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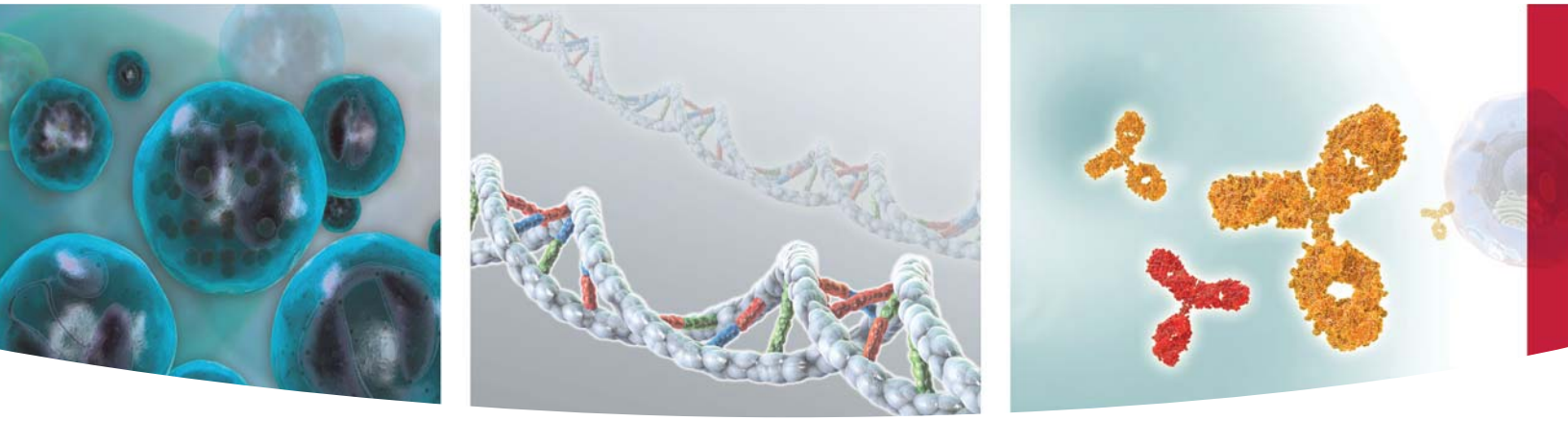
PharmacoScan™ Assay 24-Array Format

Manual Protocol

UserGuide



eBioscience GeneChip USB

P/N 703286 Rev. 2

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Contents

Chapter 1

About the PharmacoScan™ Solution

Overview

Developed in collaboration with experts across the field of pharmacogenomics, PharmacoScan™ Solution is the industry's broadest content genetic analysis system specifically designed to provide insight into the absorption, distribution, metabolism, and excretion (ADME) and transport of commonly prescribed medicines. By interrogating more than 4,600 markers in nearly 1,200 genes known to play a role in drug metabolism, traditional clinical researchers will gain unprecedented understanding into an individual's ability to process those drugs with high evidence for genetic association, as well as those markers where moderate, low, preliminary and unknown evidence exists. PharmacoScan Solution utilizes the proven GeneTitan® Multi-Channel Instrument, a system that is preferred worldwide by genetic researchers requiring efficient workflow, high throughput, economic pricing and lot-to-lot consistency required to support multi-year data collection and analysis efforts.

Introduction to the PharmacoScan™ Assay 24-Array Format Manual Protocol

PharmacoScan™ Assay 24-Array Format Manual Protocol is available as a bundled kit that includes the arrays, reagents and consumables needed for processing four 24-format plates, each having 22 samples and 2 controls.

PharmacoScan interrogates SNPs, indels and copy number variation (CNV) in a single assay workflow. Starting with genomic DNA, the samples are processed by performing a manual target preparation protocol followed by automated processing of the array plates on the GeneTitan MC Instrument.

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

PharmacoScan provides pharmacogenomic variation information for more than 4,600 ADME markers in nearly 1,200 genes. This content is sourced from globally endorsed consortium databases including, but not limited to CPIC, PharmGKB, and PharmaADME. Also included on PharmacoScan are high value markers for human leukocyte antigen (HLA) imputation, markers for killer cell immunoglobulin-like receptors (KIR), markers for human ancestry identification (AIM), a marker GWAS backbone, and markers for sample ID and tracking. The combination of these high value markers, in addition to PharmacoScan's ability to precisely call variants in critical genes on a microarray, compliments Thermo Fisher Scientific's current solutions for pharmacogenomics using the Real-Time PCR OpenArray and Ion AmpliSeq NGS Panels for Targeted Sequencing platforms.

PharmacoScan is a multiplex genotyping assay which combines the proven Axiom chemistry in a 24 sample format with the incorporation of a multiplex PCR step to overcome some of the complexities associated with genotyping highly homologous markers. PharmacoScan software and algorithm developments include an allele translation and phenotyping tool and copy number aware genotyping. Array plates are processed on a GeneTitan® MC Instrument controlled by Affymetrix GeneChip Command Console® 4.3 or higher. The resulting CEL files are analyzed by Axiom™ Analysis Suite 2.0 or higher, or by Affymetrix Power Tools 1.19 or newer.

PharmacoScan™ Assay 24-Array Format Manual Protocol

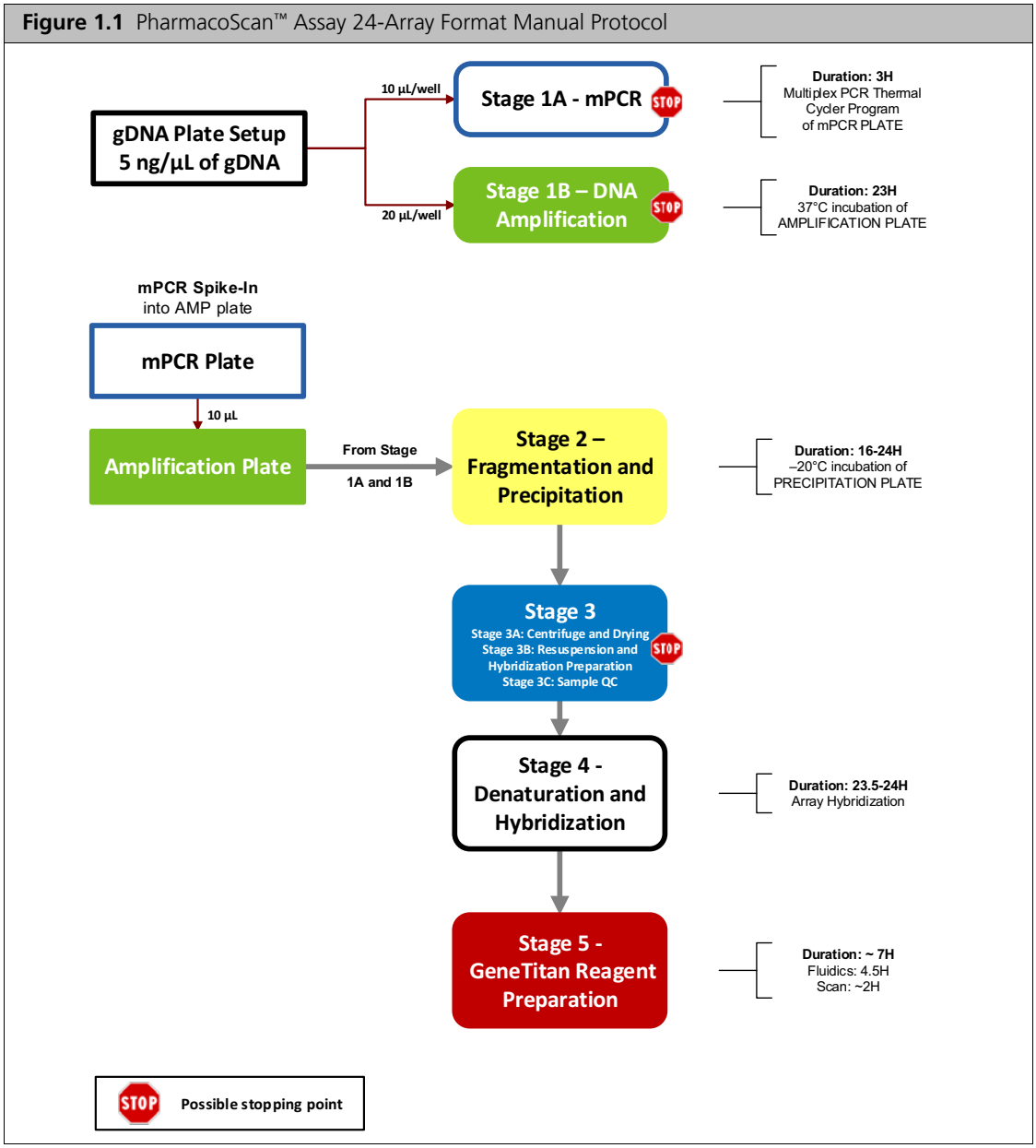
Running the PharmacoScan Assay 24-Array Format Manual Protocol requires the following sets of steps:

1. Genomic DNA Prep—Resulting in samples that meet requirements spelled out in Chapter 2, *Genomic DNA Preparation and Requirements* on page 11.
2. A multiplex PCR step (mPCR) followed by target preparation of the samples (see Chapter 4, *Target Preparation* on page 38).
3. Array Processing, done with
   - GeneTitan MC Instrument
   - GeneTitan Instrument Control software
   - AGCC Portal software

   See Chapter 5, *Array Processing with the GeneTitan® Multi-Channel Instrument* on page 96.

A list of the required equipment and supplies for running the PharmacoScan Assay 24-Array Format Manual Protocol can be found in the *PharmacoScan™ Assay 24-Array Format Manual Protocol Site Preparation Guide*, P/N 703287.

Figure 1.1 PharmacoScan™ Assay 24-Array Format Manual Protocol



Related Documentation

- *PharmacoScan™ Assay 24-Array Format Manual Protocol Site Preparation Guide*, P/N 703287
- *PharmacoScan™ Assay 24-Array Format Manual Protocol QRC*, P/N 703288
- *Axiom® Genotyping Solution Data Analysis Guide*, P/N 702961
- *GeneTitan® MC Protocol for Axiom 2.0 Array Plate Processing QRC*, P/N 702988
- *GeneTitan® Multi-Channel Instrument User Guide*, P/N 08-0308
- *GeneTitan® Multi-Channel Instrument Site Preparation Guide*, P/N 08-0305
- *Axiom™ Analysis Suite User Guide*, P/N 703307
- *Affymetrix® GeneChip® Command Console® 4.0 User Guide*, P/N 702569
- *Affymetrix® Genotyping Console™ 4.2 User Guide*, P/N 702982

Safety Warnings and Precautions

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

WARNING: The following components contain harmful or toxic ingredients:

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

In all cases customers should use adequate local and general ventilation in order to minimize airborne concentrations.

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

Precautions

1. PHARMACOSCAN ARRAYS AND PLATES ARE FOR RESEARCH USE ONLY; NOT FOR DIAGNOSTIC PROCEDURES.
2. Avoid microbial contamination, which may cause erroneous results.
3. **WARNING**: All biological specimens and materials with which they come into contact should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. This includes adherence to the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030) for blood-derived and other samples governed by this act. Never pipet by mouth. Avoid specimen contact with skin and mucous membranes.
4. **CAUTION**: Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic reagents.
5. Exercise care to avoid cross-contamination of samples during all steps of this procedure, as this may lead to erroneous results.
6. Use powder-free gloves whenever possible to minimize introduction of powder particles into sample or array plates.
7. **CAUTION**: Use care when handling the Scan Tray as it has protruding guiding posts that may be sharp and can stick out of the pouch if not handled carefully.

Chapter 2

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 PharmacoScan™ Reagent Kit 4x24 Reactions (Table 3.1 on page 18). The kit contains two Control gDNAs, Control DNA 1 and Control DNA 2. This DNA meets the requirements outlined below, and both Control DNAs must be included on every plate for data analysis purposes. The size and purity of sample gDNA can be compared with those of the control DNA to assess sample quality.

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

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

Sources of Genomic DNA

The following sources of human gDNA have been successfully tested in the PharmacoScan Assay 24-Format Manual Assay with DNA that meets the above requirements.

- Blood
- Cell line

Other sample types have not been validated in this assay and are not currently supported.

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 13.
- DNA must not be degraded.
  The approximate average size of gDNA may be assessed on a 1% agarose gel using an appropriate size standard control. Approximately 90% of the DNA must be greater than 10 Kb in size. Control DNA can be run on the same gel for side-by-side comparison.

Special Requirements

Pre-Amplification Area

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

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

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

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

We recommend this quality control step to 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	Thermo Fisher Scientific (formerly Life Technologies™ /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 for Gel Analysis

- Loading a DNA mass of 10 ng to 20 ng per well is recommended. If lower amounts are loaded, omission of the loading dye is recommended in order to improve visualization. Loading ≥25 ng gDNA per well can improve the image.
- Add 3 µL of 0.1X of *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.

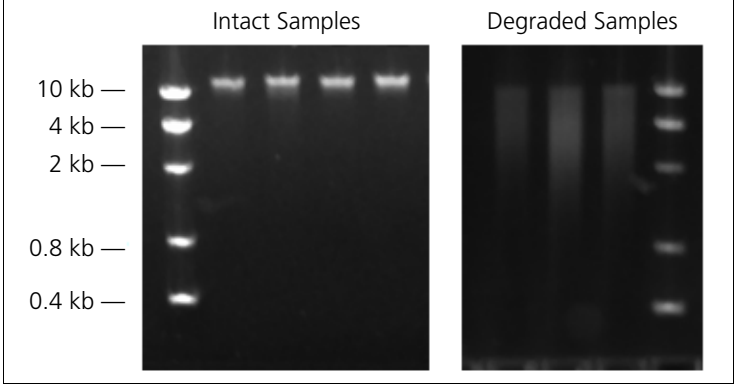
To Run a 48-lane 1% Agarose E-Gel:

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. Adjust the run time to ~27 min.
4. Insert the 48 well 1% Agarose E-Gels into the slot.
5. Remove the combs.
6. Load 20 µL from the above plate onto two 48 well 1% agarose E-Gels.
7. Load 15 µL of diluted High Range DNA Marker (1:3 dilution or ~0.34 X from stock) into all marker wells (as needed).
8. Fill all empty wells with water.
9. Push the Power/Prg button again (it will change from red to green).

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

Figure 2.1 shows gel images of intact gDNA (that is suitable for use in the PharmacoScan™ Assay 24-Array Format Manual Protocol) and degraded gDNA samples. Customers whose gDNA is degraded (similar to the image in Figure 2.1) should perform a test experiment to investigate the performance of their samples in the PharmacoScan Assay 24-Array Format Manual Protocol prior to beginning any large scale genotyping projects.

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).

Genomic DNA Preparation

This step needs to be done before proceeding with the mPCR and DNA amplification stages. The genomic DNA (gDNA) you will process using the PharmacoScan Assay 24-Array Format Manual Protocol should meet the general requirements listed earlier in this chapter. The amount of gDNA is 50 ng for the mPCR step and 100 ng for the PharmacoScan whole-genome amplification step.

Table 2.2 Input Requirements for PharmacoScan Assay 24-Array Format Manual Protocol

Assay Step	Volume per Well	Input Mass per Well	gDNA Concentration
Stage 1A: mPCR	10 µL	50 ng	5 ng/µL
Stage 1B: DNA Amplification	20 µL	100 ng	5 ng/µL

To Prepare gDNA:

1. Thaw Samples and Controls

2. Quantitate and Dilute gDNA

3. Aliquot the Diluted Samples and the Controls DNA 1 and DNA 2

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 2.3 are required for this stage.

Table 2.3 Equipment and Consumables Required for *Genomic DNA Preparation*

Quantity	Item
As required	Adhesive seals for plates
1	Ice bucket, filled with ice
1 each	Pipettes: ▪ Single-channel P10 or P20 ▪ Optional: multi-channel P10 or P20
As required	Pipette tips
1	Plate, deep well: ABGene 96 Square Well Storage; AB-0932
1	Plate centrifuge
1	96 well PCR plate (Bio-Rad HSS-9641 for ABI 9700, ABI Veriti™, ABI ProFlex™, and Bio-Rad HSP-9631 for Eppendorf Master™ Cycler pro S)
1	Plate spectrophotometer (required only if no OD measurements available for samples)
1	Vortexer

Reagents

The reagents listed in Table 2.4 are required for this stage.

Table 2.4 Reagents Required for *Genomic DNA Preparation*

Reagent	Supplier	Part Number
▪ Control DNA 1 and Control DNA 2 (PharmacoScan Module B)	Affymetrix	902897
▪ Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Affymetrix	75793
▪ Quanti-iT PicoGreen dsDNA Assay kit	Life Technologies	P7589

1. Thaw Samples and Controls

Thaw the components listed below to room temperature:

- gDNA samples
- Control DNA 1 and Control DNA 2 (from PharmacoScan Module B).

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 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 Test Sample gDNA:

1. Gently vortex (50% maximum) and spin the gDNA.
2. Quantitate each sample (*e.g.*, using the Quant-iT™ PicoGreen® dsDNA Kit).
3. Using reduced EDTA TE buffer, dilute each sample to a concentration of 5 ng/µL.
4. Seal, vortex and spin.

NOTE: Do NOT dilute the Control DNA 1 or Control DNA 2 from PharmacoScan Module B (P/N 902897). They are already at the working concentration.

NOTE: We strongly recommend you determine your sample concentrations using the Quant-iT PicoGreen assay by Life Technologies (P/N P7589). Sample concentration determined by UV absorbance is often inaccurate and can yield different results.

3. Aliquot the Diluted Samples and the Controls DNA 1 and DNA 2

Next, the samples and controls are placed in a deep well plate for amplification and into a 96-well PCR plate for mPCR:

NOTE: Make sure gDNA is well mixed before plating.

IMPORTANT: Control DNA 1 and Control DNA 2 are required for assay performance. Both controls must be included on mPCR and Amplification Sample Plates and placed in indicated wells. Controls need to be run every time assay is performed.

Amplification Sample Plate

- ABgene 96 Square Well Storage; AB-0932.

Aliquot Diluted Samples and Controls to Columns 5, 7, and 9 of the Deep Well Plate:

1. Aliquot 20 µL of each diluted gDNA sample to columns 5, 7, and 9 of the ABgene deep well plate as shown in Figure 2.2.
2. Pipet 20 µL of Control DNA 1 to well G09 and 20 µL of Control DNA 2 to well H09.
3. Seal and spin.

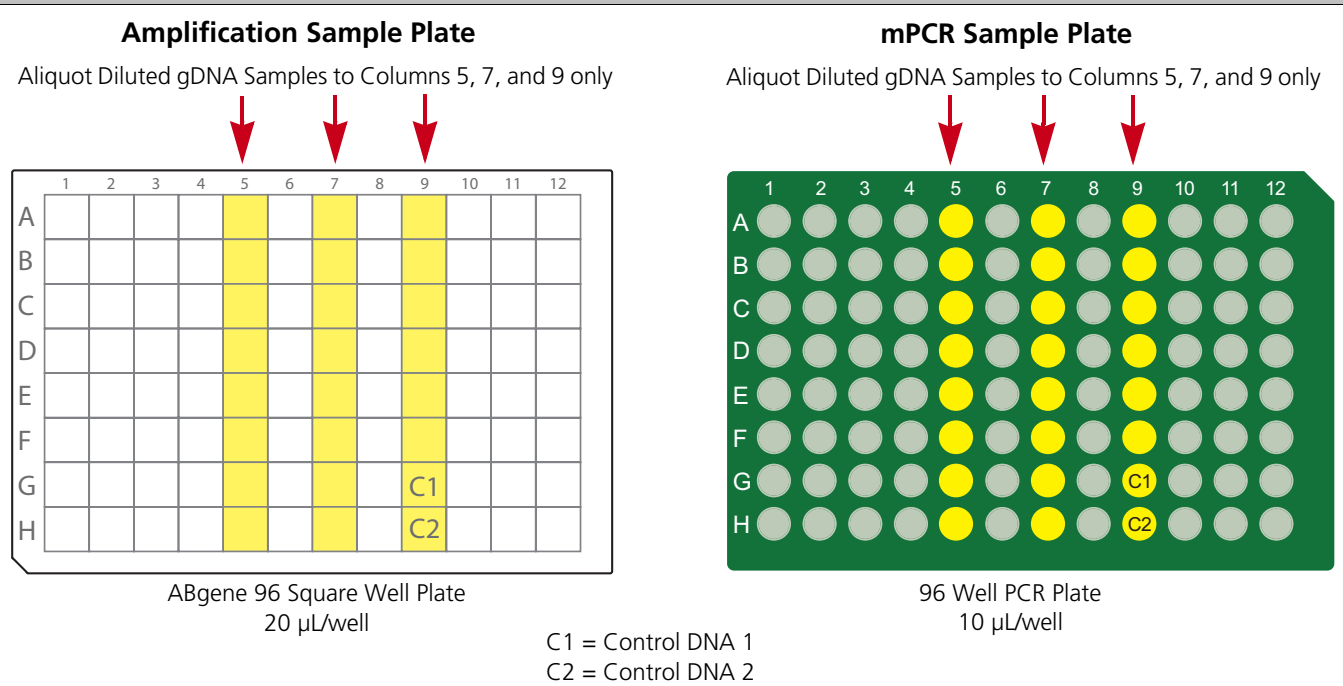
mPCR Sample Plate

- Bio-Rad 96-well plate; HSS-9641 for ABI 9700, ABI Veriti, ABI ProFlex, Bio-Rad 96 well plate; HSP-9631 for Eppendorf Mastercycler pro S

Aliquot Diluted Samples and Controls to Columns 5, 7, and 9 of the mPCR Sample Plate:

1. Aliquot 10 µL of each diluted gDNA sample to columns 5, 7, and 9 of 96-well PCR plate as shown in Figure 2.2.
2. Pipet 10 µL of Control DNA 1 to well G09 and 10 µL of Control DNA 2 to well H09.
3. Seal and spin.

Figure 2.2 Aliquoting to Columns 5, 7, and 9



4. Freeze or Proceed

At this point you can:

- Store the sample plate at –20°C, or
- Proceed to DNA Amplification for Manual Target Prep. See Chapter 4, *Target Preparation* on page 38.

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

5. Create a Batch Registration File

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

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

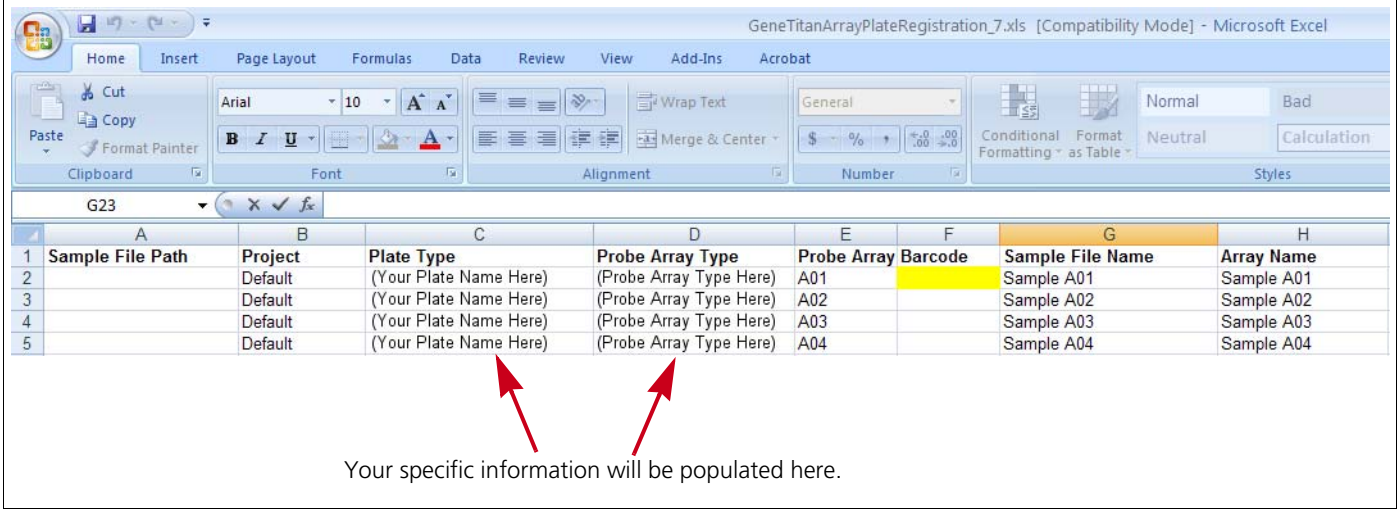
- Data file generation during imaging.
- Tracking the experimental results for each sample loaded onto an array plate.

Detailed instructions for creating this file are located in Appendix C, *Registering Samples in Affymetrix GeneChip® Command Console®* on page 145. See also Figure 2.3 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 2.3 Example of a Batch Registration File



Chapter 3

Preparation Before You Start

Introduction

This manual assay format allows the user to run the PharmacoScan™ Assay for 24 Samples four (4) times using one PharmacoScan™ Reagent Kit 4x24 Reactions and one QIAGEN Multiplex PCR *Plus* Kit (P/N 206152), which must be purchased separately. 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 PharmacoScan Assay.

One key item this manual assay format requires is the use of disposable divided reservoirs with a "trough within a trough" design, which maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent.

A list of all equipment and resources required for the PharmacoScan Assay is in the *PharmacoScan™ Assay 24-Array Format Manual Protocol Site Preparation Guide*, P/N 703287.

PharmacoScan™ Reagent Kit 4x24 Reactions, Arrays, and GeneTitan® Consumables Required

The table below lists the PharmacoScan reagents and GeneTitan consumables required to process four PharmacoScan 24F Array Plates. The table also lists the QIAGEN Multiplex PCR kit required for the PharmacoScan assay. Please refer to the *PharmacoScan™ Assay 24-Array Format Manual Protocol Site Preparation Guide*, P/N 703287 for detailed information regarding the necessary materials required to run the PharmacoScan Assay.

Table 3.1 PharmacoScan™ Assay Arrays, Reagents, and GeneTitan Consumables Required

Part Number	Description	Quantity
902994	PharmacoScan™ 24F Array Plate	4
901606	Axiom® GeneTitan® Consumables Kit	4
902908	PharmacoScan™ Reagent Kit 4x24 Reactions	1
206152	QIAGEN Multiplex PCR *Plus* Kit, 100 Reactions	1

Requirements and Recommendations

This section describes requirements and recommendations for facilities and equipment needed to perform the PharmacoScan Assay 24-Array Format Manual Protocol.

Room Temperature

When referred to in the PharmacoScan Assay 24-Array Format Manual Protocol, room temperature is 18 to 25˚C.

Special Requirements

Amplification Staging Area

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

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

Fume Hood

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

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

Control Requirements

A negative control is not required for this assay.

Two controls are required for proper data analysis. These controls, Control DNA 1 and Control DNA 2, are included in the PharmacoScan Assay Reagent Kit 4x24 Reactions.

Plate Requirements and Recommendations

The following types of plates are required for performing manual target preparation. Refer to the *PharmacoScan™ Assay 24-Array Format Manual Protocol Site Preparation Guide,* P/N 703287, for vendor information.

- ABgene 96 Square Well Storage Plate, 2.2 mL
- Bio-Rad Hard Shell Semi-skirted 96-well plate, P/N HSS-9641 for the ABI 9700, ABI Veriti, and ABI ProFlex thermal cyclers. Use the Bio-Rad Hard Shell Low-profile 96-well plate, P/N HSP-9631 for the Eppendorf Mastercycler pro S. Refer to the *PharmacoScan™ Assay 24-Array Format Manual Protocol Site Preparation Guide,* P/N 703287, for vendor information.
- 96-well UV Star Plates, 370 µL/well

Thermal Cycler Recommendations and Protocols

The following thermal cyclers are recommended for the PharmacoScan Assay 24-Array Format Manual Protocol:

- ABI 9700 (with gold-plated or silver block)
- ABI Veriti
- ABI ProFlex
- Eppendorf Mastercycler pro S

IMPORTANT: Always use the heated lid option when programming protocols. The PharmacoScan mPCR protocol was validated using the "9600 Mode" on the ABI 9700, ABI Veriti, and ABI ProFlex thermal cyclers. The "Safe" mode was used for the Eppendorf Mastercycler pro S. Refer to the manufacturer's instructions for instrument programming.

Figure 3.1 PharmacoScan mPCR Thermal Cycler Protocol (Stage 1A)

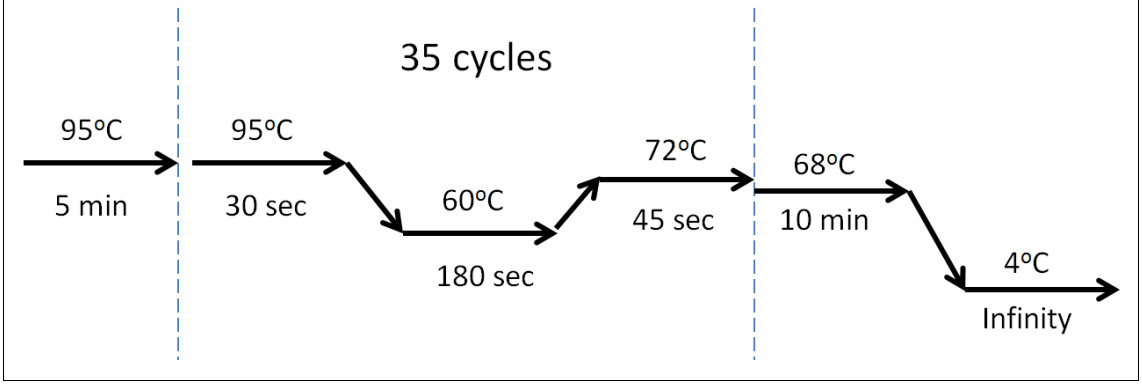
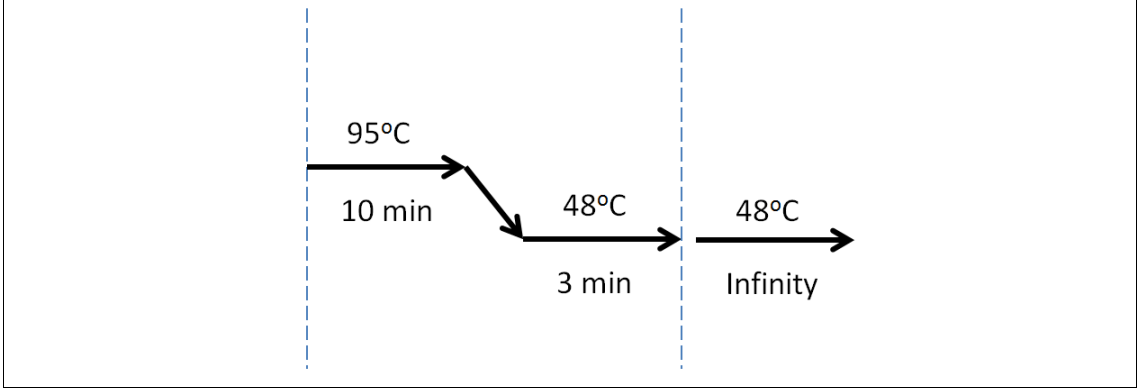


Figure 3.2 PharmacoScan Denature Thermal Cycler Protocol (Stage 4)



WARNING: Evaporation during denaturation can negatively impact assay performance. Use the recommended thermal cycler consumables and sealing film to eliminate condensation and evaporation.

PharmacoScan Assay 24-Array Format Manual Protocol has been validated with the ABI 9700 (with gold-plated or silver block) ABI Veriti, ABI ProFlex, and Eppendorf Mastercycler pro S. Use of other thermal cyclers may result in assay failure and may violate the array and reagent replacement policy.

Thermal Cycler Consumables

Table 3.2 provides details into the consumables to be used with the ABI 9700 thermal cycler.

Table 3.2 Thermal Cycler Consumables for the PharmacoScan Assay 24-Array Format Manual Protocol

Thermal Cycler Model	PCR Plate Type	Seal
ABI 9700	▪ BioRad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (P/N HSS-9641)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
ABI Veriti	▪ BioRad Hard-Shell Full Height 96-well Semi-Skirted PCR Plate (P/N HSS-9641)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
ABI ProFlex	▪ BioRad Hard-Shell Full Height 96-well Semi-Skirted PCR Plate (P/N HSS-9641)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)
Eppendorf Mastercycler pro S	▪ BioRad Hard-Shell Low Profile 96-well Full-Skirt PCR Plate (P/N HSP-9631)	MicroAmp Clear Adhesive Film from Applied Biosystems (P/N 4306311)

Oven Recommendations

The following ovens are recommended:

- ED 56 drying oven by BINDER (replaces BINDER Model ED 53)
  Refer to the *PharmacoScan Assay 24-Array Format Manual Protocol Site Preparation Guide,* P/N 703287, for vendor information.
- Affymetrix GeneChip Hyb Oven 645

NOTE: The GeneChip® Hybridization Oven 640 is currently not supported with the PharmacoScan Assay 24-Array Format Manual Protocol; however, if you want to utilize it in the workflow please contact your Field Service Engineer (FSE) or Affymetrix Technical Support regarding the compatibility of this oven with the PharmacoScan Assay 24-Array Format Manual Protocol.

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

Plate Centrifuge

One plate centrifuge is required for the PharmacoScan Assay 24-Array Format Manual Protocol. Refer to the *PharmacoScan Assay 24-Array Format Manual Protocol Site Preparation Guide,* P/N 703287, for an appropriate plate centrifuge that can be used. When centrifuging and drying pellets as instructed under *Stage 3A: Centrifuge Precipitation Plate and Dry the DNA Pellet* on page 64, the centrifuge must be able to spin down plates at:

- Rcf: 3200 x*g* (4000 RPM for the Eppendorf 5810R with the rotor configuration described in the *PharmacoScan Assay 24-Array Format Manual Protocol Site Preparation Guide,* P/N 703287).
- Temperature: 4°C and room temperature.

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.

Plate Shakers

We recommend using one of the following shakers listed in Table 3.3.

Table 3.3 Shakers

Shaker	Supplier	Part Number
Thermo Scientific™ Compact Digital Microplate Shaker	Thermo Scientific	88880023
Jitterbug™	Boekel Scientific	Model 130 000

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.

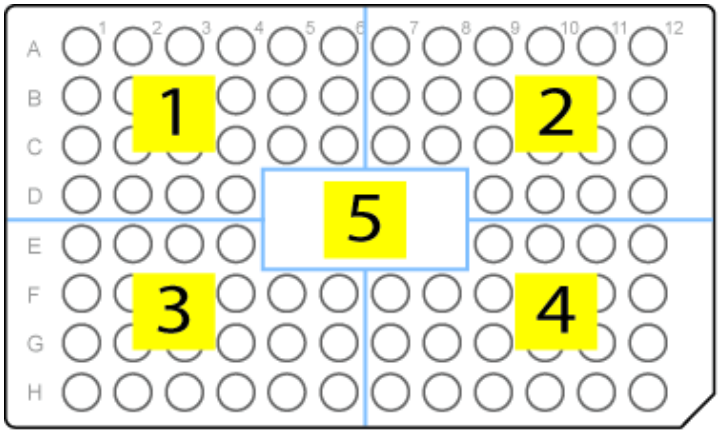
  Blot-dry—Prior to sealing plates, we recommend checking the top of the plate to make sure that there are no droplets. If droplets are present, blot-dry the top of the plate before sealing to ensure a tight seal.

  A. To remove droplets prior to sealing overlay a sheet of Kimwipe across the top of the plate and gently pat down to dry.

  B. Lift the sheet off the plate and discard. Confirm the top of the plate is dry and seal the plate as usual.

- **Vortex:**
  - **Plates:**
    - For deep well plates (such as ABgene 2.2 mL square well storage plates), vortex 5 seconds in each sector for a total of 5 sectors (Figure 3.3).
    - For PCR plates vortex 2 seconds in each sector for a total of 5 sectors (Figure 3.3).
  - **Reagent Vials: 3** times, 1 sec each time.
- **Spin**—when instructed to spin plates or reagent vials, follow these guidelines unless otherwise instructed (for example, when centrifuging and drying pellets, see Step 2 in the section *Stage 3A: Centrifuge Precipitation Plate and Dry the DNA Pellet* on page 64).
  - **Plates**:
    - Spin at 1000 rpm for 30 sec at room temperature.
    - Do not spin for more than 1 min.
  - **Reagent Vials**: 3 sec.

Figure 3.3 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 11 for more information.

About the Reagents and Master Mix Preparation

PharmacoScan Reagent Kit 4x24 Reactions Components

IMPORTANT: This kit includes Module 5.

- **Module 5: Pouch 1 of 2, –25°C to –15°C: P/N 902796**

  Contains an extra tube of Axiom Ligate Enzyme for back-up purposes in the event that it is needed.
- **Module 5: Pouch 2 of 2, 2°C to 8°C: P/N 902797**

  Contains 3 Axiom Hold Buffer bottles that should be used to prepare the Scan Tray for second, third, and fourth plate.

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

IMPORTANT: The PharmacoScan Assay 24-Array Format Manual Protocol is compatible only with reagents from a PharmacoScan Reagent Kit 4x24 Reactions. These reagents are not interchangeable with reagents from other Affymetrix reagent kits, such as SNP 6.0, DMET Plus, etc.

QIAGEN Reagents

QIAGEN Multiplex PCR *Plus* Kit

QIAGEN Multiplex PCR *Plus* Kit (P/N 206152) is used with PharmacoScan Reagent Kit 4x24 Reactions to process 24 samples four times. The QIAGEN kit configuration is as follows:

- 3 tubes of 0.85 mL of Multiplex PCR Master Mix, 2X
- 1 tube of 2 mL of Q-Solution, 5X
- 2 tubes of 1.9 mL of RNase-free Water
- 1 tube of 1.2 mL of CoralLoad Dye, 10X

NOTE: The CoralLoad Dye will not be needed for PharmacoScan Assay 24-Array Format Manual Protocol and can be discarded.

QIAGEN kit mPCR reagents can be freeze-thawed multiple times without affecting assay performance; however, it is convenient to use one tube of 2X Master Mix for one 24-format assay plate. After each use, the tube with remaining 2X QIAGEN Master Mix should be returned to the kit and stored at –20˚C. The fourth assay plate can be processed by thawing and pooling the Master Mix remaining in these three tubes. It is recommended to use 1 tube of Water and Q-Solution to process all four PharmacoScan 24F Array Plates. Freeze unused reagents after each use.

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 a pipette of appropriate size for the volume of liquid being transferred (Table 3.4).

Table 3.4 Recommended Pipette Sizes

Pipette Size	Recommended Volume Range
Single channel P20 / 8-channel P20	1-20 µL
Single channel P200 / 8 and 12-channel P200	20-200 µL
Single channel P1000 / 8-channel P1200	200-1000 µL

- 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 may impact the timing of the protocol and may adversely impact the assay. Pipette substitution may violate the terms of the PharmacoScan Assay 24-Array Format Manual Protocol and array replacement policy.
- Always use pipettes that have been calibrated.
- It is essential that you be proficient with the use of single- and multi-channel pipettes. To familiarize yourself with the use of multi-channel pipettes, we strongly recommend practicing several times before processing actual samples. Use water and reagent reservoirs 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.

Multi-Channel Pipettes

Use 8 or 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.

Using the Divided Reservoir

The PharmacoScan Assay 24-Array Format Manual Protocol utilizes disposable divided reservoirs with a "trough within a trough" design which maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent. During the assay, dispense reagents into either the 8-channel, 4-channel, or both sides, as indicated in Figure 3.4.

Figure 3.4 Dispense Reagents into Divided Reservoir

Fill Both Channels

- Isopropanol
- Hold Buffer

8-Channel Side

- Denat MM
- Amp MM
- Frag MM
- Precip MM
- Hyb MM
- Stain 1 MM
- Neutral Soln
- Stop Soln
- Resuspension Buffer
- Gel Diluent
- Water (for QC)

4-Channel Side

- Stabilize MM
- Stain 2 MM
- Ligation MM

Freeze-Thaw Instructions

Excess volume of the PharmacoScan Reagent Kit may be stored in a freezer at –25°C to –15°C or a refrigerator at 2°C to 8°C to be used in subsequent experiments for up to 60 days after initial use (Table 3.5). Affymetrix recommends that reagents not exceed three freeze-thaw cycles. Please monitor the freeze-thaw cycles of the reagents by following the guidelines below.

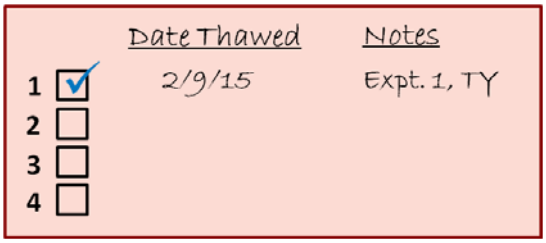
IMPORTANT: PharmacoScan Module A and PharmacoScan Module B reagents are packaged for single-use only and any remaining reagent should be discarded.

Mark Reagent Pouches, Tubes and Bottles to Track Use

To keep track of usage, we recommend that users mark the pouch while the reagents are thawing.

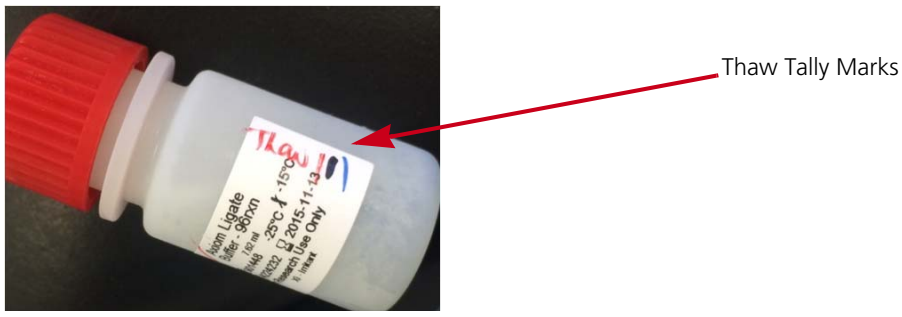
- Using a permanent marker, label the module pouch with "Thaw #1: XX/XX/XX" and any other useful information (i.e., experiment name, user name, etc.).

Figure 3.5 Example of Labeling a Reagent Pouch



- Using a permanent marker, make a tally mark on each reagent tube or bottle to indicate how many times the reagent has been thawed.

Figure 3.6 Example of a properly marked reagent bottle that has been thawed 3 times.



- After the experiment, gather all PharmacoScan reagents.
- Place all tubes and bottles back in the appropriate pouch and place in proper storage temperature. See Table 3.5.

Table 3.5 Reagent Storage Temperature

Storage Temperature	Module 1	Module 2-1	Module 2-2	Module 4-1	Module 4-2
2°C to 8°C			✓		✓
–25°C to –15°C	✓	✓		✓	

NOTE: QIAGEN Multiplex PCR *Plus* Kit reagents are stored at –20°C. Refer to page 24 for recommendations on freeze-thawing components.

Equipment, Consumables, Labware, and Reagents Required

Equipment Required for the PharmacoScan Assay 24-Array Format Manual Protocol

Thermal Cycler

Refer to *Thermal Cycler Recommendations and Protocols* on page 20

Oven

Refer to *Oven Recommendations* on page 21

Plate Centrifuge

Refer to *Plate Centrifuge* on page 21

Plate Shaker

Refer to *Plate Shakers* on page 22

Consumables Required for PharmacoScan™ Assay 24-Array Format Manual Protocol

Table 3.6 Consumables Required for PharmacoScan™ Assay 24-Array Format Manual Protocol

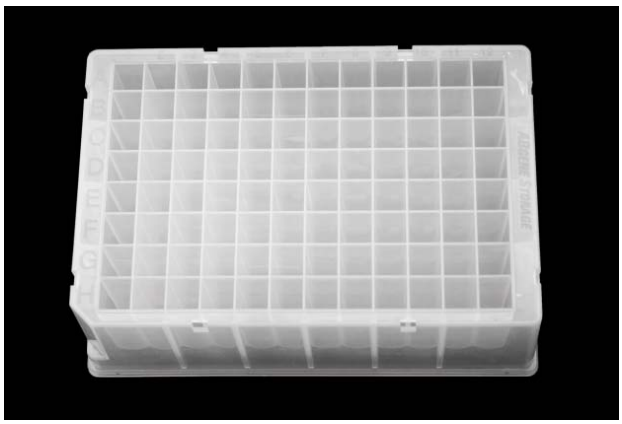
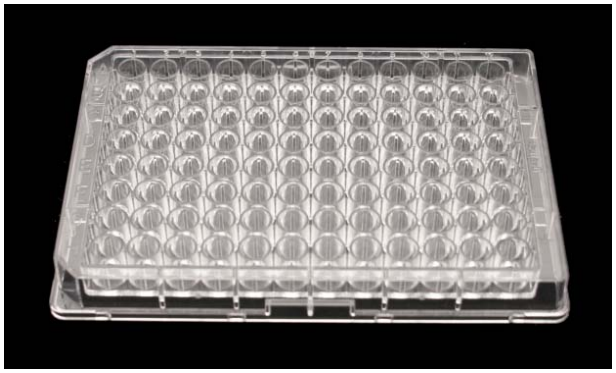
Labware	Supplier and Part Number	Labware Image
ABgene 96 Square Well Storage Plate, 2.2 mL ABgene Storage Plate, 96-well, 2.2 mL, square well, conical	Thermo Fisher Scientific (formerly Life Technologies) P/N AB-0932	
OD Plate Greiner UV-Star® 96 well plates	Sigma-Aldrich, VWR International, Fisher Scientific P/N 655801 E&K P/N 25801	

Table 3.6 Consumables Required for PharmacoScan™ Assay 24-Array Format Manual Protocol (Continued)

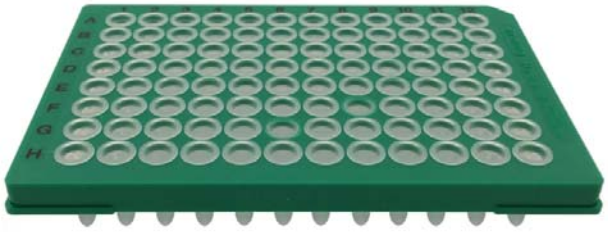
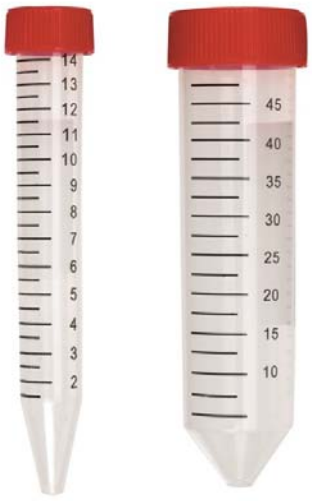
Labware	Supplier and Part Number	Labware Image
Bio-Rad Hard Shell 96-well plate Bio-Rad Hard-Shell® Low-Profile 96-Well Skirted PCR Plates **NOTE:** Please refer to Table 3.2 for the PCR plate type recommended for your specific thermal cycler.	Bio-Rad P/N HSP-9631	
96 Half-Skirt Plate Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plates **NOTE:** Please refer to Table 3.2 for the PCR plate type recommended for your specific thermal cycler.	BioRad P/N HSS-9641	
1.7 mL Microcentrifuge Tubes, DNAse and RNAse-free	Common labware - order through your preferred labware supplier	
8-well strip tubes with caps, DNAse and RNAse-free	Common labware - order through your preferred labware supplier	
50 mL and 15 mL Conical-bottom Centrifuge Tubes, Polypropylene	Various	

Table 3.6 Consumables Required for PharmacoScan™ Assay 24-Array Format Manual Protocol (Continued)

Labware	Supplier and Part Number	Labware Image
Zerostat Anti-static Gun and Ion-Indicator Cap	Milty Zerostat, Affymetrix P/N 74-0014	
96-well Block Cooling Chamber for 0.2 mL tubes, 96 holes (4 for 1.5 mL & 6 for 0.5 mL tubes), Dim.: 6 1/8″L x 3 1/8″W x 1″ H	Diversified Biotech P/N CHAM-1000	
25 mL Reagent Reservoir with Divider	Thermo Fisher Scientific P/N 8095	

GeneTitan® MC Instrument Consumables

All consumables for the GeneTitan MC Instrument are provided by Affymetrix. Table 3.7 provides guidance on the consumables that are shipped with the array plate.

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

Table 3.7 PharmacoScan™ GeneTitan Tray Consumables (from the Axiom® GeneTitan® Consumables Kit, P/N 901606)

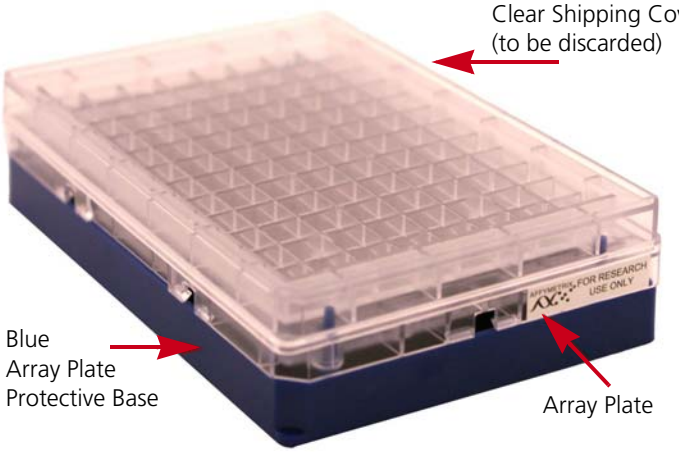
Item	Part Number	Labware Image	Information
PharmacoScan 24F Array Plate	902994 Box 550745 Pouch	**NOTE:** Array plate is not included in the Axiom® GeneTitan® Consumables Kit.  	24-Array Plate: ▪ Comprised of three parts: clear plastic cover, array plate, and blue array plate protective base. ▪ The clear plastic cover for the array plate protects the array plate during transport. Discard after opening pouch. ▪ The array plate must always be kept in the blue array plate protective base at all times. The blue array plate protective base in the package holds the array and protects it from damage or exposure to dust.

Table 3.8 Axiom® GeneTitan® MC Instrument Consumables (from the Axiom® GeneTitan® Consumables Kit, P/N 901606)

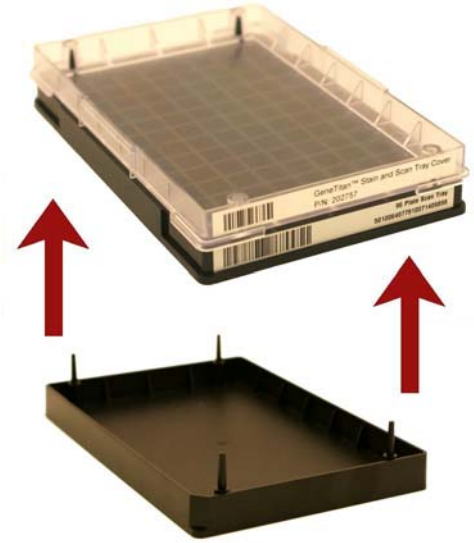
Item	Part Number	Labware Image	Information
Scan Tray	900746 Box 501006 Pouch	  ▪ The Scan Tray must be loaded into the GeneTitan Instrument with the Scan Tray Cover only. ▪ Do not load the Scan Tray with the protective base still on.	96-Plate Scan Tray: ▪ Comprised of three parts: scan tray, black protective base, and a scan tray cover. ▪ The black scan tray protective base in the package protects the glass bottom of the scan tray from damage before it is loaded into the GeneTitan MC Instrument. ▪ The scan tray cover protects the contents in the scan tray and must be deionized before used. See Appendix D, *Deionizing Procedure for GeneTitan® Trays and Covers* on page 148. ▪ Remove the black scan tray protective base before loading the scan tray with the scan tray cover into the GeneTitan MC Instrument.
Black Scan Tray Protective Base, shown without the Scan Tray with cover			▪ The black scan tray protective base in the package is used to protect the bottom of 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 set aside the protective base from the scan tray before loading.

Table 3.8 Axiom® GeneTitan® MC Instrument Consumables (from the Axiom® GeneTitan® Consumables Kit, P/N 901606) (Continued)

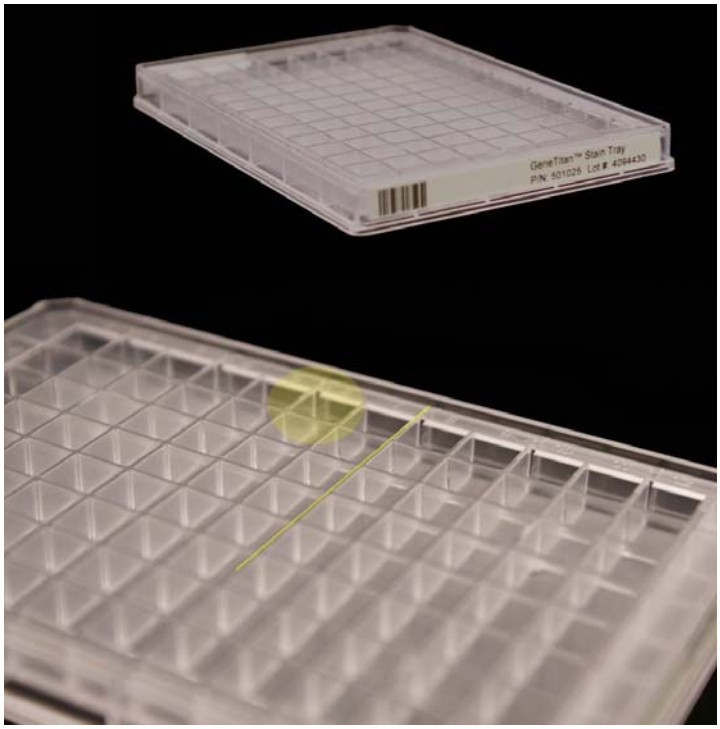
Item	Part Number	Labware Image	Information
Scan Tray with cover, shown without the black protective base			▪ The GeneTitan scan tray must be loaded with the scan tray cover into the GeneTitan MC Instrument. ▪ **Do not load the scan tray with the protective base**.
GeneTitan 5 Stain Trays Kit	4249910 Kit 501025 Tray		▪ The GeneTitan Stain Tray Kit comes with 5 stain trays packaged in zip-top bags to keep them free of dust. ▪ The GeneTitan stain trays are barcoded and the trays have separator walls that are flush with the frame of the stain tray, as shown by the yellow line and the yellow oval in the lower photo.

Table 3.8 Axiom® GeneTitan® MC Instrument Consumables (from the Axiom® GeneTitan® Consumables Kit, P/N 901606) (Continued)

Item	Part Number	Labware Image	Information
GeneTitan® Stain and Scan Tray Cover	202757		▪ The GeneTitan stain and scan tray covers prevent evaporation of the stains in stain trays and the array holding buffer in the scan tray. ▪ All stain and scan trays must be placed in the GeneTitan MC Instrument with the GeneTitan stain tray cover. ▪ All tray covers must be deionized to remove static electricity prior to placing the cover on the tray. ▪ See the section *Deionizing Procedure for GeneTitan® Trays and Covers* on page 148 for the anti-static procedure.
GeneTitan stain tray shown with the stain tray cover	Tray 501025 Cover 202757		
Hybridization Tray	900747		▪ After aliquoting the denatured Hyb Ready samples into the hybridization tray, the tray should be immediately loaded into the GeneTitan MC 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.

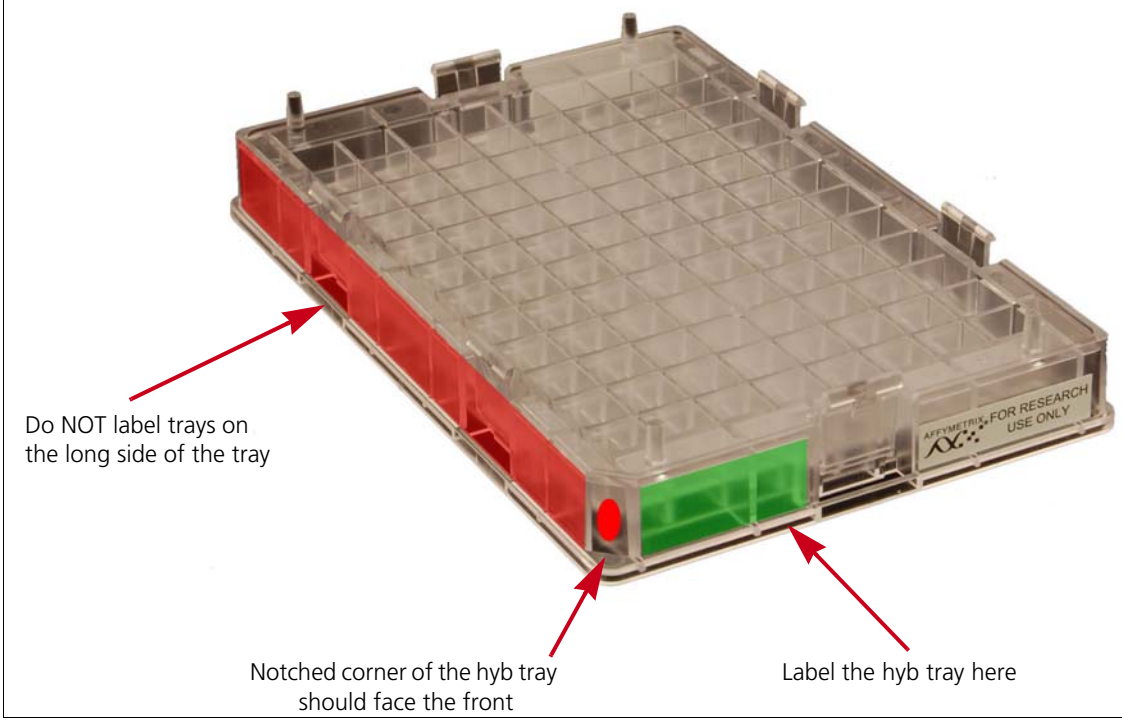
Proper labeling for hyb trays and reagent trays is described in:

- *Labeling for Hyb Trays*, below
- *Labeling for Stain Trays* on page 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 3.7. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.

Figure 3.7 Labeling GeneTitan Hyb Trays

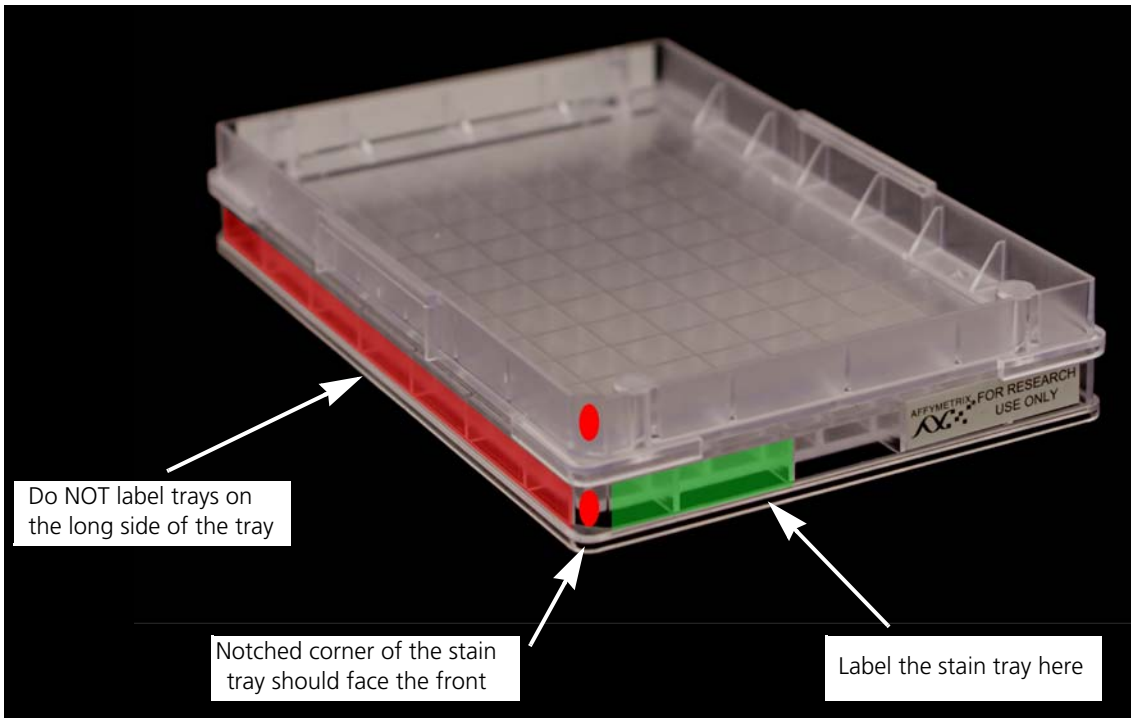


CAUTION: Writing on the wrong side of the hyb tray, or on the wrong part of the long side, may interfere with the operation of sensors in the GeneTitan MC Instrument.

Labeling for Stain Trays

You may label the stain trays on the **left side of the front of the tray** as shown in Figure 3.8. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.

Figure 3.8 Labeling GeneTitan Stain Tray



(see *Stage 5: GeneTitan® Reagent Preparation* on page 79 for detailed information).

Reagent Kit for the PharmacoScan™ Assay 24-Array Format Manual Protocol

The PharmacoScan Assay 24-Array Format Manual Protocol uses the PharmacoScan Reagent Kit 4x24 Reactions (P/N 902908). One PharmacoScan Reagent Kit 4x24 Reactions was developed to process four 24 Format samples. Kits consist of 7 modules for different stages of the assay with some modules having both 4˚C and –20˚C pouches. There are specific instructions for which reagents are needed and how to treat them within each stage.

Table 3.9 PharmacoScan™ Reagent Kit 4x24 Reactions, P/N 902908 (Sufficient for four 24-Array Format Plates)

Component		Storage
Module 1: P/N 901711 ▪ Axiom Denat Soln 10X ▪ Axiom Neutral Soln ▪ Axiom Water	▪ Axiom Amp Soln ▪ Axiom Amp Enzyme	–25°C to –15°C
Module 2: Pouch 1 of 2: P/N 901528 ▪ Axiom Frag Enzyme ▪ Axiom 10X Frag Buffer ▪ Axiom Precip Soln 2	▪ Axiom Hyb Buffer ▪ Axiom Hyb Soln 1	–25°C to –15°C
Module 2: Pouch 2 of 2: P/N 901529 ▪ Axiom Frag Diluent ▪ Axiom Frag Rxn Stop ▪ Axiom Precip Soln 1	▪ Axiom Resusp Buffer ▪ Axiom Hyb Soln 2	2°C to 8°C
Module 3 ▪ Axiom Wash Buffer A: PN 901446 (8 bottles per kit) ▪ Axiom Wash Buffer B: PN 901447 (4 bottles per kit)	▪ Axiom Water: PN 901578 (4 bottles per kit)	room temperature
Module 4: Pouch 1 of 2: P/N 901278 ▪ Axiom Ligate Buffer ▪ Axiom Ligate Enzyme ▪ Axiom Ligate Soln 1	▪ Axiom Probe Mix 1 ▪ Axiom Stain Buffer ▪ Axiom Stabilize Soln	–25°C to –15°C
Module 4: Pouch 2 of 2: 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	2°C to 8°C
Module 5: Pouch 1 of 2: P/N 902796 ▪ Axiom Ligate Enzyme		–25°C to –15°C
Module 5: Pouch 2 of 2: P/N 902797 ▪ Axiom Hold Buffer (3 bottles)		2°C to 8°C
PharmacoScan Module A: P/N 902896 ▪ 10X mPCR primers (4 tubes, use 1 per array)		–25°C to –15°C
PharmacoScan Module B: P/N 902897 ▪ Control DNA 1 (4 tubes, use 1 per array) ▪ Control DNA 2 (4 tubes, use 1 per array)		–25°C to –15°C

Chapter 4

Target Preparation

Introduction

Target preparation for the PharmacoScan™ Assay 24-Array Format Manual Protocol enables you to perform target preparation to process 24 samples at a time. The protocol is performed in two parts:

- Part 1: Target Preparation as described in this chapter
- Part 2: Array Processing is performed on the GeneTitan® Multi-Channel (MC) Instrument
  Array handling and processing protocols require the use of a GeneTitan MC Instrument, as described in Chapter 5, *Array Processing with the GeneTitan® Multi-Channel Instrument* on page 96.

IMPORTANT: Read all the instructions in Chapter 3, *Preparation Before You Start* on page 18, before performing manual target preparation.

A list of all equipment and resources required for the PharmacoScan Assay 24-Array Format Manual Protocol is in the *PharmacoScan Assay 24-Array Format Manual Protocol Site Preparation Guide*, P/N 703287.

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

For the PharmacoScan Assay 24-Array Format Manual Protocol, target preparation as well as GeneTitan Reagent Preparation use only **columns 5, 7, and 9** of the plates and trays consumables.

Stage 1A: Multiplex PCR (mPCR)

IMPORTANT: Before proceeding to mPCR or DNA Amplification, perform the gDNA preparation described in **Chapter 2, *Genomic DNA Preparation and Requirements* on page 11.**

The following steps are necessary to perform mPCR:

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

Duration

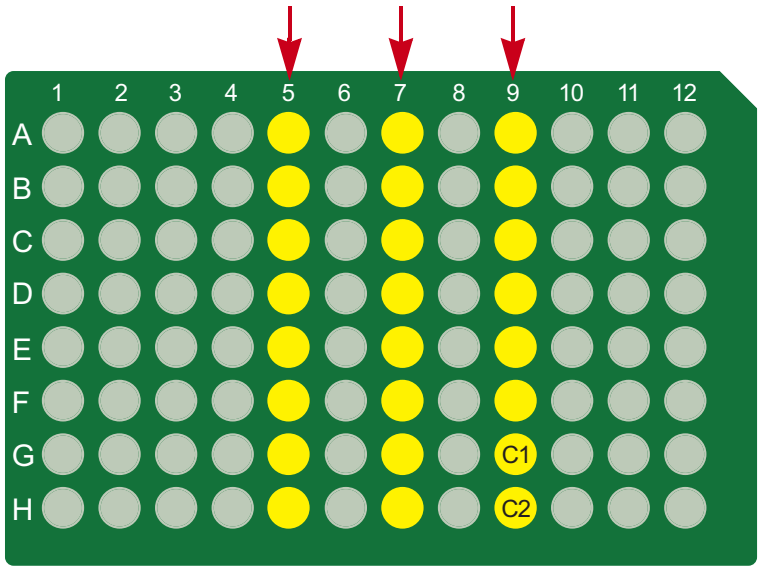
For 24 samples:

- Time to thaw materials: 0.5 hr
- Hands-on time: approximately 0.5 hr
- Thermal Cycler run time: approximately 3 hr
- Total time required: approximately 4 hr

Input Required

The mPCR Sample Plate, with 10 µL of sample diluted to a concentration of 5 ng/µL in columns 5, 7, and 9 of a 96-well PCR plate compatible with your thermal cycler.

Figure 4.1 Aliquoting to Columns 5, 7, and 9



See *Genomic DNA Preparation* on page 14 for more information.

Equipment, Consumables and Reagents Required

Equipment and Consumables

The equipment and consumables listed in Table 4.1 are required for this stage.

Table 4.1 Equipment and Consumables Required for Stage 1A: mPCR

Quantity	Consumable Item
As required	Adhesive seals for 96-well plate-Applied Biosystems MicroAmp clear adhesive film
1	Marker, fine point, permanent
1	Microfuge tube holder
1	Strip tube holder
1	96-well plate holder
1	1.7 mL microfuge tube (RNase/DNase-free)
1	8- or 12- well strip tubes with caps (RNase/DNase-free)
Quantity	**Equipment**
1	Vortex for plates and microtubes
1	Mini microfuge with microtube adapter
1	Mini microfuge with strip tube adapter
1	Aluminum plate block cooled to 4°C
1	Plate centrifuge
1	Ice bucket with ice
1	Thermal Cycler programmed with the **PharmacoScan mPCR** protocol (see *Thermal Cycler Recommendations and Protocols* on page 20).
1 Each	Rainin Pipettes: ▪ Single channel P200 ▪ Single channel P1000 ▪ Multi-channel P200
As Needed	Pipette tips

Reagents Required

Table 4.2 Reagents Required for Stage 1A: mPCR

From the PharmacoScan Reagent Kit 4x24 Reactions	Module
10X Primer Mix, 1 tube	PharmacoScan Module A, P/N 902896
From QIAGEN Multiplex PCR *Plus* Kit (100)*	**Part Number 206152**
2X Multiplex PCR Master Mix (1 tube)	
5X Q-Solution (1 tube)	
RNase-free Water (1 tube)	

* *CoralLoad Dye in kit is not needed for PharmacoScan Assay 24-Array Format Manual Protocol.*

1: Prepare for mPCR

To Prepare for mPCR

1. Power on the thermal cycler programmed with **PharmacoScan mPCR** protocol (refer to Table 3.1 on page 20). Make sure the heated lid option has been selected.
2. Thaw the mPCR Reagents and prepare the mPCR Reaction Plate.

To Thaw and Prepare the Reagents:

1. If the gDNA mPCR Sample Plate was frozen, thaw at room temperature on the benchtop, vortex, and pulse-spin.
2. Place mPCR Sample Plate on ice or a cold aluminum block once thawed.

> **IMPORTANT: gDNA samples must be 10 µL volume at a concentration of 5 ng/µL in columns 5, 7, and 9 of 96-well PCR plate (see Genomic DNA preparation, *mPCR Sample Plate* on page 16).**

3. Thaw the following reagents on the benchtop at room temperature and place on ice once thawed:
   - From –20°C stored Affymetrix PharmacoScan™ Reagent Kit 4x24 Reactions (P/N 902908), PharmacoScan Module A (P/N 902896):
     - 1 tube of 10X mPCR primers
   - From –20°C stored QIAGEN Multiplex PCR *Plus* Kit (P/N 206152):
     - 1 vial of QIAGEN Multiplex PCR Master Mix, 2X
     - 1 vial Q-Solution, 5X
     - 1 vial RNase-free Water

> **IMPORTANT:**
> - **Make sure reagents are thoroughly mixed prior to use.**
> - **Vortex water and Q-Solution.**
> - **Master Mix should be thoroughly mixed by inverting tube 10 times. DO NOT VORTEX.**

2: Prepare the mPCR Master Mix

1. Label a 1.7 mL microfuge tube *mPCR*.
2. To the *mPCR* tube, add the reagents listed in Table 4.3 as follows:
   A. Add water, Q-Solution, and primers to tube. Cap.
   B. Vortex briefly. Pulse spin.
   C. Add the QIAGEN 2X Master Mix to the tube.
   D. Mix thoroughly but gently, by setting P1000 to 700 µL and pipetting the reaction mixture up and down five times. Pulse spin.

Table 4.3 mPCR Master Mix

Reagent	1 Reaction	28 Reactions (>16% extra)
RNase-free Water	2 µL	56 µL
Q-solution	4 µL	112 µL
10X mPCR Primer Mix	4 µL	112 µL
2X QIAGEN Multiplex PCR Master Mix	20 µL	560 µL
Total	**30 µL**	**840 µL**

3. The resulting mPCR Master Mix should be kept in ice and added to the mPCR sample plate as soon as possible after preparation.

3: Set up the mPCR Reaction Plate

1. Confirm the 96-well PCR sample plate is labeled, *mPCR* along with date and any desired experimental details.
2. Place the plate in an aluminum block which had been stored at 4°C.
3. Transfer 100 µL of the mPCR Master Mix to each of the 8 wells of a strip tube.
4. Cap the strip tubes and pulse spin.
5. Use a P-200 mutli-channel pipette to carefully transfer 30 µL of mPCR Master Mix into columns 5, 7, and 9 of the mPCR plate. Final volume of each well is 40 µL.
6. Seal plate with adhesive seal, ensuring seal is firmly pressed down to prevent sample contamination during mixing and evaporation during PCR cycling.
7. Vortex plate for 2 sec in each quadrant twice (refer to *Seal, Vortex, and Spin* on page 22).
8. Spin down at 2000 rpm for 30 sec.
9. Return plate to cold aluminum block until plate can be loaded onto thermal cycler (Note: Load plate onto thermal cycler within five minutes).

4: Freeze QIAGEN Reagents

1. Working quickly, mark and date QIAGEN reagent tubes and return them to kit stored in –20°C freezer (refer to *Freeze-Thaw Instructions* on page 27).
2. Discard any remaining 10X Primer Mix and Control DNAs in appropriate waste container.

IMPORTANT: Do not reuse 10X Primer Mix or Control DNA 1 or Control DNA 2 reagents. These are intended as single use reagents. Sufficient quantities (4 individual tubes) are provided for processing 4 x 24F array plates per kit.

5: Run the PharmacoScan mPCR Thermal Cycler Protocol

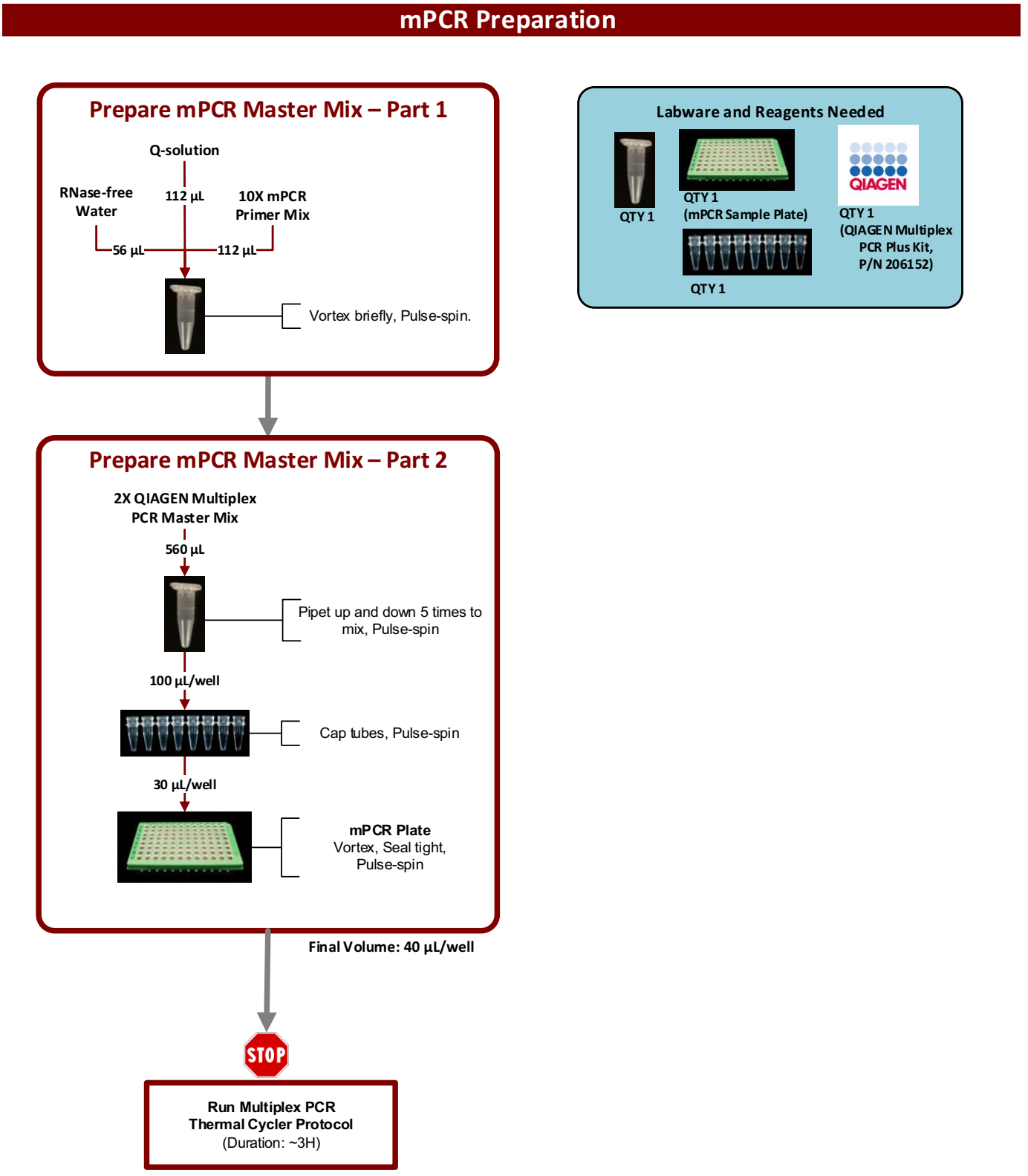
1. Place mPCR on thermal cycler and run **PharmacoScan mPCR** protocol (refer to Figure 3.1 on page 20).

NOTE: The mPCR Sample Plate is now referred to as the mPCR Reaction Plate from this point forward.

6: Freeze mPCR Reaction Plate or Proceed

1. After the **PharmacoScan mPCR** thermal cycler protocol is complete, remove the plate from thermal cycler, pulse spin, and store plate at –20°C. Ensure plate is well sealed to prevent evaporation during storage.
2. The PharmacoScan suggested workflow is to proceed to *Stage 1B: DNA Amplification* on page 45 after the mPCR Reaction Plate has been placed on the thermal cycler.
3. If the Amplification Step has already been completed, and it is desired to proceed to *Stage 2: Fragmentation and Precipitation* on page 52, then the mPCR Reaction Plate can be left at 4°C for several hours until needed for mPCR Spike-In (on page 55).

Figure 4.2 Stage 1A: mPCR Preparation Workflow Diagram



Stage 1B: DNA Amplification

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

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

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

Duration

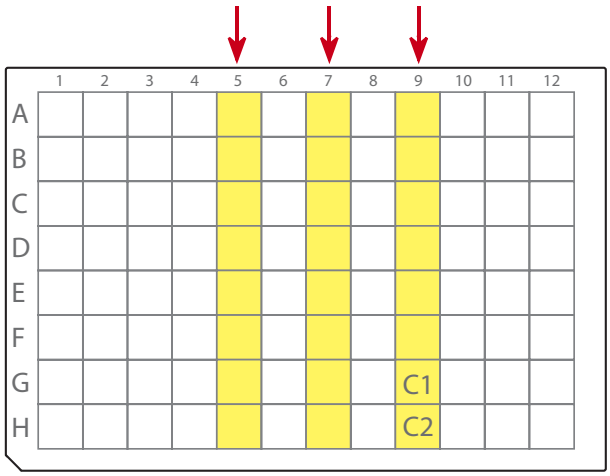
For 24 samples:

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

Input Required

The gDNA Sample Plate, with 20 µL of each gDNA diluted to a concentration of 5 ng/µL in columns 5, 7, and 9 of an **ABgene 96 square well storage plate, 2.2 mL**.

Figure 4.3 Aliquoting to Columns 5, 7, and 9



See *Genomic DNA Preparation* on page 14 for more information.

Equipment, Consumables and Reagents Required

Equipment and Consumables

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

Table 4.4 Equipment and Consumables Required for Stage 1B: DNA Amplification

Quantity	Item
As required	Adhesive seals for 96-well plate - Applied Biosystems MicroAmp Clear adhesive film
1	Cooler, chilled to –20°C 
1	Microcentrifuge tube holder
1	15 mL tube holder
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1 each	Rainin Pipettes: ▪ Single-channel P200 ▪ Single-channel P1000 ▪ Multi-channel P20 ▪ Multi-channel P200 ▪ Multi-channel P1200
As needed	Pipette tips
As needed	Pipette, serological ▪ 5 x 1/10 mL (VWR P/N 89130-896) ▪ 10 x 1/10 mL (VWR P/N 89130-898)
1	Pipet aid
1	Plate centrifuge, at room temperature
1	Oven, set at 37°C
1	1.7 mL polypropylene microcentrifuge tube (RNAse/DNAse-free)
1	15 mL conical tube
1	Vortexer
1	Timer
3	Matrix™ 25 mL divided Reagent Reservoir P/N 8095

Reagents Required

Table 4.5 Reagents Required for Stage 1B: DNA Amplification

From the PharmacoScan™ Reagent Kit 4x24 Reactions	Module
Axiom Denat Soln 10X	Module 1, –20°C P/N **901711**
Axiom Neutral Soln	
Axiom Amp Soln	
Axiom Water	
Axiom Amp Enzyme	

1: Prepare for DNA Amplification

To Prepare for DNA Amplification

1. Set an incubator/oven temperature at 37°C.
   We recommend using one of these ovens:
   - Binder ED 56
   - Affymetrix GeneChip® 645 Hybridization Oven (turn rotation on to 15 rpm)
2. Set the centrifuge temp to room temperature.
3. Thaw and Prepare the Reagents and Sample Plate.

To Thaw and Prepare the Reagents:

1. Thaw the Sample Plate on the benchtop at room temperature and pulse-spin.

   IMPORTANT:
   - **gDNA samples must be brought to room temperature before proceeding with denaturation.**
   - **gDNA samples must be 20 µL volume of each gDNA at a concentration of 5 ng/µL in columns 5, 7, and 9 of an ABgene 96 square well storage plate, 2.2 mL (see *Genomic DNA Preparation* on page 14).**

2. Thaw the following reagents in a small water bath on the benchtop at room temperature (small water bath: small tray or container, such as a pipet tip box, filled with fresh filtered water):
   - Axiom Denat Soln 10X
   - Axiom Neutral Soln
   - Axiom Amp Soln
   - Axiom Water
   - Leave the Axiom Amp Enzyme in the cooler in the freezer until ready to use.
3. Vortex all reagents (except Axiom Amp Enzyme), then place at room temperature.
   - Axiom Amp Soln: Vortex for 30 sec to thoroughly mix.
   - Axiom Neutral Soln: Vortex for 30 sec to thoroughly mix.
   - Axiom Denat Soln 10X: Vortex and pulse-spin before use.
   - Axiom Amp Enzyme: Gently invert and flick the tube 3 times to mix and pulse-spin just before use.

   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. The bottles can also be thawed in a dish with Millipore water. The Axiom Amp Soln must be thoroughly mixed before use.

4. Label the 1.7 mL microcentrifuge tube and the 15 mL conical tube as indicated in the table below:

Table 4.6 Labeling Tubes

Label	Tube Size	Temperature	Contents
▪ *D MM*	1.7 mL	leave tube at room temperature	Denaturation Master Mix
▪ *Amp MM*	15 mL	leave tube at room temperature	Amplification Master Mix

5. Label three Matrix 25 mL divided Reagent Reservoirs (P/N 8095) as indicated in the table below:

Table 4.7 Labeling Reagent Reservoirs for DNA Amplification

Label	Temperature	Contents	Reservoir Side
▪ *D MM*	Leave reservoir at room temperature	Denaturation Master Mix	8-channel
▪ *N Soln*	Leave reservoir at room temperature	Neutralization Solution	8-channel
▪ *Amp MM*	Leave reservoir at room temperature	Amplification Master Mix	8-channel

2: Prepare the Denaturation Master Mix

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

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

Table 4.8 Preparing Denaturation Master Mix (*D MM*)

Reagent	per Sample	Master Mix 24+
To the 1.7 mL tube marked *D MM*, add:		
Axiom Denat Soln 10X	2 µL	116 µL
Axiom Water	18 µL	1044 µL
Total Volume	**20 µL**	**1160 µL**

2. Vortex, pulse spin, and leave at room temperature.

3: Add Denaturation Master Mix to Samples

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

1. Pulse-spin the Sample Plate.

   Remember: Samples must be at room temperature for this step.
2. Using a P1000, gently pipet the Denaturation Master Mix into the 8-channel side of the reagent reservoir marked *D MM*.
3. Carefully remove the seal from the Sample Plate and discard the seal.
4. Using a P20 8-channel pipette, add **20 µL of Denaturation Master Mix** to each sample in columns 5, 7, and 9 (Total Volume: 40 µL/well).
   - Pipet directly into the liquid of each well. Do not mix by pipetting up and down.
   - Change tips between each addition.
   - This plate is now known as the Denaturation Plate.
5. Seal and vortex the Denaturation Plate. Start the timer for a **10 minute incubation** after vortexing.

6. Pulse-spin the Denaturation Plate at room temperature.

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

7. Visually examine the volume in each well.
   A. Keep a record of any wells that visually appear to have a particularly low or high volume; these samples may need to be repeated.
   B. Do **NOT** stop to measure volumes; proceed without delay.
8. Complete the **10 minute incubation** on the benchtop at room temperature.
   While completing the incubation at room temperature, prepare the Neutralization Soln as described in Step 1 on page 49.
9. After incubation **immediately** add the Neutralization Soln as described in *4: Add Neutralization Solution to Samples* on page 49.

4: Add Neutralization Solution to Samples

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

1. Measure 3.64 mL of Axiom Neutral Soln and slowly pipet the reagent into the 8-channel side of the reagent reservoir marked *N Soln*.
2. Carefully remove the seal from the Denaturation Plate and discard the seal.
3. Using a P200 8-channel pipette, add **130 µL of Axiom Neutral Soln** to each sample (Total Volume: 170 µL/well).
   - Pipet down the wall of each well. Change tips between each addition.
   - The plate is now known as the Neutralization Plate.
4. Seal, vortex, and pulse-spin the Neutralization Plate.
5. Visually examine the volume in each well (should be ~170 µL/well) and:
   A. Keep a record of any wells that visually appear to have a particularly low or high volume; these samples may need to be repeated.
   B. Do **NOT** stop to measure volumes.
6. Proceed immediately to *5: Prepare the Amplification Master Mix* on page 49.

5: Prepare the Amplification Master Mix

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

1. Per Table 4.9, pipet the appropriate amount of PharmacoScan Amp Soln into the 15 mL tube labeled *Amp MM* at room temperature.

Table 4.9 Amplification Master Mix (*Amp MM*)

Reagent	Per Sample	Master Mix
To the 15 mL tube marked *Amp MM*, add:		
Axiom Amp Soln	225 µL	6.75 mL
Axiom Amp Enzyme	5 µL	150 µL
Total Volume	**230 µL**	**6.90 mL**

2. Remove the Axiom Amp Enzyme from the freezer and place in a portable cooler at –20°C.
   A. Invert and flick the Axiom Amp Enzyme tube three times, then pulse-spin.
   B. Per Table 4.9 on page 49, add the appropriate amount of Axiom Amp Enzyme to the tube labeled *Amp MM*.
   C. Vortex the Amplification Master Mix well, invert the tube 2 times, and then vortex again.

6: Add Amplification Master Mix to Samples

1. *Slowly* pour the Amplification Master Mix to the 8-channel side of the reagent reservoir labeled *Amp MM*.
2. Carefully remove the seal from the Neutralization Plate and discard the seal.
3. Using a P1200 8-channel pipette, ***slowly*** add **230 µL Amplification Master Mix** to each sample of the Neutralization Plate.
   - Pipet down the wall of the well (Total Volume: 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.
4. Seal tightly, vortex twice, and spin the Amplification Plate for one minute at 1000 rpm (as described in *Seal, Vortex, and Spin* on page 22).
5. Place the sealed Amplification Plate in an oven set at **37**°C and leave **undisturbed for 23 ±1 hr**.

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

7: Freeze Reagents

Store remaining Module 1 reagents for future use. Follow guidelines presented in the section *Freeze-Thaw Instructions* on page 27.

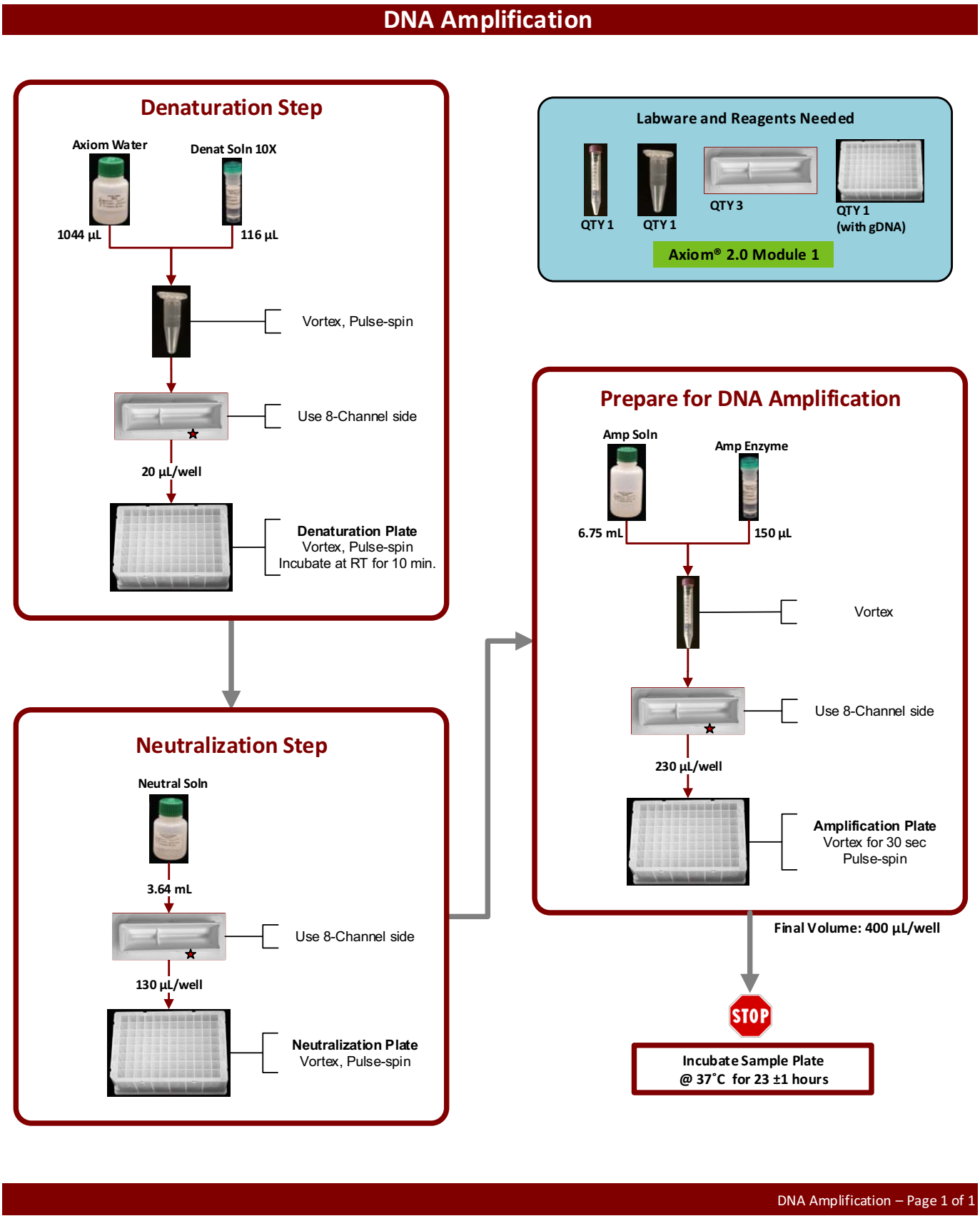
8: Freeze or Proceed

After the incubation finishes, you can either:

- Proceed to *Stage 2: Fragmentation and Precipitation* on page 52.
- Store the Amplification Plate at –20°C.

NOTE: If freezing, do not perform the stop amplification reaction step or the mPCR Spike-In before you store the Amplification Plate at –20°C. The Stop Amplification Reaction step and mPCR Spike-In will be performed after thawing the frozen plate, as described in *1: Prepare for Fragmentation and Precipitation* on page 53.

Figure 4.4 Stage 1B: Amplification Workflow Diagram



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

- mPCR Reaction Plate from *Stage 1A: Multiplex PCR (mPCR)* on page 39.
- Amplification Plate from *Stage 1B: DNA Amplification* on page 45.

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	Freezer set to –20°C (Designate a shelf where the precipitation plates can be left undisturbed)
1	Cooler, chilled to –20°C
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1 each	Rainin Pipettes: ▪ Single channel P1000 ▪ Single channel P200 ▪ Multi-channel P20 ▪ Multi-channel P200 ▪ Multi-channel P1200
As needed	Pipette tips for pipettes listed above
1	Pipet-aid
As needed	Pipette, serological ▪ 5 x 1/10 mL ▪ 10 x 1/10 mL
1	Plate centrifuge set at room temp
1	Mini microcentrifuge (microfuge with microtube rotor)

Table 4.10 Equipment and Consumables Required for Stage 2: Fragmentation and Precipitation

Quantity	Item
2-3	Ovens (see *Oven Recommendations* on page 21): ▪ One oven set at 37°C ▪ One oven set to 65°C
2	15 mL conical tube
4	Matrix™ 25 mL divided Reagent Reservoir P/N 8095
1	Vortexer

Reagents Required

Table 4.11 Reagents Required for Stage 2: Fragmentation and Precipitation

Reagent	Module
From the PharmacoScan Reagent Kit 4x24 Reactions	
Axiom Frag Enzyme (leave at –20°C until ready to use)	Module 2-1, –20°C P/N 901528
Axiom 10X Frag Buffer	
Axiom Precip Soln 2	
Axiom Frag Diluent	Module 2-2, 2–8°C P/N 901529
Axiom Frag Rxn Stop	
Axiom Precip Soln 1	
User-supplied - *Refer to the PharmacoScan Assay 24-Array Format Manual Protocol Site Preparation Guide*, P/N 703287	
Isopropanol (2-Propanol), 99.5%	24 samples: 20 mL per 24-array format plate

1: Prepare for Fragmentation and Precipitation

Set Ovens and Centrifuge

1. Set two incubators/ovens as follows, preferably the night before:
   - One oven set at 37°C. Use an oven that can sustain a constant temperature of 37°C and has a temperature accuracy of ±1°C.
   - One oven set at 65°C.
2. Set the centrifuge temp to room temperature.

TIP: Keep a set of balance plates ready to minimize any time delays before spinning the Fragmentation Plate in-between steps.

Thaw and Prepare the Amplified DNA Samples, mPCR Reaction Plate, and Reagents

Thaw and Prepare the Amplified DNA Sample Plate

If the Plate of Amplified DNA Samples is Frozen (skip this step if the Amplified Sample Plate was not frozen at the end of the previous stage):

1. Place the deep-well plate in a small water bath.
   For example, pour fresh filtered 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 the plate 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 re-seal the plate using 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 mPCR Reaction Plate

If the mPCR Reaction Plate is frozen:
(skip this step if the mPCR Reaction Plate was not frozen at the end of Stage 1A):

1. Thaw the plate at room temperature for about 30 minutes or until all samples are thawed.
2. Spin down the plate at 1000 rpm for 30 sec.
3. To avoid cross-contamination of wells during vortexing, remove the seal and tightly re-seal the plate using a fresh seal.
4. Vortex the plate for 10 sec to thoroughly mix.
5. Spin at 1000 rpm for 30 sec.

Thaw and Prepare the Reagents

To Thaw and Prepare the Fragmentation Reagents:

1. Axiom 10X Frag Buffer:
   - Thaw on the benchtop at room temperature then place on ice.
   - Vortex before use.
2. Axiom Frag Diluent:
   - Place on ice.
   - Vortex and pulse-spin before use.
3. Frag Enzyme: Leave at –20°C until ready to use. Just before use, gently flick the tube 3 times to mix and pulse-spin.

To Thaw and Prepare the Precipitation Reagents:

1. Axiom Precip Soln 1
   - Place on benchtop to warm to room temperature.
   - Vortex before use.
2. Axiom Precip Soln 2:
   - Thaw on the bench top at room temperature then place on ice.
   - Vortex and pulse-spin before use.
3. Isopropanol (user-supplied)
   - Keep at room temperature.

Label Tubes And Reagent Reservoirs

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

Table 4.12 Label Conical Tubes

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

2. Label four Matrix 25 mL divided Reagent Reservoirs (P/N 8095) as indicated in the table below.

Table 4.13 Label Reagent Reservoirs for Fragmentation and Precipitation

Label	Temperature	Contents
▪ Frg MM	Leave reservoir at room temperature	Fragmentation Master Mix
▪ *Stop*	Leave reservoir at room temperature	Frag Rxn Stop
▪ *Precip MM*	Leave reservoir at room temperature	Precipitation Master Mix
▪ *ISO*	Leave reservoir at room temperature	Isopropanol

2: mPCR Spike-In to Amplification Plate

- If proceeding directly from the end of *Stage 1B: DNA Amplification* on page 45, remove the Amplification Plate from the 37°C oven.
- If working with a thawed Amplification Plate, change the seal, vortex, and pulse-spin the Amplification Plate.

! IMPORTANT: Make sure that the mPCR Reaction Plate has been thoroughly mixed before adding to Amplification Plate.

1. Carefully transfer 10 µL of the mPCR reaction into the corresponding well of the Amplification Plate. Pipette should be submerged in sample and mixing performed by pipetting up and down a few times. Ensure complete liquid transfer from pipette tip.
2. Seal plate well. Ensure the seal is securely attached to the plate to minimize evaporation during next steps.
3. Thoroughly mix by vortexing plate for 30 seconds and pulse spin.
4. Immediately proceed to next step, Incubate Samples in Pre-heated Ovens

3. Incubate Samples in Pre-heated Ovens

Stop the DNA Amplification Reaction:

1. Place the Amplification Plate in the 65°C oven.
2. Incubate for 20 minutes.

Prepare for Fragmentation:

1. Place the Amplification Plate in the 37°C oven:
   - Remove the Amplification Plate from the 65°C oven and check the seal. Press on the seal, if needed, then transfer the plate to the 37°C oven.
2. Incubate for 45 minutes.

4: Prepare the 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 4.14 Fragmentation Master Mix

Reagent	per Sample	Master Mix 24+
To the 15 mL tube marked *Frg MM*, add:		
Axiom 10X Frag Buffer	45.7 µL	1.69 mL
Axiom Frag Diluent	10.3 µL	381 µL
Axiom Frag Enzyme	1.0 µL	37 µL
Total Volume	**57 µL**	**2.11 mL**

   - Add the reagents from Table 4.14 to the *Frg MM* tube in the order shown, using appropriate single channel 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 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. Using a P1000 pipet, slowly transfer the Fragmentation Master Mix in the 8-channel side of the reagent reservoir labeled *Frg MM* placed at room temperature.

5: Add the Fragmentation Master Mix to Samples

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 8-channel pipette to add **57 µL of Fragmentation Master Mix** to each reaction. Do not mix by pipetting up and down.
   - Change tips after each addition.
   - After adding the Fragmentation Master Mix to the plate, the plate is now known as the Fragmentation Plate.
4. Seal the Fragmentation Plate and vortex twice.
5. Start the timer for **30 min**.
6. Pulse-spin the Fragmentation Plate in the plate centrifuge at room temperature.

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 *6: Add the Stop Solution to the Samples*, below.

6: Add the Stop Solution to the Samples

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

1. A few minutes before the end of the 30 minute incubation period, measure 988 µL of the Axiom Frag Rxn Stop solution and transfer into the 8-channel side of the reagent reservoir labeled *Stop*.
2. Remove the Fragmentation Plate from the oven and place on the bench top at room temperature.
3. At the **end of the 30 minute fragmentation incubation period**, carefully remove the seal from the Fragmentation Plate and discard the seal.
4. Using a P20 8-channel pipette, end the fragmentation reaction by adding **19 µL of Stop Solution** to each reaction. Do not mix by pipetting up and down.
   - Pipet directly into the liquid of each well.
   - Change tips after each addition.
   - Proceed immediately to the next step.
5. Seal and vortex and do a quick spin at 1000 rpm.
6. Leave the Fragmentation Plate on the benchtop while you prepare the Precipitation Master Mix.

7: Prepare the Precipitation Master Mix

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

1. Prepare Precipitation Master Mix in the 15 mL conical tube labeled *Precip MM*.

Table 4.15 Precipitation Master Mix

Reagent	per Sample	Master Mix 24+
To the 15 mL tube marked *Precip MM*, add:		
Axiom Precip Soln 1	238 µL	6.19 mL
Axiom Precip Soln 2	2 µL	52 µL
Total Volume	**240 µL**	**6.24 mL**

NOTE: Use a 5 mL serological pipette to pipet PharmacoScan Precip Soln 1.

2. Vortex the *Precip MM* tube and place on benchtop at room temperature.
3. Pour the Precipitation Master Mix into the 8-channel side of the reagent reservoir labeled *Precip MM*.
4. Carefully remove the seal from the Fragmentation Plate and discard the seal.
5. Using a P1200 8-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, vortex, and pulse-spin the Precipitation Plate.

8: Prepare and Add Isopropanol to Precipitation Plate

1. Remove the Precipitation Plate from the centrifuge and place on the benchtop at room temperature.
2. Pour 20 mL of isopropanol into both sides of the reagent reservoir labeled *ISO*.
3. Carefully remove the seal from the Precipitation Plate and discard the seal.
4. Using a P1200 8-channel pipette, add **600 µL isopropanol** to each sample and mix well by pipetting up and down 6-7 times within the solution. Observe the solution while it is within the tips - it should look homogeneous after pipetting 5-7 times. If not, repeat mixing a few more times until the solution looks homogeneous.

   Do not vortex the plate after isopropanol addition to avoid cross-contamination of the samples.

   Change the tips after each addition.
5. Blot the top of the plate with a Kimwipe and seal tightly with a MicroAmp Adhesive Seal.

9: Freeze the Precipitation Plate Overnight

Carefully transfer the Precipitation Plate into the –20˚C freezer and **incubate overnight (16-24 hrs)**.

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

10: Freeze Reagents

Store remaining Module 2-1 and Module 2-2 reagents for future use. Follow guidelines presented in the section *Freeze-Thaw Instructions* on page 27.

Store the mPCR Plate at –20˚C for optional QC purposes if desired. See Appendix F, *mPCR Quality Control Gel Protocol* on page 163.

Figure 4.5 mPCR Spike-In Flowchart

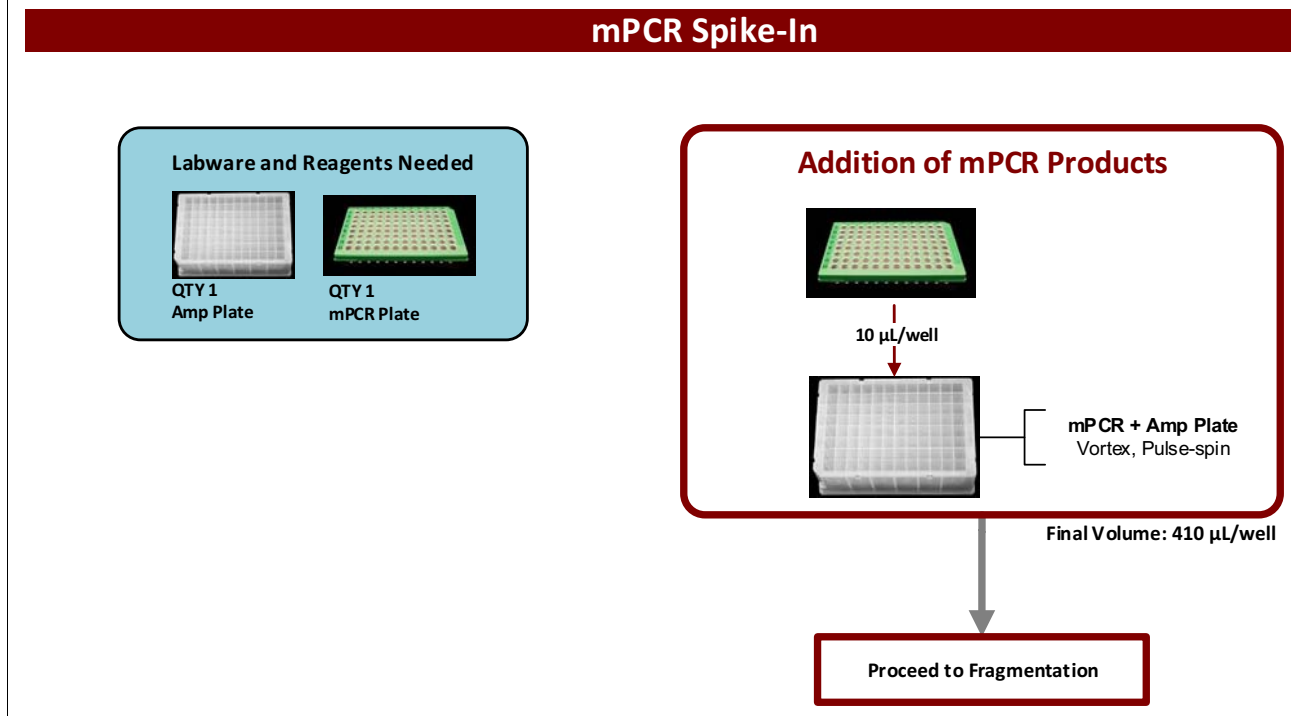
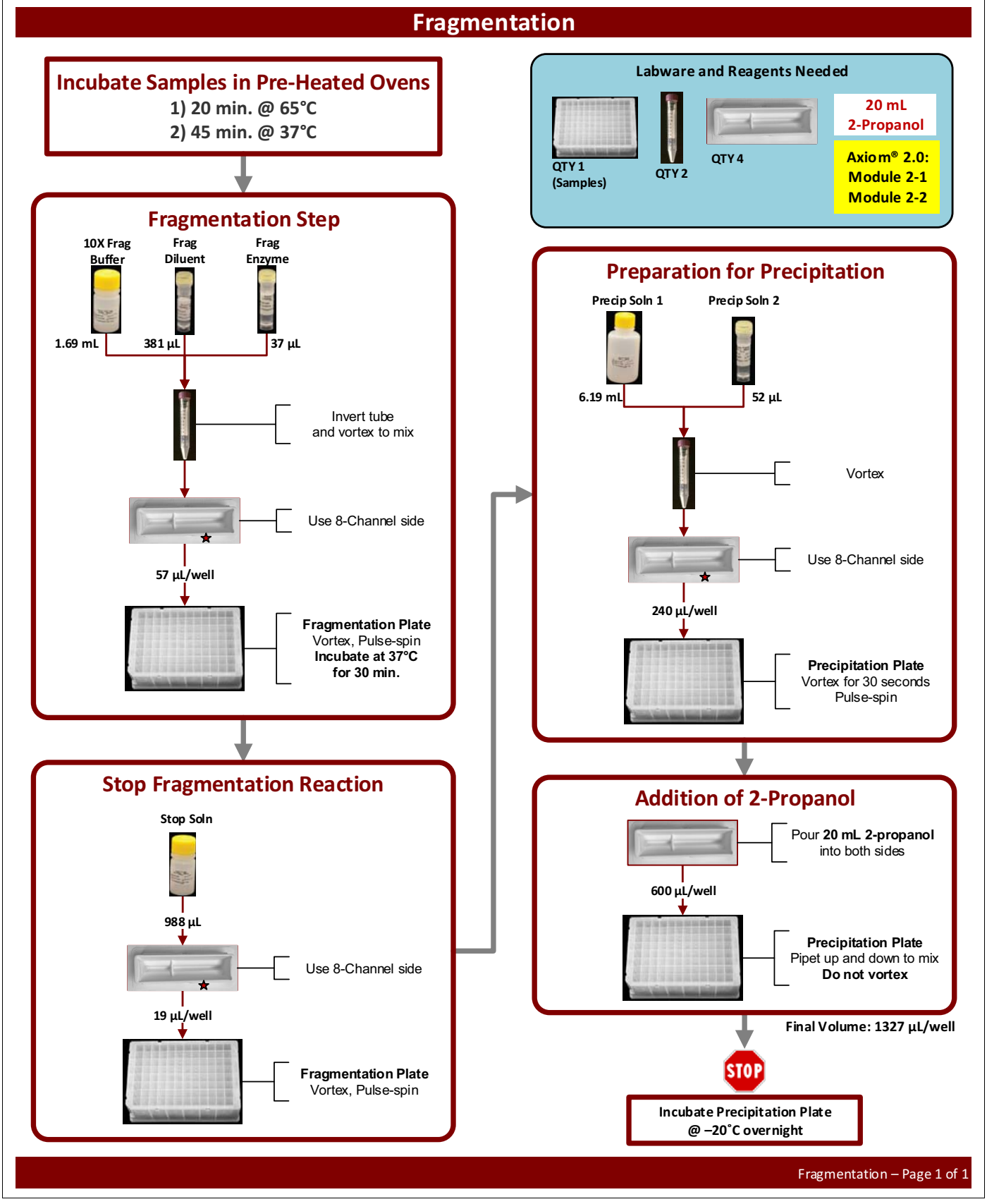


Figure 4.6 Stage 2: Fragmentation & Precipitation



Stage 3: Centrifuge and Drying, Resuspension and Hybridization Preparation, and Sample QC

This stage requires the following sets of steps:

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

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

Duration

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

Total: 2.5 hr

Input Required

Precipitation Plate from *Stage 2: Fragmentation and Precipitation* on page 52.

Equipment, Consumables, and Reagents Required

The equipment and consumables listed in Table 4.16 are required for this stage.

Table 4.16 Equipment and Consumables Required for Stage 3: Drying, Resuspension and QC

Quantity	Item
As required	Adhesive seals for 96-well plates
1	Marker, fine point, permanent
1 each	Rainin Pipettes: ▪ Single channel P20 ▪ Single channel P-100 ▪ Multi-channel P20 ▪ Multi-channel P-200

Table 4.16 Equipment and Consumables Required for Stage 3: Drying, Resuspension and QC (Continued)

Quantity	Item
As needed	Pipette tips for pipettes listed above
2	Any 96-well PCR plate for making the dilutions: ▪ QC Dilution Plate ▪ Gel Samples Plate
1	Bio-Rad Hard-Shell Semi-Skirted PCR P/N HSS-9641 (for ABI 9700 thermal cycler) ▪ 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	15 mL conical tube
1	5 mL Serological Pipette
1	Pipet aid
1	Shaker, either: ▪ Thermo Scientific™ Compact Digital Microplate Shaker ▪ Jitterbug
1	Vortexer
4	Matrix™ 25 mL divided Reagent Reservoir P/N 8095

Reagents Required

Table 4.17 Reagents Required for Stage 3: Drying, Resuspension and QC

Reagent	Module
From the PharmacoScan Reagent Kit 4x24 Reactions	
Axiom Hyb Buffer	Module 2-1, –20°C P/N 901528
Axiom Hyb Soln 1	
Axiom Resusp Buffer	Module 2-2, 2–8°C P/N 901529
Axiom Hyb Soln 2	
Other Reagents Required for QC steps (optional)	
Gel Diluent, 5 mL of 1000-fold dilution of TrackIt™ Cyan/Orange Loading Buffer (see Appendix A, *Fragmentation Quality Control Gel Protocol* on page 133 for dilution instructions.)	
Gel Sample Plate	
15 fold dilution of TrackIt™ 25 bp DNA Ladder (P/N 10488-022)	
Nuclease-free water, ultrapure MB Grade, 5 mL (P/N 71786; for OD and Dilution Plate preparation)	

Gels and Related Materials Required

At the end of this stage, verifying the fragmentation reaction is highly recommended. See Appendix A, *Fragmentation Quality Control Gel Protocol* on page 133 for the required gel and related materials.

Examining the mPCR Reaction Plate by agarose gel electrophoresis is optional and recommended only if assay troubleshooting is required.

Stage 3A: Centrifuge Precipitation Plate and Dry the DNA Pellet

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

To Centrifuge and Dry the DNA Pellets:

1. Turn the oven on and preheat to **37**°C.

   Use an oven that can sustain a constant temperature of 37°C and has a temperature accuracy of ±1°C (we recommend the Binder ED 56). If using an Affymetrix GeneChip Hyb Oven, set the rotation speed to 15 rpm to distribute heat.

2. Transfer the Precipitation Plate from the –20°C freezer to a pre-chilled centrifuge. Centrifuge the plate **for 40 min at 4°C** at 3200 x*g* (4000 RPM for the Eppendorf 5810R centrifuge with the rotor configuration described in the *PharmacoScan Assay 24-Array Format Manual Protocol Site Preparation Guide*, P/N 703287).

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

3. Immediately after the 40 min centrifugation period, empty the liquid from each 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 plates on a pile of Kimwipes on a bench and allow them to drain for 5 min. Transfer the plate to a new pile of Kimwipes twice during the 5 min period.
4. Turn the plate right side up and place in an oven for 20 min at 37°C to dry.
   - Tightly seal the plate upon completion

   NOTE: If using an Affymetrix 645 oven:
   - **Place the plate on the bottom of the oven. Plates do not rotate.**
   - **Turn off the rotor during the 20 min drying time.**

5. Do one of the following:
   - Proceed directly to *Stage 3B: Resuspension and Hybridization Preparation* on page 65, even if some droplets of liquid remain. Leave the sample plate at room temperature. It is helpful to begin preparing reagents for Stage 3B while centrifuging and drying pellets.
   - Store the plate for resuspension later in the same day:
     - Tightly seal the plate.
     - If resuspension will be carried within 4 hours, keep the plate at room temperature.
   - Store the plate for resuspension on another day:
     - Tightly seal the plate.
     - Store the plate at –20°C.

Stage 3B: Resuspension and Hybridization Preparation

1: Prepare for Resuspension and Hybridization Preparation

To Prepare for Resuspension and Hybridization

1. Set the centrifuge to room temperature.

2: Prepare DNA Pellets and Warm The Resuspension Buffer

IMPORTANT: The plate of pelleted DNA and resuspension reagent must be at room temperature before proceeding with this step.

The equilibration of the plate of pelleted DNA and resuspension buffer to room temperature (18-25°C) is very critical for the success of the PharmacoScan Target Prep. When any of these are cooler than room temperature, pellets may not resuspend completely. This may result in compromised assay performance. Please note following guidelines on how to work with plates with fresh, cold, or frozen pellets:

DNA Pellet Types:

- Fresh Pellets: A plate with fresh pellets can be kept at room temperature if proceeding with the Resuspension and Hybridization Preparation protocol within 4 hours.
- Cold Pellets: A plate with fresh pellets that are not processed within 4 hours can be transferred to a refrigerator (2-8°C) if processed during the same day. However, it is critical to equilibrate the plate to room temperature for at least 30 minutes before proceeding with the Resuspension and Hybridization Preparation protocol.
- Frozen Pellets: A plate with frozen pellets must be pre-equilibrated at room temperature for at least 1.5 hour before proceeding with the Resuspension and Hybridization Preparation protocol.

Warm the Resuspension Buffer:

- The Resuspension buffer needs at least 1 hour to equilibrate to room temperature.

3: Thaw and Prepare the Reagents

To Thaw and Prepare the Reagents:

1. Thaw Axiom Hyb Soln 1 on the benchtop at room temperature.
2. Warm Axiom Resusp Buffer, Axiom Hyb Buffer, and Axiom Hyb Soln 2 on the benchtop at room temperature for at least one hour.
3. Vortex the Axiom Resusp Buffer and the Axiom Hyb Buffer. Keep at room temperature.
4. Vortex and pulse-spin Axiom Hyb Soln 1 and Axiom Hyb Soln 2 before use.

4: Label Tubes and Reservoirs

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

Table 4.18 Label Tube

Label	Tube Size	Temperature	Contents
▪ *Hyb MM*	15 mL	Room Temperature in Fume Hood	Hybridization Master Mix

2. Label two Matrix 25 mL divided Reagent Reservoirs (P/N 8095) as indicated in the table below.

Table 4.19 Label Reagent Reservoirs for Resuspension and Hybridization Preparation

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

5: Add Resuspension Buffer to DNA Pellets

NOTE: If a plate was stored at –20°C after drying the pellets, it must be allowed plate to sit at room temperature for 1.5 hour before carrying out resuspension.

NOTE: Make sure the Resusp Buffer has equilibrated to room temperature before adding to dry pellets in Step 3, below.

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

1. Using a P-1000, carefully pipet 1.4 mL of Axiom Resusp Buffer into the 8-channel side of the reagent reservoir labeled *Resus*.
2. Carefully remove the seal from the Precipitation Plate and discard the seal.
3. Using a P200 8-channel pipette, transfer **35 µL Axiom Resusp Buffer** to each well in Columns 5, 7, and 9 of the Precipitation Plate. Avoid touching the pellets with the pipette tips.
   - Change pipette tips after each addition.
   - After adding Resuspension buffer, the plate is known as the Resuspension Plate.
4. Seal the Resuspension Plate.

6: Resuspension of DNA Pellets

1. Place the sealed Resuspension Plate on one of the following shakers:
   - Thermo Scientific™ Compact Digital Microplate Shaker: at **speed 900 rpm for 10 min**
   - Jitterbug: at **speed 7 for 10 min**
2. Inspect the Resuspension Plate from the bottom. If the pellets are not dissolved, repeat Step 1.
3. Quickly spin at 1000 rpm.

7: Prepare the Hybridization Master Mix

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

1. While the Resuspension Plate is shaking, prepare the Hybridization Master Mix in the *Hyb MM* 15 mL tube.
   A. Add the reagents in Table 4.20 to the *Hyb MM* tube in the order shown.

Table 4.20 Hybridization Master Mix

Reagent	per Sample	Master Mix 24+
To the 15 mL tube labeled *Hyb MM*, add:		
Axiom Hyb Buffer	70.5 µL	2.26 mL
Axiom Hyb Soln 1	0.5 µL	16 µL
Axiom Hyb Soln 2	9 µL	288 µL
Total Volume	**80 µL**	**2.56 mL**

   B. Vortex twice to mix.

8: Prepare the Hyb Ready Sample Plate

To Prepare the Hyb Ready Sample Plate

1. Choose a 96-well plate that will be compatible with the thermo cycler model that will be used for sample denaturation. See Table 3.2 on page 21 for information on thermo cycler consumables.
2. Label the 96-well PCR plate as Hyb Ready [Sample ID].
3. Set a P200 8-channel pipette to 45 µL (this is slightly higher than the volume of the sample in each well of the Resuspension Plate).
4. Using the P200 pipette, transfer the entire contents of each well in columns 5, 7, and 9 of the Resuspension Plate to the corresponding columns of the labeled Hyb Ready Plate.
   - Change pipette tips after each transfer.
5. Pour the Hyb Master Mix into the 8-channel side of the reagent reservoir labeled Hyb MM.
6. Using a P200 8-channel pipette, add 80 µL of the Hyb Master Mix to each well of columns 5, 7, and 9 the Hyb Ready Plate.
   - Change pipette tips after each addition.
7. Seal, vortex twice, and pulse-spin.

9: Freeze or Proceed

At this point you can:

- Proceed to *Stage 3C: Recommended: Perform Quantitation and Fragmentation QC Checks* (highly recommended), below; or
- Proceed to *Stage 4: Denaturation and Hybridization*; or
- Store the Hyb Ready samples at –20˚C.
- Store remaining Module 2-1 and Module 2-2 reagents for future use. Follow the guidelines presented in the section *Freeze-Thaw Instructions* on page 27.

Stage 3C: Recommended: Perform Quantitation and Fragmentation QC Checks

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

1: Prepare for Sample QC

To Prepare for Sample QC:

Prepare the Reagents

Obtain the Reagents for Sample QC:

1. 5 mL of nuclease-free water for the water reservoir.
2. 5 mL of Gel Diluent
   The Gel Diluent is a 1000-fold dilution of the TrackIt Cyan/Orange Loading Buffer (Invitrogen) as described in *Diluting the TrackIt™ Cyan/Orange Loading Buffer and 25 bp Ladder* on page 134.
3. 90 µL of a 15-fold dilution of TrackIt™ 25 bp DNA Ladder, P/N 10488-022, Thermo Fisher Scientific
4. One E-Gel 48 Agarose Gel, 4% Agarose, P/N G8008-04, Thermo Fisher Scientific

Label Reservoirs

Label two Matrix 25 mL divided Reagent Reservoirs (P/N 8095) as indicated below:

- Label one reservoir as H_2O
- Label the second reservoir as Loading Dye

Table 4.21 Label Reagent Reservoirs for QC

Label	Temperature	Contents
▪ *NF H2O*	Leave reservoir at room temperature	Nuclease-free Water
▪ *Loading Dye*	Leave reservoir at room temperature	Diluted Loading dye

Prepare Sample QC Plates

1. Label two 96-well PCR plates for making the dilutions:
   - Label one plate as *Dil QC*
   - Label the second plate as *Gel QC*
2. Obtain one 96-well UV Star, 370 µL/well plate

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

2: Perform QC Checks

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

1. Prepare Dilution QC Plate and OD Plate.
   Pour 5 mL nuclease-free water into the 8-channel side of the reagent reservoir labeled *NF H20*. The water will be used to make the QC Dilution Plate and the OD Plate.
   A. Add 33 µL nuclease-free water to each well in column 5, 7 and 9 of the *Dil QC* Plate.
   B. Add 90 µL nuclease-free water to each well in column 5, 7 and 9 of the OD Plate.

2. Prepare the Dilution QC Plate:
   A. Transfer **3 µL of the Hyb Ready sample** from each well of the *Hyb Ready Plate* to the corresponding well of the *Dil QC* Plate. Change pipette tips after each transfer.
   B. Seal, vortex, and pulse-spin.
3. Prepare OD Plate:
   A. Carefully remove the seal from the QC Dilution Plate and discard the seal.
   B. Transfer **10 µL of each Dil QC sample** to the to the corresponding wells of the 96-well UV Star plate. Change pipette tips after each transfer.
   C. Mix by pipetting up and down.
      - Change pipette tips after each addition.
      - Final sample mass dilution is 120-fold.

   See Appendix B, *Sample Quantitation after Resuspension* on page 136 for more information on performing the Sample Quantitation.
4. Prepare Gel QC Plate:
   A. Pour 5 mL Gel Diluent into the 8-channel side of the reagent reservoir labeled *Loading Dye*.
   B. Add **120 µL gel diluent** to each well in column 5, 7 and 9 of the *Gel QC* Plate.
   C. Transfer **3 µL of each Dil QC sample** to the corresponding wells of the *Gel QC* Plate. Change pipette tips after each transfer.
   D. Seal, vortex, and pulse-spin the plate.
5. Run gel as described in Appendix A, *Fragmentation Quality Control Gel Protocol* on page 133.

   After the QC checks, the Dilution QC Plate, OD Plate, and remaining Gel QC samples can be discarded once satisfactory results from the gel and OD 260 readings have been obtained.

3: 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.

Figure 4.7 Stage 3: Resuspension, #1 of 2

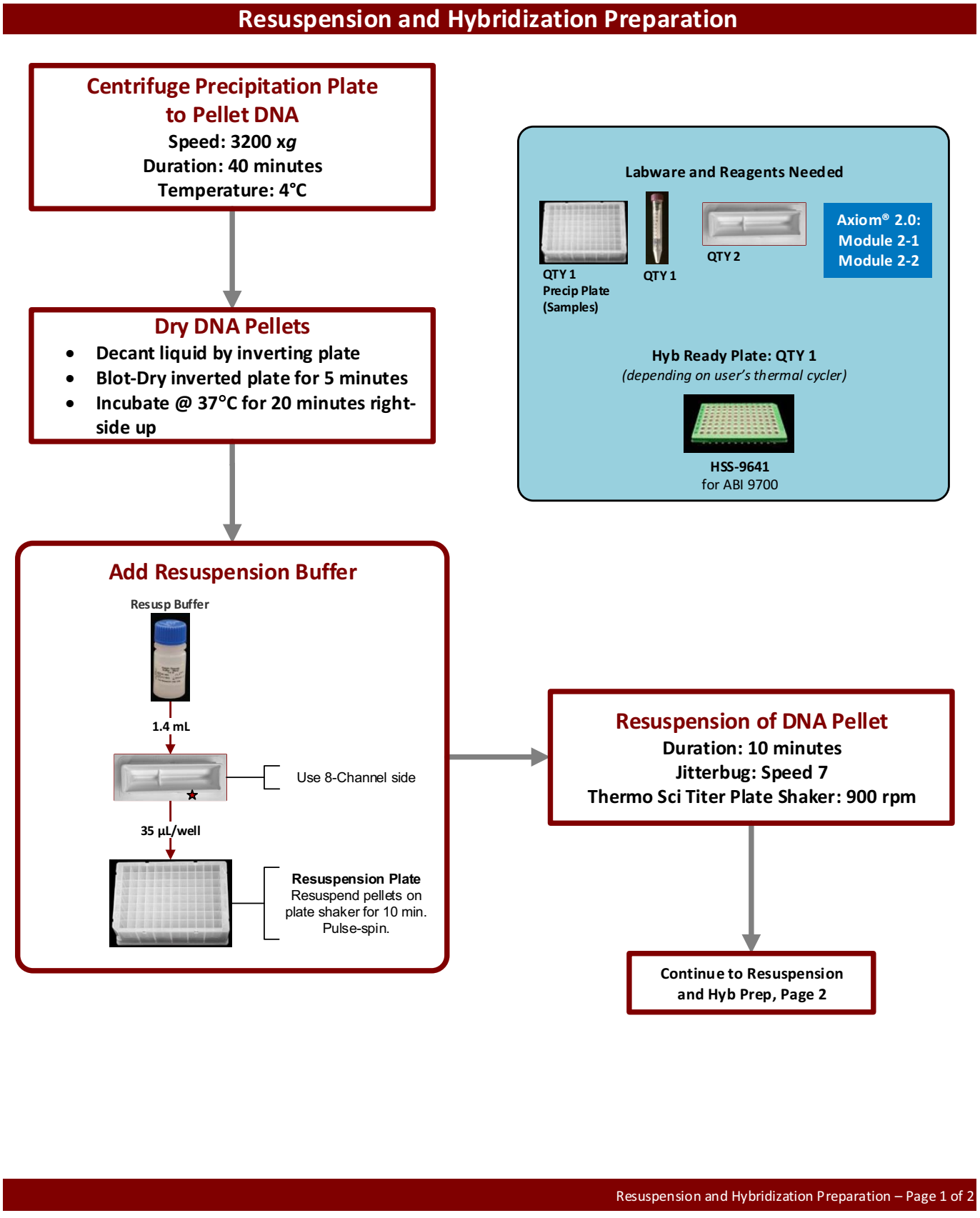


Figure 4.8 Stage 3: Resuspension, #2 of 2

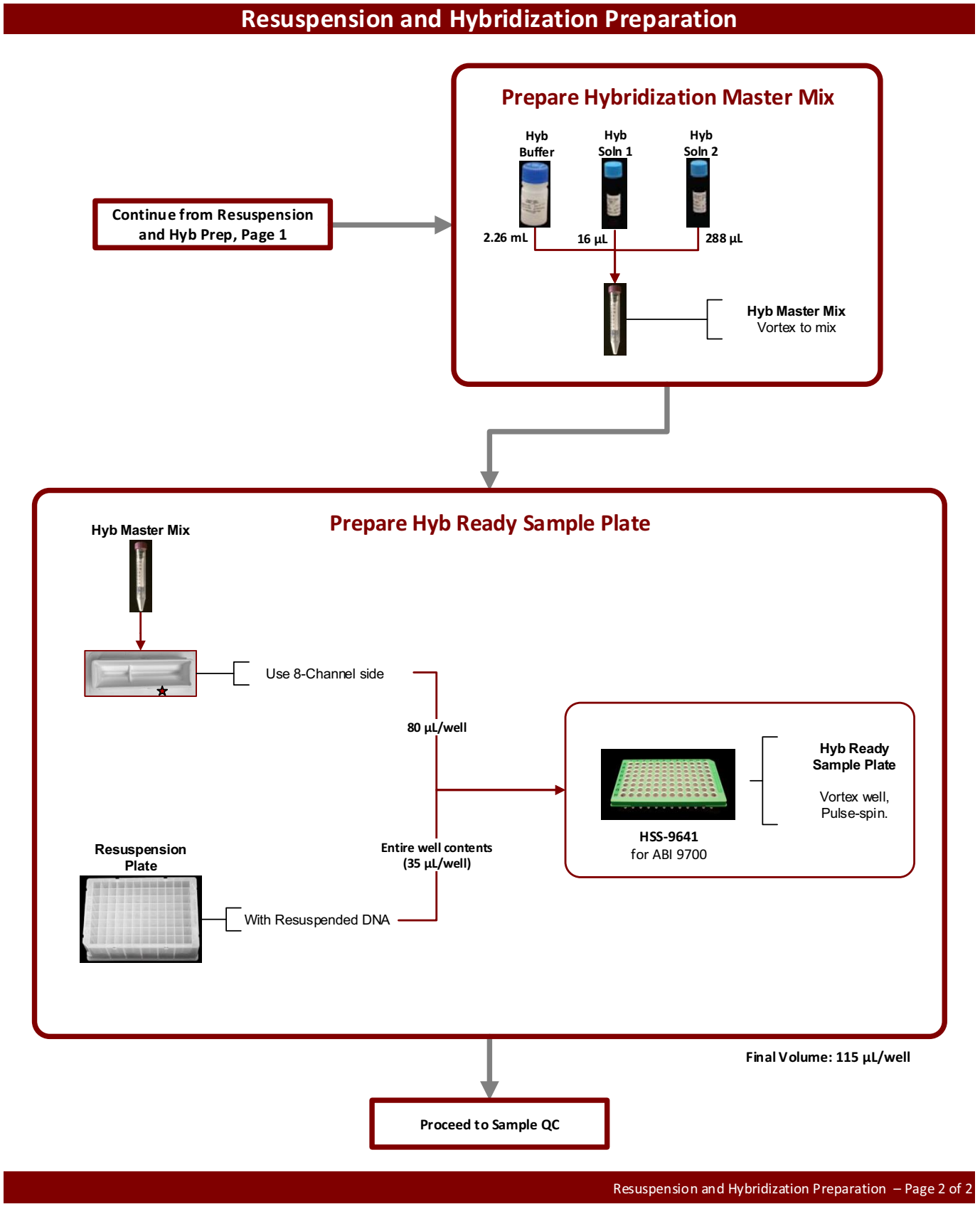
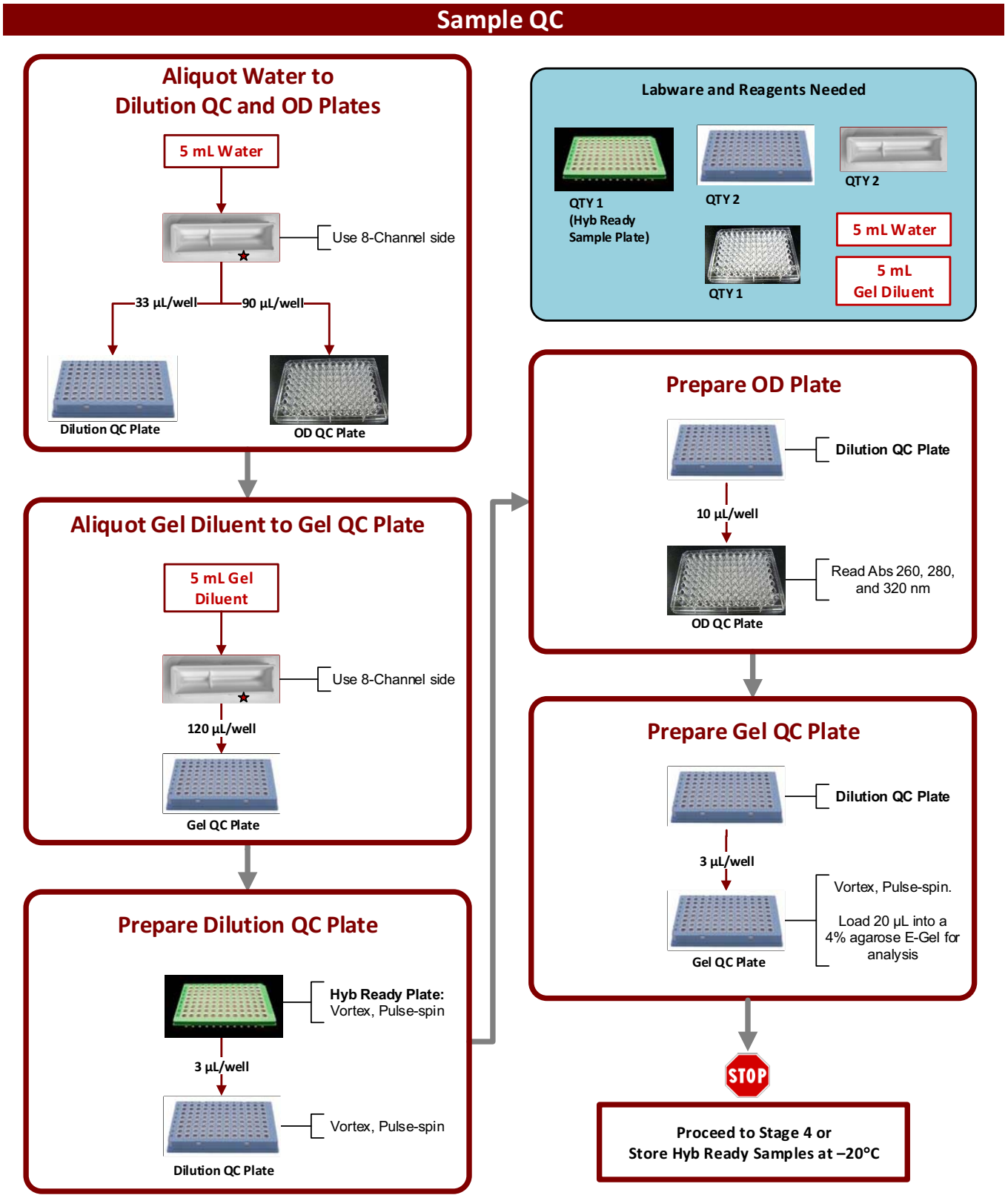


Figure 4.9 Stage 3: QC



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.

This stage requires the following sets of steps:

1: Prepare for Denaturation and Hybridization on page 75
2: Prepare Hyb Ready Samples Stored at –20°C on page 75
3: Prepare the GeneTitan® MC Instrument on page 75
4: Denature the Hyb Ready Sample Plate on page 76
5: Prepare Hybridization Tray and Load into GeneTitan® MC Instrument on page 76
4: Store Remaining Reagents on page 92

To Perform Stage 4:

If the Hyb Ready Plate was stored at –20°C, go to *2: Prepare Hyb Ready Samples Stored at –20°C* on page 75.

If you are proceeding directly from the end of Stage 3 on page 69, go to *3: Prepare the GeneTitan® MC Instrument* on page 75.

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

Duration

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

Required Input from Previous Stage

- Hyb Ready Sample Plate

Equipment, Consumables, and Reagents Required

The following thermal cycler is recommended:

- ABI 9700

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

The thermal cycler needs to be programmed with the **PharmacoScan Denature** protocol (see *Thermal Cycler Recommendations and Protocols* on page 20).

Table 4.22 Equipment Required for Stage 4: Denaturation and Hybridization

Quantity	Equipment	
1	GeneTitan MC Instrument	
1	Rainin P200 8-channel Pipette	
As needed	Pipette tips	
1	Thermal Cycler	Appropriate thermal cycler, programmed with the **PharmacoScan Denature** protocol (see *Thermal Cycler Recommendations and Protocols* on page 20).
1	96 well metal chamber warmed in a 48°C oven*	

**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 4.23 Consumables Required for Stage 4: Denaturation and Hybridization

Quantity	Consumable	Part Number
1	▪ One PharmacoScan™ 24F Array Plate in a protective base	P/N 902994
1	▪ Hyb Tray*	P/N 500867

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

Table 4.24 Reagents Required from the PharmacoScan Reagent Kit 4x24 Reactions

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

1: Prepare for Denaturation and Hybridization

To Prepare for Denaturation and Hybridization

1. Preheat the 96-well metal chamber in a 48°C oven.
2. **Allow array plate to equilibrate to room temperature for a minimum of 25 minutes.**
   A. Leave the array plate in the pouch at room temperature, for a minimum of 25 minutes, before opening and loading on the GeneTitan MC Instrument to allow the plate to come to room temperature.
   B. At the end of the array warm up time, open the pouch and scan the array plate barcode into the Batch Registration file as described in Appendix C, *Registering Samples in Affymetrix GeneChip® Command Console®* on page 145.

   ▲ **WARNING: Do not remove the array plate from the protective base or touch the surface of any arrays.**
3. Power up the thermal cycler and prepare for the **PharmacoScan Denature** protocol to run with the heated lid option selected.

2: 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.

3: Prepare the GeneTitan® MC Instrument

Before you denature your Hyb Ready samples, ensure that the GeneTitan MC Instrument is ready for use by following the instructions given in Chapter 5, *Stage 2: Hybridization* on page 105 and Appendix C, *Registering Samples in Affymetrix GeneChip® Command Console®* on page 145.

A brief summary of the steps which may need to be performed is:

1. Prepare the reagents from Module 3 as described in Table 4.25:

Table 4.25 Reagents from Module 3

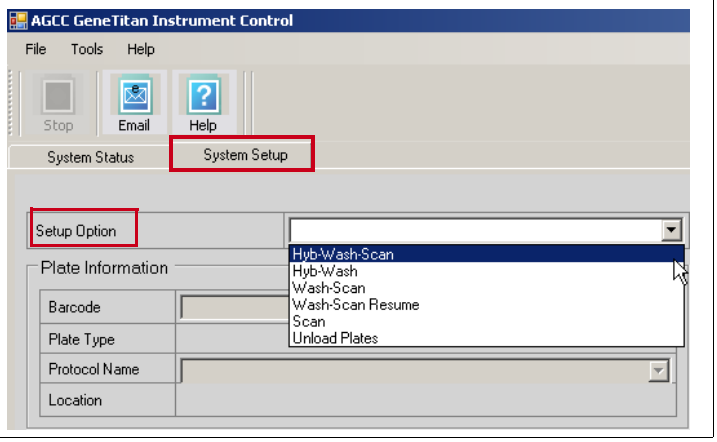
Reagent	Temp Out of Module*	Treatment
Axiom Wash Buffer A (P/N 901446)	Room Temp	Invert 2-3X for mixing before filling GT bottle
Axiom Wash Buffer B (P/N 901447)	Room Temp	Invert 2-3X for mixing before filling GT bottle
Axiom Water (P/N 901578)	Room Temp	N/A

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

2. Launch AGCC and select AGCC GeneTitan Control.

3. 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 sample.
4. Select the System Setup tab (Figure 4.10).

Figure 4.10 Setup Options for Processing Array Plates



5. Configure the software as follows:
   A. Setup Option: **Hyb-Wash-Scan**.
   B. Click **Next**.
   C. Plate information:
      - Barcode: Scan or manually enter the PharmacoScan array plate barcode and click **Next**.
      - Protocol Name: Select the protocol name and click **Next**.
6. Fill the Wash A, Wash B and Rinse bottles.
7. Empty the Waste bottle.

4: Denature the Hyb Ready Sample Plate

1. Make sure the thermal cycler is powered on and the **PharmacoScan Denature** protocol with the heated lid option has been selected.
2. Open the lid of the thermal cycler and place the sealed Hyb Ready Plate on the thermal cycler. Check the integrity of the seal as evaporation during denaturation can negatively impact assay performance.
3. Close the lid.
4. Start the **PharmacoScan Denature** protocol, described on *Thermal Cycler Recommendations and Protocols* on page 20).

5: Prepare Hybridization Tray and Load into GeneTitan® MC Instrument

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

1. After the **PharmacoScan Denature** protocol has completed, remove the Hyb Ready Plate from the thermal cycler 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 seal from Hyb Ready Plate and discard.
4. Remove the hyb tray (from Axiom GeneTitan Consumables Kit) from packaging.

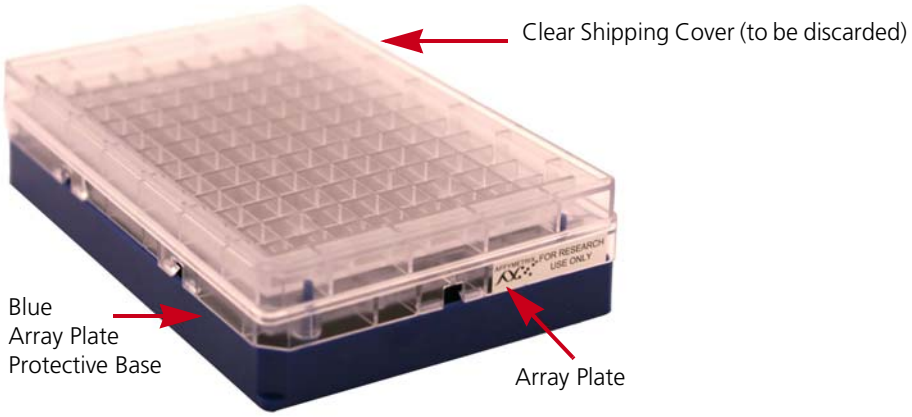
5. Label the hyb tray. See the note below and Figure 3.7 on page 35 for more information.

IMPORTANT: It is critical that you write only on the proper location of the hyb tray (on the edge in front of wells A1 and B1) as illustrated in Figure 3.7 on page 35. Do NOT write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure.

IMPORTANT: Do not confuse hyb trays with stain trays.

6. Place the hyb tray under the fume hood.
7. Using a P200 8-channel pipette set at **105 µL**, slowly transfer the denatured samples in columns 5, 7, and 9 of the Hyb Ready Plate into the corresponding wells of the hyb tray. Dispense to the first stop to avoid creating bubbles.
   - Change pipette tips after each transfer; discard the tip even if it shows some volume left.
   - Ensure that there are no air bubbles present in the hyb tray. Puncture any air bubbles that you see using a clean pipette tip.
   - There is no need to spread the sample around the bottom of the hyb tray wells. Sample distribution across the well will occur when the array plate is stacked together with the hyb tray by the GeneTitan MC Instrument.
8. Load the array plate and hyb tray into the GeneTitan MC Instrument (see *Load a PharmacoScan™ Array Plate and Hyb Tray Onto the GeneTitan® MC Instrument* on page 110).

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



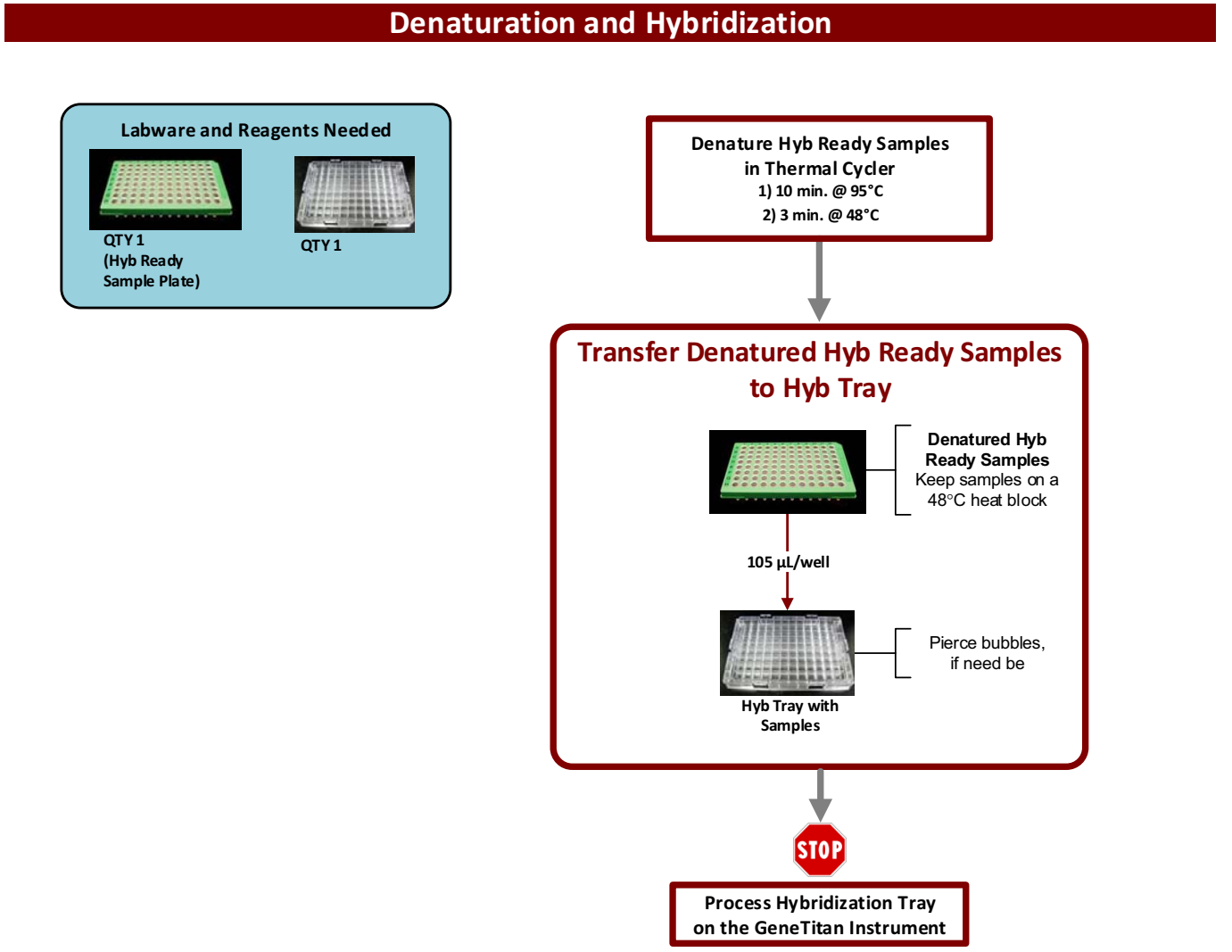
Load the hyb tray on the right side without any covering. The hyb tray should not have any bubbles.

IMPORTANT: After the GeneTitan MC Instrument has stacked the array plate and hyb tray, the instrument will extend the drawer. Manually check the stacking by gently pressing the six latching points to confirm that the two parts are clamped properly, and check underneath the arrays to make sure there are no bubbles. If bubbles are found, gently tap the plate on top and the bubbles should disappear. Do NOT tip/tilt the array plate/hyb tray sandwich while inspecting the bottom for bubbles. See Step 3 on page 113 for detailed instructions.

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

Near the end of the 23.5 to 24 hour hybridization period in the GeneTitan MC, proceed to *Stage 5: GeneTitan® Reagent Preparation* on page 79.

Figure 4.11 Stage 4: Denaturation and Hybridization



Stage 5: GeneTitan® Reagent Preparation

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

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

IMPORTANT: The reagent trays prepared in this step, *Stage 5: GeneTitan® Reagent Preparation* are for the continued processing of an PharmacoScan array plate that:

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

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

To prepare the reagent trays for the GeneTitan MC Instrument:

1: Prepare for GeneTitan® Reagent Preparation on page 82

2: Prepare the Stain, Ligation, and Stabilization Master Mixes on page 85

3: Aliquot Master Mixes and Hold Buffer into Trays on page 88

4: Store Remaining Reagents on page 92

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

Table 4.26 Reagent Trays Required for the PharmacoScan Assay 24-Array Format Manual Protocol on the GeneTitan® MC Instrument

Type of Tray	Quantity	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 4.27 Equipment Required for Stage 5: Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan® MC Instrument

Quantity	Equipment		
1	GeneTitan MC Instrument		
1	Ice bucket with ice		
As Needed	Kimwipes		
As Needed	Markers		
1	Cooler for enzyme		
1	Zerostat Anti-static Gun		
1	Microcentrifuge		
1	Pipetaid		
1 each	Rainin Pipettes—single channel ▪ P200 ▪ P1000	Rainin Pipettes—8-channel: ▪ P200	Rainin Pipettes—12-channel: ▪ P200
1	Vortexer		

Table 4.28 Consumables Required for Stage 5: Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan® MC Instrument

Quantity	Consumable	Part Number
As required	Aluminum foil (optional)	
1 kit includes: 1 5 6	GeneTitan Consumables Kit ▪ Scan Tray ▪ Stain Tray ▪ Covers for trays	Affymetrix P/N 901606 P/N 501006 P/N 501025 P/N 202757
1	Pipette, serological ▪ 5 x 1/10 mL (VWR P/N 89130-896)	
As required for pipettes listed in Table 4.27	Pipette tips	
5	Matrix™ 25 mL divided Reagent Reservoir	P/N 8095
4	15 mL conical tube	

Reagents Required

Table 4.29 PharmacoScan Reagents Required for Stain and Ligation Stage

Reagent	Module
Axiom Ligate Buffer	Module 4-1, –20°C P/N 901278
Axiom Ligate Enzyme	
Axiom Ligate Soln 1	
Axiom Probe Mix 1	
Axiom Stain Buffer	
Axiom Stabilize Soln	
Axiom Ligate Soln 2	Module 4-2, 2-8°C P/N 901276
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*	
Axiom Ligate Enzyme (extra tube for back-up purposes)	Module 5-1, –25°C to –15°C P/N 902796
Axiom Hold Buffer (3 bottles)	Module 5-2, 2°C to 8°C P/N 902797

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

1: Prepare for GeneTitan® Reagent Preparation

Thaw and Prepare the Reagents

NOTE: Ligation Buffer and Ligation Solution 2 require approximately 30 to 40 min to thaw on the benchtop at room temperature.

Table 4.30 Reagents Required for GeneTitan® MC Instrument Reagent Tray Preparation

Module	Reagent	Thaw on Benchtop, Then Place on Ice	Place on Ice	Place on Benchtop at Room Temperature
Module 3 Room Temperature	Axiom Wash Buffer A			✓
	Axiom Wash Buffer B			✓
	Axiom Water (1 bottle required)			✓
Module 4-1 –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 4-2 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† (1 bottle required)‡			✓

* *An extra tube of Ligate Enzyme is in Module 5-1 in the event that it is needed.*

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

‡ *Axiom Hold Buffer for preparing the Scan Tray for the 2nd, 3rd, and 4th plate are provided in Module 5-2.*

Preparing Axiom Wash A and Axiom Stabilize Diluent

During storage of the Axiom Wash A and Axiom Stabilize Diluent (in Module 4-2 stored 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 benchtop 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.

To Prepare the Stabilize Diluent:

If crystals are observed in the Axiom Stabilize Diluent:

1. Vortex and Spin.
2. Look for precipitate.
   If any:
   - Warm tube to room temperature and vortex again.

Preparing Axiom Ligate Buffer

White precipitate is sometimes observed when the Axiom Ligate Buffer is thawed.

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

To Prepare the Axiom Ligate Buffer:

1. Place on the benchtop at room temperature for 30 min. This bottle can also be thawed in a dish with room temperature Millipore water.
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. If precipitate is still present after hand warming proceed with the protocol below.

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 4-1 at –20°C on the benchtop 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 4-2 as follows:
   A. Gently flick each tube 2 to 3 times to mix, then spin.
   B. Place reagents on ice, except for the *Hold Buffer*, *Ligate Soln 2* and *Water*— leave these reagents on the benchtop at room temperature.

Label the Master Mix Tubes

1. Mark the side of each tube with one of designations shown in Table 4.31.

Table 4.31 Labeling Master Mix Tubes for Stain, Ligation, and Stabilization Reagents

Conical Tube	Number of Tubes	Tube Designation	Contents	Place Tube:
15 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

Label the Reagent Reservoirs

1. Label five Matrix 25 mL divided Reagent Reservoirs (P/N 8095) as indicated in the table below.

Table 4.32 Labeling Reagent Reservoirs

Reservoir Designation	Contents
S1	▪ Stain 1 Master Mix
S2	▪ Stain 2 Master Mix
Stbl	▪ Stabilization Master Mix
Lig	▪ Ligation Master Mix
Hold	▪ Hold Buffer

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

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 15 mL tube labeled *S1* in the order shown in Table 4.33. This recipe will provide enough for both *S1* reagent trays.

Table 4.33 Stain 1 Master Mix

Reagent	per Array	Master Mix 24+
To the tube marked *S1*, add:		
▪ Axiom Wash A	201.6 µL	5.24 mL
▪ Axiom Stain Buffer	4.2 µL	109.2 µL
▪ Axiom Stain 1-A	2.1 µL	54.6 µL
▪ Axiom Stain 1-B	2.1 µL	54.6 µL
Total	**210 µL (105 µL x 2)**	**5.46 mL**

2. Gently invert the tube 10 times to mix. Do not vortex.
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 single-channel pipettes to add reagents to the 15 mL tube labeled *S2* in the order shown in Table 4.34.

Table 4.34 Stain 2 Master Mix

Reagent	per Array	Master Mix 24+
To the tube marked *S2*, add:		
▪ Axiom Wash A	100.8 µL	2.62 mL
▪ Axiom Stain Buffer	2.1 µL	54.6 µL
▪ Axiom Stain 2-A	1.05 µL	27.3 µL
▪ Axiom Stain 2-B	1.05 µL	27.3 µL
Total	**105 µL**	**2.73 mL**

2. Gently invert the *S2 MM* tube 10 times to mix. Do not vortex.
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 single-channel pipettes to add reagents to the 15 mL tube labeled *Stbl* in the order shown in Table 4.35.

Table 4.35 Stabilization Master Mix

Reagent	per Array	Master Mix 24+
To the tube marked *Stbl*, add:		
▪ Axiom Water	93.19 µL	2376 µL
▪ Axiom Stabilize Diluent	10.50 µL	268 µL
▪ Axiom Stabilize Soln	1.31 µL	33.4 µL
Total	**105 µL**	**2678 µL**

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

Prepare Ligation Master Mix

Important Note About the Second Tube of PharmacoScan Ligate Enzyme:
Module 5-1 contains an extra tube of Axiom Ligate Enzyme which has been provided for back-up purposes in the event that it is needed. If there is insufficient volume of the Ligate Enzyme for the preparation of the fourth (4th) 24 Sample Ligation Master Mix, discard the tube and its contents and proceed to use the second tube of Axiom Ligate Enzyme (Module 5-1). The remaining contents of the second tube of Ligate Enzyme can then be discarded or stored in the freezer for future use.

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 single-channel pipettes to add reagents to the 15 mL tube labeled *Lig* in the order shown in Table 4.36.

Table 4.36 Ligation Master Mix Preparation—Stage 1

Reagent	per Array	Master Mix 24+
To the tube marked *Lig*, add:		
▪ Axiom Ligate Buffer	66.15 µL	1753 µL
▪ Axiom Ligate Soln 1	13.12 µL	348 µL
▪ Axiom Ligate Soln 2	3.15 µL	83.5 µL
Sub–Total	**82.42 µL**	**2184 µL**

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 Ligate 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 4.37.

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

Table 4.37 Ligation Master Mix Preparation—Stage 2

Reagent	per Array	Master Mix 24+
▪ Ligation Master Mix from Stage 1	82.42 µL	2184 µL
▪ Axiom Probe Mix 1	10.5 µL	278 µL
▪ Axiom Probe Mix 2	10.5 µL	278 µL
▪ Axiom Ligate Enzyme	1.58 µL	42 µL
Total	**105 µL**	**2783 µL**

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 Hold Buffer into Trays

Label the Trays

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

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

IMPORTANT: **It is critical that you write only on the proper side of the front edge of stain trays, as illustrated in Figure 3.8 on page 36. The front edge of the tray is the short side with the lettering A through H. Do NOT write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and lids.**

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

Deionize Trays and Covers

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

See Appendix D, *Deionizing Procedure for GeneTitan® Trays and Covers* on page 148 for the recommended technique.

About Aliquoting Reagents to Trays

Stain Trays: Only fill columns 5, 7, and 9 with ligation, staining, and stabilization reagents.

Scan Tray: It is important to fill all 96 wells with Hold Buffer. The scan tray has an open-bottom design, so it is very important that all 96 wells of the scan tray receive 150 µL of Hold Buffer.

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

For all trays, pipet 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 aliquoting master-mixes**

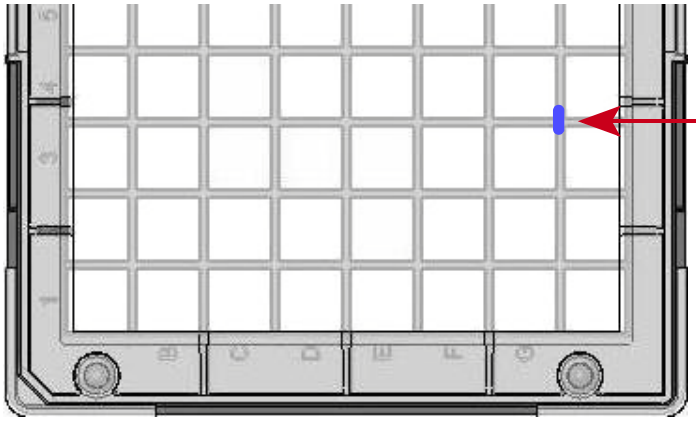
When aliquoting ligation, staining, and stabilization reagents to the trays, it is not necessary to spread the reagent to each corner of the well. The reagent will spread evenly when the array plate is inserted into the reagent tray during processing with the GeneTitan MC Instrument.

Stain 1 Master Mix

To Aliquot the Stain 1 Master Mix:

1. Pour the S1 Master Mix into the 8-channel side of the reagent reservoir marked *S1*, placed on the bench top at room temperature.
2. Load a P200 8-channel pipette with 8 new pipette tips and aliquot **105 µL per well** to columns 5, 7, and 9 of 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 4.12).

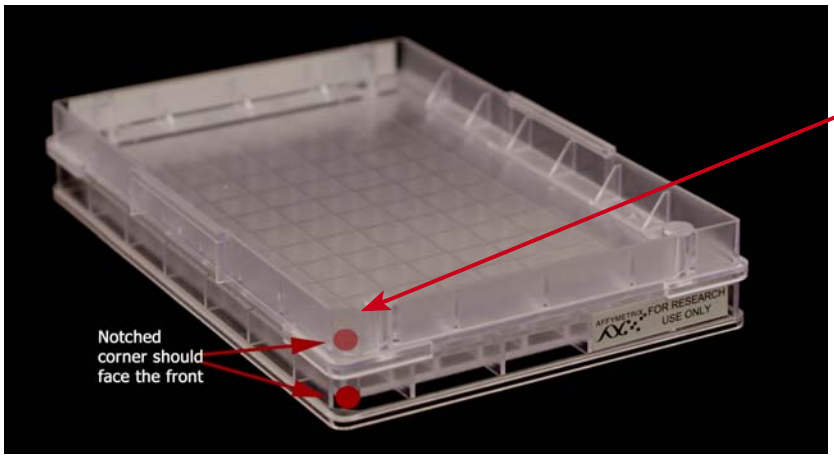
Figure 4.12 Well Dividers in Trays.



4. Place covers on the *S1* trays. Orient cover correctly on the tray with the notched corners together (Figure 4.13).

! 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 4.13 Placing Cover on Stain Tray



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

Stain 2 Master Mix

To Aliquot the Stain 2 Master Mix:

1. Carefully pipet or pour the Stain 2 Master Mix into the 4-channel side of the reagent reservoir marked *S2*, placed on the bench top at room temperature.
2. Load a P200 8-channel pipette with 4 new pipette tips and aliquot **105 µL per well** to columns 5, 7, and 9 of the *S2* tray. Dispense to the first stop only to avoid creating bubbles.
   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 4.13).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Stabilization Master Mix

To Aliquot the Stabilization Master Mix:

1. Carefully pipet or pour the Stabilization Master Mix into the 4-channel side of the reagent reservoir marked *Stbl*, placed on the bench top at room temperature.
2. Load a P200 8-channel pipette with 4 new pipette tips and aliquot **105 µL per well** to columns 5, 7, and 9 of the *Stbl* tray. Dispense to the first stop only to avoid creating bubbles.
   You do not need to change pipette tips between additions of the Stabilization Master Mix.
3. If:
   - bubbles are present, puncture them with a pipette tip.
   - droplets of liquid splashed onto the well dividers, blot the top of the tray with a Kimwipe.
4. Place a cover on the tray. Orient cover correctly on the tray with the notched corners together (Figure 4.13).

Ligation Master Mix

To Aliquot the Ligation Master Mix:

1. Carefully pipet or pour the Ligation Master Mix into the 4-channel side of the reagent reservoir marked *Lig*, placed on the bench top at room temperature.
2. Load a P200 8-channel pipette with 4 new pipette tips and aliquot **105 µL per well** to columns 5, 7, and 9 of the *Lig* tray. Dispense to the first stop only to avoid creating bubbles.
   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 4.13).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Axiom Hold Buffer

NOTE: Module 5-2 has 3 Axiom Hold Buffer bottles that should be used to prepare the Scan Tray for second, third, and fourth plate.

To Aliquot the Axiom Hold Buffer to the Scan Tray:

1. Ensure that the Axiom Hold Buffer has equilibrated to room temperature. Vortex and then pour the Hold Buffer into both the 4-channel and 8-channel side of the reagent reservoir labeled *Hold*, placed on the bench top at room temperature.
2. Remove the scan tray from its pouch.
3. Remove the scan tray cover, but leave the scan tray on its protective black base.
4. Prepare the barcoded scan tray cover (P/N 202757) that came with the scan tray by completing the deionization procedure described in Appendix D, *Deionizing Procedure for GeneTitan® Trays and Covers* on page 148. Place the cover as shown in Figure 4.15 on page 92 to prevent dust or static from accumulating on the bottom of the cover.
   - Use a 12-channel P200 pipette with new pipette tips to aliquot **150 µL to EACH of the 96 wells** of the 96 Plate 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.
5. If droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove.
6. Cover the tray by orienting the notched corner of the scan tray cover over the notched edge of the tray and the flat side of the cover against the scan tray (Figure 4.14).

IMPORTANT: The scan tray has an open-bottom design, so it is very important that all 96 wells of the scan tray receive 150 µL of Axiom Hold Buffer.

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 118 for instructions on loading the reagent trays.

Figure 4.14 Scan Tray with the Clear Cover and Protective Base

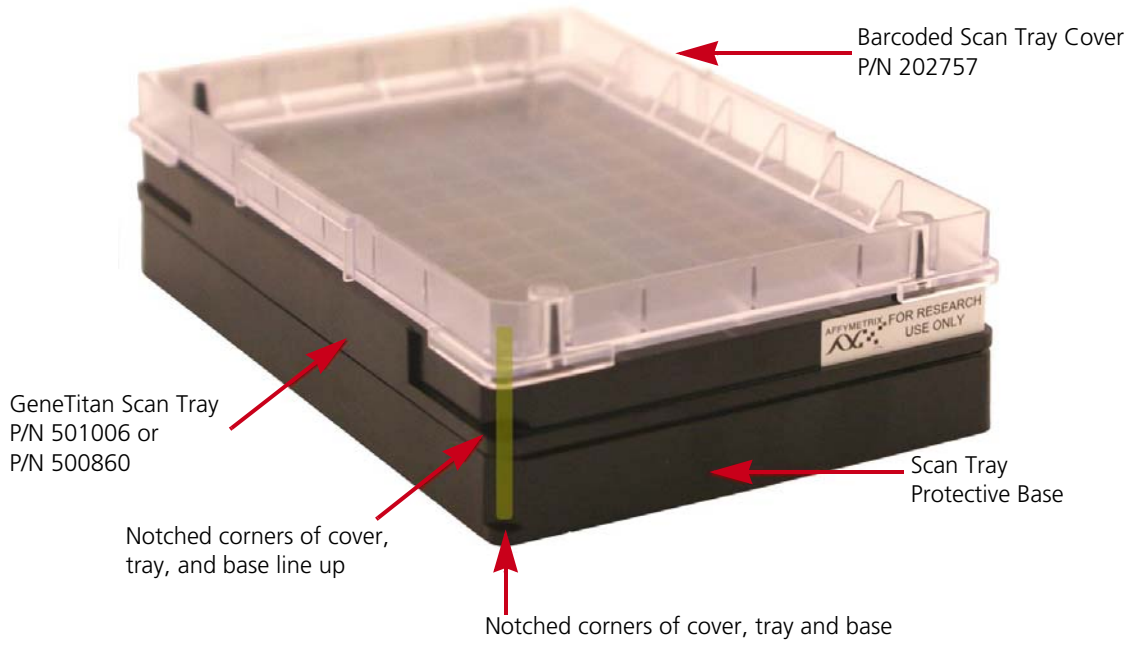


Figure 4.15 Loading the Scan Tray with Hold Buffer

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

4: Store Remaining Reagents

Store remaining Module 4-1 and Module 4-2 reagents for future use. Follow the guidelines presented in the section *Freeze-Thaw Instructions* on page 27.

Figure 4.16 Stage 5: GeneTitan Reagent Preparation, #1 of 3

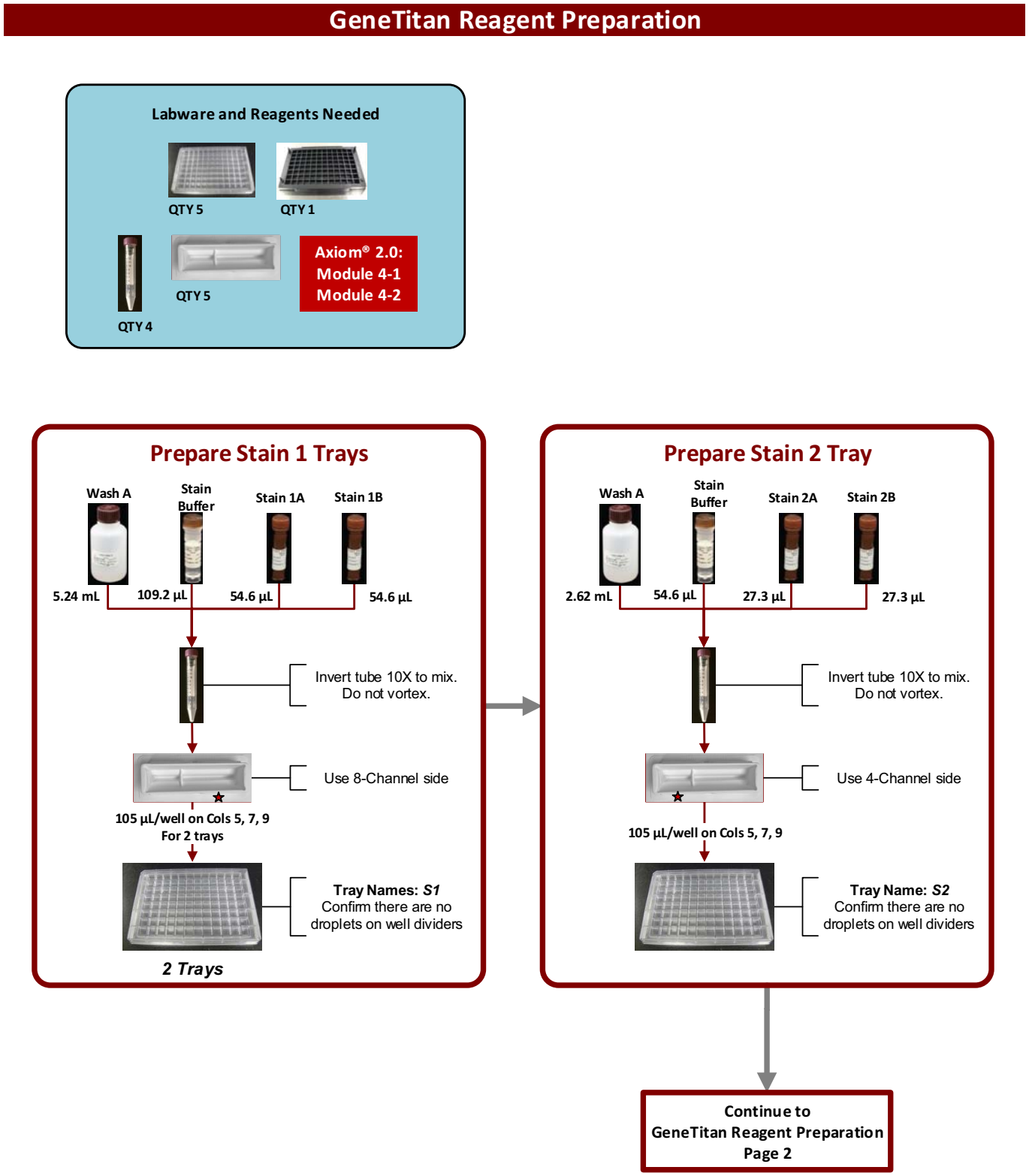


Figure 4.17 Stage 5: GeneTitan Reagent Preparation, #2 of 3

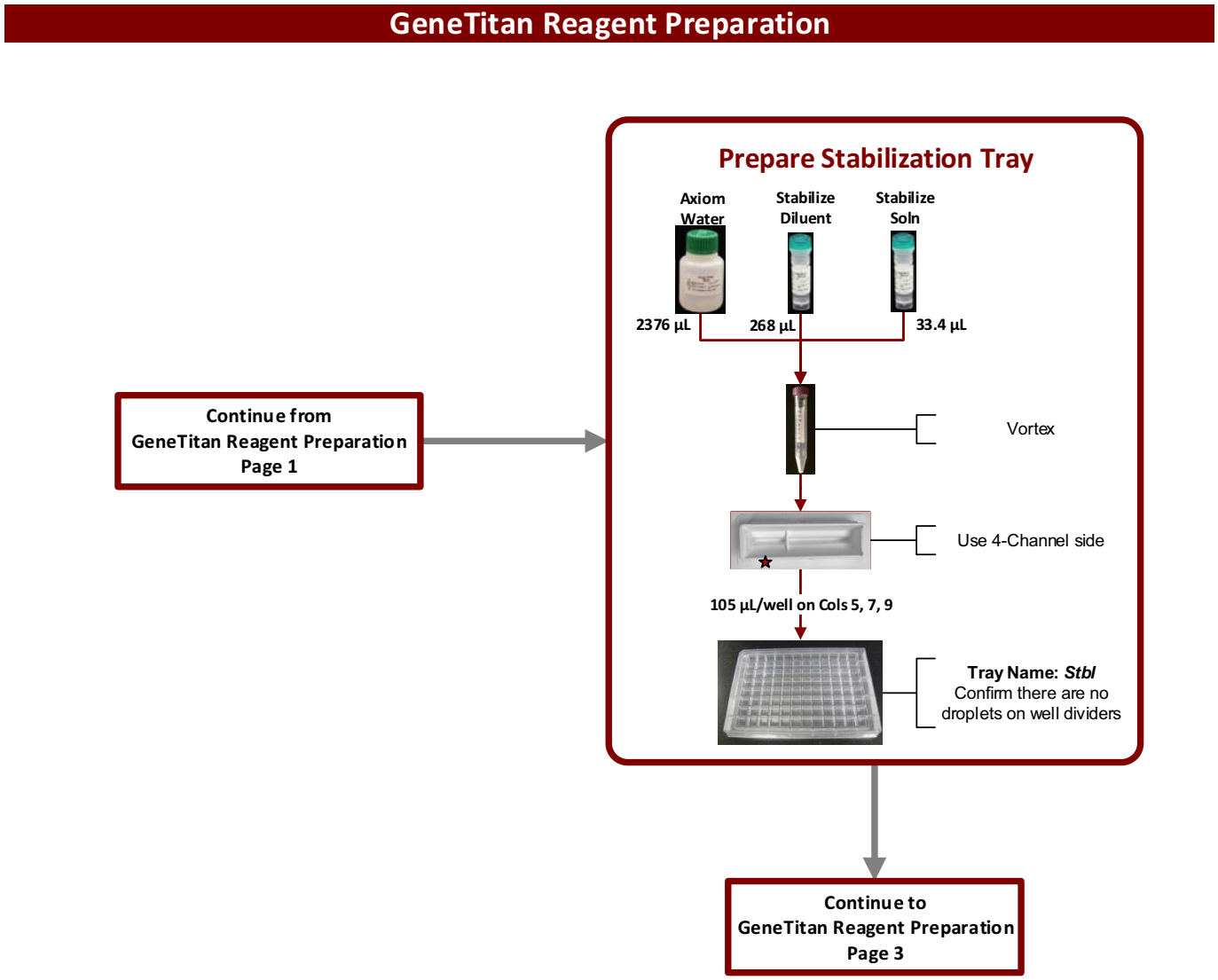
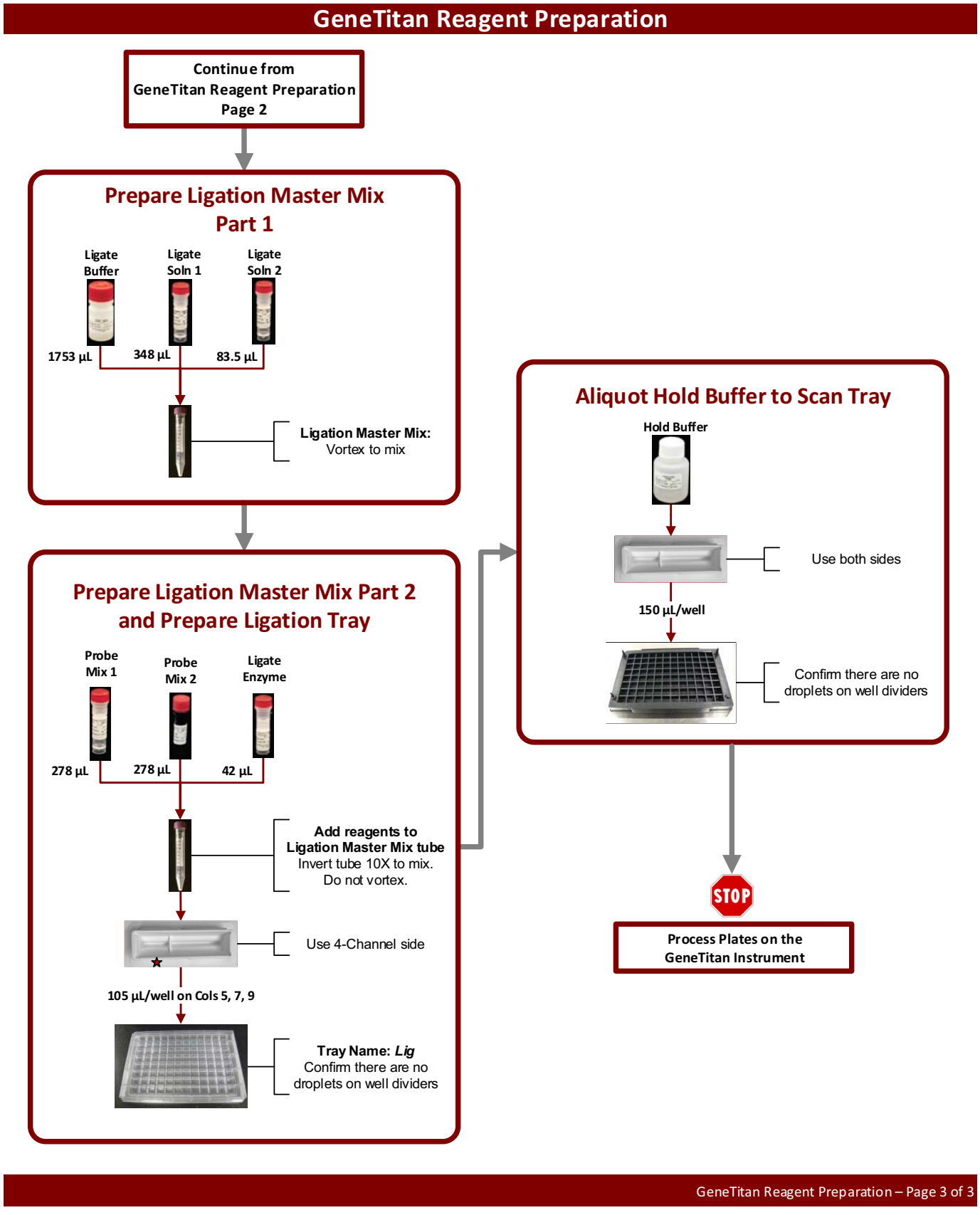


Figure 4.18 Stage 5: GeneTitan Reagent Preparation, #3 of 3



Chapter 5

Array Processing with the GeneTitan® Multi-Channel Instrument

The PharmacoScan™ Assay 24-Array Format Manual Protocol is designed for processing 24 samples at a time. The protocol is performed in two sets of steps:

- Target Preparation, performed on the lab bench without advanced automation. See Chapter 4, *Target Preparation* on page 38.
- Array processing, performed on the GeneTitan® Multi-Channel (MC) Instrument.

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

Before Using the GeneTitan® MC Instrument

Proper Tray Alignment and Loading

Proper alignment and loading of plates, covers and trays is critical when using the GeneTitan MC Instrument. Each plate, cover and tray has one notched corner. The notched corner of plates, trays, covers and bases must be in vertical alignment with each other, and placed in position A1 per the Tray Alignment guide inside each GeneTitan MC drawer (Figure 5.1 on page 97 and Figure 5.2 on page 98).

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

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

NOTE: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. Refer to Appendix E, *GeneTitan® Multi-Channel Instrument Care* on page 151 for the message displayed to the user and the procedure for replacing the filters.

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

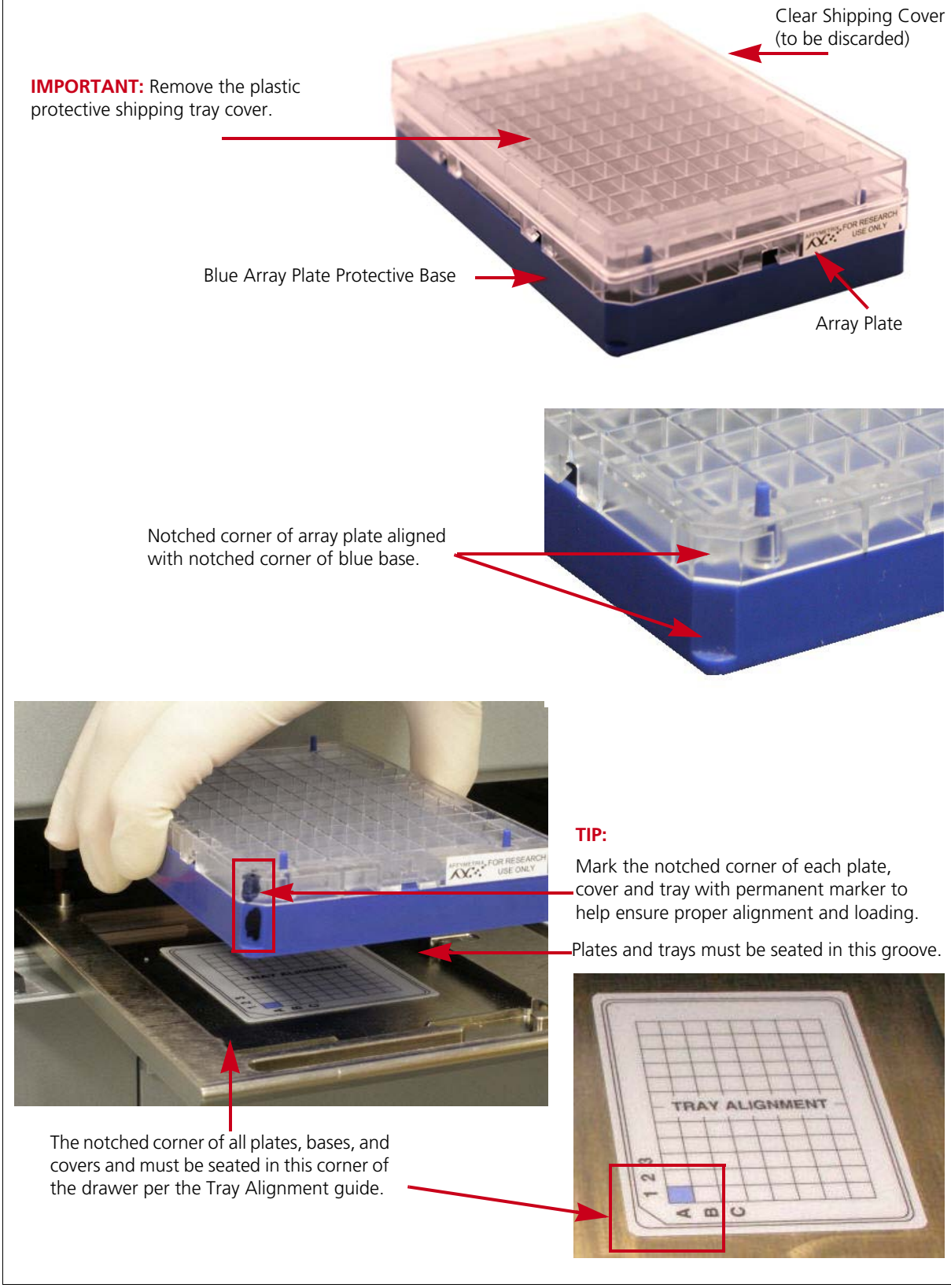
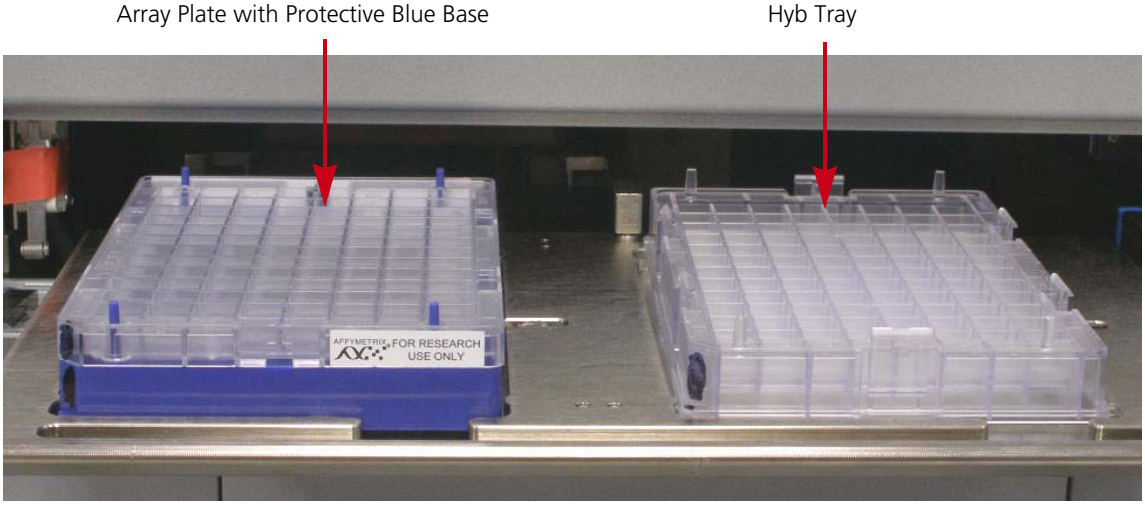


Figure 5.2 Array Plate with Protective Blue Base and the Hyb Tray Aligned and Properly Loaded into Drawer 6

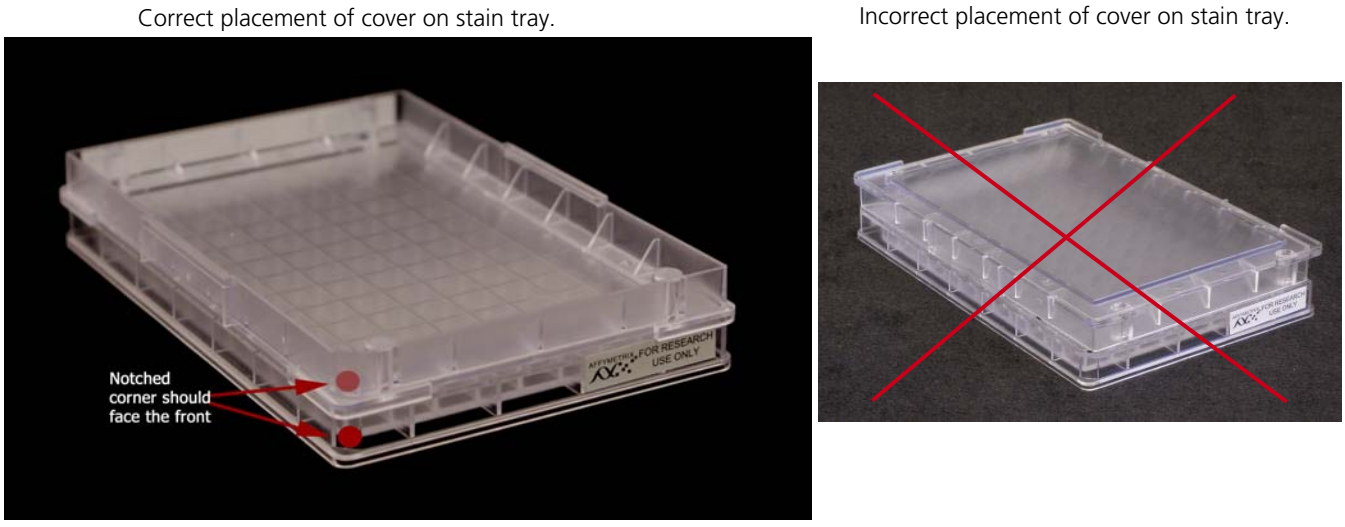


IMPORTANT: When you install the consumables, ensure that the fingers are retracted. Do not lay the consumables on top of the drawer fingers - this indicates that the instrument is not functioning correctly. Please notify your Field Service Engineer if the fingers do not retract automatically. You should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



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:

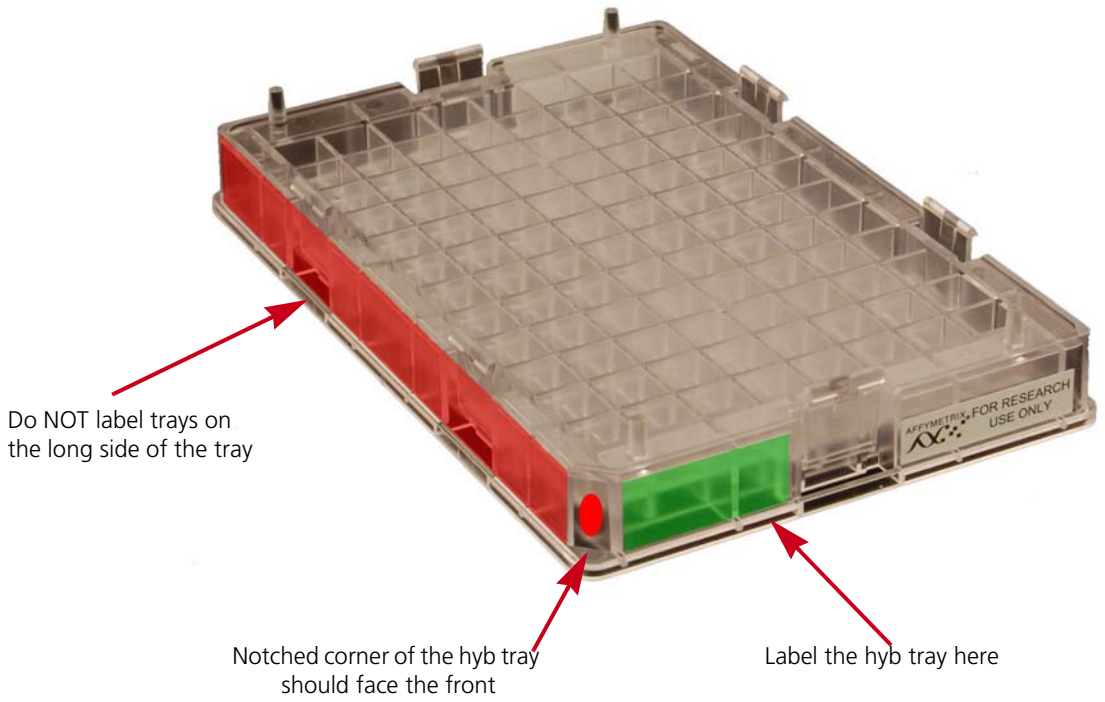
- *Labeling for Hyb Trays*, below
- *Labeling for Stain Trays* on page 100

IMPORTANT: Do not confuse hyb trays with stain trays.

Labeling for Hyb Trays

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

Figure 5.4 Labeling GeneTitan Hyb Trays

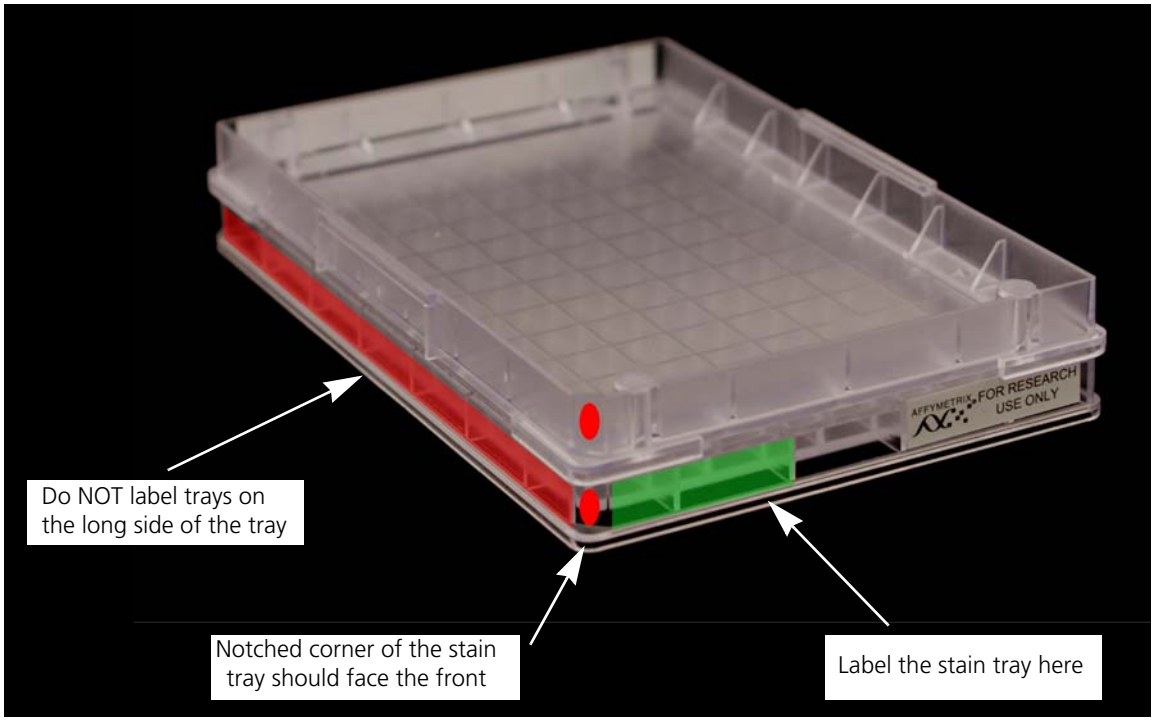


CAUTION: Writing on the wrong side of the hyb tray, or on the wrong part of the long side, may interfere with the operation of sensors in the GeneTitan MC Instrument.

Labeling for Stain Trays

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

Figure 5.5 Labeling GeneTitan Stain Tray (Stain Tray shown with Lid)



E-mail and Telephone Notifications from the GeneTitan® MC Instrument

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

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

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

- Starts
- Completes
- Aborts
- Encounters an error

GeneTitan® MC Instrument Lamp

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

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

Refer to the *GeneTitan® MC Instrument User Guide*, P/N 08-0308, or Appendix E, *GeneTitan® Multi-Channel Instrument Care* on page 151 of this user guide for details on replacing the lamp.

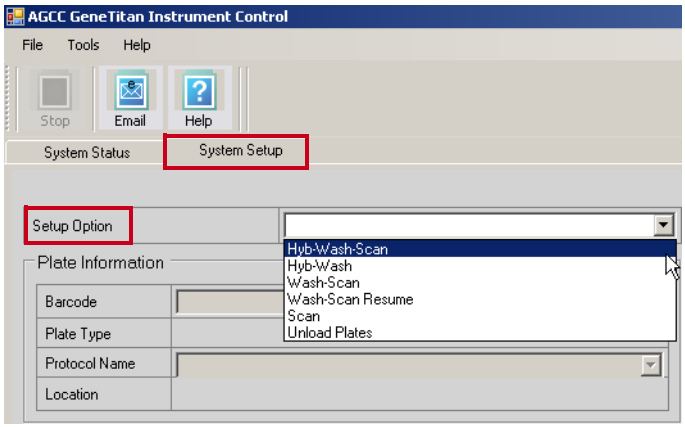
Refer to the *GeneTitan® MC Instrument User Guide*, P/N 08-0308, for the Lambda LS and Smart controller system. The Lamp and the controller should NEVER be switched ON or OFF manually. The GeneTitan MC Instrument control software manages the lamp activity and will switch the lamp ON and OFF as required. 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 Instrument.

- *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 24 samples = 23.5 hr
- *Wash*: samples on arrays are ligated, washed, stained and fixed.
  - Duration for 24 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 E, *GeneTitan® Multi-Channel Instrument Care* on page 151 for the message displayed to the user and the procedure for replacing the filters

- *Scan*: The array plate is moved to the imaging device in the GeneTitan MC Instrument and each array is scanned.
  - Duration for 24 samples = 2 hrs

Hyb-Wash

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

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

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

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

When Ready to Scan the Array Plate:

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

Wash-Scan

Use this option if:

- It was necessary to hybridize the array plate in an oven separate from the GeneTitan MC Instrument.
- You wish to bypass the Hybridization step and perform only the Wash/Stain and Scan steps.

Wash-Scan-Resume

Use this option if:

- Fluidics processing has been interrupted (e.g., a power failure occurs at your facility).

Scan

Use this option:

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

Unload Plates

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

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

Aborting a Process

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

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

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

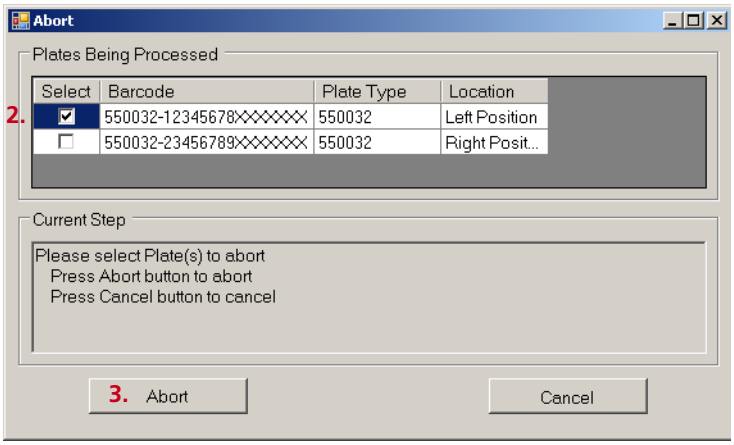
Figure 5.7 Manually Aborting an Array Plate

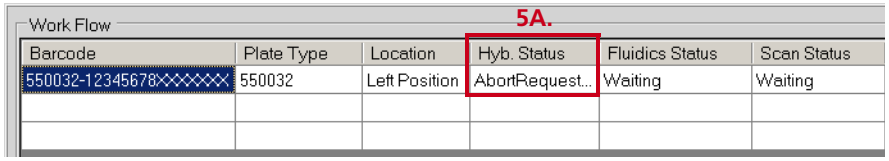
To abort array plate processing:

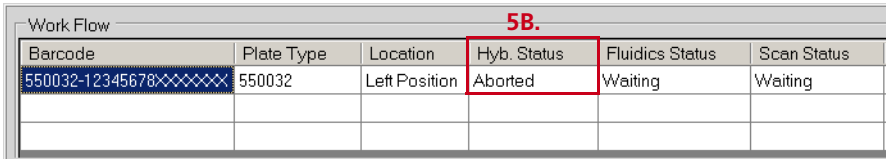
1. Click the **Stop** button.
2. Select the array plate that you want to abort.
3. Click **Abort**.
4. Click **Yes**.
5. Wait until the status of the array plate in the WorkFlow window changes from *AbortRequest…* to *Aborted* (5A and 5B).
6. Once aborted, retrieve the array plate and other related consumables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

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

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







Stage 1: Create and Upload Batch Registration File

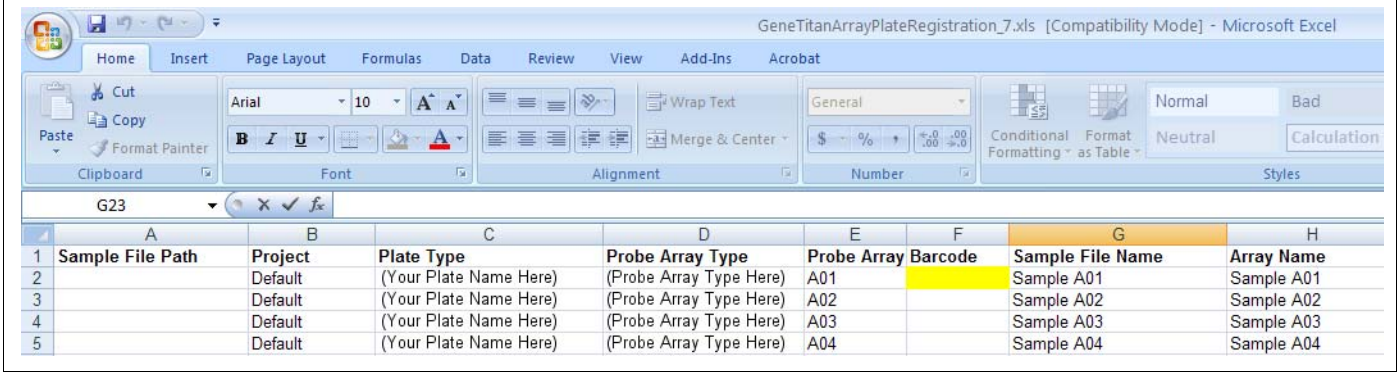
In the AGCC software, you must create and upload a Batch Registration file before you begin *Stage 2: Hybridization* on page 105 (example shown in Figure 5.8). This file contains information critical for:

- 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 C, *Registering Samples in Affymetrix GeneChip® Command Console®* on page 145 for detailed instructions.)
2. In AGCC, select the array plate format (24 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 105.

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



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

Stage 2: Hybridization

Reagents Required

Reagents Required

Table 5.1 Reagents Required from the PharmacoScan™ Reagent Kit 4x24 Reactions

Reagent	Module
Axiom Wash Buffer A (both bottles; 1L), P/N **901446**	Module 3, Room Temperature
Axiom Wash Buffer B, P/N **901447**	
Axiom Water, P/N **901578**	

- PharmacoScan 24-array plate is required for this step. Prior to inserting this plate into the GeneTitan MC Instrument for hybridization, the array plate should be brought to room temperature as described on Step A on page 75.
- A hybridization tray containing denatured samples (from Step 8 on page 77 in Chapter 4) is also required for this step. The denatured samples should be transferred to the hyb tray only after the GeneTitan MC Instrument is ready for loading the hyb tray in the *Loading Tray Consumables onto the GeneTitan® MC Instrument* on page 120.

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 E, *GeneTitan® Multi-Channel Instrument Care* on page 151 for the message displayed to the user and the procedure for replacing the filters

IMPORTANT: Please do not close the scanner application by right-clicking on it and choosing the "Close" option. This will cause the scanner application to exit abnormally and cause undue delay in processing the next plate. The correct way to close the application is described in *Shutting Down the GeneTitan® MC Instrument* on page 126.

Figure 5.9 Launching AGCC and initializing the GeneTitan MC Instrument.

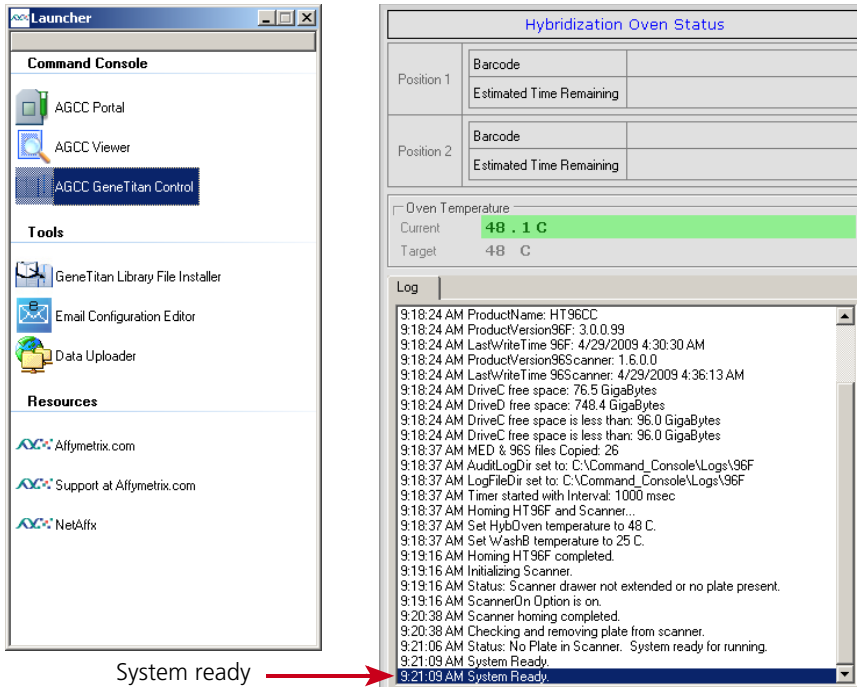
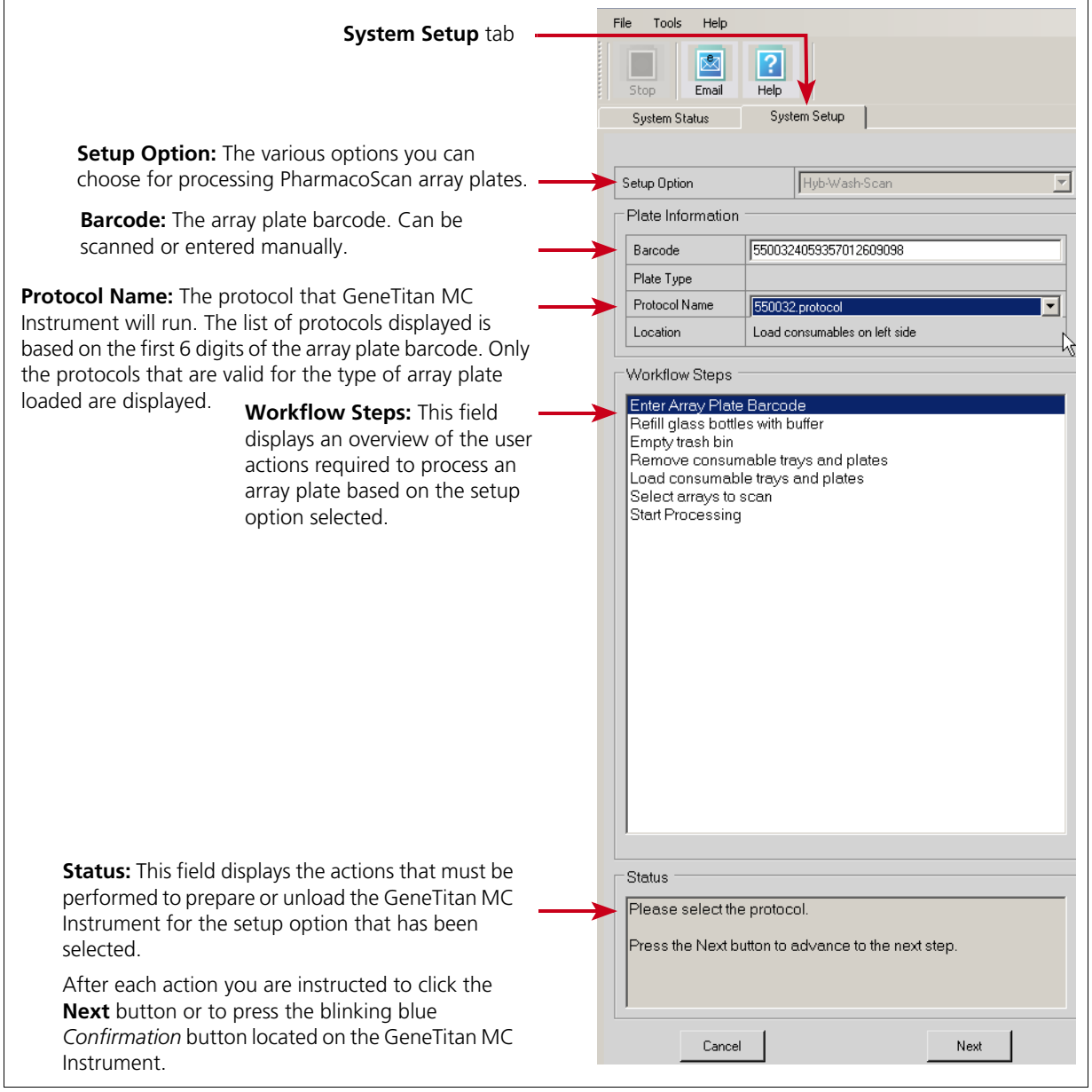


Figure 5.10 System Setup Tab and the Information Displayed in this Pane



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 101.
   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 PharmacoScan array plates.

- **24 PharmacoScan array plate requires ~20 GB**

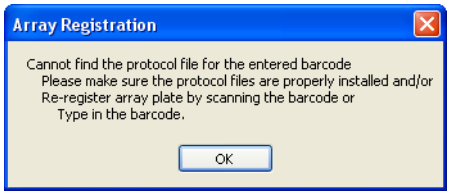
C. Plate Information:

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

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

  550XXX <barcode> = Affymetrix 24-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 the GeneTitan MC Instrument. If a library file must be installed exit the GeneTitan Instrument, install libraries and relaunch.

- **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.

  550XXX.protocol = for Affymetrix 24-array plate barcodes

4. Complete the remaining workflow steps as follows:

A. **Refill bottles with buffer** (Figure 5.12 on page 109)

1) Fill these bottles:
   - Wash A: fill with PharmacoScan Wash Buffer A—keep at 2L full
   - Wash B: fill with PharmacoScan Wash Buffer B—Use all 600 mL of Wash B from the reagent kit per plate. Fill to 1L mark when processing two plates on the same day.
   - Rinse: fill with PharmacoScan 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 array plate. All 600 mL of the Wash buffer B from the PharmacoScan Reagent Kit 4x24 Reactions 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 1L mark only. Wash A keep at 2L. We strongly recommend refilling these bottles every time you are prompted to do so.**

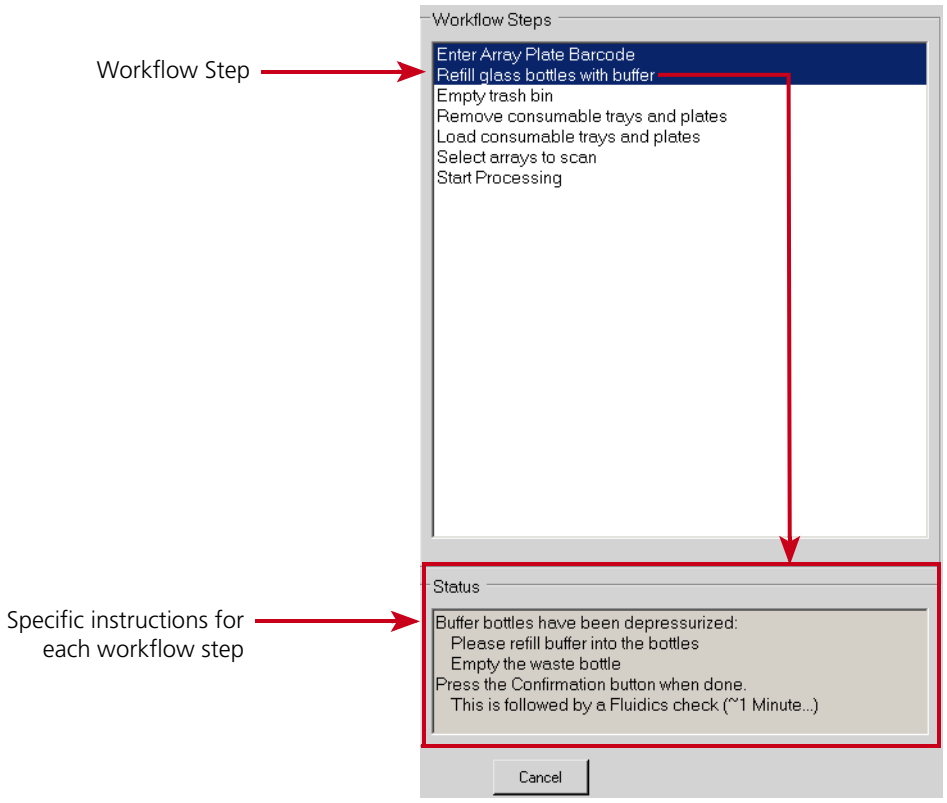
  If the volume in any of these bottles becomes too low during a run, a message is displayed (see Chapter 6, *Troubleshooting* on page 127). However, even if you fill the bottle at this time, the instrument may not be able to successfully complete the step that was in progress.

- **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 PharmacoScan Reagent Kit).**

2) Empty the waste bottle.

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

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."

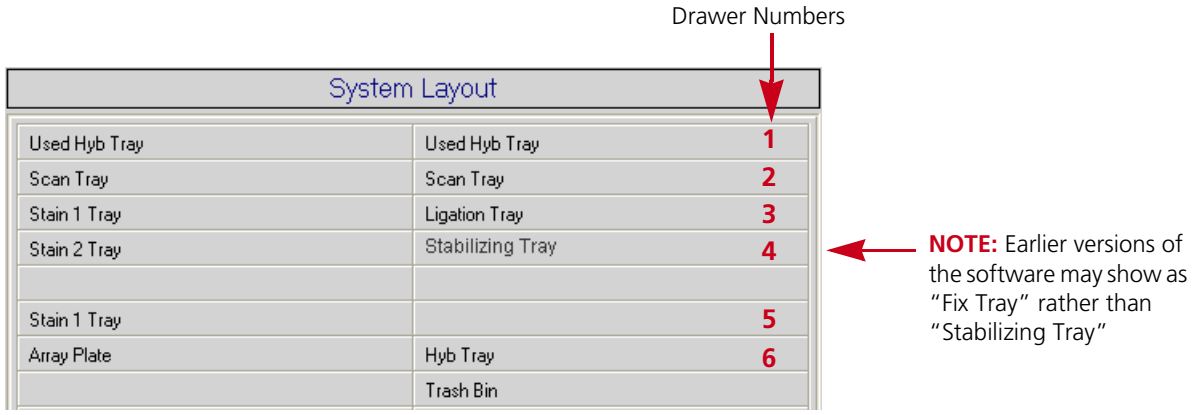
2) Press the **Confirmation** button to continue.

D. Continue to *Load a PharmacoScan™ Array Plate and Hyb Tray Onto the GeneTitan® MC Instrument* on page 110.

Load a PharmacoScan™ Array Plate and Hyb Tray Onto the GeneTitan® MC Instrument

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

Figure 5.13 System Layout—Location of Plates Inside the GeneTitan MC Instrument



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

1. When drawer 6 opens, load the array plate and hyb tray as follows:

   A. Examine the wells of the hyb tray for bubbles; puncture any bubbles with a pipette tip.

   IMPORTANT: Removing bubbles at this step greatly reduces the chance of bubbles under the arrays when the hyb tray and the PharmacoScan 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 111).

The array plate must be loaded on its protective blue base, as shown in Figure 5.15 on page 111 below. The clear plastic cover on top of the array plate SHOULD NOT be loaded in the GeneTitan MC Instrument. See Figure 5.1 on page 97 for more details on the correct way of loading the array plate.

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

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

Figure 5.14 Array Plate Packaging

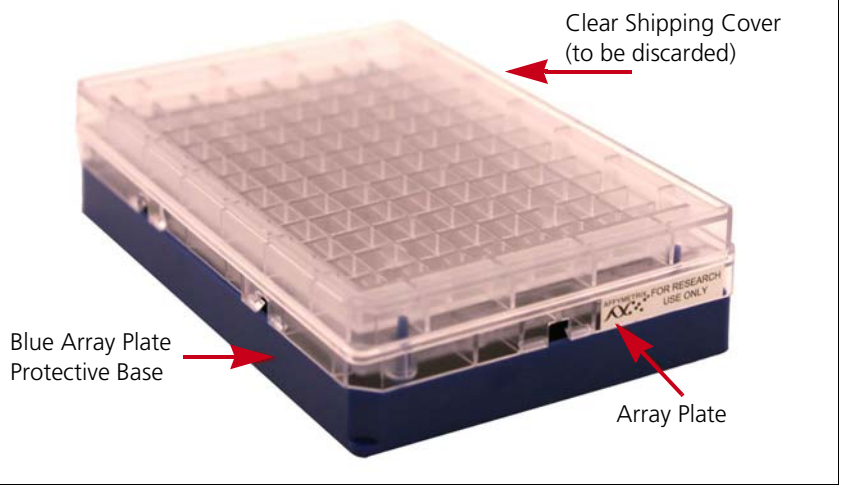
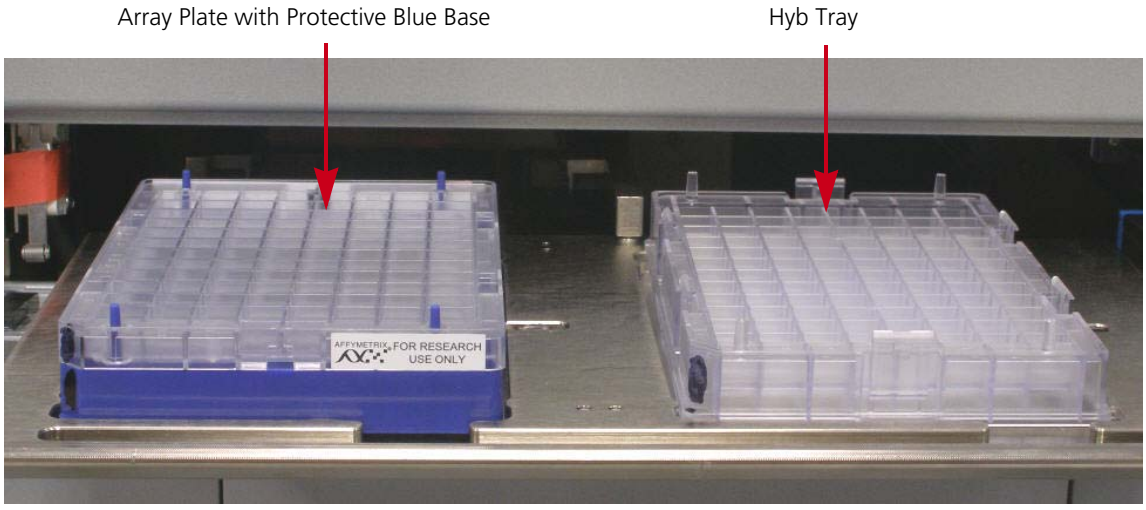


Figure 5.15 Array Plate with Protective Blue Base and the Hyb Tray Properly Loaded into Drawer 6.



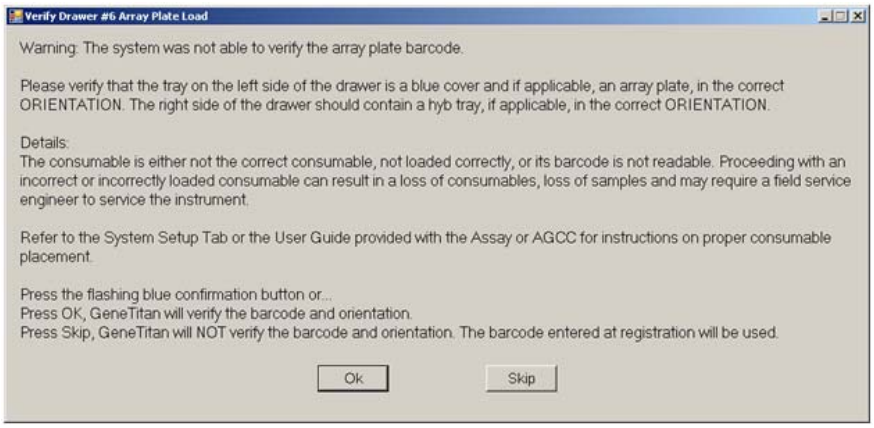
D. Load the array plate **with the protective blue base** on the left side of the drawer (Figure 5.15).

! CAUTION: The notched corner of each plate, cover and tray must be aligned. When loading onto the GeneTitan MC Instrument, the notched edge plates, covers and trays must be aligned as indicated by the Tray Alignment guide in the drawer (Figure 5.15 on page 111).

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

E. Press the **Confirmation** button.

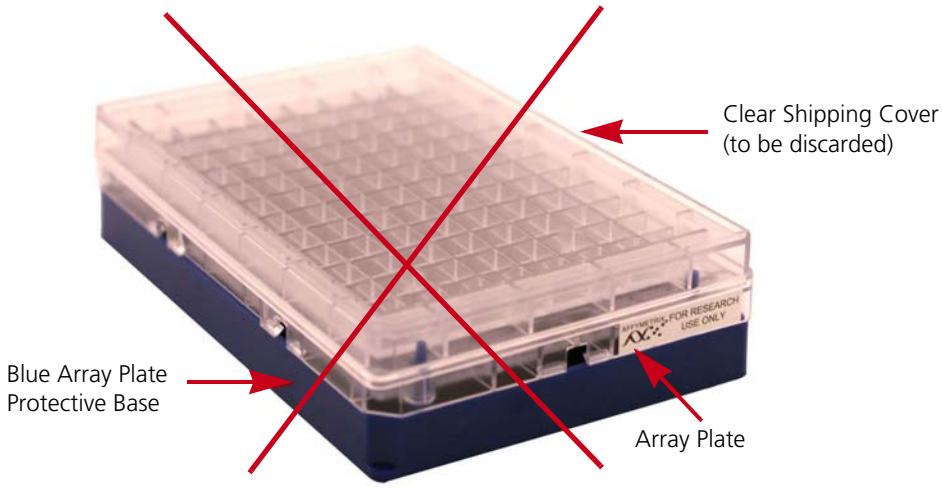
Figure 5.16 Barcode Error Message



When you load the array plate on the 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).

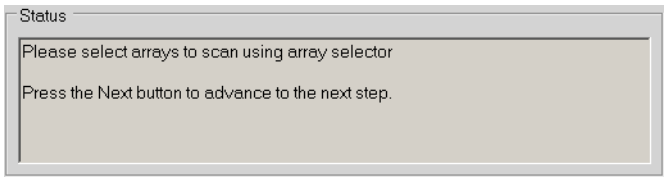
- Click **OK** to retry and check the loading of the array plate; or
- Click **Skip** if the instrument has problems reading the barcode and after verifying that the trays have been placed in the proper orientation.

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 97.

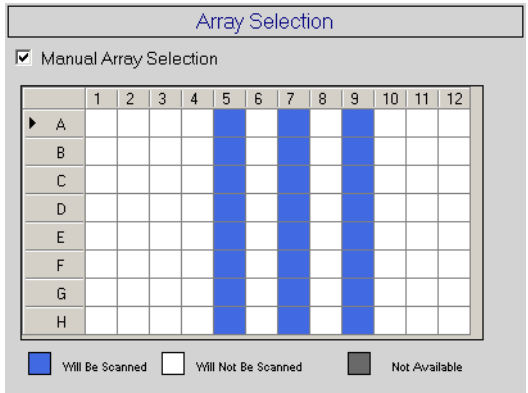


F. Select the arrays to scan (instructions in Figure 5.17).
By default, all arrays are selected.

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

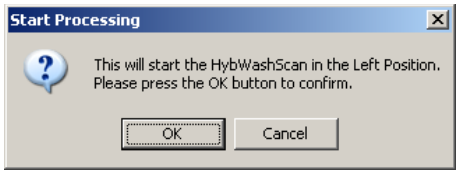
1. Click one box
2. Hold down the **Ctrl** key
3. Click another box in the same column

Group of arrays:

1. Click one box
2. Hold down the **Shift** key
3. Click and drag the mouse

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

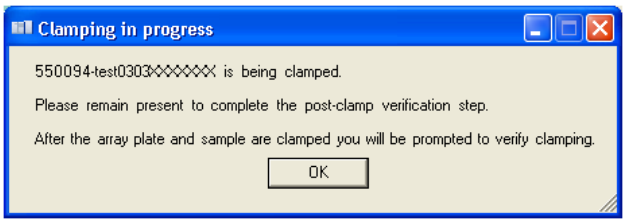
Figure 5.18 Click OK to Start Processing the First Array Plate and Hyb Tray



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

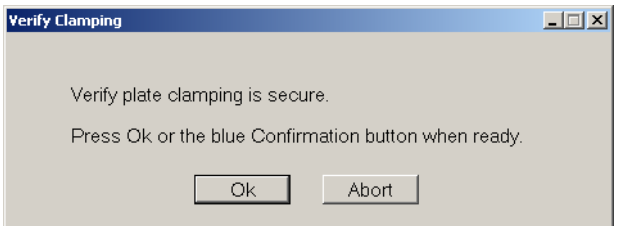
The software starts the process for clamping the array plate to the hybridization tray. Press **OK** on the dialog shown in Figure 5.19 and wait for the drawer to open before retrieving the array plate and hybridization tray combo for inspection. The sandwich of the array plate and hybridization tray needs to be manually inspected before the array processing can begin. Once clamping is complete the dialog shown in Figure 5.20 on page 113 will be displayed. If you do not press OK in Figure 5.19 the dialog box will go away without intervention and Figure 5.20 on page 113 will be displayed.

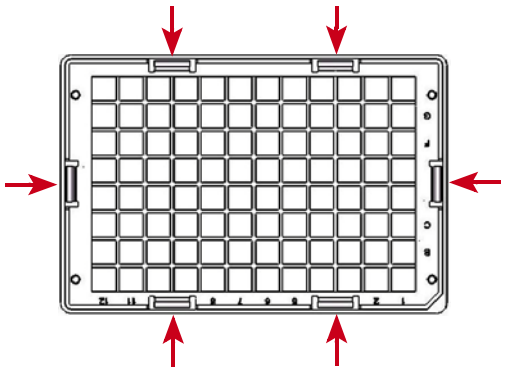
Figure 5.19



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

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





Clamping Points on PharmacoScan Array Plate and Hyb Tray

Array Plate

Hyb Tray

Notched Corners

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

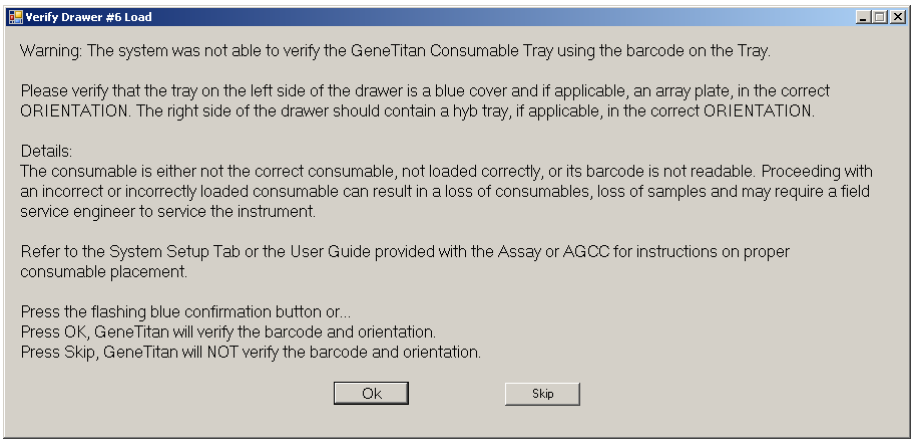
Figure 5.21 Array Plate Hyb Sandwich

B. Inspect the bottom of the plate stack for bubbles under the arrays—**do NOT invert the plates**.

C. If bubbles are present, gently tap the plate until the bubbles move out from under the arrays—**do NOT unclamp the plate stack**.

D. Return the plate stack to the drawer, and press the **Confirmation** button to proceed. The message in Figure 5.22 may be displayed again if plate orientation is incorrect or if the hyb tray barcode cannot be read. Click **OK** to proceed.

Figure 5.22 Verification Message



Load a Second PharmacoScan™ Array Plate and Hyb Tray Onto the GeneTitan® MC Instrument

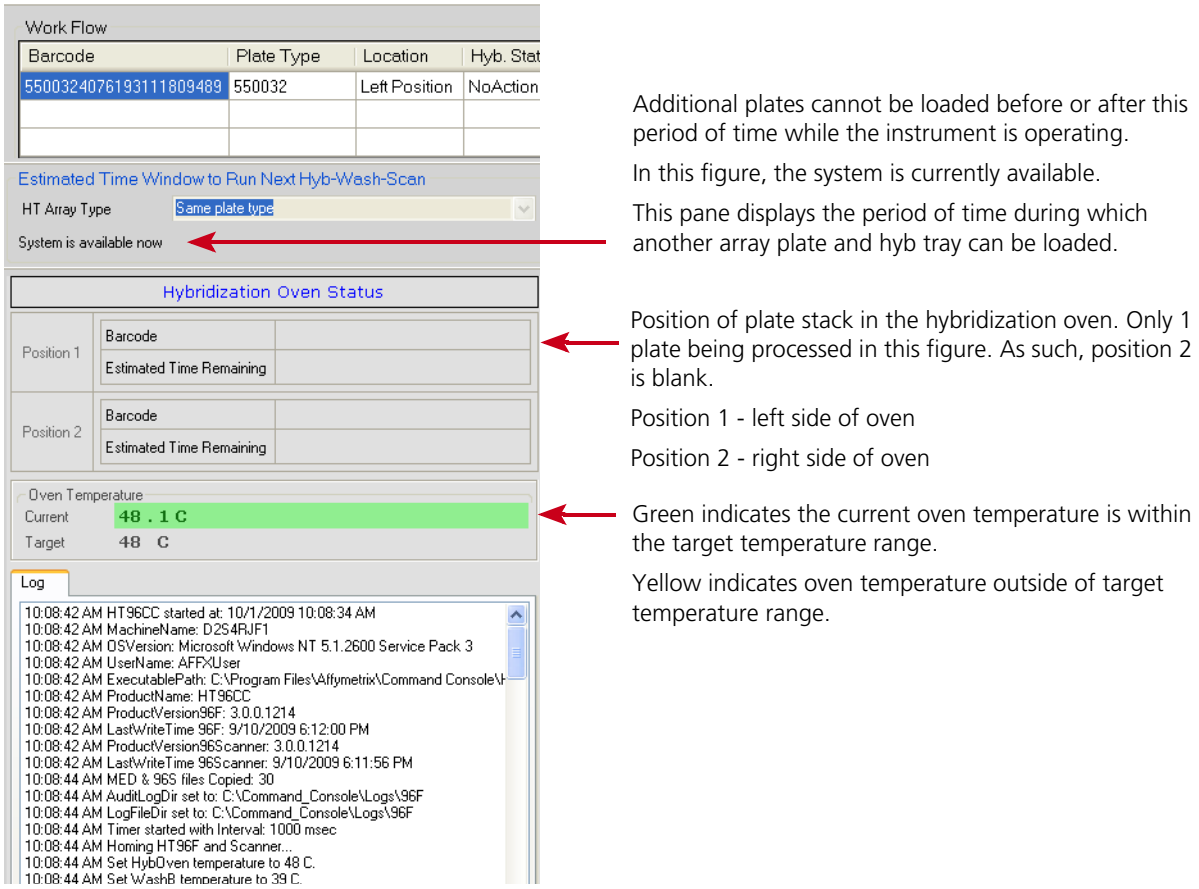
When You Can Load a Second Array Plate and Hyb Tray

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

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

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

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

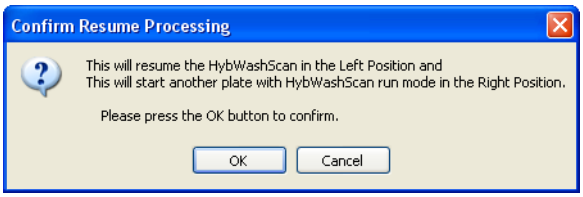


1. Select the System Setup tab.
2. Load an PharmacoScan array plate and hyb tray in the same manner that you loaded the previous plate and tray.
   - **A.** Scan or manually enter the PharmacoScan array plate barcode, then click **Next**.
   - **B.** Load the PharmacoScan array plates **with the blue base** and the hyb tray **without the cover**, then press the Confirmation button.
   - **C.** Select the arrays to scan, then click **Next**.

D. Ensure that the plates are clamped securely when prompted, then press the **Confirmation** button.

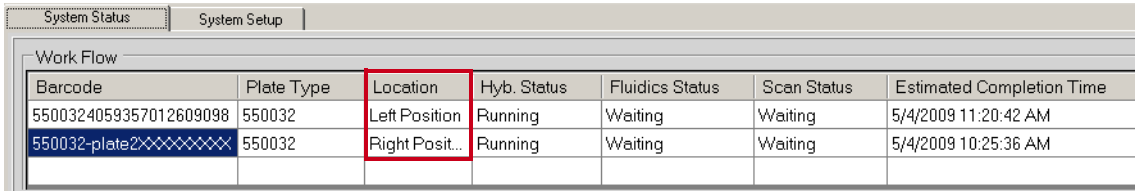
E. Click **OK** when prompted to resume plate processing (Figure 5.24).

Figure 5.24 Confirm Resume Processing Prompt



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

Figure 5.25 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 PharmacoScan Wash Buffer A up to 2L. ▪ Wash Bottle B – fill with PharmacoScan Wash Buffer B to the 1L mark. ▪ Rinse – fill with PharmacoScan Water to the 1L 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 properly. 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**, The GeneTitan MC Instrument will verify the barcode and orientation. Press **Skip**, The GeneTitan MC Instrument will NOT verify the barcode and orientation. The barcode entered at registration will be used.		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 5.26).

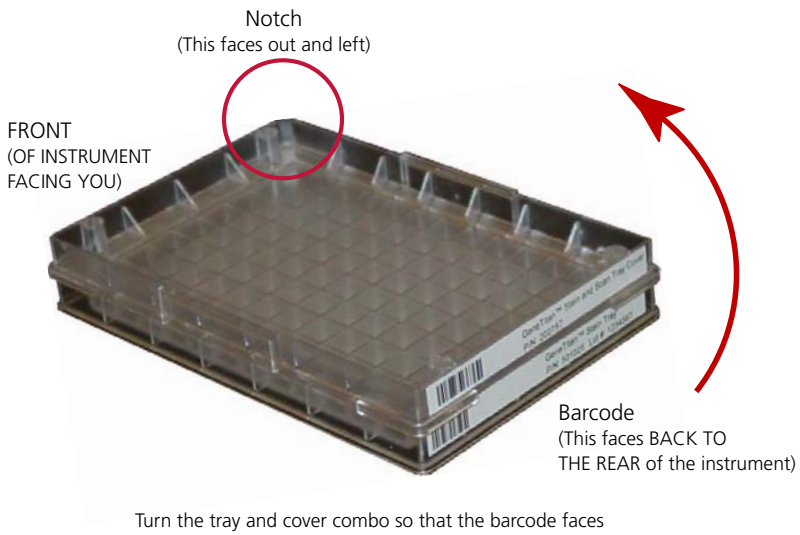
CAUTION: Do not remove the scan tray from its protective black base. Leave the scan tray in the base until loaded onto the GeneTitan MC Instrument. When handling the scan tray, the bottom glass surface of the tray should not be touched.

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

Proper Installation of the GeneTitan® Tray Consumables

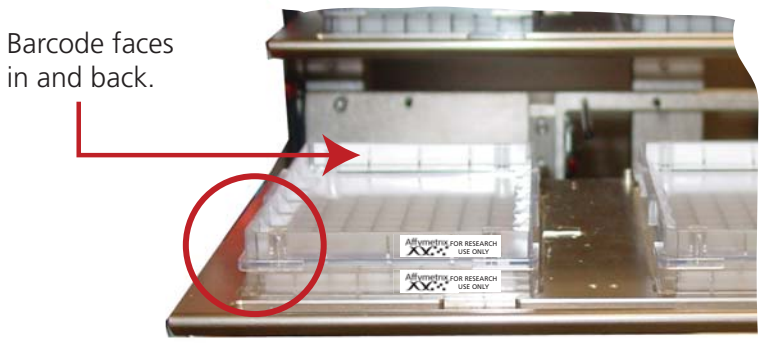
It is very important that you install the GeneTitan tray consumables in the proper orientation. The barcode faces into the instrument.

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



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 5.28 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.
The Affymetrix logo and "For Research Use Only" faces out.

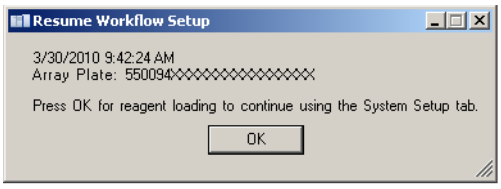
NOTE: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. Refer to Appendix E, *GeneTitan® Multi-Channel Instrument Care* on page 151 for the message displayed to the user and the procedure for replacing the filters.

Loading Tray Consumables onto the GeneTitan® MC Instrument

To Load Trays onto the GeneTitan MC Instrument:

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

Figure 5.29 The Resume Workflow Setup Message



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

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

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

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

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

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

CAUTION:

- **Orient trays as indicated by the guide inside the drawer. Improper orientation may cause the run to fail.**
- **Remove the protective black base from the scan tray immediately prior to loading Figure 5.30 on page 122).**
- **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

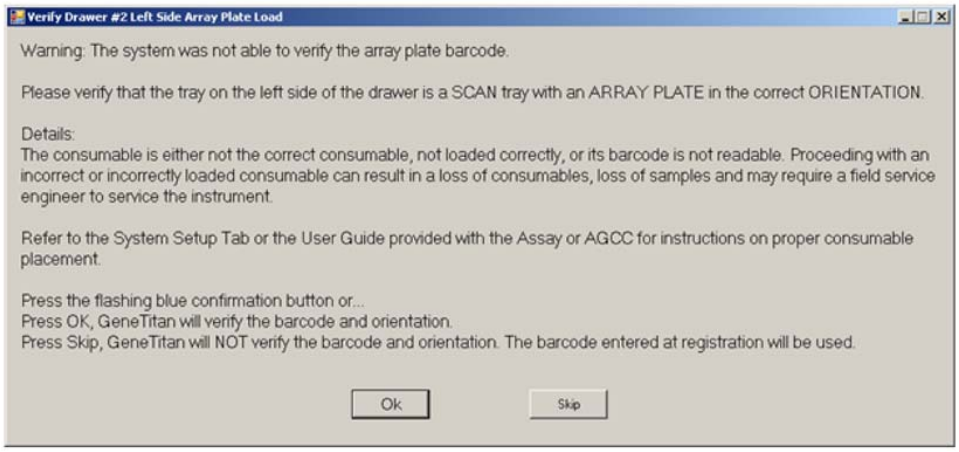
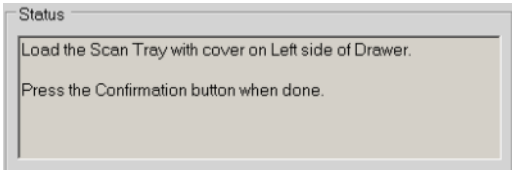
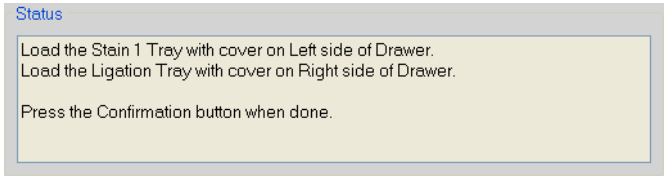
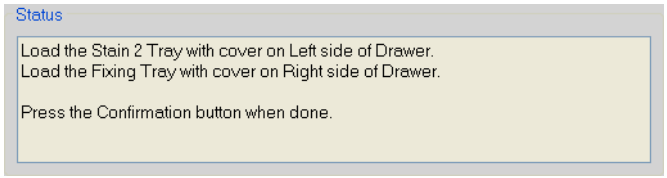
Loading Sequence by Drawer Number	Left	Right
	NOTE: If the software is unable to verify the barcode on the scan tray and the scan tray cover, the software will display the following error message 	
2	**Scan Tray with cover—do not load the** protective ***black base*** (left side of drawer as indicated in Status window) Figure 5.30 on page 122 	
3	Stain Tray with Stain 1	Ligation Tray Figure 5.31 on page 123 
4	Stain Tray with Stain 2	Stbl Tray Figure 5.32 on page 123 

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

Loading Sequence by Drawer Number	Left	Right
5	Stain Tray with Stain 1	Empty

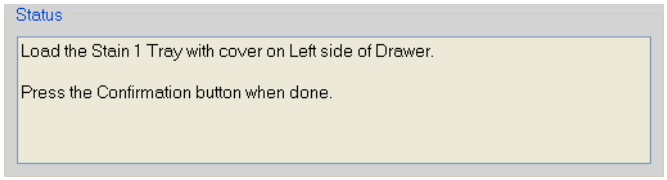
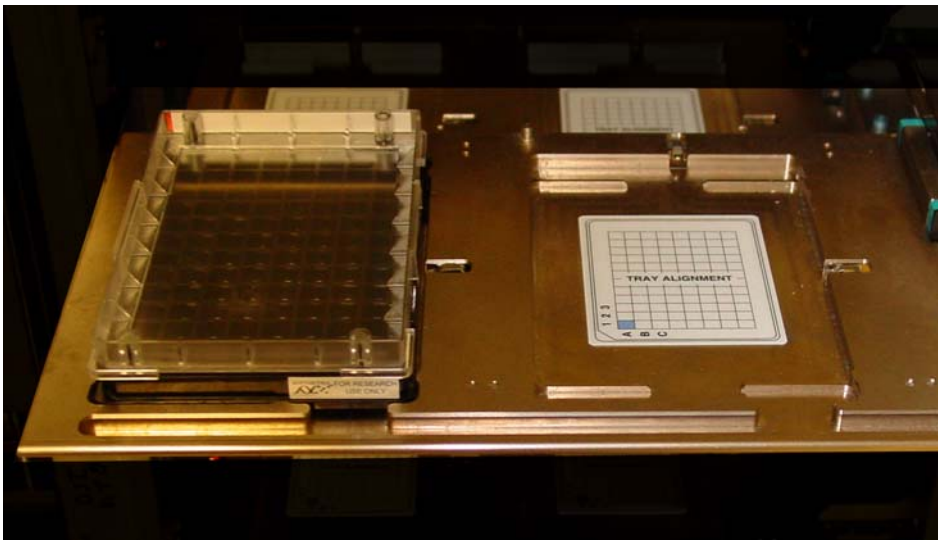


Figure 5.30 Scan Tray loaded in Drawer 2.

Scan tray with cover loaded in drawer 2.



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

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

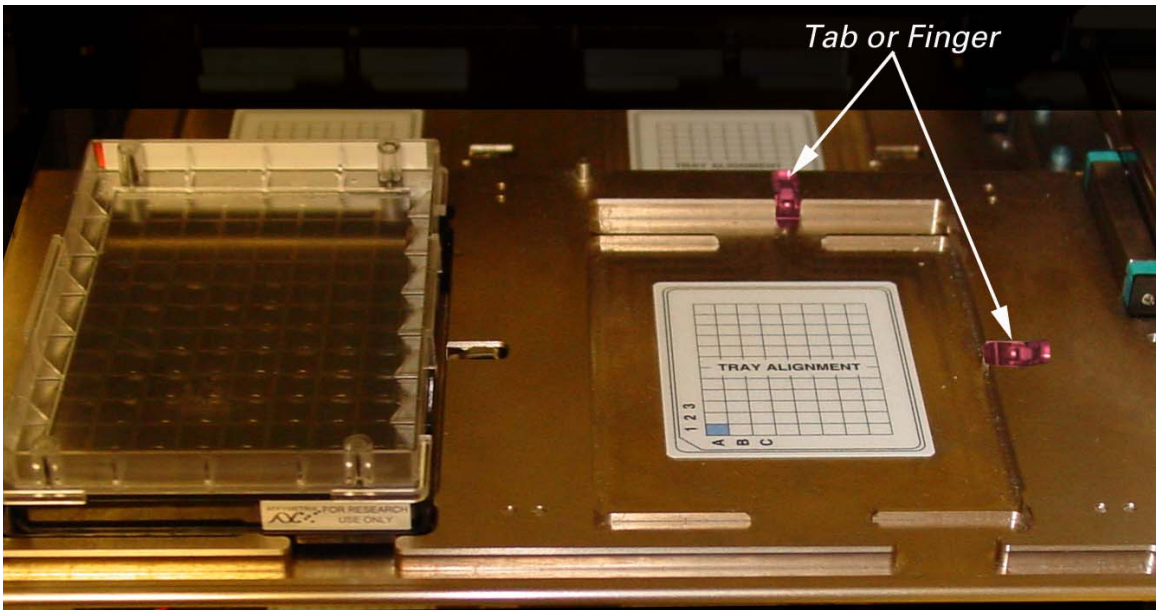


Figure 5.31 Stain 1 Tray and Ligation Tray Loaded in Drawer 3

Stain 1 Tray (left) and Ligation Tray (right) loaded in drawer 3

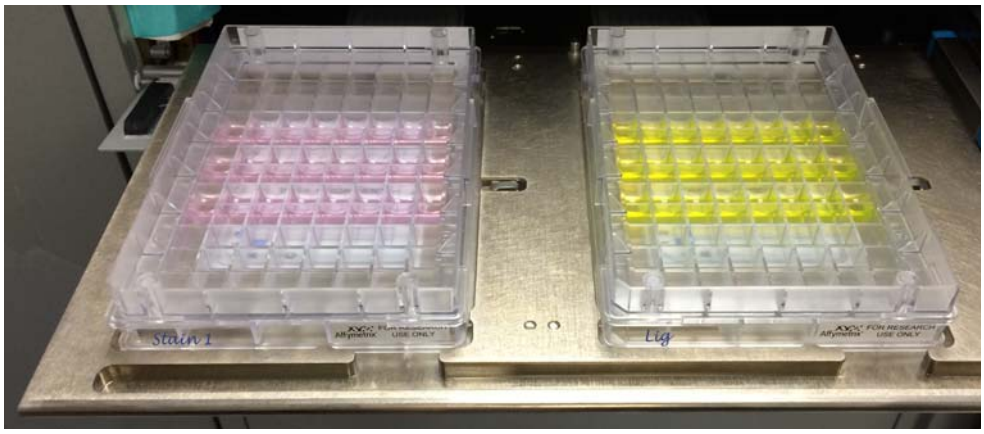


Figure 5.32 Stain 2 Tray and Stbl Tray Loaded in Drawer 4

Stain 2 Tray (left) and Stbl Tray (right) loaded in drawer 4

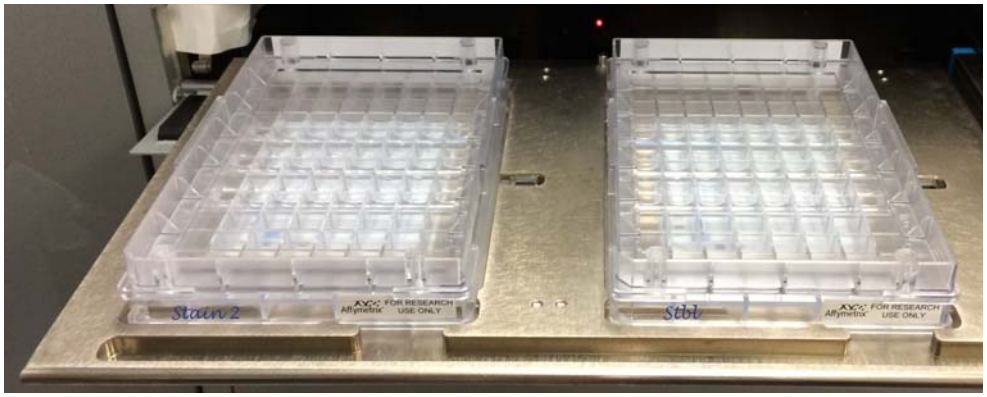
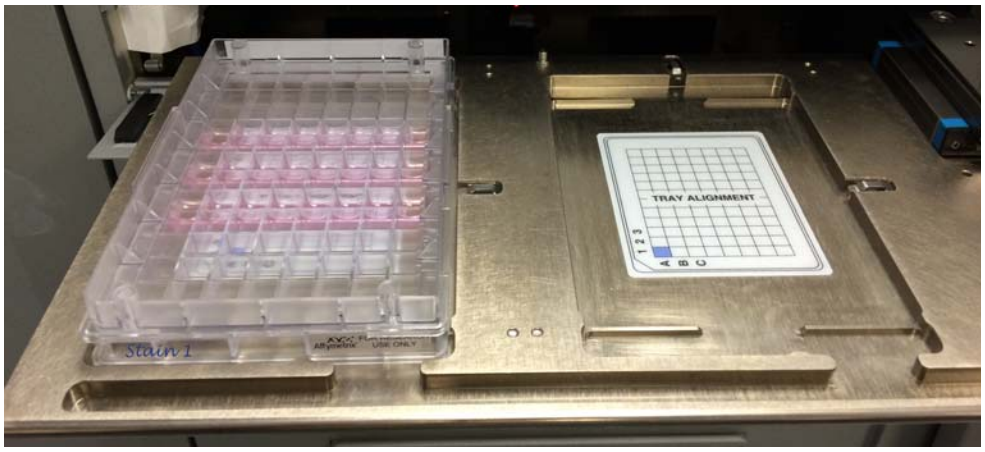


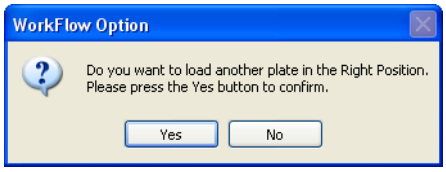
Figure 5.33 Stain 1 Tray Loaded in Drawer 5

Stain 1 Tray loaded in drawer 5



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

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



4. Follow the prompts and:
   - **A.** Setup Option: select **Setup Another Run**, then click **Next**.
   - **B.** Scan or manually enter the PharmacoScan array plate barcode, then click **Next**.
   - **C.** Select a protocol, then click **Next**.
   - **D.** When drawer 6 opens:
     1) Remove the blue cover from the previous PharmacoScan array plate.
     2) Load a new PharmacoScan array plate and new blue base on the left; load a new hyb tray on the right.
     3) Press the **Confirmation** button.
   - **E.** Click **OK** when prompted (Figure 5.35).

Figure 5.35 Confirm Resume Processing Message

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

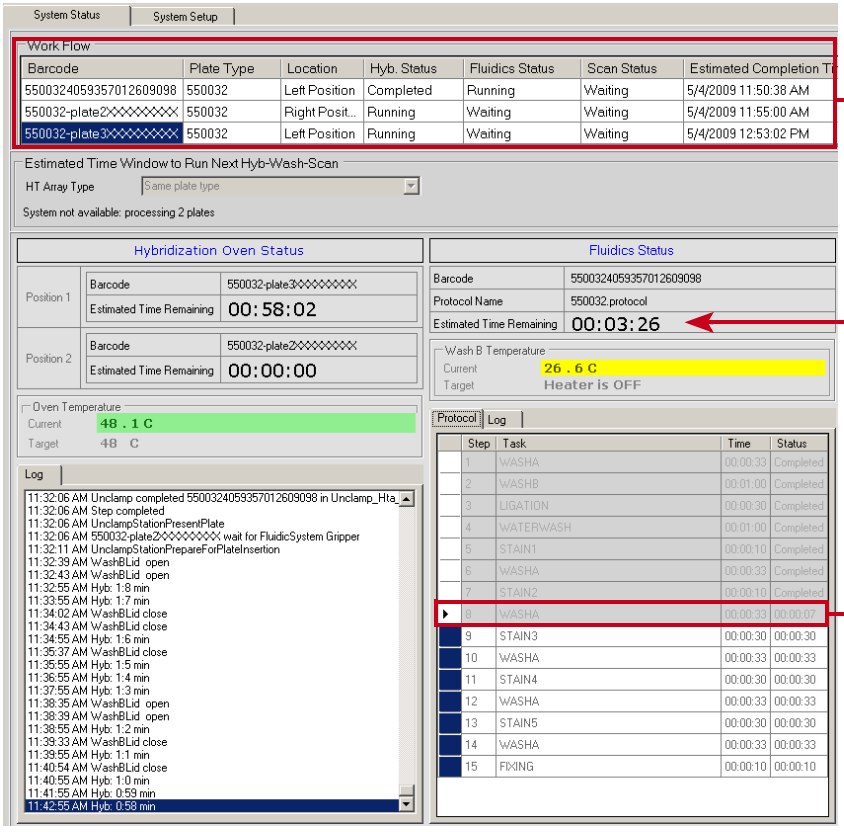
When processing resumes:

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

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



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

Estimated Completion Time is for the current process.

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

Step currently executing in Fluidics.

Continuing the Workflow

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

When the scanning process begins, the window shown in Figure 5.37 is displayed. This window must remain open while PharmacoScan array plates are being scanned.

CAUTION:

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

Figure 5.37 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.

Shutting Down the GeneTitan® MC Instrument

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

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

To Shutdown the GeneTitan MC Instrument:

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

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

Chapter 6

Troubleshooting

GeneTitan® Multi-Channel Instrument

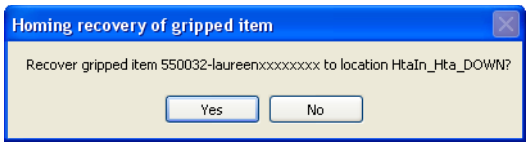
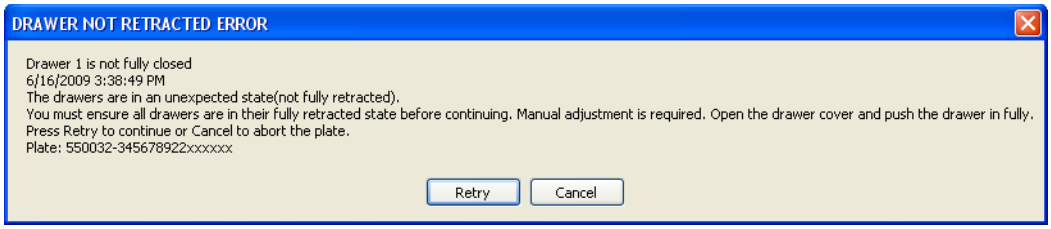
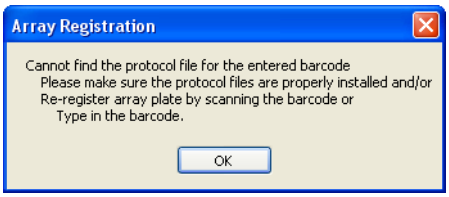
Refer to the *GeneTitan® Multi-Channel Instrument User Guide*, P/N 08-0306 for further troubleshooting information.

Table 6.1 GeneTitan® Multi-Channel Instrument Troubleshooting Guidelines for the PharmacoScan™ Assay 24-Array Format Manual Protocol

Problem	Possible Causes	Possible Actions
Plate trapped in GeneTitan Multi-Channel 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 Multi-Channel 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 Multi-Channel Instrument and finish processing using the Wash/Scan process.
FAILED messages	See *Failed Messages* on page 129	
FLUIDIC DIAGNOSTIC messages	See *Fluidic Diagnostic Messages* on page 129.	
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 132.

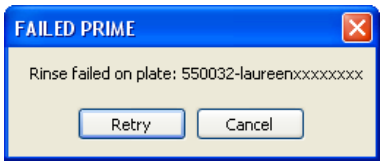
Miscellaneous Messages

Table 6.2 Miscellaneous messages and recommended actions

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

Failed Messages

Table 6.3

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 a PharmacoScan array plate. All 600 mL of the Wash buffer B from the PharmacoScan Reagent Kit 4x24 Reactions 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 6.4 Problem messages

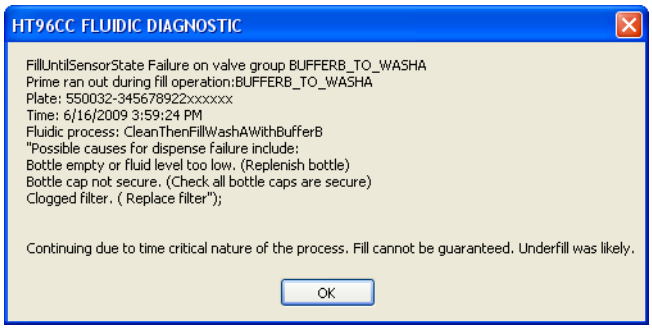
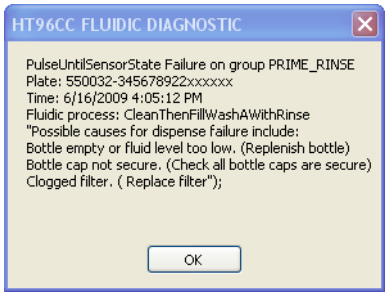
Problem and Possible Causes	
	About this message: ▪ BUFFERX = Buffer bottle A, B or Rinse ▪ WASHX = Wash A or B reservoir in the fluidics station. Recommended actions: ▪ Replenish fluid level in the Rinse or Wash Bottle B to the 1L mark. Do not overfill. ▫ Only replenish bottles when prompted by the 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 2L. ▪ Secure the bottle cap. ▪ Replace the filter Instructions for filter replacement in the *GeneTitan® Multi-Channel Instrument User Guide*, 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 Guide*, P/N 08-0306.

Table 6.4 Problem messages (Continued)

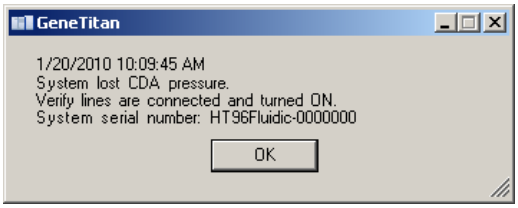
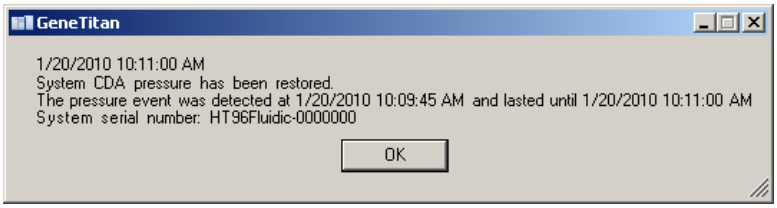
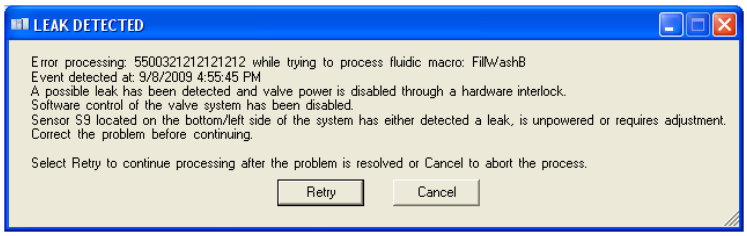
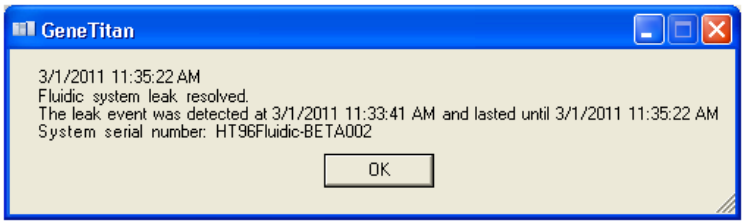
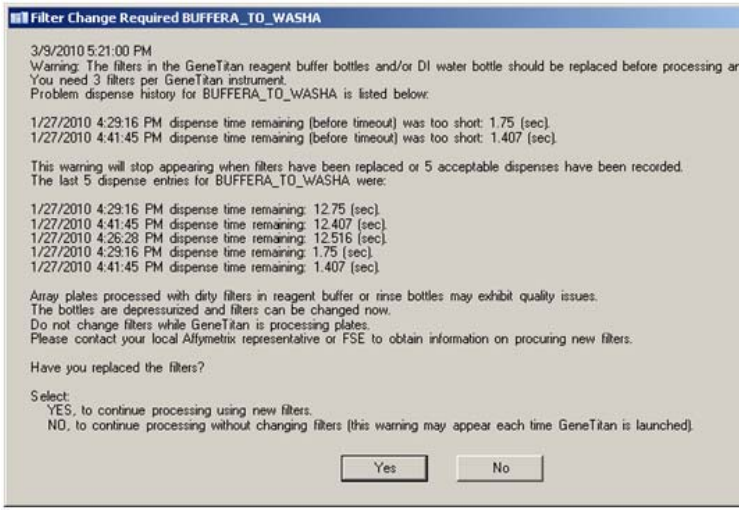
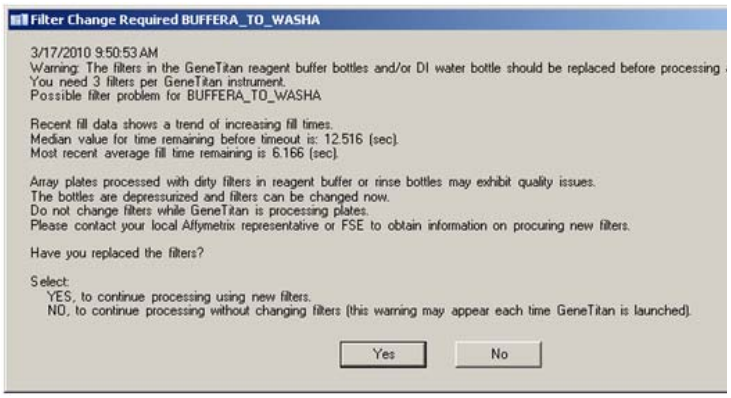
Problem and Possible Causes	
When the instrument experiences a loss in Clean Dry Air (CDA) pressure, the software will display the warning message.  When the pressure is detected again, a dialog message confirming the availability of CDA pressure is displayed. 	Possible Causes Please verify that the facility CDA or the portable CDA compressor is in working condition. Refer to the GeneTitan MC Site Preparation 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.
Leak Detected Leak checks are performed at application startup and any time a fluidic process (priming filling draining etc.) is performed. The leak detection is a hard-wired sensor which will shut off fluid flow without software control. Leaks are normally confined to the drip pan located inside the system.  	Causes: ▪ System malfunction ▪ User killing the application using task manager during a fill operation resulting in application exit without stopping flow. Solution: Contact Affymetrix field support. The system cannot be used for any fluidic processing until this is resolved.

Table 6.4 Problem messages (Continued)

Problem and Possible Causes	
Leak Resolved 	This message is displayed when the leak is resolved (meaning the sensor LED is again lit up). If the original leak detected message was not acknowledged it will be automatically removed from the GUI and replaced by the following message. It will remain displayed until another leak is detected or the user acknowledges it by pressing OK. To resolve this issue complete the following tasks ▪ Verify all internal and external tubing is connected and clean ▪ Verify wash reservoirs are clean ▪ Verify all bottle caps are secure and that no bottle cap is crimping a supply line. ▪ Verify vacuum is working properly ▪ Do not refill bottles or empty waste except when prompted to by the GeneTitan application. ▪ Contact your facility group to ensure CDA is supplied to your GeneTitan system. Contact Affymetrix Field Service to have the sensor adjusted or replaced if the problem persists even after correcting for the usual causes outlined above.
Filter Error Messages  	The filters in the GeneTitan fluidics bottles (Wash A, Wash B and DI Water) need to be replaced when the filters are worn out. The software displays warning message boxes for the filter in each reagent bottle when it detects a problem or shows a trend of increased fill times during fluid fill operations. If an error is detected as described above, then a message box titled "Filter Change Required" is displayed along with the information on the specific dispense operation. You should change all three filters when a warning is displayed for any one of the three filters. Refer to the section *Replacing the Filter* on page 154 in Appendix E.

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 PharmacoScan 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 Multi-Channel 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 a drawer 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.

Appendix A

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	Various	—
Pipettes, multi- and single-channel P20, single-channel P200	Various	—
Plate centrifuge	Various	—
Vortex	Various	—

E-Gels and Reagents

Table A.2 E-Gel and Reagents Required

Item	Supplier	Part Number
Mother E-Base Device	Thermo Fisher Scientific (formerly Life Technologies™ /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 Consumables Required

Item	Supplier	Part Number
Adhesive film – use one of the following: ▪ MicroAmp Clear Adhesive Film ▪ Microseal 'B' Film	 Thermo Fisher Scientific Bio-Rad	 4306311 MSB1001
50 mL Conical Tube	Various	—
Pipette Tips	Same brand as pipette	—

Diluting the TrackIt™ Cyan/Orange Loading Buffer and 25 bp Ladder

The following recipe is for preparing a large batch of the Gel Diluent, a 1000-fold dilution of the TrackIt Cyan-Orange Loading Buffer:

To Dilute the TrackIt Cyan/Orange Loading Buffer (P/N 10482-028, Thermo Fisher Scientific):

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

To Dilute the TrackIt 25bp Ladder (P/N 10488-022, Thermo Fisher Scientific):

The following recipe is for preparing a 15-fold dilution of the Invitrogen TrackIt 25 bp DNA Ladder:

1. In a 1.7mL microcentrifuge tube, add 6 µL of TrackIt 25 bp DNA Ladder to 84 µL nuclease-free water. Total volume: 90 µL.
2. Vortex tube to mix well. Pulse-spin to get droplets down.

NOTE: The recipe has enough volume to fill 4 marker wells of one E-Gel® *48* 4% agarose gel. Scale up as needed if running multiple gels.

Fragmentation QC Gel Protocol

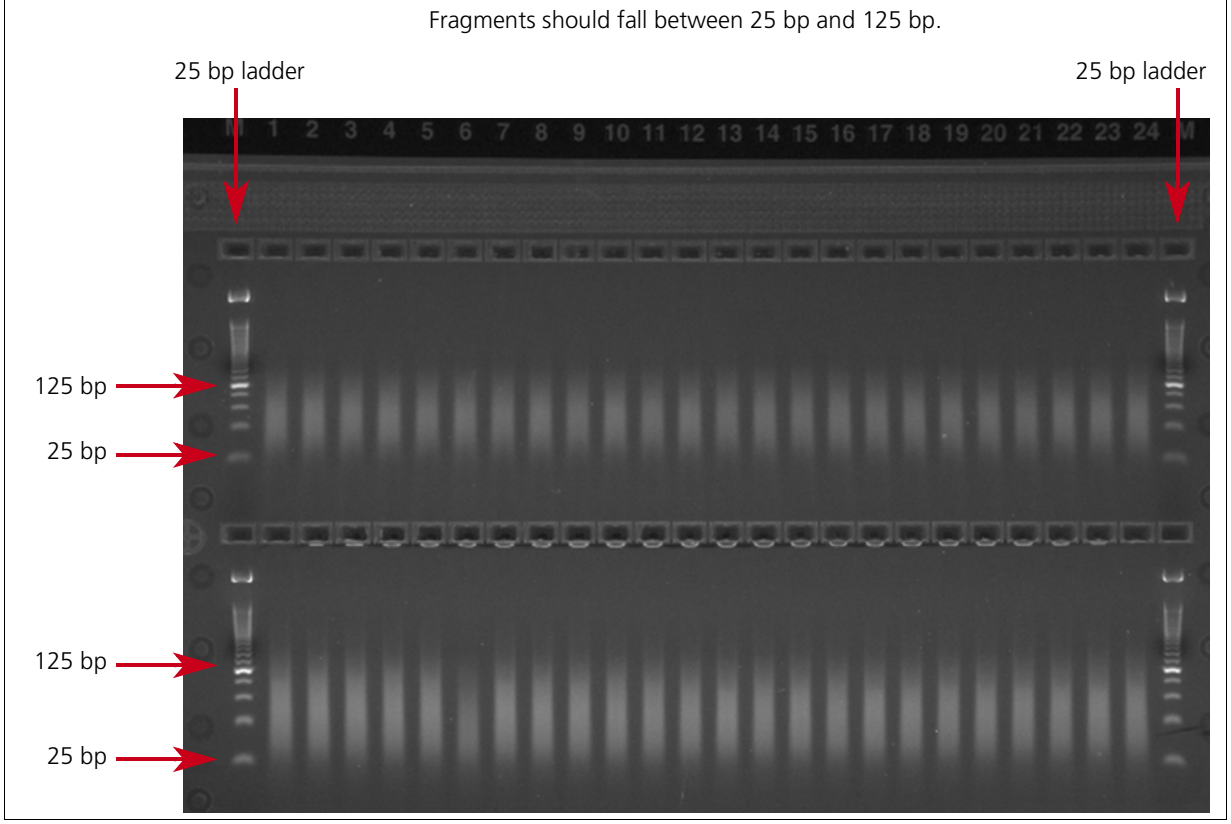
This protocol is based on running QC gels for 24 samples:

To Run a Fragmentation QC Gel:

1. Tightly seal the Gel QC plate.
2. Vortex the plate for 3 sec. Pulse-spin to get droplets down.
3. Connect an E-Base™ device(s) to an electrical outlet.
4. Push the Power/Prg button on each to ensure the program is in EG mode (not EP mode).
5. Take the gel out of the pouch and remove the combs.
6. Place the E-Gel® 48 gel into an E-Base unit.
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. Image the gel.

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



Appendix B

Sample Quantitation after Resuspension

Protocol for Sample Quantitation after Resuspension

Equipment Required

The following equipment is required for this protocol.

Table B.1 Equipment Required for Sample Quantitation After Resuspension

Quantity	Item
1	DTX 880 Multimode Detector with Genomic Filter Slide

Quantitate the Diluted Samples

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

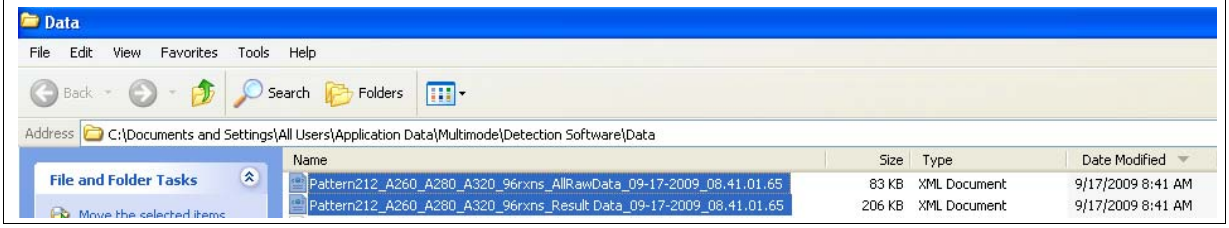
For OD quantitation, readings should be taken at wavelengths of 260, 280, and 320 nm. See *Suggested Protocol for OD Quantitation Using the DTX 880* on page 138 for more information.

To Quantitate the Diluted Samples Prepared for OD Quantitation:

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

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

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

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

OD Yield Assessment Guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the PharmacoScan Assay 24-Array Format Manual Protocol. If the median yield for the plate is < 1000 µg DNA per sample:

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

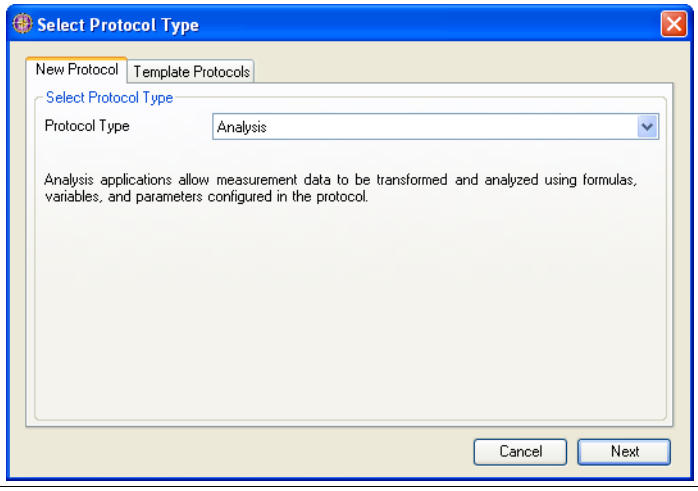
This DNA yield corresponds to an A260 value of approximately 0.49 and an A260-A320 value of approximately 0.42.

Suggested Protocol for OD Quantitation Using the DTX 880

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

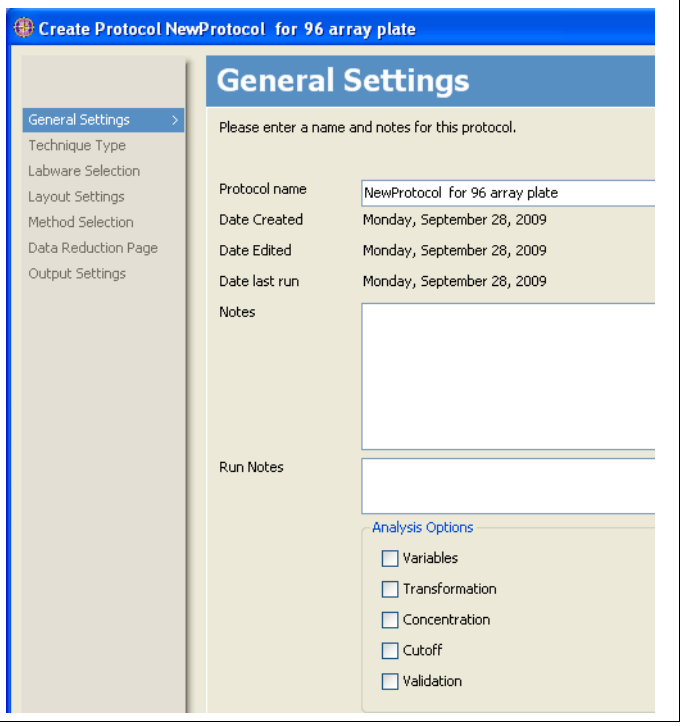
Protocol Type—Analysis

Figure B.3 Protocol Type



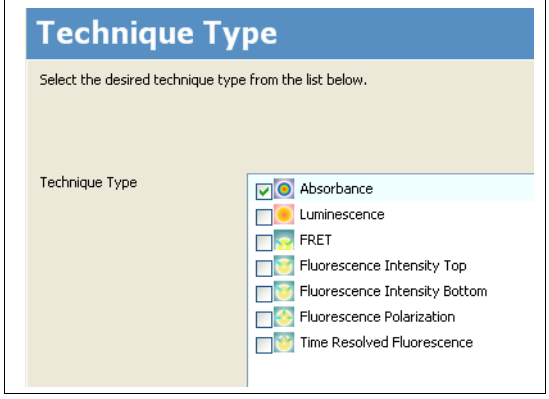
General Settings—enter a name for the protocol

Figure B.4 General Settings



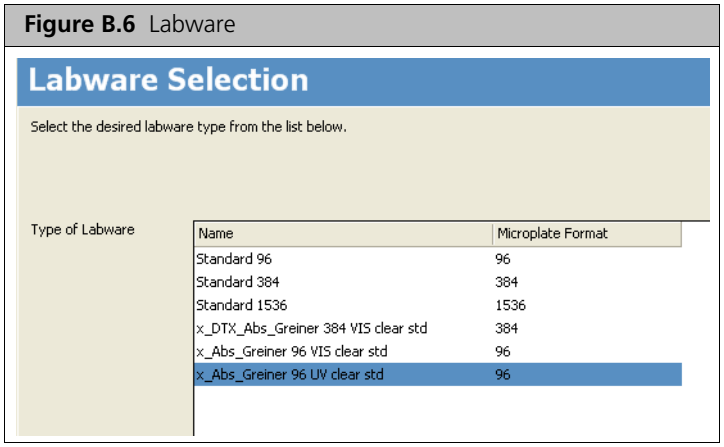
Technique Type—select Absorbance.

Figure B.5 Technique Type



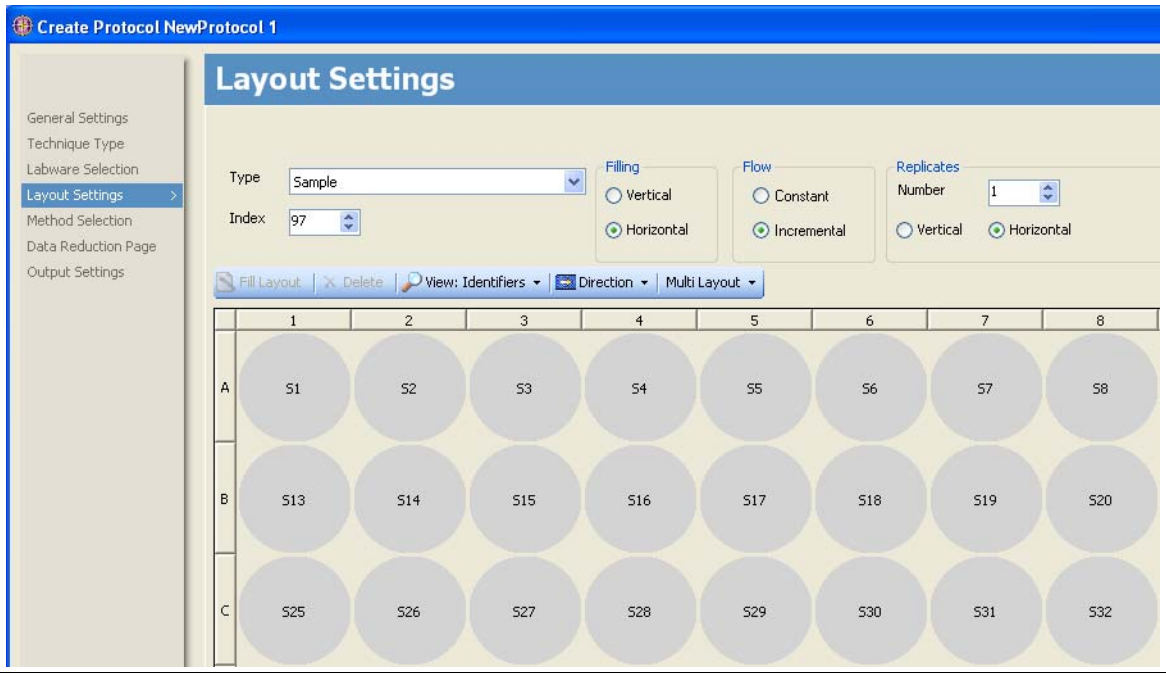
Labware—x_Abs_Greiner 96 UV clear std (96 microplate format)

Figure B.6 Labware



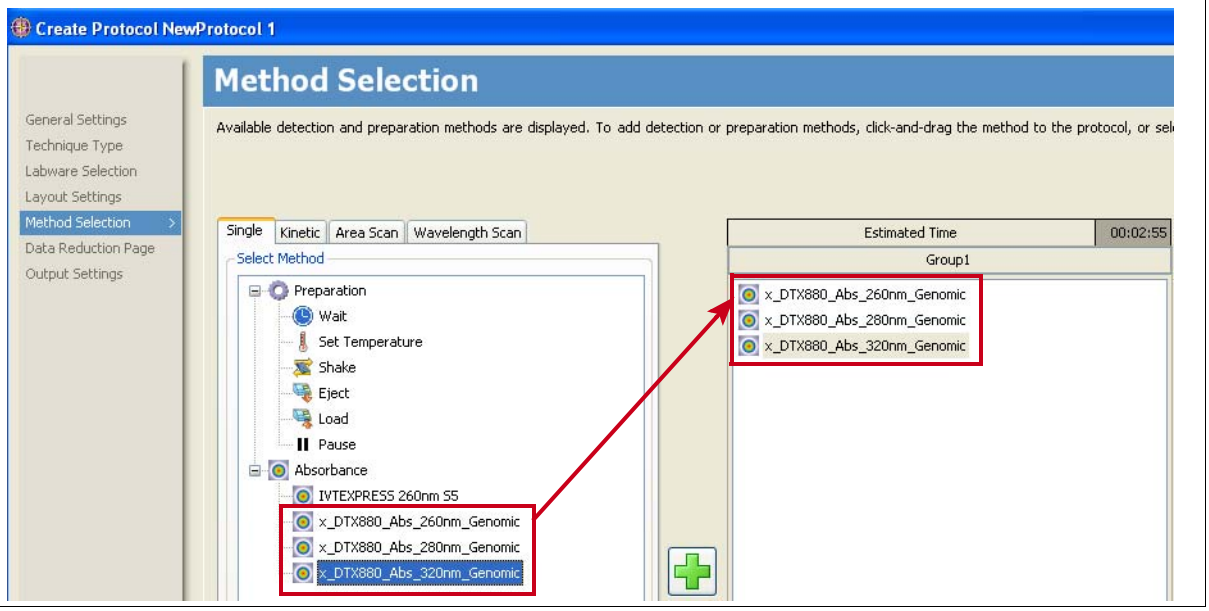
Layout Settings—as appropriate for 96-array plates

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

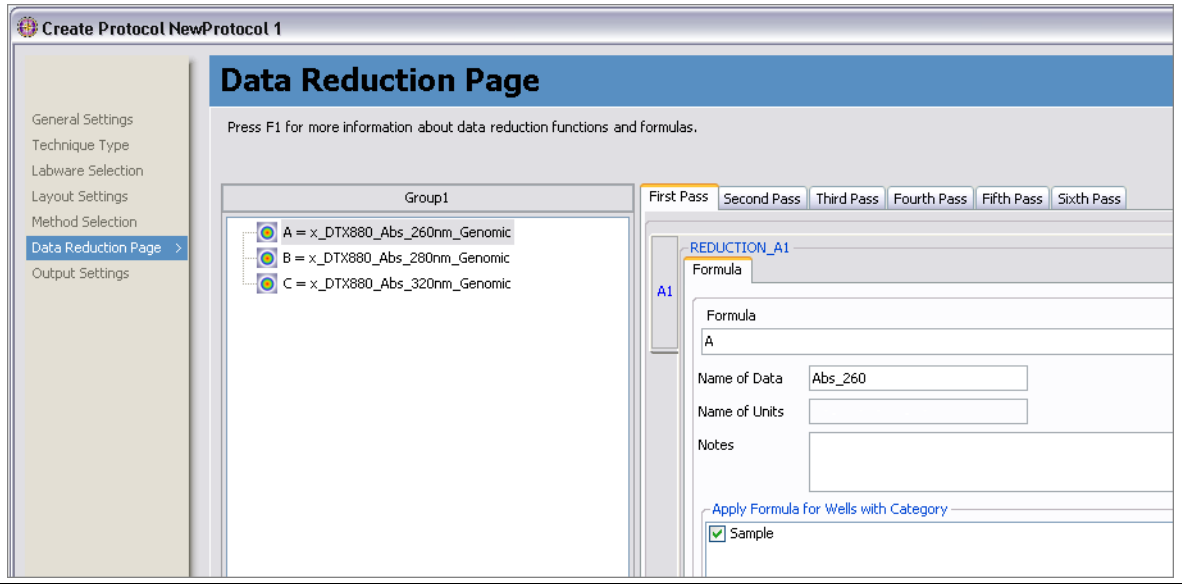


Figure B.10 Data Reduction Page—Second Pass

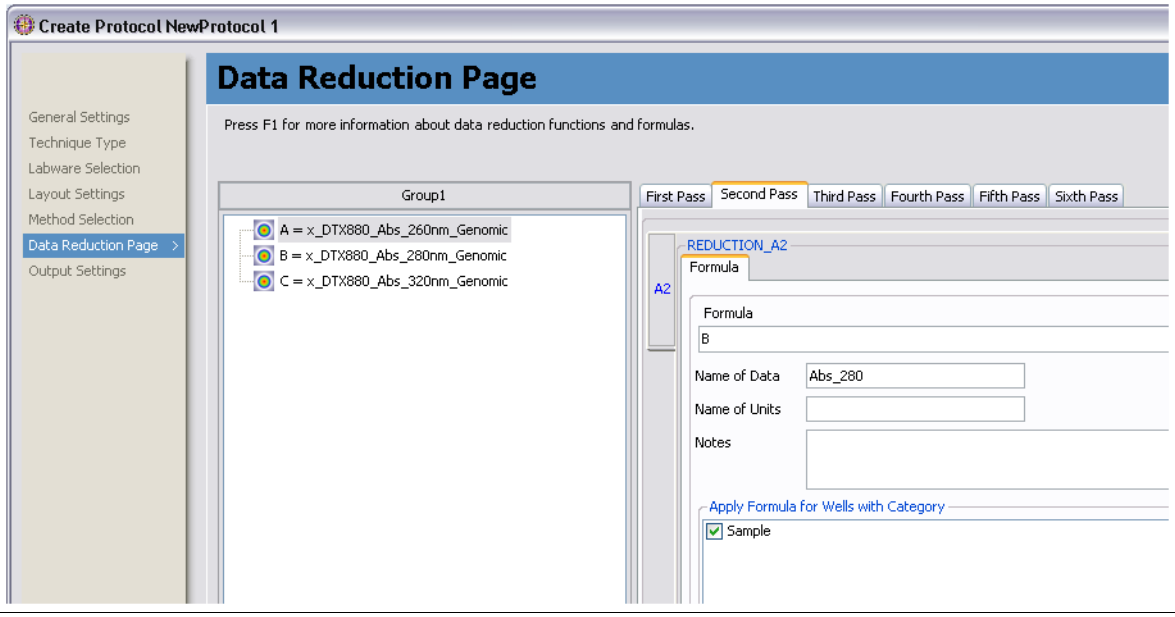


Figure B.11 Data Reduction Page—Third Pass

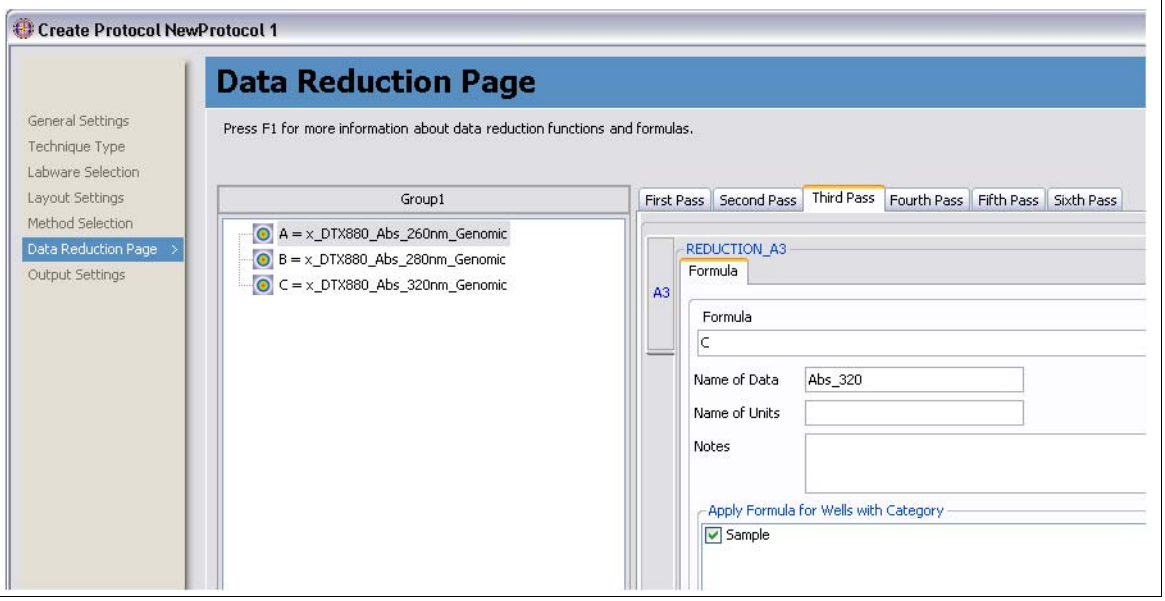


Figure B.12 Data Reduction Page—Fourth Pass

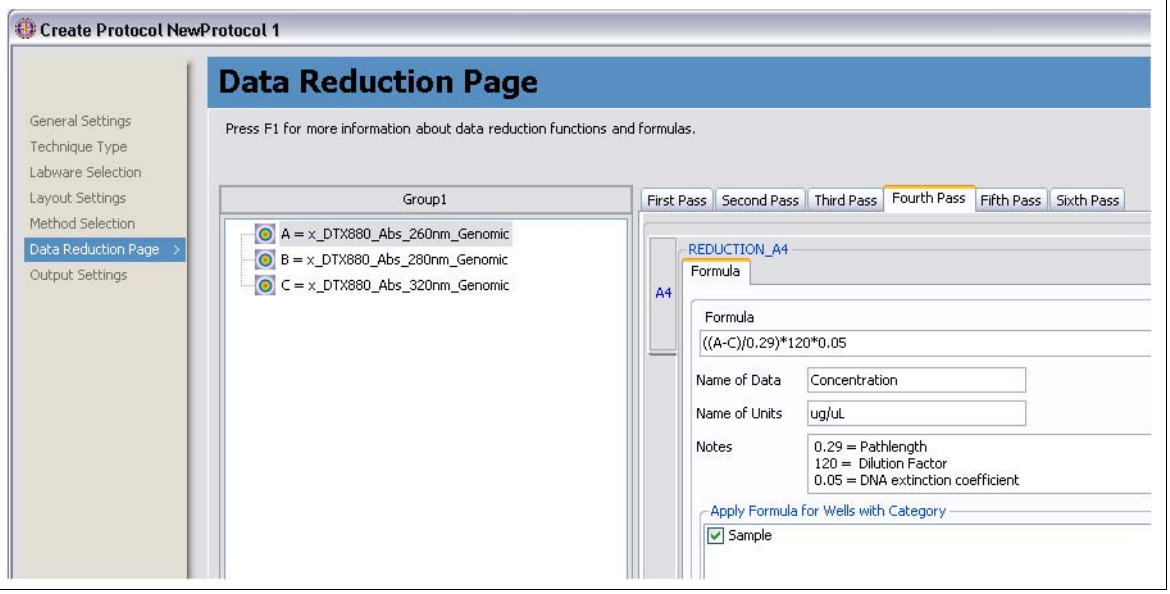


Figure B.13 Data Reduction Page—Fifth Pass

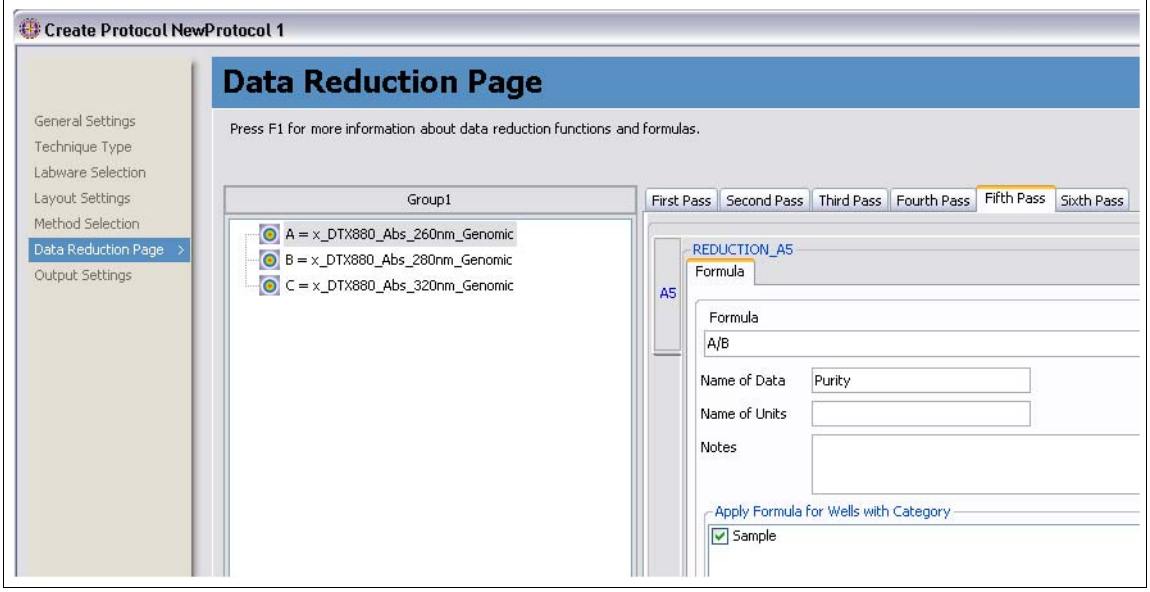
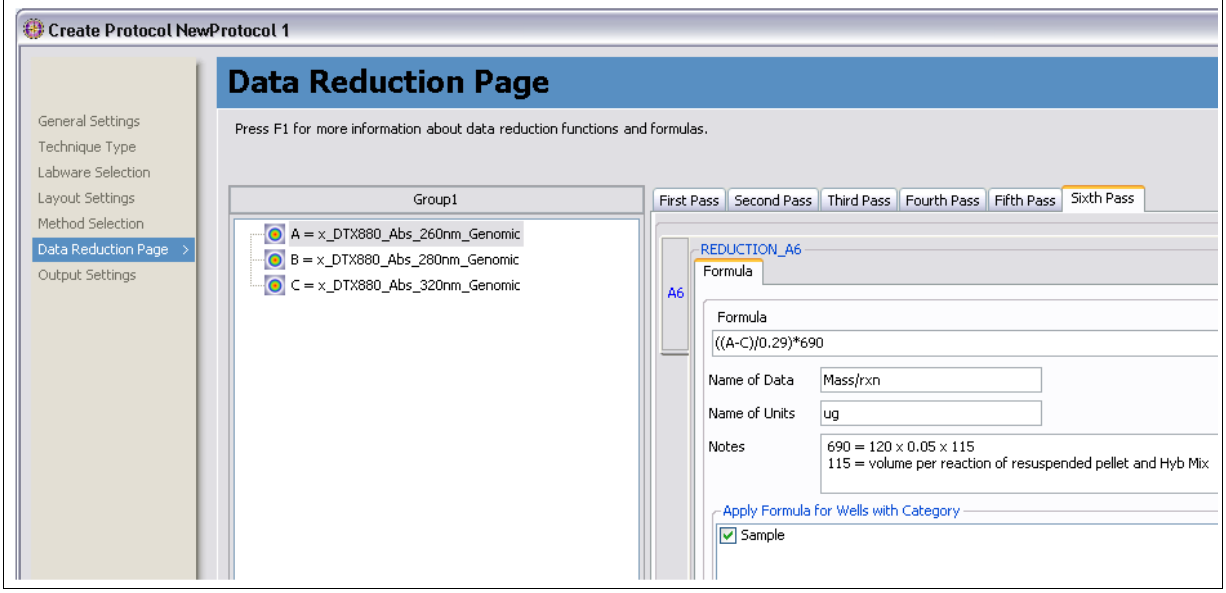
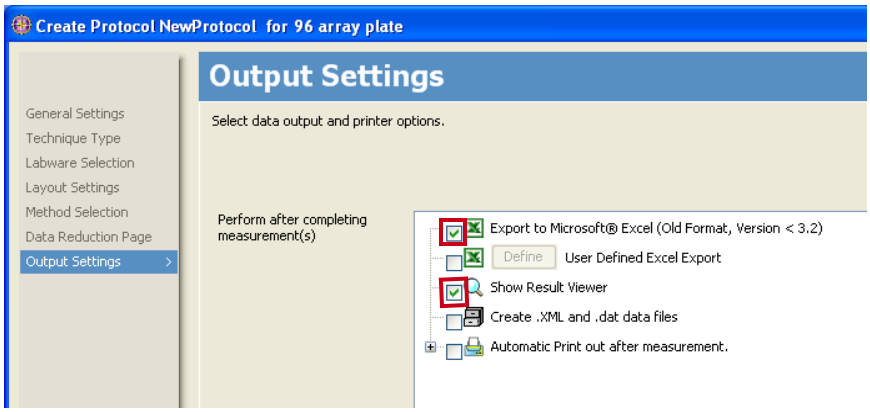


Figure B.14 Data Reduction Page—Sixth Pass



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

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

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

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

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

P = the optical path length (measured in cm) 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 *PharmacoScan Assay 24-Array Format Manual Protocol Site Preparation Guide*, P/N 703287, 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 137 and against *OD Yield Assessment Guidelines* on page 137.

Appendix C

Registering Samples in Affymetrix GeneChip® Command Console®

Creating a GeneTitan® Array Plate Registration File

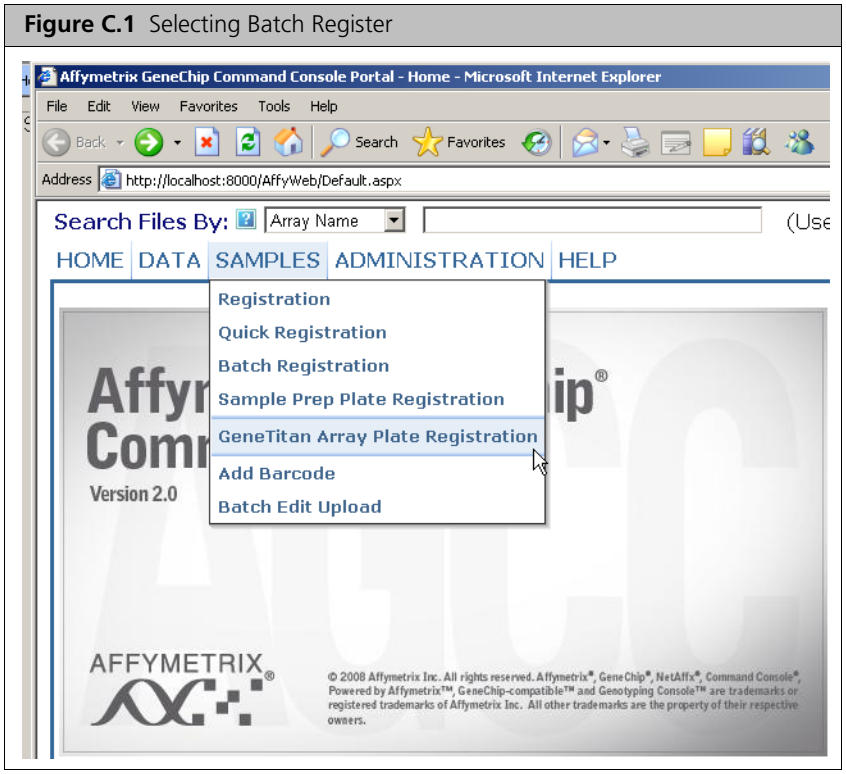
A GeneTitan Array Plate Registration file is a Microsoft Excel spreadsheet that includes information on the samples you are processing on a single array plate. This information includes the array plate format, the array plate barcode, and sample file names so that you can track the samples that are loaded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

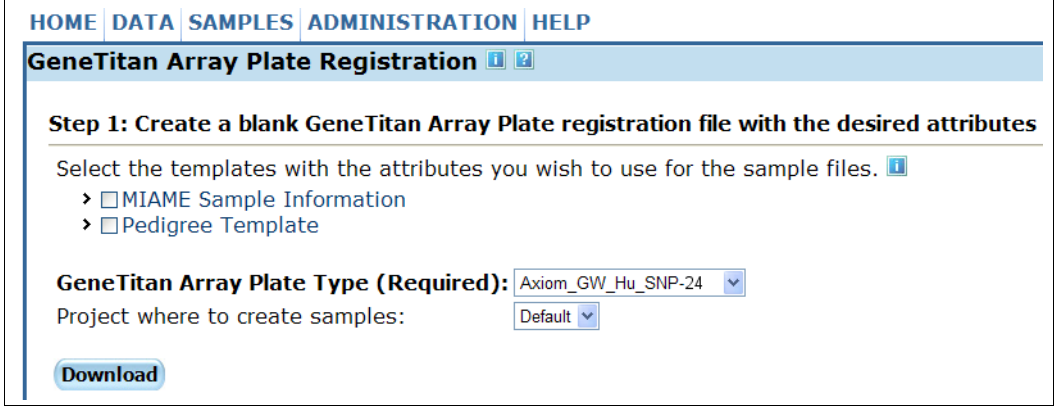
1. In AGCC Portal, open the **Samples** menu and select **GeneTitan Array Plate Registration**.

Figure C.1 Selecting Batch Register



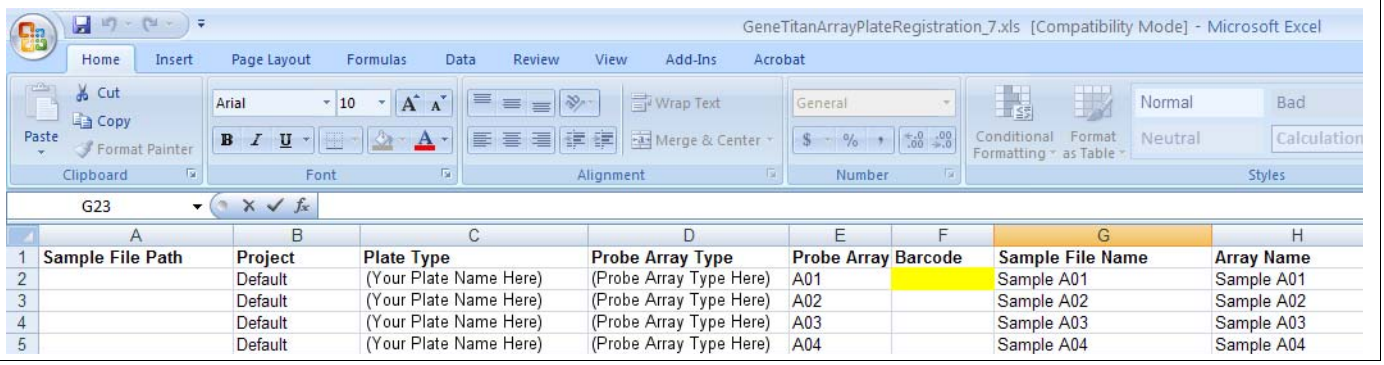
2. Step 1—Figure C.2 on page 146:
   - **A.** Select the array plate type.
   - **B.** Click **Download**.

Figure C.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 C.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	(Your Plate Name Here)	(Probe Array Type Here)	A01		Sample A01	Sample A01
3		Default	(Your Plate Name Here)	(Probe Array Type Here)	A02		Sample A02	Sample A02
4		Default	(Your Plate Name Here)	(Probe Array Type Here)	A03		Sample A03	Sample A03
5		Default	(Your Plate Name Here)	(Probe Array Type Here)	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 C.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 C.5 is displayed.

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

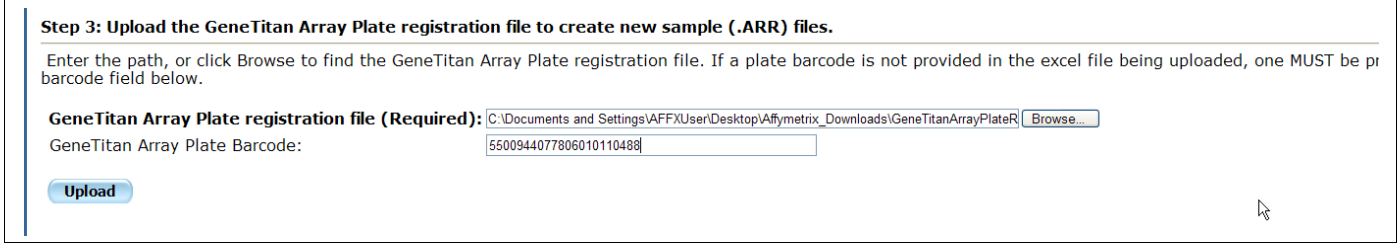
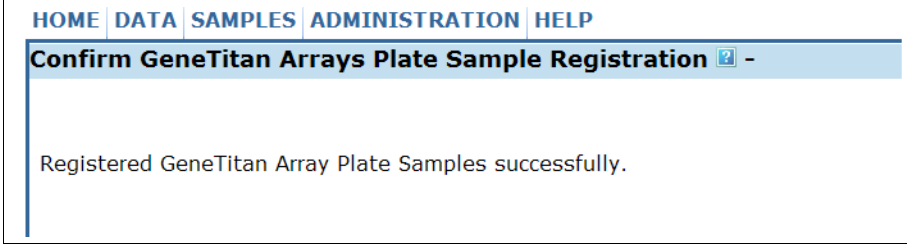


Figure C.5 Array plate samples successfully registered.



Appendix D

Deionizing Procedure for GeneTitan® Trays and Covers

We recommend the use of the Zerostat 3 Anti-Static Gun (P/N 74-0014) to deionize GeneTitan® MC Instrument stain tray trays and lids.

IMPORTANT: Except for the Axiom® array plates, scan tray and the hybridization tray, you must deionize all GeneTitan stain trays, stain tray covers and scan tray cover 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 D.1, Figure D.2 and Figure D.3.

Deionize the inner surface of each tray and cover:

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

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

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

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

Deionization Procedure

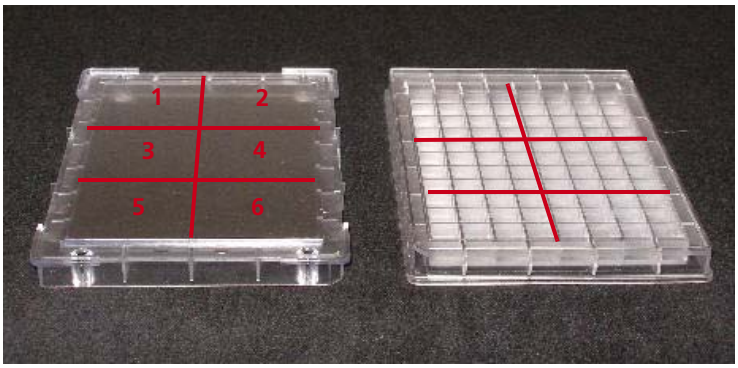
The following process provides guidance on how to use the anti-static gun on the stain tray, and stain and scan tray covers only. See Figure D.3.

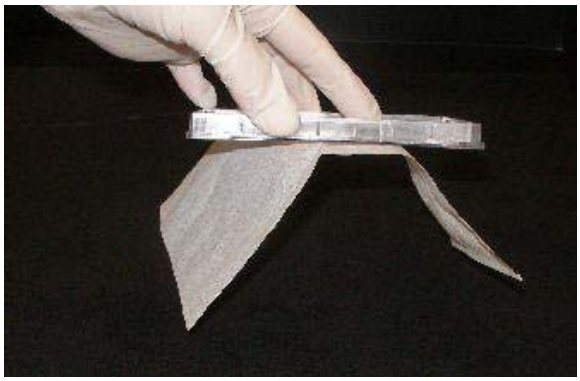
WARNING: The deionization steps 4 and 5 will damage the Axiom arrays on the plate. Before using the anti-static gun, ensure that the Axiom array plates remain in their protective pouch and placed away from the deionization area. You must place the scan tray and hybridization tray away from the area where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

1. Treat the plate or lid as if it were divided into 6 sections (see Figure D.3), 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 tray and pull the trigger. Ensure that a stream of ionized particles settles on all wells of the stain tray 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.
5. 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).
6. Place the treated cover or tray on the Kimwipe and lift it up (see Figure D.3).
7. Do one of the following:
   - If the Kimwipe does not cling to the plastic, proceed with the protocol.
   - If the Kimwipe still clings to the plastic, then perform steps 3 and 4 again. If it continues to cling to the plastic, test the device using the ion-indicator cap to confirm that the unit is still releasing ions. Otherwise, it may be time to replace the unit.

Figure D.3 Removing the static charge from stain trays and lids.

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





- If a Kimwipe clings to treated surface, try the deionization procedure again.
- If the Kimwipe still clings, it may be time to replace the anti-static gun.

Ion-Indicator Cap

The ion-indicator cap is a testing device used to verify the release of ions when the anti-static gun is in use (P/N 74-0014, Figure D.3).

Testing the Zerostat 3 with the Ion-Indicator Cap

1. Insert the ion-indicator cap into the nose of the Zerostat and then slowly squeeze the release trigger (see Figure D.4).

Figure D.4 Zerostat 3 Anti-Static Gun (P/N 74-0014) with Ion-Indicator Cap to Test Functionality

Ion-Indicator Cap

The Ion-Indicator Cap is attached to the Zerostat to test the functionality of the anti-static gun.

IMPORTANT: Do not use the Ion-Indicator Cap on the Zerostat gun when deionizing trays and lids.

2. Observe the discharge through the viewing slot on the ion-indicator cap of the anti-static gun. A visible light is observed in the viewing window on the cap when charged ions are discharged.
3. If you cannot see the light, the gun may be unusable and you should replace it.
4. Each Zerostat anti-static gun is capable of 50,000 trigger operations, which is sufficient for approximately 200-250 runs on the GeneTitan MC Instrument.

! IMPORTANT: Make sure to remove the cap from the gun before deionizing a tray or cover.

Appendix E

GeneTitan® Multi-Channel Instrument Care

Introduction

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® Multi-Channel (MC) Instrument should be positioned on a sturdy level bench away from extremes in temperature and away from moving air.

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

Cleaning and Maintenance

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

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

Monthly

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

Every Six Months

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

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

Servicing the Outer Enclosure Fan Filters

Cleaning Schedule

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

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

Part details:

Affymetrix P/N: 01-0669

Number of filters required per GeneTitan instrument: 3

Figure E.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.

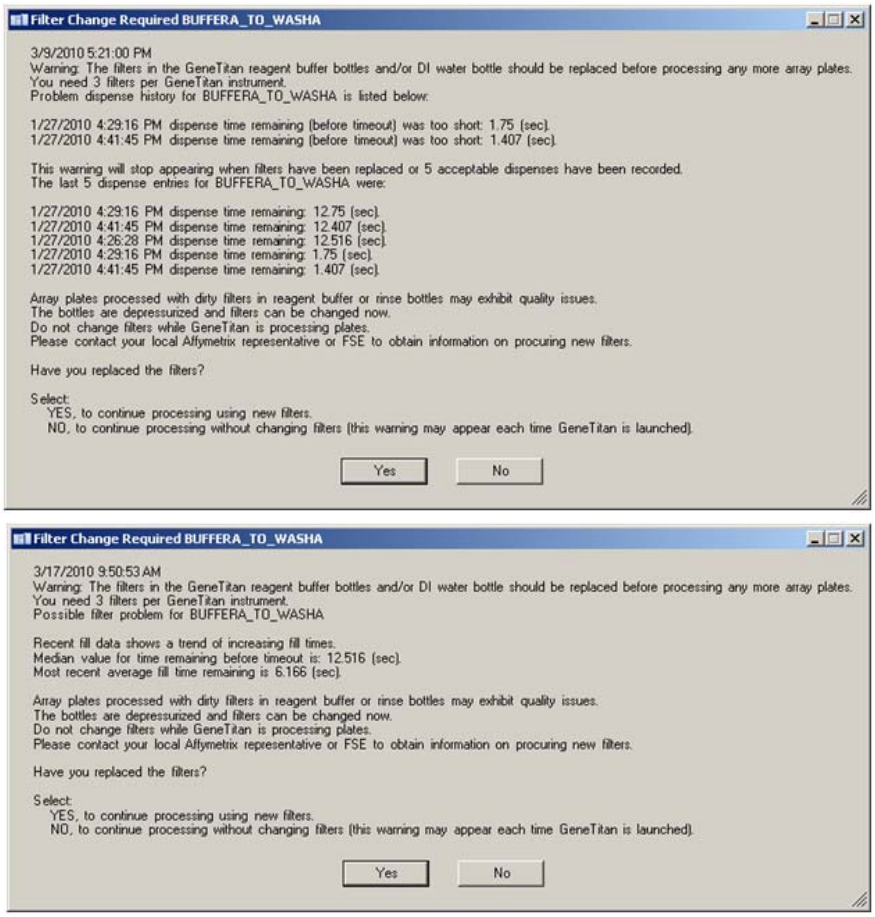
Replacing the Bottle Filters

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 E.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.

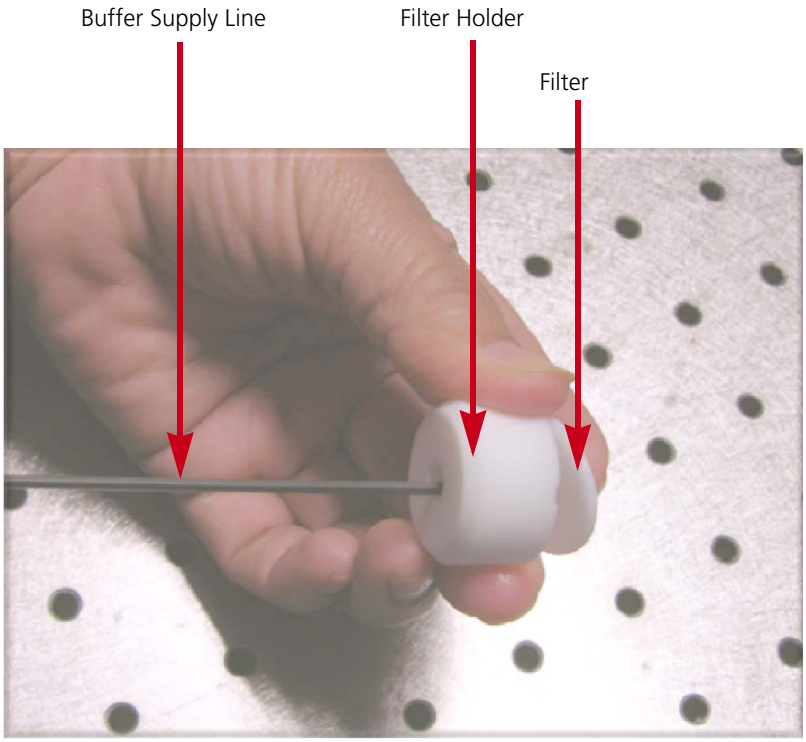
After changing the filters in all three bottles using the procedure described below, please press the **Yes** button to continue. If you choose to ignore the error message, press the **No** button. This warning message will be displayed each time AGCC instrument control software is launched. You may 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 E.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.

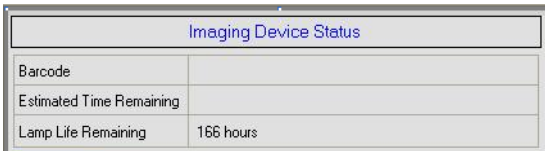
Affymetrix Part Number: 01-0740

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

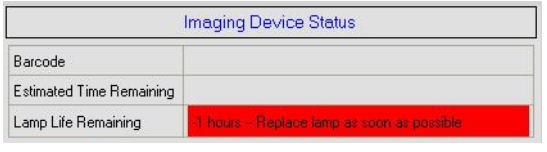
The Imaging Status pane displays lamp life and Imaging Device status notices for the GeneTitan MC. In normal operation, the pane displays the hours of life left in the lamp:

Figure E.4 Lamp Life Above Tolerance



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

Figure E.5 Lamp Life Above Tolerance



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

Figure E.6 Imaging Device Offline

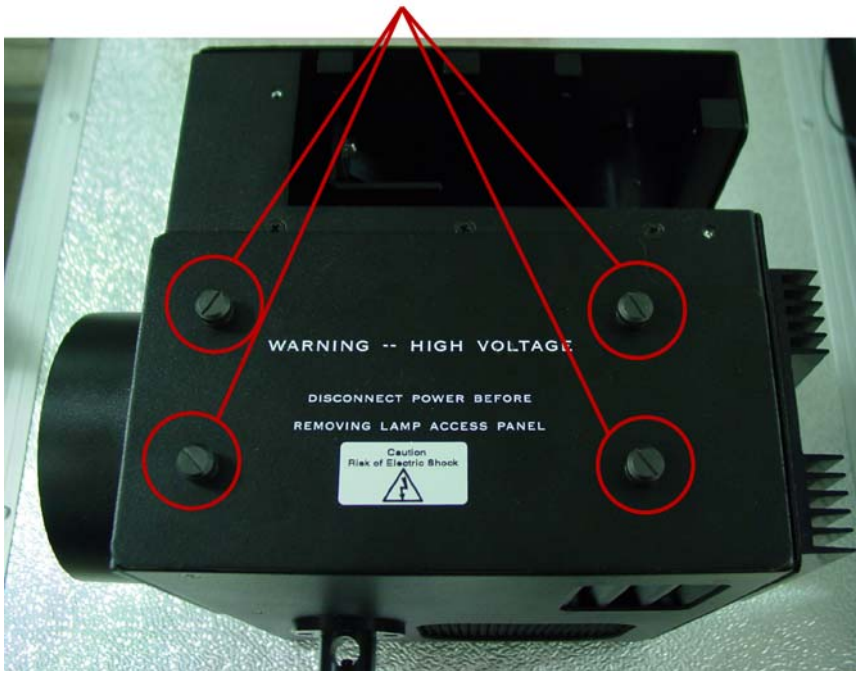
NOTE: The 300 Watt Xenon lamp in the GeneTitan MC instrument is warranted for 500 hours. The instructions to replace the lamp are available on the following page. After changing the lamp, it is necessary to reset the lamp life clock manually.

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

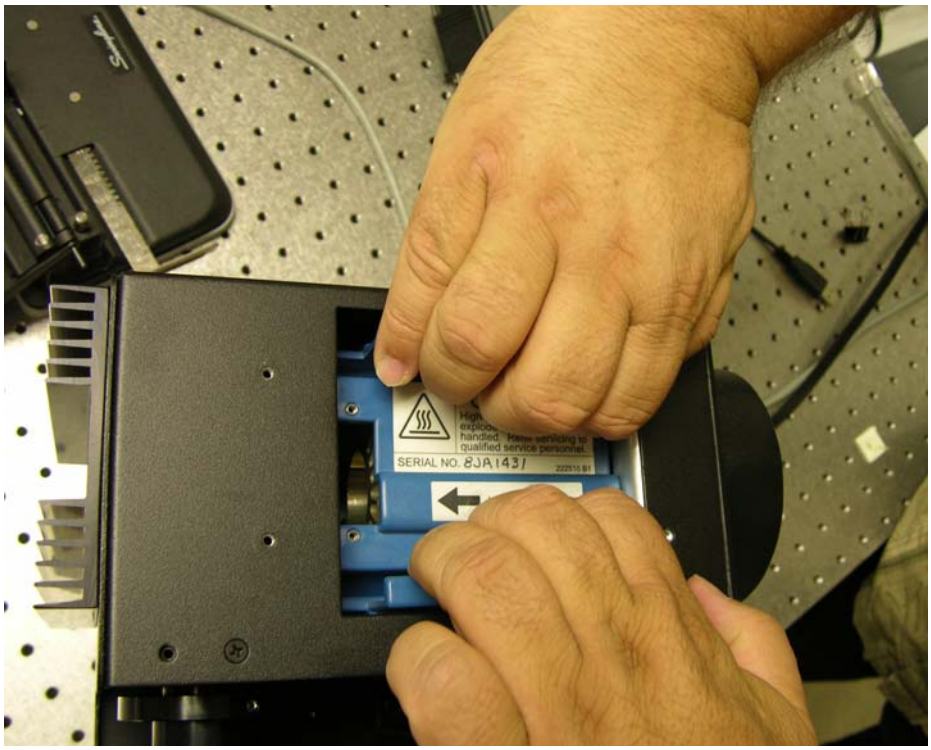
1. Unscrew the four retaining bolts. They should be finger tight (Figure F.7).

Figure E.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 E.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 E.9 The Reflecting Mirror

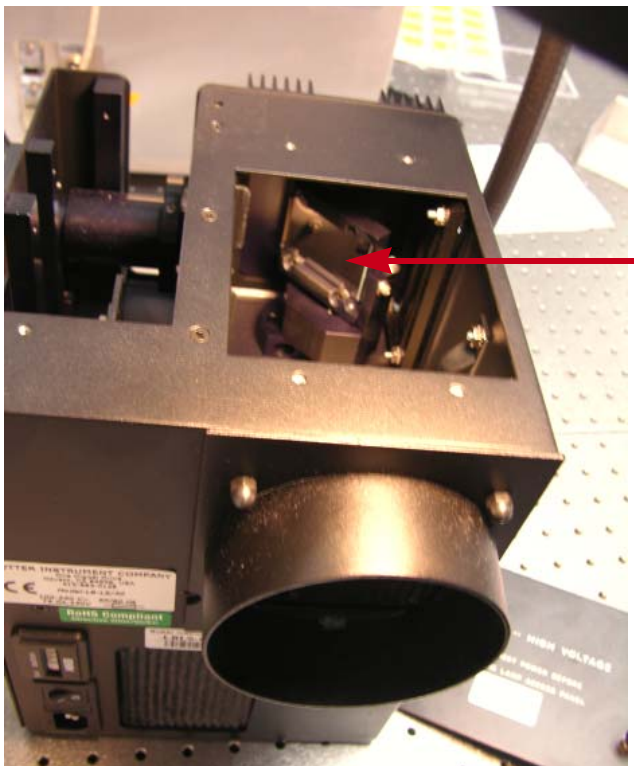
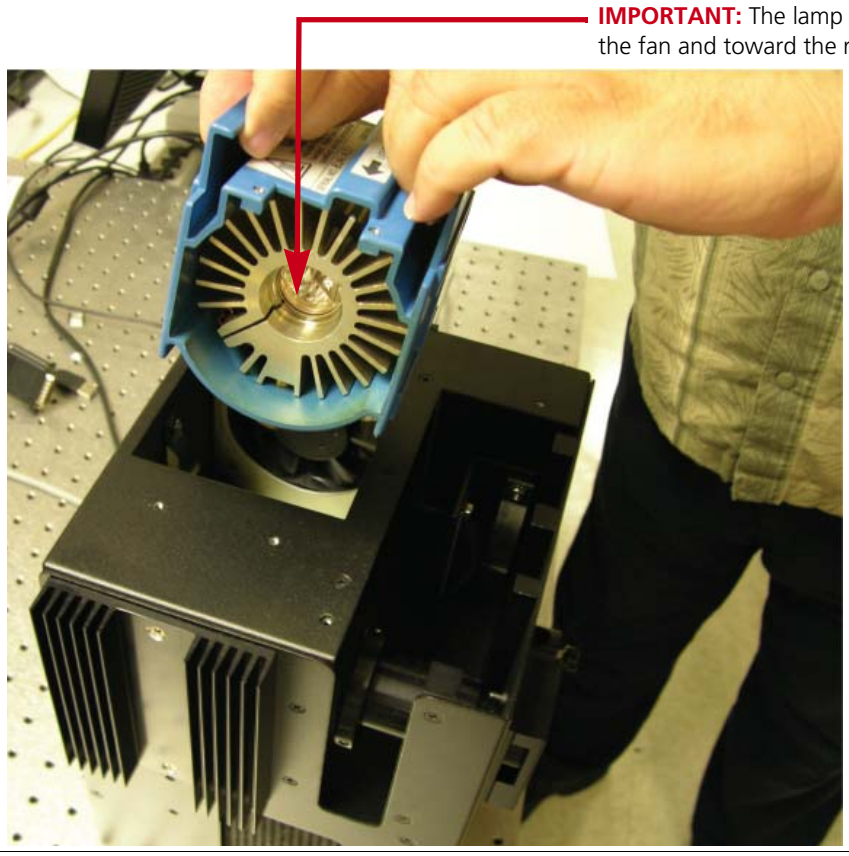


Figure E.10 Inserting the Lamp

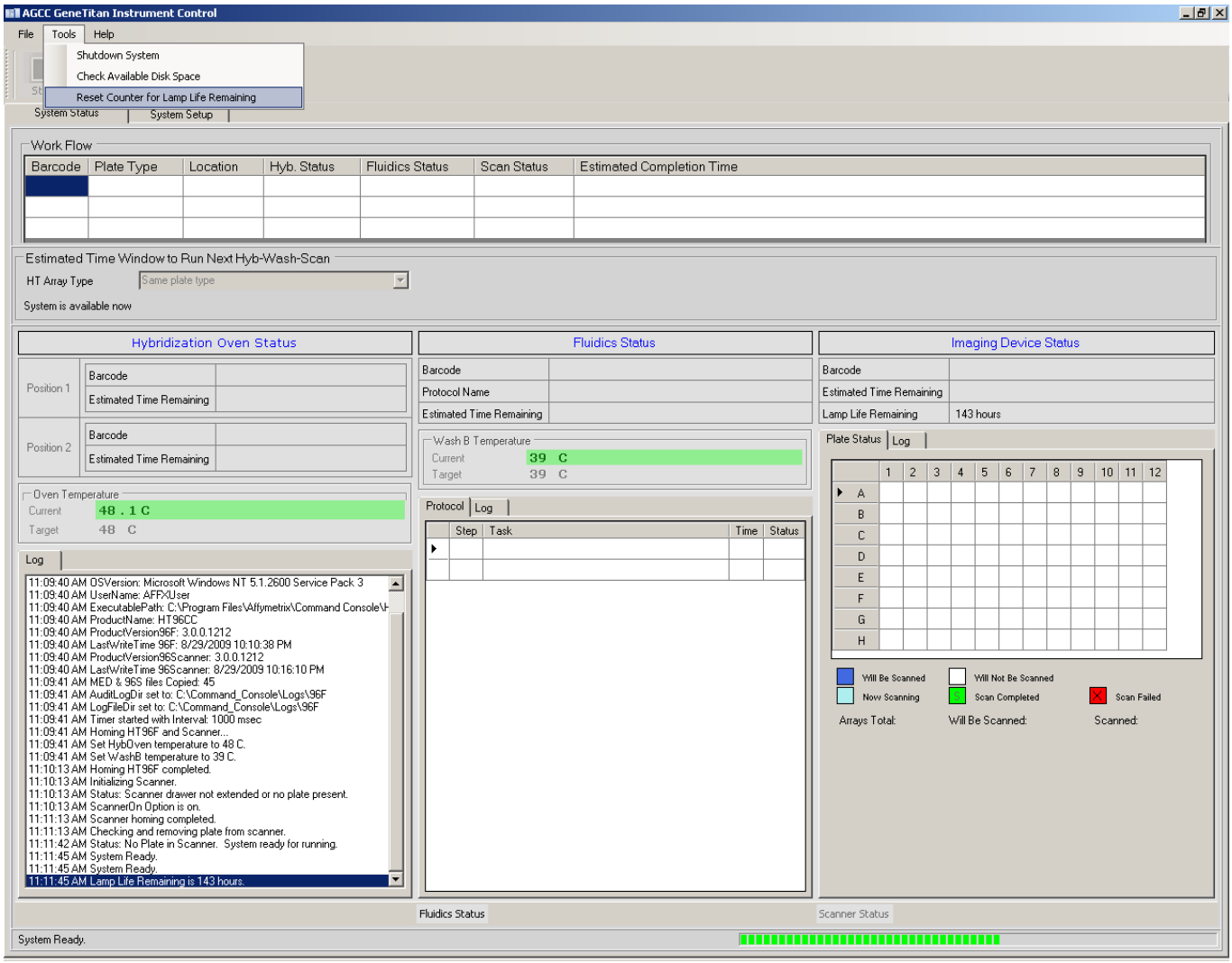


Resetting the Lamp Counter

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.

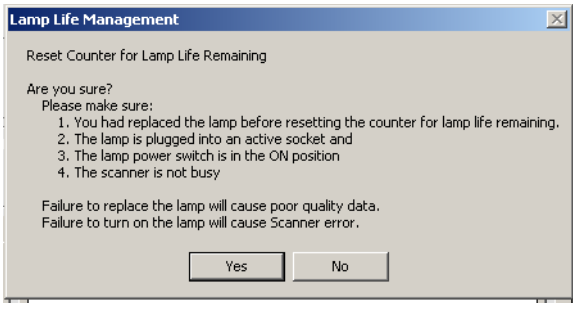
1. On the software application click **Tools → Reset Counter for Life Remaining** (Figure F.11).

Figure E.11 Inserting the Lamp



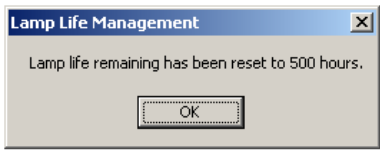
2. The software will display a message that allows you to change your mind.

Figure E.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 E.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.

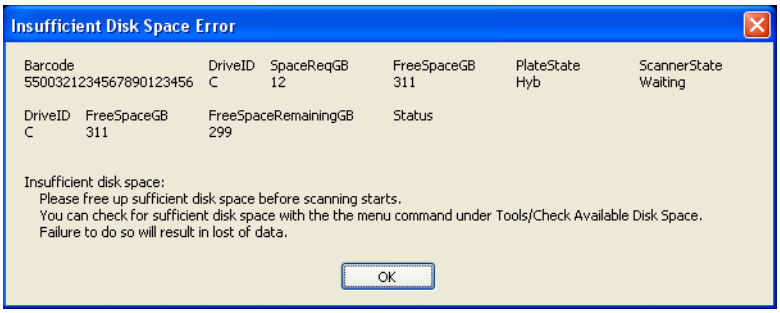
For software errors that do not involve hardware crashes the most common solution is to shut down the application and then restart it. If the same error occurs shut down both the application and the computer and then restart. If it still occurs shut down the entire unit and then restart.

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 E.14 Insufficient Disk Space Notice



If you see this notice, you will need to free up sufficient disk space before imaging starts.

Appendix F

mPCR Quality Control Gel Protocol

Protocol for Running an mPCR Quality Control Gel

NOTE: This is an optional procedure. It is meant only as a QUALITATIVE examination of the mPCR reaction to confirm that amplification has occurred. Gene copy number differences will result in differences in DNA band patterns and amplicon intensities, and therefore sample to sample variation maybe observed.

Equipment Required

Table F.1 Equipment Required

Item	Supplier	Part Number
Gel imager	Various	—
Pipette, multi- and single-channel	Various	—
Plate centrifuge	Various	—
Vortex	Various	—

E-Gels and Reagents

Table F.2 E-Gel and Reagents Required

Item	Supplier	Part Number
Mother E-Base Device	Thermo Fisher Scientific (formerly Life Technologies™ /Invitrogen)	EB-M03
Daughter E-Base Device		EB-D03
E-Gel® *48* 2% agarose gels		G8008-02
TrackIt™ Cyan/Orange Loading Buffer		10482-028
NEB 50 bp DNA Ladder	New England BioLabs	N3236S
Reduced EDTA TE Buffer (10 mM Tris-HCL PH 8.0, 0.1 mM EDTA)	Affymetrix	75793
Diluted TrackIt Cyan/Orange Loading Buffer	Refer to *Diluting the TrackIt™ Cyan/Orange Loading Buffer and 25 bp Ladder* on page 134	

Consumables

Table F.3 Consumables Required

Item	Supplier	Part Number
Adhesive film – use one of the following:		
▪ MicroAmp Clear Adhesive Film	Thermo Fisher Scientific (formerly Life Technologies)	4306311
▪ Microseal 'B' Film	Bio-Rad	MSB1001
Pipette Tips	Same brand as pipette	—
96-well PCR plate	Various	various

Prepare NEB 50 bp DNA Ladder

Dilute the NEB 50 bp Ladder (P/N N3236S, New England BioLabs):

The following recipe is for preparing a 250-fold dilution of the NEB 50 bp DNA Ladder (4 ng/µL final concentration):

1. In a 1.7 mL microcentrifuge tube, add 1 µL of 50 bp DNA Ladder to 249 µL of 1000-fold diluted TrackIt dye.
2. Vortex tube to mix well. Pulse-spin to get droplets down.

Preparing mPCR Samples for Gel Analysis

1. Thaw mPCR Reaction plate on benchtop at room temperature.
2. Ensure plate seal is secure, vortex plate, and pulse spin.
3. Dilute mPCR reaction samples 120-fold by:
   A. First dilute mPCR samples 12-fold in buffer.
   Transfer 2 µL of the mPCR reaction into 22 µL of low EDTA TE. Seal plate. Vortex. Pulse-spin.
   B. Then dilute samples another 10-fold in loading dye to prepare “mPCR Gel QC plate”.
   Transfer 2 µL of the 12-fold diluted mPCR reactions into 18 µL of 1000-fold diluted TrackIt dye.

mPCR QC Gel Protocol

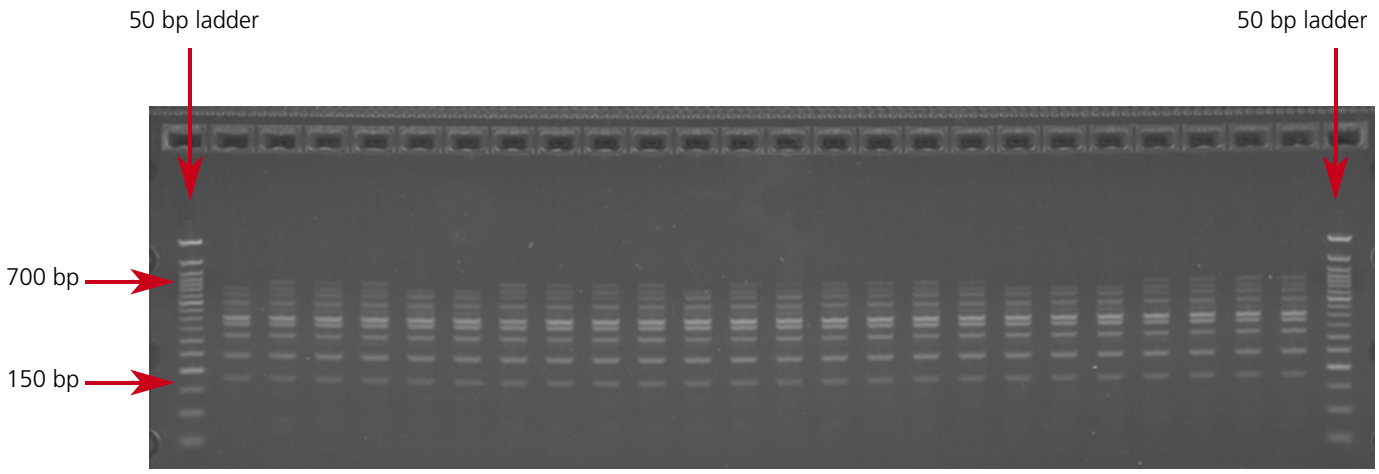
This protocol is based on running QC gels for 24 samples.

To Run mPCR QC Gel:

1. Tightly seal the *mPCR Gel QC plate*.
2. Vortex the plate for 3 sec. Pulse-spin to get droplets down.
3. Connect an E-Base™ device(s) to an electrical outlet.
4. Push the Power/Prg button on each to ensure the program is in EG mode (not EP mode).
5. Take the gel out of the pouch and remove the combs.
6. Place the E-Gel® 48 gel into an E-Base unit.
7. Load 15 µL from each well of the mPCR Gel QC plate onto the gels.
8. Load 15 µL of diluted 50 bp ladder into the marker wells (M).
9. Load 15 µL nuclease-free water into any unused wells.
10. Run the gels for 25 min.
11. Image the gel.

mPCR QC Gel images should look similar to the gel shown in Figure F.1.

Figure F.1 Example of a Typical mPCR QC E-Gel



NOTE: Variation in DNA band patterns and intensities maybe observed from sample to sample due to gene copy number differences. The mPCR QC Gel is meant to be a qualitative examination of the mPCR reaction to confirm that amplification has occurred for each sample.

All samples were amplified and show DNA bands that fall between 150 bp and 700 bp.