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PharmacoScan™ Assay 96-Array Format Manual Workflow

USER GUIDE

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Products: PharmacoScan™ Reagent Kit

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Products: PharmacoScan™ Array Plates

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3	17 May 2018	Includes an option for a three-hour DNA precipitation step to enable faster assay turnaround time.
2	30 January 2018	Two additional sample types have been validated and added to the document: saliva and buccal cell.
1	06 June 2017	New publication.

Corporate entity

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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 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 96-Array Format Manual Workflow

PharmacoScan™ Assay 96-Array Format Manual Workflow is available as a bundled kit that includes the arrays, reagents and consumables needed for processing one 96-format plate, each having 94 samples and two controls.

PharmacoScan interrogates biallelic as well as multiallelic 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 Applied Biosystems 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 OpenArray™ Real-Time PCR and Ion Ampliseq™ NGS panels.

PharmacoScan is a multiplex genotyping assay which combines the proven Axiom chemistry 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 Applied Biosystems™ GeneChip™ Command Console™ 4.3 or higher. The resulting CEL files are analyzed by Axiom™ Analysis Suite 3.0 or higher, or by Applied Biosystems Microarray Power Tools 1.19 or newer.

For further information, see to "Related documentation" on page 214.

PharmacoScan™ Assay 96-Array Format Manual Workflow

Running the PharmacoScan Assay 96-Array Format Manual Workflow 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 15.
2. A multiplex PCR step (mPCR) followed by target preparation of the samples (see Chapter 4, "Target preparation" on page 42).
3. Array Processing, done with
   - GeneTitan MC Instrument
   - GeneTitan Instrument Control software
   - GCC Portal software

   See Chapter 5, "Array processing with the GeneTitan™ Multi-Channel Instrument" on page 103.

A list of the required equipment and supplies for running the PharmacoScan Assay 96-Array Format Manual Workflow can be found in the *PharmacoScan™ Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460.

What's new

This revision of the user guide introduces the option for a three-hour DNA precipitation step to enable a faster assay turnaround time, going from sample to CEL file generation within 72 hours. The three-hour precipitation step shortens *Stage 2: Fragmentation and precipitation* to enable the operator to advance to *Stage 3: Centrifuge and drying, resuspension and hybridization preparation, and sample QC* followed by *Stage 4: Denaturation and hybridization* all on day 2 of the assay workflow. See Figure 1. Note that this workflow option requires approximately nine hours to complete these combined day 2 activities (fragmentation to initiation of hybridization on the GeneTitan MC Instrument).

In addition, a recommended workflow is presented to support the processing of three plates per week using the shortened DNA precipitation step. See Chapter 7 for details regarding equipment and operator assumptions to support this workflow.

The standard PharmacoScan Assay workflow, in which the DNA is precipitated overnight, provides a convenient stopping point to support single operator assay execution of one plate within an eight-hour workday. A recommended workflow is presented to support the processing of three plates per week using the standard overnight DNA precipitation. See Chapter 6 for details regarding equipment and operator assumptions to support this workflow.

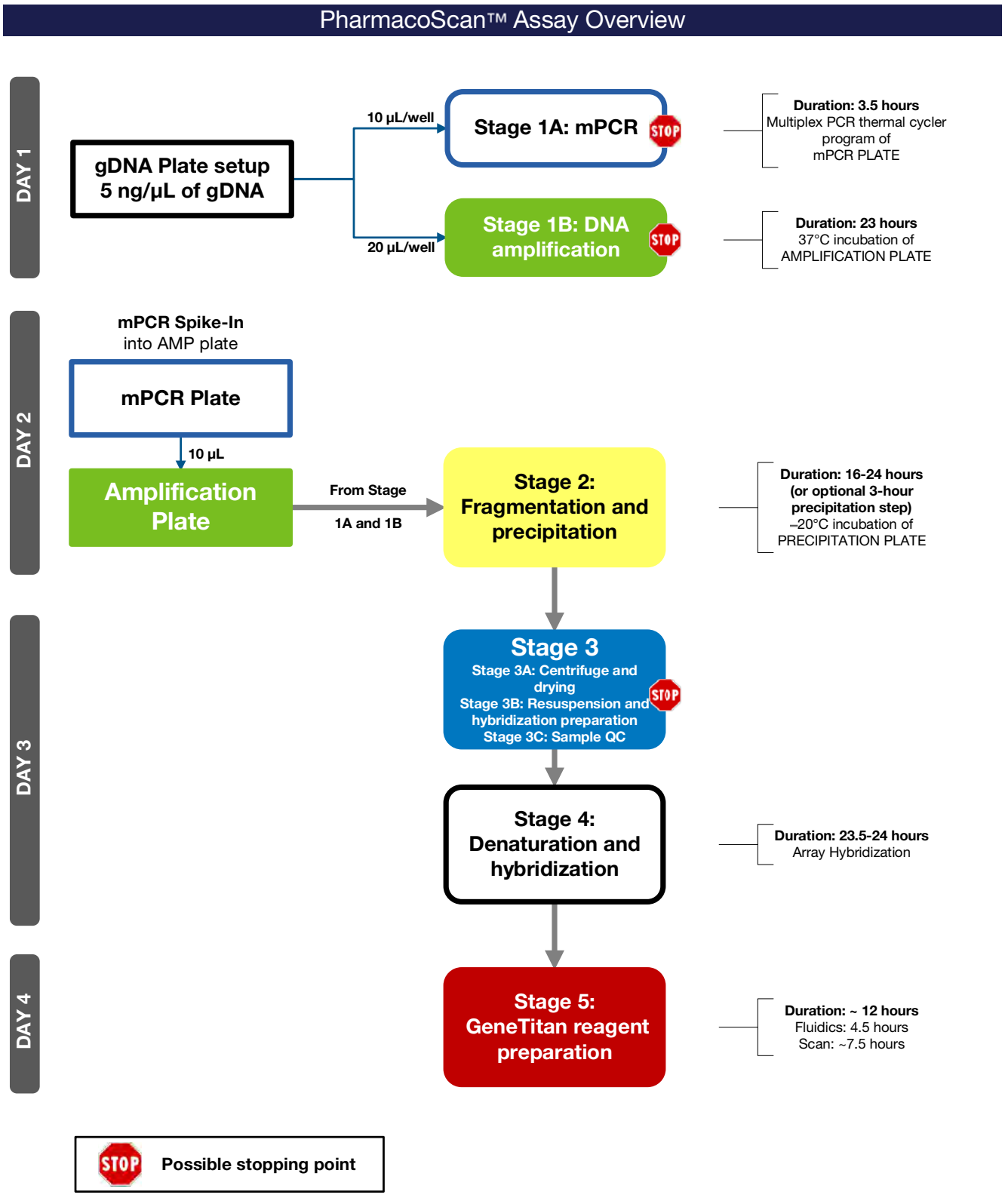

Figure 1 PharmacoScan™ Assay 96-Array Format Manual Workflow

Multiple plate workflows

Thermo Fisher Scientific provides workflows that allow you to run a set of samples and array plates through the protocol using a minimum of personnel and a fourty-hour week. The timing of steps is critical because of the following constraints:

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

These limitations require careful timing. For detailed information, see "Process three PharmacoScan™ array plates per week using an overnight precipitation step" on page 142. In addition, a recommended workflow using the three-hour precipitation is presented in Chapter 7.

2 Genomic DNA preparation and requirements

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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 use the PharmacoScan™ Reagent Kit 96 Reactions (Cat. No. 913025, see Table 5 on page 23). 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.

Sources of genomic DNA

The following sources of human gDNA have been successfully tested in the PharmacoScan Assay 96-Array Format Manual Workflow with DNA that meets the above requirements.

- Blood
- Saliva
- Buccal cell
- 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 18.
- 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.

  Note: DNA size integrity is important for successful assay performance. It is strongly advised to assess gDNA by gel electrophoresis as described below. This is of particular importance for DNA extracted from saliva and buccal cells, sample types prone to DNA degradation.

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 24, however these areas may be combined due to space and equipment limitations.

Assess the quality of genomic DNA using 1% agarose E-gels

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

Equipment and reagents recommended

Table 1 E-Gel® and reagents required

Item	Supplier	Cat. No.
Mother E-Base Device	Thermo Fisher Scientific	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 (RediLoad dye diluted 10-fold with nuclease-free water) 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.

Run a 48-lane 1% Agarose E-Gel

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

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

Figure 2 shows gel images of intact gDNA (that is suitable for use in the PharmacoScan™ Assay 96-Array Format Manual Workflow) and degraded gDNA samples. Customers whose gDNA is degraded (similar to the image in Figure 2) should perform a test experiment to investigate the performance of their samples in the

PharmacoScan Assay 96-Array Format Manual Workflow prior to beginning any large scale genotyping projects.

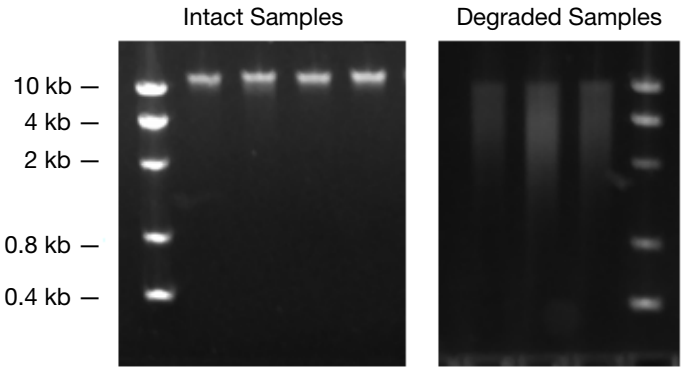

Figure 2 Gel images showing intact gDNA and degraded gDNA

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 60 minutes.
3. Centrifuge at 12,000 x *g* in a microcentrifuge at room temperature for 20 minutes
4. Remove supernatant and wash pellet with 80% ethanol.
5. Centrifuge at 12,000 x *g* at room temperature for 5 minutes
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 process using the PharmacoScan Assay 96-Array Format Manual Workflow 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 Input requirements for PharmacoScan Assay 96-Array Format Manual Workflow

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

Major steps 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 an Array Plate 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 3 are required for this stage.

Table 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: multichannel 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 Applied Biosystems 9700, Applied Biosystems Veriti™, Applied Biosystems 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 4 are required for this stage.

Table 4 Reagents required for "Genomic DNA preparation"

Reagent	Supplier	Cat. No.
• Control DNA 1 and Control DNA 2 (PharmacoScan Module B)	Thermo Fisher Scientific	Part No. 912897
• Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Thermo Fisher Scientific	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).

Thaw samples and controls using one of the methods below:

- Place items on benchtop for one hour, or
- 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. Remove the sample plate and/or sample tube from the water bath and wipe-dry using lab wipes. Ensure the outside is completely dry before opening the sample plate or tube to minimize any contamination, which can lead to reaction failure.

2. Quantitate and dilute 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 (Part No. 912897). 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 (Cat. No. 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: Ensure 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; Cat. No. AB-0932.

Aliquot diluted samples and controls to the deep well plate:

1. Aliquot 20 µL of each diluted gDNA sample to the ABgene deep well plate as shown in Figure 3.
2. Pipet 20 µL of Control DNA 1 to well G12 and 20 µL of Control DNA 2 to well H12.
3. Seal and spin.

mPCR sample plate

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

Aliquot diluted samples and controls to the mPCR sample plate:

1. Aliquot 10 µL of each diluted gDNA sample to the 96-well PCR plate as shown in Figure 3.
2. Pipet 10 µL of Control DNA 1 to well G12 and 10 µL of Control DNA 2 to well H12.
3. Seal and spin.

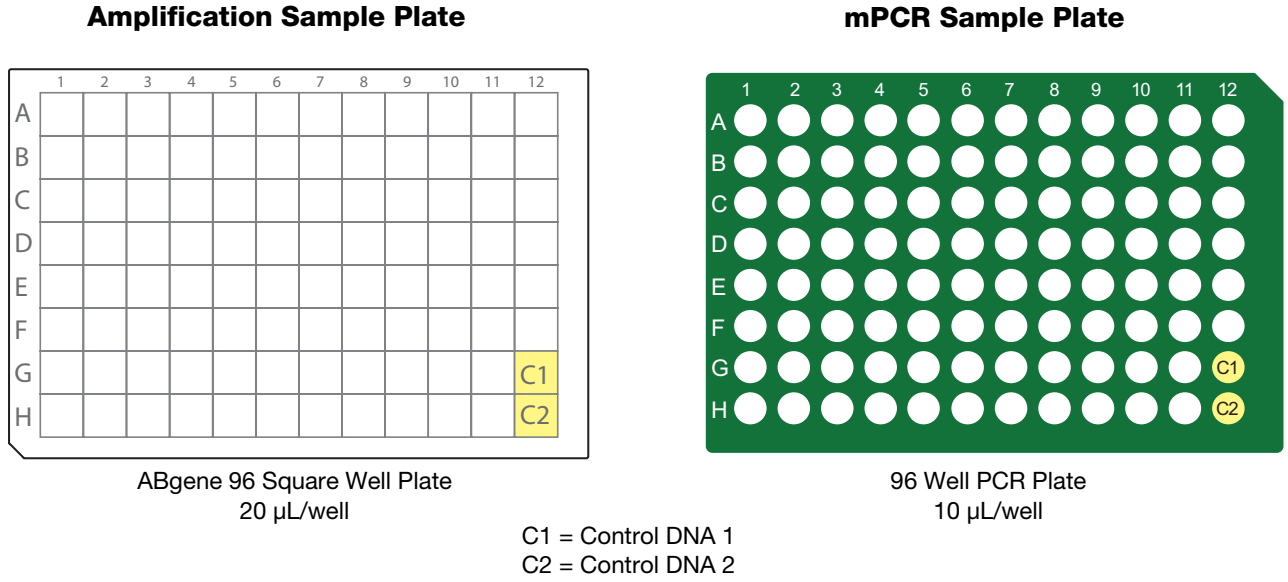

Figure 3 Aliquoting genomic DNA

4. Freeze or proceed

At this point you can:

- Store the sample plate at –20°C, or
- Proceed to DNA Amplification for Manual Target Preparation. See Chapter 4, "Target preparation" on page 42.

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

5. Create an Array Plate Registration file

IMPORTANT! It is important to create and upload a GeneTitan Array Plate Registration file with your sample information prior to loading the array plate and hybridization 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, scan the array plate barcode and upload the file to Applied Biosystems GeneChip Command Console (GCC) v4.3 or higher.

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, "Register Samples in GeneChip™ Command Console™" on page 189. See also Figure 4 for an example of a GeneTitan Array Plate Registration file.

1. Open GCC 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 is scanned when you are ready to load the array plate and samples onto the GeneTitan MC Instrument for processing.

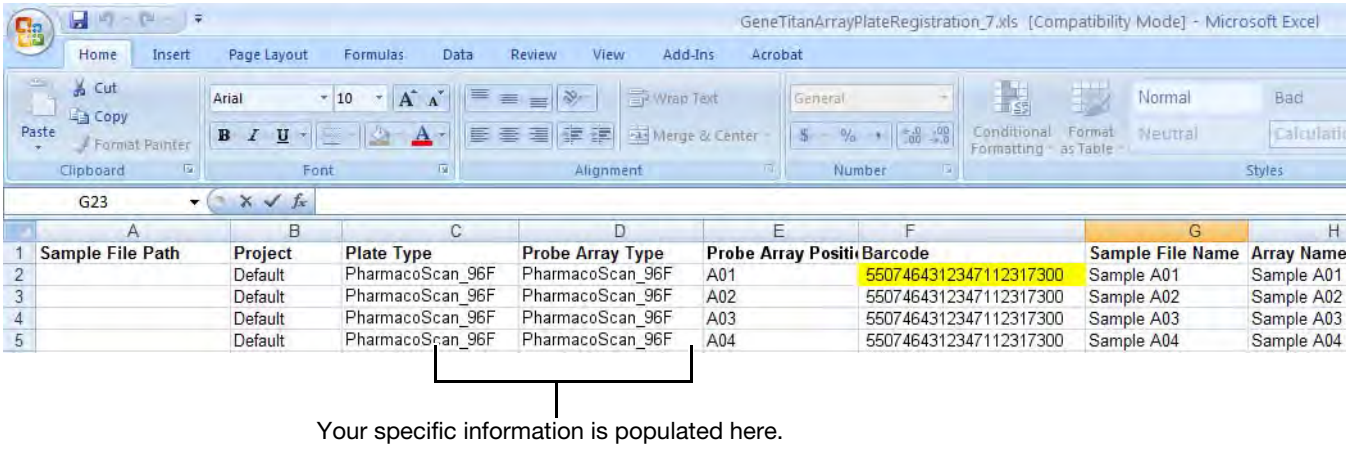

Figure 4 Example of a GeneTitan Array Plate Registration file

3 Preparation before you start

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Introduction

This manual assay format allows the user to run the PharmacoScan™ Assay for 96 samples using one PharmacoScan™ Reagent Kit 96 Reactions (Cat. No. 913025) and one QIAGEN Multiplex PCR *Plus* Kit (Cat. No. 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 workflow requires is the use of disposable 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 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460.

PharmacoScan™ Reagent Kit 96 Reactions, arrays, and GeneTitan™ consumables required

The table below lists the PharmacoScan reagents and GeneTitan consumables required to process one PharmacoScan 96F Array Plate. The table also lists the QIAGEN Multiplex PCR kit required for the PharmacoScan assay. See the *PharmacoScan™ Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460 for detailed information regarding the necessary materials required to run the PharmacoScan Assay.

Table 5 PharmacoScan™ Assay arrays, reagents, and GeneTitan consumables required

Cat. No	Description	Quantity
903160	PharmacoScan™ 96F Array Plate	1
901606	Axiom™ GeneTitan™ Consumables Kit	1
913025	PharmacoScan™ Reagent Kit 96 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 96-Array Format Manual Workflow.

Room temperature

When referred to in the PharmacoScan Assay 96-Array Format Manual Workflow, room temperature is 18°C 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 96 Reactions.

Plate requirements and recommendations

The following types of plates are required for performing manual target preparation. See the *PharmacoScan™ Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460, for supplier information.

- ABgene 96 Square-Well Storage Plate, 2.2 mL
- Bio-Rad Hard Shell Semi-skirted 96-well plate, Cat. No. HSS-9641 for the Applied Biosystems 9700, Applied Biosystems Veriti, and Applied Biosystems ProFlex thermal cyclers. Use the Bio-Rad Hard Shell Low-profile 96-well plate, Cat. No. HSP-9631 for the Eppendorf Mastercycler pro S. See the *PharmacoScan™ Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460, for supplier information.
- 96-well UV-Star® plates, 370 µL/well

Thermal cycler recommendations and protocols

The following thermal cyclers are recommended for the PharmacoScan Assay 96-Array Format Manual Workflow:

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

Note: Two validated thermal cyclers are required if running the three plate/week manual target preparation workflow.

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

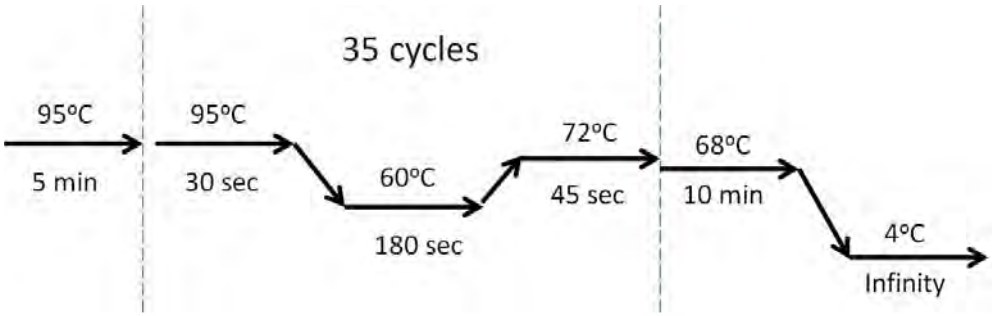


Figure 5 PharmacoScan mPCR thermal cycler protocol (Stage 1A)

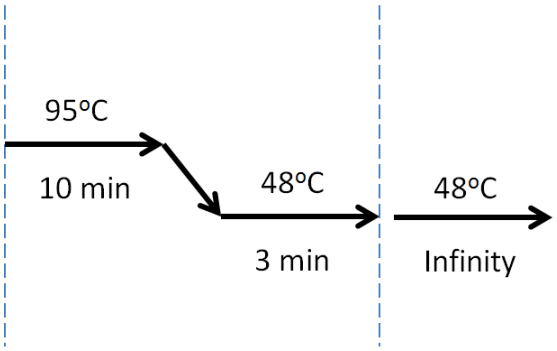


Figure 6 PharmacoScan Denature thermal cycler protocol (Stage 4)

⚠ **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 96-Array Format Manual Workflow has been validated with the Applied Biosystems 9700 (with gold-plated or silver block) Applied Biosystems Veriti, Applied Biosystems 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 6 provides details into the consumables to be used with the Applied Biosystems 9700 thermal cycler.

Table 6 Thermal cycler consumables for the PharmacoScan Assay 96-Array Format Manual Workflow

Thermal cycler model	PCR plate type	Seal
Applied Biosystems 9700	• BioRad Hard-Shell Full-Height 96-Well Semi-Skirted PCR Plate (Cat. No. HSS-9641)	MicroAmp™ Clear Adhesive Film from Thermo Fisher Scientific (Cat. No. 4306311)
Applied Biosystems Veriti	• BioRad Hard-Shell Full Height 96-well Semi-Skirted PCR Plate (Cat. No. HSS-9641)	MicroAmp™ Clear Adhesive Film from Thermo Fisher Scientific (Cat. No. 4306311)
Applied Biosystems ProFlex	• BioRad Hard-Shell Full Height 96-well Semi-Skirted PCR Plate (Cat. No. HSS-9641)	MicroAmp™ Clear Adhesive Film from Thermo Fisher Scientific (Cat. No. 4306311)
Eppendorf Mastercycler pro S	• BioRad Hard-Shell Low Profile 96-well Full-Skirt PCR Plate (Cat. No. HSP-9631)	MicroAmp™ Clear Adhesive Film from Thermo Fisher Scientific (Cat. No. 4306311)

Oven recommendations

The following ovens are recommended:

- BINDER ED 56 Drying and Heating Chamber. See the *PharmacoScan™ Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460, for ordering information.
- Applied Biosystems GeneChip Hybridization Oven 645

  Note: The GeneChip™ Hybridization Oven 640 is currently not supported with the PharmacoScan Assay; however, if you want to utilize it in the workflow contact your Field Service Engineer (FSE) or Technical Support regarding the compatibility of this oven with the PharmacoScan Assay 96-Array Format Manual Workflow.

  – If using a GeneChip Hybridization Oven, set the rotation speed to 15 rpm to aid in even heat distribution.

  – For either GeneChip Hybridization Oven, plates are placed in the bottom of the oven. To avoid interfering with the rotation apparatus, do not stack plates in the oven.

Plate centrifuge

One plate centrifuge is required for the PharmacoScan Assay 96-Array Format Manual Workflow. See the *PharmacoScan Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460, 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 69, the centrifuge must be able to spin down plates at:

- Rcf: 3,200 x *g* (4,000 rpm for the Eppendorf 5810R with the rotor configuration described in the *PharmacoScan Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460).
- 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 7.

Table 7 Shakers

Shaker	Supplier	Cat. No.
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 prevents 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 ensure 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 laboratory tissue 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 at maximum speed for 5 seconds in each sector for a total of five sectors (Figure 7).
    - For PCR plates vortex two seconds in each sector for a total of five sectors (Figure 7).
  - **Reagent Vials:** three times at maximum speed, one second each time.

  Note: In the procedures, "vortex twice" means to repeat the vortexing step.

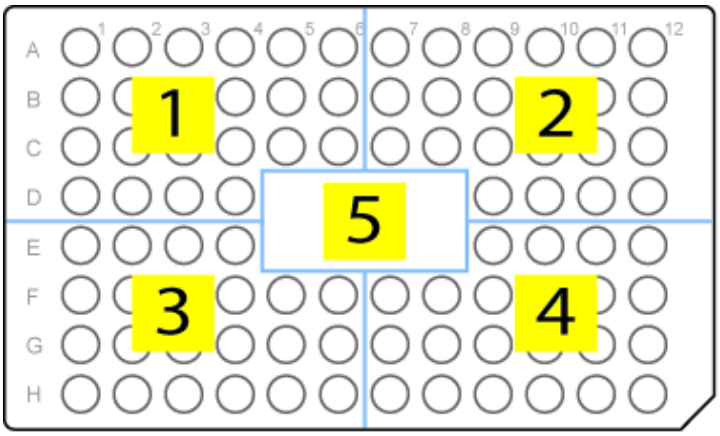


Figure 7 Vortexing plates

- **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 69).
  - **Plates**:
    - Spin at 1,000 rpm for 30 seconds at room temperature.
    - Do not spin for more than one minute.
  - **Reagent Vials**: three seconds.

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.

See Chapter 2, "Genomic DNA preparation and requirements" on page 15 for more information.

About the reagents and master mix preparation

PharmacoScan Reagent Kit 96 Reactions components

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

IMPORTANT! The PharmacoScan Assay 96-Array Format Manual Workflow is compatible only with reagents from a PharmacoScan Reagent Kit 96 Reactions. These reagents are not interchangeable with reagents from other reagent kits, such as CytoScan™, SNP 6.0, DMET Plus, etc.

QIAGEN reagents

QIAGEN Multiplex PCR *Plus* Kit

QIAGEN Multiplex PCR *Plus* Kit (Cat. No. 206152) is used with PharmacoScan Reagent Kit 96 Reactions to process 96 samples. The QIAGEN kit configuration is as follows:

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

Note: The CoralLoad Dye is not needed for PharmacoScan Assay 96-Array Format Manual Workflow and can be discarded.

All three tubes of 2x Multiplex PCR Master Mix are needed to process one 96F array plate, but only one tube of Water and one tube of Q-Solution is required.

Reagents from other suppliers

- Use only fresh reagents from the recommended suppliers 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 8).

Table 8 Recommended pipette sizes

Pipette size	Recommended volume range
Single channel P20 / 12-channel P20	1-20 µL
Single channel P200 / 12-channel P200	20-200 µL
Single channel P1000 / 12-channel P1200	200-1,000 µL

- We recommend the use of Rainin pipettes and tips. Thermo Fisher Scientific has only verified the use of Rainin multichannel 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 96-Array Format Manual Workflow and array replacement policy.
- Always use pipettes that have been calibrated.
- It is essential that you be proficient with the use of single and multichannel pipettes. To familiarize yourself with the use of multichannel 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 are not 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.

Multichannel 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.

Matrix™ 25 mL reagent reservoirs

The PharmacoScan 96-Array Format Manual Workflow requires the use of disposable reservoirs with a “trough within a trough” design. This special design maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent.

Figure 8 Dispense reagents from Matrix™ 25 mL reagent reservoirs

Equipment, consumables, labware, and reagents required

Equipment required

Thermal cycler

See "Thermal cycler recommendations and protocols" on page 25.

Oven

See "Oven recommendations" on page 26.

Plate centrifuge

See "Plate centrifuge" on page 26.

Plate shaker

See "Plate shakers" on page 27.

Consumables required

Table 9 Consumables required for PharmacoScan™ Assay 96-Array Format Manual Workflow

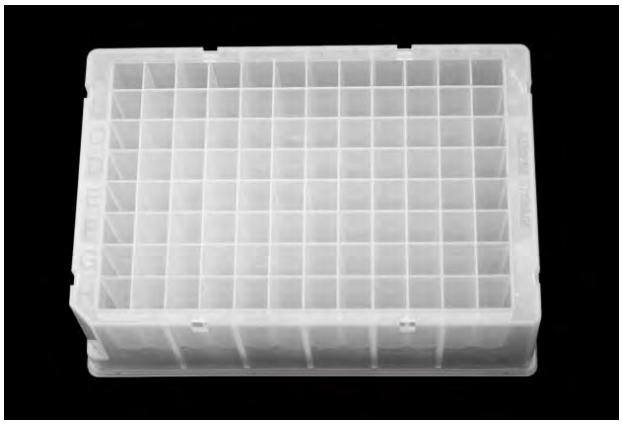
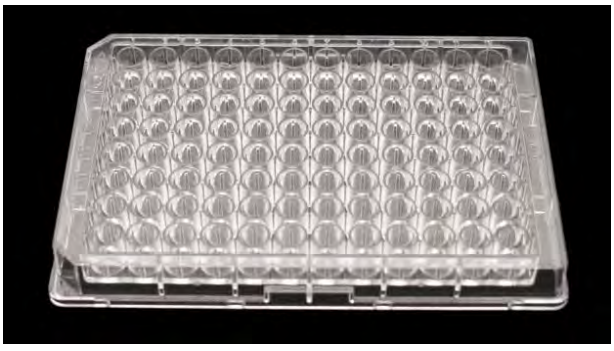
Labware	Supplier and Cat. No.	Image
ABgene 96 Square Well Storage Plate, 2.2 mL ABgene Storage Plate, 96-well, 2.2 mL, square well, conical	Thermo Fisher Scientific (formerly Life Technologies) Cat. No. AB-0932	
OD Plate Greiner UV-Star® 96 well plates	Sigma-Aldrich, VWR International, Fisher Scientific Cat. No. 655801 E&K Cat. No. 25801	

Table 9 Consumables required for PharmacoScan™ Assay 96-Array Format Manual Workflow (Continued)

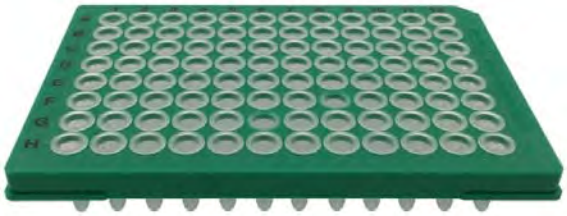
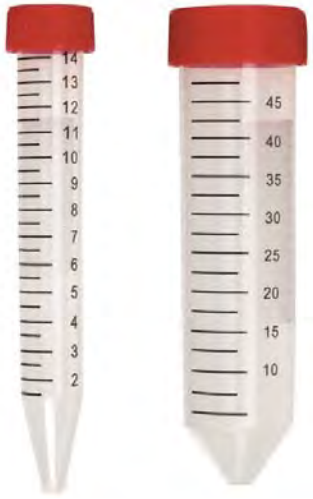
Labware	Supplier and Cat. No.	Image
Bio-Rad® Hard-Shell® 96-well plate Bio-Rad Hard-Shell Low-Profile 96-Well Skirted PCR Plates **Note:** See Table 6 for the PCR plate type recommended for your specific thermal cycler.	Bio-Rad Cat. No. HSP-9631	
96 Half-Skirt Plate Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plates **Note:** See Table 6 for the PCR plate type recommended for your specific thermal cycler.	BioRad Cat. No. HSS-9641	
1.7 mL microcentrifuge tubes, DNAse and RNAse-free	Common labware - order through your preferred labware supplier	
50 mL and 15 mL conical-bottom centrifuge tubes, Polypropylene	Various	

Table 9 Consumables required for PharmacoScan™ Assay 96-Array Format Manual Workflow (Continued)

Labware	Supplier and Cat. No.	Image
Zerostat Anti-static Gun and Ion-Indicator Cap	Milty Zerostat, Thermo Fisher Scientific Cat. No. 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 Cat. No. CHAM-1000	
Thermo Scientific™ Matrix™ Reagent Reservoirs, 25 mL	Thermo Scientific Cat. No. 8093-11	
100 mL reagent reservoir	VWR Cat. No. 89092-836	

GeneTitan™ MC Instrument consumables

All consumables for the GeneTitan MC Instrument are provided by Thermo Fisher Scientific. Table 10 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 10 PharmacoScan™ GeneTitan tray consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606)

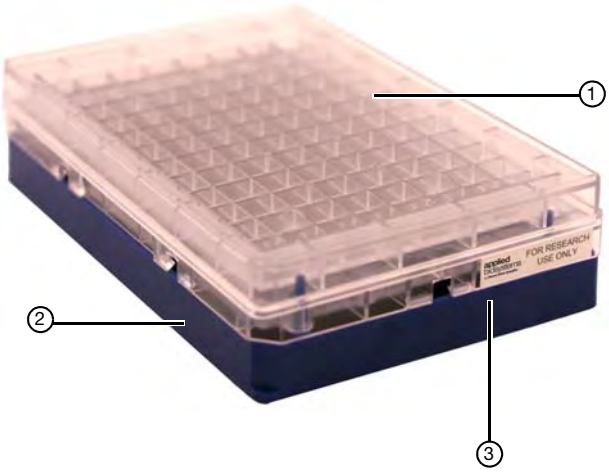
Item	Cat. No.	Image	Information
PharmacoScan 96F Array Plate	903160 Box 550746 Pouch	 **Note:** Array plate is not included in the Axiom™ GeneTitan™ Consumables Kit.  ① Clear tray shipping cover (to be discarded) ② Array plate protective base ③ Array plate	96-array plate: • Comprised of three parts: 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 11 Axiom™ GeneTitan™ MC Instrument Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606)

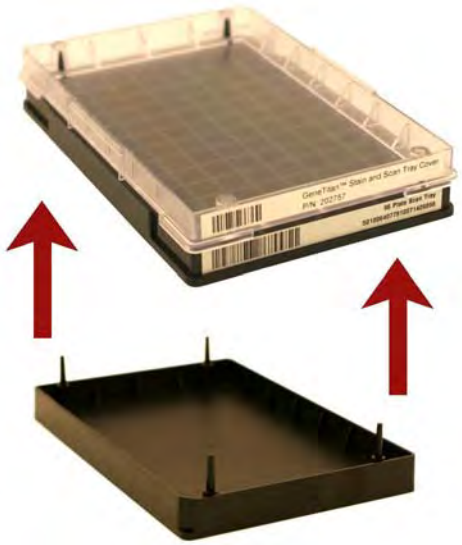
Item	Cat. No.	Labware image	Information
Scan Tray	900746 Box 501006 Pouch	  • The scan tray must be loaded into the GeneTitan Instrument with the scan tray cover only. • Do not load the scan tray with the protective base.	96-Plate scan tray: • Comprised of three parts: scan tray, black protective base, 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 192. • Remove the black scan tray protective base before loading the scan tray with the scan tray cover into the GeneTitan MC Instrument.

Table 11 Axiom™ GeneTitan™ MC Instrument Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606) (Continued)

Item	Cat. No.	Labware image	Information
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.
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**.

Table 11 Axiom™ GeneTitan™ MC Instrument Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606) (Continued)

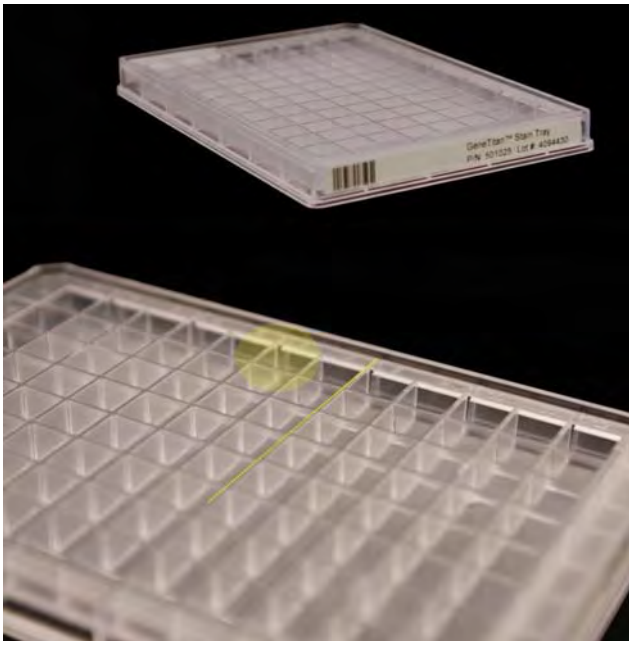
Item	Cat. No.	Labware image	Information
GeneTitan 5 Stain Trays Kit	4249910 Kit 501025 Tray		• The GeneTitan Stain Tray Kit comes with 5 stain trays packaged in zip-top bags to keep them free of dust. • The GeneTitan stain trays are barcoded and the trays have separator walls that are flush with the frame of the stain tray, as shown by the yellow line and the yellow oval in the lower photo.
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 192 for the anti-static procedure.

Table 11 Axiom™ GeneTitan™ MC Instrument Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606) (Continued)

Item	Cat. No.	Labware image	Information
GeneTitan stain tray shown with the stain tray cover	Tray 501025 Cover 202757		
Hybridization tray	900747		• After aliquoting the denatured hybridization 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.

Label GeneTitan™ hybridization and reagent trays

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

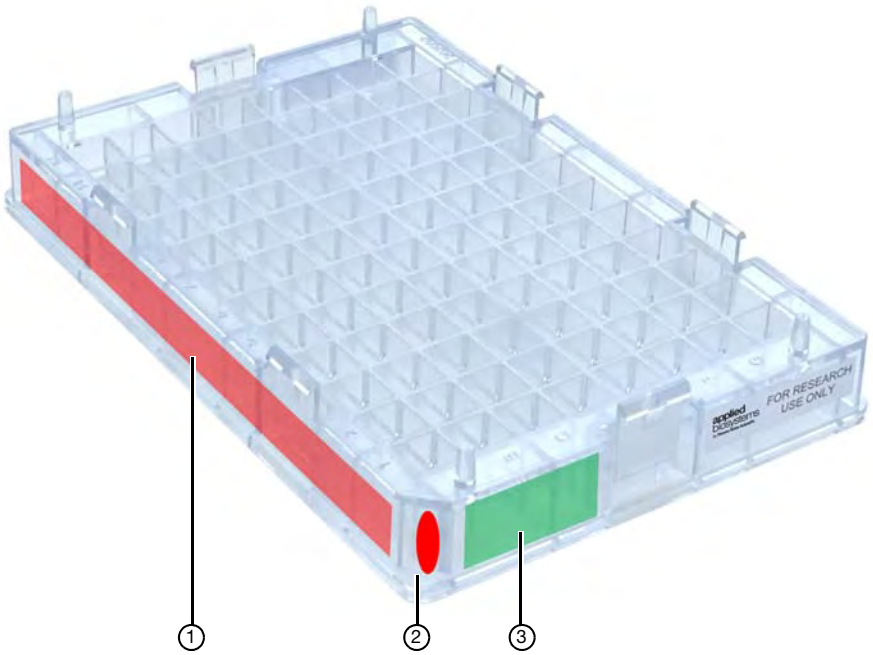
IMPORTANT! It is critical that you write only on the proper locations of the proper sides of hybridization and stain trays. Do **NOT** write in any other location, as this can interfere with sensors inside the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of covers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

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

- "Labeling for hybridization trays" on page 38
- "Labeling for stain trays" on page 40

Labeling for hybridization trays

You may label the hybridization tray on the front part of the **short side of the tray, next to the notch at the left,** as shown in Figure 9. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.



① Do NOT label trays on the long side of the tray.

② Notched corner of the hybridization tray should face the front.

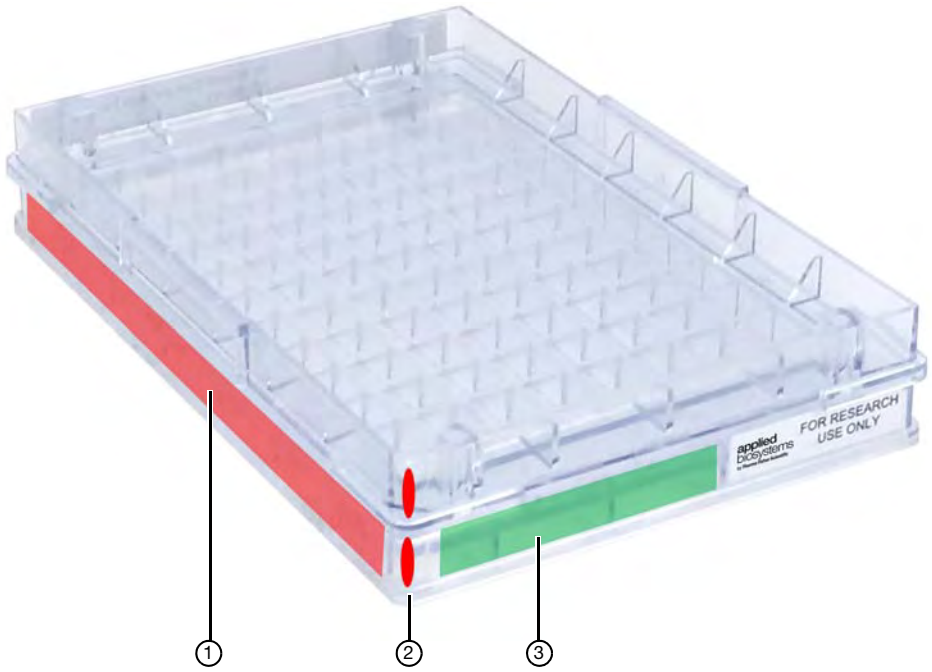
③ Label the hybridization tray in this area.

Figure 9 Correct area to label GeneTitan hybridization trays.

⚠ **CAUTION!** Writing on the wrong side of the hybridization 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 10. The correct side is closest to the notched corner, corresponding to the A1 through F1 wells.



① Do NOT label trays on the long side of the tray.
② Notched corner of the stain tray should face the front.
③ Label the stain tray here.

Figure 10 Labeling GeneTitan Stain Tray (stain tray shown with cover)

See "Stage 5: GeneTitan™ reagent preparation" on page 86 for detailed information.

Reagent kit for the PharmacoScan™ Assay 96-Array Format Manual Workflow

The PharmacoScan Assay 96-Array Format Manual Workflow uses the PharmacoScan Reagent Kit 96 Reactions (Cat. No. 913025). One PharmacoScan Reagent Kit 96 Reactions was developed to process one 96-array format plate. 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 12 PharmacoScan™ Reagent Kit 96 Reactions, Cat. No. 913025[1]

Component	Storage
Module 1: Part No. 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: Part No. 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: Part No. 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: Part No. 901446 (2 bottles per kit) • Axiom Wash Buffer B: Part No. 901447 (1 bottles per kit) • Axiom Water: Part No. 901578 (1 bottle per kit)	room temperature
Module 4: Pouch 1 of 2: Part No. 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: Part No. 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
PharmacoScan Module A: Part No. 912896 • 10X mPCR primers	–25°C to –15°C
PharmacoScan Module B: Part No. 912897 • Control DNA 1 • Control DNA 2	–25°C to –15°C

[1] Sufficient for processing one PharmacoScan 96-array format plate.

4 Target preparation

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Introduction

Target preparation for the PharmacoScan™ Assay 96-Array Format Manual Workflow enables you to perform target preparation to process 96 samples at a time. The protocol is performed in two parts:

- Part 1: Manual 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 103.

IMPORTANT! Read all the instructions in Chapter 3, "Preparation before you start" on page 23, before performing manual target preparation.

A list of all equipment and resources required for the PharmacoScan Assay 96-Array Format Manual Workflow is in the *PharmacoScan Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460.

Using the manual target preparation protocol, three array plates can be processed per work week for a total of 288 arrays. See Chapter 6, "Process three PharmacoScan™ array plates per week using an overnight precipitation step" on page 142 or Chapter 7, "Process three PharmacoScan™ array plates per week using a three-hour precipitation step" on page 156 for further information.

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 15.

The following steps are necessary to perform mPCR:

"1: Prepare for mPCR" on page 45

"2: Prepare the mPCR master mix" on page 46

"3: Set up the mPCR reaction plate" on page 46

"4: Run the PharmacoScan mPCR thermal cycler protocol" on page 47

"5: Freeze mPCR Reaction Plate or proceed" on page 47

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 24 for more information.

Duration

For 96 samples:

- Time to thaw materials: 30 minutes
- Hands-on time: approximately 30 minutes
- Thermal Cycler run time: approximately 3.5 hours
- Total time required: approximately 4.5 hours

Input required

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

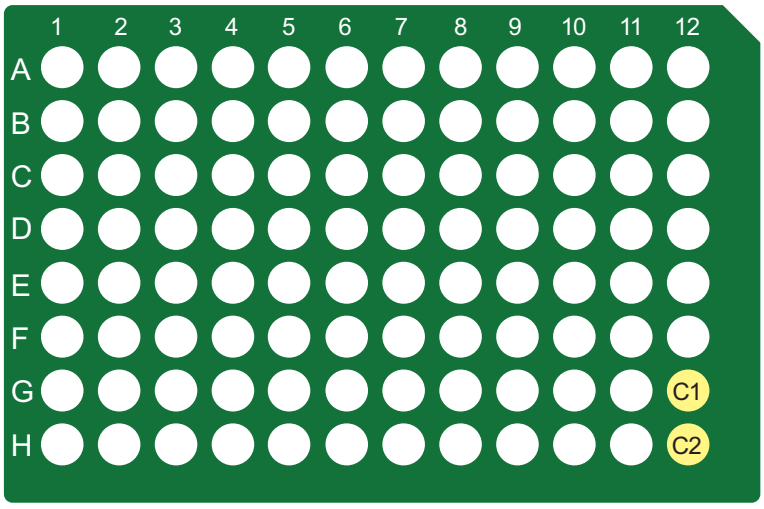


Figure 11 mPCR sample plate

See "Genomic DNA preparation" on page 19 for more information.

Equipment, consumables, and reagents required

Equipment and consumables

The equipment and consumables listed in Table 13 are required for this stage.

Table 13 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	96-well plate holder
1	15 ml conical tube (RNase/DNase-free)
Quantity	**Equipment**
1	Vortex
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 25).
1 Each	Rainin Pipettes: • Single channel P200 • Single channel P1000 • Multichannel P200
As Needed	Pipette tips

Reagents required

Table 14 Reagents Required for Stage 1A: mPCR

From the PharmacoScan Reagent Kit 96 Reactions	Module
10X Primer Mix, one tube	PharmacoScan Module A, Part No. 912896
From QIAGEN Multiplex PCR *Plus* Kit (100)[1]	**Cat. No. 206152**
2X Multiplex PCR Master Mix (three tubes)	
5X Q-Solution (one tube)	
RNase-free Water (one tube)	

[1] CoralLoad Dye in kit is not needed for PharmacoScan Assay 96-Array Format Manual Workflow.

1: Prepare for mPCR

Prepare for mPCR

1. Power on the thermal cycler programmed with **PharmacoScan mPCR** protocol (See Figure 5 on page 25). Ensure the heated lid option has been selected.
2. Thaw the mPCR Reagents and prepare the mPCR Reaction Plate.

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 after thawed.

IMPORTANT! gDNA samples must be 10 µL volume at a concentration of 5 ng/µL in a 96-well PCR plate (see Genomic DNA preparation, "mPCR sample plate" on page 21).

3. Thaw the following reagents on the benchtop at room temperature and place on ice after thawed:
   - From –20°C stored Applied Biosystems PharmacoScan™ Reagent Kit 96 Reactions (Cat. No. 913025), PharmacoScan Module A (Part No. 912896):
     - One tube of 10X mPCR primers
   - From –20°C stored QIAGEN Multiplex PCR *Plus* Kit (Cat. No. 206152):
     - Three vials of QIAGEN Multiplex PCR Master Mix, 2X
     - One vial Q-Solution, 5X
     - One vial RNase-free Water

IMPORTANT!
- Ensure reagents are thoroughly mixed prior to use.
- Vortex water and Q-Solution.
- Master Mix should be thoroughly mixed by inverting tube ten times. DO NOT VORTEX.

2: Prepare the mPCR master mix

1. Label a 15 mL conical tube *mPCR*.
2. To the *mPCR* tube, add the reagents listed in Table 15 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. It is necessary to use all three vials supplied by QIAGEN. It is recommended to set a P1000 single-channel pipette to 800 uL and remove this volume from the first vial. Transfer solution to the 15-mL tube. Change tips, and repeat this step for the remaining two vials.
   d. Mix thoroughly but gently, inverting tube end-over-end ten times. Pulse spin.

Table 15 mPCR Master Mix

Reagent	1 Reaction	120 Reactions
RNase-free Water	2 µL	240 µL
Q-solution	4 µL	480 µL
10X mPCR Primer Mix	4 µL	480 µL
2X QIAGEN Multiplex PCR Master Mix	20 µL	2,400 µL
Total	**30 µL**	**3,600 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. Carefully pour the prepared mPCR Master Mix into a 25 mL reservoir.
4. Use a P200 mutli-channel pipette to carefully transfer 30 µL of mPCR Master Mix into the mPCR plate. Final volume of each well is 40 µL.
5. Seal plate with adhesive seal, ensuring seal is firmly pressed down to prevent sample contamination during mixing and evaporation during PCR cycling.
6. Vortex plate for two seconds in each quadrant twice (See "Seal, vortex, and spin" on page 27).
7. Spin down at 2,000 rpm for 30 seconds.
8. Return plate to cold aluminum block until plate can be loaded onto thermal cycler (Note: Load plate onto thermal cycler within five minutes).
9. Discard all leftover reagents, including any remaining unused QIAGEN reagents.

4: Run the PharmacoScan mPCR thermal cycler protocol

1. Place mPCR on thermal cycler and run **PharmacoScan mPCR** protocol (see Figure 5 on page 25).

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

5: 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 49 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 57, then the mPCR Reaction Plate can be left at 4°C for several hours until needed for mPCR Spike-In (on page 61).

Note: An mPCR QC Gel can be run for qualitative evaluation of the mPCR reaction prior to the mPCR Spike-In step during Fragmentation. See Appendix F, "mPCR quality control gel protocol" on page 208 for protocol details.

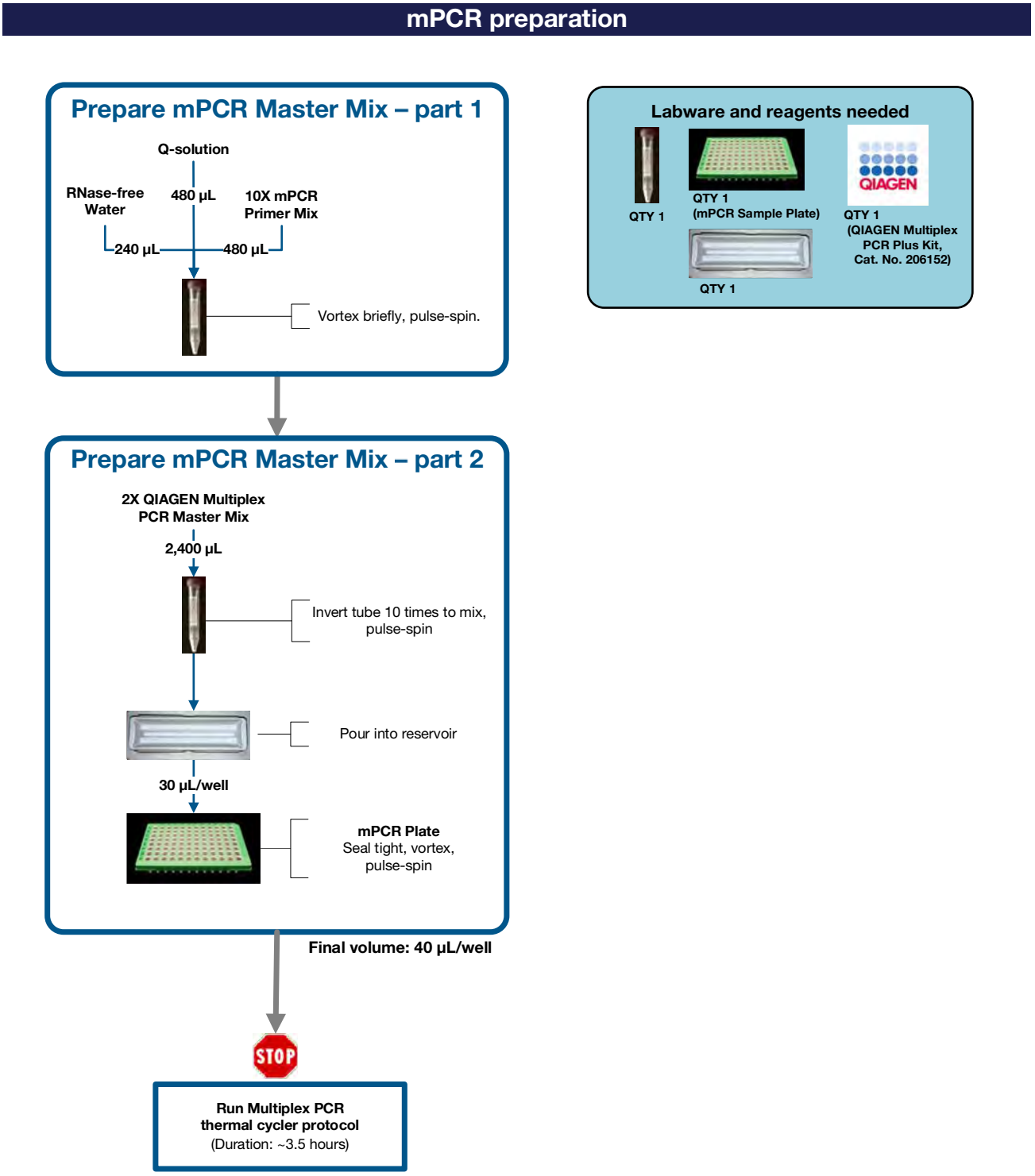


Figure 12 Stage 1A: mPCR Preparation workflow diagram

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 15.

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

"1: Prepare for DNA amplification" on page 51
"2: Prepare the Denaturation Master Mix" on page 52
"3: Add Denaturation Master Mix to samples" on page 53
"4: Add Neutralization Solution to samples" on page 53
"5: Prepare the Amplification Master Mix" on page 54
"6: Add Amplification Master Mix to samples" on page 54
"7: Freeze or proceed" on page 55

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 24 for more information.

Duration

For 96 samples:

- Time to thaw materials: 60 minutes
- Hands-on time: approximately 30 minutes
- Incubation at 37°C: 23 ±1 hour
- Total time required: approximately 24.5 hours

Input required

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

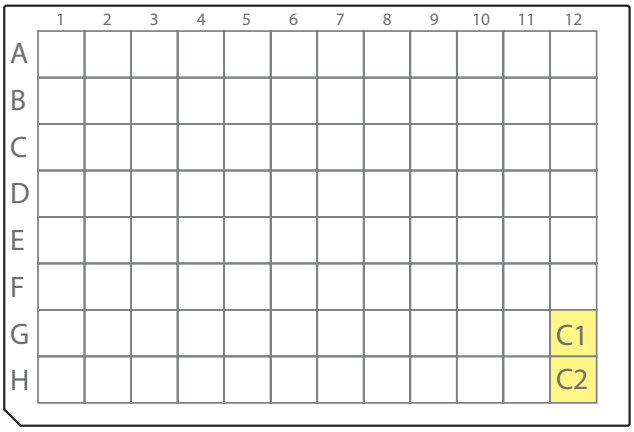


Figure 13 Whole genome amplification sample plate

See "Genomic DNA preparation" on page 19 for more information.

Equipment, consumables, and reagents required

Equipment and consumables

The equipment and consumables listed in Table 16 are required for this stage.

Table 16 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 and 50 mL tube holders
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1 each	Rainin Pipettes: • Single-channel P200 • Single-channel P1000 • Multichannel P20 • Multichannel P200 • Multichannel P1200
As needed	Pipette tips
As needed	Pipette, serological • 5 x 1/10 mL (VWR Cat. No. 89130-896) • 10 x 1/10 mL (VWR Cat. No. 89130-898)
1	Pipet aid
1	Plate centrifuge, at room temperature
1	Oven, set at 37°C
1	50 mL conical tube
1	15 mL conical tube
1	Vortexer
1	Timer
3	Matrix™ 25 mL Reservoir Cat. No. 8093-11

Reagents required

Table 17 Reagents required for Stage 1B: DNA amplification

From the PharmacoScan™ Reagent Kit 96 Reactions	Module
Axiom Denat Soln 10X	Module 1, –20°C Part No. 901711
Axiom Neutral Soln	
Axiom Amp Soln	
Axiom Water	
Axiom Amp Enzyme	

1: Prepare for DNA amplification

1. Set an incubator/oven temperature at 37°C.

   We recommend using one of these ovens:

   - Binder ED 56
   - Applied Biosystems 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.

Thaw and prepare the reagents

1. Thaw the Sample Plate on the benchtop at room temperature. Vortex, pulse spin, and leave plate at room temperature.

   IMPORTANT!

   - gDNA samples must be brought to room temperature before proceeding with denaturation.
   - gDNA samples must be **20 µL** volume of each gDNA at a concentration of **5 ng/µL** in an ABgene 96 square well storage plate, 2.2 mL (see "Genomic DNA preparation" on page 19).
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 seconds to thoroughly mix.
   - Axiom Neutral Soln: Vortex for 30 seconds to thoroughly mix.
   - Axiom Denat Soln 10X: Vortex and pulse-spin before use.
   - Axiom Amp Enzyme: Gently invert and flick the tube three times to mix and pulse-spin just before use.

Note: Allow ~60 minutes for Axiom Amp Soln to thaw on the benchtop at room temperature. If the solution is not completely thawed after 60 minutes, 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 15 mL and 50 mL conical tubes as indicated in the table below:

Label	Tube size	Temperature	Contents
• *D MM*	15 mL	leave tube at room temperature	Denaturation Master Mix
• *Amp MM*	50 mL	leave tube at room temperature	Amplification Master Mix

5. Label three Matrix 25 mL reservoirs (Cat. No. 8093-11) as indicated in the table below:

Label	Temperature	Contents
• *D MM*	Leave reservoir at room temperature	Denaturation Master Mix
• *N Soln*	Leave reservoir at room temperature	Neutralization Solution
• *Amp MM*	Leave reservoir at room temperature	Amplification Master Mix

2: Prepare the Denaturation Master Mix

Carry out the following steps at room temperature.

1. Following the table below, dilute the appropriate volume of Axiom Denat Soln 10X using the Axiom Water.

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked *D MM*, add:		
Axiom Denat Soln 10X	2 µL	400 µL
Axiom Water	18 µL	3.6 mL
Total volume	**20 µL**	**4.0 mL**

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

3: Add Denaturation Master Mix to 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 or pour the Denaturation Master Mix into the reagent reservoir marked *D MM*.
3. Carefully remove the seal from the Sample Plate and discard the seal.
4. Using a P20 12-channel pipette, add **20 µL of Denaturation Master Mix** to each sample in (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 **ten minute incubation** after vortexing.
6. Pulse-spin the Denaturation Plate to 1,000 at room temperature.

   Note: The quick spin time is included in the ten 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 might need to be repeated.
   b. Do **NOT** stop to measure volumes; proceed without delay.
8. Complete the **ten 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 53.
9. After incubation **immediately** add the Neutralization Soln as described in "4: Add Neutralization Solution to samples" on page 53.

4: Add Neutralization Solution to samples

Carry out the following steps at room temperature.

1. Pour the Axiom Neutral Soln into the reagent reservoir marked *N Soln*.
2. Carefully remove the seal from the Denaturation Plate and discard the seal.
3. Using a P200 12-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 might need to be repeated.
   b. Do **NOT** stop to measure volumes.
6. Proceed immediately to "5: Prepare the Amplification Master Mix" on page 54.

5: Prepare the Amplification Master Mix

Carry out the following steps at room temperature.

1. Per Table 18, pipet the appropriate amount of PharmacoScan Amp Soln into the 50 mL tube labeled *Amp MM* at room temperature.

   Tip: The Amp Soln is a viscous solution. To ensure the Amp Soln reagent transfer is accurate:

   - Pipet slowly.
   - Allow bubbles generated from mixing to settle at the top before pipetting.
   - Use a 10 mL serological pipette to transfer the Amp Soln into the Amp MM tube.

   Table 18 Amplification Master Mix (*Amp MM*)

Reagent	per sample	Master Mix
To the 50 mL tube marked *Amp MM*, add:		
Axiom Amp Soln	225 µL	26.0 mL
Axiom Amp Enzyme	5 µL	578 µL
Total volume	**230 µL**	**26.58 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 18, add the appropriate amount of Axiom Amp Enzyme to the tube labeled *Amp MM*.
   c. Vortex the Amplification Master Mix well, invert the tube two times, and then vortex again.

6: Add Amplification Master Mix to samples

1. *Slowly* pour the Amplification Master Mix into the reagent reservoir labeled *Amp MM*.
2. Carefully remove the seal from the Neutralization Plate and discard the seal.
3. Using a P1200 12-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. Blot the top of the plate with a Kimwipe laboratory tissue, seal tightly, vortex twice, and spin the Amplification Plate for one minute at 1,000 rpm (as described in "Seal, vortex, and spin" on page 27).
5. Place the sealed Amplification Plate in an oven set at **37**°C and leave **undisturbed for 23 ±1 hour.**

   Note: If using a GeneChip™ Hybridization Oven, place the plate on the bottom of the oven. Plates do not rotate. Set the rotor for 15 rpm speed. See "Oven recommendations" on page 26 for more information.

7: Freeze or proceed

After the incubation finishes, you can either:

- Proceed to "Stage 2: Fragmentation and precipitation" on page 57.
- 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 is performed after thawing the frozen plate, as described in "1: Prepare for fragmentation and precipitation" on page 59.

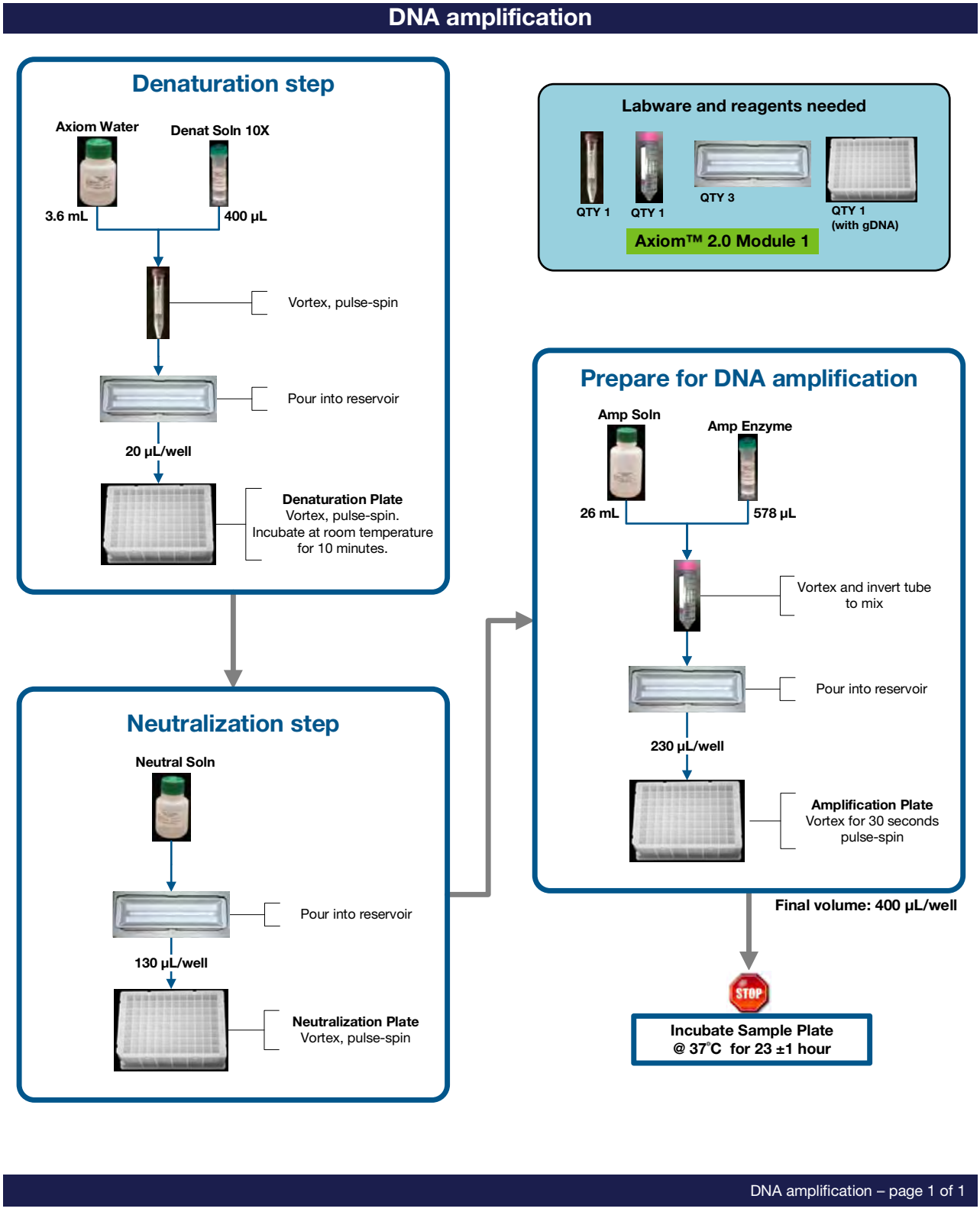


Figure 14 Stage 1B: Amplification workflow diagram

Stage 2: Fragmentation and precipitation

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

"1: Prepare for fragmentation and precipitation" on page 59
"2: mPCR Spike-in to Amplification Plate" on page 61
"3: Incubate samples in pre-heated ovens" on page 61
"4: Prepare the Fragmentation Master Mix" on page 61
"5: Add the Fragmentation Master Mix to samples" on page 62
"6: Add the Stop Solution to the samples" on page 62
"7: Prepare the Precipitation Master Mix" on page 63
"8: Prepare and add isopropanol to Precipitation Plate" on page 63
"9: Freeze the Precipitation Plate" on page 64
"10: Store mPCR plate" on page 64

Duration

Total time: approximately two hours (plus time for precipitation incubation at –20°C, three hours or overnight).

Input required

- mPCR Reaction Plate from "Stage 1A: Multiplex PCR (mPCR)" on page 43.
- Amplification Plate from "Stage 1B: DNA amplification" on page 49.

Equipment, consumables and reagents required

Equipment and consumables

The equipment and consumables listed in Table 19 are required for this stage.

Table 19 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 • Multichannel P20 • Multichannel P200 • Multichannel 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

Table 19 Equipment and consumables required for Stage 2: Fragmentation and precipitation (Continued)

Quantity	Item
1	Plate centrifuge set at room temperature
1	Mini microcentrifuge (microfuge with microtube rotor)
2-3	Ovens (see "Oven recommendations" on page 26): • One oven set at 37°C • One oven set to 65°C
1	15 mL conical tube and holder
3	Matrix™ 25 mL Reagent Reservoir Cat. No. 8093-11
1	100 mL reagent reservoir
1	Vortexer

Reagents required

Table 20 Reagents required for Stage 2: Fragmentation and precipitation

Reagent	Module
From the PharmacoScan Reagent Kit 96 Reactions	
Axiom Frag Enzyme (leave at –20°C until ready to use)	Module 2-1, –20°C Part No. 901528
Axiom 10X Frag Buffer	
Axiom Precip Soln 2	
Axiom Frag Diluent	Module 2-2, 2–8°C Part No. 901529
Axiom Frag Rxn Stop	
Axiom Precip Soln 1	
User-supplied - See the *PharmacoScan Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460	
Isopropanol (2-Propanol), 99.5%	96 samples: 65 mL per plate

1: Prepare for fragmentation and precipitation

Set ovens and centrifuge

1. Set the incubators/ovens.
   a. If you are running one plate per week, you need to 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.
   b. If you are running the three-hour precipitation workflow, an additional oven set to 48°C is recommended.
   c. If you are running the three-plate per week manual target preparation workflow, three ovens are recommended. See Chapter 6, "Process three PharmacoScan™ array plates per week using an overnight precipitation step" on page 142, or Chapter 7, "Process three PharmacoScan™ array plates per week using a three-hour precipitation step" on page 156 for further information.
2. Set the centrifuge temp to room temperature.

   Note: 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

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 minutes until all wells have thawed.
3. Spin down the plate at 1,000 rpm for 30 seconds.
4. To avoid cross-contamination of wells during vortexing:
   a. Remove the seal and blot the top of the plate with a Kimwipe laboratory tissue.
   b. Tightly re-seal the plate using a fresh seal.
5. Vortex the plate for 30 seconds to thoroughly mix.
6. Spin at 1,000 rpm for 30 seconds.

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 1,000 rpm for 30 seconds.
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 ten seconds to thoroughly mix.
5. Spin at 1,000 rpm for 30 seconds.

Thaw and prepare the reagents

Prepare reagents as shown below at the start of the 65°C incubation of the Amplification Plate.

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

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 and keep at room temperature.
   - 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 tube as indicated in the table below:

Label	Tube size	Temperature	Contents
• *Frg MM*	15 mL	Place tube on ice	Fragmentation Master Mix

2. Label four reagent reservoirs as indicated in the table below.

Label	Reservoir size	Temperature	Contents
• Frg MM	25 mL	Leave at room temperature	Fragmentation Master Mix
• *Stop*	25 mL	Leave at room temperature	Frag Rxn Stop
• *Precip MM*	25 mL	Leave at room temperature	Precipitation Master Mix
• *ISO*	100 mL	Leave at room temperature	Isopropanol

2: mPCR Spike-in to Amplification Plate

- If proceeding directly from the end of "Stage 1B: DNA amplification" on page 49, 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! Ensure 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. Transfer the Amplification Plate from the 65°C oven to the 37°C oven:
   - Press on the seal, if needed.
2. Incubate for 45 minutes.

4: 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 21 Fragmentation Master Mix

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked *Frg MM*, add:		
Axiom 10X Frag Buffer	45.7 µL	6.0 mL
Axiom Frag Diluent	10.3 µL	1.35 mL
Axiom Frag Enzyme	1.0 µL	131 µL
Total volume	**57 µL**	**7.48 mL**

- Add the reagents from Table 21 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 two to three 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. Slowly pour the Fragmentation Master Mix in 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 12-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 minutes**

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

   ⚠ **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 can 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

Carry out the following steps at room temperature.

1. A few minutes before the end of the 30 minute incubation period, pour the Axiom Frag Rxn Stop solution in the 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 12-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 1,000 rpm.
6. Leave the Fragmentation Plate on the benchtop while you prepare the Precipitation Master Mix.

7: Prepare the Precipitation Master Mix

Carry out the following steps at room temperature.

1. Prepare Precipitation Master Mix by adding 218 µL of Axiom Precipitation Solution 2 directly to the Axiom Precipitation Solution one bottle. *Precip MM*.

Table 22 Precipitation Master Mix

Reagent	per sample	Master Mix 96+
Add Axiom Precip Solution 2 directly to the Axiom Precipitation Solution one bottle.		
Axiom Precip Soln 1	238 µL	26 mL
Axiom Precip Soln 2	2 µL	218 µL
Total volume	**240 µL**	**26.22 mL**

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

   Note: After adding the Precipitation Master Mix, the plate is now known as the Precipitation Plate.
6. Seal, 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 65 mL of isopropanol into the 100 mL reagent reservoir labeled *ISO*.
3. Carefully remove the seal from the Precipitation Plate and discard the seal.
4. Using a P1200 12-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 laboratory tissue and seal tightly with a MicroAmp Adhesive Seal.

9: Freeze the Precipitation Plate

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

A new option for DNA target precipitation is to incubate the plate in the –20°C freezer for three hours, instead of overnight. This shortened precipitation allows you to proceed to "Stage 3: Centrifuge and drying, resuspension and hybridization preparation, and sample QC" followed by "Stage 4: Denaturation and hybridization"on day 2 of the assay workflow.

IMPORTANT! The three-hour DNA precipitation workflow extends the day 2 assay schedule. Approximately nine hours are required to complete Stage 2 through Stage 4 (Figure 1 on page 13). Review Chapter 3 and Chapter 4 for timing details.

Note: It is recommended to designate a shelf in a –20°C freezer where the plates can be left undisturbed. In addition, the freezer should not be subjected to frequent temperature excursions.

10: Store mPCR plate

Store the mPCR Plate at –20°C for optional QC purposes if desired. See Appendix F, "mPCR quality control gel protocol" on page 208.

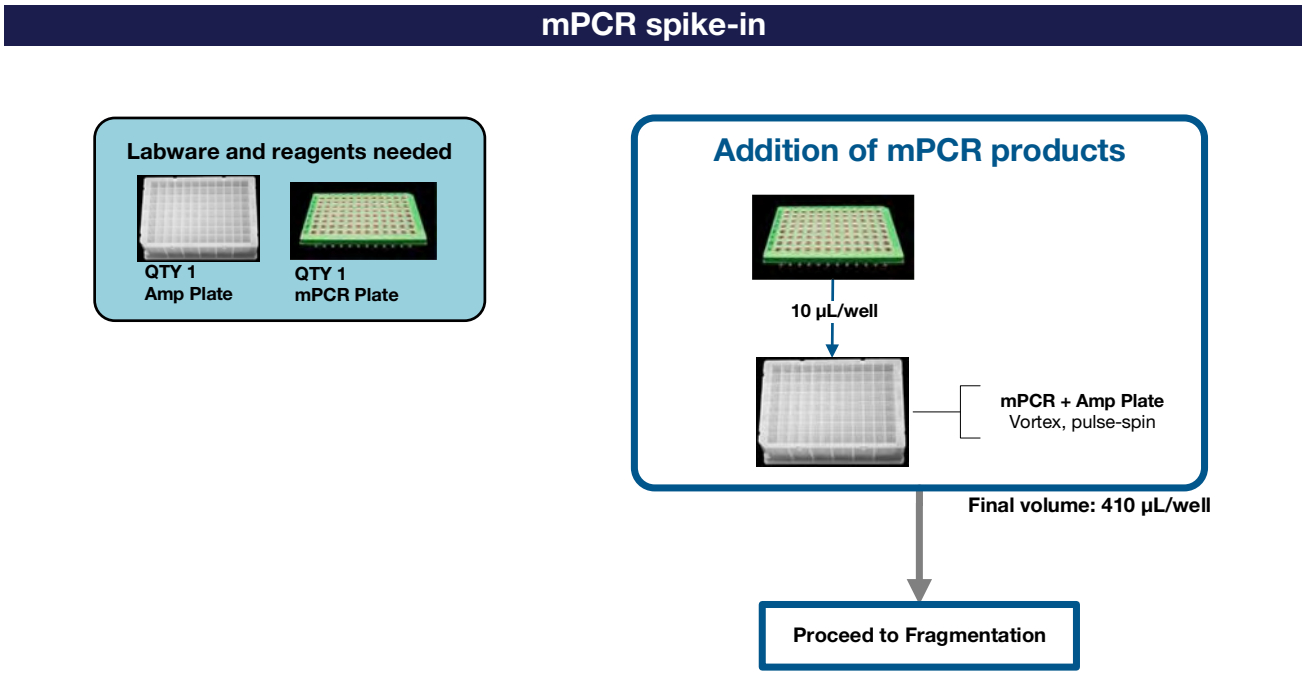


Figure 15 mPCR Spike-In flowchart

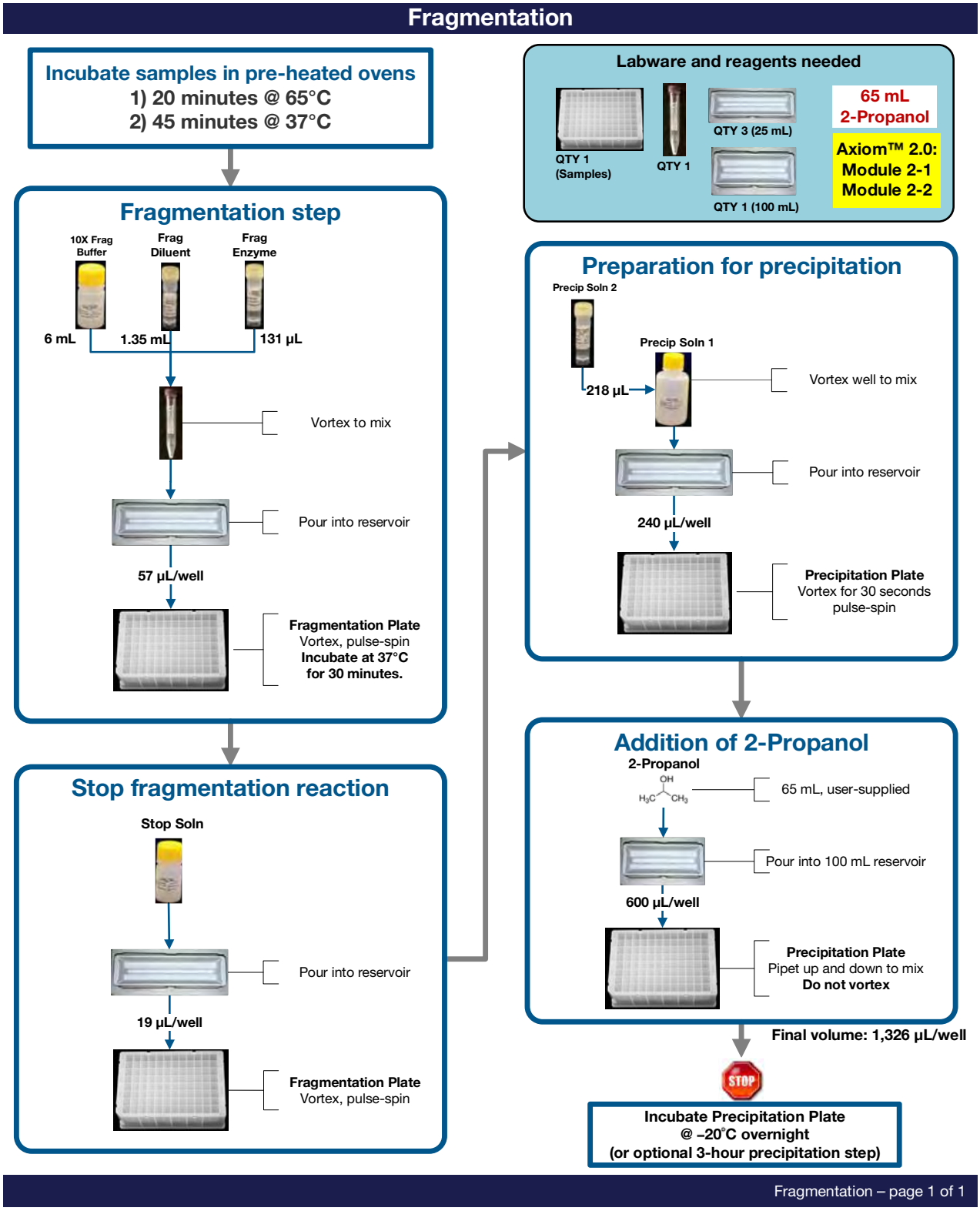


Figure 16 Stage 2: Fragmentation and precipitation

Stage 3: Centrifuge and drying, resuspension and hybridization preparation, and sample QC

This stage requires the following sets of steps:

"Stage 3A: Centrifuge precipitation plate and dry the DNA pellet" on page 69
"Stage 3B: Resuspension and hybridization preparation" on page 71
- "1: Prepare for resuspension and hybridization preparation" on page 71
- "2: Prepare DNA pellets and warm the resuspension buffer" on page 71
- "3: Thaw and prepare the reagents" on page 71
- "4: Label tubes and reservoirs" on page 72
- "5: Add Resuspension Buffer to DNA pellets" on page 72
- "6: Resuspension of DNA pellets" on page 72
- "7: Prepare the Hybridization Master Mix" on page 73
- "8: Prepare the Hyb Ready Sample Plate" on page 73
- "9: Freeze or proceed" on page 73

"Stage 3C: Perform quantitation and fragmentation QC checks" on page 74
- "1: Prepare for sample QC" on page 74
- "2: Perform QC checks" on page 75
- "3: Freeze or proceed" on page 75

⚠ **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: 80 minutes
- Resuspension and hybridization mix preparation: 25 minutes
- Gel QC and OD: 45 minutes

Total: 2.5 hours

Input required

Precipitation Plate from "Stage 2: Fragmentation and precipitation" on page 57.

Equipment, consumables, and reagents required

The equipment and consumables listed in Table 23 are required for this stage.

Table 23 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 P100 • Single channel P000 • Multichannel P20 • Multichannel P200
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 Cat. No. HSS-9641 (for Applied Biosystems 9700, Applied Biosystems Veriti™, Applied Biosystems ProFlex™ thermal cyclers), HSP-9631 (for Eppendorf Mastercycler proS 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	10 mL Serological Pipette
1	Pipet aid
1	Shaker, either: • Thermo Scientific™ Compact Digital Microplate Shaker • Jitterbug
1	Vortexer
4	Matrix™ 25 mL Reagent Reservoir, Cat. No. 8093-11

Reagents required

Table 24 Reagents required for Stage 3: Drying, resuspension, and sample QC

Reagent	Module
From the PharmacoScan Reagent Kit 96 Reactions	
Axiom Hyb Buffer	Module 2-1, –20°C Part No. 901528
Axiom Hyb Soln 1	
Axiom Resusp Buffer	Module 2-2, 2–8°C Part No. 901529
Axiom Hyb Soln 2	
Other reagents required for QC steps (optional)	
Gel Diluent, 5 mL of 1,000-fold dilution of TrackIt™ Cyan/Orange Loading Buffer (see Appendix A, "Fragmentation quality control gel protocol" on page 177 for dilution instructions.)	
Gel Sample Plate	
15 fold dilution of TrackIt™ 25 bp DNA Ladder (Cat. No. 10488-022)	
Nuclease-free water, ultrapure MB Grade, 5 mL (Cat. No. 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 177 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.

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 a GeneChip Hybridization 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 minutes at 4°C** at 3,200 x *g* (4,000 rpm for the Eppendorf 5810R centrifuge with the rotor configuration described in the *PharmacoScan Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460).

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

   ⚠ **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 can result in cracked plates, loss of sample, unbalanced centrifugation, damage to the instrument and possible physical injury.

3. Immediately after the 40 minute 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 clean waste container and allow the liquid to drain. Collect liquid and dispose of liquid according to local, state, and federal regulations.
   c. While still inverted, gently press the plates on a pile of Kimwipe laboratory tissues on a bench and allow them to drain for 5 minutes. Transfer the plate to a new pile of tissues twice during the 5 minute period.
4. Turn the plate right side up and place in an oven for 20 minutes at 37°C to dry.
   - Tightly seal the plate upon completion

   Note: If using a GeneChip Hybridization Oven 645:
   - Place the plate on the bottom of the oven. Plates do not rotate.
   - Turn off the rotor during the 20 minute drying time.

5. Do one of the following:
   - Proceed directly to "Stage 3B: Resuspension and hybridization preparation" on page 71, 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 is carried within 4 hours, keep the plate at room temperature.
     - If resuspension is carried out in more than 4 hours, store the plate in a refrigerator (2-8°C).
   - 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

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 critical for the success of the PharmacoScan target preparation. When any of these are cooler than room temperature, pellets might not resuspend completely. This can result in compromised assay performance. Note following guidelines on how to work with plates with fresh, cold, or frozen pellets:

Pellets

- **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.

Resuspension and hybridization reagents

- Resuspension buffer, hybridization buffer, Hyb Soln 1, and Hyb Soln 2 need at least 60 minutes to equilibrate to room temperature.

3: 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.

Label	Tube size	Temperature	Contents
• *Hyb MM*	15 mL	Room temperature in fume hood	Hybridization Master Mix

2. Label two Matrix 25 mL Reagent Reservoirs (Cat. No. 8093-11) as indicated in the table below.

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 hours before carrying out resuspension.

Note: Ensure the Resusp Buffer has equilibrated to room temperature before adding to dry pellets in Step 3, below.

Carry out the following steps at room temperature.

1. Pour Axiom Resusp Buffer into the reagent reservoir labeled *Resus*.
2. Carefully remove the seal from the Precipitation Plate and discard the seal.
3. Using a P200 12-channel pipette, transfer **35 µL Axiom Resusp Buffer** to each well of the Precipitation Plate. 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 ten minutes.**
   - Jitterbug: at **speed 7 for ten minutes.**
2. Inspect the Resuspension Plate from the bottom. If the pellets are not dissolved, repeat Step 1.
3. Quickly spin at 1,000 rpm.

7: Prepare the Hybridization Master Mix

⚠ **CAUTION!** It is recommended that the remainder of the steps in this stage be performed 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 the table below to the *Hyb MM* tube in the order shown using serological and single-channel pipettes as needed.

Reagent	per sample	Master Mix 96+
To the 15 mL tube labeled *Hyb MM*, add:		
Axiom Hyb Buffer	70.5 µL	7.8 mL
Axiom Hyb Soln 1	0.5 µL	55.6 µL
Axiom Hyb Soln 2	9 µL	1.0 mL
Total volume	**80 µL**	**8.86 mL**

   b. Vortex twice to mix.

8: Prepare the Hyb Ready Sample Plate

1. Choose a 96-well plate that is compatible with the thermal cycler model used for sample denaturation. See Table 6 on page 26 for information on thermal cycler consumables.
2. Label the 96-well PCR plate as Hyb Ready [Sample ID].
3. Set a P200 12-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 of the Resuspension Plate to the corresponding wells of the labeled Hyb Ready Plate.
   - Change pipette tips after each transfer.
5. Pour the Hyb Master Mix into the reagent reservoir labeled Hyb MM.
6. Using a P200 12-channel pipette, add 80 µL of the Hyb Master Mix to each well of 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: Perform quantitation and fragmentation QC checks" (highly recommended), below; or
- Proceed to "Stage 4: Denaturation and hybridization"; or
- Store the hybridization ready samples at –20°C.

Stage 3C: 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

Prepare the reagents

Obtain the reagents for sample QC:

1. 15 mL of nuclease-free water for the water reservoir.
2. 15 mL of Gel Diluent.

   The Gel Diluent is a 1,000-fold dilution of the TrackIt Cyan/Orange Loading Buffer (Invitrogen) as described in "Dilute the TrackIt™ Cyan/Orange Loading Buffer and 25 bp ladder" on page 178.
3. 90 µL of a 15-fold dilution of TrackIt™ 25 bp DNA Ladder, Cat. No. 10488-022, Thermo Fisher Scientific.
4. One E-Gel 48 Agarose Gel, 4% Agarose, Cat. No. G8008-04, Thermo Fisher Scientific.

Label reservoirs

Label two Matrix 25 mL Reagent Reservoirs (Cat. No. 8093-11) as indicated below:

Label	Temperature	Contents
• *H2O*	Leave reservoir at room temperature	Nuclease-free Water
• *Gel Dil*	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

Carry out the following steps at room temperature.

1. Prepare Dilution QC Plate and OD Plate:
   a. Pour 15 mL nuclease-free water into the reagent reservoir labeled *H20*. The water is used to make the QC Dilution Plate and the OD Plate.
   b. Add 33 µL nuclease-free water to each well of the *Dil QC* Plate.
   c. Add 90 µL nuclease-free water to each well of the OD Plate.
2. Prepare the Dilution QC Plate:
   a. Transfer **3 µL of the hybridization 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 180 for more information on performing the Sample Quantitation.
4. Prepare Gel QC Plate:
   a. Pour 15 mL of Gel Diluent into the reagent reservoir labeled *Gel Dil*.
   b. Add **120 µL gel diluent** to each well 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 177.

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

3: Freeze or proceed

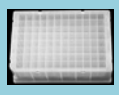
At this point you can:

- Proceed to "Stage 4: Denaturation and hybridization", below; or
- Store the hybridization ready samples at –20°C.

Resuspension and hybridization preparation

Centrifuge Precipitation Plate to pellet DNA
Speed: 3,200 x *g*
Duration: 40 minutes
Temperature: 4°C

Labware and reagents needed

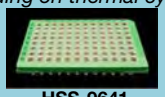

QTY 1
Precip Plate
(Samples)


QTY 1


QTY 2

Axiom™ 2.0:
Module 2-1
Module 2-2

Hyb Ready Plate: QTY 1
(depending on thermal cycler used)


HSS-9641
for Applied Biosystems 9700

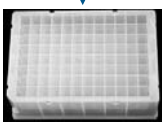
Dry DNA pellets
- **Decant liquid by inverting plate**
- **Blot-Dry inverted plate for 5 minutes**
- **Incubate @ 37°C for 20 minutes right-side up**

Add Resuspension Buffer

Resusp Buffer


Pour into reservoir

35 µL/well


Resuspension Plate
Resuspend pellets on plate shaker for 10 minutes. Pulse-spin.

Resuspension of DNA pellet
Duration: 10 minutes
Jitterbug: Speed 7
Thermo Scientific Titer Plate Shaker: 900 rpm

Continue to Resuspension and hybridization preparation, Page 2

Figure 17 Stage 3: Resuspension, #1 of 2

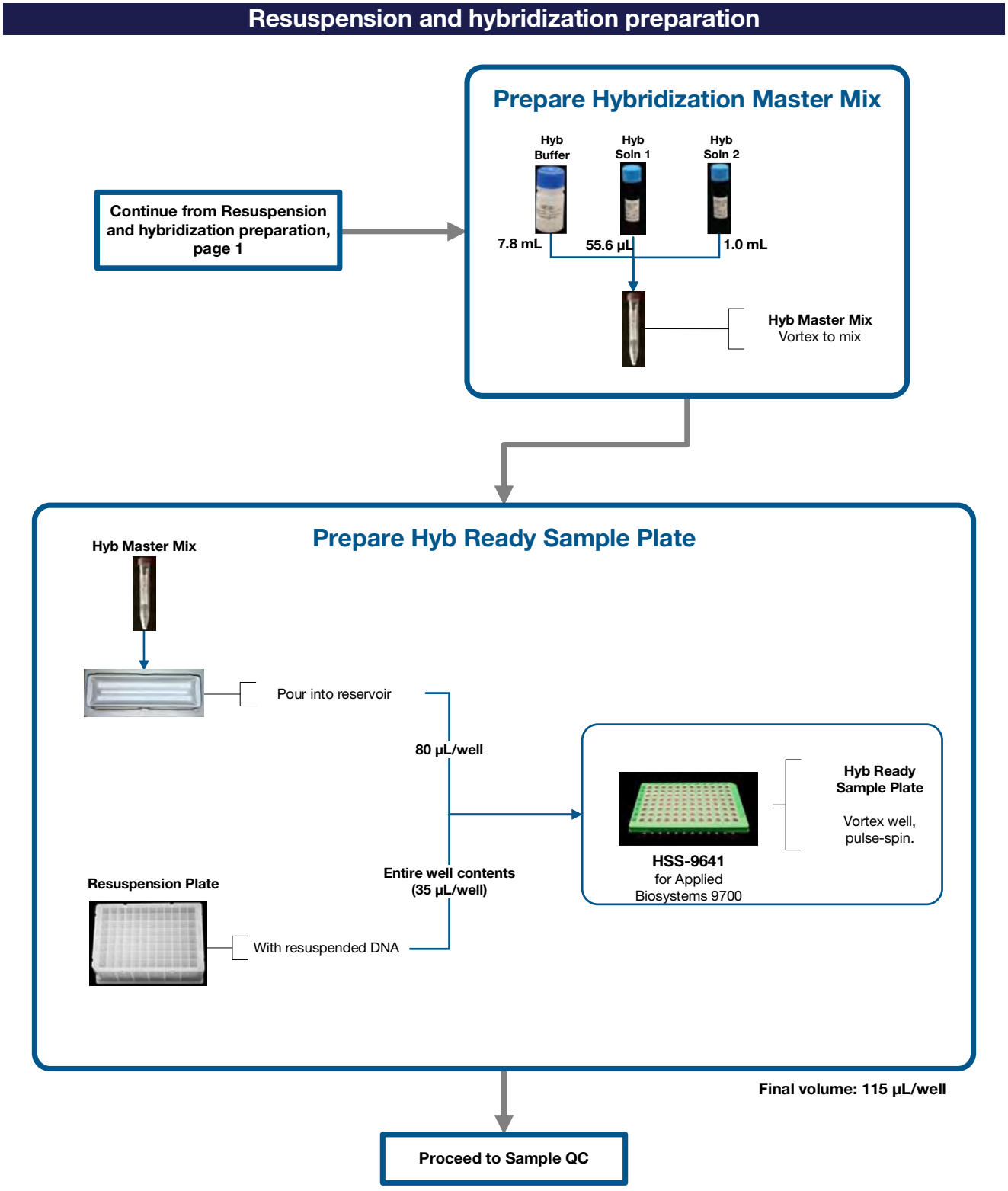


Figure 18 Stage 3: Resuspension, #2 of 2

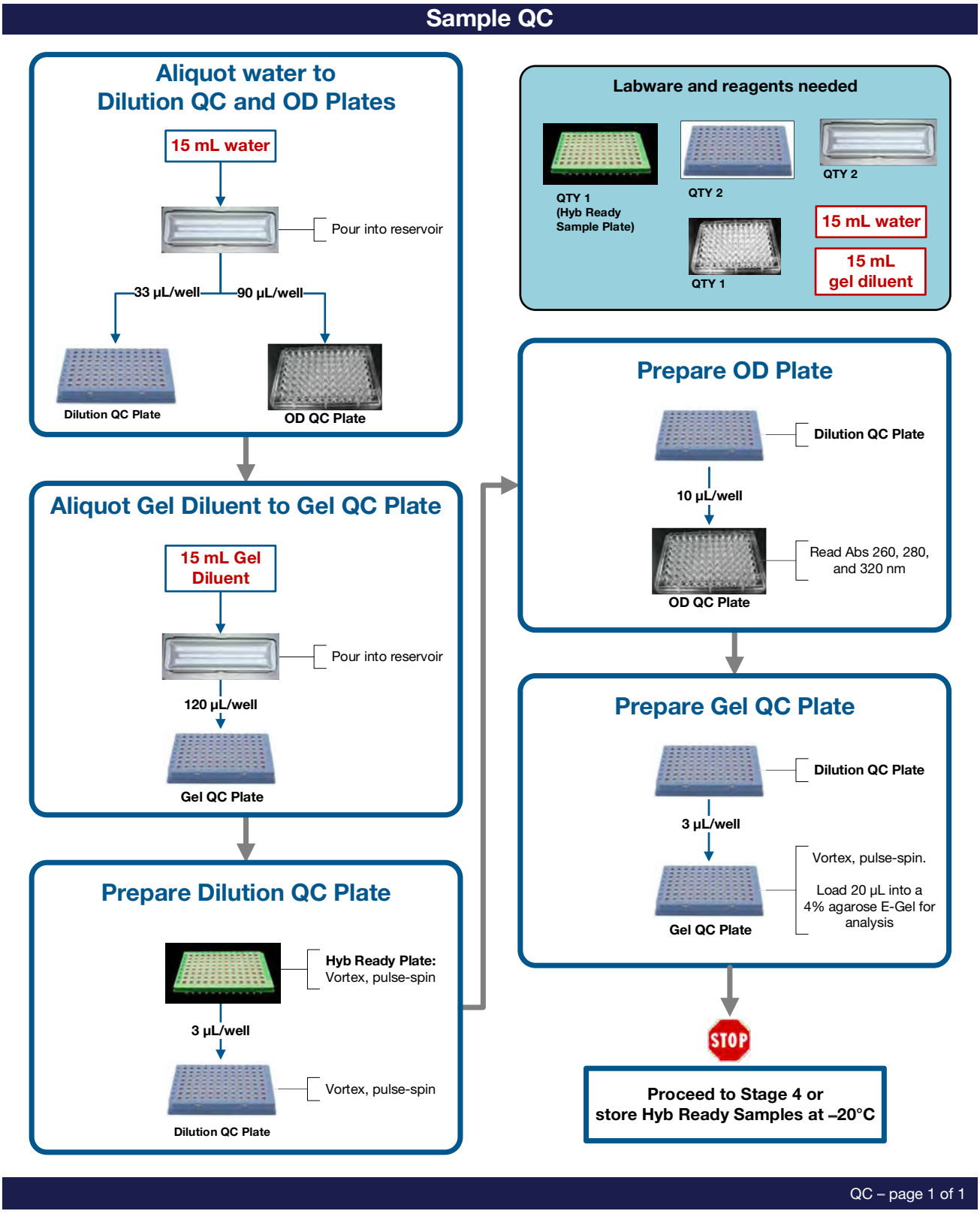


Figure 19 Stage 3: QC

Stage 4: Denaturation and hybridization

You proceed to Stage 4 in one of two ways:

- Directly from Stage 3 without interruption.
- With hybridization 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 81.

"2: Prepare Hyb Ready Samples stored at –20°C" on page 81.

"3: Prepare the GeneTitan™ MC Instrument" on page 81.

"4: Denature the Hyb Ready Sample Plate" on page 82.

"5: Prepare hybridization tray and load into GeneTitan™ MC Instrument" on page 83.

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 81.
- If you are proceeding directly from the end of Stage 3 on page 75, go to "3: Prepare the GeneTitan™ MC Instrument" on page 81.

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 hybridization time

Required input from previous stage

- Hyb Ready Sample Plate

Equipment, consumables, and reagents required

The following thermal cyclers are recommended:

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

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 25).

Table 25 Equipment required for Stage 4: Denaturation and hybridization

Quantity	Equipment
1	GeneTitan MC Instrument
1	Rainin P200 12-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 25).
1	96 well metal chamber warmed in a 48°C oven[1] 

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

Table 26 Consumables required for Stage 4: Denaturation and hybridization

Quantity	Consumable	Cat. No.
1	• One PharmacoScan™ 96F Array Plate in a protective base	Cat. No. 903160
1	• Hybridization tray[1]	Part No. 500867

[1] 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 (Cat. No. 901606). The hybridization tray is available in the Axiom™ GeneTitan™ Consumables Kit.

Table 27 Reagents required from the PharmacoScan Reagent Kit 96 Reactions

Reagent	Module	Part No.
Axiom Wash Buffer A (both bottles; 1 L)	Module 3, Room temperature	901446
Axiom Wash Buffer B		901447
Axiom Water		901578

1: 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 GeneTitan Array Plate Registration file as described in Appendix C, "Register Samples in GeneChip™ Command Console™" on page 189.

   ⚠ **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

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. Ensure 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 laboratory tissue.
   c. Use a fresh seal and tightly reseal the plate.
3. Vortex the Hyb Ready Plate briefly, then spin at 1,000 rpm for 30 seconds.
4. Place the Hyb Ready Plate at room temperature.

3: Prepare the GeneTitan™ MC Instrument

Before you denature your hybridization 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 113 and Appendix C, "Register Samples in GeneChip™ Command Console™" on page 189.

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

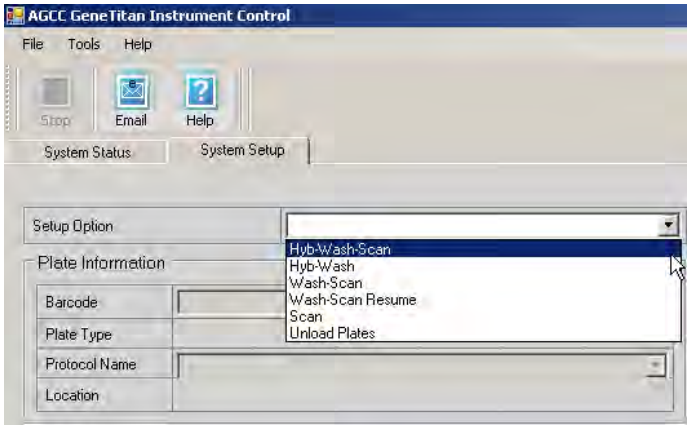
1. Prepare the reagents from Module 3 as described in the table below:

Reagent	Temperature[1]	Treatment
Axiom Wash Buffer A (Part No. 901446)	Room temperature	Invert 2-3X for mixing before filling GT bottle
Axiom Wash Buffer B (Part No. 901447)	Room temperature	Invert 2-3X for mixing before filling GT bottle
Axiom Water (Part No. 901578)	Room temperature	None

[1] Indicates the temperature the reagent is held at immediately after removal from module.

2. Launch GCC and select GCC GeneTitan Control.

3. Upload your sample registration file now.
   If you do not upload your samples before scanning the array plate barcode, the software assigns names to your sample.
4. Select the **System Setup** tab.



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. Ensure 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 25).

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.

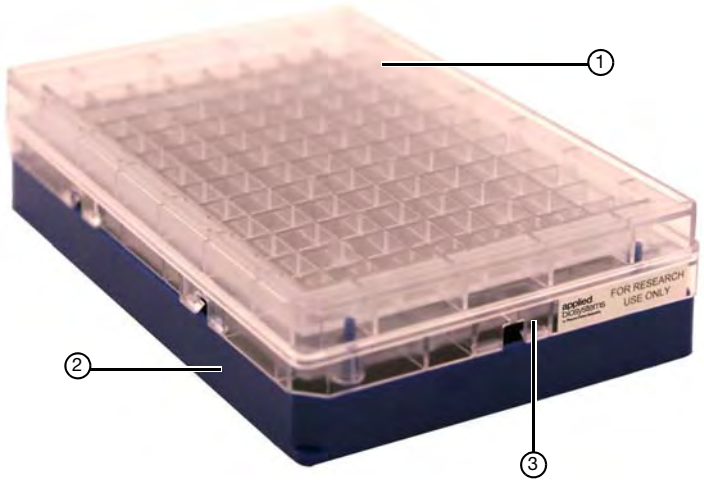
1. After the **PharmacoScan Denature** protocol has completed, remove the Hyb Ready Plate from the thermal cycler and place into a 96-well metal chamber that has been pre-warmed in an oven at 48°C.
2. Move the metal chamber containing the denatured Hyb Ready Plate to a fume hood.
3. Remove seal from Hyb Ready Plate and discard.
4. Remove the hybridization tray (from Axiom GeneTitan Consumables Kit) from packaging.
5. Label the hybridization tray. See the important note below and Figure 9 on page 39 for more information.

   IMPORTANT! It is critical that you write only on the proper location of the hybridization tray (on the edge in front of wells A1 and B1) as illustrated in Figure 9 on page 39. 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 hybridization trays with stain trays.

6. Place the hybridization tray under the fume hood.
7. Using a P200 12-channel pipette set at **105 µL**, slowly transfer the denatured samples in the Hyb Ready Plate into the corresponding wells of the hybridization 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 hybridization 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 hybridization tray wells. Sample distribution across the wells occurs when the array plate is stacked together with the hybridization tray by the GeneTitan MC Instrument.
8. Load the array plate and hybridization tray into the GeneTitan MC Instrument (see "Load a PharmacoScan™ array plate and hybridization tray onto the GeneTitan™ MC Instrument" on page 118).

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.



① Clear tray shipping cover (to be discarded)
② Array plate protective base
③ Array plate

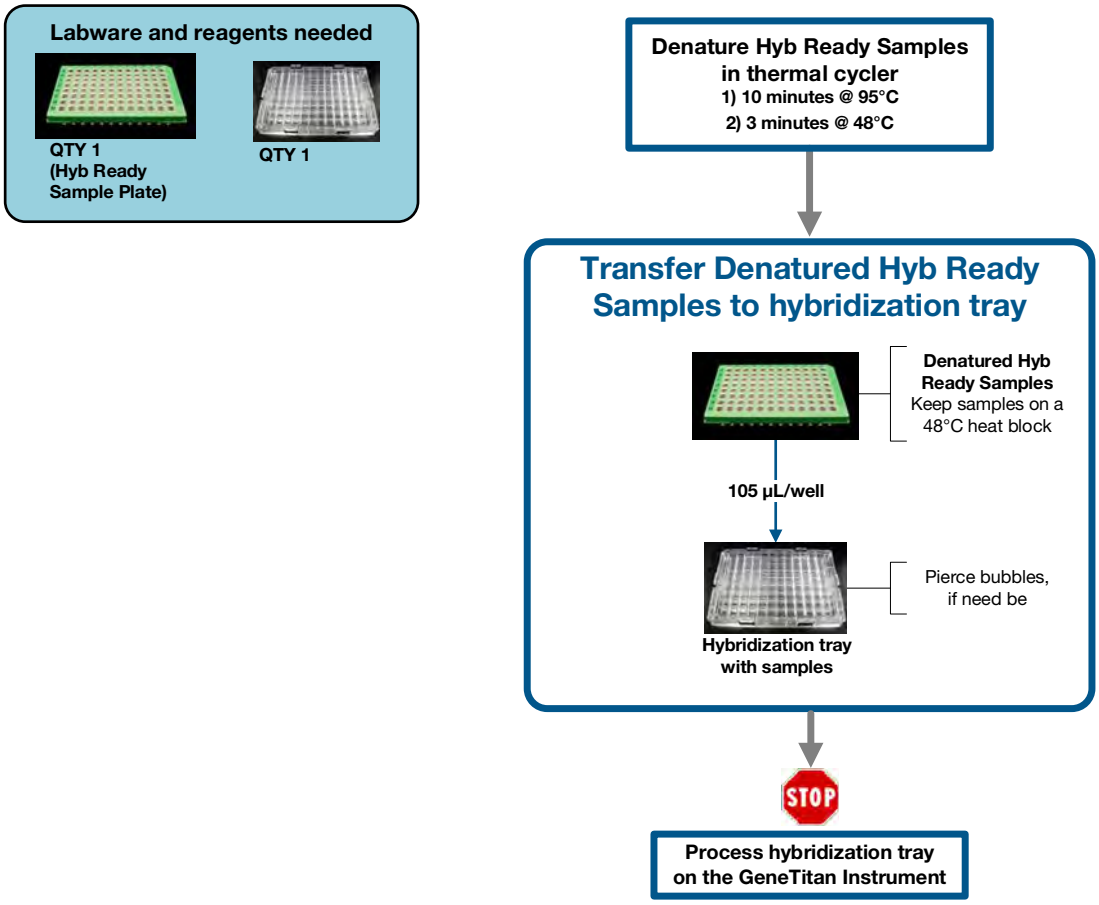
Load the hybridization tray on the right side without any covering. The hybridization tray should not have any bubbles.

IMPORTANT! After the GeneTitan MC Instrument has stacked the array plate and hybridization tray, the instrument extends 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 ensure 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/hybridization tray sandwich while inspecting the bottom for bubbles. See Step 3 on page 122 for detailed instructions.

Hybridization continues 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 86.

Denaturation and hybridization



Denaturation and hybridization – page 1 of 1

Figure 20 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. After prepared, these plates must be loaded onto the instrument as soon as possible and should not be stored.

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

1: Prepare for GeneTitan™ reagent preparation on page 89

2: Prepare the Stain, Ligation, and Stabilization Master Mixes on page 92

3: Aliquot master mixes and Hold Buffer into trays on page 94

The reagents and trays required are as follows:

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 28 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	Kimwipe laboratory tissues
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—12-channel: • P200
1	Vortexer

Table 29 Consumables required for Stage 5: Manually preparing ligation, staining, and stabilization reagent trays for the GeneTitan™ MC Instrument

Quantity	Consumable	Cat. No.
As required	Aluminum foil (optional)	
1 kit includes: 1 5 6	GeneTitan Consumables Kit • Scan Tray • Stain Tray • Covers for trays	Cat. No. 901606 Part No. 501006 Part No. 501025 Part No. 202757
 1 1	Pipette, serological 5 x 1/10 mL 10 x 1/10 mL	 VWR Cat. No. 89130-896 VWR Cat. No. 89130-898
As required for pipettes listed in Table 28	Pipette tips	
5	Matrix™ 25 mL Reagent Reservoir	Cat. No. 8093-11
4	15 mL conical tube	

Reagents required

Table 30 PharmacoScan reagents required for stain and ligation stage

Reagent	Module
Axiom Ligate Buffer	Module 4-1, –20°C Part No. 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 Part No. 901276
Axiom Probe Mix 2[1]	
Axiom Wash A	
Axiom Stain 1-A[1]	
Axiom Stain 1-B[1]	
Axiom Stain 2-A[1]	
Axiom Stain 2-B[1]	
Axiom Stabilize Diluent	
Axiom Water	
Axiom Hold Buffer[1]	

[1] 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 minutes to thaw on the benchtop at room temperature.

Table 31 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 4-1 –20°C	Axiom Ligate Buffer			✓ - for 30 minutes
	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 minutes
	Axiom Probe Mix 2[1]		✓	
	Axiom Wash A			✓ - for 30 minutes
	Axiom Stain 1-A[1]		✓	
	Axiom Stain 1-B[1]		✓	
	Axiom Stain 2-A[1]		✓	
	Axiom Stain 2-B[1]		✓	
	Axiom Stabilize Diluent		✓	
	Axiom Water			✓
	Axiom Hold Buffer			✓

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

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 okay and does not adversely impact assay performance. Follow the instructions below to resuspend any precipitate before use.

Prepare the Axiom Wash A

1. Vortex the bottle for 30 seconds.
2. Place on the benchtop at room temperature for 30 minutes
3. Examine the reagent for precipitate (look into the top of the bottle).
4. If precipitate is still present, vortex again for 30 seconds.

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.

Prepare the Axiom Ligate Buffer

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

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

1. Place on the benchtop at room temperature for 30 minutes 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 seconds.
4. Re-examine the buffer for precipitate.
5. If precipitate is still present, warm the bottle with your hands and vortex again for 30 seconds.
6. If precipitate is still present after hand warming proceed with the protocol below.
7. Leave the Axiom Ligate Buffer on the benchtop until ready to use.

Prepare the remaining reagents

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 two to three 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 the table below.

Conical tube	Number of tubes	Tube designation	Contents	Place tube:
50 mL	1	*S1*	• Stain 1 Master Mix	On ice
15 mL	1	*S2*	• Stain 2 Master Mix	On ice
15 mL	1	*Stbl*	• Stabilization Master Mix	On ice
15 mL	1	*Lig*	• Ligation Master Mix	On ice

Label the reagent reservoirs

1. Label five Matrix 25 mL Reagent Reservoirs (Cat. No. 8093-11) as indicated in the table below.

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

1. Use appropriate serological and single-channel pipettes to add reagents to the 50 mL tube labeled *S1* in the order shown in the table below. This recipe provides enough for both *S1* reagent trays.

Reagent	per array	Master Mix 96+
To the tube marked *S1*, add:		
• Axiom Wash A	201.6 µL	22.2 mL
• Axiom Stain Buffer	4.2 µL	463 µL
• Axiom Stain 1-A	2.1 µL	231 µL
• Axiom Stain 1-B	2.1 µL	231 µL
Total	**210 µL (105 µL x 2)**	**23.13 mL**

2. Gently invert the tube ten 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

1. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *S2* in the order shown in the table below.

Reagent	per array	Master Mix 96+
To the tube marked *S2*, add:		
• Axiom Wash A	100.8 µL	11.1 mL
• Axiom Stain Buffer	2.1 µL	231 µL
• Axiom Stain 2-A	1.05 µL	115.6 µL
• Axiom Stain 2-B	1.05 µL	115.6 µL
Total	**105 µL**	**11.56 mL**

2. Gently invert the *S2 MM* tube ten 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

1. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Stbl* in the order shown in the table below.

Reagent	per array	Master Mix 96+
To the tube marked *Stbl*, add:		
• Axiom Water	93.19 µL	10.3 mL
• Axiom Stabilize Diluent	10.50 µL	1.16 mL
• Axiom Stabilize Soln	1.31 µL	144.8 µL
Total	**105 µL**	**11.61 mL**

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

Prepare Ligation Master Mix

The Ligation Master Mix is prepared in two stages.

Begin preparing the Ligation Master Mix (Stage 1)

1. Place the 15 mL conical tube marked *Lig* on ice.
2. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Lig* in the order shown in the table below.

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

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

Finish preparing the Ligation Master Mix (Stage 2)

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 the table below.

   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.

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

3. Gently invert ten 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 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 location of the stain/reagent trays (on the edge in front of wells A1 to F1) as illustrated in Figure 10 on page 40. 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 covers onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and covers.

IMPORTANT! Do not confuse hybridization 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 192 for the recommended technique.

About aliquoting reagents to trays

IMPORTANT! Always aliquot reagents to the bottom of the tray. Avoid touching the sides or the top of the wells with the pipette tips. Droplets close to or on the top of the well dividers can cause the cover 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.

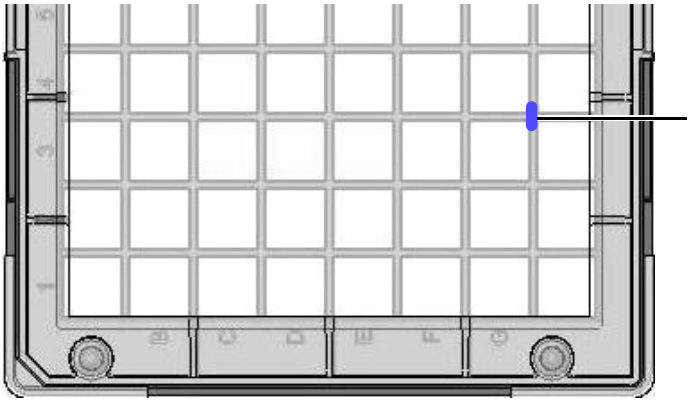
IMPORTANT! Remember to deionize the stain trays and the covers before aliquoting master-mixes.

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 spreads evenly when the array plate is inserted into the reagent tray during processing with the GeneTitan MC Instrument.

Aliquot Stain 1 Master Mix

1. Pour the S1 Master Mix into the reagent reservoir marked *S1*, placed on the bench top at room temperature.
2. Load a P200 12-channel pipette with 12 new pipette tips and aliquot **105 µL per well** to both S1 trays. Dispense to the first stop only to avoid creating bubbles.

   You do not need to change pipette tips between additions of the Stain 1 Master Mix.
3. If:
   - bubbles are present, puncture them with a pipette tip.
   - droplets of liquid splashed onto the well dividers, place a Kimwipe laboratory tissue on top of the tray to blot and remove. (Figure 21).



Example of a droplet of liquid that has splashed onto the well divider of a stain tray during reagent aliquoting.

Ensure no droplets of liquid are on top of the wells dividers. Blot with a Kimwipe laboratory tissue to remove.

Figure 21 Well dividers in trays (partial tray view).

4. Place covers on the *S1* trays. Orient cover correctly on the tray with the notched corners together (Figure 22).

IMPORTANT! Leaving liquid on the top of the dividers can cause excessive evaporation or can form a seal that restricts the removal of the GeneTitan tray cover.

Figure 22 Placing cover on Stain Tray. Notched corners should face front.

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

Aliquot Stain 2 Master Mix

1. Pour the Stain 2 Master Mix into the reagent reservoir marked *S2*, placed on the bench top at room temperature.
2. Load a P200 12-channel pipette with 12 new pipette tips and aliquot **105 µL per well** to 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 laboratory tissue 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 22).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Aliquot Stabilization Master Mix

1. Pour the Stabilization Master Mix into the reagent reservoir marked *Stbl*, placed on the bench top at room temperature.
2. Load a P200 12-channel pipette with 12 new pipette tips and aliquot **105 µL per well** to 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 laboratory tissue.
4. Place a cover on the tray. Orient cover correctly on the tray with the notched corners together (Figure 22).

Aliquot Ligation Master Mix

1. Pour the Ligation Master Mix into the reagent reservoir marked *Lig*, placed on the bench top at room temperature.
2. Load a P200 12-channel pipette with 12 new pipette tips and aliquot **105 µL per well** to 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 laboratory tissue 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 22).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Aliquot 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 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 (Part No. 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 192. Place the cover as shown in Figure 24 on page 99 to prevent dust or static from accumulating on the bottom of the cover.

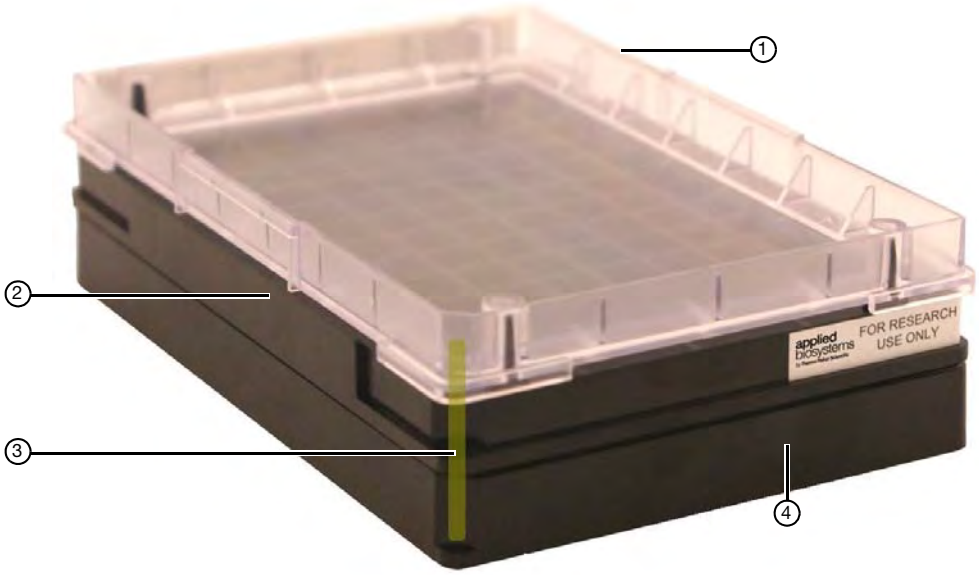
   - Use a 12-channel P200 pipette with new pipette tips to aliquot **150 µL to EACH of the 96 wells** of the 96 Plate Scan Tray. Dispense to the first stop and avoid touching the bottom of the tray.
   - You do not need to change pipette tips between additions of the Hold Buffer.

5. If droplets of liquid splashed onto the well dividers, place a Kimwipe laboratory tissue 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 23).

IMPORTANT! The scan tray has an open-bottom design, so it is 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 131 for instructions on loading the reagent trays.



① Barcoded scan tray cover, Part No. 202757
② GeneTitan scan tray, Part No. 501006 or Part No. 500860
③ Notched corners of cover, tray, and base line up
④ Scan tray protective base

Figure 23 Scan tray with the clear cover and protective base

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

Figure 24 Loading the scan tray with Hold Buffer

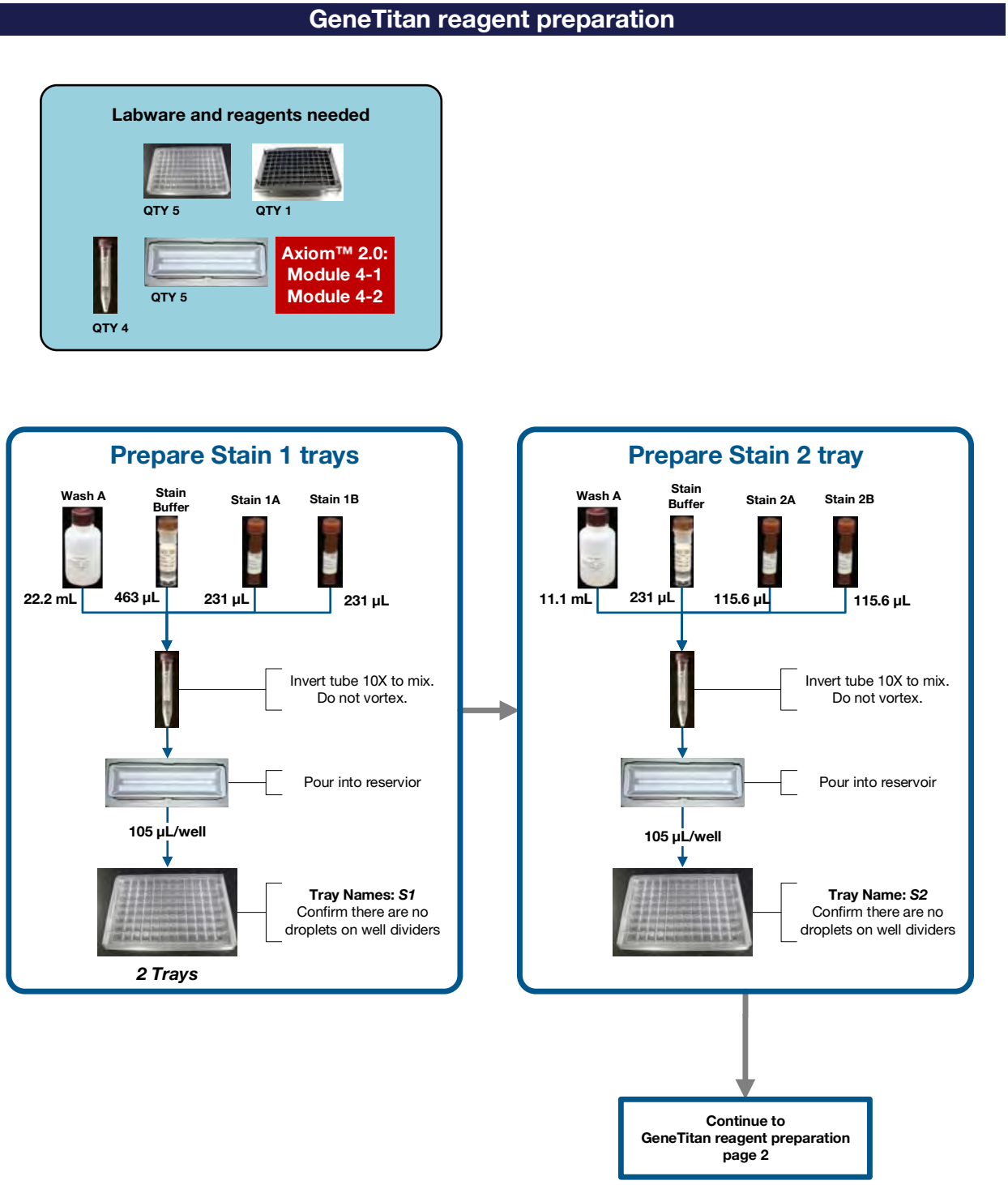


Figure 25 Stage 5: GeneTitan reagent preparation, #1 of 3

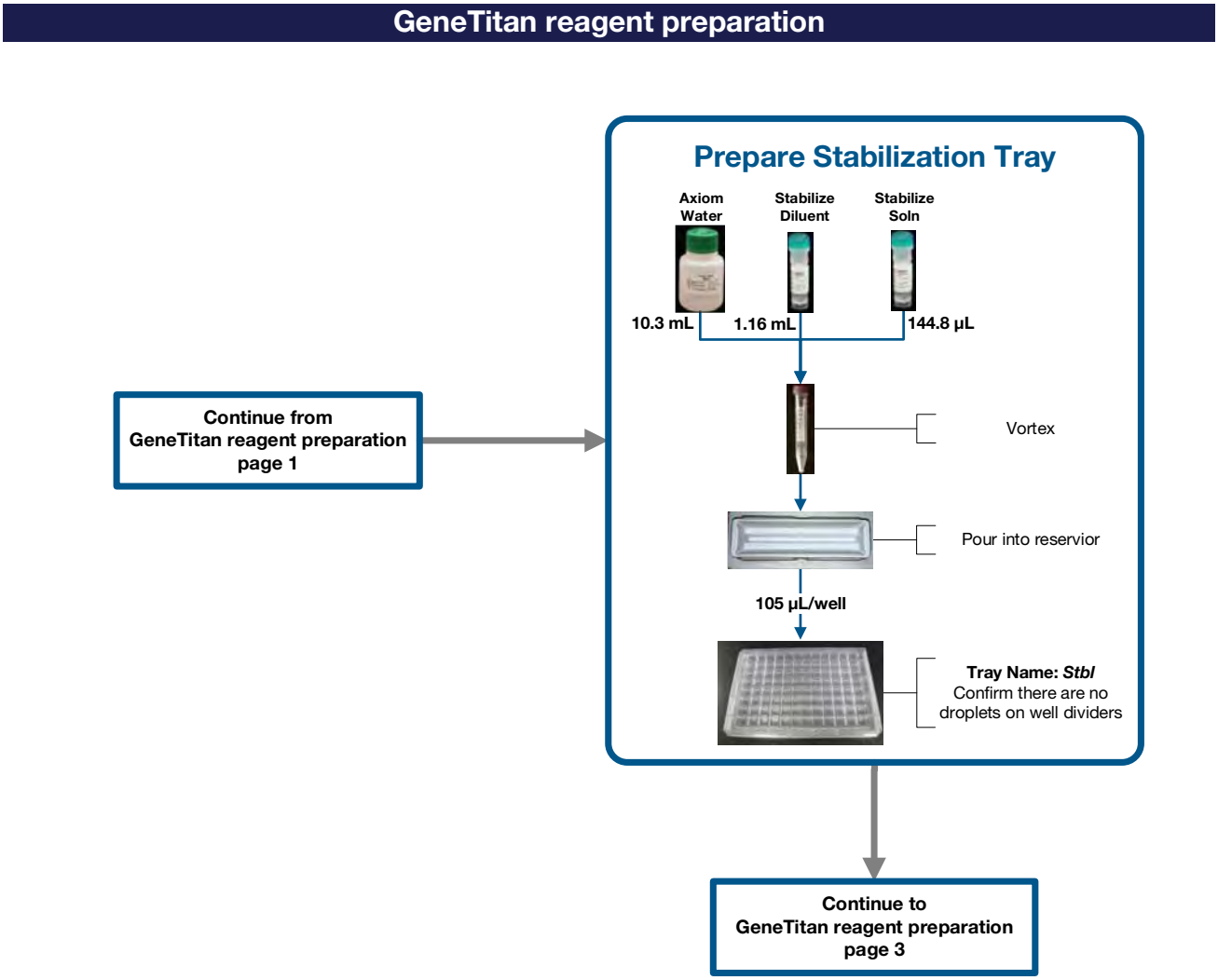


Figure 26 Stage 5: GeneTitan reagent preparation, #2 of 3

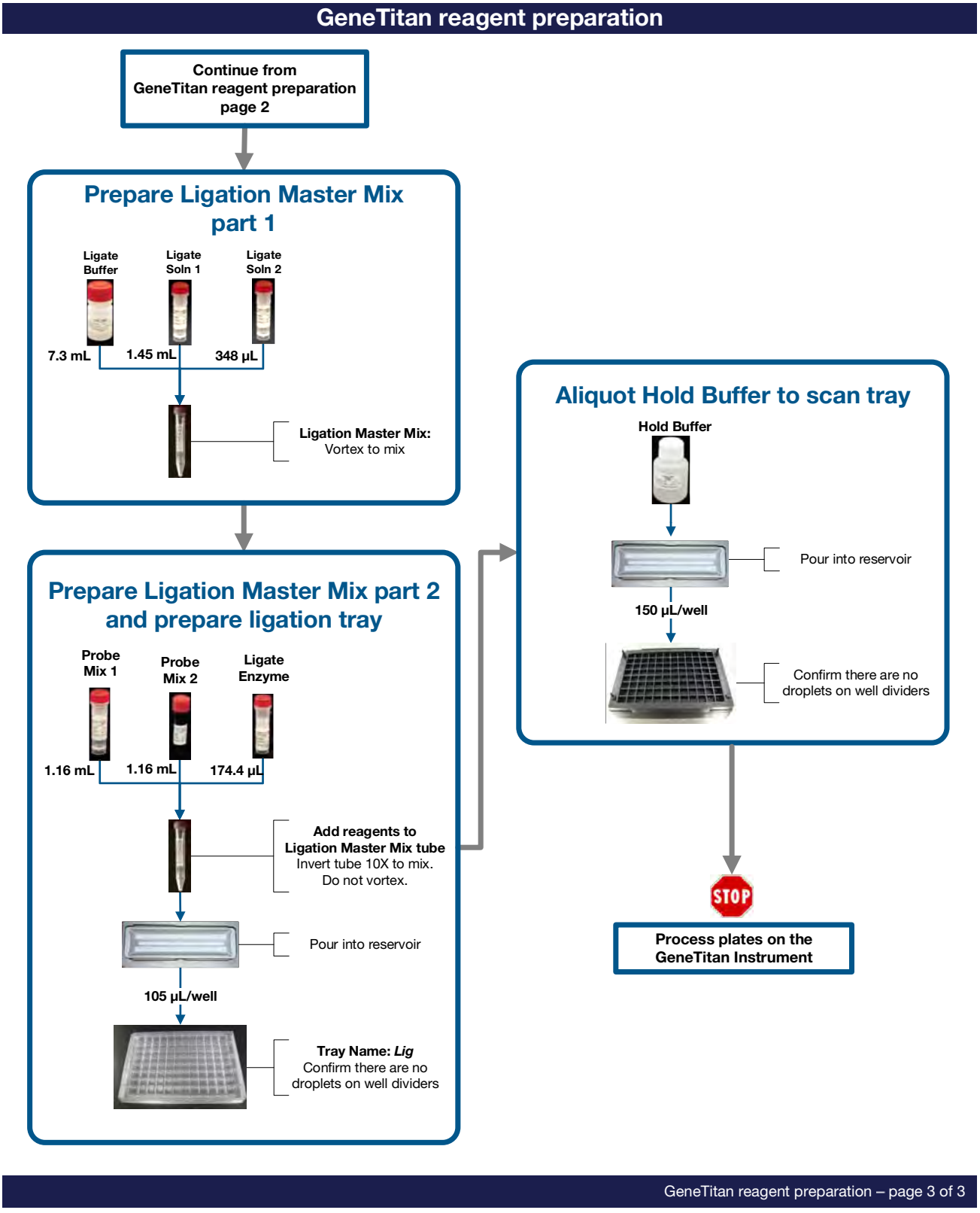


Figure 27 Stage 5: GeneTitan reagent preparation, #3 of 3

5 Array processing with the GeneTitan™ Multi-Channel Instrument

The PharmacoScan™ Assay 96-Array Format Manual Workflow is designed for processing 96 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 42.
- **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 103
  - Proper tray alignment and loading 103
  - Stain trays and covers 105
  - Label GeneTitan™ hybridization and reagent trays 106
  - Load trays onto the GeneTitan™ MC Instrument 133
- Stage 1: Create and upload GeneTitan Array Plate Registration file 112
- Stage 2: Hybridization 113
- Stage 3: Ligate, wash, stain and scan 131

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 28 on page 104 and Figure 29 on page 105).

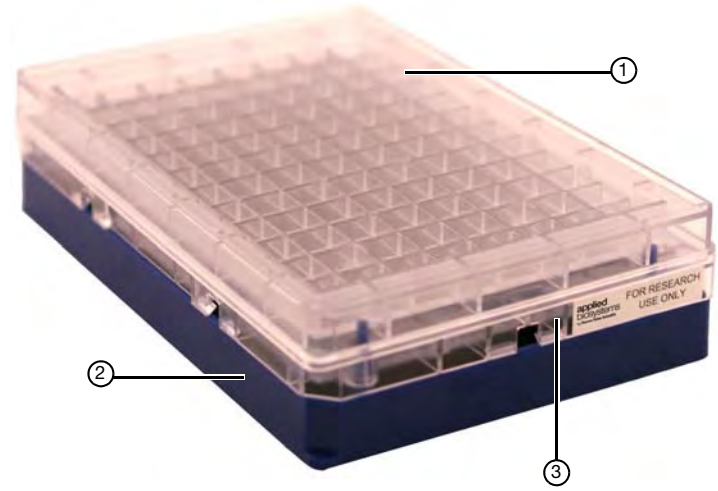
IMPORTANT! When running a multi-plate workflow, you must pay careful attention to the software prompts instructing which side of the drawer to place or remove a plate/tray.

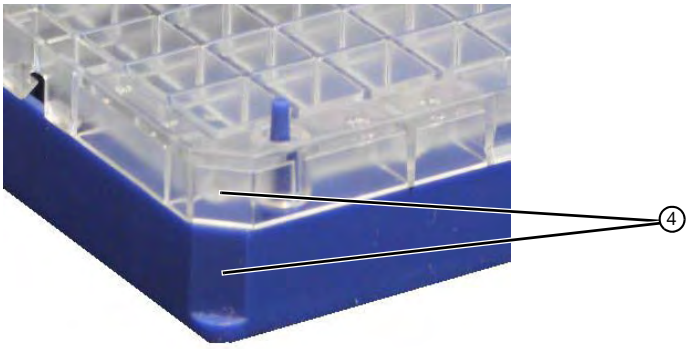
Note: Mark the notched corner of each plate, cover and tray with permanent marker to help ensure proper alignment and loading onto the GeneTitan MC Instrument.

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

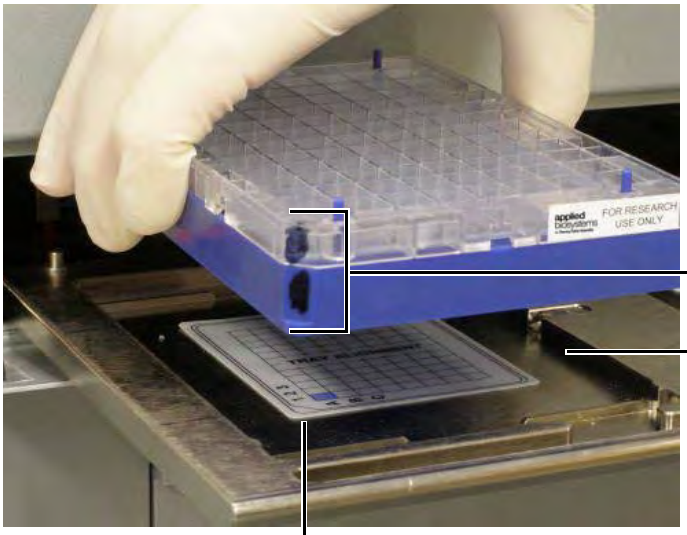
Note: The instrument control software displays a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. See Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 196 for the message displayed to the user and the procedure for replacing the filters.

IMPORTANT! Remove the plastic protective shipping tray cover.



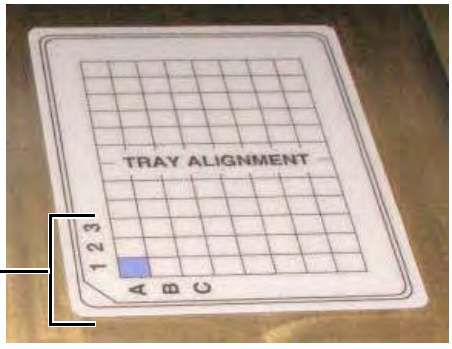


① Clear tray shipping cover (to be discarded)
② Array plate protective base
③ Array plate
④ Notched corner of array plate aligned with notched corner of blue base.



⑤ **Tip:** Mark the notched corner of each plate, cover and tray with permanent marker to help ensure proper alignment and loading.

⑥ Plates and trays must be seated in this rectangular recess.



⑦ The notched corner of all plates, bases, and covers and must be seated in this corner of the drawer per the Tray Alignment guide.

Figure 28 Proper alignment and loading of plates, covers and trays in the GeneTitan™ MC Instrument

① Array plate with protective blue base

② Hybridization tray

Figure 29 Array plate with protective blue base and the hybridization 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. 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.

Correct placement of cover on stain tray.

Notched corners of tray and cover should be aligned and facing front

Incorrect placement of cover on stain tray.

Figure 30 Placement of covers on trays

Label GeneTitan™ hybridization and reagent trays

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan MC Instrument, you 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 hybridization 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 covers onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and covers.

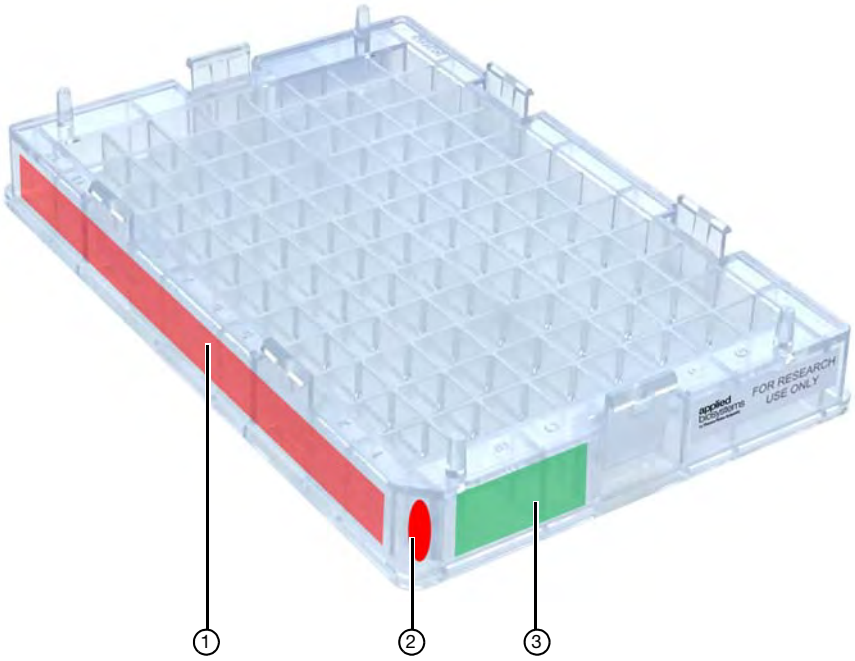
Proper labeling for hybridization trays and reagent trays is described in:

- "Labeling for hybridization trays", below
- "Labeling for stain trays" on page 107

IMPORTANT! Do not confuse hybridization trays with stain trays.

Labeling for hybridization trays

You can label the hybridization tray on the front part of the **short side of the tray, next to the notch at the left,** as shown in Figure 31. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.



① Do NOT label trays on the long side of the tray.
② Notched corner of the hybridization tray should face the front.
③ Label the hybridization tray in this area.

Figure 31 Correct area to label GeneTitan hybridization trays.

⚠ **CAUTION!** Writing on the wrong side of the hybridization tray, or on the wrong part of the long side, can interfere with the operation of sensors in the GeneTitan MC Instrument.

Labeling for stain trays

You can label the stain trays on the **left side of the front of the tray** as shown in Figure 32. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.

① Do NOT label trays on the long side of the tray.
② Notched corner of the stain tray should face the front.
③ Label the stain tray here.

Figure 32 Labeling GeneTitan Stain Tray (Stain Tray shown with cover)

Email and telephone notifications from the GeneTitan™ MC Instrument

We strongly recommend that you configure the Applied Biosystems GeneChip™ Command Console (GCC) 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. See the GCC user guide for instructions.

The types of notifications available 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.

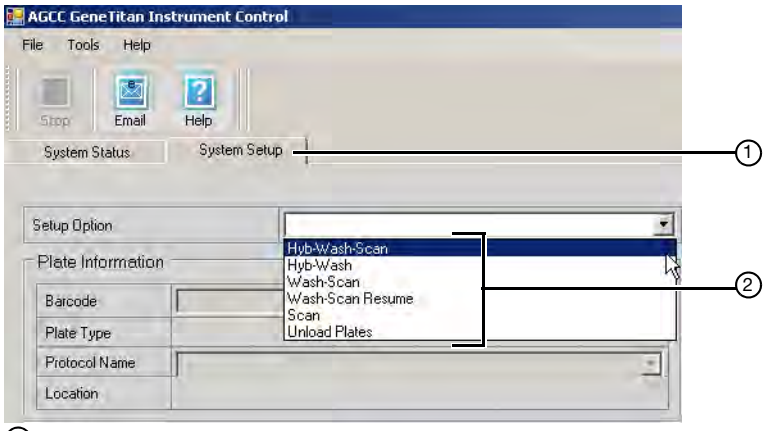
See the *GeneTitan™ MC Instrument User Guide*, Pub. No. 08-0308, or Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 196 of this user guide for details on replacing the lamp.

See the *GeneTitan™ MC Instrument User Guide*, Pub. No. 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 switches the lamp ON and OFF as required. It takes 10 minutes to warm-up the lamp. In idle mode the lamp remains ON for twohours 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. 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 33. A brief description of each option is given below.



① **System Setup** tab

② Setup options

Figure 33 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.

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

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

  Note: The instrument control software displays 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. See Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 196 for the message displayed to the user and the procedure for replacing the filters
- *Scan*: The array plate is moved to the imaging device in the GeneTitan MC Instrument and each array is scanned.
  - Duration for 96 samples = 7.5 hours

Hyb-Wash

If this setup option is selected, array plate processing stops 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 cover is required. Do not invert the plate stack. If inverted, the Hold Buffer spills 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 three days or less.

When ready to scan the array plate:

1. Keeping the plate protected from light, bring the plate to room temperature for ~50 minutes
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.

  Note: It usually takes 25-30 minutes to warm up Wash B if this option is selected.

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.
- If you want to queue a second plate for scanning. Using the **Scan** option allows you to start a second **Scan** workflow while another **Scan** workflow is already running. See "Queue a second plate for scanning" on page 126.

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.

Abort a process

If needed, you can abort the processing of one or more array plates. Instructions and an example are shown below in Figure 34.

If the instrument aborts a process, you can retrieve the array plate and related consumables as described in Figure 34. An instrument-initiated abort can occur:

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

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*.
6. After 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 remains in the "AbortRequest" state until the gripper becomes available.

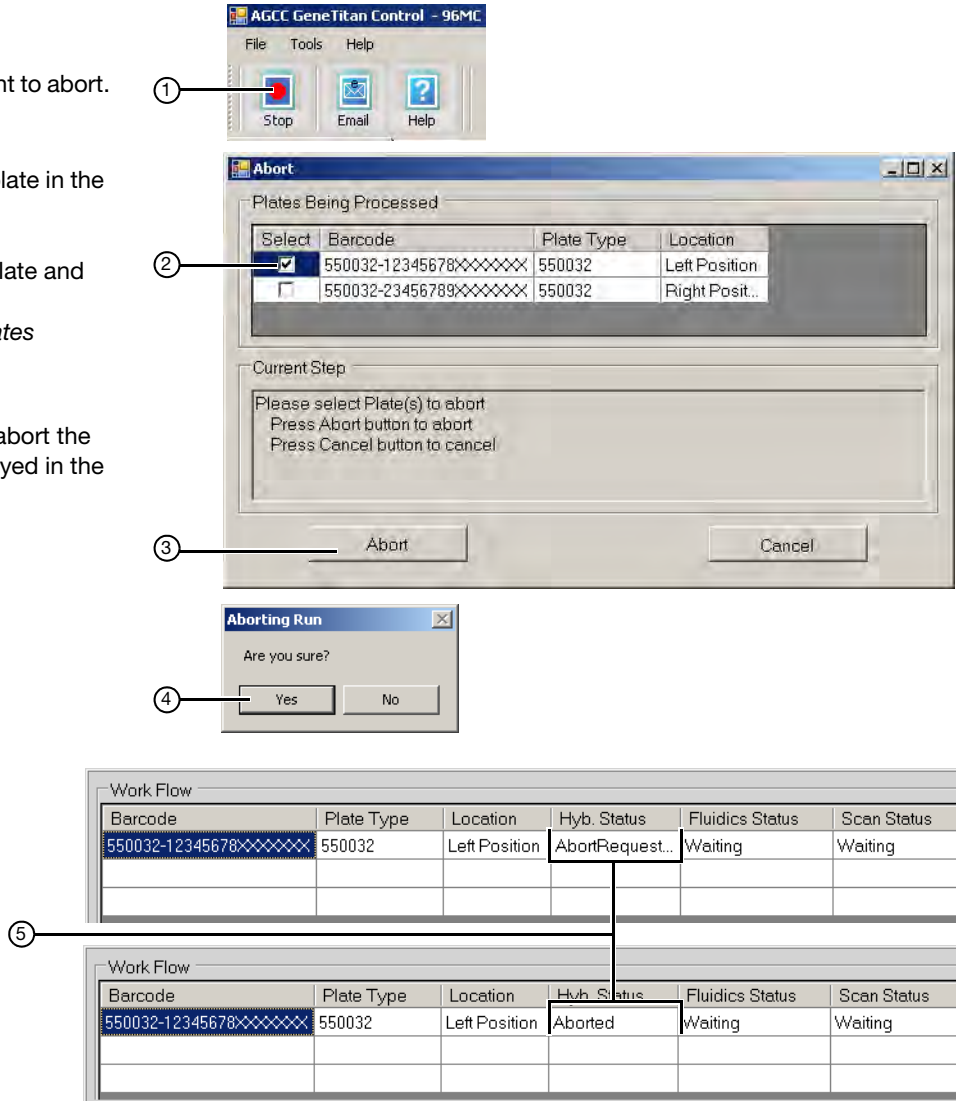


Figure 34 Manually aborting an array plate

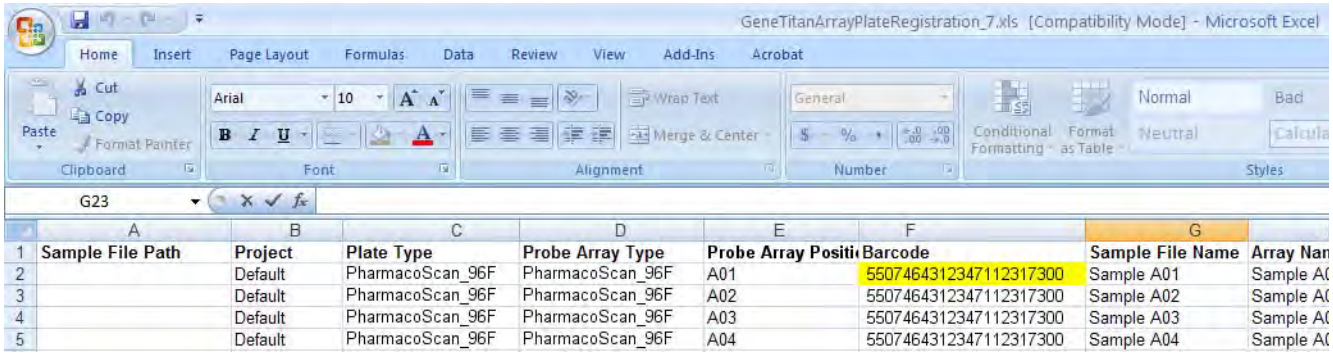
Stage 1: Create and upload GeneTitan Array Plate Registration file

You must create and upload a GeneTitan Array Plate Registration file in the GCC software before you begin "Stage 2: Hybridization" on page 113 (example shown in Figure 35). 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 an array plate registration file, create one now. (See Appendix C, "Register Samples in GeneChip™ Command Console™" on page 189 for detailed instructions.)
2. In GCC, select the array plate format (96 samples) and open a GeneTitan Array Plate 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 important to create and upload a GeneTitan Array Plate Registration file with your sample information prior to starting "Stage 2: Hybridization" on page 113.



	A	B	C	D	E	F	G	
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array Positi	Barcode	Sample File Name	Array Nar
2		Default	PharmacoScan_96F	PharmacoScan_96F	A01	550746431234711231730 0	Sample A01	Sample A(
3		Default	PharmacoScan_96F	PharmacoScan_96F	A02	550746431234711231730 0	Sample A02	Sample A(
4		Default	PharmacoScan_96F	PharmacoScan_96F	A03	550746431234711231730 0	Sample A03	Sample A(
5		Default	PharmacoScan_96F	PharmacoScan_96F	A04	550746431234711231730 0	Sample A04	Sample A(

Figure 35 Example of a GeneTitan Array Plate Registration file for an array plate

Stage 2: Hybridization

Reagents required

Reagents required

Table 32 Reagents required from the PharmacoScan™ Reagent Kit 96 Reactions

Reagent	Module	Part No.
Axiom Wash Buffer A (both bottles; 1 L)	Module 3, Room Temperature	901446
Axiom Wash Buffer B		901447
Axiom Water		901578

- PharmacoScan 96-array plate is required for this step. Prior to inserting this plate into the GeneTitan MC Instrument for hybridization, the array plate should be brought to room temperature as described below:
  a. Warm up the array plate on the benchtop before setting up hybridization on the GeneTitan MC Instrument.
  b. Remove the array plate box from the 4°C refrigerator where it is stored.
  c. Open the box and remove the pouch containing the array plate and protective base.
  d. Leave the array plate in the pouch, unopened but placed on the bench for a minimum of 25 minutes before opening and loading on the GeneTitan MC Instrument to allow the plate to come to room temperature.
  e. At the end of the array warm up time, open the pouch and scan the array plate barcode into the GeneTitan Array Plate Registration file (see "Stage 1: Create and upload GeneTitan Array Plate Registration file" on page 112).
- A hybridization tray containing denatured samples (from Step 8 on page 83 in Chapter 4) is also required for this step. The denatured samples should be transferred to the hybridization tray only after the GeneTitan MC Instrument is ready for loading the hybridization tray in the "Load trays onto the GeneTitan™ MC Instrument" on page 133.

Setup the instrument

1. Launch GCC Launcher and select **GCC GeneTitan Control** (Figure 36).

   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 displays 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. See Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 196 for the message displayed to the user and the procedure for replacing the filters.

IMPORTANT! Do not close the scanner application by right-clicking on it and choosing the "Close" option. This causes 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 "Shut down the GeneTitan™ MC Instrument" on page 141.

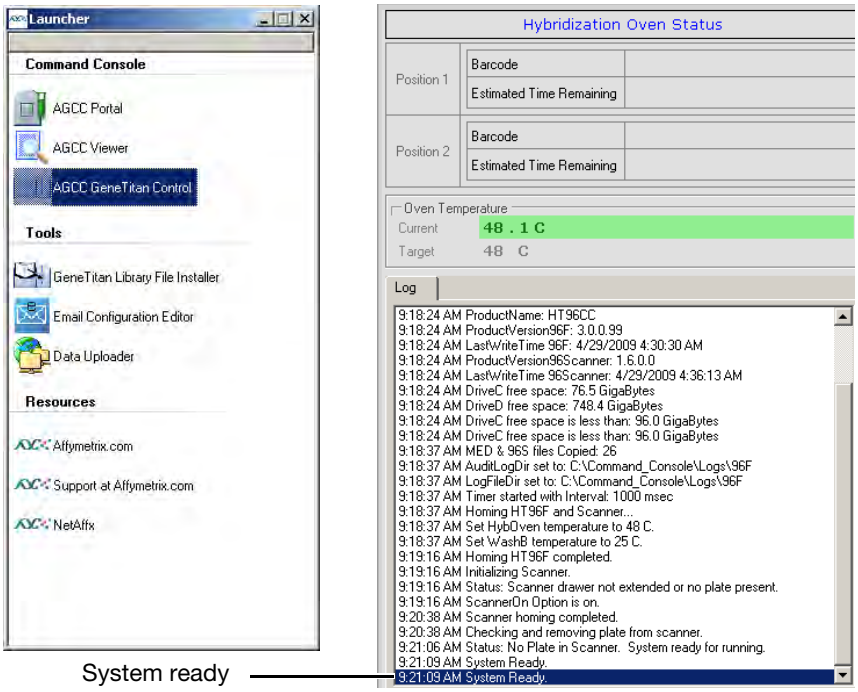


Figure 36 Launch GCC and initialize the GeneTitan MC Instrument.

System Setup tab

Setup Option: The various options you can choose for processing array plates.

Barcode: The array plate barcode. Can be scanned or entered manually.

Protocol Name: The protocol that GeneTitan MC Instrument runs. The list of protocols displayed is based on the first 6 digits of the array plate barcode. Only the protocols that are valid for the type of array plate loaded are displayed.

Workflow Steps: This field displays an overview of the user actions required to process an array plate based on the setup option selected.

Status: This field displays the actions that must be performed to prepare or unload the GeneTitan MC Instrument for the setup option that has been selected.

After each action you are instructed to click the **Next** button or to press the blinking blue *Confirmation* button located on the GeneTitan MC Instrument.

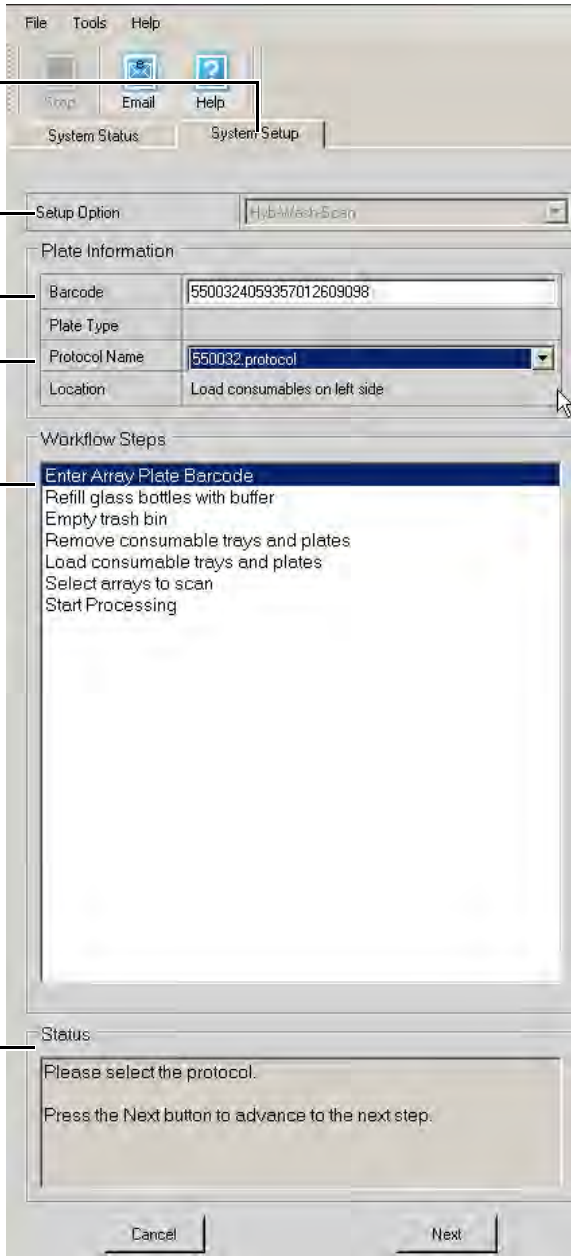


Figure 37 System Setup tab and the information displayed in this pane

2. Select the **System Setup** tab (Figure 37).
3. Configure the software as follows:
   a. Setup Option: **Hyb-Wash-Scan**

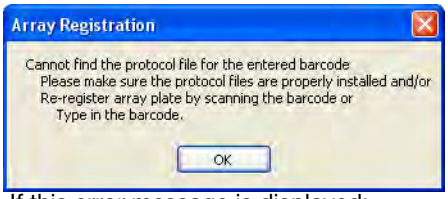
      Other options available are described under "Setup options for array plate processing" on page 108.
   b. Click **Next**.

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 are generated by eight PharmacoScan array plates. The 96F PharmacoScan array plate requires ~80 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 uses to process the plate, and the imaging device parameters required for this type of plate.



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 MC Instrument, install libraries and relaunch the GeneTitan MC Instrument.

Figure 38 Barcode error message

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

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

4. Complete the remaining workflow steps as follows:

a. **Refill bottles with buffer (**Figure 38 on page 117**)**
Fill these bottles:

- Wash A: fill with PharmacoScan Wash Buffer A—keep at 2 L full
- Wash B: fill with PharmacoScan Wash Buffer B—Use all 600 mL of Wash B from the reagent kit per plate. Fill to 1-L 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 96 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 1-L mark only. Wash A keep at 2 L. We strongly recommend refilling these bottles every time you are prompted to do so.

If the volume in any of these bottles becomes too low during a run, a message is displayed (see Chapter 8, "Troubleshooting" on page 169). However, even if you fill the bottle at this time, the instrument might 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 1-L mark (use both bottles from the PharmacoScan Reagent Kit).

b. Empty the waste bottle.

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

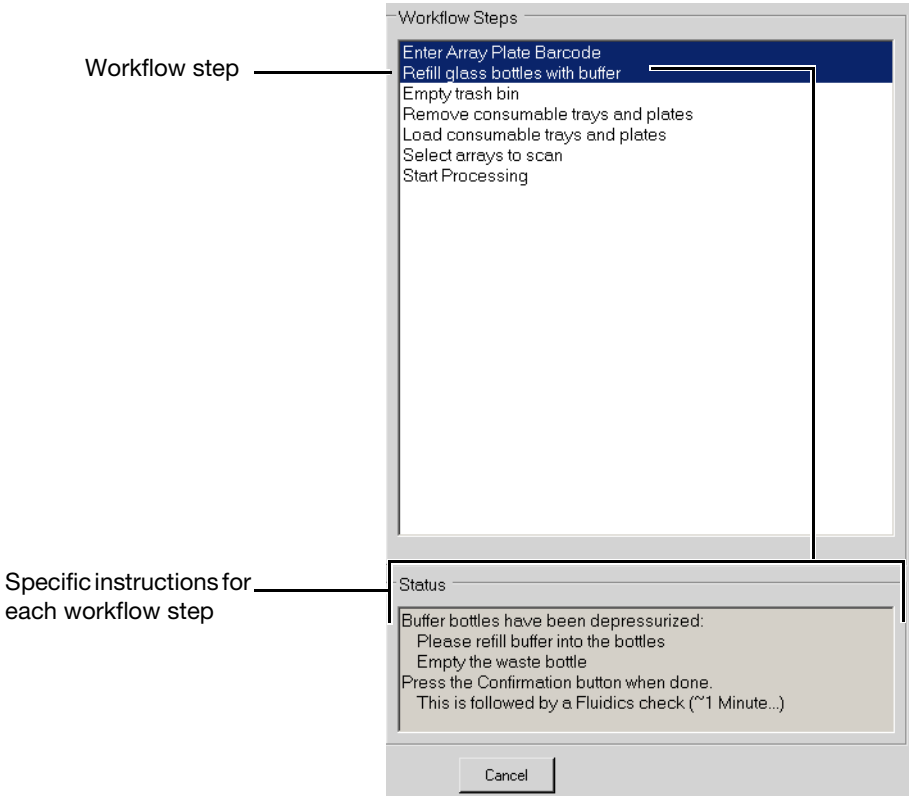


Figure 39 Example of the remaining workflow steps

d. **Empty trash bin**

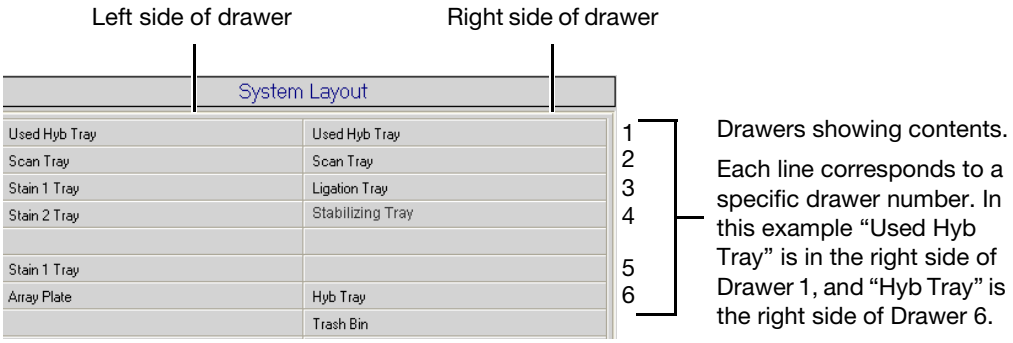
- Open the trash bin and empty.
- If already empty, the trash bin remains locked and the Status pane reads "Trash bin is empty."
- Press the **Confirmation** button to continue.

e. **Remove consumable trays and plates**
   - Remove used trays and plates when drawers open.
   - If no consumables to remove, the Status window reads "Drawers are empty."
   - Press the **Confirmation** button to continue.

f. Continue to "Load a PharmacoScan™ array plate and hybridization tray onto the GeneTitan™ MC Instrument" on page 118.

Load a PharmacoScan™ array plate and hybridization 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.



Note: Earlier versions of the software can show as "Fix Tray" rather than "Stabilizing Tray".

Figure 40 System Layout—location of plates inside the GeneTitan MC Instrument.

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

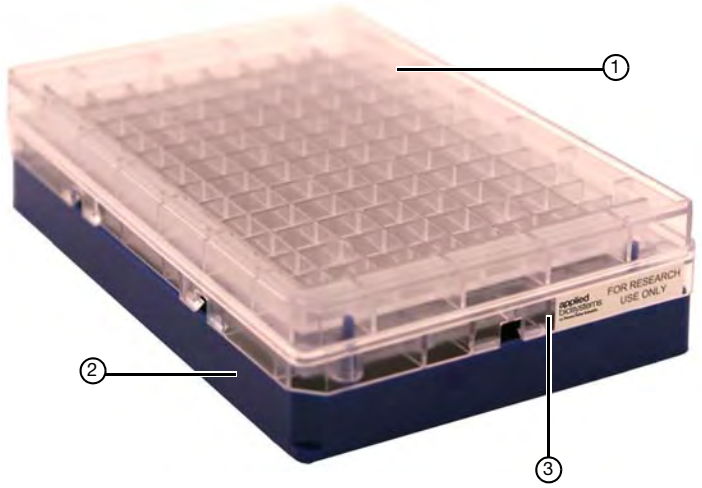
      IMPORTANT! Removing bubbles at this step greatly reduces the chance of bubbles under the arrays when the hybridization tray and the PharmacoScan array plates are clamped. Bubbles under an array can result in black spots on the array image.

   b. Load the hybridization tray **without the cover** on the right side of the drawer (Figure 42 on page 119).

      The array plate must be loaded on its protective blue base, as shown in Figure 42 on page 119 below. The clear plastic cover on top of the array plate SHOULD NOT be loaded in the GeneTitan MC Instrument. See Figure 28 on page 104 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 41).



① Clear tray shipping cover (to be discarded)
② Array plate protective base
③ Array plate

Figure 41 Array plate packaging.

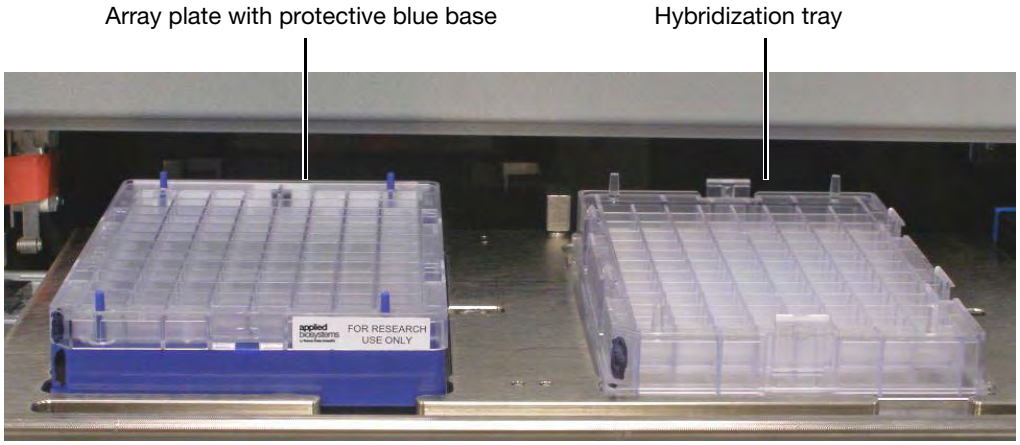


Figure 42 Array plate with protective blue base and the hybridization tray properly loaded into drawer 6.

d. Load the array plate **with the protective blue base** on the left side of the drawer (Figure 42).

> ⚠ **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.
>
> The error message shown might 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 on the GeneTitan MC Instrument.

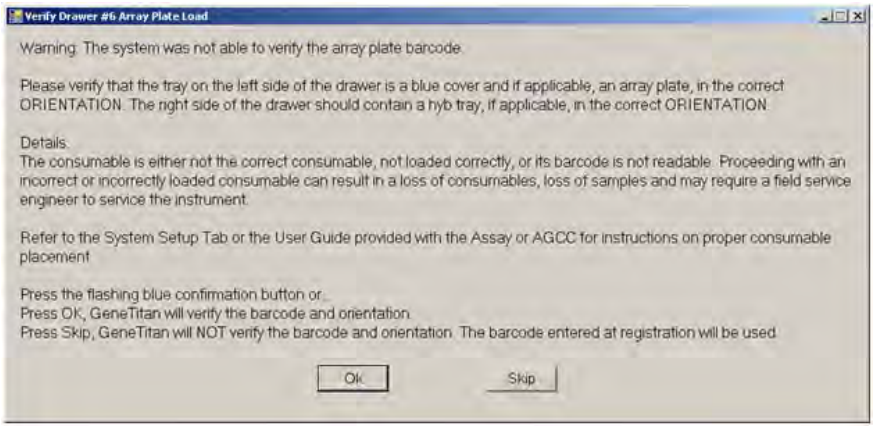
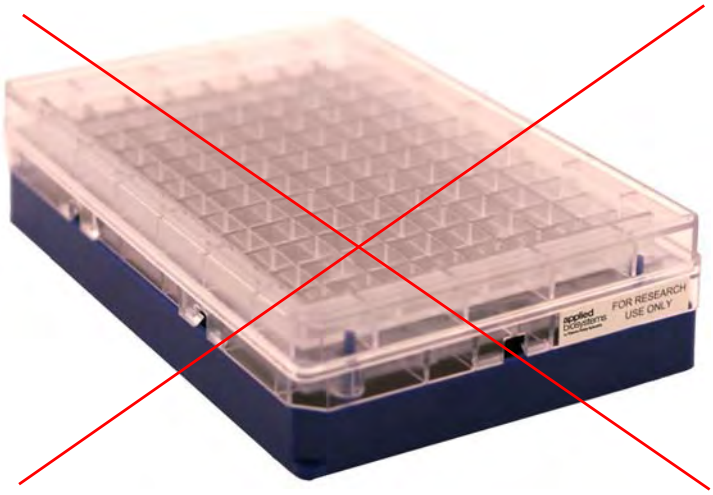


Figure 43 Barcode error message

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 pushes the tray out and prompts (Figure 43) you to load the correct plate with the proper orientation into the instrument (Figure 42).

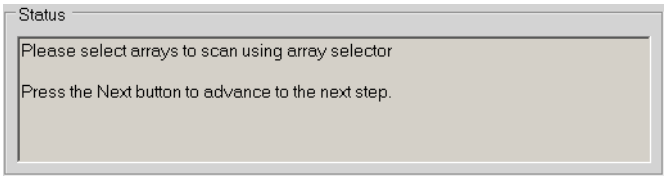
- Click **OK** to retry and check the loading of the array plate; or
- Click **Skip** if the instrument has problems reading the barcode and after verifying that the trays have been placed in the proper orientation.

IMPORTANT! Do not install a three-plate stack of trays. Confirm that you have removed the clear plastic shipping cover as shown in Figure 28 on page 104.

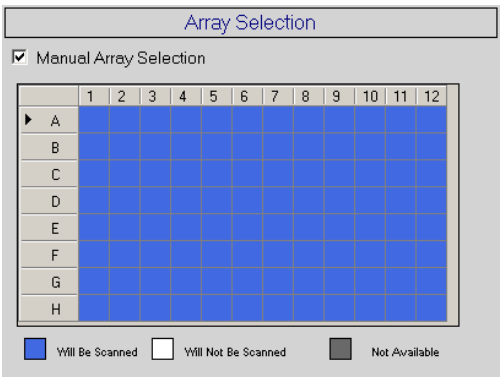


f. Select the arrays to scan (instructions in Figure 44).

By default, all arrays are selected.



Message in Status window.



Default – all arrays are selected

Single array - click one box

Multiple arrays:

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

Group of arrays:

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

Figure 44 Selecting which arrays to scan an array plate

2. Click **Next**, then click **OK** to begin processing the samples (Figure 45).

   The array plate is placed on top of the hybridization tray and clamped (now referred to as the *plate stack*).

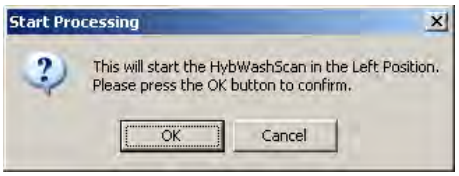


Figure 45 Click OK to start processing the first array plate and hybridization tray.

The software starts the process for clamping the array plate to the hybridization tray. Press **OK** on the dialog shown in Figure 46 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. After clamping is complete the dialog shown in Figure 47 on page 122 is displayed. If you do not press **OK** in Figure 46 the dialog box goes away without intervention and Figure 47 on page 122 is displayed.

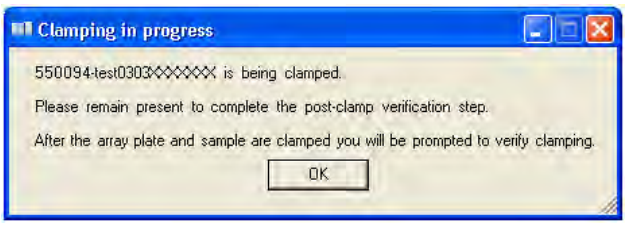
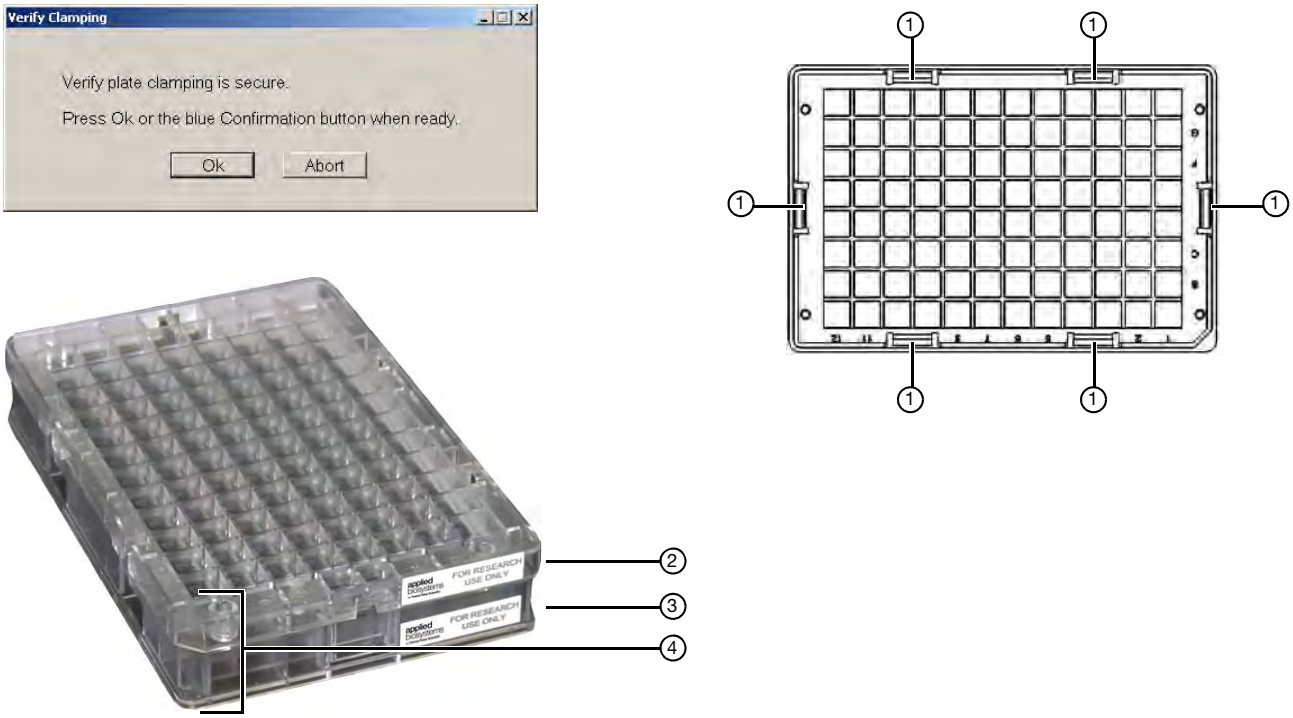


Figure 46 Clamping in progress notification

3. When drawer 6 opens and the prompt in Figure 47 is displayed:



① Clamping points on an PharmacoScan array plate and hybridization tray
② Array Plate
③ Hybridization tray
④ Notched corners

Figure 47 Location of camping points on the array plate and hybridization tray.

a. Remove the plate stack and gently press the two plates together at each clamping point.

b. Listen for a clicking sound which indicates that the plates are now clamped. No clicking sound indicates the plates are already clamped (See Figure 48 for an example of a array plate hybridization tray sandwich).

Figure 48 Array plate hybridization sandwich

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

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

e. Return the plate stack to the drawer, and press the **Confirmation** button to proceed.

   The message in Figure 49 might be displayed again if plate orientation is incorrect or if the hybridization tray barcode cannot be read. Click **OK** to proceed.

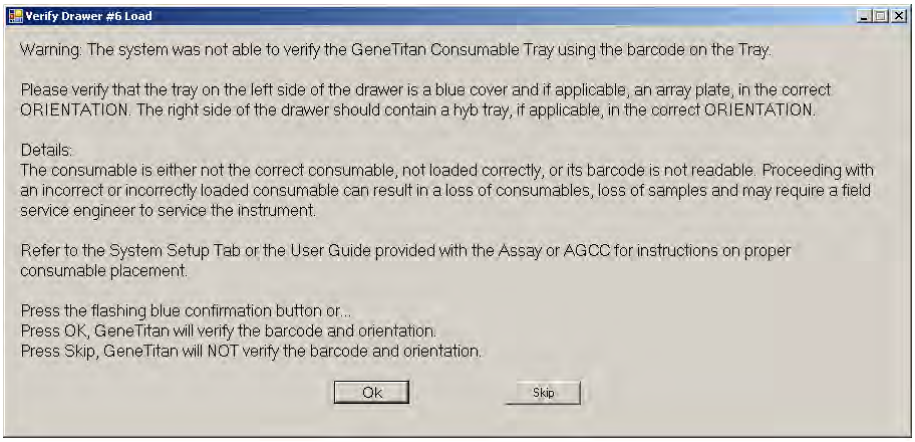


Figure 49 Verification message

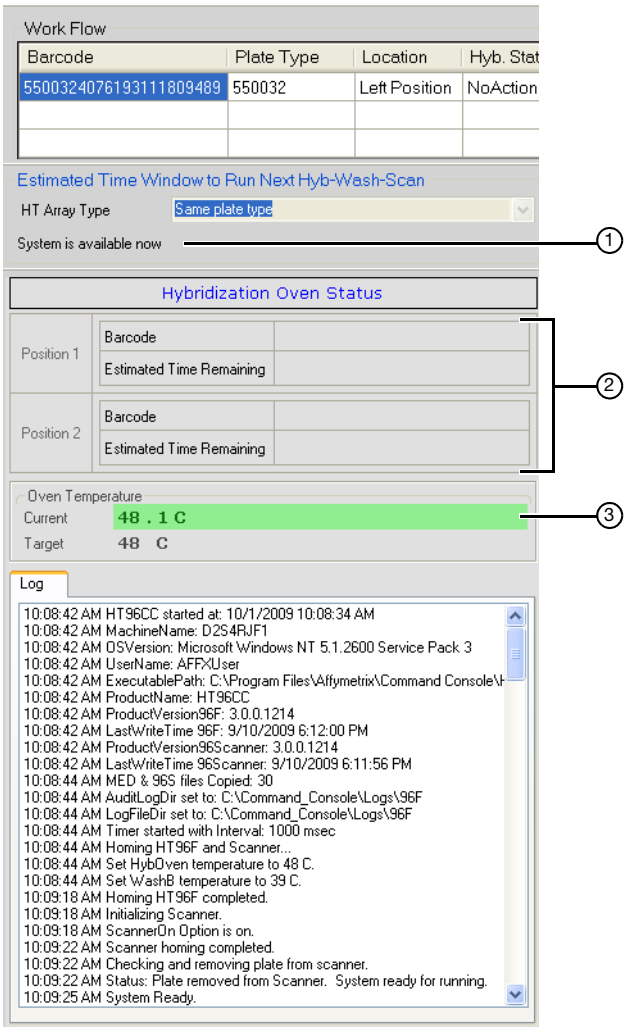
Load a second PharmacoScan™ array plate and hybridization tray onto the GeneTitan™ MC Instrument

When you can load a second array plate and hybridization tray

After processing begins, you have a specific period of time during which you can load another PharmacoScan array plate and hybridization tray. This period of time is displayed above the Hybridization Oven Status pane (Figure 50). You cannot load another hybridization tray before or after this period of time.

IMPORTANT! You must load the next array plate and hybridization tray during the period of time displayed above the Hybridization Oven Status. You cannot load another hybridization tray before or after this period of time.

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 (up to ~7.5 hours).



① This pane displays the period of time during which another array plate and hybridization tray can be loaded.

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

In this example the system is currently available.

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

Position 1 - left side of oven

Position 2 - right side of oven

③ Oven Temperature.

- Green indicates the current oven temperature is within the target temperature range.
- Yellow indicates oven temperature outside of target temperature range.

Figure 50 Loading a second hybridization tray and hybridization oven status information.

1. Select the System Setup tab.

2. Load an PharmacoScan array plate and hybridization 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 hybridization 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 51).

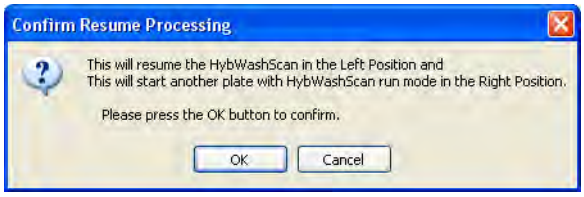
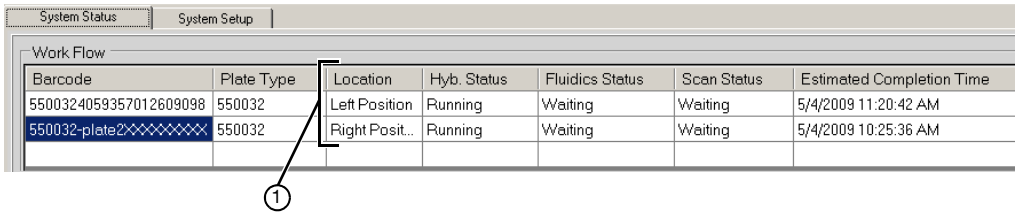


Figure 51 Confirm Resume Processing Prompt

Select the System Status tab to view PharmacoScan array plates status in the WorkFlow window (Figure 52).



① Location: Left and Right positions = the position of the scan tray in drawer 2 (left or right side of the drawer).

Figure 52 Example of the workflow window when two plates are loaded and are in the hybridization oven

Queue a second plate for scanning

Using the **Scan** option in the System Setup tab, you can start a second scan workflow while another scan workflow is already running.

1. Start the first Scan workflow in the GeneTitan Instrument. Wait until the first plate is loaded into the imaging device and starts scanning.
2. Go to the **System Setup** tab and select **Scan** from **Setup Option** drop-down list (Figure 53).
   The Setup Option drop-down list is active only after the first plate begins scanning.

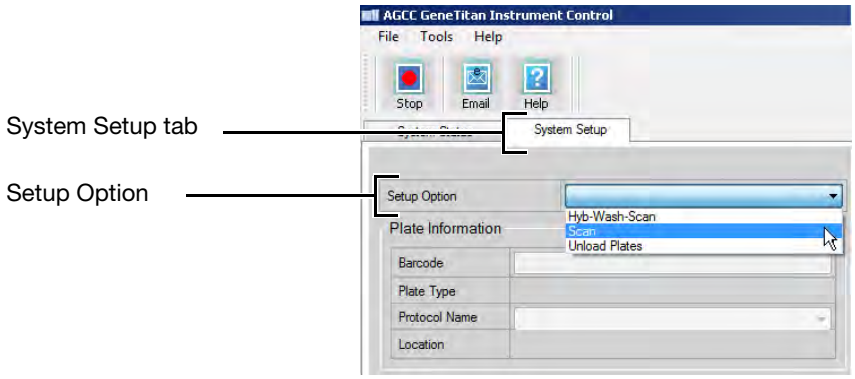


Figure 53 Scan setup option for processing a second array plate

3. Click **Next** in the lower left section of the window under the Status box.
4. Scan or manually enter the array plate barcode, then click **Next**.
5. Following the instructions in the Status box, empty the trash bin if needed and then press the GeneTitan Confirmation button to continue.
6. Place the array plate on top of a scan tray in the correct orientation such that notched corner of the array plate and scan tray are aligned.
7. Load the array plate/scan tray combo in drawer 2 of the GeneTitan Instrument, on the left or right side, as instructed in the Status box.
   - Be sure to load the array plate/scan tray combo in the correct orientation in the drawer. If needed, see Figure 28 on page 104 for further information on the proper alignment and loading of plates, covers and trays in the GeneTitan MC Instrument.
8. Press the GeneTitan Confirmation button when ready.
9. Select the arrays to scan in the Array Selection section in the upper right corner of the window, then click **Next**.
10. A Start Processing confirmation message appears (Figure 54). Click **OK** to continue.

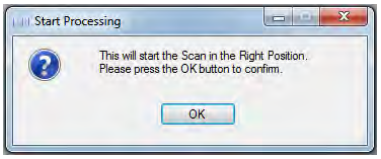


Figure 54 Start scan confirmation message

11. The second queued plate runs after the first scan finishes and the scanner is available.

Status window prompts and actions required

As a part of normal GeneTitan MC Instrument operations you might see the following status prompts. Table 33, Table 34 Table 35 and explains the necessary actions required. Table 36 and Table 37 explain possible barcode error messages and the necessary action required.

Table 33 Refilling buffer bottles and emptying the waste bottle

Status window prompt	Action required	Receptacle – reagent
Buffer bottles have been depressurized. Refill buffer into the bottles. Empty the waste bottle.	• Replenish the fluid in Wash Bottles A and B, and the Rinse bottle[1]. • Empty the Waste Bottle. • Press the *Confirmation* button to continue.	• Wash Bottle A: fill with Axiom Wash Buffer A up to 2 L. • Wash Bottle B: fill with Axiom Wash Buffer B to the 1-L mark. • Rinse: fill with Axiom Water to the 1-L mark. Do not overfill these bottles.

[1] Every time you are prompted to refill the buffer bottles, the system runs a fluidics check (duration ~1 minute).

Table 34 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 can not open it. Continue the process by pressing the blue confirmation button	—

Table 35 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.	—

Table 36 Loading the array plate and hybridization tray; Barcode Error Messages.

Status window prompt	Action required	Reagent – receptacle
Load array plate tray on [Left/Right] side of drawer. Load hybridization tray without cover on [Left/Right] side of drawer.	Load the array plate with the blue base and the hybridization tray in drawer 6. • **IMPORTANT:** The blue base must remain in "left side HTA in" even when empty. • **IMPORTANT:** The trays must be positioned correctly. If the trays are placed incorrectly, the software displays an error dialog box indicating the barcode could not be read. • Press the **Confirmation** button to continue.	• Hybridization tray loaded with denatured samples.
Text version of the error message **WARNING:** The system was not able to verify the array plate barcode. Verify that the tray on the left side of the drawer has a blue protective base and if applicable, an array plate, in the correct ORIENTATION. The right side of the drawer should contain a hybridization 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 can require a field service engineer to service the instrument. • See the **System Setup** tab or the user guide provided with the assay or GCC for instructions on proper consumable placement. • Press the flashing blue confirmation button or... – Press **OK**, the GeneTitan MC Instrument verifies the barcode and orientation. – Press **Skip**, the GeneTitan MC Instrument does NOT verify the barcode and orientation. The barcode entered at registration is used.		These messages are displayed if: • A plate has been loaded improperly. • The bar code is missing or obscured

Table 37 Loading the Scan tray and stain tray; barcode error messages

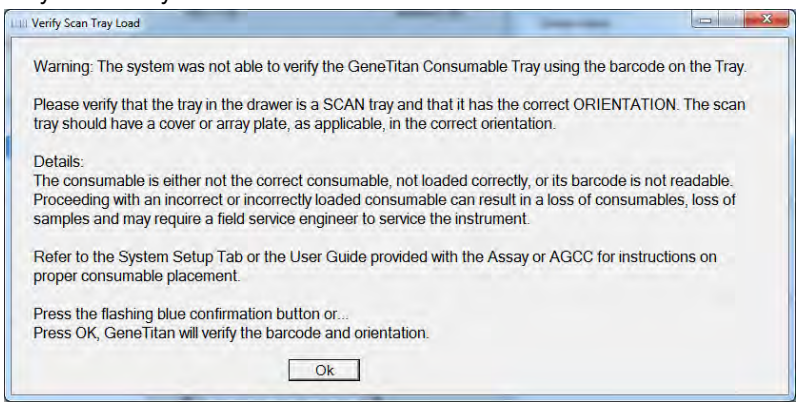
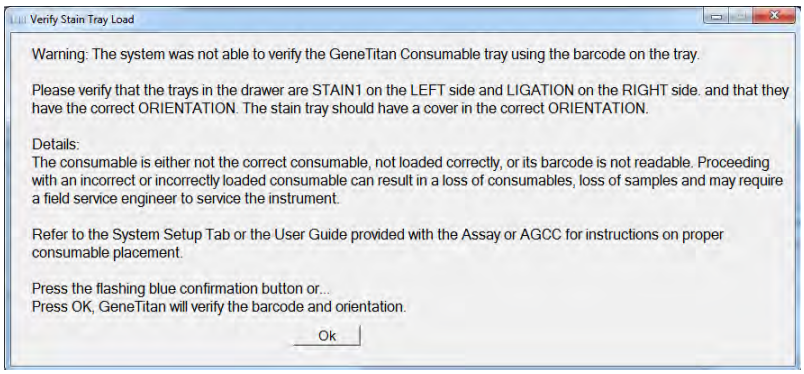
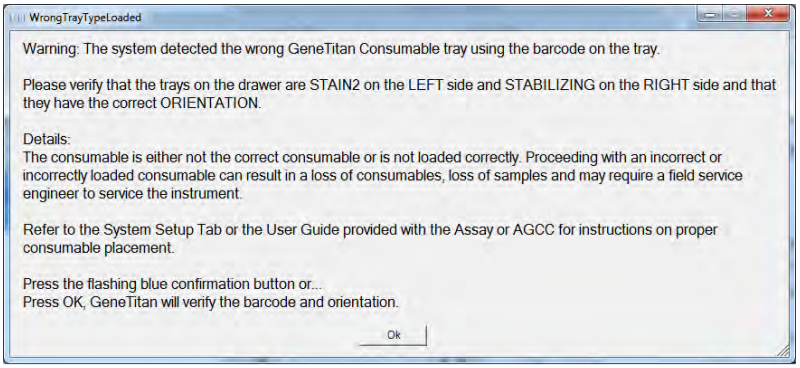
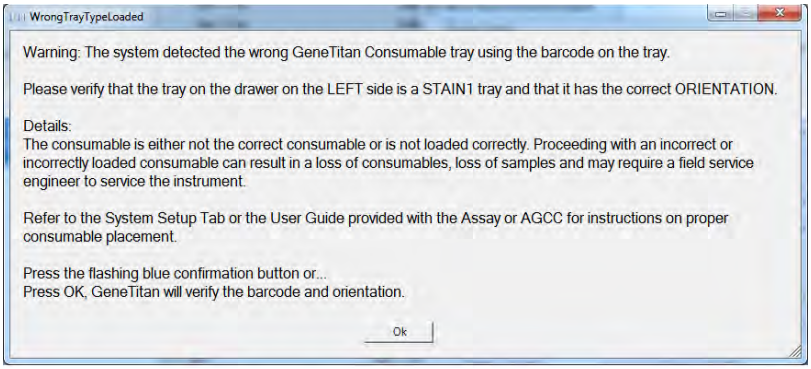
Error message	Action required
Verify Scan Tray Load 	The system was not able to verify that GeneTitan Consumable tray using the barcode on the tray. • Verify that the tray in the drawer is a Scan Tray • Verify that the Scan Tray is placed in the drawer in the correct orientation • The Scan Tray should have a cover or Array Plate, as applicable, in the correct orientation **Note:** When a tray has been correctly loaded but the system is unable to read the barcode, a **Skip** button is present in the error message allowing you the option to proceed.
Wrong Stain Trays - Drawer 3 	The system was not able to verify that GeneTitan Consumable tray using the barcode on the tray. • Verify that the trays in drawer 3 are: – STAIN 1 on the Left, and – LIGATION on the Right • Verify that the trays are placed in the drawer in the correct orientation • Verify that the trays have covers and that the covers are on the trays in the correct orientation **Note:** When a tray has been correctly loaded but the system is unable to read the barcode, a **Skip** button is present in the error message allowing you the option to proceed.

Table 37 Loading the Scan tray and stain tray; barcode error messages

Error message	Action required
Wrong Stain Trays - Drawer 4 	The system detected the wrong GeneTitan Consumable Tray using the barcode on the tray. • Verify that the trays in drawer 4 are: – STAIN 2 on the Left, and – STABILIZING on the Right • Verify that the trays are placed in the drawer in the correct orientation **Note:** When a tray has been correctly loaded but the system is unable to read the barcode, a **Skip** button is present in the error message allowing you the option to proceed.
Wrong Stain Tray - Drawer 5 	The system detected the wrong GeneTitan Consumable Tray using the barcode on the tray. • Verify that the tray in drawer 5 has: – STAIN1 on the Left • Verify that the tray is placed in the drawer in the correct orientation **Note:** When a tray has been correctly loaded but the system is unable to read the barcode, a **Skip** button is present in the error message allowing you the option to proceed.

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 55).

> ⚠ **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.

① Notched corner of the cover is aligned with the notched corner of the scan tray.
② Always leave the scan tray in its protective black base.

Figure 55 The Scan Tray with cover on the black base

Proper installation of the GeneTitan™ tray consumables

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

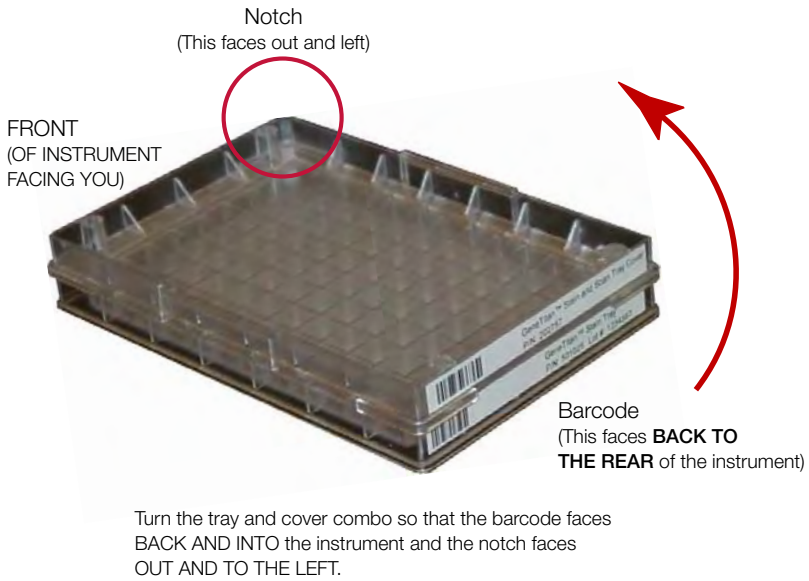


Figure 56 You must rotate and install the trays so that the barcode faces into the instrument.

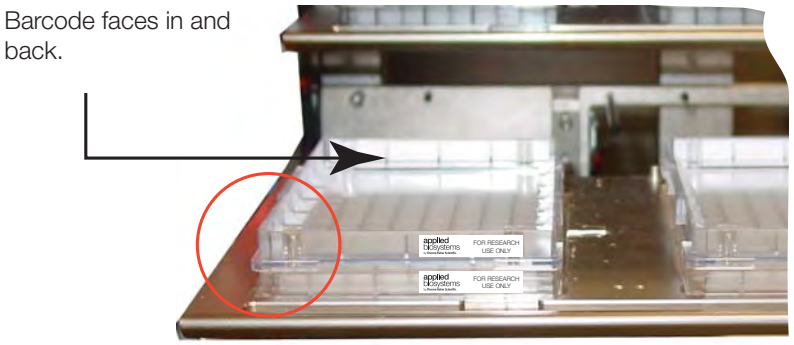


Figure 57 The proper installation of the GeneTitan tray consumables (the image shows the Stain Tray and the Stain Tray cover as an example)

Note: The instrument control software displays 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. See Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 196 for the message displayed to the user and the procedure for replacing the filters.

Load trays onto the GeneTitan™ MC Instrument

When hybridization of an PharmacoScan array plate has finished, a message (Figure 58) alerts you to resume the workflow setup. Press **OK** and the software takes you directly back to the System Setup tab.

Figure 58 The Resume Workflow Setup message

This prompt to continue into reagent load (Figure 58) occurs when the hybridization is complete. "Estimated Time Remaining" displayed under "Hybridization Oven Status" can display a time remaining of 0 to 30 minutes when the prompt occurs.

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

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

  Note: The time estimate displayed on some systems can 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 hybridization 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 primes itself again); Waste bottle—empty if needed.

      Wash bottle A—2 L. Wash Bottle B and Rinse Bottle—fill to 1-L mark only.

   b. Empty the trash bin.
   c. Remove consumable trays and plates as instructed, except for the blue base.

      Leave the blue array plate base in drawer 6 even though the base is empty.
2. Load consumable trays and plates as follows:
   a. Follow the prompts in the Status window (load sequence and prompts in Table 38).
   b. After 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 Kimwipe laboratory tissues, and reload the tray.

CAUTION!

- Orient trays as indicated by the guide inside the drawer (Figure 62 on page 137). Improper orientation can cause the run to fail.
- Remove the protective black base from the scan tray immediately prior to loading Figure 59 on page 135).
- Examine each cover for droplets of liquid after loading. Liquid on the cover can result in capillary phenomenon. As a result, the tray can stick to the cover and be lifted out of place inside the instrument.

Table 38 Sequence for Loading the Trays with Reagents

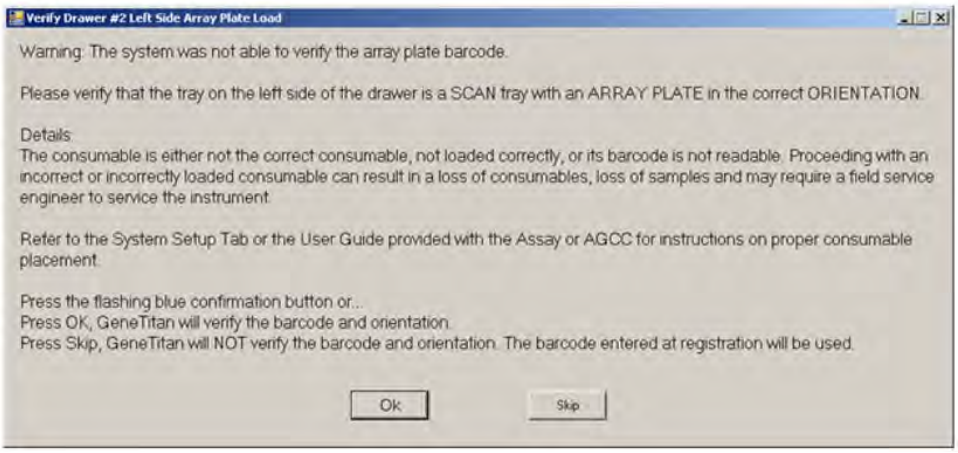
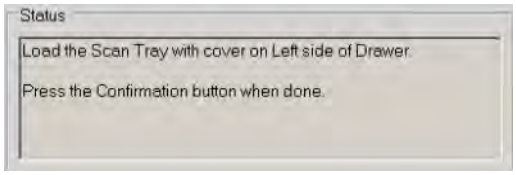
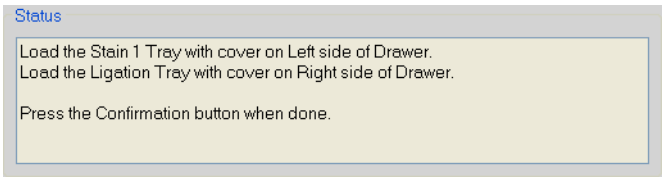
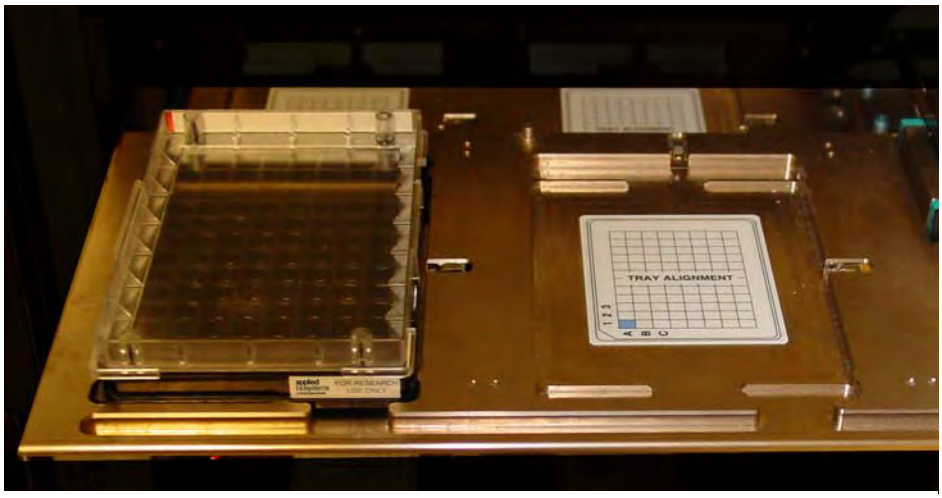
Loading sequence by drawer number	Left	Right
	Note: If the software is unable to verify the barcode on the scan tray and the scan tray cover, the software displays the following error message 	
2	**Scan Tray with cover—do not load the** protective ***black base*** (left side of drawer as indicated in Status window) Figure 59 on page 135 	
3	Stain Tray with Stain 1	Ligation Tray
	Figure 60 on page 136 	

Table 38 Sequence for Loading the Trays with Reagents (Continued)

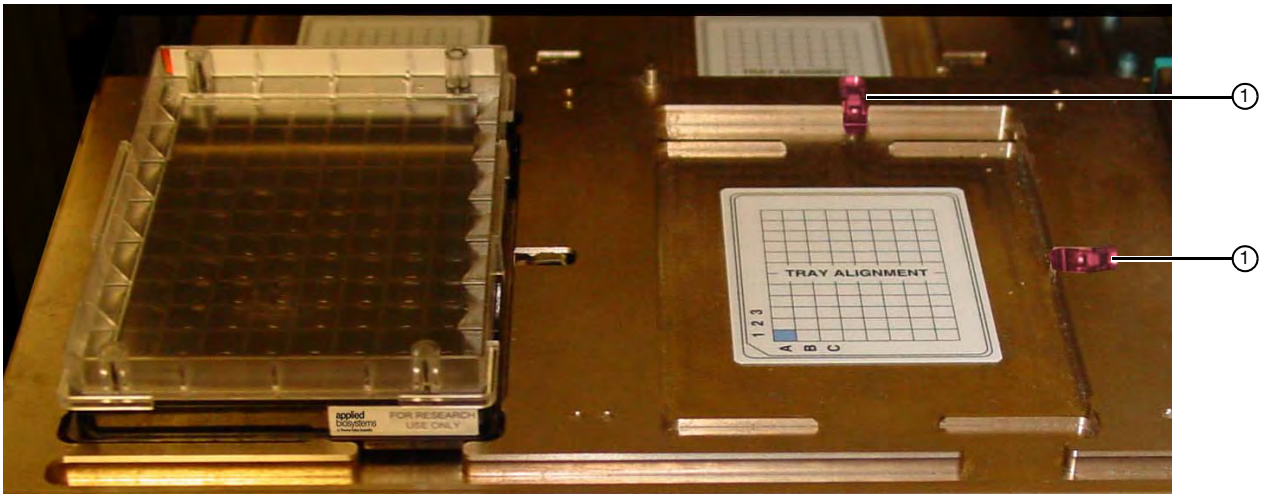
Loading sequence by drawer number	Left	Right
4	Stain Tray with Stain 2	Stbl Tray
	Figure 61 on page 137	
	Status Load the Stain 2 Tray with cover on Left side of Drawer. Load the Fixing Tray with cover on Right side of Drawer. Press the Confirmation button when done.	
5	Stain Tray with Stain 1	Empty
	Status Load the Stain 1 Tray with cover on Left side of Drawer. Press the Confirmation button when done.	



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

Figure 59 Scan Tray loaded in drawer 2.

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



① Tab or “finger” in GeneTitan drawer.

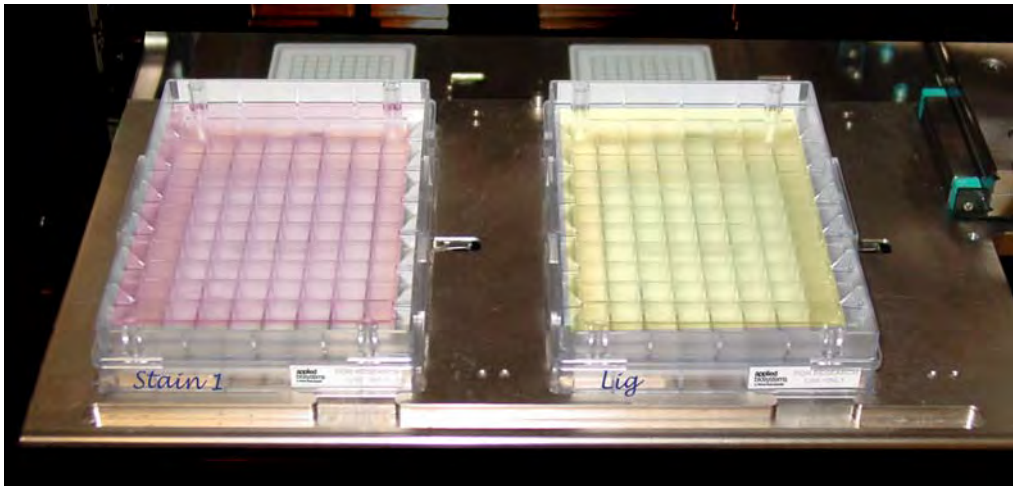


Figure 60 Stain 1 tray (left) and Ligation tray (right) loaded in drawer 3

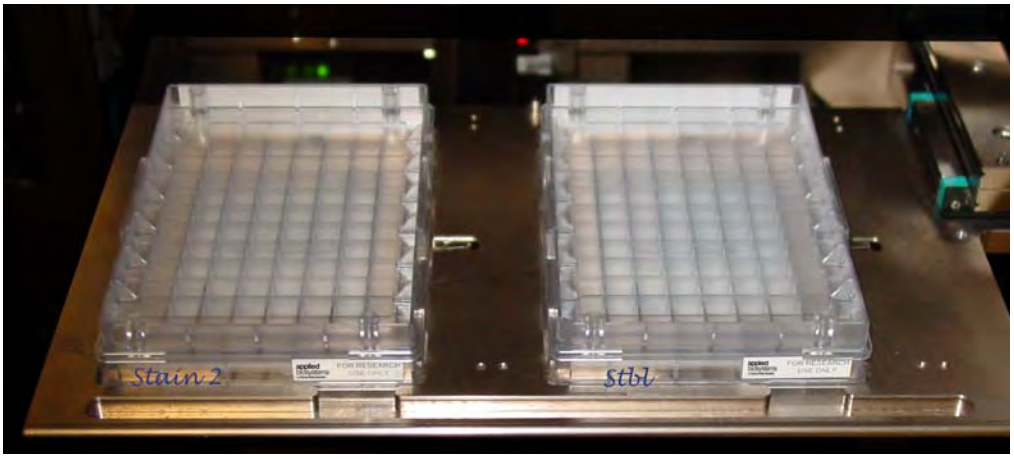


Figure 61 Stain 2 tray (left) and Stbl tray (right) loaded in drawer 4

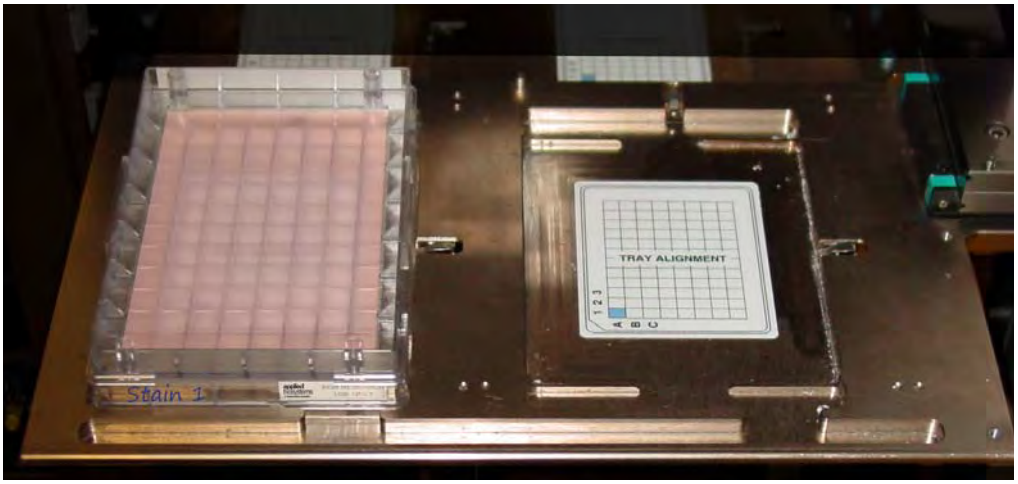


Figure 62 Stain 1 tray loaded in drawer 5

3. At the prompt shown in Figure 63, click *Yes* to load another PharmacoScan array plate and hybridization tray.

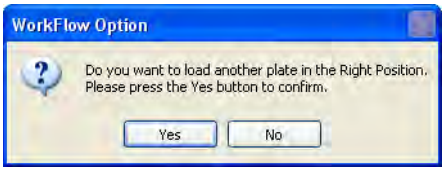


Figure 63 Prompt asking to load another plate. Right or left position determined by the position of PharmacoScan array plates already in the GeneTitan MC Instrument.

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:
   - Remove the blue cover from the previous PharmacoScan array plate.
   - Load a new PharmacoScan array plate and new blue base on the left; load a new hybridization tray on the right.
   - Press the **Confirmation** button.

e. Click **OK** when prompted (Figure 64).

Figure 64 Confirm Resume Processing message

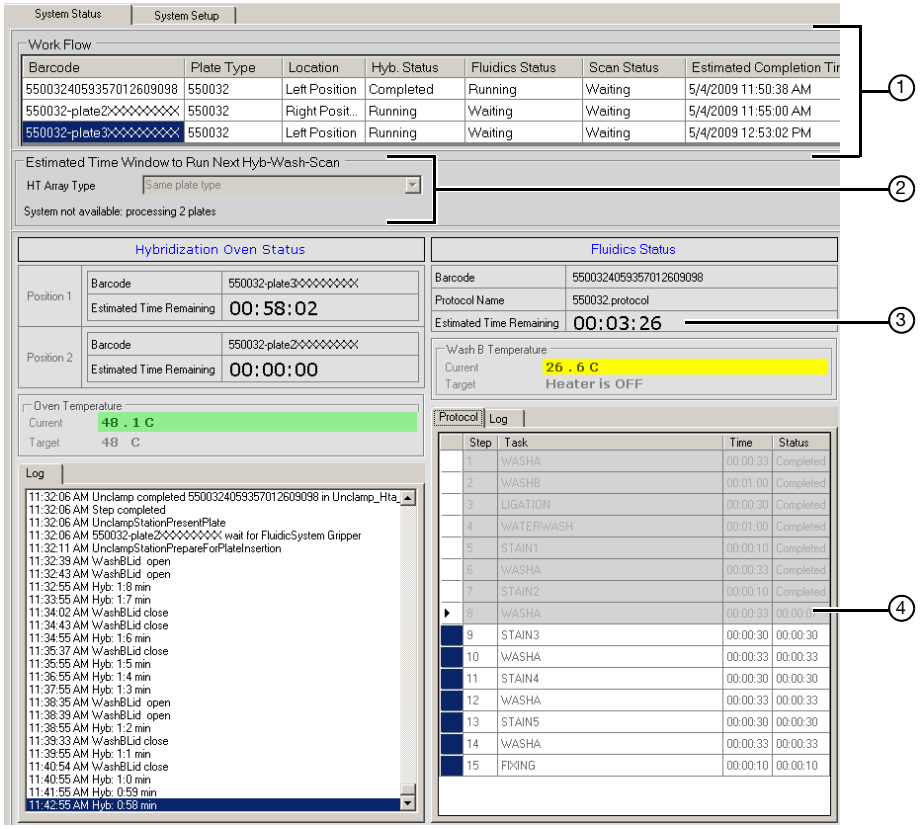
f. When drawer 6 opens, confirm that the plate stack is securely clamped, then press the **Confirmation** button.

The following is a description of array plate movements in the GeneTitan MC Instrument as users execute a multi-plate workflow.

1. The plate stack which has finished hybridization is moved from the hybridization oven to drawer 1 (temporarily).
2. The new plate stack in drawer 6 is moved to the hybridization oven.
3. The plate stack currently in drawer 1 (see Step 1) is moved to the unclamping station where it is unclamped and moved into the fluidics section of the GeneTitan MC Instrument.

   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 65 is an example of how the System Status Workflow window appears when three PharmacoScan array plates are being processed.



① **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: two in the hybridization oven and one in fluidics.
Estimated Completion Time is for the current process.

② Status area: Current status indicates that another (4th) plate cannot be added to the GeneTitan hybridization oven because both oven slots are currently in use.

③ **Estimated Time Remaining** for fluidics is adjusted as necessary. Adjustments can be due to process interruptions such as a drawer being opened.

④ Step currently executing in fluidics.

Figure 65 Example of the System Status window—three PharmacoScan array plates are being processed.

Continue the workflow

After 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 66 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 halts the scanning process. You can minimize this window if needed without creating any interference to the imaging.
- Do not manually, or through the GCC transfer utility, move any data associated with the current plate that is being processed/scanned. Transferring data can dramatically slow scanning and can cause the computer to freeze.

Figure 66 Scan Control window

Shut 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.

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 GCC software if it does not close automatically.

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

6 Process three PharmacoScan™ array plates per week using an overnight precipitation step

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

- Overview of the three-plate workflow for manual target preparation 143
- Thaw frozen plates of amplified DNA 147
- Manual target preparation and array processing 148

When using the PharmacoScan Assay 96-Array Format Manual Workflow, one to two people can process up to three PharmacoScan 96-array format plates in one forty-hour work week. This chapter describes the timing of the steps for each sample and array plate that are required to perform this workflow.

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

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

- Chapter 4, "Target preparation" on page 42.
- Chapter 5, "Array processing with the GeneTitan™ Multi-Channel Instrument" on page 103.

Overview of the three-plate workflow for manual target preparation

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

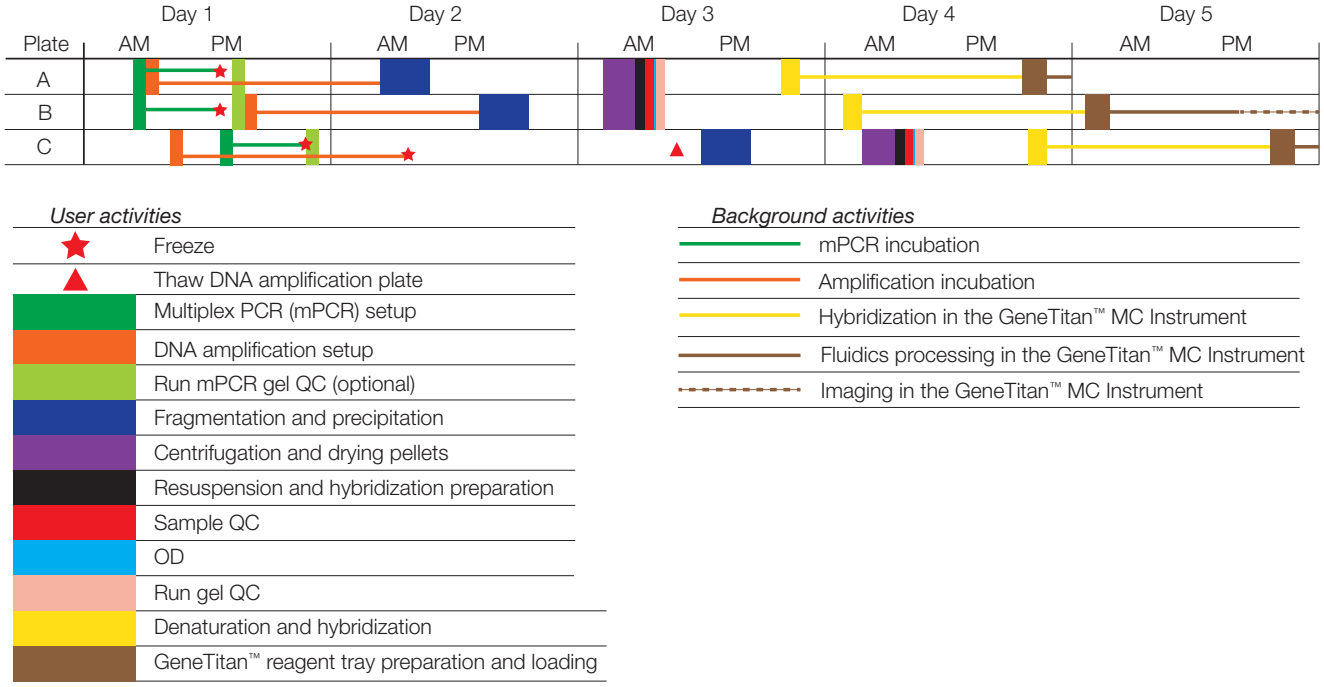


Figure 67 Full week activities for the PharmacoScan Assay 96-Array Format Manual Workflow

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

In order to process three plates during a 40-hour week, the steps must be performed in the order and with the timing described in this chapter. On day 1, it is recommended that one person be responsible for setting up the whole genome amplification for all three plates and that another person take care of setting up and running mPCR for all three plates. It is also helpful to have two people working together on day 3 and day 4.

Table 39 Daily steps for manual target preparation workflow

Day	Activities	Plates
1	• Amplify three plates of genomic DNA • mPCR setup and incubation for three plates • mPCR Gel QC for three plates (optional) • Freeze mPCR reaction plates until needed for mPCR spike-in during fragmentation	• A, B, and C • A, B, and C • A, B, and C • A, B, and C
2	• Fragment and precipitate two plates amplified on day 1 • Freeze one plate of amplified DNA for fragmentation later in the week	• A, B • C
3	• Fragment and precipitate one plate • Centrifuge, dry, resuspend and QC two plates precipitated on day 2 • Denature and begin hybridization for one plate on the GeneTitan MC Instrument	• C • A, B • A
4	• Centrifuge, dry, resuspend and QC plates precipitated on day 3 • Denature and begin hybridization for two plates on the GeneTitan MC Instrument • GeneTitan reagent trays preparation and loading	• C • B, C • A
5	• GeneTitan reagent trays preparation and loading	• B, C

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

These constraints are described in more detail in:

- "Timing issues for manual target preparation" on page 145.
- "Timing issues for GeneTitan™ MC Array Processing" on page 146.

Timing issues for manual target preparation

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

Table 40 Time required for manual target preparation

Manual preparation	Hands-on time required	Total preparation time[1]	Incubation/hybridization/ processing
"Stage 1A: Multiplex PCR (mPCR)" "Stage 1B: DNA amplification"	30 minutes 30 minutes	60 minutes 90 minutes	~3.5 hours 23 ±1 hour
"Stage 2: Fragmentation and precipitation"	2 hours	2 hours	Overnight Precipitation
"Stage 3: Centrifuge and drying, resuspension and hybridization preparation, and sample QC"			
• "Stage 3A: Centrifuge precipitation plate and dry the DNA pellet"	30 minutes	80 minutes	N/A
• "Stage 3B: Resuspension and hybridization preparation"	25 minutes	25 minutes	N/A
• "Stage 3C: Perform quantitation and fragmentation QC checks"	45 minutes	45 minutes	N/A
"Stage 4: Denaturation and hybridization"	25 minutes	45 minutes	23.5–24 hours hybridization
"Stage 5: GeneTitan™ reagent preparation"	60 minutes	90 minutes	Additional time for processing: 96 arrays: 12.5 hours

[1] Total preparation time includes reagent thawing time and hands-on time.

Timing issues for GeneTitan™ MC Array Processing

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

The hybridization time for the PharmacoScan 96-Array Format Manual Workflow on the GeneTitan MC Instrument is 23.5 to 24 hours (Table 41). This provides a 30-minute window during which you are prompted by the instrument control software to load the reagents required for washing and staining.

Table 41 Time required for array plate processing on the GeneTitan MC Instrument

Steps on the GeneTitan MC Instrument	Time required
Hybridization of two plates in one day • First plate loaded at 9:30 a.m. • Second plate loaded at 5:00 p.m.	23.5 hours each plate
Loading reagent trays	15 minutes
Fluidics	5 hours each plate
Imaging[1]	up to 7.5 hours depending on array format

[1] For labs that run several array plate formats, imaging times can vary.

Change oven temperatures for the three plate workflow

Multiple ovens are required for manual target preparation. If you are running the three plate/week workflow, three ovens are recommended. Table 42 lists the different temperatures required for each step. Though only two ovens are strictly required, we recommend maintaining separate 37°C ovens for the amplification and fragmentation stages to avoid confusion of plates and to minimize excess opening and closing of oven doors during incubation periods. Table 43 provides a list of suggested settings for three ovens when performing the three plate/week workflow.

Table 42 Oven temperatures needed for each step of the workflow

Workflow step	Oven temp
Amplification	37°C
Stopping amplification	65°C
Pre-fragmentation incubation	37°C
Fragmentation incubation	37°C
Drying	37°C
Hybridization[1]	48°C

[1] For preheating of the 96-well metal chamber for hybridization transfer.

Table 43 Suggested settings for ovens when performing three plate/week manual target preparation workflow

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

Thermal cycler requirements for three plate workflow

At least two thermal cyclers are needed to conduct the three plate workflow. Multiplex PCR runs for all three plates are performed on day 1, and each run is approximately 3.5 hours. See Chapter 3 for a list of validated thermal cyclers.

Thaw frozen plates of amplified DNA

1. Place the deep well plate in a small water bath.

   For example, pour Millipore water into a small tray. Place the frozen plate in the water in the tray.
2. Leave the plate in the water bath for ~50 minutes until all wells have thawed.
3. Spin down at 1,000 rpm for 30 seconds.
4. To avoid cross-contamination of wells during vortexing:
   a. Remove the seal and blot the top of the plate with a Kimwipe laboratory tissue.
   b. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 seconds to thoroughly mix (see guidelines described in "Seal, vortex, and spin" on page 27).
6. Spin at 1,000 rpm for 30 seconds.

Manual target preparation and array processing

Day 1

Note: The day 1 schedule is written for two people working together. One person sets up the whole genome amplification for all three plates, and the other person sets up and runs the mPCR reactions for all three plates. When the mPCR run is complete, the mPCR QC gel can be run (optional), and then the plates are frozen until needed.

- On this day you start whole genome amplification of the three plates; each plate must incubate 23 ±1 hours after amplification begins.
- All amplifications should be set up on day 1 to allow for a 23 ±1 hours amplification incubation for each plate and to minimize movement between pre-amplification and post-amplification areas.
- Begin thawing the amplification reagents, particularly the Axiom 2.0 Amp Soln, 60 minutes prior to the start of each reaction.
- On this day you set up and run the multiplex PCR for the three plates. Two thermal cyclers are required.
- mPCR QC Gels (optional) can be run for plate A, plate B, and plate C.
- Freeze all three mPCR reaction plates until needed for mPCR spike-in during Fragmentation.

IMPORTANT! Amplification preparation should take place in an Amplification Staging Room or dedicated area such as biosafety hood with dedicated pipettes, tips, vortex, etc. See "Amplification staging area" on page 24 for more information.

Table 44 Day 1 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Activity	Plate	Approximate times[1]		Hands-on duration
		Start time	End time	
mPCR setup	A and B	9:30 a.m.	10:30 a.m.	30 minutes
DNA amplification	A	9:30 a.m.	11:00 a.m.	30 minutes
DNA amplification	C	10:30 a.m.	12:00 p.m.	30 minutes
mPCR setup	C	1:00 p.m.	2:00 p.m.	30 minutes
DNA amplification	B	1:30 p.m.	3:00 p.m.	30 minutes
mPCR QC gel (optional)	A and B	2:00 p.m.	2:30 p.m.	30 minutes
mPCR QC gel (optional)	C	5:30 p.m.	6:00 p.m.	30 minutes
Freeze mPCR reaction plates (–20°C)	A, B, C	End of mPCR run (and after aliquot removed if performing optional mPCR gel QC).		

[1] Approximate start time indicates start of thawing of reagents.

See "Stage 1A: Multiplex PCR (mPCR)" on page 43 -- "Stage 1B: DNA amplification" on page 49 for more information on the protocol.

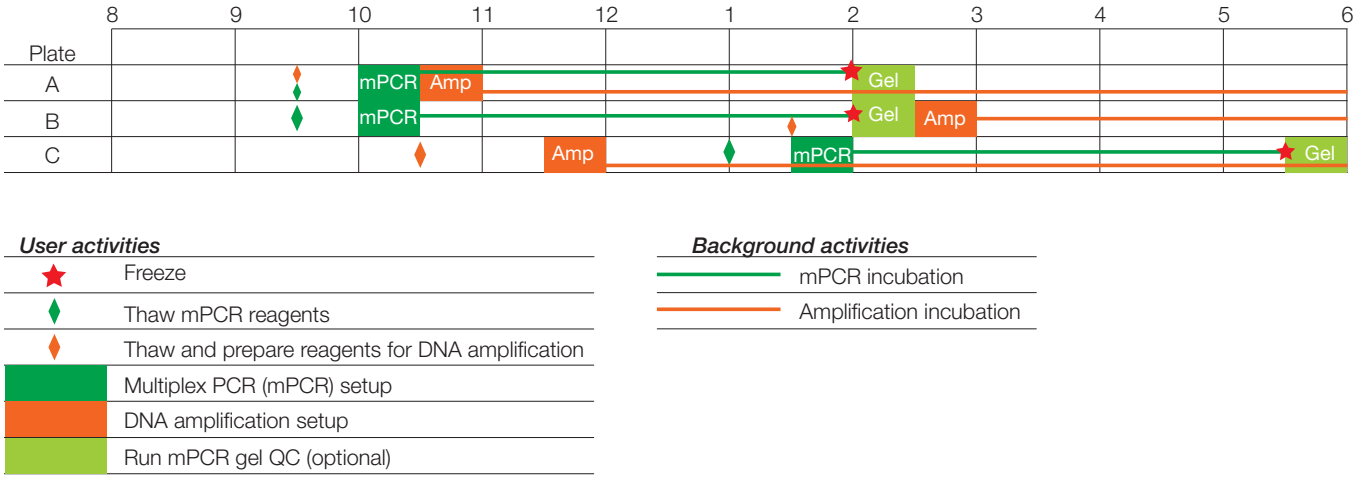


Figure 68 Day 1 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Day 2

- Table 45 shows the steps that need to be performed on the second day.
- Plates A and B are fragmented and precipitated on day 2 without freezing to preserve a 23-hour amplification incubation.
- Precipitation is carried out at –20°C overnight.

IMPORTANT! Store plate C at –20°C immediately after the end of the 23-hour amplification reaction (without performing the 65°C stop amplification reaction step).

Table 45 Day 2 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Activity	Plate	Approximate times		Duration
		Start time	End time	
Thaw mPCR reaction plate	A	9:30 a.m.	10:00 a.m.	30 minutes
Fragment and precipitate	A	10:00 a.m.	12:00 p.m.	2 hours
Freeze (–20°C) amplification plate	C	11:00 a.m.	—	Overnight
Thaw mPCR reaction plate	B	1:30 p.m.	2:00 p.m.	30 minutes
Fragment and precipitate	B	2:00 p.m.	4:00 p.m.	2 hours

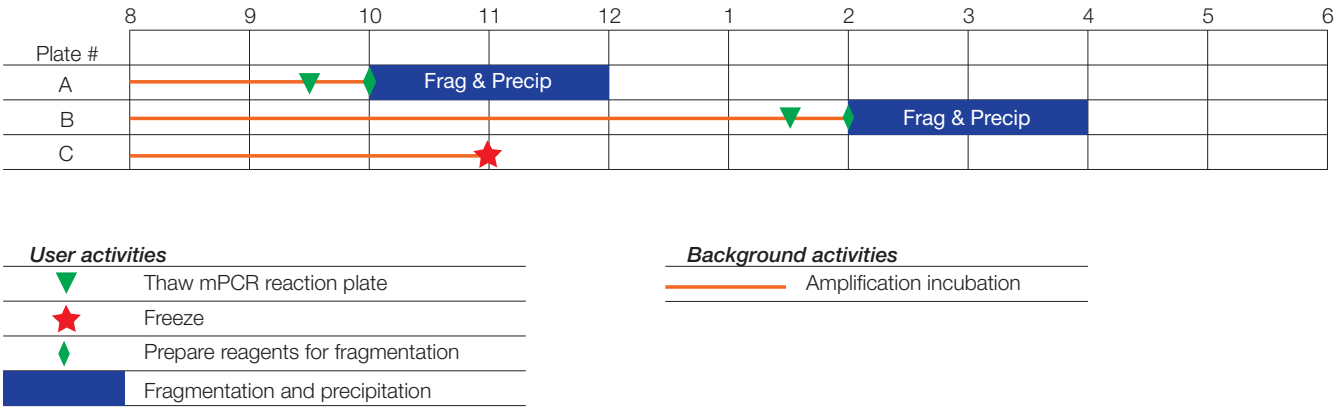


Figure 69 Day 2 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Day 3

- Centrifuge, dry, resuspend, and QC plates A and B.
- Thaw plate C (see "Thaw frozen plates of amplified DNA" on page 147).
- Fragment (including the 65°C stop amplification reaction step) and precipitate plate C.
- Perform denaturation on plate A.
- Transfer plate A samples to hybridization tray A.
- Load hybridization tray A and array plate into GeneTitan MC Instrument and begin hybridization.

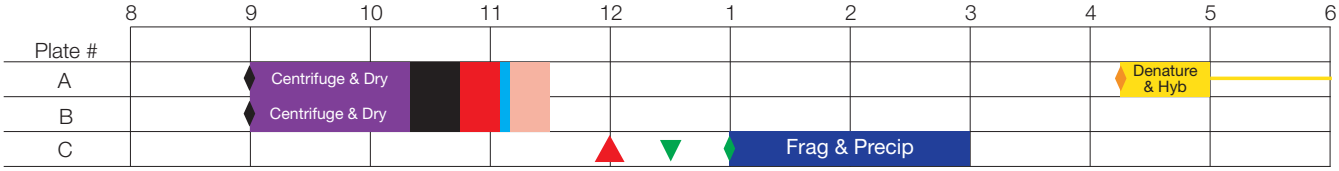
⚠ **WARNING!** The hybridization tray preparation should take place under a running fume hood.

IMPORTANT! Amplified plates that are frozen must be thawed and thoroughly mixed by following the procedure under "Thaw frozen plates of amplified DNA" on page 147.

Table 46 Day 3 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Activity	Plate	Approximate times		Duration
		Start time	End time	
Centrifuge and dry	A, B	9:00 a.m.	10:20 a.m.	80 minutes
Resuspension and hybridization preparation	A, B	10:20 a.m.	10:45 a.m.	25 minutes
Sample QC	A, B	10:45 a.m.	11:05 a.m.	20 minutes
Sample quantitation (OD)[1]	A, B	11:05 a.m.	11:10 a.m.	5 minutes
Frag gel QC run	A, B	11:05 a.m.	11:30 a.m.	25 minutes
Thaw plate C	C	12:00 p.m.	1:00 p.m.	60 minutes
Thaw mPCR reaction plate	C	12:30 p.m.	1:00 p.m.	30 minutes
Fragment and precipitate	C	1:00 p.m.	3:00 p.m.	2 hours
Denaturation and hybridization	A	4:15 p.m.	5:00 p.m.	45 minutes setup, 23.5–24 hours hybridization

[1] Sample Quantitation runs concurrently with frag gel QC run. Load the gel QC plate first, then read the OD QC plate.



User activities	
▼	Thaw mPCR reaction plate
◆	Prepare reagents for resuspension and hybridization prep
◆	Prepare reagents for fragmentation
◆	Warm array plate to room temperature
▲	Thaw DNA amplification plate
	Fragmentation and precipitation
	Centrifugation and drying pellets
	Resuspension and hybridization preparation
	Sample QC
	Sample quantitation—OD
	Fragmentation gel QC run
	Denaturation and hybridization

Background activities	
—	Hybridization in the GeneTitan™ MC Instrument

Figure 70 Day 3 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Day 4

- Denaturation of Samples/Load array plate and hybridization tray in the GeneTitan MC Instrument for plates B and C.
- Centrifuge, dry, resuspend, and QC plate C.
- GeneTitan reagent trays preparation and loading for plate A.

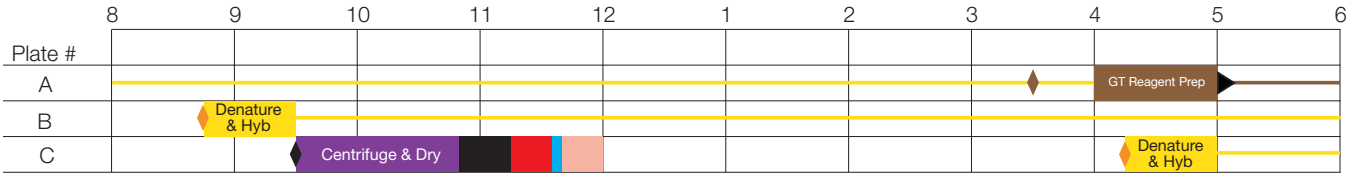
⚠ **WARNING!** The hybridization tray preparation should take place under a running fume hood.

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

Table 47 Day 4 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Activity	Plate	Approximate times		Duration
		Start time	End time	
Denaturation and hybridization	B	8:45 a.m.	9:30 a.m.	45 minutes setup, 23.5–24 hours hybridization
Centrifuge and dry	C	9:30 a.m.	10:50 a.m.	80 minutes
Resuspension and hybridization preparation	C	10:50 a.m.	11:15 a.m.	25 minutes
Sample QC	C	11:15 a.m.	11:35 a.m.	20 minutes
Sample quantitation (OD)[1]	C	11:35 a.m.	11:40 a.m.	5 minutes
Fragmentation gel QC run	C	11:35 a.m.	12:00 p.m.	25 minutes
GeneTitan reagent preparation and loading	A	3:30 p.m.	5:00 p.m.	90 minutes
Denaturation and hybridization	C	4:15 p.m.	5:00 p.m.	45 minutes setup, 23.5–24 hours hybridization

[1] Sample Quantitation runs concurrently with frag gel QC run. Load the gel QC plate first, then read the OD QC plate.



User Activities	
♦	Warm array plate to room temperature
♦	Prepare reagents for resuspension and hybridization prep
♦	Thaw reagents for GeneTitan reagent tray preparation
♦	Warm array plate to room temperature
▶	Load array plate into GTMC, begin wash-scan
	Centrifugation and drying pellets
	Resuspension and hybridization preparation
	Sample QC
	Sample quantitation—OD
	Fragmentation gel QC run
	GeneTitan™ reagent tray preparation and loading
	Denaturation and hybridization

Background Activities
Hybridization in the GeneTitan™ MC Instrument
Fluidics processing in the GeneTitan™ MC Instrument

Figure 71 Day 4 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Day 5

- GeneTitan reagents preparation and loading for plates B and C.

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

Table 48 Day 5 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

Activity	Plate	Approximate times		Duration
		Start time	End time	
GeneTitan reagent tray preparation and loading	B	8:00 a.m.	9:30 a.m.	90 minutes
GeneTitan reagent tray preparation and loading	C	3:30 p.m.	5:00 p.m.	90 minutes

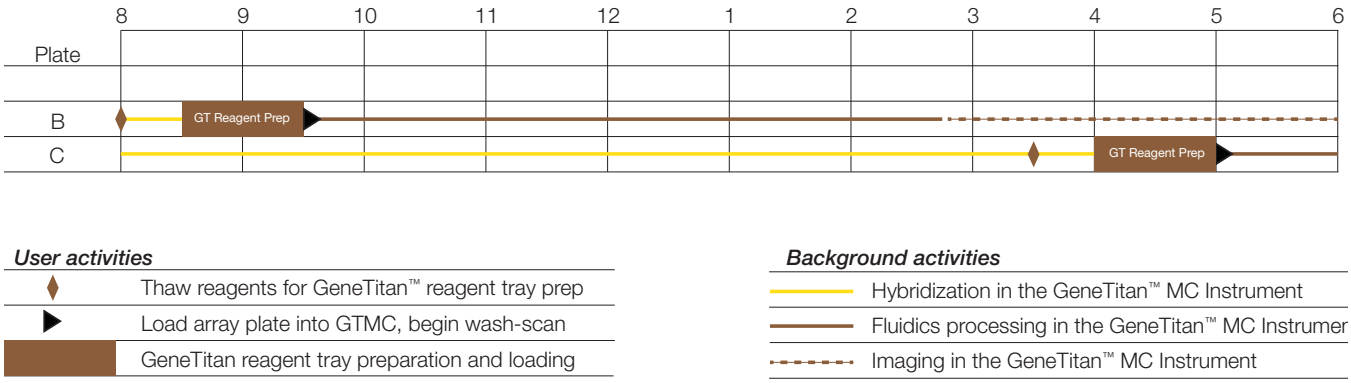


Figure 72 Day 5 activities for the PharmacoScan Assay 96-Array Format Manual Workflow

7 Process three PharmacoScan™ array plates per week using a three-hour precipitation step

The three plate per week workflow using the newly validated three-hour precipitation step is described in the following sections:

- Overview of the three-plate workflow using a three-hour precipitation step for manual target preparation 157
- Thaw frozen plates of amplified DNA 161
- Manual target preparation and array processing 162

The PharmacoScan Assay 96-Array Format Manual Workflow using the three-hour DNA precipitation step allows faster assay turnaround time. Using this recently validated and accelerated workflow, plate 1 CEL files are available within 72 hours, and CEL files for all three plates are available by day 5. One to two people can process three PharmacoScan 96-array format plates in one work week. This chapter describes the timing of the steps for each sample and array plate that are required to perform this workflow. Note that this workflow requires approximately nine- to ten-hour workdays.

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

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

- Chapter 4, "Target preparation" on page 42.
- Chapter 5, "Array processing with the GeneTitan™ Multi-Channel Instrument" on page 103.

Overview of the three-plate workflow using a three-hour precipitation step for manual target preparation

The figures and table below display the timing and duration of the hands-on processing necessary for performing the three plate workflow.

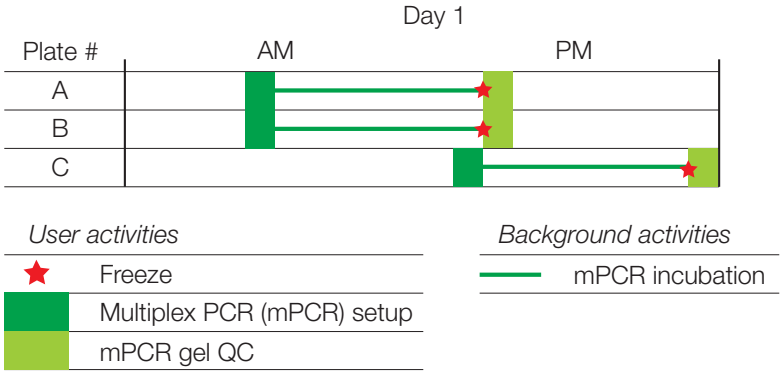


Figure 73 mPCR activities for PharmacoScan Assay three plate workflow using three-hour precipitation

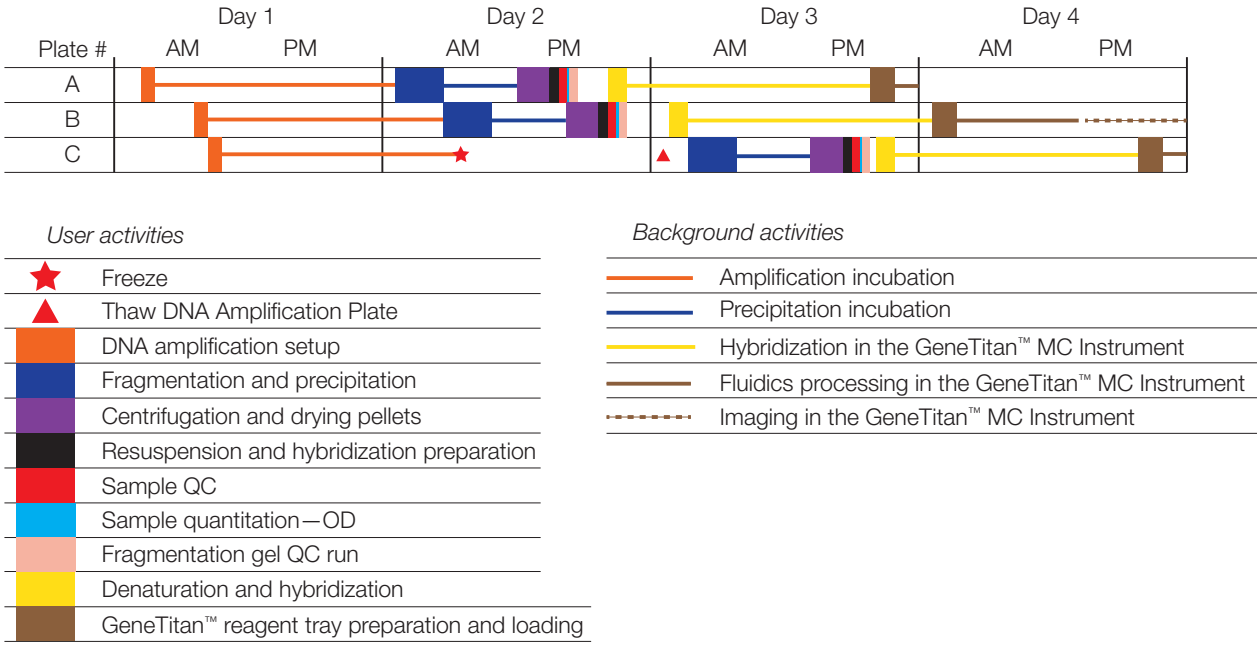


Figure 74 Full week activities for the PharmacoScan Assay 96-Array Format Manual Workflow using three-hour precipitation

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

The steps must be performed in the order and with the timing described in this chapter. On day 1, it is recommended that one person be responsible for setting up the whole genome amplification for all three plates and that another person take care of setting up and running mPCR for all three plates. It is also helpful to have two people working together on day 2 and day 3.

Table 49 Daily steps for manual target preparation workflow

Day	Activities	Plates
1	• Amplify three plates of genomic DNA • mPCR setup and incubation for three plates • mPCR Gel QC for three plates (optional) • Freeze mPCR reaction plates until needed for mPCR spike-in during fragmentation	• A, B, and C • A, B, and C • A, B, and C • A, B, and C
2	• Fragment and precipitate two plates amplified on day 1 • Freeze one plate of amplified DNA for fragmentation on day 3 • Centrifuge, dry, resuspend, and QC two plates • Denature and begin hybridization for one plate on the GeneTitan MC Instrument	• A, B • C • A, B • A
3	• Denature and begin hybridization for two plates on the GeneTitan MC Instrument • Fragment and precipitate one plate • Centrifuge, dry, resuspend, and QC one plate • GeneTitan reagent trays preparation and loading	• B, C • C • C • A
4	• GeneTitan reagent trays preparation and loading for two plates	• B, C

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

These constraints are described in more detail in:

- "Timing issues for manual target preparation" on page 159.
- "Timing issues for GeneTitan™ MC Array Processing" on page 160.

Timing issues for manual target preparation

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

Table 50 Time required for manual target preparation

Manual preparation	Hands-on time required	Total preparation time[1]	Incubation/hybridization/ processing
"Stage 1A: Multiplex PCR (mPCR)" "Stage 1B: DNA amplification"	30 minutes 30 minutes	60 minutes 90 minutes	~3.5 hours 23 ±1 hour
"Stage 2: Fragmentation and precipitation"	2 hours	2 hours	Three-hour precipitation
"Stage 3: Centrifuge and drying, resuspension and hybridization preparation, and sample QC"			
• "Stage 3A: Centrifuge precipitation plate and dry the DNA pellet"	30 minutes	80 minutes	N/A
• "Stage 3B: Resuspension and hybridization preparation"	25 minutes	25 minutes	N/A
• "Stage 3C: Perform quantitation and fragmentation QC checks"	45 minutes	45 minutes	N/A
"Stage 4: Denaturation and hybridization"	25 minutes	45 minutes	23.5–24 hours hybridization
"Stage 5: GeneTitan™ reagent preparation"	60 minutes	90 minutes	Additional time for processing: 96 arrays: 12.5 hours

[1] Total preparation time includes reagent thawing time and hands-on time.

Timing issues for GeneTitan™ MC Array Processing

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

The hybridization time for the PharmacoScan 96-Array Format Manual Workflow on the GeneTitan MC Instrument is 23.5 to 24 hours (Table 51). This provides a 30-minute window during which you are prompted by the instrument control software to load the reagents required for washing and staining.

Table 51 Time required for array plate processing on the GeneTitan MC Instrument

Steps on the GeneTitan MC Instrument	Time required
Hybridization of two plates in one day • First plate loaded at 9:30 a.m. • Second plate loaded at 6:00 p.m.	23.5 hours each plate
Loading reagent trays	15 minutes
Fluidics	5 hours each plate
Imaging[1]	up to 7.5 hours depending on array format

[1] For labs that run several array plate formats, imaging times can vary.

Change oven temperatures for the three plate workflow

Multiple ovens are required for manual target preparation. If you are running the three plate/week workflow, three ovens are recommended. Table 52 lists the different temperatures required for each step. Table 53 provides a list of suggested settings for three ovens when performing the three plate/week workflow.

Table 52 Oven temperatures needed for each step of the workflow

Workflow step	Oven temperature
Amplification	37°C
Stopping amplification	65°C
Pre-fragmentation incubation	37°C
Fragmentation incubation	37°C
Drying	37°C
Hybridization[1]	48°C

[1] For preheating of the 96-well metal chamber for hybridization transfer.

Table 53 Suggested settings for ovens when performing three plate/week manual target preparation workflow using three-hour precipitation

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

Thermal cycler requirements for three plate workflow

At least two thermal cyclers are needed to conduct the three plate workflow. Multiplex PCR runs for all three plates are performed on day 1, and each run is approximately 3.5 hours. See Chapter 3 for a list of validated thermal cyclers.

Thaw frozen plates of amplified DNA

1. Place the deep well plate in a small water bath.

   For example, pour Millipore water into a small tray. Place the frozen plate in the water in the tray.
2. Leave the plate in the water bath for ~50 minutes until all wells have thawed.
3. Spin down at 1,000 rpm for 30 seconds.
4. To avoid cross-contamination of wells during vortexing:
   a. Remove the seal and blot the top of the plate with a Kimwipe laboratory tissue.
   b. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 seconds to thoroughly mix (see guidelines described in "Seal, vortex, and spin" on page 27).
6. Spin at 1,000 rpm for 30 seconds.

Manual target preparation and array processing

Day 1

Note: The day 1 schedule is written for two people working together. One person sets up the whole genome amplification for all three plates, and the other person sets up and runs the mPCR reactions for all three plates. When the mPCR run is complete, the mPCR QC gel can be run (optional), and then the plates are frozen until needed.

- On this day you start whole genome amplification of the three plates: each plate must incubate 23 ±1 hours after amplification begins.
- All amplifications should be set up on day 1 to allow for a 23 ±1 hour amplification incubation for each plate and to minimize movement between pre-amplification and post-amplification areas.
- Begin thawing the amplification reagents, particularly the Axiom 2.0 Amp Soln, 60 minutes prior to the start of each reaction.
- On this day you set up and run the multiplex PCR for the three plates. Two thermal cyclers are required.
- mPCR QC Gels (optional) can be run for plate A, plate B, and plate C.
- Freeze all three mPCR reaction plates until needed for mPCR spike-in during Fragmentation.

IMPORTANT! Amplification preparation should take place in an Amplification Staging Room or dedicated area such as biosafety hood with dedicated pipettes, tips, vortex, etc. See "Amplification staging area" on page 24 for more information.

Table 54 Day 1 activities for the PharmacoScan Assay 96-Array Format Manual Workflow using three-hour precipitation

Activity	Plate	Approximate times[1]		Hands-on duration
		Start time	End time	
mPCR setup	A and B	8:30 a.m.	9:30 a.m.	30 minutes
DNA amplification	A	8:00 a.m.	9:30 a.m.	30 minutes
DNA amplification	B	10:00 a.m.	11:30 a.m.	30 minutes
mPCR setup	C	12:00 p.m.	1:00 p.m.	30 minutes
DNA amplification	C	10:30 a.m.	12:00 p.m.	30 minutes
mPCR QC gel (optional)	A and B	1:00 p.m.	2:00 p.m.	30 minutes
mPCR QC gel (optional)	C	4:30 p.m.	5:30 p.m.	30 minutes
Freeze mPCR reaction plates (–20°C)	A, B, C	End of mPCR run (and after aliquot removed if performing optional mPCR gel QC)		

[1] Approximate start time indicates start of thawing of reagents.

See "Stage 1A: Multiplex PCR (mPCR)" on page 43 and "Stage 1B: DNA amplification" on page 49 for more information on the protocol.

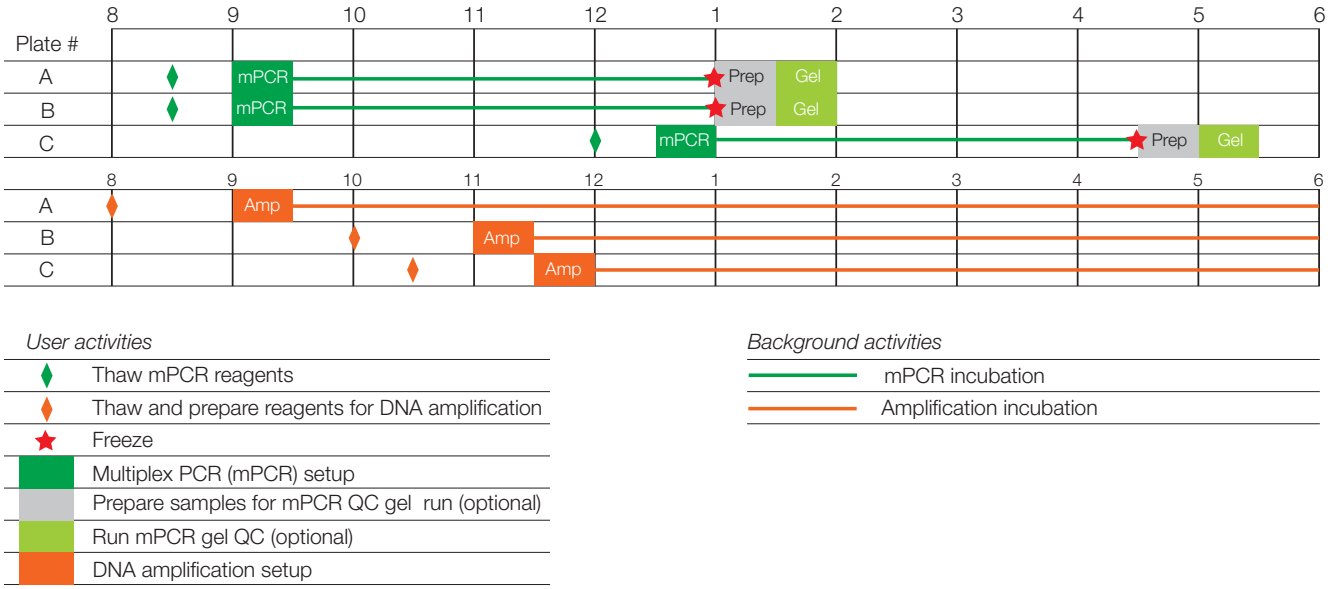


Figure 75 Day 1 activities for the PharmacoScan Assay 96-Array Format Manual Workflow using three-hour precipitation

Day 2

- It is recommended that two people work together for day 2 activities.
- Table 55 shows the steps that need to be performed on the second day.
- Plates A and B are fragmented and precipitated on day 2 without freezing to preserve a 23-hour amplification incubation.
- Precipitation is carried out at –20°C for three hours.
- Centrifuge, dry, resuspend, and QC plates A and B.
- Perform denaturation and hybridization for plate A.

IMPORTANT! Store plate C at –20°C immediately after the end of the 23-hour amplification reaction (without performing the 65°C stop amplification reaction step).

Table 55 Day 2 activities for the PharmacoScan Assay 96-Array Format Manual Workflow using the three-hour precipitation

Activity	Plate	Approximate times		Duration
		Start time	End time	
Thaw mPCR reaction plate	A	8:00 a.m.	8:30 a.m.	30 minutes
Fragment and precipitate	A	8:30 a.m.	10:30 a.m.	2 hours
Thaw mPCR reaction plate	B	10:00 a.m.	10:30 a.m.	30 minutes
Incubate Precipitation plate at –20°C	A	10:30 a.m.	1:30 p.m.	3 hours
Fragment and precipitate	B	10:30 a.m.	12:30 p.m.	2 hours
Freeze (–20°C) amplification plate	C	11:00 a.m.	—	Overnight
Incubate Precipitation plate at –20°C	B	12:30 p.m.	3:30 p.m.	3 hours
Centrifuge and dry	A	1:30 p.m.	2:50 p.m.	80 minutes
Resuspension and hybridization preparation	A	2:50 p.m.	3:15 p.m.	25 minutes
Sample QC	A	3:15 p.m.	3:35 p.m.	20 minutes
Centrifuge and dry	B	3:30 p.m.	4:50 p.m.	80 minutes
Sample quantitation (OD)[1]	A	3:35 p.m.	3:40 p.m.	5 minutes
Frag gel QC run	A	3:35 p.m.	4:00 p.m.	25 minutes
Resuspension and hybridization preparation	B	4:50 p.m.	5:15 p.m.	25 minutes
Sample QC	B	5:15 p.m.	5:35 p.m.	20 minutes
Denaturation and hybridization	A	5:15 p.m.	6:00 p.m.	45 minutes set up, 23.5-24 hours hybridization
Sample quantitation (OD)[1]	B	5:35 p.m.	5:40 p.m.	5 minutes
Frag gel QC run	B	5:35 p.m.	6:00 p.m.	25 minutes

[1] Sample Quantitation runs concurrently with frag gel QC run. Load gel QC plate first, then read the OD QC plate.

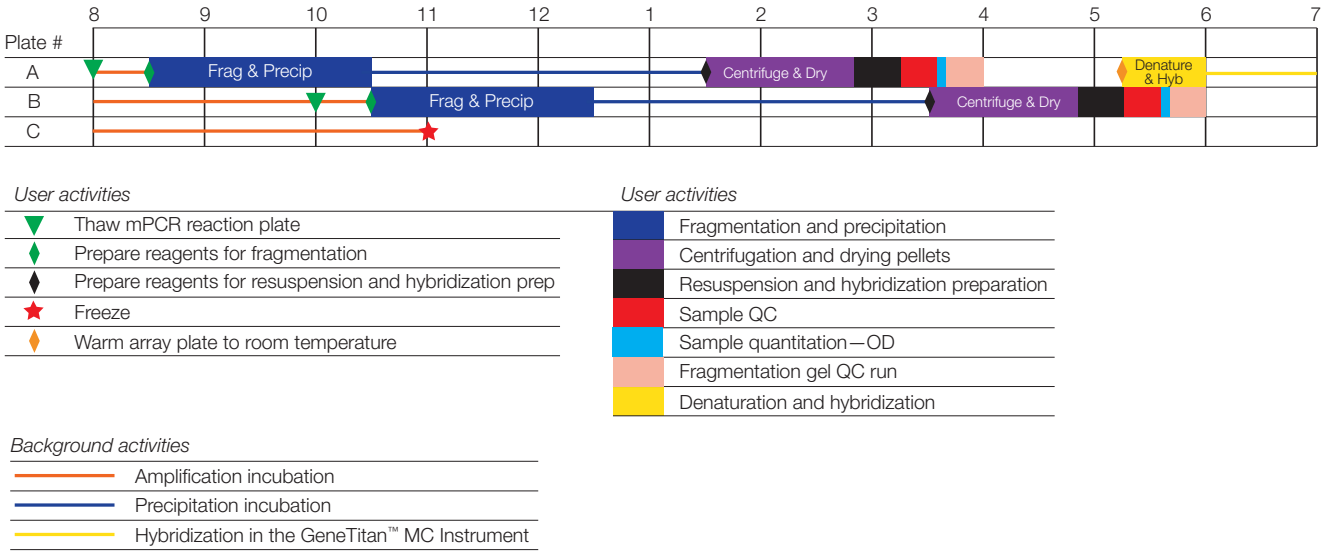


Figure 76 Day 2 activities for the PharmacoScan Assay 96-Array Format Manual Workflow using three-hour precipitation

Day 3

- It is recommended that two people work together for day 3 activities.
- Perform denaturation and hybridization of plate B.
- Thaw plate C (see "Thaw frozen plates of amplified DNA" on page 161).
- Fragment (including the 65°C stop amplification reaction step) and precipitate plate C.
- Centrifuge, dry, resuspend, and QC plate C.
- GeneTitan reagent trays preparation and loading for plate A.
- Perform denaturation and hybridization for plate C.

⚠ **WARNING!** The hybridization tray preparation should take place under a running fume hood.

IMPORTANT! Amplified plates that are frozen must be thawed and thoroughly mixed by following the procedure under "Thaw frozen plates of amplified DNA" on page 161.

Table 56 Day 3 activities for the PharmacoScan Assay 96-Array Format Manual Workflow using three-hour precipitation

Activity	Plate	Approximate times		Duration
		Start time	End time	
Denaturation and hybridization	B	8:45 a.m.	9:30 a.m.	45 minutes set up, 23.5-24 hours hybridization
Thaw DNA amplification plate C	C	8:30 a.m.	9:30 a.m.	60 minutes
Thaw mPCR reaction plate	C	9:00 a.m.	9:30 a.m.	30 minutes
Fragment and precipitate	C	9:30 a.m.	11:30 a.m.	2 hours
Incubate Precipitation plate at –20°C	C	11:30 a.m.	2:30 p.m.	3 hours
Centrifuge and dry	C	2:30 p.m.	3:50 p.m.	80 minutes
Resuspension and hybridization preparation	C	3:50 p.m.	4:15 p.m.	25 minutes
Sample QC	C	4:15 p.m.	4:35 p.m.	20 minutes
GeneTitan reagent tray preparation and loading	A	4:30 p.m.	6:00 p.m.	90 minutes
Sample quantitation (OD)[1]	C	4:35 p.m.	4:40 p.m.	5 minutes
Frag gel QC run	C	4:35 p.m.	5:00 p.m.	25 minutes
Denaturation and hybridization	C	5:15 p.m.	6:00 p.m.	45 minutes setup, 23.5–24 hours hybridization

[1] Sample Quantitation runs concurrently with frag gel QC run. Load gel QC plate first, then read the OD QC plate.

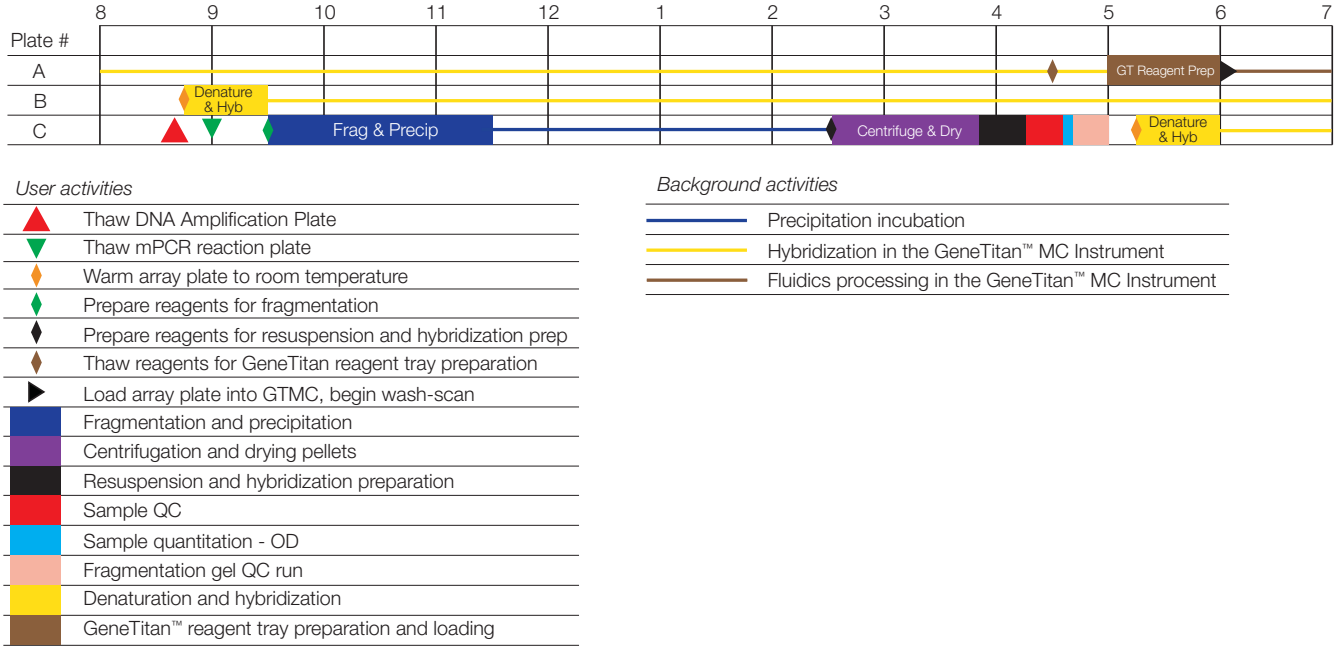


Figure 77 Day 3 activities for the PharmacoScan Assay 96-Array Format Manual Workflow using three-hour precipitation

Day 4

- GeneTitan reagent trays preparation and loading for plates B and C.

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

Table 57 Day 4 activities for the PharmacoScan Assay 96-Array Format Manual Workflow using three-hour precipitation

Activity	Plate	Approximate times		Duration
		Start time	End time	
GeneTitan reagent tray preparation and loading	B	8:00 a.m.	9:30 a.m.	90 minutes
GeneTitan reagent tray preparation and loading	C	4:30 p.m.	6:00 p.m.	90 minutes

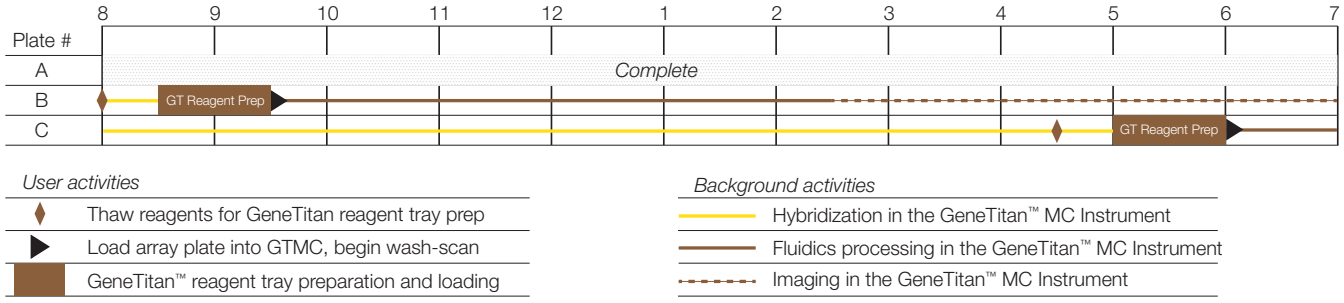


Figure 78 Day 4 activities for the PharmacoScan Assay 96-Array Format Manual Workflow using three-hour precipitation

8 Troubleshooting

GeneTitan™ Multi-Channel Instrument

See the *GeneTitan™ Multi-Channel Instrument User Guide,* Pub. No. 08-0308 for further troubleshooting information.

Table 58 GeneTitan™ Multi-Channel Instrument Troubleshooting Guidelines for the PharmacoScan™ Assay 96-Array Format Manual Workflow

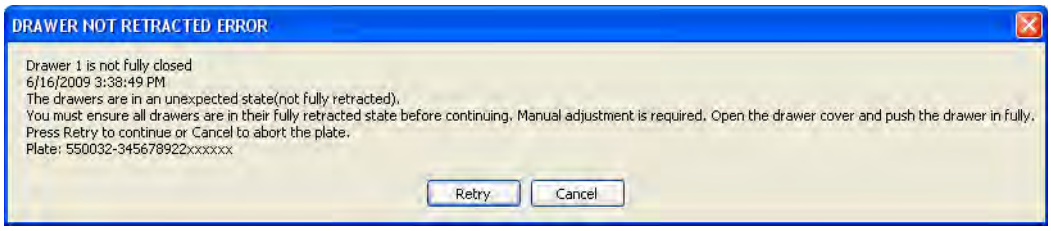
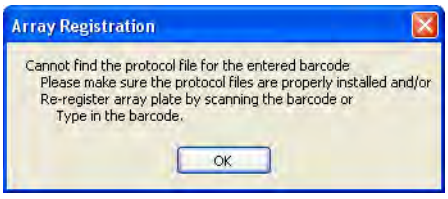
Problem	Possible causes	Possible actions
Plate trapped in GeneTitan Multi-Channel Instrument.	• Plate (or plate with cover) not properly loaded in drawer. • Cut edge of cover and plate not aligned. • Gripper failed to retrieve plate. • System requires adjustment.	1. Restart the GeneTitan Multi-Channel Instrument. 2. Run the setup option *Unload Plates* 3. If the plate remains trapped in the instrument, call Thermo Fisher Scientific 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 hybridization 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 GCC 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 hybridization 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.

Table 58 GeneTitan™ Multi-Channel Instrument Troubleshooting Guidelines for the PharmacoScan™ Assay 96-Array Format Manual Workflow

Problem	Possible causes	Possible actions
FAILED messages	See "GeneTitan MC Instrument messages that appear when the instrument has a fluidics problem" on page 172	
FLUIDIC DIAGNOSTIC messages	See "Fluidic diagnostic messages" on page 172.	
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 176.

Miscellaneous messages

Table 59 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 occurs. Homing does not complete and you are not able to use the system. The item held by the gripper is moved to either: • Drawer 2—plates and trays • Trash Bin—covers The drawer names 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 hybridization 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 hybridization 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.

Fluidic diagnostic messages

GeneTitan MC Instrument messages that appear when the instrument has a fluidics problem

Table 60

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 oaky, but cleanup is not 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 96 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 61 Problem messages

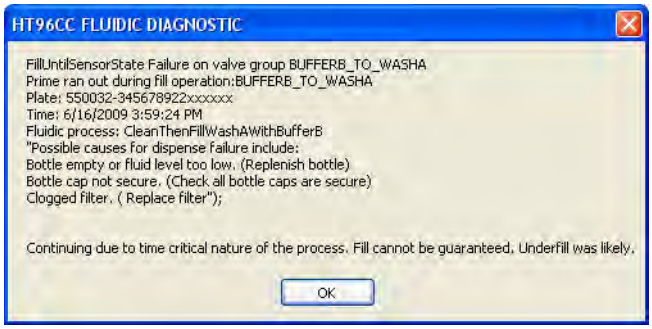
Problem and possible causes	
	About this message: • BUFFERX = Buffer bottle A, B or Rinse • WASHX = Wash A or B reservoir in the fluidics station. Recommended actions: • Replenish fluid level in the Rinse or Wash Bottle B to the 1-L mark. Do not overfill. – Only replenish bottles when prompted by the UI. Replenishing during fluidic processing can cause system malfunction including overflowing inside the system and more problems. The only thing to do while a plate is running is to ensure bottle caps are secure. • Replenish fluid level in Wash Bottle A to 2 L. • Secure the bottle cap. • Replace the filter Instructions for filter replacement in the *GeneTitan™ Multi-Channel Instrument User Guide*, Pub. No. 08-0308. If the problem persists, call Thermo Fisher Scientific support.

Table 61 Problem messages (Continued)

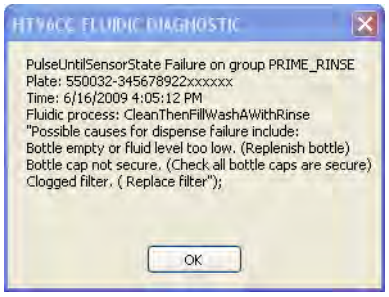
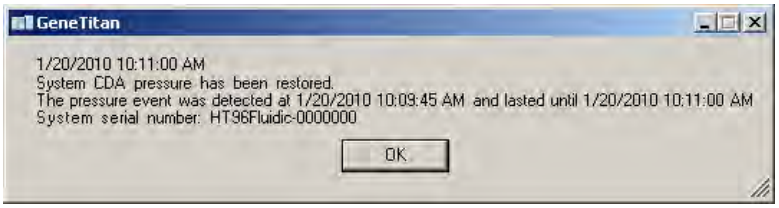
Problem and possible causes	
	The typical cause is an unsecure bottle cap. If the failure is detected during priming, the instrument pauses and waits for the problem to be corrected. If the failure is detected during another process, and if the cause is a clogged filter, wait until the end of the run to replace the filter. Instructions for filter replacement in the *GeneTitan™ Multi-Channel Instrument User Guide*, Pub. No. 08-0308.
When the instrument experiences a loss in Clean Dry Air (CDA) pressure, the software displays the warning message.  When the pressure is detected again, a dialog message confirming the availability of CDA pressure is displayed. 	Possible causes Verify that the facility CDA or the portable CDA compressor is in working condition. See 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.

Table 61 Problem messages (Continued)

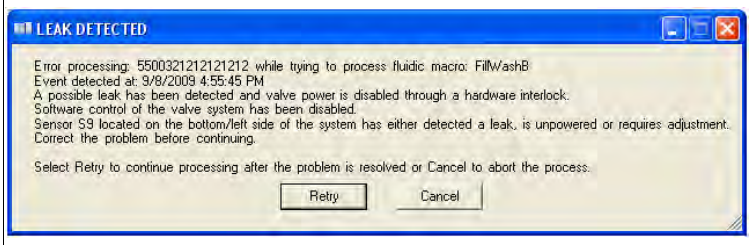
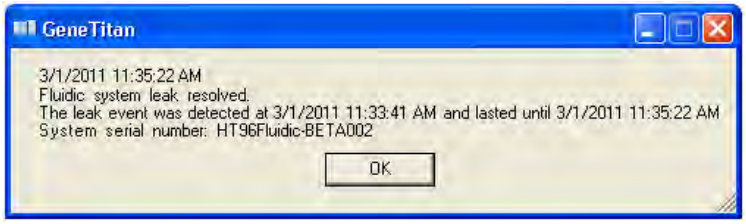
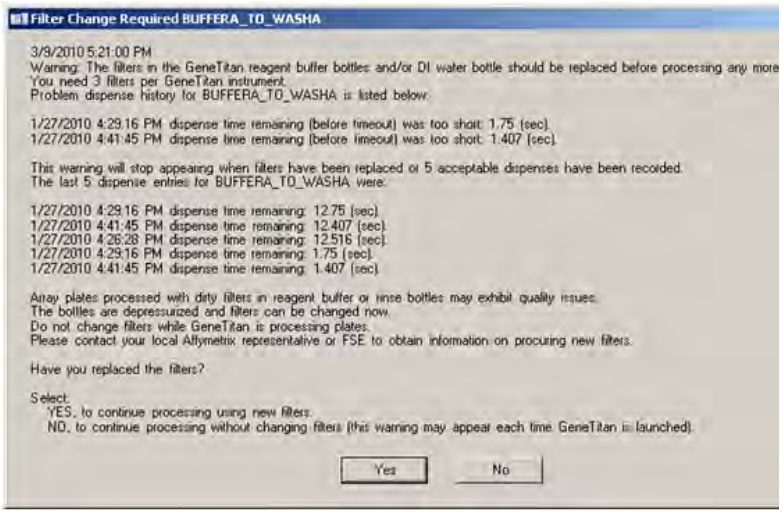
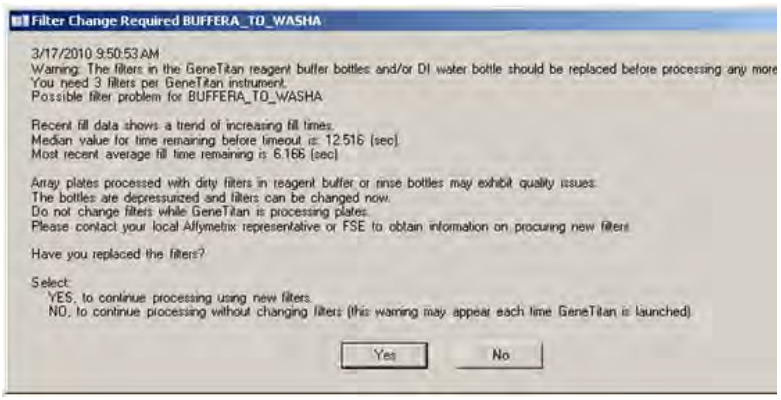
Problem and possible causes	
Leak Detected Leak checks are performed at application startup and any time a fluidic process (priming filling draining etc.) is performed. The leak detection is a hard-wired sensor which shuts off fluid flow without software control. Leaks are normally confined to the drip pan located inside the system.  	Causes: • System malfunction • Operator killing the application using task manager during a fill operation resulting in application exit without stopping flow. Solution: Contact Thermo Fisher Scientific field support. The system cannot be used for any fluidic processing until this is resolved.
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 is automatically removed from the GUI and replaced by the following message. It remains displayed until another leak is detected or the operator 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 an authorized Field Service Engineer to have the sensor adjusted or replaced if the problem persists even after correcting for the usual causes outlined above.

Table 61 Problem messages (Continued)

Problem and possible causes	
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. See the section "Replace the filter" on page 199 in Appendix E.

Wash/Scan Resume

If a run is aborted during fluidics processing, the instrument places 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 are 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 hour 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-hybridization 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 forces an unload of the aborted plate(s)

System-initiated

- Power interruption
- Plate loaded incorrectly
- Equipment malfunction

The system aborts the processing. Follow the instructions displayed in the user interface.

User-initiated

Can abort processing of individual array plates.

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

A Fragmentation quality control gel protocol

Protocol for running a fragmentation quality control gel

Equipment required

Table 62 Equipment required

Item	Supplier	Cat. No.
Gel imager	Various	—
Pipettes, multi- and single-channel P20, single-channel P200	Various	—
Plate centrifuge	Various	—
Vortex	Various	—

E-Gels and reagents

Table 63 E-Gel and reagents required

Item	Supplier	Cat. No.
Mother E-Base Device	Thermo Fisher Scientific	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
Nuclease-free water		71786

Consumables

Table 64 Consumables required

Item	Supplier	Cat. No.
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	—
1.7 mL microfuge tube	Various	—

Dilute the TrackIt™ Cyan/Orange Loading Buffer and 25 bp ladder

The following recipe is for preparing a large batch of the Gel Diluent, a 1,000-fold dilution of the TrackIt Cyan-Orange Loading Buffer:

To dilute the TrackIt Cyan/Orange Loading Buffer (Cat. No. 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 (Cat. No. 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

Running one E-Gel® 48 4% agarose gel to sample a 96 well plate is recommended. A suggested sampling pattern is to load the gel with the following wells from the 96 well Gel QC Plate:

- Row A, C, E, G, or
- Row B, D, F, H

If processing multiple plates, sampling different wells from each plate can be helpful in monitoring assay processing performance.

Run a fragmentation QC gel

1. Tightly seal the Gel QC Plate produced during "Stage 3C: Perform quantitation and fragmentation QC checks".
2. Vortex the plate for one second each corner and one second in the center at the maximum setting; spin at 1,000 rpm for 30 seconds.
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 minutes
11. Image the gel.

Fragmentation QC gel images should look similar to the gel shown in Figure 79.

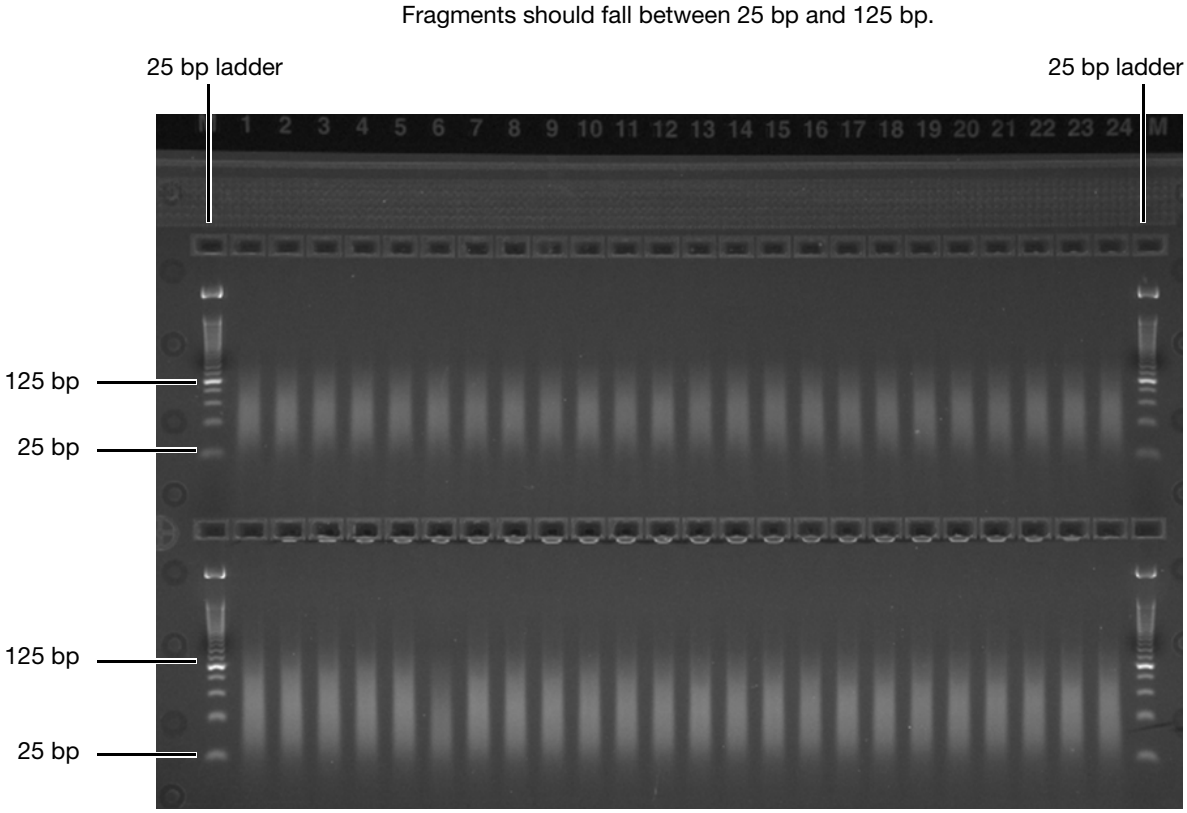


Figure 79 Example of a typical fragmentation QC E-gel

B

Sample quantitation after resuspension

- Protocol for sample quantitation after resuspension 180
- Suggested protocol for OD quantitation using the DTX 880 182
- Perform sample quantitation on a plate reader other than the DTX880 188

Protocol for sample quantitation after resuspension

Equipment required

The following equipment is required for this protocol.

Table 65 Equipment required for sample quantitation after resuspension

Quantity	Item
1	DTX 880 Multimode Detector with Genomic Filter Slide

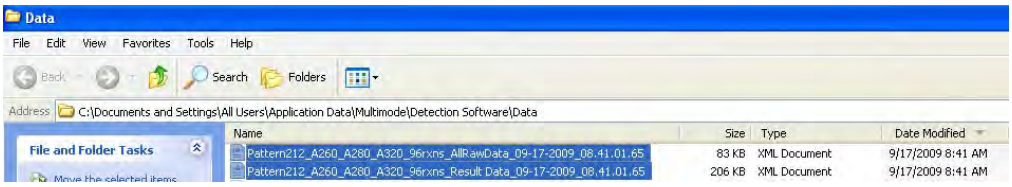
Quantitate the diluted samples

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 182 for more information.

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. Open the ResultData file with Microsoft® Excel® to view and assess the OD readings. RawData file information is included in the ResultData file.



Assess the OD readings

If using the formula provided in this appendix, the raw data is included in the final Result Data file. Figure 80 is an example of a Result Data file. Your OD readings should be similar to those displayed below.

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

Figure 80 Example of Result Data file with acceptable OD readings

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 96-Array Format Manual Workflow. If the median yield for the plate is < 1,000 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 0.50.

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

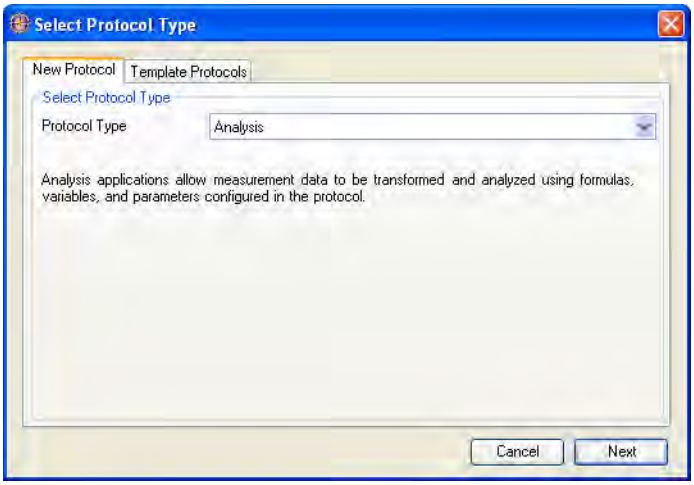


Figure 81 Protocol Type

General Settings—enter a name for the protocol

Figure 82 General Settings

Technique Type—select Absorbance.

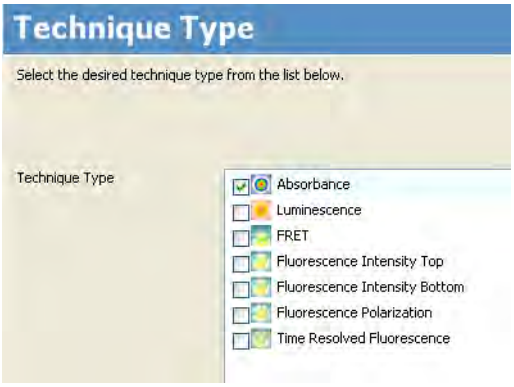


Figure 83 Technique Type

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

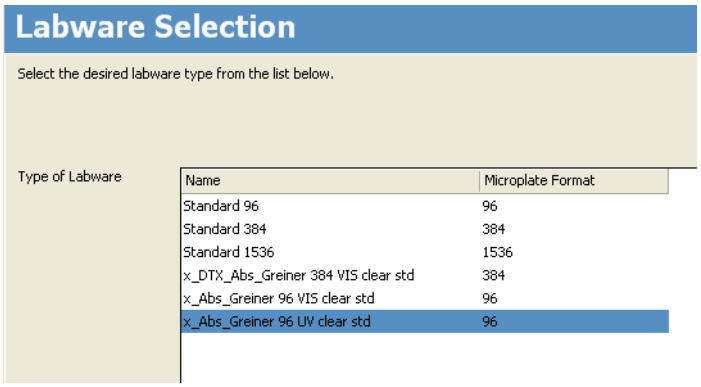


Figure 84 Labware

Layout Settings—as appropriate for 96-array plates

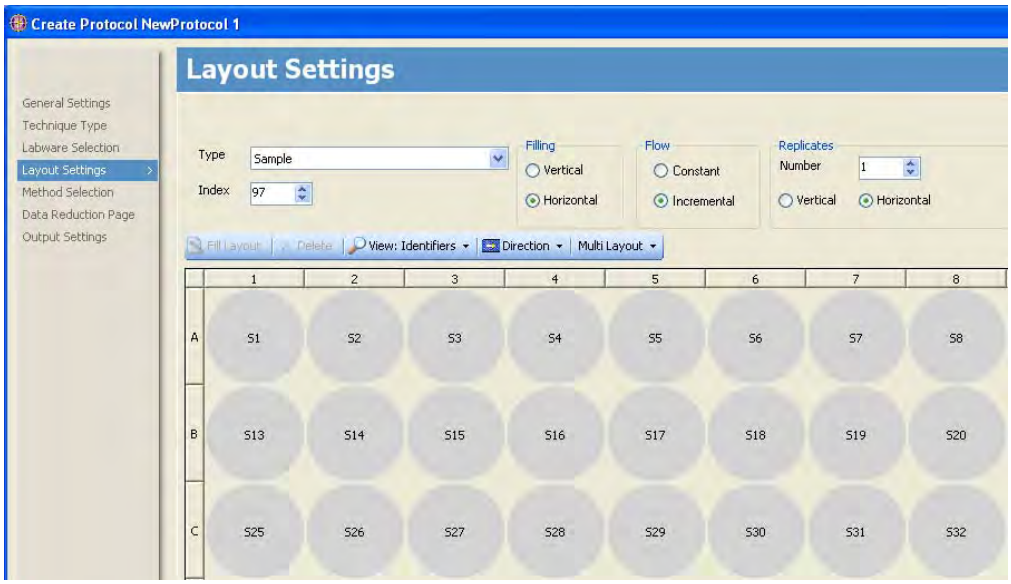


Figure 85 Layout Settings

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

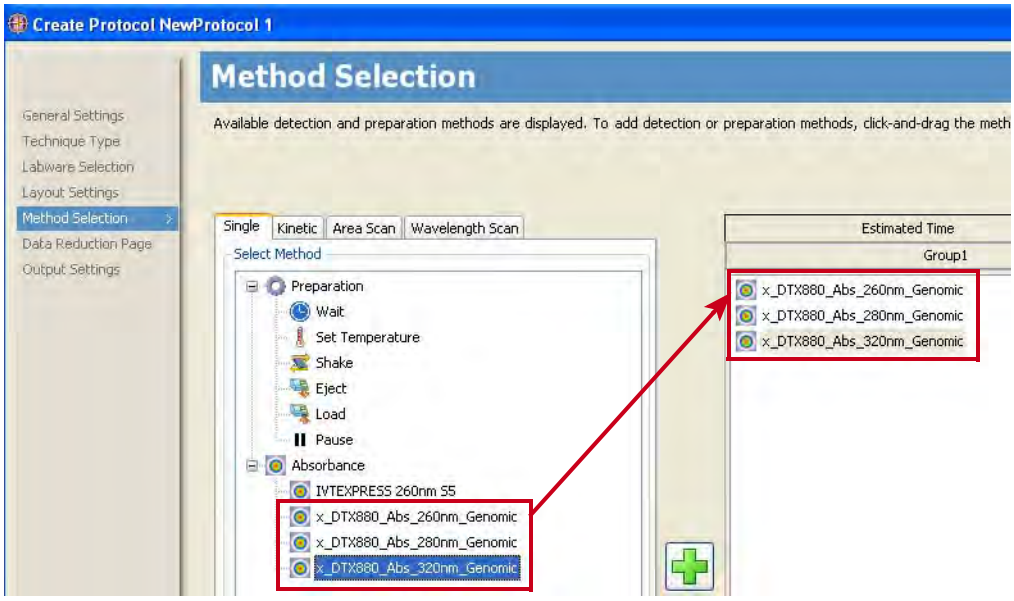


Figure 86 Method Selection

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.

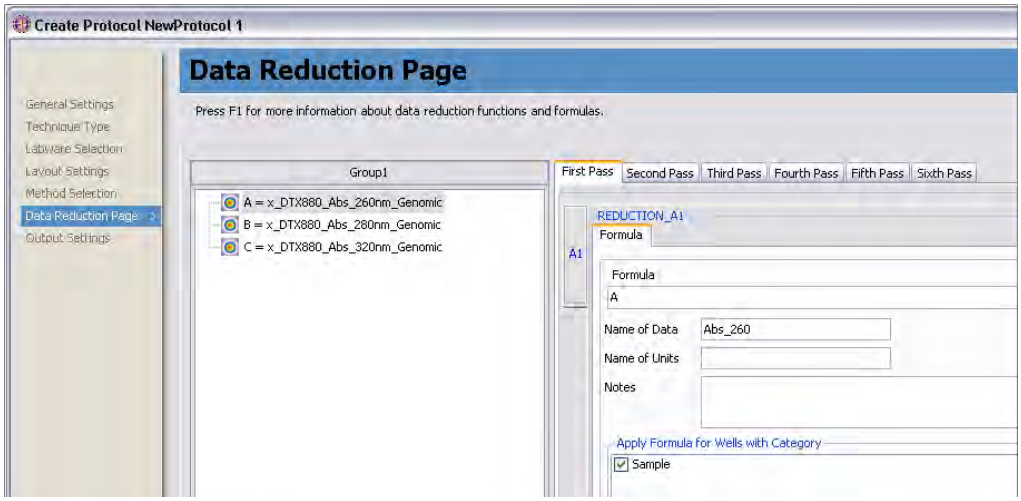


Figure 87 Data Reduction Page—First Pass

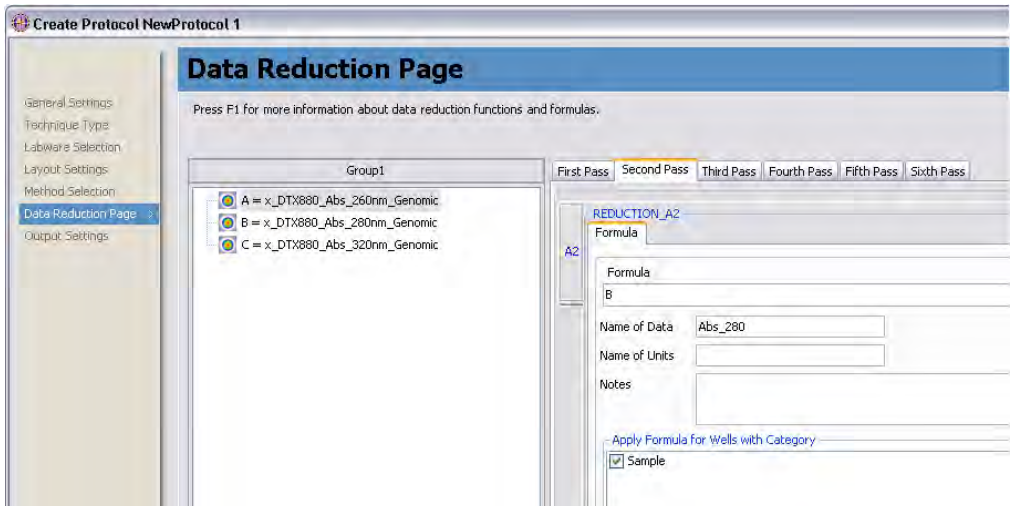


Figure 88 Data Reduction Page—Second Pass

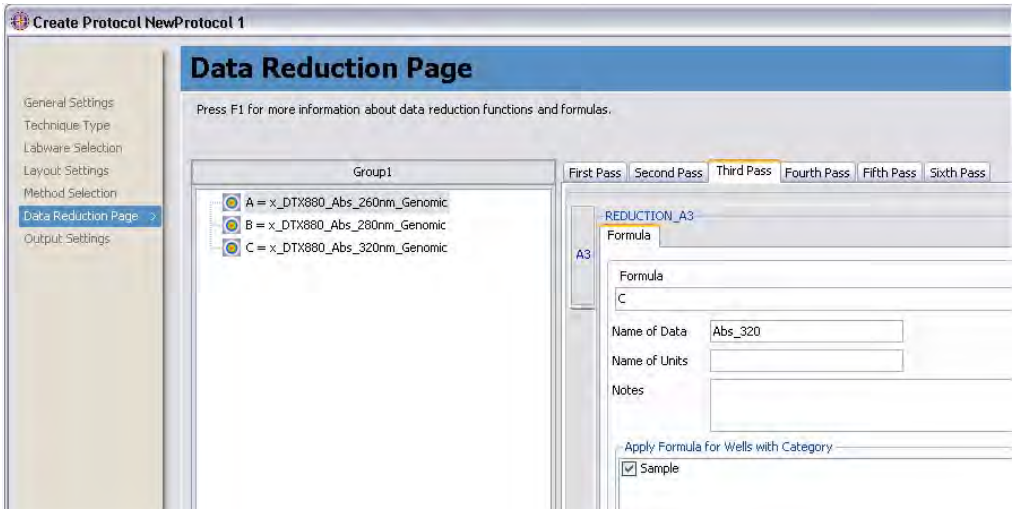


Figure 89 Data Reduction Page—Third Pass

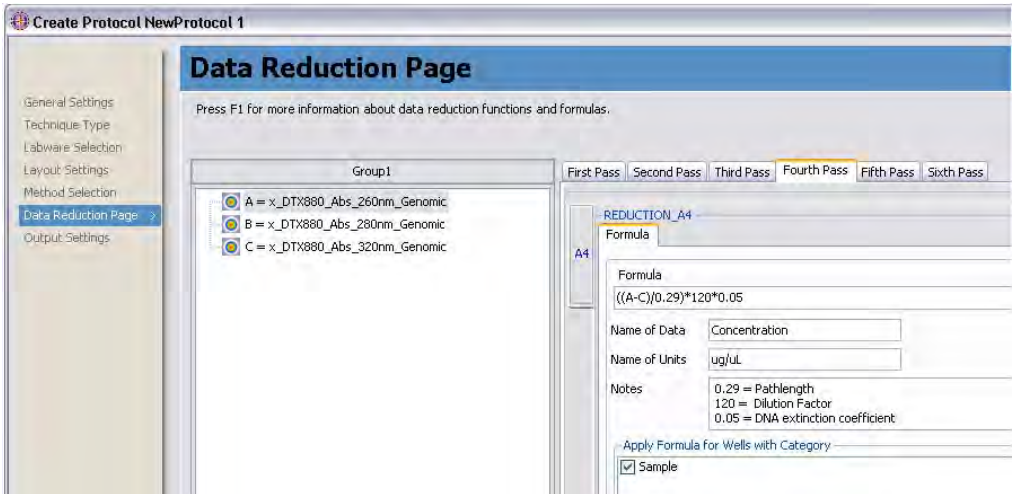


Figure 90 Data Reduction Page—Fourth Pass

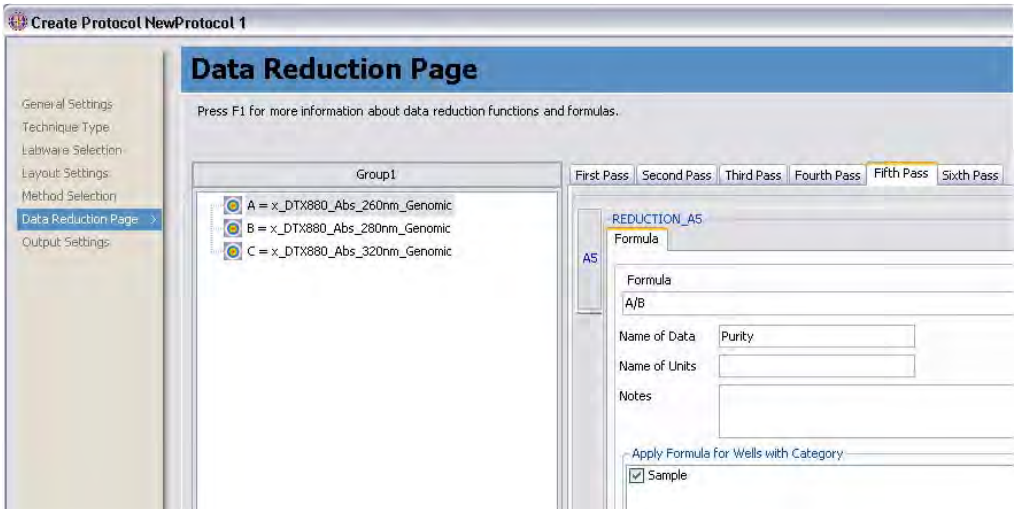


Figure 91 Data Reduction Page—Fifth Pass

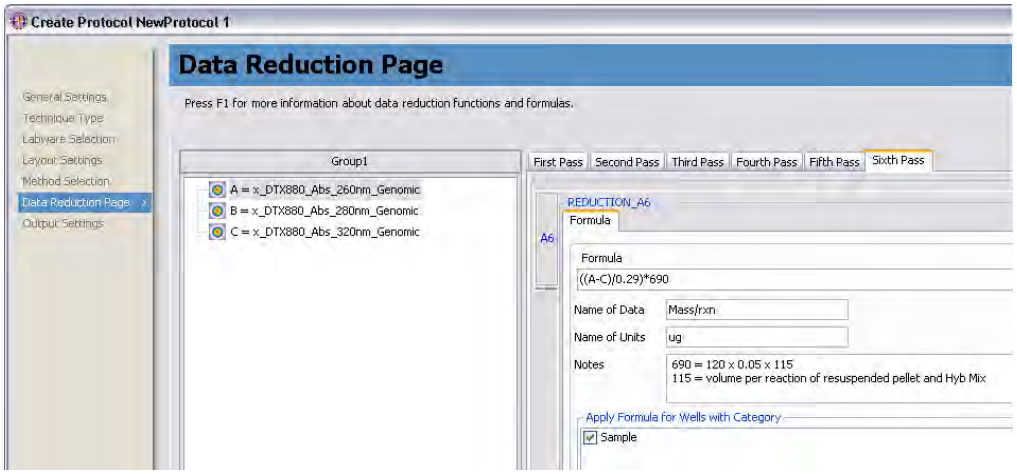


Figure 92 Data Reduction Page—Sixth Pass

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

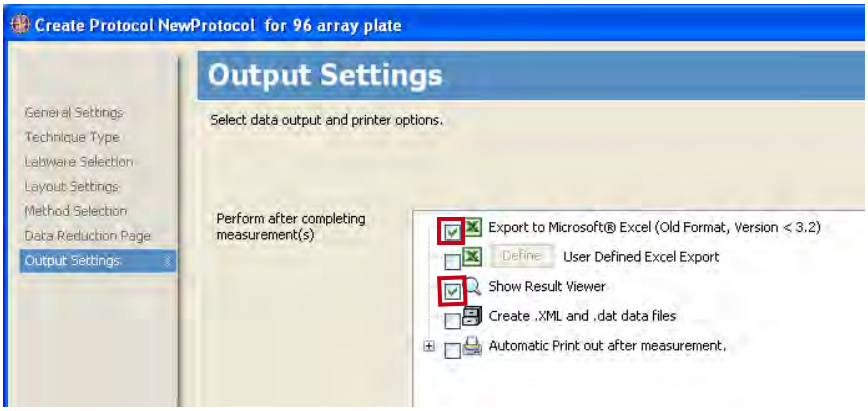


Figure 93 Output Settings

Save the protocol.

Perform 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 workflow)

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 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. 703460, 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 80 on page 181 and against "OD yield assessment guidelines" on page 181.

Register Samples in GeneChip™ Command Console™

Create 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).

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

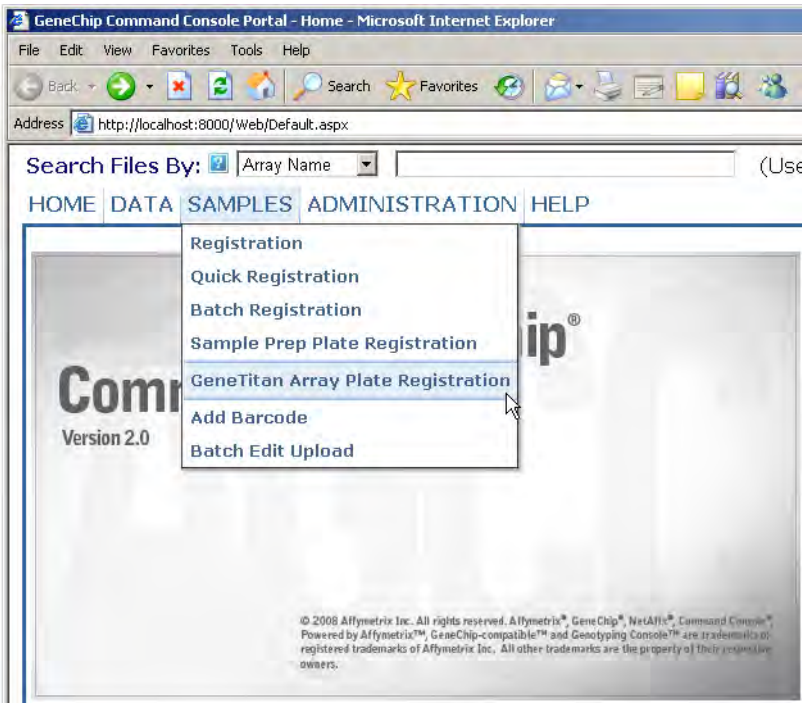


Figure 94 Selecting GeneTitan Array Plate Registration

2. Step 1—Figure 95 on page 190:
   a. Select the array plate type.
   b. Click **Download**.

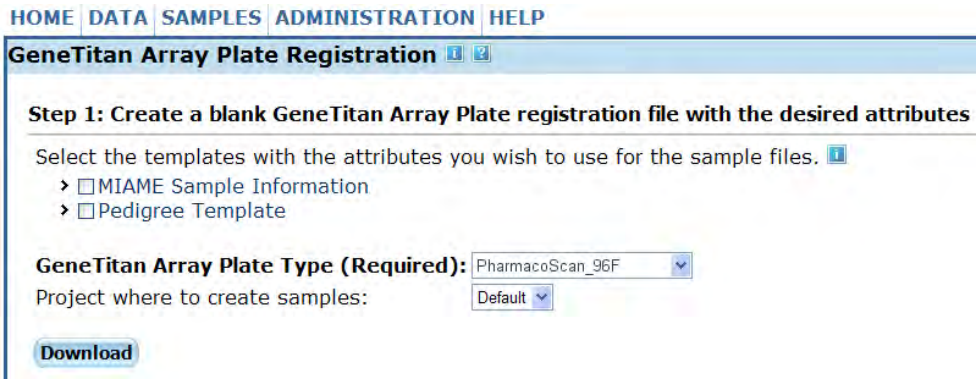
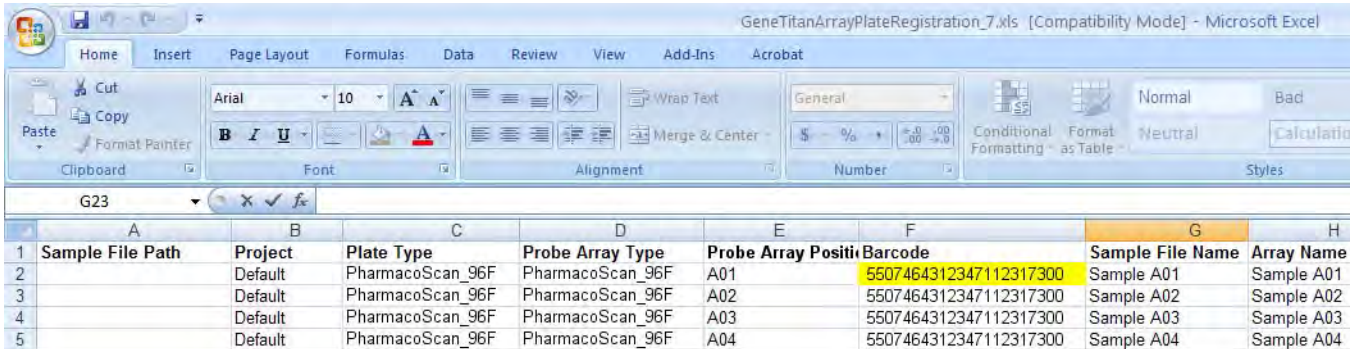


Figure 95 Selecting the type of array plate to be processed

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.



	A	B	C	D	E	F	G	H
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array Positi	Barcode	Sample File Name	Array Name
2		Default	PharmacoScan_96F	PharmacoScan_96F	A01	550746431234711231730O	Sample A01	Sample A01
3		Default	PharmacoScan_96F	PharmacoScan_96F	A02	550746431234711231730O	Sample A02	Sample A02
4		Default	PharmacoScan_96F	PharmacoScan_96F	A03	550746431234711231730O	Sample A03	Sample A03
5		Default	PharmacoScan_96F	PharmacoScan_96F	A04	550746431234711231730O	Sample A04	Sample A04

Figure 96 Entering sample information into a GeneTitan Array Plate Registration file.

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 97), 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 98 is displayed.

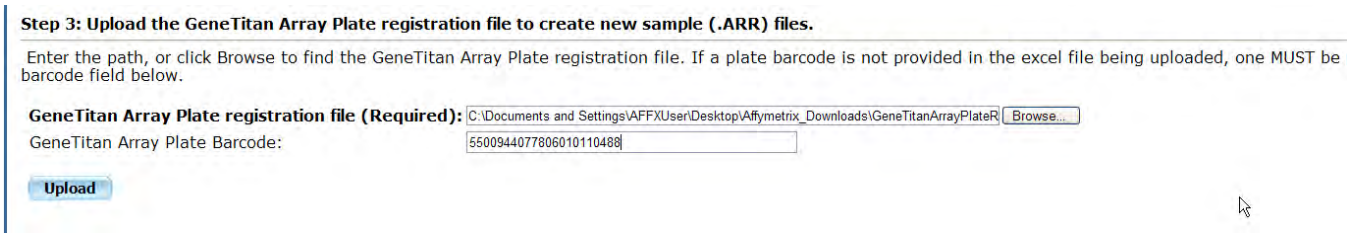


Figure 97 Uploading the array plate registration file to GCC.

Figure 98 Array plate samples successfully registered.

D

Deionizing procedure for GeneTitan™ trays and covers

We recommend the use of the Zerostat 3 Anti-Static Gun (Cat. No. 74-0014) to deionize GeneTitan™ MC Instrument stain tray trays and covers.

IMPORTANT! Except for the 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 99, Figure 100 and Figure 101.

Deionize the inner surface of each tray and cover:

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

⚠ **CAUTION!** Do not deionize the scan tray or hybridization tray.

Figure 99 Scan Tray with Cover. Deionize only the cover.

Figure 100 Stain Tray with Cover. Deionize the cover and the tray.

Deionization procedure

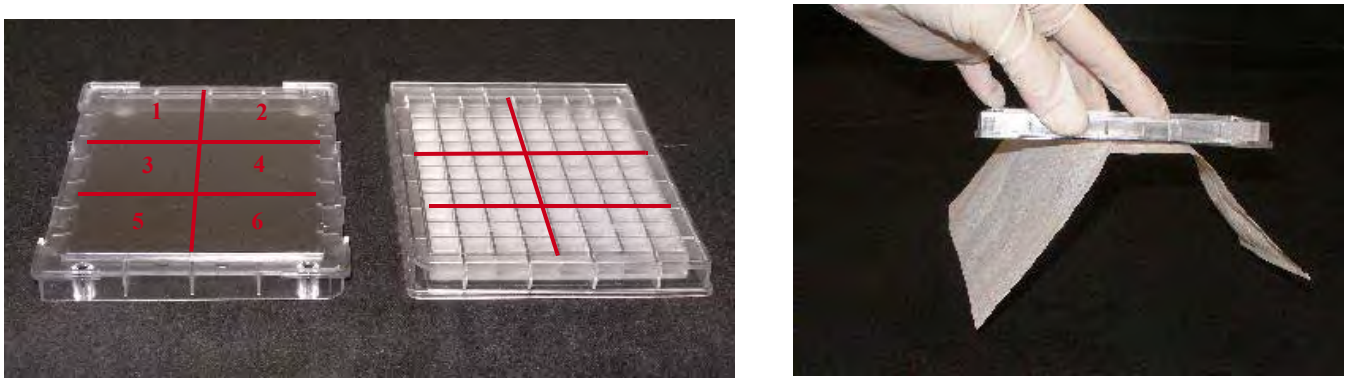
The following process provides guidance on how to use the anti-static gun on the stain tray, and stain and scan tray covers only. See Figure 101.

⚠ **WARNING!** The deionization steps 4 and 5 damage the arrays on the plate. Before using the anti-static gun, ensure that the 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 cover as if it were divided into six sections (see Figure 101), and deionize as follows.
2. Place a Kimwipe laboratory tissue 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 three 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 laboratory tissue.
5. Aim the anti-static gun (Cat. No. 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 three 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 laboratory tissue and lift it up (see Figure 101).
7. Do one of the following:
   - If the tissue does not cling to the plastic, proceed with the protocol.
   - If the tissue 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 might be time to replace the unit.

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



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

Figure 101 Removing the static charge from stain trays and covers.

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 (Cat. No. 74-0014, Figure 101).

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 102).

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 covers.

Figure 102 Zerostat 3 Anti-Static Gun (Cat. No. 74-0014) with ion-indicator cap to test functionality.

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 might 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! Ensure to remove the cap from the gun before deionizing a tray or cover.

E

GeneTitan™ Multi-Channel Instrument care

- Introduction 196
- Cleaning and maintenance 196
- Troubleshooting 205

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 is off or unused overnight or longer. This prevents 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 can 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.

Service the outer enclosure fan filters

Schedule

The GeneTitan fan filter cartridge (Figure 103) should be cleaned at least every 90 days of service. Note that in some service locations, the presence of excessive dust or particulate matter can 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: Thermo Fisher Scientific P/N: 01-0669
- Number of filters required per GeneTitan instrument: 3

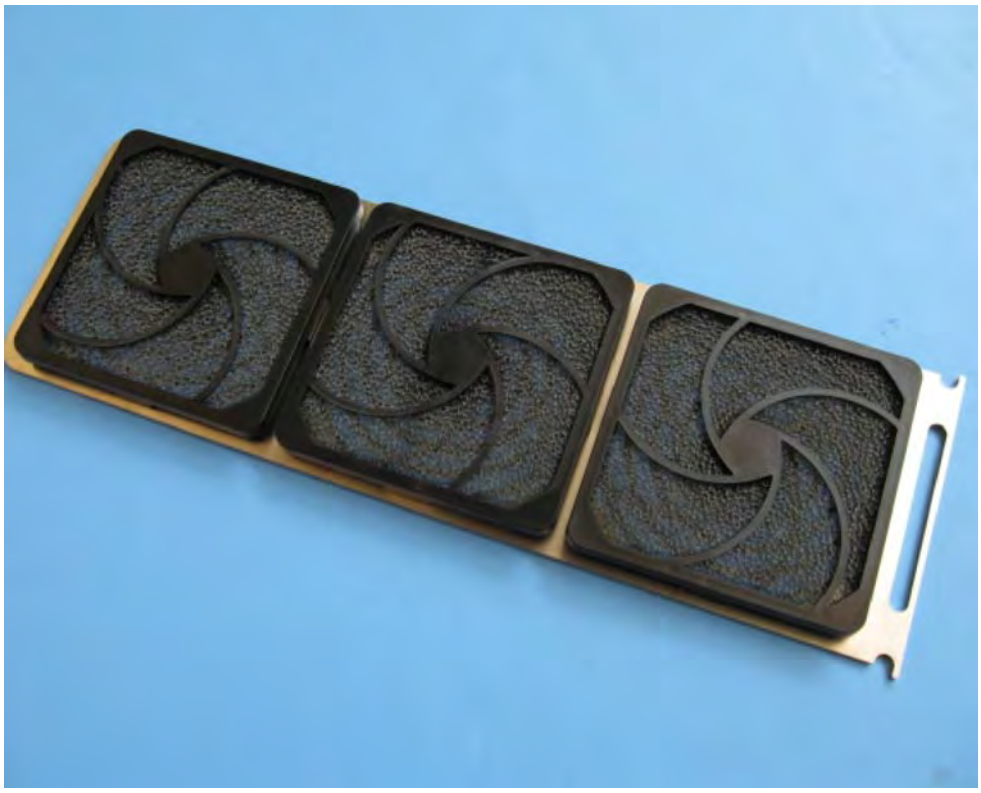


Figure 103 The GeneTitan filter cartridge

Clean the GeneTitan filter cartridge

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.

Replace the bottle filters

The bottles used in GeneTitan Instrument contain a filter to remove particulates that can 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 104) 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.

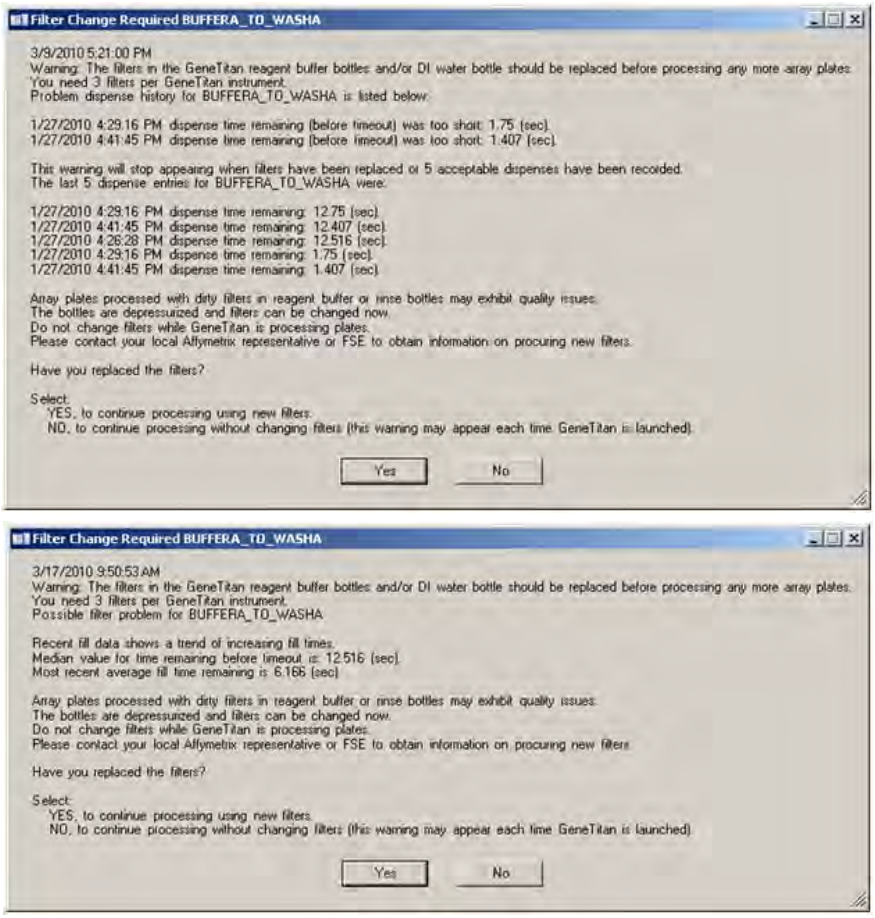


Figure 104 Filter Change Required Messages

The message boxes displayed in Figure 104 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.

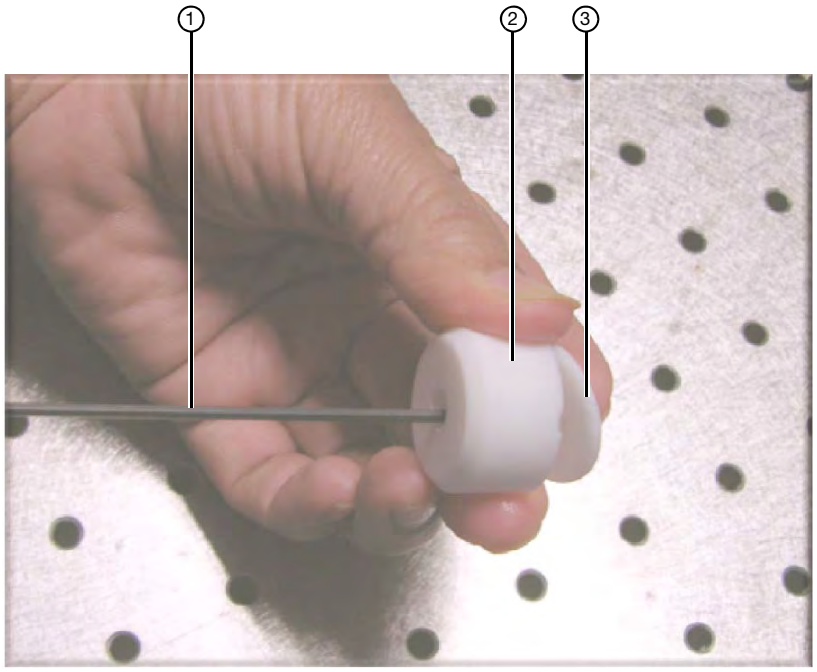
Note: The reagent bottles are depressurized when this warning message is displayed. It is safe to change the filters in all three fluidic bottles when this message is displayed.

After changing the filters in all three bottles using the procedure described below, press the **Yes** button to continue. If you choose to ignore the error message, press the **No** button. This warning message is displayed each time GCC instrument control software is launched. You can 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: Thermo Fisher Scientific P/N: 01-0671



① Buffer supply line
② Filter holder
③ Filter

Figure 105 Replacing the filter

Remove and inspect 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.

Replace 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.

Replace 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 alerts you to the requirement to replace the lamp. This procedure is simple but you must follow good health and safety precautions.

- Thermo Fisher Scientific Cat. No. 01-0740

IMPORTANT! 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 106):

Figure 106 Lamp Life above tolerance

It displays a red or yellow notice when the lamp life is getting short (Figure 107):

Figure 107 Lamp Life above tolerance

It also displays a red notice when the Imaging Device is offline (Figure 108):

Figure 108 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

Remove the xenon lamp

1. Unscrew the four retaining bolts. They should be finger tight (Figure 109).

Figure 109 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 110). You must use both hands to remove the lamp successfully. Apply equal pressure on each side of the lamp and gently lift.

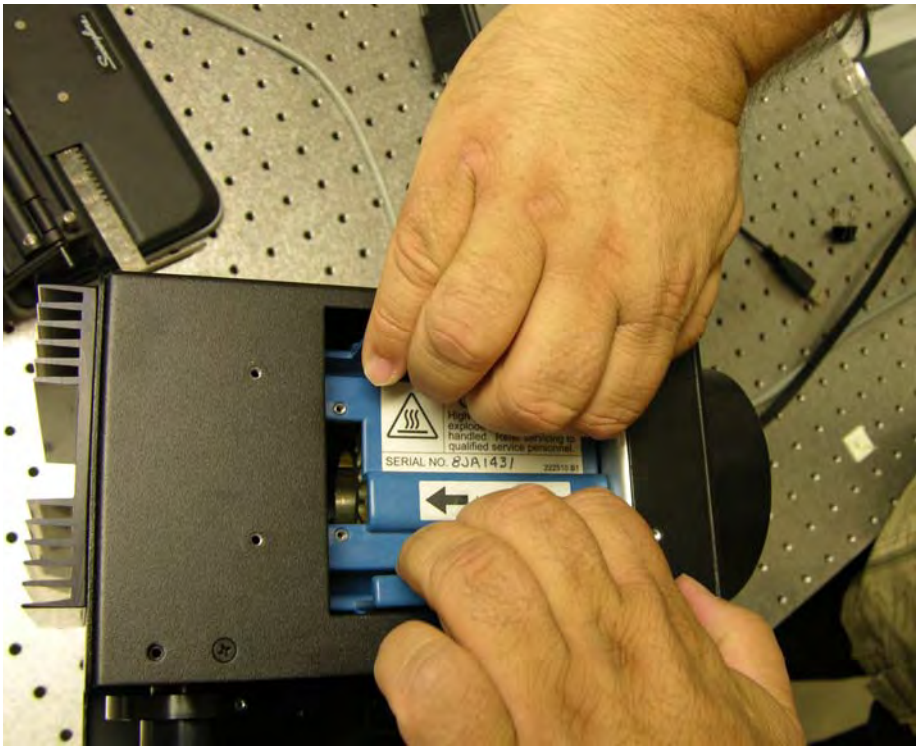


Figure 110 Lift out the lamp

Replace 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 111) and vertically insert the lamp (Figure 112).
2. Replace the warning cover and hand tighten the bolts (Figure 109).

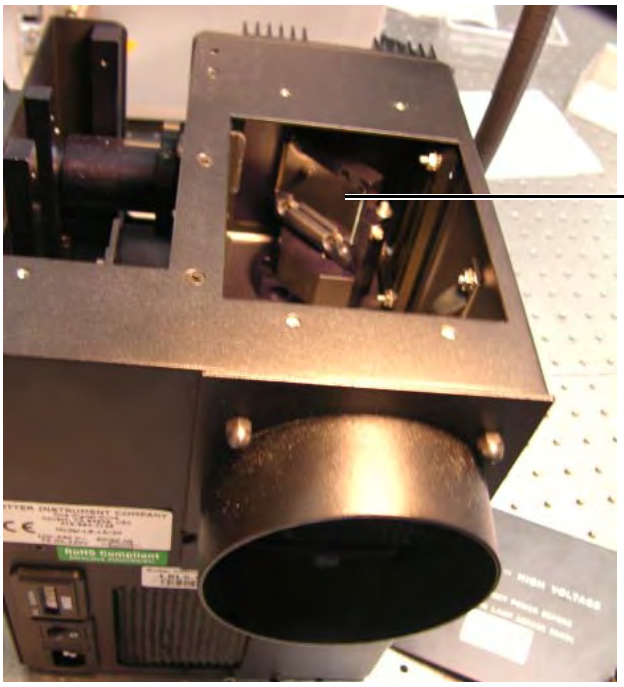


Figure 111 The reflecting mirror

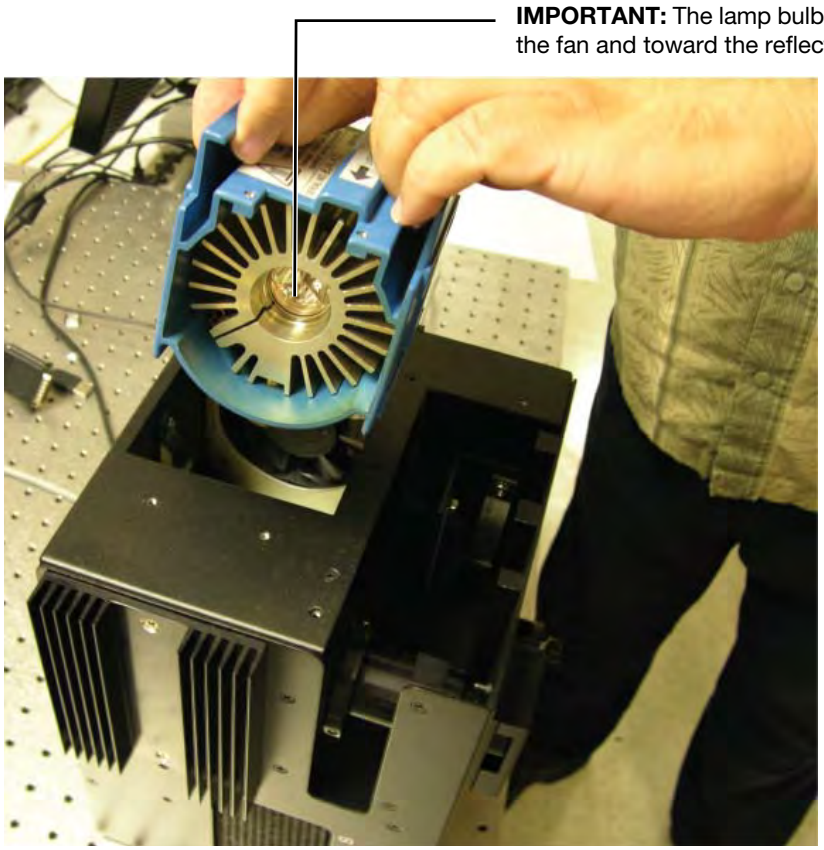


Figure 112 Insert the lamp

Reset 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 113).

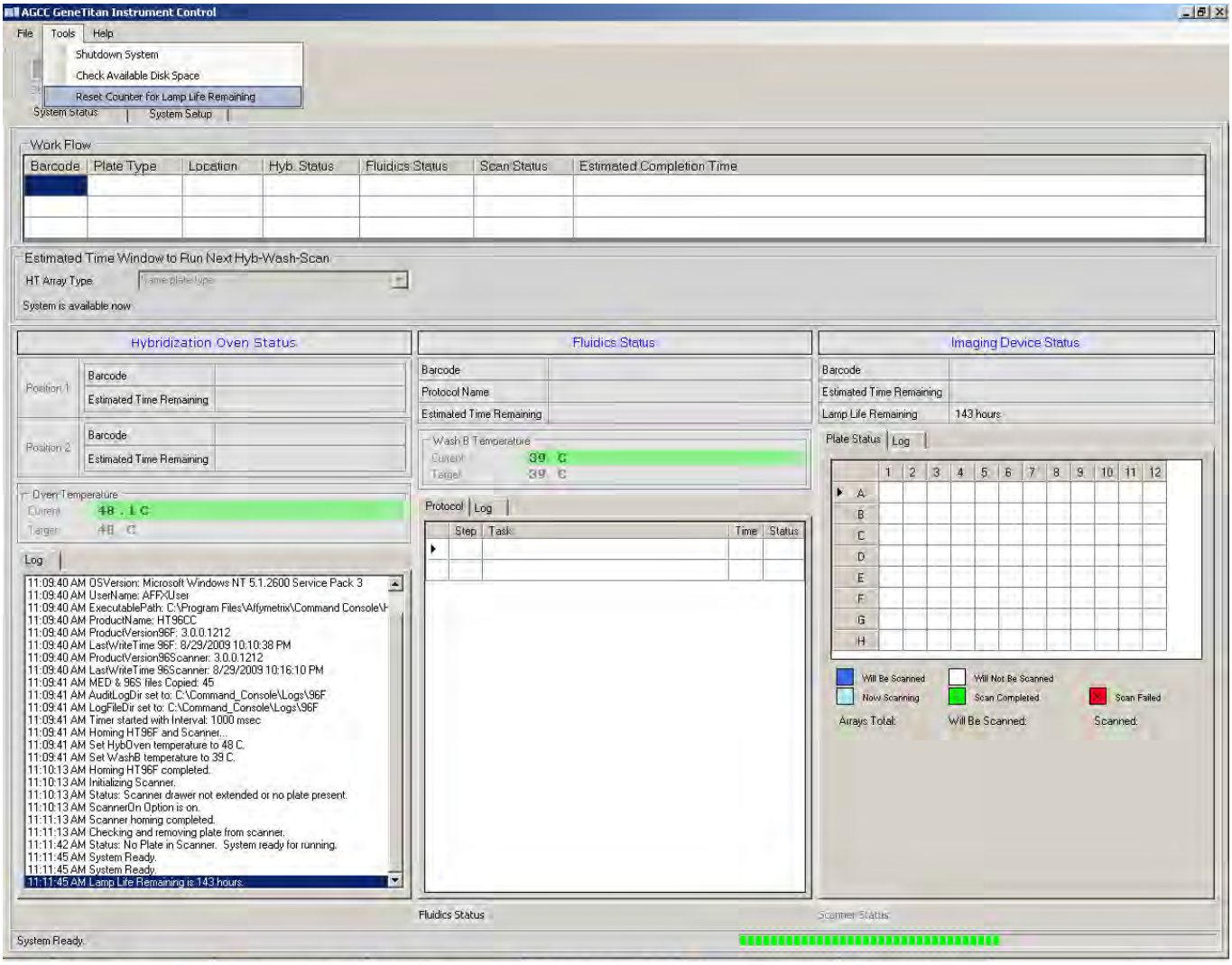


Figure 113 Inserting the Lamp

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

Figure 114 Are you sure?

3. Click **Yes** if you want to reset the counter. The software displays a message that confirms that the software has reset the counter (Figure 115).

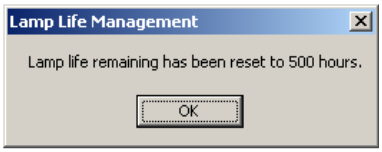


Figure 115 The counter is reset

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 Thermo Fisher Scientific 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 GCC 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 can be requested by your field application scientist (FAS), field service engineer (FSE), or when contacting Technical Support.

GCC log files

The following files apply to the GeneTitan Instruments. All the GCC 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 (DEC).
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 GCC files

Your FAS and/or FSE can 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. Ensure there are no duplicate library files, as these can cause problems.
3. GCC 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 GCC 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.

GCC 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. Thermo Fisher Scientific might 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)
      - 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).
      - 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 Thermo Fisher Scientific 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 (Figure 116) when:

- You first initialize the software and instrument
- You select arrays for imaging.

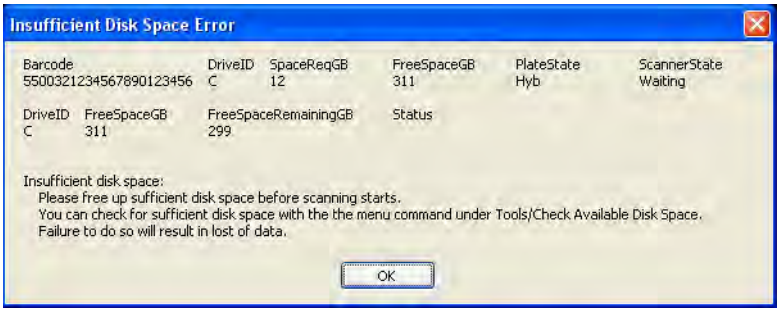


Figure 116 Insufficient Disk Space notice

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

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 result in differences in DNA band patterns and amplicon intensities, and therefore sample to sample variation can be observed.

Equipment required

Table 66 Equipment required

Item	Supplier	Cat. No.
Gel imager	Various	—
Pipette, multi- and single-channel	Various	—
Plate centrifuge	Various	—
Vortex	Various	—

E-Gels and reagents

Table 67 E-Gel and reagents required

Item	Supplier	Cat. No.
Mother E-Base Device	Thermo Fisher Scientific	EB-M03
Daughter E-Base Device		EB-D03
E-Gel® *48* 2% agarose gels		G8008-02
TrackIt™ Cyan/Orange Loading Buffer		10482-028
Reduced EDTA TE Buffer (10 mM Tris-HCL PH 8.0, 0.1 mM EDTA)		75793
NEB 50 bp DNA Ladder	New England BioLabs	N3236S
Diluted TrackIt Cyan/Orange Loading Buffer	See "Dilute the TrackIt™ Cyan/Orange Loading Buffer and 25 bp ladder" on page 178	

Consumables

Table 68 Consumables Required

Item	Supplier	Cat. No.
Adhesive film – use one of the following: • MicroAmp Clear Adhesive Film • Microseal 'B' Film	 Thermo Fisher Scientific Bio-Rad	 4306311 MSB1001
Pipette Tips	Same brand as pipette	—
96-well PCR plate	Various	Various
1.7 mL microcentrifuge tube	Various	Various

Prepare NEB 50 bp DNA ladder

Dilute the NEB 50 bp ladder (Cat. No. 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 1,000-fold diluted TrackIt dye.
2. Vortex tube to mix well. Pulse-spin to get droplets down.

Prepare 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 1,000-fold diluted TrackIt dye.

Run the mPCR QC gel protocol

This protocol is based on running QC gels for 96 samples. Two E-Gel® 48 2% agarose gels is needed.

1. Tightly seal the *mPCR Gel QC plate*.
2. Vortex the plate for three seconds. 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 minutes
11. Image the gel.

mPCR QC Gel images should look similar to the gel shown in Figure 117.

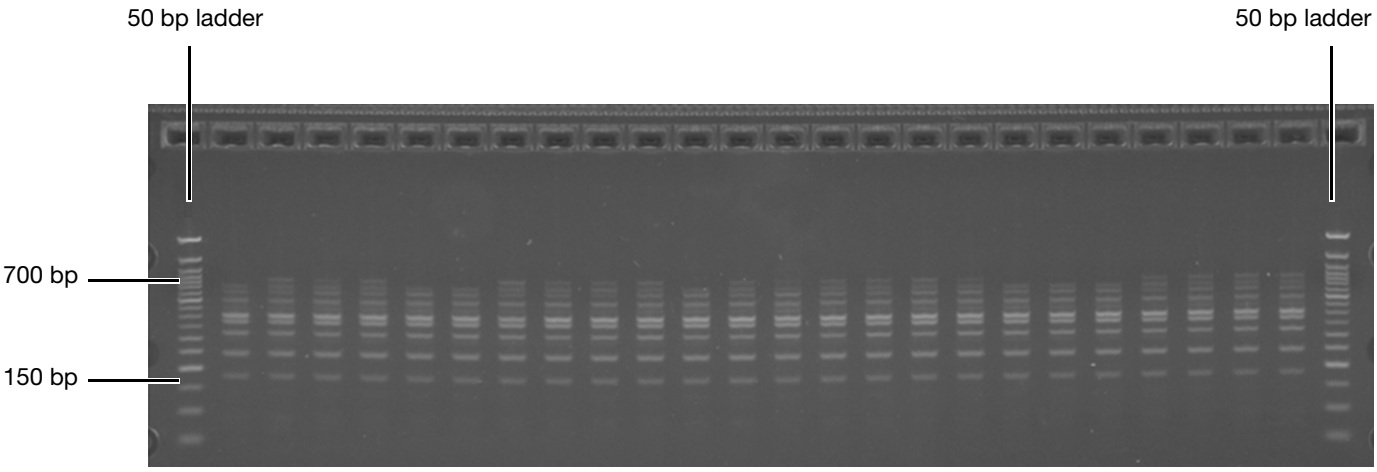


Figure 117 Example of a typical mPCR QC E-gel

Note: Variation in DNA band patterns and intensities can be 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.

Safety

⚠ **WARNING! GENERAL SAFETY**. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and support" section in this document.

⚠ **WARNING!** The following components contain harmful or toxic ingredients:

- Axiom Stabilize Soln: 8% gluteraldehyde
- Axiom Hyb Soln 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.

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.

Chemical safety

⚠ **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/ provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/ CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Table 69

Document	Publication number	Description
PharmacoScan™ Assay 96-Array Format Manual Workflow Site Preparation Guide	703460	Provides guidance on reagents, instruments, and supplies required to run the PharmacoScan Assay 96-Array Format Manual Workflow.
PharmacoScan™ Assay 96-Array Format Manual Workflow Quick Reference	703461	An abbreviated reference for the target preparation step of the PharmacoScan Assay 96-Array Format Manual Workflow. This quick reference document is intended for experienced users.
PharmacoScan™ Assay 24-Array Format Manual Workflow User Guide	703286	This user guide provides comprehensive instructions on running the PharmacoScan Assay 24-Array Format Manual Workflow.
PharmacoScan™ Assay 24-Array Format Manual Workflow Site Preparation Guide	703287	Provides guidance on reagents, instruments, and supplies required to run the PharmacoScan Assay 24-Array Format Manual Workflow.
PharmacoScan™ Assay 24-Array Format Manual Workflow Quick Reference	703288	An abbreviated reference for the target preparation step of the PharmacoScan Assay 24-Array Format Manual Workflow. This quick reference document is intended for experienced users.
Axiom™ Genotyping Solution Data Analysis Guide	702961	This guide provides information and instructions for analyzing Axiom genotyping array data. It includes the use of Axiom™ Analysis Suite, Power Tools (APT) and SNPolisher R package to perform quality control analysis (QC) for samples and plates, SNP filtering prior to downstream analysis, and advanced genotyping methods.
GeneTitan™ MC Protocol for Axiom 2.0 Array Plate Processing Quick Reference	702988	A quick reference document for experienced GeneTitan users processing Axiom and PharmacoScan array plates.
GeneTitan™ Multi-Channel Instrument User Guide	08-0308	The GeneTitan Multi-Channel (MC) Instrument automates array processing from target hybridization to data generation by combining a hybridization oven, fluidics processing, and state-of-the art imaging device into a single bench-top instrument. This document detailing the use, care, and maintenance for the GeneTitan MC Instrument.

Table 69

Document	Publication number	Description
GeneTitan™ Multi-Channel Instrument Site Preparation Guide	08-0305	Provides guidance on creating and maintaining the proper environment required for the GeneTitan Multi-Channel Instrument.
Analysis and software		
Axiom™ Genotyping Solution Data Analysis Guide	702961	This guide provides information and instructions for analyzing Axiom genotyping array data. It includes the use of Axiom™ Analysis Suite, Power Tools (APT) and SNPolisher R package to perform quality control analysis (QC) for samples and plates, SNP filtering prior to downstream analysis, and advanced genotyping methods.
Applied Biosystems ™ GeneChip™ Command Console™ Software User Guide	702569	This user guide provides instructions on using Applied Biosystems GeneChip Command Console Software (GCC) used to control GeneChip instrument systems. Command Console Software provides an intuitive set of tools for instrument control and data management used in the processing of arrays.
Axiom™ Analysis Suite 3.0 User Guide	703307	This user guide provides instructions on using Axiom™ Analysis Suite—a single-source software package to enable complete genotyping analysis of all Axiom and PharmacoScan arrays.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

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- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, contact Life Technologies at **thermofisher.com/support**.