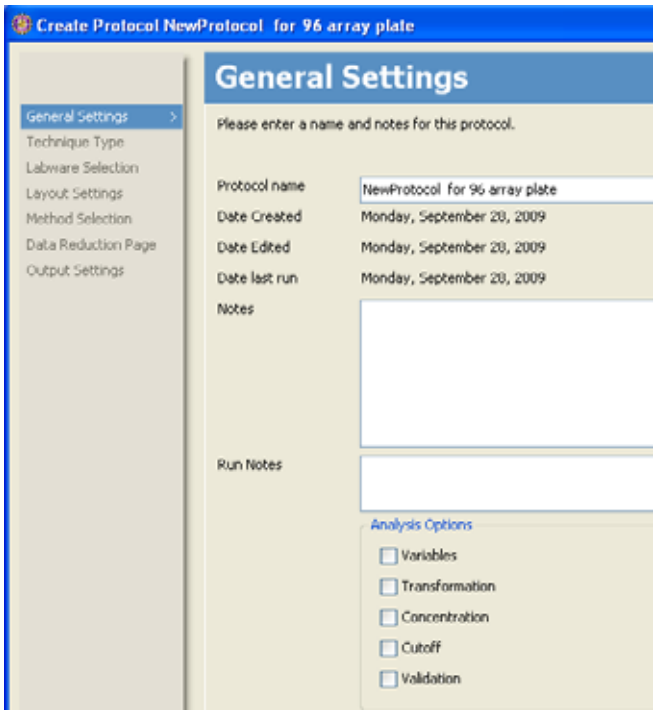
Protocol Type — Analysis

Figure B.3 Protocol Type



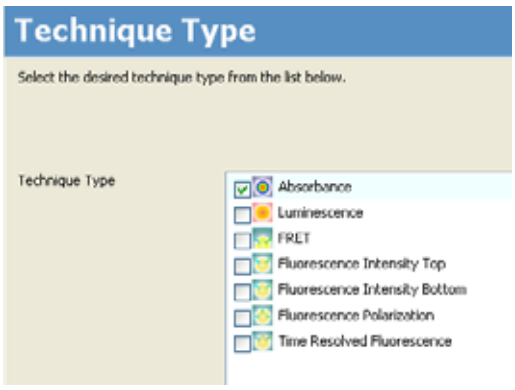
General Settings — enter a name for the protocol

Figure B.4 General Settings



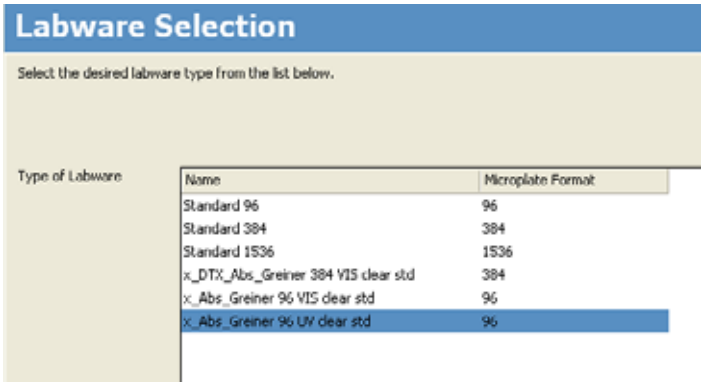
Technique Type — select Absorbance.

Figure B.5 Technique Type



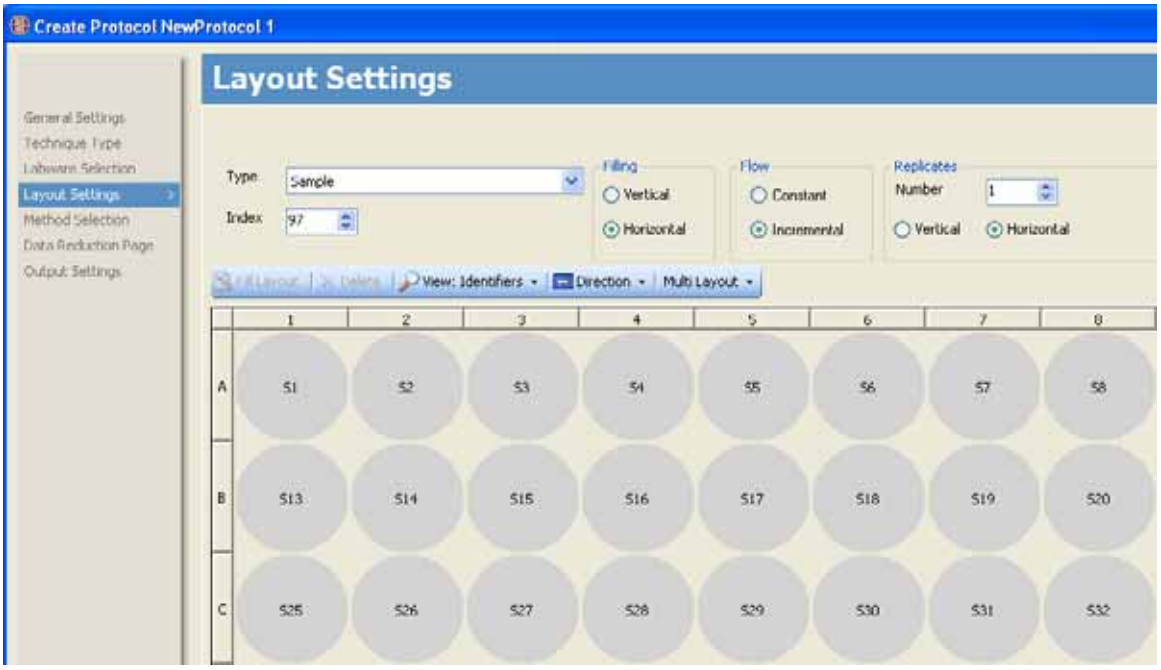
Labware — x_Abs_Greiner 96 UV clear std (96 microplate format)

Figure B.6 Labware



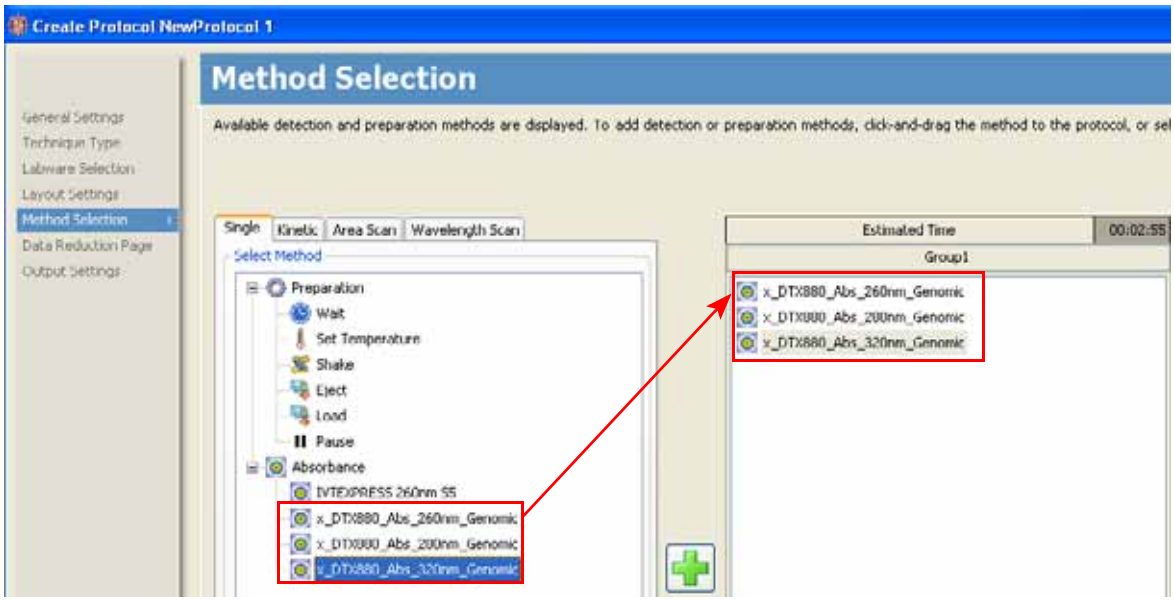
Layout Settings — as appropriate for 96-array plates

Figure B.7 Layout Settings



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

Figure B.8 Method Selection



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

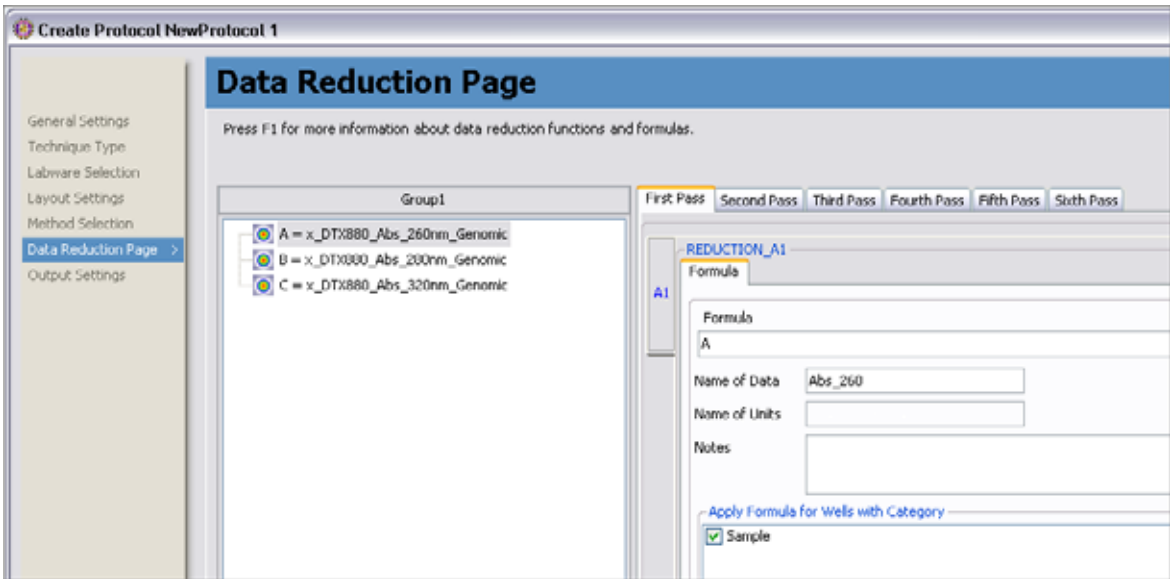


Figure B.10 Data Reduction Page — Second Pass

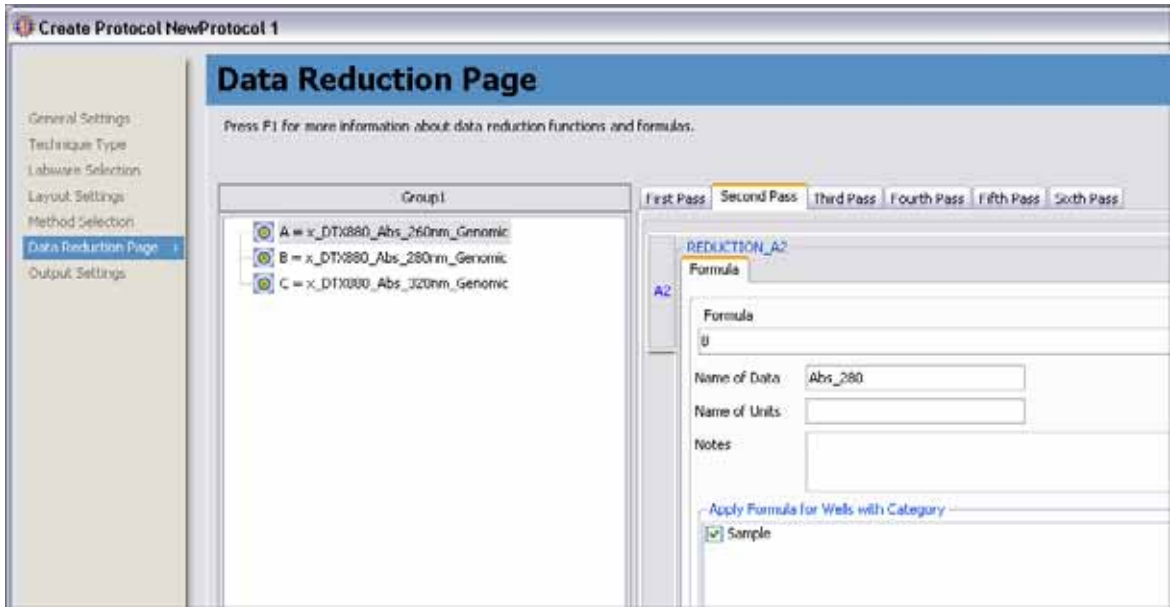


Figure B.11 Data Reduction Page — Third Pass

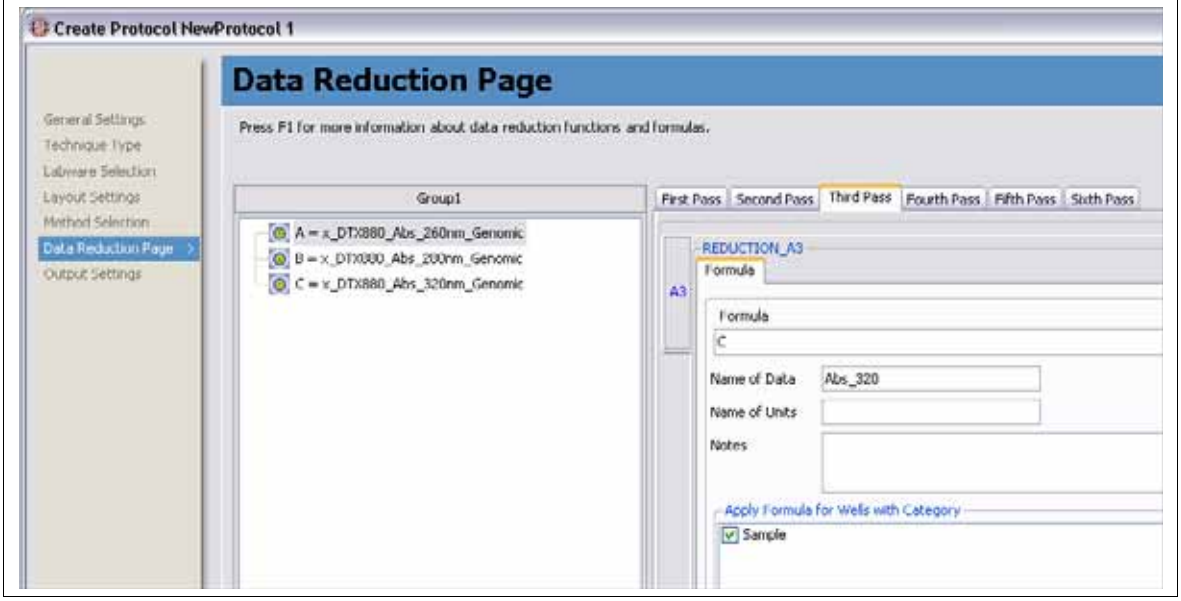


Figure B.12 Data Reduction Page — Fourth Pass

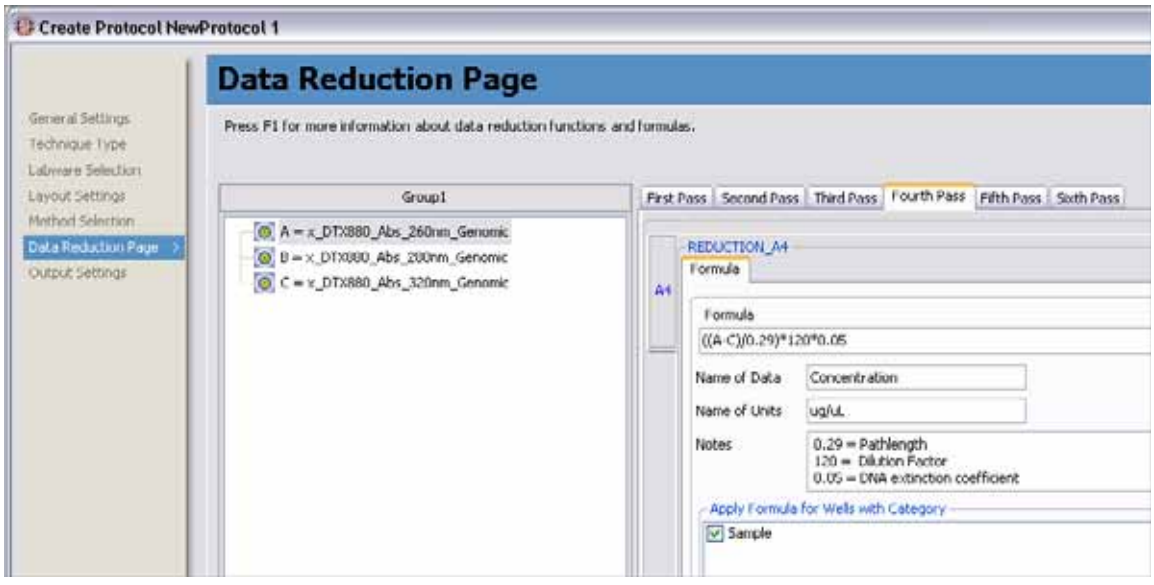


Figure B.13 Data Reduction Page — Fifth Pass

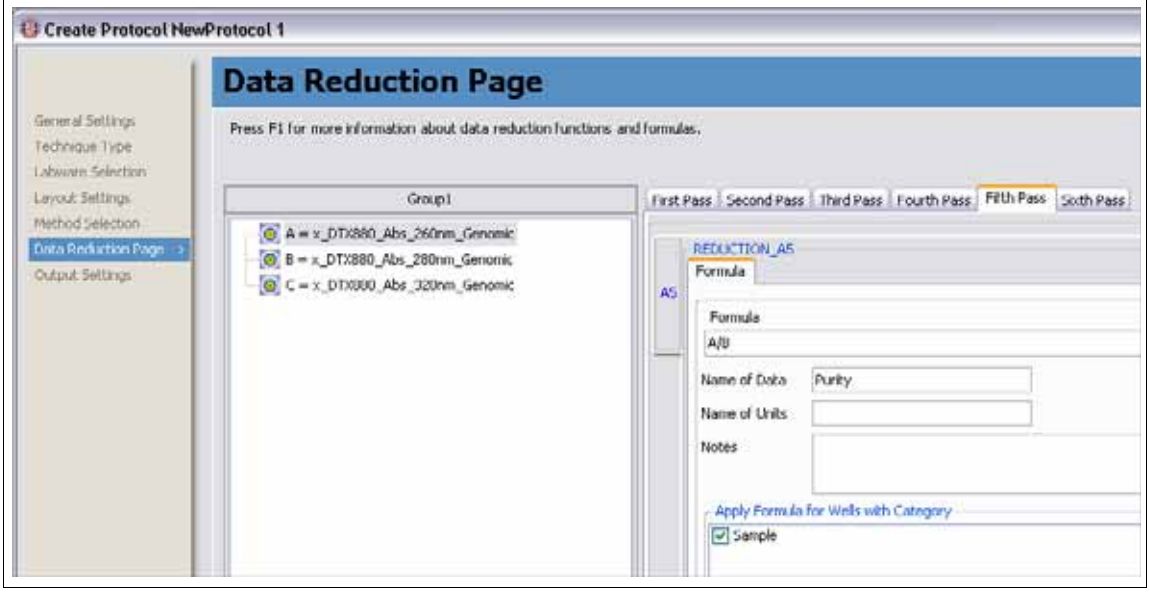
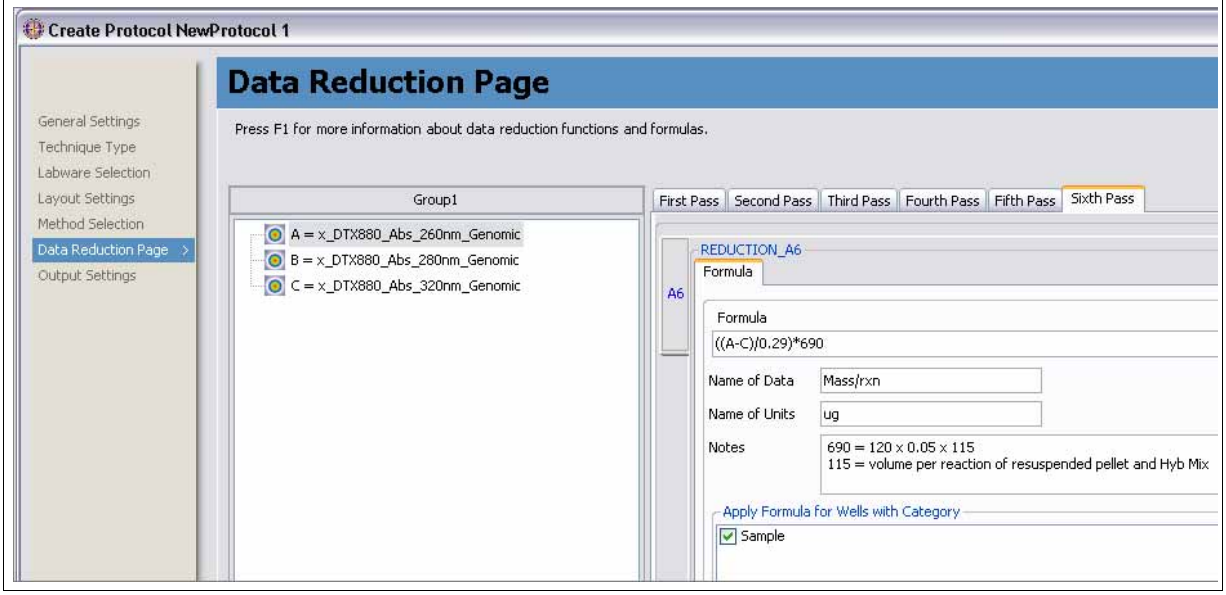
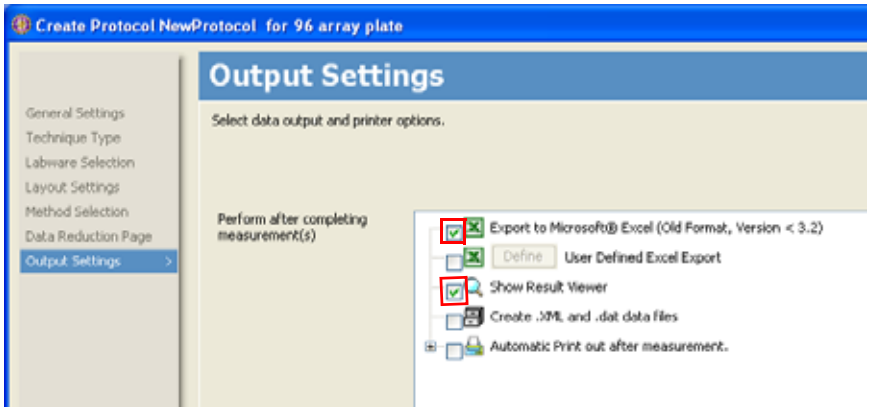


Figure B.14 Data Reduction Page — Sixth Pass



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

Figure B.15 Output Settings



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

B = 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 Site Prep Guide,* P/N 702858, 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 226 and against *OD Yield Assessment Guidelines* *on page 226*.

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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 Genotyping AssayGenotyping 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 plate can be stored at -20C 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, P100
- PCR plate, BIO-RAD, P/N HSP9631

  or

  - PCR Plate HSS-9601 stacked on a Costar serocluster round-bottom plate for the off-deck ABI 9700 and ABI 2720 thermal cycler when using the automated target prep methods

  or

  - PCR Plate HSS- 9601 plate for use with manual target prep
- Axiom™ Genome-Wide or Custom myDesign™ Array Plate Kit (96 array)

Reagents

- Modules 3 and 4 of the Axiom 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

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 ~45 µL.
4. To recover all of the remaining material from the Hyb Tray:
   A. Add 50 µL 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 Plate:
   - 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 128*)

   ! 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.
   B. When hybridization is complete, prepare a new set of reagent plates 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 134*)
   C. Transfer the reagent plates 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

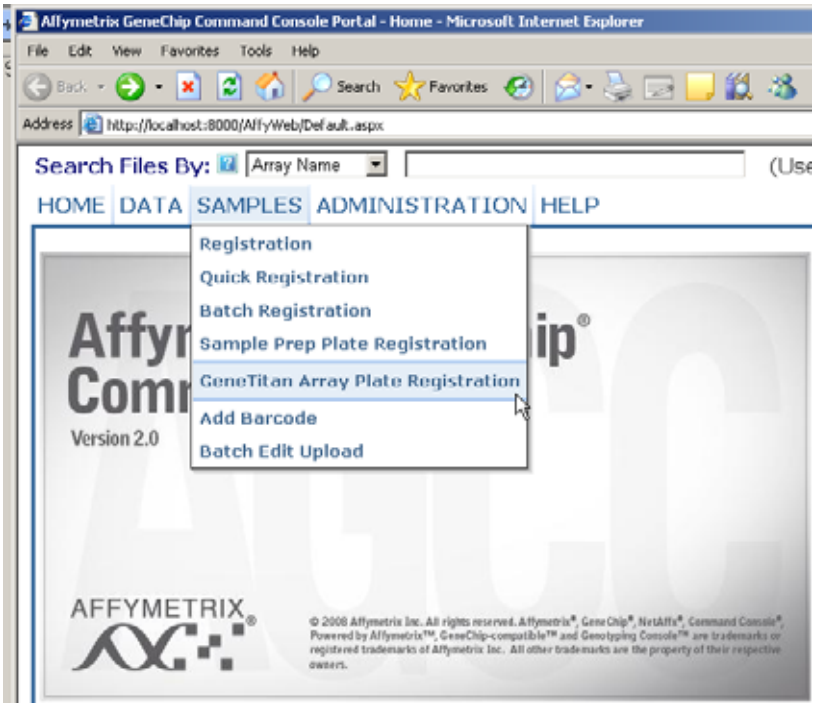
A GeneTitan Array Plate Registration file is a Microsoft Excel spreadsheet that includes information on the samples you are processing on a single array plate. This information includes the array plate format, the array plate barcode, and sample file names so that you can track the samples that are loaded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

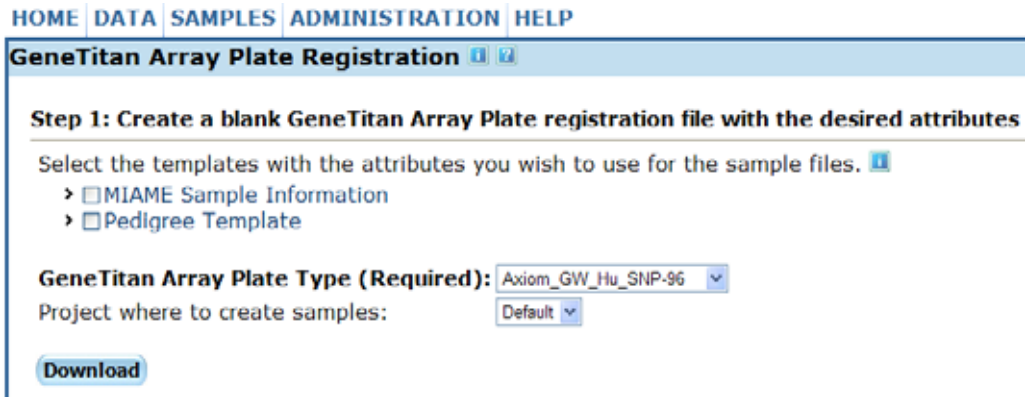
1. In AGCC Portal, open the **Samples** menu and select **GeneTitan Array Plate Registration**.

Figure D.1 Selecting Batch Register



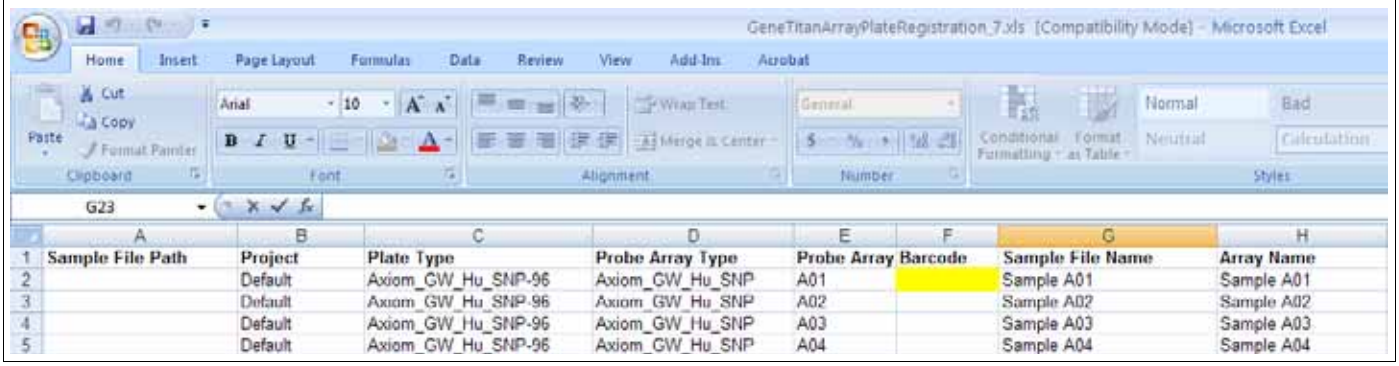
2. Step 1 — Figure D.2 on page 238:
   - **A.** Select the array plate type.
   - **B.** Click **Download**.

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



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.

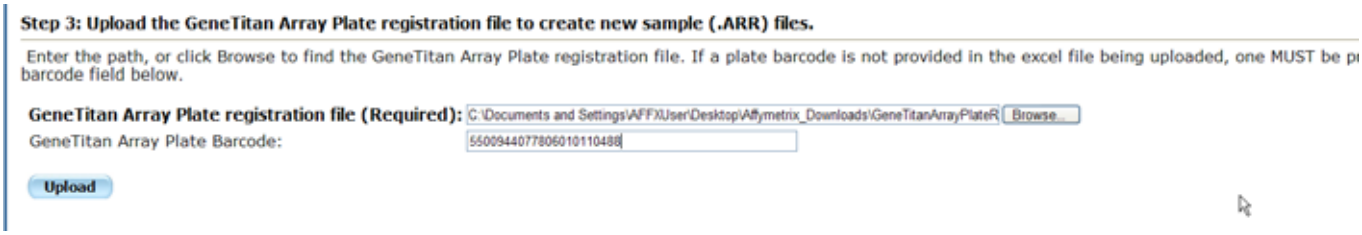


Figure D.5 Array plate samples successfully registered.

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Deionization Procedure for GeneTitan Trays and Covers

Use the following technique to destatic GeneTitan MC Stain Tray 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 (PN 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 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.**

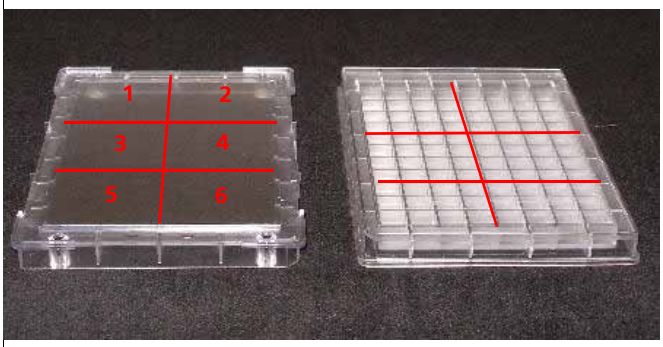
1. Treat the plate or lid as if it were divided into 6 sections, and deionize as follows.
2. Place a Kimwipe on the benchtop.
3. Place the stain tray on a table top. Use the anti-static gun to aim at the center of each of the six sections on a 96-well or 24-well cover or tray and pull the trigger. Ensure 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).

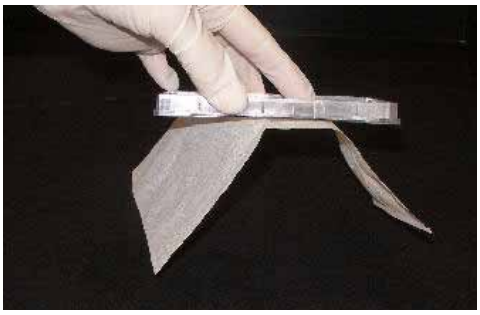
5. Place the treated cover or tray on the Kimwipe and lift it up.
6. Do one of the following:
   - If the Kimwipe does not adhere to the plastic, proceed with the step.
   - If the Kimwipe adheres to the plastic, then perform steps 3 and 4 again. If it continues to adhere to the plastic, then the gun 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.

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GeneTitan® MC 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® 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.

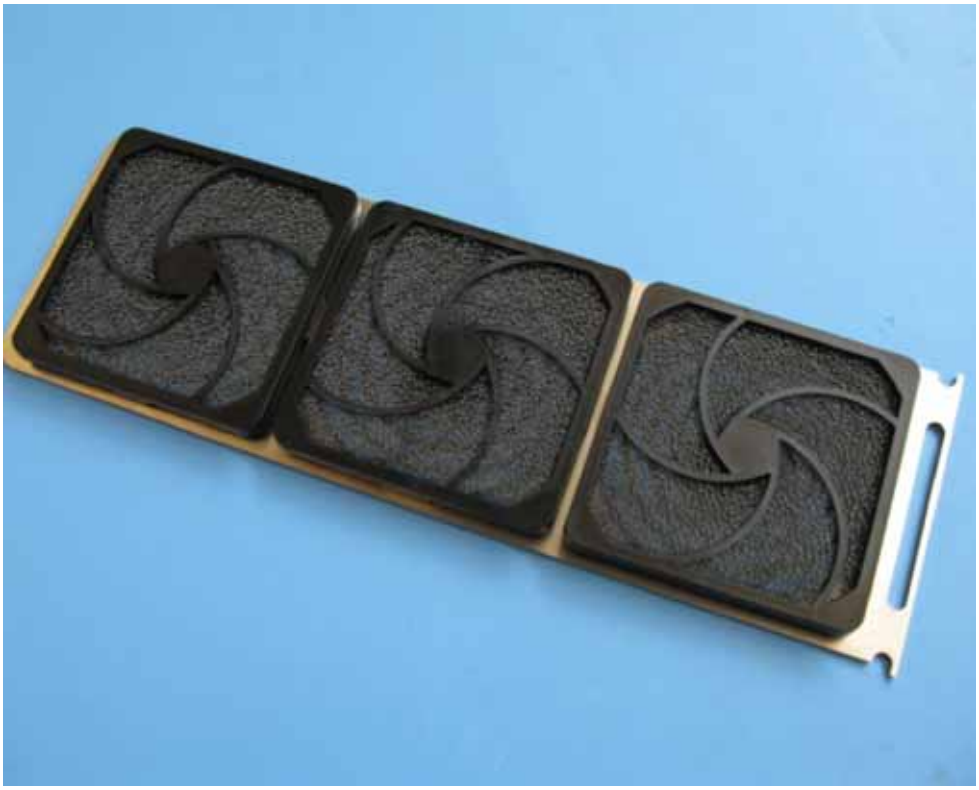
A plugged filter cartridge can cause excessive temperatures within the machine that can cause unwanted evaporation on test media.

Part details:

Affymetrix P/N: 01-0669

Number of parts per assembly: 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. Submerse 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.

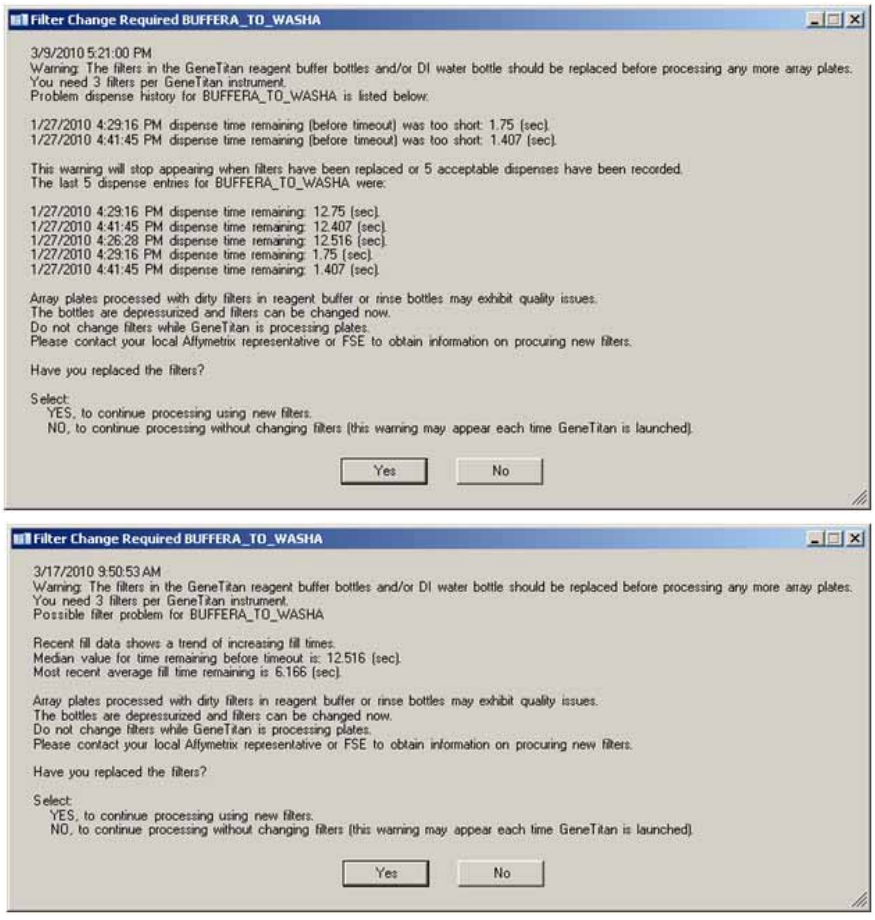
Replacing the Bottle Filters

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.

Figure F.2 Filter change required messages



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.

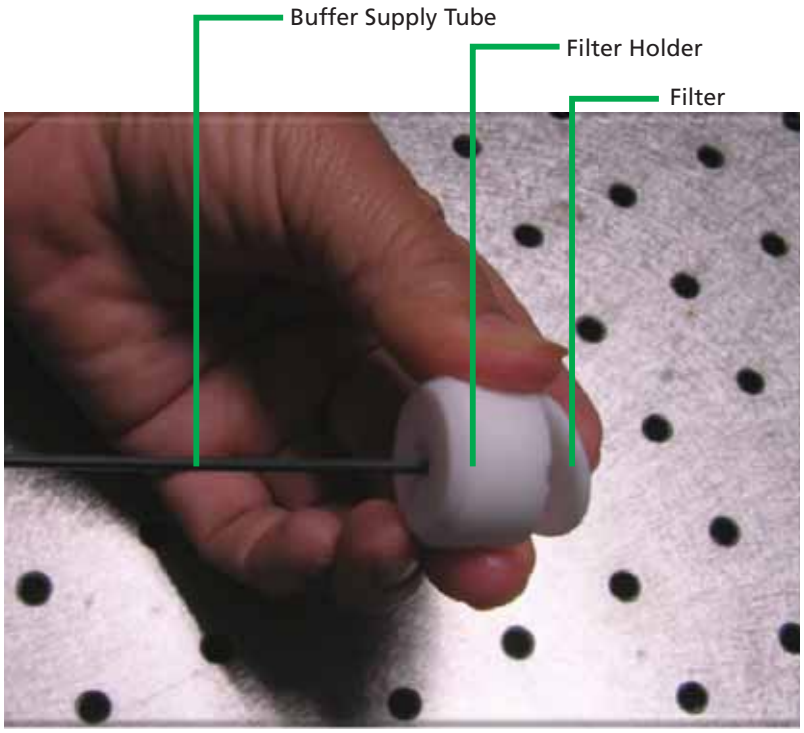
After changing the filters in all three bottles using the procedure described below, please press the **Yes** button to continue. If you choose to ignore the error message, press the **No** button. This warning message will be displayed each time AGCC instrument control software is launched. You may 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

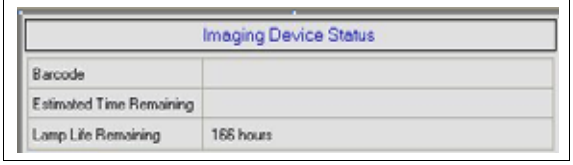
IMPORTANT: Please DO NOT try to replace the lamp when a plate is being processed either in the fluidics or scanner system.

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

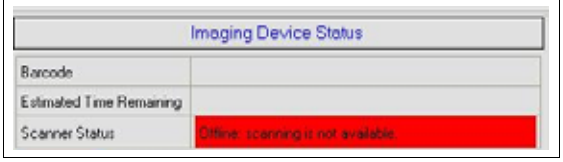


It displays a red or yellow notice when the lamp life is getting short:

Figure F.5 Lamp Life above tolerance

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

Figure F.6 Imaging Device Offline



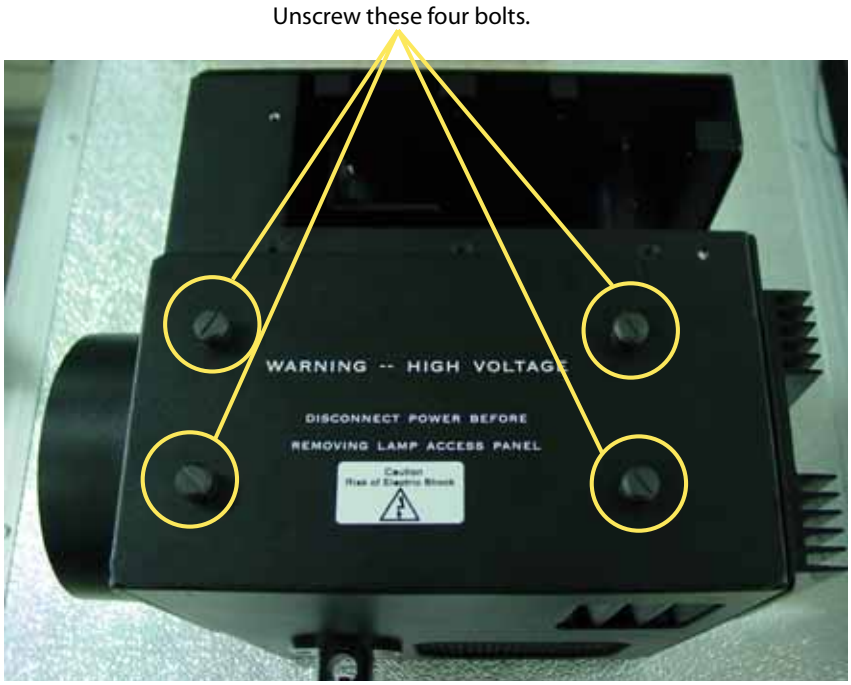
NOTE: The 300 Watt Xenon lamp in the GeneTitan MC instrument is warranted for 500 hours. The instructions to replace the lamp are available on the following page. After changing the lamp, it is necessary to reset the lamp life clock manually.

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

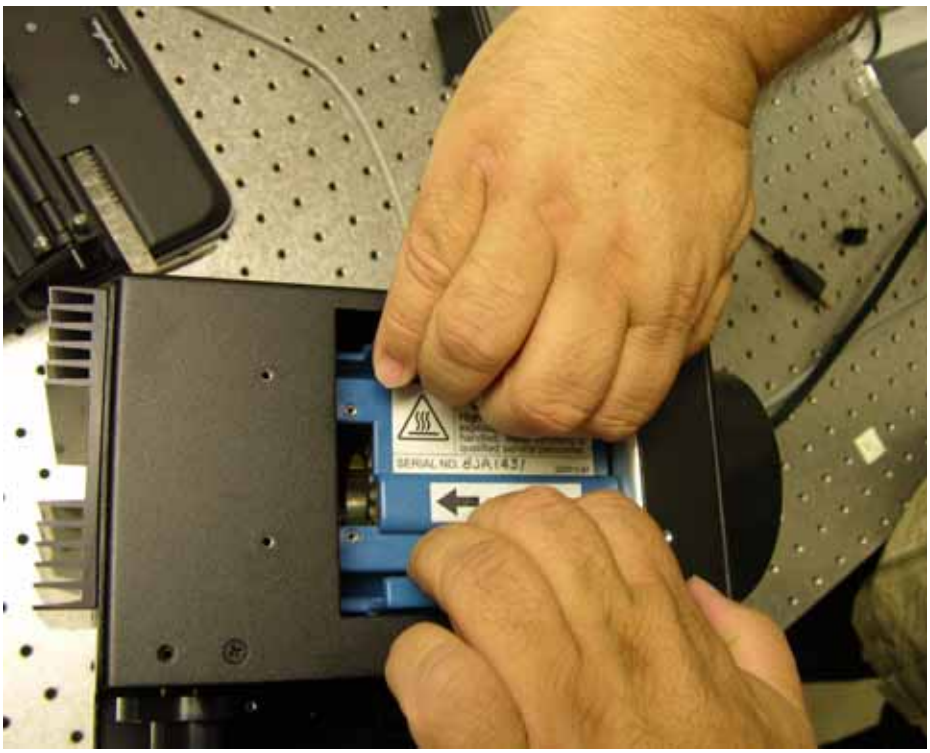
1. Unscrew the four retaining bolts. They should be finger tight (Figure F.7).

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

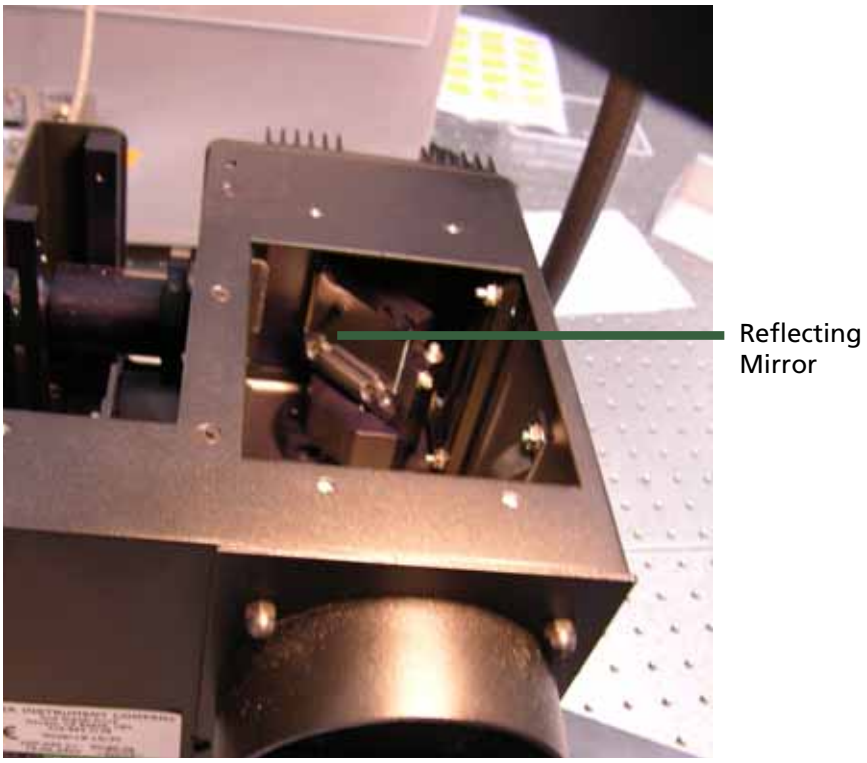
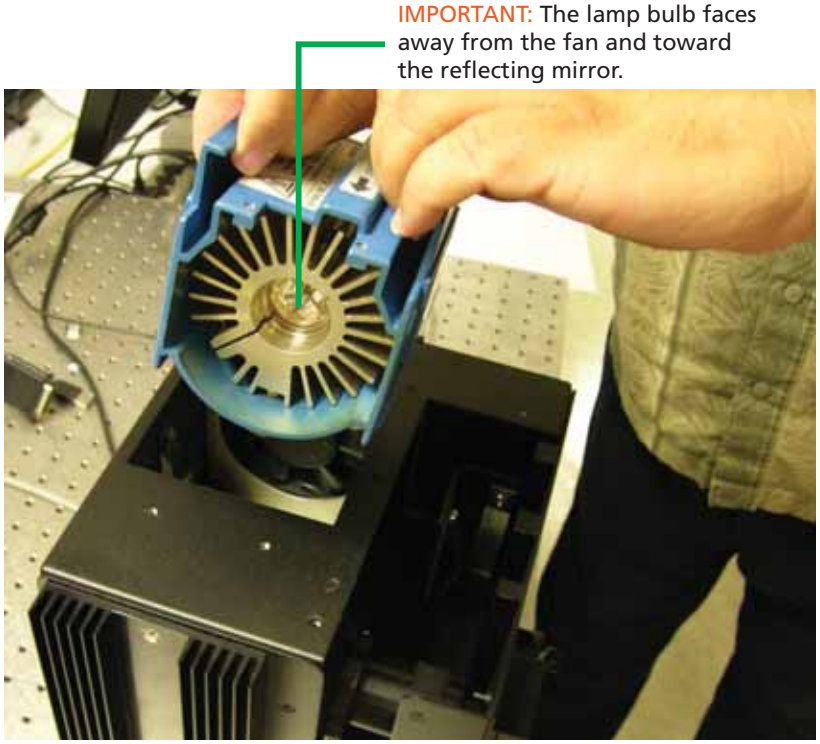


Figure F.10 Inserting the Lamp

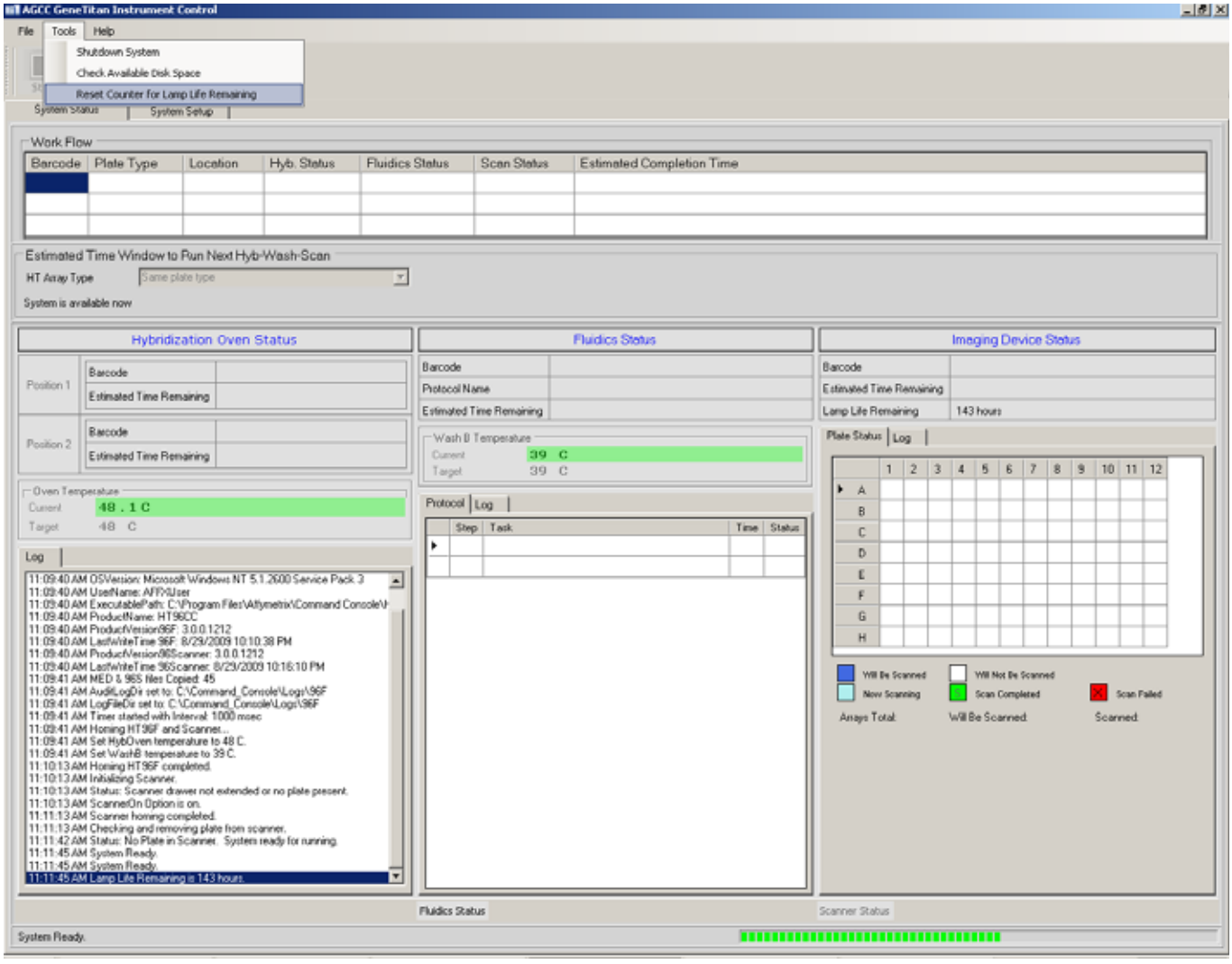


Resetting the Lamp Counter

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.

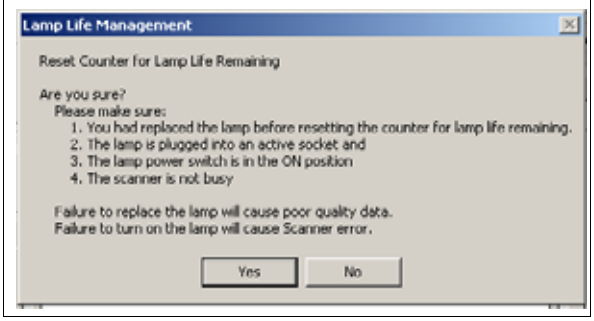
1. On the software application click **Tools** → **Reset Counter for Life Remaining** (Figure F.11).

Figure F.11 Inserting the Lamp



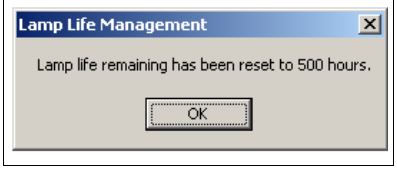
2. The software will display a message that allows you to change your mind.

Figure F.12 Are you Sure?



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).

Figure F.13 The counter is reset.



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.

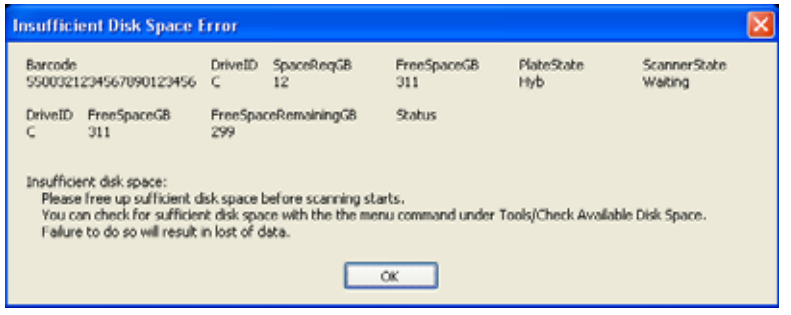
For software errors that do not involve hardware crashes the most common solution is to shut down the application and then restart it. If the same error occurs shut down both the application and the computer and then restart. If it still occurs shut down the entire unit and then restart.

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.

Figure F.14 Insufficient Disk Space Notice



If you see this notice, you will need to free up sufficient disk space before imaging starts.

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