Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow

USER GUIDE

for use with:
Axiom™ PharmacoFocus™ Mini 96-Array Plate
Axiom™ PharmacoFocus™ Mini 96-Array Plate Core
Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit

Catalog Numbers 952380, 952424, and 952389

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ThermoFisher SCIENTIFIC

Thermo Fisher Scientific Baltics UAB |
V.A. Graiciuno 8, LT-02241 |
Vilnius, Lithuania

Products:
Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit

Affymetrix Pte Ltd |
7 Gul Circle #2M-01 |
Keppel Logistics Building |
Singapore 629563

Products:
Axiom™ PharmacoFocus™ Mini 96-Array Plate
Axiom™ PharmacoFocus™ Mini 96-Array Plate Core

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. MAN0019172

Revision	Date	Description
C.0	12 October 2020	• Added Axiom™ PharmacoFocus™ Mini 96-Array Plate Core product information. • Added Bio-Rad HSP9601 as an option to Bio-Rad HSP9631. • Added ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate as an option to Eppendorf™ DeepWell™ Plate 96. • Clarified assay plate naming in Stage 4.
B.0	18 August 2020	Added Axiom™ GeneTitan™ 384HT Consumables Kit (Cat. No. 952385), clarified workflow procedures, and standardized assay plate names.
A.0	29 June 2020	New publication.

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1 Overview

About the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow

Axiom™ PharmacoFocus™ Assay is a targeted pharmacogenomic research solution designed to provide scientific insight into the absorption, distribution, metabolism, and excretion (ADME) and transport of commonly prescribed medicines. By interrogating ~2,000 markers in ~150 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.

The Axiom™ PharmacoFocus™ Assay uses the proven GeneTitan™ Multi-Channel (MC) 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 multiyear data collection and analysis efforts.

About the Axiom™ PharmacoFocus™ Assay

The Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow is available as a bundled kit that includes the arrays and reagents needed for processing one Axiom™ PharmacoFocus™ Mini 96-Array Plate, each having 94 samples and 2 controls.

The Axiom™ PharmacoFocus™ Assay interrogates biallelic and multiallelic single nucleotide polymorphisms (SNPs), simple insertion/deletions (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 hybridization-ready targets are then transferred to the GeneTitan™ MC Instrument for automated, hands-free processing including hybridization, staining, washing, and imaging.

The Axiom™ PharmacoFocus™ Assay provides researchers with pharmacogenomic variation information for ~2,000 markers in nearly 150 genes, including critical alleles in high value genes inclusive of CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2C8, CYP2D9, GSTM1 and SULT1A1. This content is

sourced from globally endorsed consortium databases including, but not limited to, CPIC, PharmGKB, and PharmaADME and with emphasis on variants with highest levels of evidence per PharmGKB. Also included on Axiom™ PharmacoFocus™ Array Plates are high value markers for human leukocyte antigen (HLA) imputation, markers for human ancestry identification (AIM), and markers for sample ID and tracking. Axiom™ PharmacoFocus™ Array Plates are offered in 2 configurations: Axiom™ PharmacoFocus™ Array Plates, featuring star allele reporting, and Axiom™ PharmacoFocus™ Array Plates Core, without the star alleles reports.

The Axiom™ PharmacoFocus™ Assay is a multiplex genotyping assay that combines the proven Axiom™ chemistry with the incorporation of a multiplex PCR step to overcome some complexities associated with genotyping highly homologous markers. Axiom™ PharmacoFocus™ 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™ GCC 4.3 or later. The resulting CEL files are analyzed by Axiom™ Analysis Suite 5.1.1 or later, or by Applied Biosystems™ Array Power Tools 2.11.3 or later.

For more information, see “Related documentation” on page 217.

Assay steps

Running the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow requires the following steps:

1. Genomic DNA preparation—Resulting in samples that meet requirements outlined in “Genomic DNA preparation” on page 19.
2. A multiplex PCR step (mPCR) followed by target preparation of the samples. See Chapter 4, “Multiplex PCR and target preparation”.
3. Array Processing using
   - GeneTitan™ MC Instrument
   - GeneTitan™ Instrument Control software
   - GCC Portal software

   See Chapter 5, “Process array plates with the GeneTitan™ Multi-Channel Instrument”.

A list of the required equipment and supplies for running the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow can be found in the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0019173.

Overview of the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow

gDNA plate setup 5 ng/µL of gDNA			
Day 1	▼ — ▼		
	10 µL/well	20 µL/well	10 µL/well from the gDNA Sample Plate is used for Stage 1A, and 20 µL/well from the gDNA Sample Plate is used for Stage 1B.
Target preparation			
Day 1	Stage 1A: Perform multiplex PCR (mPCR)	Stage 1B: Amplify the genomic DNA	~4.5 hours
	▼	▼	🛑 23 ±1 hour amplification incubation at 37°C. Optional stopping point.
Day 2	mPCR Product Plate	Amplification Plate	10 µL mPCR spike-in to Amplification Plate
	▼	▼	
Day 2	Stage 2: Fragment and precipitate the DNA		Approximate time: 16–24 hours (or optional 3-hour precipitation step) –20°C incubation of Precipitation Plate
	▼		
Day 3	Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC		🛑 Optional stopping point.
	▼		
Day 3	Stage 4: Transfer, denature, then hybridize the denatured samples		23.5 to 24-hour array hybridization in the GeneTitan™ MC Instrument.
	▼		
Day 4	Stage 5: Prepare the GeneTitan™ reagents and trays		
	▼		
Array processing			
Day 4	Chapter 5, Process array plates with the GeneTitan™ Multi-Channel Instrument *Array processing is completed with the GeneTitan™ MC Instrument and GeneChip™ Command Console™ v4.3 or later.*		Fluidics: ~5 hours Scan: ~1.5 hours

Multiplate workflows

Thermo Fisher Scientific supports high-throughput workflows that allow you to run a set of samples and array plates through the protocol using a minimum number of personnel in a 40-hour week. The timing of steps is critical because of the following limits:

- Incubation for DNA amplification is 22–24 hours.
- Hybridization in the GeneTitan™ MC Instrument is 23.5–24 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.

For more information, contact your local support representative.

Assay features

The *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow User Guide* provides instructions for manual target preparation and processing of Axiom™ PharmacoFocus™ Mini 96-Array Plates on the GeneTitan™ MC Instrument.

Target preparation for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow is done in 96-array format. The hybridization-ready target is hybridized onto a partially populated 384-array format array plate, referred to as mini 96 layout. The switch from a 96-array format to a 384-array format occurs when the user transfers the hybridization-ready samples after thermal denaturation from a 96-array format PCR plate to a 384-array format GeneTitan™ Hybridization Tray.

Figure 1 shows the mini 96-array format plate layout. The arrays are glued only to the quadrant 1 positions of a 384-array format plate. Quadrant 1 refers to the odd column well positions in rows A/C/E/G/I/K/M/O.

Figure 1 Mini 96-array format plate (bottom view).

1 Position A1

GeneTitan™ reagent tray barcodes

GeneTitan™ MC Instrument consumables and Applied Biosystems™ GeneChip™ Command Console™ v4.3 or higher are required for the preparation of the Axiom™ 2.0 stain reagents. Each tray has a unique part number and barcode that offers traceability. These trays have the following labels and barcodes:

Figure 2 GeneTitan™ reagent tray barcodes and color-coded labels.

1. Stain 1 Tray—Part No. 501279
2. Stain 2 Tray—Part No. 501394
3. Ligation Tray—Part No. 501398
4. Stabilization Tray—Part No. 501396

The unique barcodes along with the GeneChip™ Command Console™ v4.3 or later software prevents users from making errors when placing the trays in the GeneTitan™ MC Instrument during stage 3 of the array processing. See “Stage 3: Ligate, wash, stain, and scan” on page 126 for instructions.

When manually plating the GeneTitan™ stain reagents, it is critical that the trays are filled with the reagent that corresponds to that particular stain tray. Stain trays filled with the incorrect reagent may lead to failure of the assay on the GeneTitan™ MC Instrument.

After the trays have been prepared, ensure that the trays are placed in the appropriate drawer location in the GeneTitan™ MC Instrument. Failure to place the proper tray in the correct location results in an error and the GeneTitan™ MC Instrument will not proceed with the processing of the trays. See “Proper tray alignment and placement” on page 168 for instructions.

GeneChip™ Command Console™ v4.3 or later also offers the facility for queuing a second plate for scanning before the first scan is complete. The software automatically moves the second plate into the scanner when the first plate has completed scanning. See “Queue a second plate for scanning” on page 125 for instructions.

2 Prepare genomic DNA

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 uniform amplification, the gDNA must be of high quality, and must be free of contaminants that can affect the enzymatic reactions to be performed.

The Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389) is used for this protocol. The kit contains 2 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 evaluate sample quality.

Assay performance can vary for gDNA samples that do not meet the general requirements. However, the reliability of any given result must be evaluated 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 laboratories at Thermo Fisher Scientific for DNA that meets the requirements for the Axiom™ PharmacoFocus™ Assay.

- Whole blood
- Buccal cell
- Saliva
- Cell lines

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

Note: DNA derived from formalin-fixed paraffin-embedded (FFPE) blocks must not be used with this assay.

General requirements

- Starting DNA must be double-stranded for accurate concentration determination.
- DNA must be of high purity. DNA must be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (that is, EDTA). The gDNA extraction/purification method must create DNA that is salt-free because high concentrations of particular salts can also inhibit enzyme reactions. DNA purity 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 in "Genomic DNA cleanup" on page 19.
- DNA must not be degraded. The average size of gDNA can be evaluated 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 comparison.

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

Special requirements

Preamplification 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 preamplification room or area separate from the main laboratory.

This preamplification area requires 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, however these areas may be combined due to space and equipment limitations.

Evaluate the quality of genomic DNA with 1% agarose E-Gel™

We recommend this quality control step to evaluate the quality of the gDNA before starting the assay.

Equipment and reagents required

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Mother E-Base™ Device	EBM03
Daughter E-Base™ Device (optional for running multiple gels in parallel)	EBD03
E-Gel™ *48* Agarose Gels, 1%	G800801
*Redi*Load™ Loading Buffer	750026
E-Gel™ 96 High Range DNA Marker	12352019

Guidelines for gDNA Sample Plate preparation

The following guidelines are recommended when preparing the gDNA Sample 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 to improve visualization. Loading ≥25-ng gDNA per well can improve the image.
- Add 3 µL of 0.1X of *Redi*Load™ Loading Buffer (*Redi*Load™ Loading Buffer dye diluted 10-fold with nuclease-free water) dye to each sample.
- Bring each sample to a total volume of 20 µL using nuclease-free water. For example, if the volume of genomic DNA is 5 µL, add 3 µL of *Redi*Load™ Loading Buffer, and bring to 20 µL total by adding 12 µL of water.
- Seal, vortex, and centrifuge briefly.

Run a 48 lane 1% agarose E-Gel™

1. Power on the E-Base™ Device (red light).
2. Push the **Power/Prg** button to ensure that the gel base is in **EG** mode (not EP).
3. Insert the E-Gel™ *48* Agarose Gels, 1% into the slot.
4. Remove 2 combs.
5. Load 20 µL of gDNA samples onto the E-Gel™ *48* Agarose Gels, 1%.
6. If needed, load 15 µL of diluted E-Gel™ 96 High Range DNA Marker (1:3 dilution or ~0.34X from stock) into all marker wells.
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 ready for imaging.

E-Gel™ results

The following figure shows gel images of intact gDNA (that is appropriate for use in the Axiom™ PharmacoFocus™ Assay) and degraded gDNA samples. For gDNA that is degraded perform a test experiment to investigate the performance of the samples in the Axiom™ PharmacoFocus™ Assay before starting any large-scale genotyping projects.

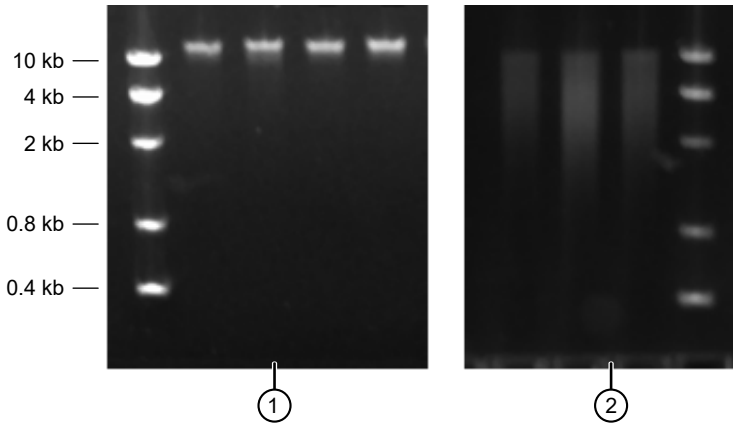


Figure 3 Gel images with intact gDNA and degraded gDNA.

① Intact samples ② Degraded samples

Genomic DNA extraction/purification methods

Genomic DNA extraction and purification methods that meet the general requirements that are outlined are expected to yield successful results. Methods that include boiling or strong denaturants are not acceptable because the DNA would be made single-stranded and can no longer be accurately quantified 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, then incubate at –20°C for 1 hour.
3. Centrifuge at 12,000 × *g* in a microcentrifuge at room temperature for 20 minutes.
4. Remove supernatant, then wash pellet with 80% ethanol.
5. Centrifuge at 12,000 × *g* at room temperature for 5 minutes.
6. Remove the 80% ethanol, then repeat the 80% ethanol wash one more time.
7. Resuspend the pellet in Low EDTA TE Buffer (10-mM Tris-HCl pH 8.0, 0.1 mM EDTA).

Genomic DNA preparation

This step must be done before proceeding with the mPCR and DNA amplification stages.

The genomic DNA (gDNA) you process using the Axiom™ PharmacoFocus™ Assay must meet the general requirements that are listed earlier in this chapter. The amount of gDNA is 50 ng for the mPCR step and 100 ng for the Axiom™ PharmacoFocus™ whole-genome amplification step.

Genomic DNA input requirements

Sample type	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

Time required

Allow 30–60 minutes for reagents to thaw and 30 minutes for setup.

Equipment, consumables, and reagents required

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Equipment and consumables required

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	96-deepwell plate: • ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate (Cat. No. AB0932) • Eppendorf™ DeepWell™ Plate 96 (Cat. No. 13-864-302)
1	96-well PCR plate: • Bio-Rad HSS9641 for Applied Biosystems™ GeneAmp™ PCR System 9700, Applied Biosystems™ Veriti™ Thermal Cycler, and Applied Biosystems™ ProFlex™ PCR System • Bio-Rad HSS9641, Bio-Rad HSP9631, or Bio-Rad HSP9601 for the Eppendorf™ Mastercycler™ pro S
1	Plate centrifuge
1	Plate spectrophotometer (required only if no OD measurements are available for samples)
1	Vortexer

Reagents required

Reagent	Source
From the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389)	
Control DNA 1 and Control DNA 2 (from Module B—Control DNA)	Part No. 912897
User-supplied	
Low EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	75793
Quant-iT™ PicoGreen™ dsDNA Assay Kit	P7589

Thaw samples and controls

Thaw the following components to room temperature.

- gDNA samples
- Control DNA 1 and Control DNA 2

To thaw, either:

- Place items on the benchtop for 60 minutes.
- Thaw in a water bath.
  a. Fill a small plastic dish with ultra-pure water. Do not overfill to prevent the level of the water overflowing when the sample tubes or plates are placed in the bath.
  b. Thaw the sealed gDNA Sample Plate and control DNA tubes for 30 minutes.
  c. Remove the gDNA Sample Plate and/or control DNA tubes from the water bath and wipe-dry using laboratory tissue. Ensure that the outside is dry before opening the gDNA Sample Plate or tube to minimize any contamination, which can lead to reaction failure.

Quantify and dilute test sample gDNA

1. Gently vortex (50% maximum), then centrifuge the gDNA.
2. *Recommendation*: Quantify each sample (for example, using the Quant-iT™ PicoGreen™ dsDNA Assay Kit).
3. Using Low EDTA TE Buffer, dilute each sample to a concentration of 5 ng/µL.
4. Seal, vortex, then centrifuge.

Note: Do not dilute the Control DNA 1 or Control DNA 2. They are already at the working concentration.

Note: We strongly recommend that you determine the sample concentrations using the Quant-iT™ PicoGreen™ dsDNA Assay Kit (Cat. No. P7589). Sample concentration that is determined by UV absorbance is often inaccurate and can yield different results.

Aliquot the diluted samples and the controls

The samples and controls are placed in a deep-well plate for DNA amplification and into a 96-well PCR plate for mPCR.

Note: Ensure that 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 Sample Plate and Amplification Sample Plate. Controls must be run every time the assay is performed.

Aliquot diluted samples and the controls to the deepwell Amplification Sample Plate

One of the following 96-deepwell plates is required for this procedure:

- ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate
- Eppendorf™ DeepWell™ Plate 96

1. Aliquot 20 µL of each diluted gDNA sample to the 96-deepwell plate. See Figure 4.
2. Pipet 20 µL of Control DNA 1 to well G12 and 20 µL of Control DNA 2 to well H12.
3. Seal, then centrifuge.

Aliquot diluted samples and controls to the mPCR Sample Plate

Use the appropriate 96-well PCR plate depending on the type of thermal cycler used.

- Bio-Rad™ 96-well PCR Plate (HSS9641): for Applied Biosystems™ GeneAmp™ PCR System 9700, Applied Biosystems™ Veriti™ Thermal Cycler, Applied Biosystems™ ProFlex™ System
- Bio-Rad™ 96-well PCR Plate (HSP9631 or HSP9601): for Eppendorf™ Mastercycler™ pro S

1. Aliquot 10 µL of each diluted gDNA sample to the 96-well PCR plate.
   See Figure 4.
2. Pipet 10 µL of Control DNA 1 to well G12 and 10 µL of Control DNA 2 to well H12.
3. Seal, then centrifuge.

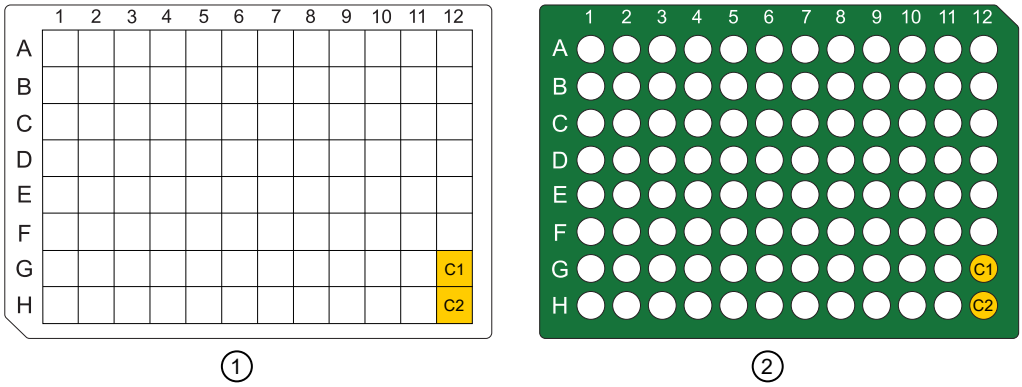


Figure 4 Aliquot genomic DNA to the Amplification Sample Plate and the mPCR Sample Plate.

- C1 = Control DNA 1
- C2 = Control DNA 2

(1) Amplification Sample Plate: 96-deepwell plate, 20 µL/well.
(2) mPCR Sample Plate: 96-well PCR plate, 10 µL/well.

Note: Analysis software will be able to identify controls regardless of well position. We recommend placement of controls in wells G12 and H12 for ease of use.

Freeze or proceed

Do one of the following:

- Store the sample plates at –20°C.
- Proceed to DNA amplification for manual target preparation. See Chapter 4, “Multiplex PCR and target preparation”.

Note: If proceeding immediately to mPCR and DNA amplification, you can leave the sample plates at room temperature.

GeneTitan™ Array Plate Registration file

Each array plate has a barcode for tracking and each row and column number identifies an individual array. The GeneTitan™ Array Plate Registration file is where you enter the sample information for each individual array of the array plate to be run. It is important to create and upload a GeneTitan™ Array Plate Registration file *before* loading the array plate and hybridization tray onto the GeneTitan™ Multi-Channel (MC) Instrument. We recommend that you create, but not upload, this file while you prepare your plate of genomic DNA. When samples are ready for hybridization, scan the array plate barcode and upload the file to Applied Biosystems™ GeneChip™ Command Console™ (GCC).

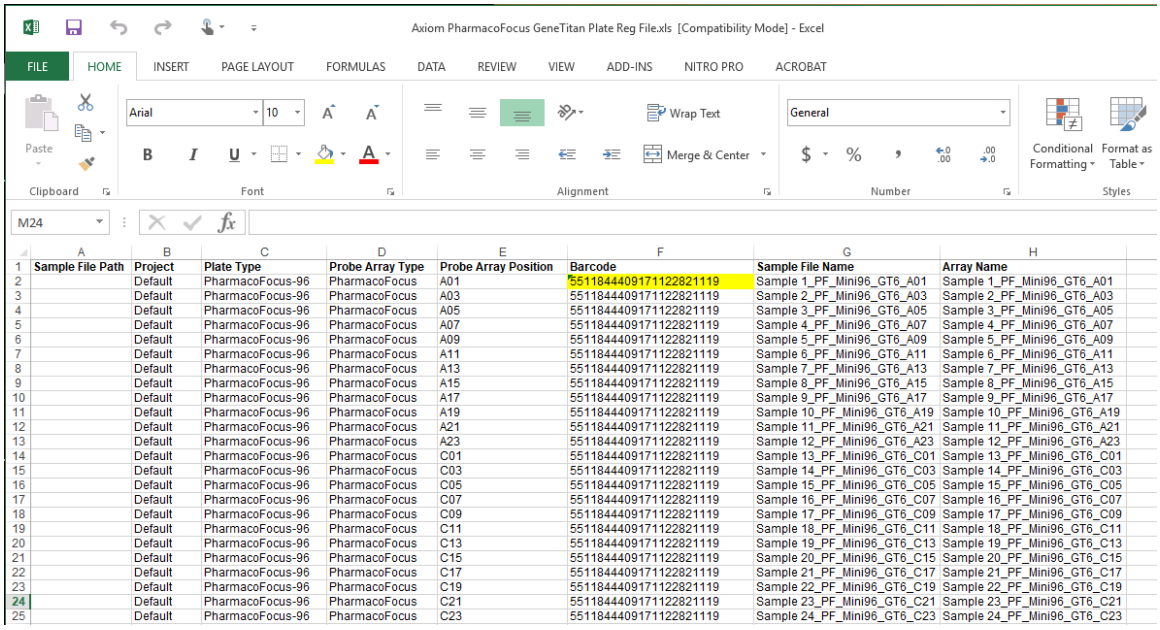
Create and save a GeneTitan™ Array Plate Registration file

This procedure creates and saves a GeneTitan™ Array Plate Registration file but does not upload the file to GeneChip™ Command Console™. The array plate and hybridization tray are scanned, and the GeneTitan™ Array Plate Registration file is uploaded when you are ready to load the plate and samples onto the GeneTitan™ MC Instrument for processing.

1. From the **Launcher** window, open **GCC Portal** ▸ **Samples** ▸ **GeneTitan™ Array Plate Registration**.
2. In the **GeneTitan Array Plate Registration** window, click to select a registration file template to use.
3. Select the **GeneTitan™ Array Plate Type** from the dropdown list.
4. Select the project for the sample files.
5. Click **Download**.
6. In the **Samples** tab of the **GeneTitan™ Array Plate Registration** window, enter a unique name for each sample and any additional information.

   For more information on the **GeneTitan™ Array Plate Registration** file, see *GeneChip™ Command Console™ User Guide* (Pub. No. 702569).
7. Save the file. Do not upload the file at this point.

Details for the array plate and hybridization tray scanning steps, and the GeneTitan™ Array Plate Registration file uploading steps are in Chapter 5, “Process array plates with the GeneTitan™ Multi-Channel Instrument”.



	A	B	C	D	E	F	G	H
1	**Sample File Path**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array Position**	**Barcode**	**Sample File Name**	**Array Name**
2		Default	PharmacoFocus-96	PharmacoFocus	A01	551184440917112282119	Sample 1_PF_Mini96_GT6_A01	Sample 1_PF_Mini96_GT6_A01
3		Default	PharmacoFocus-96	PharmacoFocus	A03	551184440917112282119	Sample 2_PF_Mini96_GT6_A03	Sample 2_PF_Mini96_GT6_A03
4		Default	PharmacoFocus-96	PharmacoFocus	A05	551184440917112282119	Sample 3_PF_Mini96_GT6_A05	Sample 3_PF_Mini96_GT6_A05
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8		Default	PharmacoFocus-96	PharmacoFocus	A13	551184440917112282119	Sample 7_PF_Mini96_GT6_A13	Sample 7_PF_Mini96_GT6_A13
9		Default	PharmacoFocus-96	PharmacoFocus	A15	551184440917112282119	Sample 8_PF_Mini96_GT6_A15	Sample 8_PF_Mini96_GT6_A15
10		Default	PharmacoFocus-96	PharmacoFocus	A17	551184440917112282119	Sample 9_PF_Mini96_GT6_A17	Sample 9_PF_Mini96_GT6_A17
11		Default	PharmacoFocus-96	PharmacoFocus	A19	551184440917112282119	Sample 10_PF_Mini96_GT6_A19	Sample 10_PF_Mini96_GT6_A19
12		Default	PharmacoFocus-96	PharmacoFocus	A21	551184440917112282119	Sample 11_PF_Mini96_GT6_A21	Sample 11_PF_Mini96_GT6_A21
13		Default	PharmacoFocus-96	PharmacoFocus	A23	551184440917112282119	Sample 12_PF_Mini96_GT6_A23	Sample 12_PF_Mini96_GT6_A23
14		Default	PharmacoFocus-96	PharmacoFocus	C01	551184440917112282119	Sample 13_PF_Mini96_GT6_C01	Sample 13_PF_Mini96_GT6_C01
15		Default	PharmacoFocus-96	PharmacoFocus	C03	551184440917112282119	Sample 14_PF_Mini96_GT6_C03	Sample 14_PF_Mini96_GT6_C03
16		Default	PharmacoFocus-96	PharmacoFocus	C05	551184440917112282119	Sample 15_PF_Mini96_GT6_C05	Sample 15_PF_Mini96_GT6_C05
17		Default	PharmacoFocus-96	PharmacoFocus	C07	551184440917112282119	Sample 16_PF_Mini96_GT6_C07	Sample 16_PF_Mini96_GT6_C07
18		Default	PharmacoFocus-96	PharmacoFocus	C09	551184440917112282119	Sample 17_PF_Mini96_GT6_C09	Sample 17_PF_Mini96_GT6_C09
19		Default	PharmacoFocus-96	PharmacoFocus	C11	551184440917112282119	Sample 18_PF_Mini96_GT6_C11	Sample 18_PF_Mini96_GT6_C11
20		Default	PharmacoFocus-96	PharmacoFocus	C13	551184440917112282119	Sample 19_PF_Mini96_GT6_C13	Sample 19_PF_Mini96_GT6_C13
21		Default	PharmacoFocus-96	PharmacoFocus	C15	551184440917112282119	Sample 20_PF_Mini96_GT6_C15	Sample 20_PF_Mini96_GT6_C15
22		Default	PharmacoFocus-96	PharmacoFocus	C17	551184440917112282119	Sample 21_PF_Mini96_GT6_C17	Sample 21_PF_Mini96_GT6_C17
23		Default	PharmacoFocus-96	PharmacoFocus	C19	551184440917112282119	Sample 22_PF_Mini96_GT6_C19	Sample 22_PF_Mini96_GT6_C19
24		Default	PharmacoFocus-96	PharmacoFocus	C21	551184440917112282119	Sample 23_PF_Mini96_GT6_C21	Sample 23_PF_Mini96_GT6_C21
25		Default	PharmacoFocus-96	PharmacoFocus	C23	551184440917112282119	Sample 24_PF_Mini96_GT6_C23	Sample 24_PF_Mini96_GT6_C23

Figure 5 Example of a GeneTitan™ Array Plate Registration file for Axiom™ PharmacoFocus™ Mini 96-Array Plate.

Note: The GeneTitan™ Array Plate Registration file for the Axiom™ PharmacoFocus™ Mini 96-Array Plate Core has a different **Plate Type** and **Probe Array Type** listed than the ones shown in example above.

3 Assay preparation

This chapter describes the procedures, equipment, and materials required for running the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow. To ensure operator safety and assay performance, operators must be familiar with this content before starting target preparation.

The manual assay format allows the user to run the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow for 96 samples using 1 Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit and 1 QIAGEN™ Multiplex PCR *Plus* Kit (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 manual target preparation. It is essential that you familiarize yourself with the information in this section prior to running the Axiom™ PharmacoFocus™ Assay.

The manual assay requires 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 Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow is provided in the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0019173.

Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit, arrays, and GeneTitan™ consumables required

This table lists the PharmacoFocus™ reagents and GeneTitan™ consumables required to process 1 Axiom™ PharmacoFocus™ Mini 96-Array Plate. The table also lists the QIAGEN™ Multiplex PCR *Plus* Kit required to run the Axiom™ PharmacoFocus™ Assay.

Quantity[1]	Description	Source
Axiom™ PharmacoFocus™ Mini 96-Array Plate products		
1[1]	Axiom™ PharmacoFocus™ Mini 96-Array Plate	952380
1	Axiom™ PharmacoFocus™ Assay Mini 96 Kit	952396
1	Axiom™ PharmacoFocus™ Assay Mini 96 Training Kit	952398
Axiom™ PharmacoFocus™ Mini 96-Array Plate Core products		
1[1]	Axiom™ PharmacoFocus™ Mini 96-Array Plate Core	952424
1	Axiom™ PharmacoFocus™ Assay Core Mini 96 Kit	952425
1	Axiom™ PharmacoFocus™ Assay Core Mini 96 Training Kit	952426
Common products for both array types		
1[1]	Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit	952389
1[1]	Axiom™ 384HT GeneTitan™ High Volume Consumables Kit (supports processing 5 array plates) or Axiom™ GeneTitan™ 384HT Consumables Kit (supports processing 1 array plate)	902629 or 952385
1[1]	QIAGEN™ Multiplex PCR *Plus* Kit (100)	206152

[1] Required to process 1 mini 96-array plate.

Requirements and recommendations

This section describes requirements and recommendations for facilities and equipment needed to perform the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow.

Room temperature

When referred to in the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow, room temperature is 18–25°C.

Special requirements

Preamplification/amplification staging 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 preamplification room or in an area separate from the main laboratory.

This preamplification area must 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.

Fume hood

At specific steps in the protocol, we recommend the use of adequate local or general ventilation to keep airborne concentrations low. A fume hood is suggested to achieve the desired concentration. A fume hood is recommended for several steps of this assay.

Control requirement

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 Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit.

Plate requirements for manual target preparation

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

See *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0019173, for supplier information.

- 96-deepwell plate: ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate or Eppendorf™ DeepWell™ Plate 96, 2 mL
- Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641) for the following thermal cyclers:
  - Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block)
  - Applied Biosystems™ Veriti™ Thermal Cycler
  - Applied Biosystems™ ProFlex™ PCR System
- Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (Cat. No. HSP9631 or HSP9601) for the following thermal cycler:
  - Eppendorf™ Mastercycler™ pro S
- Greiner Bio-One™ UV-Star™ 96-Well UV Spectroscopy Microplate, 370 µL/well (Cat. No. 07-000-407)

Thermal cycler recommendations and protocols

We have verified the performance of this assay using the following thermal cyclers in their 96-well metal chamber configurations. The use of other thermal cyclers may result in assay failure and may violate the array and reagent replacement policy. For more information, see "PCR plate type by thermal cycler for the mPCR step" on page 29.

- Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block)
- Applied Biosystems™ Veriti™ Thermal Cycler
- Applied Biosystems™ ProFlex™ System
- Eppendorf™ Mastercycler™ pro S

Note: Two verified thermal cyclers are required if running the 3-plate/week manual target preparation workflow.

IMPORTANT! Always use the heated lid option when programming protocols. See the appropriate thermal cycler user guide for programming information.

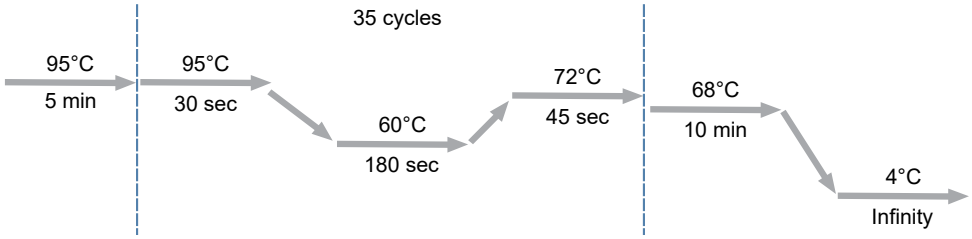


Figure 6 PharmacoFocus mPCR thermal cycler protocol (Stage 1A).

The mPCR step of the Axiom™ PharmacoFocus™ Assay has been verified with the Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block) Applied Biosystems™ Veriti™ Thermal Cycler, Applied Biosystems™ ProFlex™ System, and Eppendorf™ Mastercycler™ pro S. Use of other thermal cyclers for this stage can result in assay failure and violate the array and reagent replacement policy.

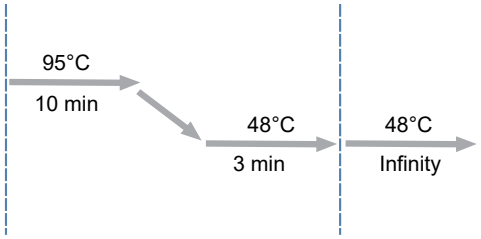


Figure 7 PharmacoFocus Denature thermal cycler protocol (Stage 4).

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

PCR plate type by thermal cycler for the mPCR step

The following table provides details about the consumables to be used with each thermal cycler when executing the mPCR step.

Thermal cycler model	PCR plate type	Seal[1]
Applied Biosystems™ GeneAmp™ PCR System 9700	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641)	MicroAmp™ Clear Adhesive Film (Cat. No. 4306311)
Applied Biosystems™ Veriti™ Thermal Cycler	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641)	MicroAmp™ Clear Adhesive Film (Cat. No. 4306311)
Applied Biosystems™ ProFlex™ PCR System	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641)	MicroAmp™ Clear Adhesive Film (Cat. No. 4306311)
Eppendorf™ Mastercycler™ pro S	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (Cat. No. HSP9631 or HSP9601)	MicroAmp™ Clear Adhesive Film (Cat. No. 4306311)

[1] Microseal™ 'B' PCR Plate Sealing Film from Bio-Rad™ (Cat. No. MSB1001) can be used instead of MicroAmp™ Clear Adhesive Film for the Applied Biosystems™ thermal cyclers.

Oven recommendations

The following ovens are recommended. See the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Site Preparation Guide* (Pub. No. MAN0019173) for supplier information.

- BINDER™ ED 56 drying oven
- GeneChip™ Hybridization Oven 645
  The GeneChip™ Hybridization Oven 640 is currently not supported with the Axiom™ PharmacoFocus™ Assay. If you intend to use the GeneChip™ Hybridization Oven 640 in the workflow, first contact your field service engineer (FSE) or Thermo Fisher Scientific Technical Support regarding the compatibility of this oven with the Axiom™ PharmacoFocus™ Assay.
  - If using a GeneChip™ Hybridization Oven, set the rotation speed to 15 rpm to aid in even heat distribution.
  - For the GeneChip™ Hybridization Oven, place plates 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 Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow. We recommend the plate centrifuges listed in "Plate centrifuge recommendations" on page 30.

When centrifuging and drying pellets, the centrifuge must meet the following requirements:

- Temperature: 4°C.
- Rcf: 3,200 × *g* with an appropriate rotor-bucket combination, or 4,000 rpm for the Eppendorf™ Centrifuge 5810 R configuration described below.

  Relative centrifugal force (rcf) can be calculated as follows:

  rcf = (1.118 × 10-5) R S2 where R is the radius of the rotor in centimeters and S is the speed of the centrifuge in revolutions per minute.

In addition, the bottom of the rotor buckets should be soft rubber to ensure that the deep-well plates do not crack. Do not use buckets that sit plates directly on a metal or hard plastic bottom. For the Eppendorf™ Centrifuge 5810 R, do not use the A-4-62 rotor with a WO-15 hard bottom plate carrier.

Plate centrifuge recommendations

The following plate centrifuges are recommended for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow.

✓	Item	Supplier	Source
☐	Sorvall™ Legend™ XTR Centrifuge (refrigerated), with: ☐ TX-750 4 + 750mL Swinging Bucket Rotor ☐ Microplate/Flask Carriers	Thermo Fisher Scientific	75004539 (bench model) 75004520 (230 V, 50 Hz, floor model) 75004521 (120 V, 60 Hz, floor model) 75004523 (230 V, 50–60 Hz, USA and Canada, floor model) 75003180 (rotor) 75003795 (set of 2 carriers) 75003617 (set of 4 carriers)
	Eppendorf™ Centrifuge 5810 R, with: ☐ Rotor A-4-81, with 4 MTP/Flex buckets	Fisher Scientific™	022625551 (230 V, 50–60 Hz) 022625501 (120 V, 50–60 Hz, 15 A) 022625101 (120 V, 50–60 Hz, 20 A) 022638807 (rotor)
	Allegra™ 25R Benchtop Centrifuge, Refrigerated, with S5700 Swinging-Bucket Rotor	Beckman Coulter™	369434 (230 V, 50–60 Hz) 369435 (200 V, 50–60 Hz) 369436 (230 V, 50 Hz) 368954 (rotor)

Plate shakers

We recommend using one of the following shakers.

Item	Source
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific™ 88880023 or 88880024
Jitterbug™	Boekel Scientific™ 130000 (115V) 130000-2 (230V)

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 the following:

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

Guidelines for use

Guidelines for handling plates and tubes

Unless otherwise stated in the protocol, adhere to the following guidelines when instructed to seal, vortex, and centrifuge plates or reagent tubes.

- **Seal plates**: We recommend using MicroAmp™ Clear Adhesive Film to seal your plates.

  IMPORTANT! Always ensure that your plates are tightly sealed. A tight seal prevents sample loss and cross-well contamination.

  Blot-dry: Before sealing plates, check 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.
  - To remove droplets before sealing, overlay a sheet of laboratory tissue across the top of the plate and gently pat down to dry.
  - Lift the sheet off the plate and discard. Ensure that the top of the plate is dry and seal the plate as usual.
- **Vortex reagent vials**:
  - Vortex 3 times for 1 second each time at the maximum setting.
- **Vortex plates**:
  - Vortex deep-well plates for 5 seconds in each of the 5 sectors. See the sectors in Figure 8.
  - Vortex PCR plates such as the Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted and the Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted for 2 seconds in each of the 5 sectors.

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

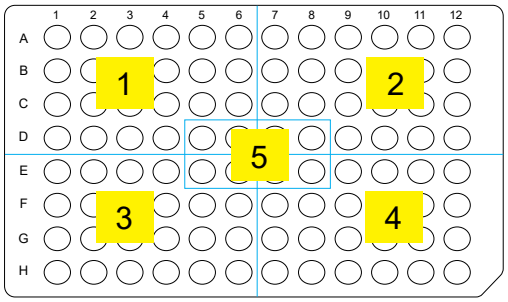


Figure 8 The 5 plate sectors.

- **Centrifuge**: When instructed to centrifuge plates or reagent vials, follow these guidelines unless otherwise instructed.
  - **Plates**:
    - Centrifuge plate to 1,000 rpm at room temperature.
    - Do not centrifuge for more than 1 minute.
  - **Reagent vials**: Briefly centrifuge for 3 seconds.

Sample quantification

This protocol has been optimized using a PicoGreen™ assay to determine genomic DNA concentrations. Other quantification methods such as UV absorbance can give different readings. Therefore, correlate readings from other methods to the equivalent PicoGreen™-determined concentration.

See Chapter 2, “Prepare genomic DNA”.

About the reagents and master mix preparations

- About the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit components:
  - Caps on the vials are color-coded according to assay stage.
  - Properly store all enzyme reagents, especially enzyme-containing vials. Improper storage methods can profoundly affect activity.
- QIAGEN reagents. QIAGEN™ Multiplex PCR *Plus* Kit (Cat. No. 206152) is used with Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit to process 96 samples. The 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 the Axiom™ PharmacoFocus™ Assay and can be discarded.

  All 3 tubes of 2x Multiplex PCR Master Mix are needed to process one 96F array plate, but only 1 tube of water and 1 tube of Q-Solution is required.
- About reagents from other suppliers:
  - Use only fresh reagents from the recommended vendors to help eliminate changes in pH or the salt concentration of buffers.
  - Consult the appropriate SDS for reagent storage and handling requirements.
- About the master mix preparations:
  - 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, then a volume error has been made or the pipettes are not accurate. We recommend that you stop and repeat the experiment.

    The volume of master mixes prepared are designed to provide consistent handling of reagents and consistent assay results. The percent overage of different master mixes can differ, depending on the reagent volumes involved.
- About reagents at the laboratory bench:
  - Properly chill essential equipment such as reagent coolers before use.
  - Ensure that the 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.

Pipette recommendations

- Use a pipette of appropriate size for the volume of liquid being transferred.

Pipette size	Recommended volume range
Single channel P20 / 8- or 12-channel P20	1-20 µL
Single channel P200 / 8- or 12-channel P200	20-200 µL
Single channel P1000 / 12-channel P1200	200-1,000 µL

- Always use pipettes that have been calibrated.
- Proficiency with the use of single and multichannel pipettes is essential. 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 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 plates or trays.

- Use single-channel pipettes for volumes less than or equal to 2 mL. For volumes between 1 mL and 2 mL, add the reagent in 2 portions with a fresh tip for each portion.
- Use serological pipettes for volumes greater than 2 mL.
- Usually, 25-mL or 50-mL serological pipettes do not fit into the mouths of the reagent bottles. Multiple transfers using 5-mL or 10-mL serological pipettes are recommended.

Multichannel pipettes

Use 12-channel pipettes when adding master mix or transferring 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™ Reagent Reservoirs

The Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow requires the use of disposable reservoirs with a "trough within a trough" design. This design maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent.

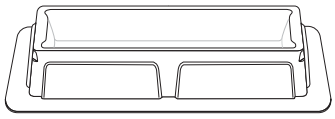
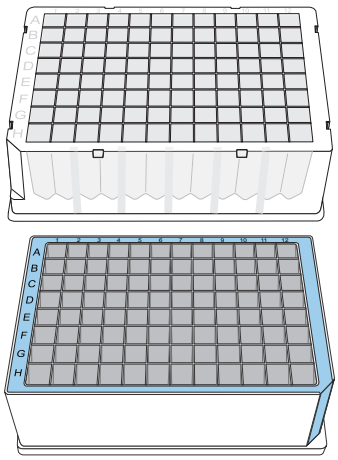
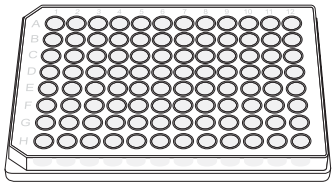
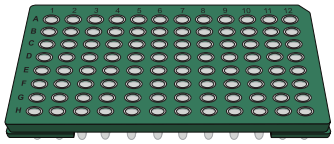
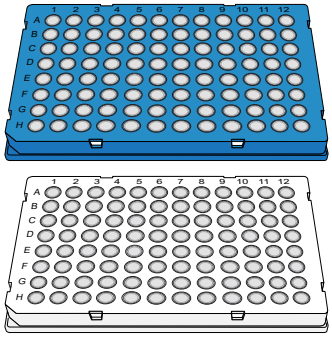


Figure 9 Dispense reagents from Matrix™ 25-mL reagent reservoirs.

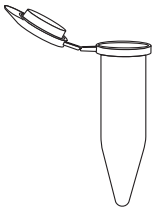
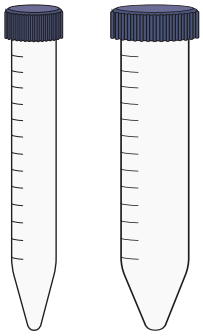
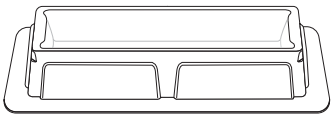
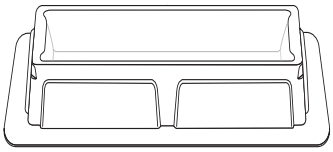
Required materials

Labware and consumables required

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Labware	Source	Image
96-deepwell plate • ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate, or • Eppendorf™ DeepWell™ Plate 96, 2,000 µL	• Fisher Scientific™ AB0932 • Fisher Scientific™ 13-864-302	
Greiner Bio-One™ UV-Star™ 96-Well UV Spectroscopy Microplate	Fisher Scientific™ 07-000-407	
Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted **Note:** See "PCR plate type by thermal cycler for the mPCR step" on page 29 for the PCR plate type recommended for the specific thermal cycler you are using.	Bio-Rad™ HSS9641	
Hard-Shell™ 96-Well PCR Plate, low profile, full skirted **Note:** See "PCR plate type by thermal cycler for the mPCR step" on page 29 for the PCR plate type recommended for the specific thermal cycler you are using.	Bio-Rad™ HSP9631, or Bio-Rad™ HSP9601	

(continued)

Labware	Source	Image
1.7-mL microcentrifuge tubes, DNAse and RNAse-free	**MLS**	
15-mL and 50-mL conical-bottom centrifuge tubes, polypropylene	**MLS**	
Matrix™ Reagent Reservoir, 25 mL	8093 (10 bags of 10), 8093-11 (pack of 100 in one bag)	
Matrix™ Reagent Reservoir, 100 mL	8085	
96-well metal chamber Warming or cooling chamber for 0.2 mL tubes, 96 holes (4 for 1.5 mL and 6 for 0.5 mL tubes), Dimensions: 6 1/8"L x 3 1/8"W x 1" H	Diversified Biotech™ CHAM-1000	
Adhesive film	Use one of the following: • MicroAmp™ Clear Adhesive Film 4306311 • Microseal™ 'B' PCR Plate Sealing Film Bio-Rad™, MSB1001	

Axiom™ GeneTitan™ consumables kits

Two Axiom™ GeneTitan™ consumables kits are available that contain trays that are required for processing Axiom™ mini 96-array format plates on the GeneTitan™ MC Instrument.

The Axiom™ 384HT GeneTitan™ High Volume Consumables Kit is sufficient to process 5 Axiom™ PharmacoFocus™ Mini 96-Array Plates.

Table 1 Axiom™ 384HT GeneTitan™ High Volume Consumables Kit (Cat. No. 902629).

Contents[1]	Per kit	Storage
384-Layout GeneTitan™ Stain Tray (Stain 1)	10	Room temperature
384-Layout Axiom™ Stain 2 Tray	5	
384-Layout Axiom™ Stabilization Tray	5	
384-Layout Axiom™ Ligation Tray	5	
384-Layout GeneTitan™ Hybridization Tray	5	
384-Layout GeneTitan™ Scan Tray	5	
384-Layout GeneTitan™ Scan and Stain Tray Cover	30	

[1] See Table 3 for detailed descriptions of each component.

The alternative Axiom™ GeneTitan™ 384HT Consumables Kit supports the processing of a single Axiom™ PharmacoFocus™ Mini 96-Array Plate.

Note: This user guide references only Cat. No. 902629, but Cat. No. 952385 can be used instead to process a single mini 96-array plate.

Table 2 Alternative Axiom™ GeneTitan™ 384HT Consumables Kit (Cat. No. 952385).

Contents[1]	Per kit	Storage
384-Layout GeneTitan™ Stain Tray (Stain 1)	2	Room temperature
384-Layout Axiom™ Stain 2 Tray	1	
384-Layout Axiom™ Stabilization Tray	1	
384-Layout Axiom™ Ligation Tray	1	
384-Layout GeneTitan™ Hybridization Tray	1	
384-Layout GeneTitan™ Scan Tray	1	
384-Layout GeneTitan™ Scan and Stain Tray Cover	6	

[1] See Table 3 for detailed descriptions of each component.

The following table lists and describes the Axiom™ GeneTitan™ tray consumables that are available in the Axiom™ 384HT GeneTitan™ High Volume Consumables Kit and the Axiom™ GeneTitan™ 384HT Consumables Kit.

IMPORTANT! All covers must have barcodes. Discard any cover without a barcode.

See Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation” for information on aligning and loading trays onto the GeneTitan™ MC Instrument.

Table 3 Axiom™ GeneTitan™ tray consumables.

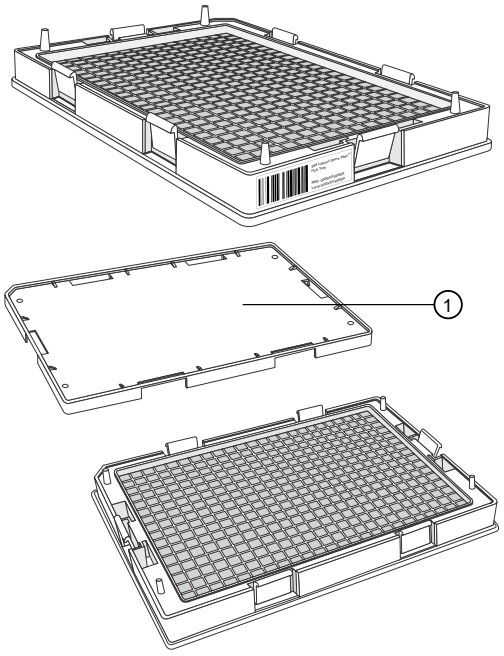
Item	Part No.	Image	Details
384-Layout GeneTitan™ Hybridization Tray	501278	 ① Hybridization tray cover to be discarded.	The 384-Layout GeneTitan™ Hybridization Trays are packaged in white pouches with the label "384 Layout GeneTitan™ Hyb Tray" ref# 501278 (pouch)/902278 (box). The hybridization trays are packaged with a protective cover that is discarded before use. 384 hybridization tray cover, Part No. 203006.

Table 3 Axiom GeneTitan tray consumables. *(continued)*

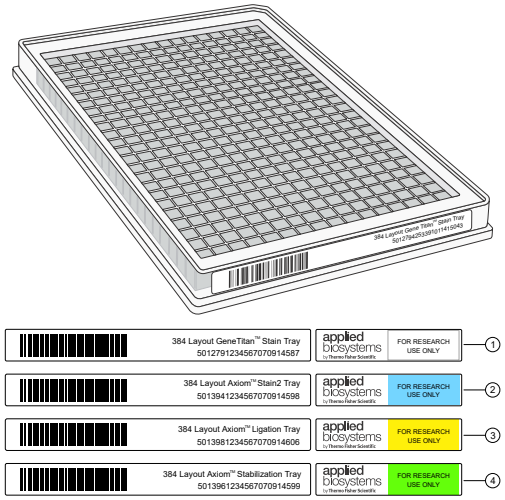
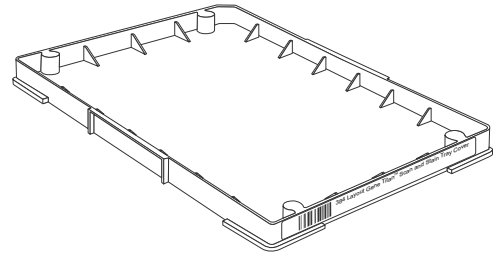
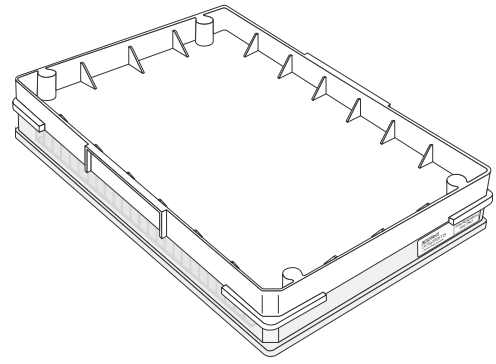
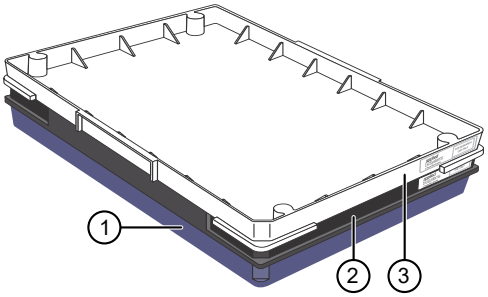
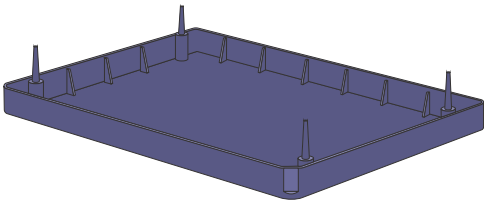
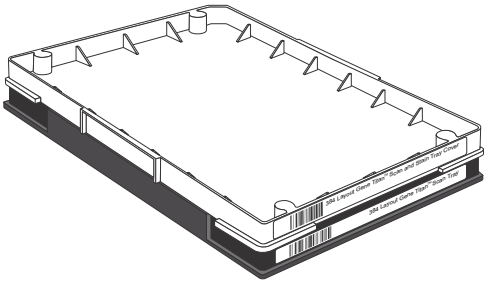
Item	Part No.	Image	Details
384 Layout GeneTitan™ Stain Trays	501279 - Stain 1 501394 - Stain 2 501398 - Ligation 501396 - Stabilization	 ① Stain 1 tray ② Stain 2 tray ③ Ligation tray ④ Stabilization tray	The stain trays are packaged in zip-top bags to keep them free of dust. Each stain tray is uniquely barcoded. **IMPORTANT!** Each stain tray is labeled with a name and an individual barcode. Ensure that you always use the appropriate tray with the corresponding reagent. Failure to do so can result in the wrong stain in the wrong location on the GeneTitan™ MC Instrument and assay failure. When transferring the trays to the GeneTitan™ MC Instrument, ensure that the trays are placed in the proper location in the drawer. Failure to do so results in an error and the GeneTitan™ MC Instrument does not proceed with processing trays.
384-Layout GeneTitan™ Scan and Stain Tray Cover	501315		The 384-Layout GeneTitan™ Scan and Stain Tray Covers are provided to prevent evaporation of the GeneTitan™ reagents in stain trays and the array holding buffer in the scan tray. The GeneTitan™ scan and stain tray covers are barcoded.
Stain tray cover, shown on top of the stain tray	501315 - Cover		The stain trays must be placed in the GeneTitan™ MC Instrument with the stain tray cover.

Table 3 Axiom GeneTitan tray consumables. *(continued)*

Item	Part No.	Image	Details
384-Layout GeneTitan™ Scan Tray[1]		 ① Scan tray protective base ② 384-Layout GeneTitan™ Scan Tray ③ Barcoded scan tray cover	The Axiom™ scan tray package includes the following: • The GeneTitan™ scan tray includes a scan tray cover. The tray cover must be used to cover the scan tray before placing the tray in the GeneTitan™ MC Instrument. • The scan tray must always be protected from damage or exposure to dust. The scan tray must always be in the blue-plate base except when loaded into the GeneTitan™ MC Instrument. • The blue scan tray protective base in the package is used to protect the bottom of the scan tray glass from damage. Remove the protective base from the scan tray before loading the scan tray with the scan tray cover in the GeneTitan™ MC Instrument.
Blue scan tray protective base	202096		The blue scan tray protective base in the package is used to protect the bottom of the scan tray glass from damage. The blue scan tray protective base is distinct from the blue array plate protective base and must not be used with the array plate. Remove the protective base from the scan tray before loading in the GeneTitan™ MC Instrument.
384-Layout GeneTitan™ Scan Tray with cover	501280 - Scan tray 501315 - Cover		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.

[1] After aliquoting the appropriate solution to each tray type, the tray must be loaded into the GeneTitan™ MC Instrument with the barcode facing away from the operator. That is, the barcode must be on the back side.

Array plate required

One of the following Axiom™ PharmacoFocus™ Mini 96-Array Plate options is required to run Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow.

Quantity	Item
1	Axiom™ PharmacoFocus™ Mini 96-Array Plate (Cat. No. 952380)
1	Axiom™ PharmacoFocus™ Mini 96-Array Plate Core (Cat. No. 952424)

Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit

Each Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389) is sufficient for 1 Axiom™ PharmacoFocus™ Mini 96-Array Plate.

Component and cap color		Storage
Module 1		–25°C to –15°C
Axiom™ 2.0 Denat Soln 10X	Axiom™ 2.0 Amp Soln	
Axiom™ 2.0 Neutral Soln	Axiom™ 2.0 Amp Enzyme	
Axiom™ Water		
Module 2—Pouch 1 of 2		–25°C to –15°C
Axiom™ Frag Enzyme	Axiom™ Hyb Buffer	
Axiom™ 10X Frag Buffer	Axiom™ Hyb Soln 1	
Axiom™ Precip Soln 2		
Module 2—Pouch 2 of 2		2°C to 8°C
Axiom™ Frag Diluent	Axiom™ Resusp Buffer	
Axiom™ Frag Rxn Stop	Axiom™ Hyb Soln 2	
Axiom™ Precip Soln 1		
Module 3		Room temperature
Axiom™ Wash Buffer A	Axiom™ Water	
Axiom™ Wash Buffer B		
Module 4—Pouch 1 of 2		–25°C to –15°C
Axiom™ Ligate Buffer	Axiom™ Probe Mix 1	
Axiom™ Ligate Enzyme	Axiom™ Stain Buffer	
Axiom™ Ligate Soln 1	Axiom™ Stabilize Soln	

(continued)

Component and cap color		Storage
Module 4—Pouch 2 of 2		2°C to 8°C
Axiom™ Ligate Soln 2	Axiom™ Stain 2-A	
Axiom™ Probe Mix 2	Axiom™ Stain 2-B	
Axiom™ Wash A	Axiom™ Stabilize Diluent	
Axiom™ Stain 1-A	Axiom™ Water	
Axiom™ Stain 1-B	Axiom™ Hold Buffer	
Module A—mPCR Primers		–25°C to –15°C
10X Primer Mix		
Module B—Control DNA		–25°C to –15°C
Control DNA 1, 5 ng/µL		
Control DNA 2, 5 ng/µL		

4 Multiplex PCR and target preparation

Introduction

Target preparation for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow enables you to perform target preparation to process 96 samples at a time. The protocol is performed in 2 parts:

- Part 1: Manual target preparation as described in this chapter.
- Part 2: Array processing is performed on the GeneTitan™ Multi-Channel (MC) Instrument. See Chapter 5, “Process array plates with the GeneTitan™ Multi-Channel Instrument”.

This manual assay format allows the user to run the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using 1 Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389) and 1 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 Axiom™ PharmacoFocus™ Assay.

Using the manual target preparation protocol, 3 array plates can be processed per work week for a total of 288 arrays.

- Chapter 6, “Three-plate workflow for Axiom™ PharmacoFocus™ Mini 96-Array Plates using an overnight DNA precipitation step”.
- Chapter 7, “Three-plate workflow for Axiom™ PharmacoFocus™ Mini 96-Array Plates using a 3-hour DNA precipitation step”.

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 Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow is in the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0019173.

Stage 1A: Perform multiplex PCR (mPCR)

IMPORTANT! Before proceeding to mPCR or DNA amplification, perform the gDNA preparation. See Chapter 2, "Prepare genomic DNA".

IMPORTANT! Preamplification preparation must take place in a dedicated area such as a biosafety hood with dedicated pipettes, tips, and vortex. See "Preamplification/amplification staging area" on page 27.

Time required

Activity	Time[1]
Thaw reagents	30 minutes
Hands-on time	~30 minutes
Thermal cycler run time	~3.5 hours
Total	**~4.5 hours**

[1] For 96 samples.

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.

See Chapter 2, "Prepare genomic DNA".

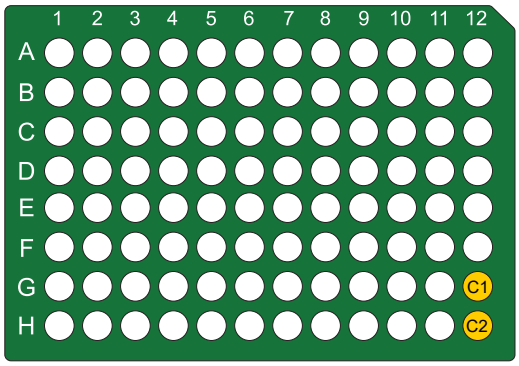


Figure 10 mPCR Sample Plate.

Materials, labware, and reagents required

Equipment and labware required for Stage 1A

Quantity	Item
Labware and consumables	
As required	Adhesive seals for 96-well plate MicroAmp™ Clear Adhesive Film
1	Marker, fine point, permanent
1	96-well plate holder
1	15-mL conical tube (RNase/DNase-free)
Equipment	
1	Vortexer
1	96-well metal chamber, cooled to 4°C
1	Plate centrifuge
1	Ice bucket with ice
1	Thermal cycler programmed with the **PharmacoFocus mPCR** protocol. See “Thermal cycler recommendations and protocols” on page 28.
1 each	Rainin™ pipettes: • Single channel P200 • Single channel P1000 • Multichannel P200
As required	Pipette tips

Reagents required for Stage 1A

Quantity	Reagent	Module
From the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit		
1 tube	◯ 10X Primer Mix	Module A—mPCR Primers, –20°C (Part No. 912896)
User supplied from the QIAGEN™ Multiplex PCR *Plus* Kit (100)[1] (Cat. No. 206152)		
3 tubes	2X Multiplex PCR Master Mix	
1 tube	5X Q-Solution	
1 tube	RNase-free Water	

[1] CoralLoad Dye in kit is not needed for Axiom™ PharmacoFocus™ Assay.

Prepare for mPCR

1. Power on the thermal cycler that is programmed with **PharmacoFocus mPCR** protocol. See Figure 6.

   Ensure that the heated lid option has been selected.

2. Thaw the mPCR reagents, then prepare the mPCR Reaction Plate.
   a. If the gDNA mPCR Sample Plate was frozen, thaw at room temperature on the benchtop, vortex, then centrifuge briefly.

   b. Place the mPCR Sample Plate on ice or on a cold 96-well metal chamber after thawed.

IMPORTANT! gDNA samples must be 10-µL volume at a concentration of 5 ng/µL in a 96-well PCR plate. See "Aliquot the diluted samples and the controls" on page 21.

3. Thaw, then prepare the reagents from Module A—mPCR Primers (Part No. 912896), of the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit.
   a. Thaw the 10X Primer Mix on the benchtop at room temperature, then place on ice.

   b. Vortex, then centrifuge briefly before use.

4. Thaw, then prepare the reagents from the QIAGEN™ Multiplex PCR *Plus* Kit.
   a. Obtain 3 tubes of QIAGEN 2X Multiplex PCR Master Mix.
      - Thaw on the benchtop at room temperature, then place on ice.
      - Thoroughly mix each tube by inverting 10 times.
      - Centrifuge briefly before use. *Do not vortex*.

   b. Obtain 1 tube of 5X Q-Solution.
      - Thaw on the benchtop at room temperature, then place on ice.
      - Vortex and centrifuge briefly before use.

   c. Obtain 1 tube of RNase-free water.
      - Thaw on the benchtop at room temperature, then place on ice.
      - Vortex and centrifuge briefly before use.

IMPORTANT!
- Ensure that reagents are thoroughly mixed before use.
- Vortex water and Q-Solution.
- Thoroughly mix the QIAGEN 2X Multiplex PCR Master Mix by inverting the tube 10 times. *Do not vortex*.

Prepare the mPCR Master Mix

1. Label a 15-mL conical tube “mPCR”.
2. To the mPCR tube, add the reagents that are listed in the following table and in the following order:
   a. Add water, Q-Solution, and the primers to the 15-mL conical tube, then cap the tube.
   b. Vortex and centrifuge briefly.
   c. Add 2,400 µL of the QIAGEN™ 2X Master Mix to the 15-mL conical tube.
      - It is necessary to use all 3 vials that are supplied by QIAGEN™.
      - Set a P1000 single-channel pipette to 800 µL, then remove this volume from the first vial.
      - Transfer the solution to the 15-mL conical tube. Change tips, then repeat this step for the remaining 2 vials.
   d. Mix thoroughly by gently inverting the tube end-over-end 10 times, then briefly centrifuge.

Reagent	One 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. Keep the resulting mPCR Master Mix in ice, then add to the mPCR Sample Plate as soon as possible after preparation.

Set up the mPCR Reaction Plate

1. Confirm that the 96-well PCR plate is labeled “mPCR”, then include the date and any other desired experimental details.
2. Place the plate in a 96-well metal chamber that had been stored at 4°C.
3. Carefully pour the prepared mPCR Master Mix into a 25-mL reservoir.
4. Use a P200 multichannel pipette to carefully transfer 30 µL of mPCR Master Mix into the mPCR Sample Plate.
   Final volume of each well is 40 µL.
5. Seal the plate with adhesive seal, ensuring that the seal is firmly pressed down to prevent sample contamination during mixing and evaporation during PCR cycling.
6. Vortex the plate for 2 seconds in each quadrant, twice. See Figure 8.
7. Centrifuge at 2,000 rpm for 30 seconds.
8. Return the plate to the cold 96-well metal chamber until the plate can be loaded onto the thermal cycler.
9. Load the plate onto the thermal cycler within 5 minutes.
10. Discard all leftover reagents, including any remaining unused QIAGEN™ reagents.

Run the PharmacoFocus mPCR thermal cycler protocol

Note: The mPCR Sample Plate is now known as the mPCR Reaction Plate

1. Place mPCR Reaction Plate on thermal cycler, then run the **PharmacoFocus mPCR** protocol.
   See Figure 6.
2. After the mPCR Reaction Plate has been placed on the thermal cycler, proceed to "Stage 1B: Amplify the genomic DNA" on page 51.

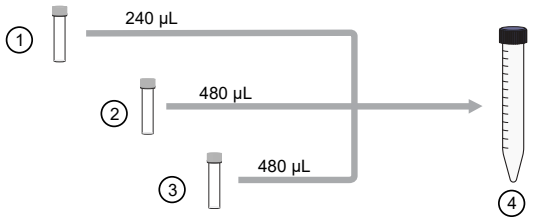
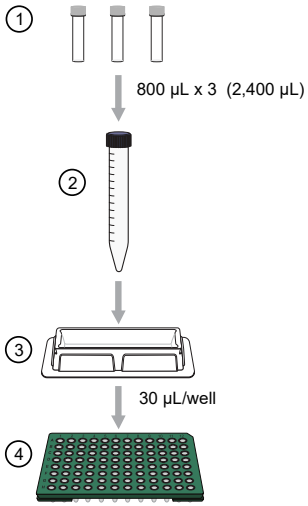
Store the mPCR Product Plate

Note: The mPCR Reaction Plate is now known as the mPCR Product Plate.

1. After the **PharmacoFocus mPCR** thermal cycler protocol is complete, remove the plate from the thermal cycler, seal, then centrifuge the plate briefly. Ensure that the plate is well sealed to prevent evaporation during storage.
2. Do one of the following:
   - For mPCR spike-in and fragmentation on another day, store the mPCR Product Plate at –20°C.
   - For mPCR spike-in and fragmentation within 4 hours, store the mPCR Product Plate in a refrigerator (2–8°C).

Note: An mPCR QC gel can be run for qualitative evaluation of the mPCR reaction before the mPCR spike-in step during fragmentation. See Appendix C, "mPCR quality control gel protocol".

Workflow for Stage 1A: Multiplex PCR

Prepare the mPCR Master Mix–part 1	
 1 RNase-free water 2 Q-solution 3 mPCR Primer Mix 4 15-mL tube	Add all components to a 15-mL tube, vortex briefly, then centrifuge briefly.
Prepare the mPCR Master Mix–part 2	
 1 Three vials of 2X QIAGEN Multiplex PCR Master Mix 2 15-mL tube from part 1 3 Matrix™ Reagent Reservoir 4 mPCR Sample Plate	1. Transfer 800 µL from 3 vials of 2X QIAGEN Multiplex PCR Master Mix into the 15-mL tube from part 1. 2. Invert tube 10 times to mix, then centrifuge briefly. 3. Pour contents of the 15-mL tube into the reservoir. 4. Add 30 µL mPCR Master Mix to each well. Seal the mPCR Reaction Plate tight, vortex, then centrifuge briefly. Final volume: 40 µL/well.
Run the Multiplex PCR thermal cycler protocol	
	Time estimate: ~3.5 hours **Note:** When the thermal cycler program is finished, the mPCR Reaction Plate is now known as the mPCR Product Plate.

Stage 1B: Amplify the genomic DNA

Before proceeding to DNA amplification, complete genomic DNA preparation. See Chapter 2, "Prepare genomic DNA".

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

IMPORTANT! Amplification preparation should take place in a dedicated area such as a biosafety hood with dedicated pipettes, tips, vortex, etc. See "Preamplification/amplification staging area" on page 27.

Time required

Activity	Time
Thaw materials	60 minutes
Hands-on time	~30 minutes
Incubation at 37°C	23 ±1 hours
Total	**~24.5 hours**

Input required

The Amplification Sample Plate, with 20 µL of each gDNA diluted to a concentration of 5 ng/µL in a 96-deepwell plate (ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate or Eppendorf™ DeepWell™ Plate 96, 2,000 µL). See Chapter 2, "Prepare genomic DNA".

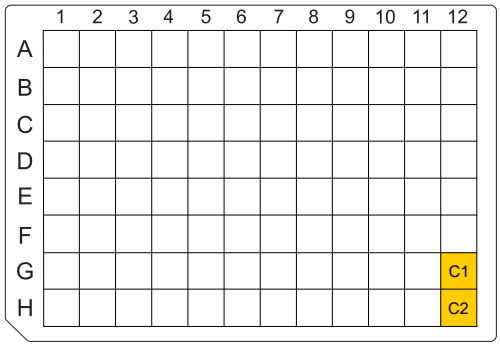


Figure 11 Whole genome amplification sample plate.

Materials, labware, and reagents required

Equipment and labware required for Stage 1B

Quantity	Item
As required	Adhesive seals for 96-well plates, MicroAmp™ Clear Adhesive Film
1	Cooler, chilled to –20°C
1	Microcentrifuge tube holder
1	Mini microcentrifuge (microfuge with microtube rotor)
1	15 mL and 50-mL tube holders
1	Marker, fine point, permanent
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™ Pipette Controller
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™ Reagent Reservoirs
As required	Laboratory tissues

Reagents required for Stage 1B

Reagent and cap color	Module
Reagents from the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389)	
Axiom™ 2.0 Denat Soln 10X	Module 1, –20°C (Part No. 901711)
Axiom™ 2.0 Neutral Soln	
Axiom™ 2.0 Amp Soln	
Axiom™ 2.0 Amp Enzyme	
Axiom™ Water	

Prepare for DNA amplification

1. Thaw the Amplification Sample Plate.
   a. Thaw the Amplification Sample Plate on the benchtop at room temperature.
   b. Vortex, briefly centrifuge, then 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 a 96-deepwell plate (ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate or Eppendorf™ DeepWell™ Plate 96, 2,000 µL. See Chapter 2, “Prepare genomic DNA”.

2. Thaw the following reagents on the benchtop at room temperature:
   - Axiom™ 2.0 Denat Soln 10X
   - Axiom™ 2.0 Neutral Soln
   - Axiom™ 2.0 Amp Soln
   - Axiom™ Water

Note: Allow ~1 hour for the Axiom™ 2.0 Amp Soln to thaw on the benchtop at room temperature. If the solution is not thawed after 1 hour, vortex briefly and return to the benchtop to complete thawing. The bottles can also be thawed in a dish with ultra-pure water. The Axiom™ 2.0 Amp Soln and the Axiom™ 2.0 Neutral Soln must be thoroughly mixed before use.

3. Leave the Axiom™ 2.0 Amp Enzyme in the freezer until ready to use.

4. Vortex all reagents (except Axiom™ 2.0 Amp Enzyme), then place at room temperature.
   - Vortex the Axiom™ 2.0 Amp Soln and Axiom™ 2.0 Neutral Soln for 30 seconds to mix thoroughly.
   - Vortex and briefly centrifuge the Axiom™ 2.0 Denat Soln 10X before placing on the deck.
   - For the Axiom™ 2.0 Amp Enzyme, just before placing on the deck gently flick the tube 3 times to mix, then centrifuge briefly.
5. Preheat the oven to 37°C.

   We recommend using one of these ovens:
   - BINDER™ ED 56
   - GeneChip™ Hybridization Oven 645 (Do not rotate plates; switch off the rotisserie in the oven.)
6. Set the centrifuge to room temperature.
7. Label the 15 mL and 50-mL conical tubes as indicated in the following table.

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

8. Label 3 Matrix™ 25-mL reservoirs (Cat. No. 809311) as indicated in the following table.

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

Prepare the Denaturation Master Mix

Carry out the following steps at room temperature.

1. To the 15-mL conical tube labeled “D MM”, add the amount of Axiom™ 2.0 Denat Soln 10X listed in the following table, then dilute it with the amount shown of Axiom™ Water from Module 1 (Part No. 901711).

Reagent and cap color	Per sample	Master mix 96+
Axiom™ 2.0 Denat Soln 10X	2 µL	400 µL
Axiom™ Water	18 µL	3.6 mL
Total volume	**20 µL**	**4.0 mL**

2. Vortex the tube, centrifuge briefly, then leave it at room temperature.

Add Denaturation Master Mix to samples

Carry out the following steps at room temperature.

1. Briefly centrifuge the sample plate.

   Note: The samples must be at room temperature for this step.

2. Gently pipet the Denaturation Master Mix using a P1000 or pour it into the reagent reservoir labeled “D MM”.

3. Carefully remove the seal from the sample plate. Discard the seal.

4. Using a P20 12-channel pipette, add 20 µL of Denaturation Master Mix to each sample. The total volume will be 40 µL/well.
   - Pipet directly into the liquid of each well.
   - Do not mix by pipetting up and down.
   - Change tips between each addition.

   Note: This plate is now the Denaturation Plate.

5. Seal and vortex the Denaturation Plate. After vortexing, start the timer for a 10-minute incubation.

6. Centrifuge the Denaturation Plate in a room-temperature centrifuge for 1 minute at 1,000 rpm.

   Note: The centrifuge time is included in the 10-minute incubation.

7. Visually examine the volume in each well. It should be 40 µL/well.
   a. Keep a record of wells that appear to have an unusually low or high volume. These samples might need to be repeated.

   b. Do *not* stop to measure volumes. Proceed without delay.

8. Complete the 10-minute incubation on the benchtop at room temperature.

   While completing the incubation at room temperature, prepare the Axiom™ 2.0 Neutral Soln as described in “Prepare for DNA amplification” on page 53.

9. After incubation, immediately add the Axiom™ 2.0 Neutral Soln. Follow the instructions in “Add Axiom™ 2.0 Neutral Soln to samples” on page 55.

Add Axiom™ 2.0 Neutral Soln to samples

Carry out the following steps at room temperature.

1. Pour the Axiom™ 2.0 Neutral Soln into the reagent reservoir labeled “N Soln”.

2. Carefully remove the seal from the Denaturation Plate. Discard the seal.

3. Using a P200 12-channel pipette, add 130 µL of Axiom™ 2.0 Neutral Soln to each sample. The total volume will be 170 µL/well.
   - Pipet down the wall of each well.
   - Change tips between each addition.

Note: The Denaturation Plate is now the Neutralization Plate.

4. Seal the Neutralization Plate, vortex it, then briefly centrifuge it.

5. Visually examine the volume in each well. It should be ~170 µL/well.
   a. Keep a record of wells that appear to have an unusually low or high volume. These samples might need to be repeated.

   b. Do *not* stop to measure volumes.

6. Proceed immediately to Amplification Master Mix preparation. See “Add Amplification Master Mix to samples” on page 57.

Prepare the Amplification Master Mix

Carry out the following steps at room temperature.

1. Add the amount of Axiom™ 2.0 Amp Soln listed in the following table into the 50-mL tube labeled “Amp MM” at room temperature.

Note: The Axiom™ 2.0 Amp Soln is a viscous solution. Follow these steps to ensure that the reagent transfer is accurate:
- Pipet slowly.
- Allow bubbles that are generated from mixing to float to the top before pipetting.
- Use a 10-mL serological pipette to transfer the Axiom™ 2.0 Amp Soln into the Amp MM tube.

Reagent and cap color	Per sample	Master mix 96+
Axiom™ 2.0 Amp Soln	225 µL	26.0 mL
Axiom™ 2.0 Amp Enzyme	5 µL	578 µL
Total volume	**230 µL**	**26.58 mL**

2. Remove the Axiom™ 2.0 Amp Enzyme from the freezer, then place it in a portable cooler at –20°C.
   a. Invert, then flick the Axiom™ 2.0 Amp Enzyme tube 3 times. Briefly centrifuge the tube.

   b. Add the amount of Axiom™ 2.0 Amp Enzyme shown in the table to the Amp MM tube.

   c. Vortex the Amplification Master Mix well, invert the tube 2 times, then vortex it again.

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. Discard the seal.
3. Using a P1200 12-channel pipette, slowly add 230 µL Amplification Master Mix to each well of the Neutralization Plate. The total volume will be 400 µL/well.
   - Pipet down the wall of the well.
   - Do not mix by pipetting up and down.
   - Change tips between each addition.

   Note: The Neutralization Plate is now the Amplification Plate.
4. Blot the top of the plate with a laboratory tissue. Seal the plate tightly, vortex twice, then centrifuge for 1 minute at 1,000 rpm.

   See “Guidelines for handling plates and tubes” on page 32.
5. Place the sealed Amplification Plate in an oven set at 37°C, then leave it undisturbed for 23 ±1 hours.

   Note: If using a GeneChip™ Hybridization Oven, place the plate on the bottom of the oven. Do not turn on the oven rotation apparatus.

Freeze the plate or proceed

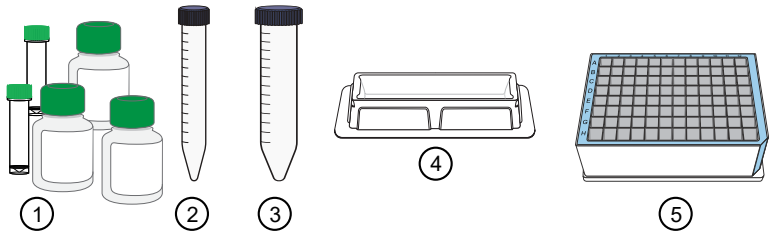
After the incubation finishes, do one of the following:

- Proceed to “Stage 2: Fragment and precipitate the DNA” on page 60.
- Store the Amplification Plate at –20°C.

Note: If freezing, do not perform the stop amplification reaction step before you store the Amplification Plate at –20°C. The stop amplification reaction step is performed after thawing the frozen plate. See “Prepare for fragmentation and precipitation” on page 61.

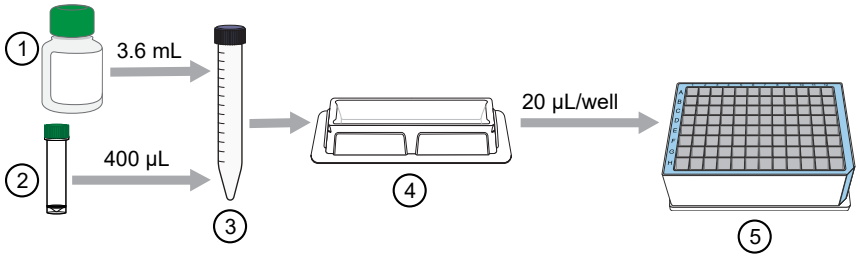
Workflow for Stage 1B: Amplify the genomic DNA

Materials required



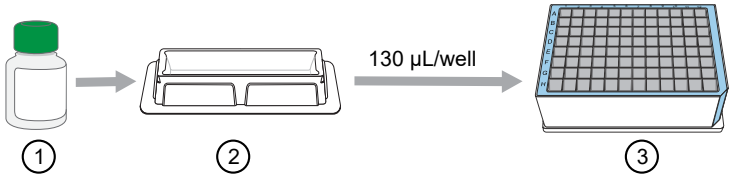
① Module 1 reagents
② Quantity = 1
③ Quantity = 1
④ Quantity = 3
⑤ Quantity = 1, Amplification Sample Plate

Denaturation step



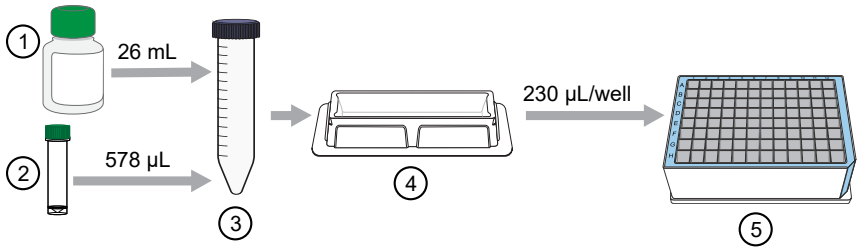
① Axiom™ Water
② Axiom™ 2.0 Denat Soln 10X
③ Vortex, then briefly centrifuge.
④ Pour into reagent reservoir.
⑤ Denaturation Plate. Vortex, briefly centrifuge, then incubate at room temperature for 10 minutes.

Neutralization step



① Axiom™ 2.0 Neutral Soln
② Pour into reagent reservoir.
③ Neutralization Plate. Vortex, then briefly centrifuge.

DNA amplification preparation



Final volume = 400 µL/well

1. Axiom™ 2.0 Amp Soln
2. Axiom™ 2.0 Amp Enzyme
3. Vortex, then invert tube to mix.
4. Pour into reagent reservoir.
5. Amplification Plate. Vortex for 30 seconds, then centrifuge briefly.

Incubate Amplification Plate offline for 23 ±1 hours at 37°C

Stage 2: Fragment and precipitate the DNA

Time required

Total time: Approximately 2 hours, plus time for precipitation incubation at –20°C, for 3 hours or overnight.

Input required

The mPCR Product Plate and the Amplification Plate from Stage 1 in a 96-deepwell plate (ABgene™ 96 Well 2.2mL Polypropylene Deepwell Storage Plate or Eppendorf™ DeepWell™ Plate 96).

Materials, labware, and reagents required

Equipment and consumables required for Stage 2

Quantity	Item
As needed	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	Fine-point permanent marker
1 each	Rainin™ pipettes • Single-channel P200 • Single-channel P1000 • Multichannel P20 • Multichannel P200 • Multichannel P1200
As needed	Pipette tips
As needed	Serological pipettes • 5 x 1/10 mL • 10 x 1/10 mL
1	Pipet-Aid™ Pipette Controller
1	Plate centrifuge set at room temperature
1	Mini microcentrifuge (microfuge with microtube rotor)

(continued)

Quantity	Item
2–3	Ovens • One set at 37°C • One set at 65°C
1	15-mL conical tube and holder
3	Matrix™ Reagent Reservoir, 25 mL
1	Matrix™ Reagent Reservoir, 100 mL
1	Vortexer

Reagents required for Stage 2

Reagent and cap color	Module
From the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389)	
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	
Isopropanol, 99.5%, 70 mL	—

Prepare for fragmentation and precipitation

Note: If the Amplification Plate or the mPCR Product Plate was frozen at the end of Stage 1, thaw the plates before beginning Stage 2. See instructions in Chapter 2 of the user guide for notes on thawing and centrifuging prior to changing the seal to avoid cross-contamination.

Set oven and centrifuge temperatures

1. Set up 2 incubators or ovens. This should be done the night before they are used.
   a. Set one oven at 37°C. Use an oven that can sustain a constant temperature of 37°C and has a temperature accuracy of ±1°C.
   b. Set one oven at 65°C.

Note: For the 3-hour precipitation workflow, 3 ovens are recommended. Set one oven at 37°C, one at 65°C, and one at 48°C in preparation for "Stage 4: Transfer, denature, then hybridize the denatured samples" on page 86.

2. Set the centrifuge to room temperature.

Note: Keep a set of balance plates ready to minimize delays before centrifuging the Fragmentation Plate between steps.

Thaw and prepare the amplified DNA samples

1. If the Amplification Plate is *not* frozen, skip to "Thaw and prepare the mPCR Product Plate" on page 62.
2. If the Amplification Plate is frozen, thaw, then prepare it as described here.
   a. Place the deep-well plate in a small bath of room-temperature ultra-pure water.
   b. Leave the plate in the water bath for ~50 minutes, until all wells have thawed.
   c. Centrifuge the plate at 1,000 rpm for 30 seconds.

   Note: To avoid cross-contamination of wells during vortexing:
   - Remove the seal and blot the top of the plate with a laboratory tissue.
   - Tightly reseal the plate with a fresh seal.

   d. Vortex the plate for 30 seconds to mix the contents.
   e. Centrifuge the plate at 1,000 rpm for 30 seconds.

Thaw and prepare the mPCR Product Plate

The following steps are for preparing the mPCR Product Plate that has been frozen. Skip this step if the mPCR Product Plate was not frozen at the end of Stage 1A.

If the mPCR Product Plate is frozen, thaw, then prepare it as described here.

1. Thaw the plate at room temperature for about 20 minutes or until all samples are thawed.
2. Centrifuge the plate at 1,000 rpm for 30 seconds.

Note: To avoid cross-contamination of wells during vortexing:
- Remove the seal.
- Tightly reseal the plate with a fresh seal.

3. Vortex the plate for 10 seconds to mix the contents.
4. Centrifuge the plate at 1,000 rpm for 30 seconds.

Thaw and prepare the reagents

1. Prepare the following fragmentation reagents:
   a. Axiom™ 10X Frag Buffer
      - Thaw on the benchtop at room temperature, then place on ice.
      - Vortex before use.

   b. Axiom™ Frag Diluent
      - Place on ice.
      - Vortex, then centrifuge briefly before use.

   c. Axiom™ Frag Rxn Stop
      - Place on the benchtop to warm to room temperature.
      - Vortex before use.

   d. Axiom™ Frag Enzyme
      - Leave at –20°C until ready to use.
      - Immediately before use, gently flick the tube 3 times to mix, then centrifuge briefly.

2. Prepare the following precipitation reagents:
   a. Axiom™ Precip Soln 1
      - Place on the benchtop to warm to room temperature.
      - Vortex before use.

   b. Axiom™ Precip Soln 2
      - Thaw on the benchtop at room temperature, then keep at room temperature.
      - Vortex, then centrifuge briefly before use.

   c. Keep the isopropanol at room temperature.

Label tubes and reagent reservoirs

1. Label the 15-mL conical tube as indicated in the following table.

Label	Temperature	Contents
Frag MM	Place tube on ice	Fragmentation Master Mix

2. Label the 4 Matrix™ Reagent Reservoirs as indicated in the following table.

Label	Reservoir size	Temperature	Contents
Frag MM	25 mL	Room temperature	Fragmentation Master Mix
Stop	25 mL	Room temperature	Frag Rxn Stop
Precip MM	25 mL	Room temperature	Precipitation Master Mix
ISO	100 mL	Room temperature	Isopropanol

Perform mPCR spike-in to the Amplification Plate

- If proceeding directly from the end of "Stage 1B: Amplify the genomic DNA" on page 51, remove the Amplification Plate from the 37°C oven.
- If working with a thawed amplified Amplification Plate, change the seal, vortex, then briefly centrifuge the plate.

IMPORTANT! Ensure that the mPCR Product Plate has been thoroughly mixed before spike-in to the Amplification Plate.

---

1. Transfer 10 µL of the mPCR products into the corresponding well of the Amplification Plate. Pipet up and down a few times to ensure complete liquid transfer from the pipette tip.
2. Seal the plate. Ensure that the seal is securely attached to the plate to minimize evaporation during the next steps.
3. Thoroughly mix by vortexing the plate for 30 seconds, then centrifuge briefly.
4. Immediately proceed to the next step ("Incubate the samples in preheated ovens" on page 64).

Incubate the samples in preheated ovens

Stop the DNA amplification reaction

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

3. Prepare reagents at the start of the 65°C incubation of the Amplification Plate.

Reagent and cap color	Module	Handling instructions
Axiom™ 10X Frag Buffer	2-1	Thaw, vortex, and keep on ice.
Axiom™ Frag Enzyme	2-1	Flick tube 3X, briefly centrifuge, then keep in –20°C cooler until ready to use.
Axiom™ Precip Soln 2	2-1	Thaw, vortex, briefly centrifuge, then keep at room temperature.
Axiom™ Frag Diluent	2-2	Vortex, briefly centrifuge, then keep on ice.
Axiom™ Frag Rxn Stop	2-2	Vortex, then keep at room temperature.
Axiom™ Precip Soln 1	2-2	Vortex, then keep at room temperature.
Isopropanol, 99.5%, 70 mL	N/A	Keep at room temperature.

4. Transfer the sample plate from the 65°C oven to the 37°C oven and incubate for 45 minutes.

Prepare for fragmentation

1. Remove the Amplification Plate from the 65°C oven, then check the seal. Press the seal, if needed.
2. Transfer the Amplification Plate to the 37°C oven.
3. Incubate for 45 minutes.

Prepare the Fragmentation Master Mix

Start making the Fragmentation Master Mix (Frag MM) 5 minutes before completion of the 37°C incubation.

1. Transfer the Axiom™ Frag Enzyme to a –20°C portable cooler until ready to use.
2. Add the reagents listed in the following table, in the order shown, to the 15-mL tube labeled “Frag MM”. Use appropriate single-channel pipettes.
   a. Just before the end of the 45-minute 37°C incubation, flick the Axiom™ Frag Enzyme tube 2 to 3 times, then centrifuge.
   b. Add the Axiom™ Frag Enzyme to the Fragmentation Master Mix at the end of the 45-minute 37°C incubation.

IMPORTANT! Leave the Axiom™ Frag Enzyme at –20°C until ready to use.

Reagent and cap color	Per sample	Master mix 96+
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**

3. Vortex the Frag MM tube twice.

4. Slowly pour the Fragmentation Master Mix into the reagent reservoir labeled “Frag MM”. Place the reservoir at room temperature.

Add 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 it on the benchtop at room temperature.

   Do *not* place the Amplification Plate on ice.

2. Carefully remove the seal from the Amplification Plate. Discard the seal.

3. Use a P200 12-channel pipette to add 57 µL of Fragmentation Master Mix to each reaction.
   - Pipet directly into the liquid of each well.
   - Do *not* mix by pipetting up and down.
   - Change tips after each addition.

   Note: The Amplification Plate is now the Fragmentation Plate.

4. Seal the Fragmentation Plate, then vortex it twice.

5. Start the timer for 30 minutes.

   Note: Keep your timer in a safe place. If the timer accidentally stops, it is helpful to have noted when the incubation started.

6. Briefly centrifuge the Fragmentation Plate at room temperature.

7. Quickly transfer the plate to the 37°C oven, then incubate for 30 minutes.

CAUTION! Watch for the end of the 30-minute incubation. *Fragmentation is an exact 30-minute incubation step.* Longer or shorter incubation times can lead to poor performance of the assay.

8. Prepare the Axiom™ Frag Rxn Stop solution a few minutes before the end of the 30-minute incubation.

   See “Add the Axiom™ Frag Rxn Stop solution to the Fragmentation Plate” on page 67.

Add the Axiom™ Frag Rxn Stop solution to the Fragmentation Plate

Carry out the following steps at room temperature.

1. A few minutes before the end of the 30-minute incubation, pour the Axiom™ Frag Rxn Stop solution into the reagent reservoir labeled “Stop”.
2. At the end of the 30-minute fragmentation incubation, remove the Fragmentation Plate from the oven. Place it on the benchtop at room temperature.
3. Carefully remove the seal from the Fragmentation Plate. Discard the seal.
4. Using a P20 12-channel pipette, end the fragmentation reaction by adding 19 µL of Axiom™ Frag Rxn Stop to each reaction.
   - Do not mix by pipetting up and down.
   - Pipette directly into the liquid of each well.
   - Change tips after each addition.
   - Proceed immediately to the next step.
5. Seal the Fragmentation Plate, then vortex and briefly centrifuge it at 1,000 rpm.
6. Leave the Fragmentation Plate on the benchtop while you prepare the Precipitation Master Mix.

Prepare the Precipitation Master Mix

Carry out the following steps at room temperature.

1. Prepare the Precipitation Master Mix (Precip MM) by adding 218 µL of Axiom™ Precip Soln 2 directly to the Axiom™ Precip Soln 1 bottle.

Reagent and cap color	Per sample	Master mix 96+
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. Place it on the benchtop at room temperature.

Add Precipitation Master Mix to samples

Carry out the following steps at room temperature.

1. Pour the Precipitation Master Mix into the reagent reservoir labeled “Precip MM”.
2. Carefully remove the seal from the Fragmentation Plate. Discard the seal.

3. Use a P1200 12-channel pipette to add 240 µL Precipitation Master Mix to each sample.
   - Rest the pipette tip against the wall of the well while delivering.
   - Do not mix by pipetting up and down.
   - Change tips after each addition.

Note: The Fragmentation Plate is now the Precipitation Plate.

4. Seal the Precipitation Plate, vortex, then briefly centrifuge.

Prepare and add isopropanol to the Precipitation Plate

1. Remove the Precipitation Plate from the centrifuge. Place it on the benchtop at room temperature.
2. Pour 65 mL of isopropanol into a 100-mL reagent reservoir labeled “ISO”.
3. Carefully remove the seal from the Precipitation Plate. Discard the seal.
4. Use a P1200 12-channel pipette to add 600-µL of isopropanol to each sample, then mix well by pipetting up and down 6–7 times.
   - Change the tips after each addition.
   - Observe the solution in the tips. It should look homogeneous after pipetting 6–7 times. If not, repeat mixing a few more times until the solution looks homogeneous.
   - Do not vortex the plate after adding the isopropanol to avoid cross-contamination of the samples.
5. Blot the top of the plate with a laboratory tissue, then seal tightly with MicroAmp™ Clear Adhesive Film.

Freeze the Precipitation Plate

Designate a shelf in a –20°C freezer where the plates can be left undisturbed. In addition, the freezer must not be subjected to frequent temperature excursions.

1. Carefully transfer the Precipitation Plate into the –20°C freezer.
2. Incubate the Precipitation Plate for the desired length of time.
   - Overnight (16–24 hours)
   - 3 hours

Note: The shortened 3-hour precipitation allows you to proceed to “Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC” on page 71 followed by “Stage 4: Transfer, denature, then hybridize the denatured samples” on page 86 on day 2 of the assay workflow.

IMPORTANT! The 3-hour DNA precipitation workflow extends the day 2 assay schedule. Approximately 9–10 hours are required to complete Stage 2 through Stage 4.

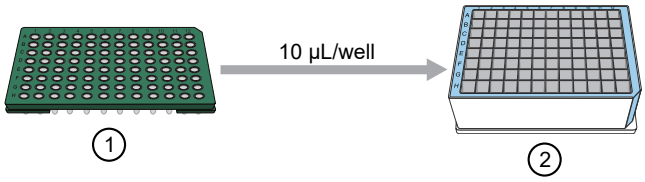
Store the mPCR Product Plate

Store the mPCR Product Plate at –20°C for optional QC.

See Appendix C, “mPCR quality control gel protocol”.

Workflow for Stage 2: mPCR spike-in to Amplification Plate

Addition of mPCR products



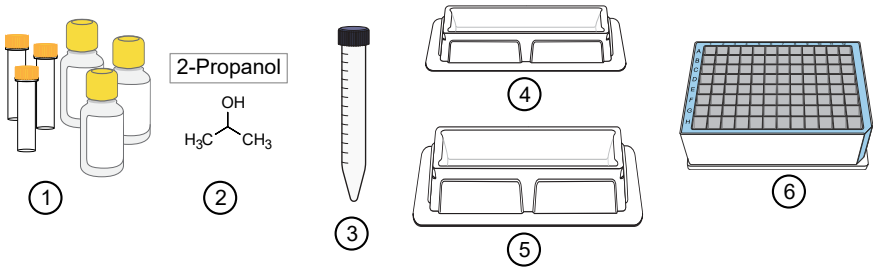
Final volume: 410 µL/well

① mPCR Product Plate
② Amplification Plate

Proceed to Stage 2: Fragmentation

Workflow for Stage 2: Fragment and precipitate the DNA

Materials required

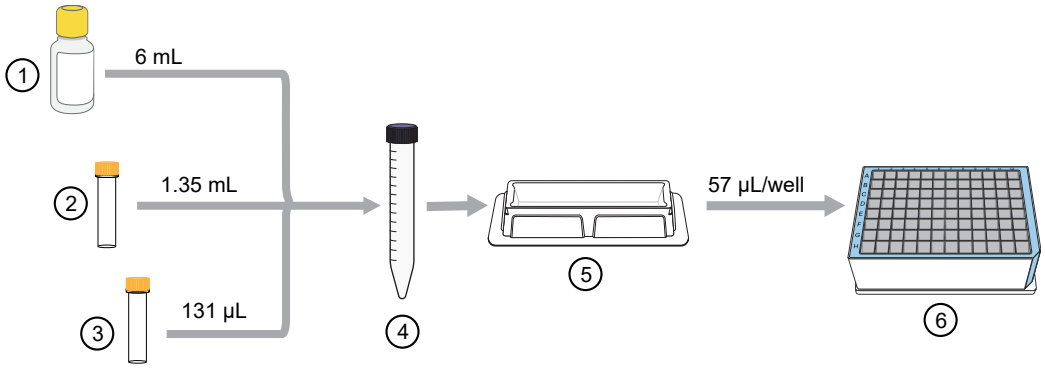


① Module 2-1 and Module 2-2 reagents
② 65 mL 2-Propanol
③ Quantity = 1
④ Quantity = 3 (25 mL)
⑤ Quantity = 1 (100 mL)
⑥ Quantity = 1, Amplification Plate with 10 µL of mPCR product spike-in

Incubate samples in pre-heated ovens

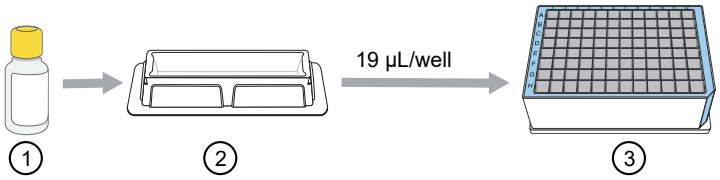
1. 20 minutes at 65°C
2. 45 minutes at 37°C

Fragmentation step



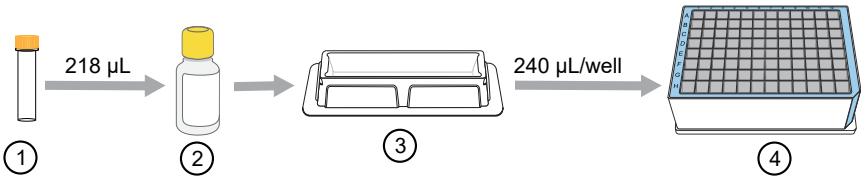
1. Axiom™ 10X Frag Buffer
2. Axiom™ Frag Diluent
3. Axiom™ Frag Enzyme
4. Vortex to mix.
5. Pour into reagent reservoir.
6. Fragmentation Plate. Vortex, briefly centrifuge, then incubate at 37°C for 30 minutes.

Stop fragmentation reaction



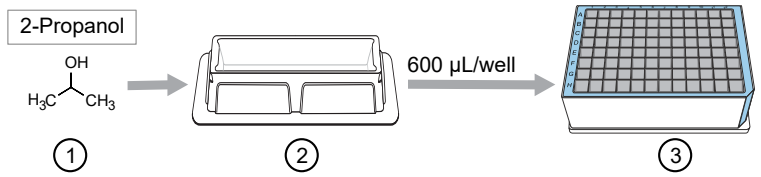
1. Axiom™ Frag Rxn Stop
2. Pour into reagent reservoir.
3. Fragmentation Plate. Vortex, then briefly centrifuge.

Precipitation preparation



1. Axiom™ Precip Soln 2
2. Axiom™ Precip Soln 1. Vortex well to mix.
3. Pour into reagent reservoir.
4. Precipitation Plate. Vortex for 30 seconds, then centrifuge briefly.

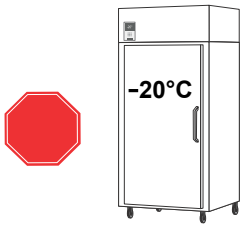
Add 2-Propanol



Final volume: 1,326 µL/well

① 65 mL 2-Propanol
② 100-mL reagent reservoir
③ Precipitation Plate. Pipet up and down to mix. Do not vortex.

Incubate Precipitation Plate at –20°C overnight (or optional 3-hour precipitation step)



Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC

CAUTION! Some steps in this stage must be performed under a fume hood.

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

Time required

Activity	Time
Centrifuge and dry plates	~1 hour 20 minutes
Resuspension and hybridization mix preparation	~25 minutes
Gel QC and OD	~45 minutes
Total	**~2.5 hours**

Input required

The Precipitation Plate from Stage 2: Fragment and precipitate the DNA.

Materials, labware, and reagents required

Equipment required for Stages 3A, 3B, and 3C

Quantity	Item
As needed	Adhesive seals for 96-well plates
1	Fine-point permanent marker
1 each	Rainin™ pipettes • Single-channel P20 • Single-channel P100 • Single-channel P1000 • Multichannel P20 • Multichannel P200
As needed	Pipette tips
2	Any 96-well PCR plate for making the dilutions • Dilution QC Plate • Gel QC Plate
1	Hyb-Ready Plate, 96-well PCR plate, one of the following: • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (Cat. No. HSP9631 or HSP9601) for Eppendorf™ Mastercycler™ pro S • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641) for Applied Biosystems™ GeneAmp™ PCR System 9700, Applied Biosystems™ Veriti™ Thermal Cycler, and Applied Biosystems™ ProFlex™ 96-well PCR System
1	OD QC Plate: Greiner Bio-One™ 96-Well UV-Star™ Plate, Flat Bottom, Chimney Style, Clear, 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™ Pipette Controller
1	Shaker
1	Vortexer
4	Matrix™ Reagent Reservoir, 25 mL

Reagents required for Stages 3A, 3B, and 3C

Reagent and cap color	Module
From the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit (Cat. No. 952389)	
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 and gel required for QC steps (optional)	
Gel Diluent, 100-fold dilution of TrackIt™ Cyan/Orange Loading Buffer. See Appendix D, “Fragmentation quality control gel protocol”.	—
E-Gel™ *48* Agarose Gels, 4%	—
Applied Biosystems™ 25 bp DNA Ladder (Cat. No. 931343) or a similar product prepared as instructed by manufacturer.	—
Nuclease-free water, ultrapure MB Grade (for OD QC Plate and Dilution QC Plate preparation)	—

Gels and related materials required

At the end of this stage, verifying the fragmentation reaction is highly recommended. See the appropriate section for required gel and related materials.

See Appendix D, “Fragmentation quality control gel protocol”.

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

Stage 3A: Centrifuge the Precipitation Plate and dry the DNA pellet

Centrifuge the Precipitation Plate and dry the DNA pellets

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

1. Preheat the oven 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 645, 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.

3. 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 that is described in the *Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0019173.

Note: If you are processing 2 plates at the same time, 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.

4. Immediately after the 40-minute centrifugation time, empty the liquid from the plate using the following steps:
   a. Carefully remove the seal from the Precipitation Plate. Discard the seal.
   b. Invert the plate over a clean waste container. Allow the liquid to drain. Collect the liquid, then discard it according to local, state, and federal regulations.
   c. While still inverted, gently press the plate on a pile of laboratory tissues on a bench. Allow it to drain for 5 minutes. Transfer the plate to a new pile of tissues twice during the 5-minute drain.

5. Turn the plate right side up and place it in an oven for 20 minutes at 37°C to dry.

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

6. Seal the plate tightly.

7. Do one of the following:

   - Proceed directly to the next stage, even if some droplets of liquid remain. See "Stage 3B: Resuspend the pellets and prepare for hybridization" on page 76. Leave the sample plate at room temperature. It is helpful to start preparing reagents for the next stage while centrifuging and drying pellets.
   - Store the Precipitation Plate for resuspension later in the same day. Tightly seal the plate.
     - If resuspension is carried out within 4 hours, keep the plate at room temperature.
     - If resuspension will be carried out after more than 4 hours, store the plate in a refrigerator at 2–8°C.
   - If resuspension will be carried out on another day, store the tightly sealed plate at –20°C.

Stage 3B: Resuspend the pellets and prepare for hybridization

Prepare for resuspension and hybridization

Set the centrifuge to room temperature.

Prepare DNA pellets and warm the Axiom™ Resusp 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 Axiom™ Resusp Buffer to room temperature (18–25°C) is critical for the success of the Axiom™ PharmacoFocus™ Assay target preparation. When the plate of DNA pellets or Axiom™ Resusp Buffer is cooler than room temperature, pellets might not resuspend completely and can compromise assay performance.

Guidelines for the DNA pellet types

Observe the following guidelines on how to work with plates with fresh, cold, or frozen pellets.

- **Fresh Pellets**: If proceeding with the resuspension and hybridization preparation protocol within 4 hours, a plate with fresh pellets can be kept at room temperature.
- **Cold Pellets**: If processed during the same day, a plate with fresh pellets that are not processed within 4 hours can be transferred to a refrigerator and kept at 2–8°C. 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 equilibrated at room temperature for at least 1.5 hours before proceeding with the resuspension and hybridization preparation protocol.

Guidelines for resuspension and hybridization reagents

The Axiom™ Resusp Buffer needs at least 60 minutes to equilibrate to room temperature.

Thaw and prepare reagents

1. Thaw the Axiom™ Hyb Soln 1 on the benchtop at room temperature.
2. Warm the Axiom™ Resusp Buffer, Axiom™ Hyb Buffer, and the Axiom™ Hyb Soln 2 on the benchtop at room temperature for at least 1 hour.
3. Vortex the Axiom™ Resusp Buffer and the Axiom™ Hyb Buffer. Keep at room temperature.
4. Vortex and briefly centrifuge the Axiom™ Hyb Soln 1 and Axiom™ Hyb Soln 2 before use.

Label tubes and reagent reservoirs

1. Label the 15-mL conical tube as indicated in the following table.

Label	Temperature	Contents
Hyb MM	Room temperature in fume hood	Hybridization Master Mix

2. Label the 2 Matrix™ Reagent Reservoirs as indicated in the following table.

Label	Reservoir size	Temperature	Contents
Resus	25 mL	Room temperature	Axiom™ Resusp Buffer
Hyb MM	25 mL	Room temperature in fume hood	Hybridization Master Mix

Add the Axiom™ Resusp Buffer to the DNA pellets

A plate stored at –20°C after drying the pellets must be allowed to sit at room temperature for 1.5 hours before carrying out resuspension.

Ensure that the Axiom™ Resusp Buffer has equilibrated to room temperature before adding it to the dry pellets in step 3.

Carry out the following steps at room temperature.

1. Pour the Axiom™ Resusp Buffer into the reagent reservoir labeled “Resus”.
2. Carefully remove the seal from the Precipitation Plate. Discard the seal.
3. Use a P200 12-channel pipette to transfer 35 µL of Axiom™ Resusp Buffer to each well of the Precipitation Plate.
   - Avoid touching the pellets with the pipette tips.
   - Change pipette tips after each addition.

   Note: The Precipitation Plate is now the Resuspension Plate.
4. Seal the plate tightly.

   Note: Blue pellets should be visible at the bottom of the wells.

Resuspend the DNA pellets

1. Place the sealed Resuspension Plate on one of the following shakers, then run for the time that is specified.
   - Thermo Scientific™ Compact Digital Microplate Shaker: 900 rpm for 10 minutes
   - Jitterbug™: Speed 7 for 10 minutes
2. Inspect the Resuspension Plate from the bottom. If the pellets are not dissolved, repeat step 1.
3. Centrifuge the plate briefly at 1,000 rpm.

Prepare the Hybridization Master Mix

CAUTION! Perform the remainder of Stage 3B under a fume hood.

1. While the Resuspension Plate is shaking, prepare the Hybridization Master Mix in the 15-mL tube labeled “Hyb MM”.
2. Add the reagents as indicated in the following table, in the order shown, to the Hyb MM tube. Use serological and single-channel pipettes.

Reagent and cap color	Per sample	Master mix 96+
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	**80 µL**	**8.86 mL**

3. Vortex the Hyb MM tube twice to mix.

Prepare the Hyb-Ready Plate

1. Select a 96-well plate that is compatible with the thermal cycler model that is used for sample denaturation. See “PCR plate type by thermal cycler for the mPCR step” on page 29.
2. Label the 96-well PCR plate “Hyb Ready [Plate ID]”.
3. Set a P200 12-channel pipette to 45 µL. This volume is slightly more than the volume of the sample in each well of the Resuspension Plate.
4. Use the P200 pipette to transfer the entire contents of each well of the Resuspension Plate to the corresponding wells of the Hyb-Ready Plate.

   Note: Change pipette tips after each addition.

5. Pour the Hybridization Master Mix into the reagent reservoir labeled “Hyb MM”.
6. Use a P200 12-channel pipette to add 80 µL of the Hybridization Master Mix to each well of the Hyb-Ready Plate.

   Note: Change pipette tips after each addition.

7. Seal the Hyb-Ready Plate, vortex it twice, then centrifuge it briefly.

Freeze or proceed

Do one of the following:

- Proceed to "Stage 3C: Perform quantification and fragmentation QC checks" on page 79. We strongly recommend performing the QC check.
- Proceed to "Stage 4: Transfer, denature, then hybridize the denatured samples" on page 86.
- Store the Hyb-Ready Plate at –20°C.

Stage 3C: Perform quantification and fragmentation QC checks

Before proceeding to "Stage 4: Transfer, denature, then hybridize the denatured samples" on page 86, we highly recommend that you perform quantification and fragmentation quality control checks.

Prepare for sample QC

Prepare the reagents

Obtain the following reagents for sample QC.

- Nuclease-free water, 15 mL, for the water reservoir
- Gel diluent, 15 mL
  Gel diluent is a 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer. See "Prepare the gel diluent" on page 187.
- 25 bp DNA Ladder prepared as instructed by the manufacturer.
  See "Dilute the 25 bp DNA Ladder" on page 191.
- Two E-Gel™ *48* Agarose Gels, 4%

Label the reagent reservoirs

Label 2 Matrix™ Reagent Reservoirs as indicated in the following table.

Label	Reservoir size	Temperature	Contents
H2O	25 mL	Room temperature	Nuclease-free Water
Gel Dil	25 mL	Room temperature	Diluted loading dye

Prepare the sample QC plates

1. Label two 96-well PCR plates for making the dilutions.
   - Label 1 plate "QC Dil".
   - Label 1 plate "Gel QC".
2. Obtain 1 Greiner Bio-One™ 96-well UV-Star™ Plate.

Perform QC checks

Carry out the following steps at room temperature.

Note: To avoid cross-contamination, change pipette tips while transferring samples from the Hyb-Ready Plate to the Dilution QC Plate.

1. Prepare the Dilution QC Plate and the OD QC Plate.
   a. Pour 15 mL of Nuclease-free Water into the reagent reservoir labeled "H20".
      The water is used to make the Dilution QC Plate and the OD QC Plate.
   b. Add 33 µL of Nuclease-free Water to each well of the Dilution QC Plate.
   c. Add 90 µL of Nuclease-free Water to each well of the OD QC Plate (96-well UV-Star™ 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 Dilution QC Plate.
      - Change pipette tips after each transfer.
   b. Seal, vortex, then briefly centrifuge the plate.
3. Prepare the OD QC Plate.
   a. Carefully remove the seal from the Dilution QC Plate. Discard the seal.
   b. Transfer 10 µL of each Dilution QC Plate sample to the corresponding wells of the OD QC Plate and mix by pipetting up and down.
      - Change pipette tips after each transfer.
   c. Mix by pipetting up and down.
      Final sample mass dilution is 120-fold.
      If needed, review the instructions on performing the sample quantification. See Appendix E, “Sample quantification after resuspension”.
4. Prepare the Gel QC Plate.
   a. Pour 15 mL of gel diluent into the reagent reservoir labeled "Gel Dil".
   b. Add 120 µL of gel diluent to each well of the Gel QC Plate.
   c. Transfer 3 µL of each Dilution QC Plate sample to the corresponding wells of the Gel QC Plate.
      - Change pipette tips after each transfer.
   d. Seal, vortex, then briefly centrifuge the plate.
5. Run the gel. See Appendix E, “Sample quantification after resuspension”.

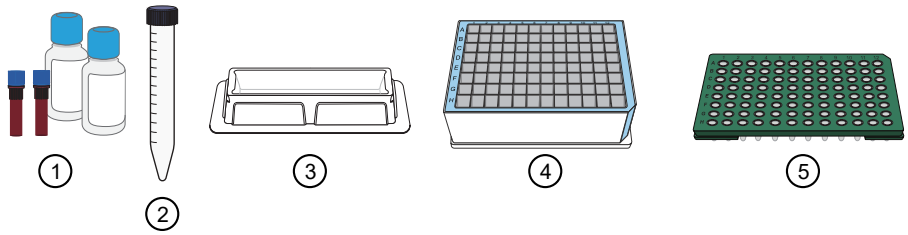
The Dilution QC Plate, OD QC Plate, and remaining Gel QC Plate samples can be discarded after you have obtained satisfactory results from the gel and OD_{260} readings.

Workflow for Stage 3A: Centrifuge the Precipitation Plate and dry the pellets

Centrifuge the Precipitation Plate to pellet DNA.
• Speed: 3,200 × *g* • Time: 40 minutes • Temperature: 4°C ▼
Dry the DNA pellets.
1. Decant liquid by inverting plate. **2.** Blot-dry inverted plate for 5 minutes. **3.** Incubate at 37°C for 20 minutes right-side up.
Continue to resuspend the pellets.

Workflow for Stage 3B: Resuspend the pellets and prepare for hybridization

Materials required



① Module 2-1 and Module 2-2 reagents
② Quantity = 1
③ Quantity = 2
④ Precipitation Plate (samples)
⑤ Hyb-Ready Plate: Quantity = 1 (Plate type is dependent on the thermal cycler used.)

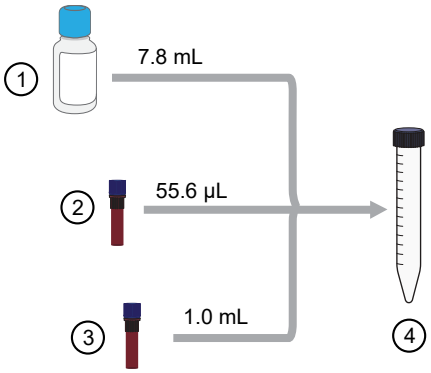
Add the Axiom™ Resusp Buffer.

① Axiom™ Resusp Buffer
② Pour into reagent reservoir.
③ Resuspension Plate. Resuspend pellets on the plate shaker for 10 minutes. Briefly centrifuge.

Resuspend the DNA pellet.

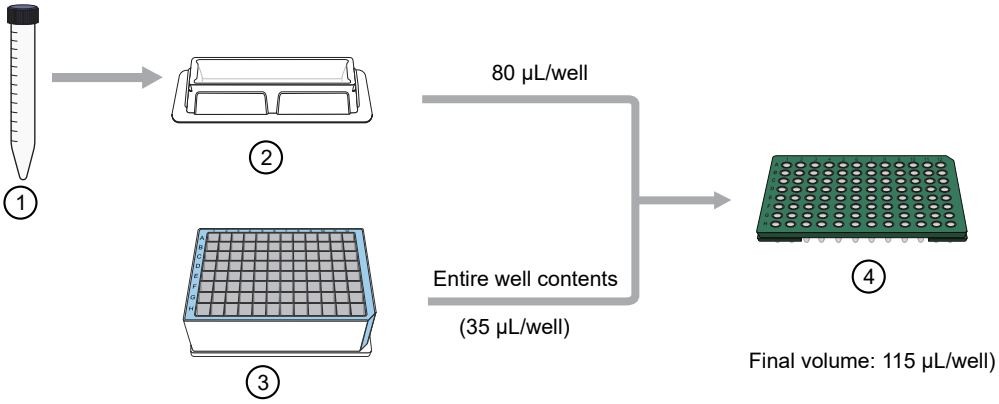
- Time: 10 minutes
- Jitterbug™: Speed 7, or Thermo Scientific™ Titer Plate Shaker: 900 rpm

Prepare the Hybridization Master Mix.



① Axiom™ Hyb Buffer
② Axiom™ Hyb Soln 1
③ Axiom™ Hyb Soln 2
④ Hybridization Master Mix. Vortex to mix.

Prepare the Hyb-Ready Plate.

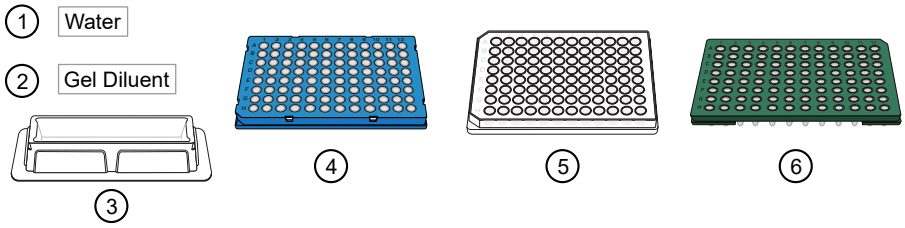


① Hybridization Master Mix.
② Pour Hybridization Master Mix into reagent reservoir.
③ Resuspension Plate with resuspended DNA.
④ Hyb-Ready Plate. Vortex well. Briefly centrifuge.

Continue to perform sample QC.

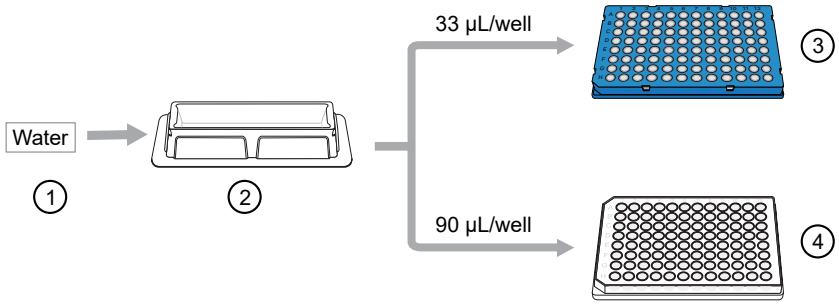
Workflow for Stage 3C: Perform sample QC

Materials required



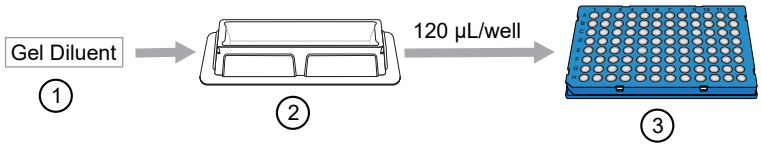
1. 15 mL water
2. 15 mL Gel diluent
3. Quantity = 2
4. Quantity = 2
5. Quantity = 1
6. Quantity = 1 (Hyb-Ready Plate)

Aliquot water to the Dilution QC Plate and the OD QC Plate.



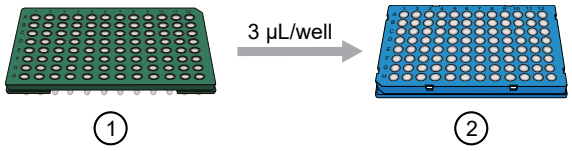
1. 15 mL water
2. Pour water into reagent reservoir.
3. Dilution QC Plate
4. OD QC Plate

Aliquot the gel diluent to the Gel QC Plate.



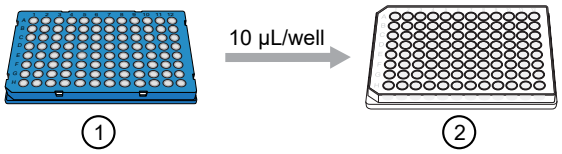
1. 15 mL Gel diluent
2. Pour the gel diluent into a reagent reservoir.
3. Gel QC Plate

Prepare the Dilution QC Plate.



① Hyb-Ready Plate. Vortex, then centrifuge briefly.
② Dilution QC Plate. Vortex, then centrifuge briefly.

Prepare the OD QC Plate.



① Dilution QC Plate
② OD QC Plate. Read Abs 260 nm, 280 nm, and 320 nm.

Prepare the Gel QC Plate.

① Dilution QC Plate
② Gel QC Plate. Vortex, then briefly centrifuge. Load 20 µL into a 4% E-Gel™ Agarose Gel for analysis.

Proceed to Stage 4, or store hybridization-ready samples at –20°C.

Stage 4: Transfer, denature, then hybridize the denatured samples

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.

Note: Before denaturing samples, ensure that the GeneTitan™ MC Instrument is ready for use.

CAUTION! GeneTitan™ Wash and rinse bottles must be filled with reagents at prompt when setting up hybridization.

CAUTION! Parts of this stage must be performed under a fume hood.

Time required

Activity	Time
Hands-on time including denaturation	~50 minutes
GeneTitan™ MC Instrument hybridization time	23.5—24 hours
Total	**24.25—24.75 hours**

Input required

The Hyb-Ready Plate from Stage 3.

Materials, labware, and reagents required

Equipment required for Stage 4

Quantity	Item
1	GeneTitan™ MC Instrument
1	Rainin™ P200 12-channel pipette
As needed	Pipette tips
1	Thermal cycler, programmed with **PharmacoFocus Denature** protocol. Use the heated lid option when setting up or running the protocol. • 95°C for 10 minutes • 48°C for 3 minutes • 48°C hold

(continued)

Quantity	Item
1	Hyb-Ready Denaturation Plate, 96-well PCR plate, one of the following: • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (Cat. No. HSP9631 or HSP9601) for Eppendorf™ Mastercycler™ pro S • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641) for Applied Biosystems™ GeneAmp™ PCR System 9700, Applied Biosystems™ Veriti™ Thermal Cycler, and Applied Biosystems™ ProFlex™ System
1	96-well metal chamber warmed in a 48°C oven[1]
1	Axiom™ PharmacoFocus™ Mini 96-Array Plate
1	384-Layout GeneTitan™ Hybridization Tray[2]

[1] The 96-well metal chamber coming out of a 48°C oven is warm to the touch. Use gloves or mitts if the metal chamber feels too hot.

[2] The Consumables for the GeneTitan™ MC Instrument are packaged separately from the array plates. The 384-Layout GeneTitan™ Hybridization Tray, along with other GeneTitan™ consumables, are included in the Axiom™ 384HT GeneTitan™ High Volume Consumables Kit (Cat. No. 902629) or Axiom™ GeneTitan™ 384HT Consumables Kit (Cat. No. 952385).

Reagents required and handling for Stage 4

Reagent	Module	Handling instructions
From the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit		
Axiom™ Wash Buffer A	Module 3, Room temperature	Invert 2-3 times for mixing before filling GeneTitan™ bottle
Axiom™ Wash Buffer B		Invert 2-3 times for mixing before filling GeneTitan™ bottle
Axiom™ Water		—

Prepare for transfer, denaturation, and hybridization

1. Preheat the 96-well metal chamber in a 48°C oven.
2. Allow the array plate to equilibrate to room temperature for at least 25 minutes.
   a. Leave the array plate in the pouch at room temperature, for at least 25 minutes to allow the plate to come to room temperature, before opening and loading in the GeneTitan™ MC Instrument.

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.
See Appendix B, "Register samples in GeneChip™ Command Console™".

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

3. Power on the thermal cycler to prepare for the **PharmacoFocus Denature** protocol to run with the heated lid option selected.

Perform Stage 4

CAUTION! Parts of this stage must be performed under a fume hood.

1. If the Hyb-Ready Plate was stored at –20°C, go to "Prepare hybridization-ready samples stored at –20°C".
2. If proceeding directly from the end of Stage 3, go to "Transfer samples from the Hyb-Ready Plate to the Hyb-Ready Denaturation Plate".

Prepare hybridization-ready samples stored at –20°C

1. Warm the Hyb-Ready Plate at room temperature for 5 minutes. It is not necessary to equilibrate the plate longer than 5 minutes.
2. Check the Hyb-Ready Plate to ensure that it is sealed well. If the plate is not sealed well:
   a. Centrifuge the plate, then carefully remove the old seal.
   b. Wipe any condensation from the top of the plate, then gently blot dry with a laboratory tissue.
   c. Tightly reseal the plate using a fresh seal.
3. Vortex the Hyb-Ready Plate for 30 seconds to mix, then centrifuge at 1,000 rpm for 30 seconds.
   It is important that the samples are thoroughly mixed prior to transfer to the Hyb-Ready Denaturation Plate.
4. Place the Hyb-Ready Plate at room temperature.

Transfer samples from the Hyb-Ready Plate to the Hyb-Ready Denaturation Plate

1. Ensure that the Hyb-Ready Plate from Stage 3 is tightly sealed.
2. Vortex the plate, then centrifuge at 1,000 rpm for 30 seconds.
3. Label a new Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted plate (or appropriate PCR plate) "Hyb-Ready Denaturation Plate".

   Use the PCR plate type that is compatible with the thermal cycler that will be used for denaturation.
4. Obtain a P200 12-channel pipette, then set it at 50 µL.
5. Transfer 50 µL of hybridization-ready sample solution from the Hyb-Ready Plate to the Hyb-Ready Denaturation Plate.
6. Tightly seal the Hyb-Ready Denaturation Plate.

   This Hyb-Ready Denaturation Plate can be stored at –20°C if the denaturation step will be performed later.

After preparing the Hyb-Ready Denaturation Plate, approximately 62 µL of sample remains in the Hyb-Ready Plate. Carefully seal the Hyb-Ready Plate and store at –20°C. After the assay is successfully completed, the plate can be discarded.

Prepare the GeneTitan™ MC Instrument

Before you denature the Hyb-Ready Denaturation Plate samples, ensure that the GeneTitan™ MC Instrument is ready for use. Follow the instructions in "Stage 2: Hybridize plates in the GeneTitan™ MC Instrument" on page 114 and Appendix B, "Register samples in GeneChip™ Command Console™". The following is a brief summary of the steps to perform.

1. Launch the GeneChip™ Command Console™ software, then select **GCC GeneTitan Control**.
2. From the Launcher window, open **GCC Portal ▸ Samples ▸ GeneTitan™ Array Plate Registration**.
3. Upload the GeneTitan™ Array Plate Registration file.

   If you do not upload your registration file before scanning the array plate barcode, the software assigns default names to your samples.

   Note: When creating the GeneTitan™ Array Plate Registration file, you can add the barcode of the hybridization tray as a sample file attribute. Adding the barcode as an attribute enables traceability in the system. See the *GeneChip™ Command Console™ User Guide*, for details on adding attributes to sample files.

   ---
4. Select the **System Setup** tab.
5. For **Setup Option**, select **Hyb-Wash-Scan**.
6. Click **Next**.

7. Complete the following in the **Plate information** section:
   a. **Barcode**: Scan or manually enter the array plate barcode, then click **Next**.
   b. **Protocol Name**: Select the protocol name, then click **Next**.
8. Fill the Wash A, Wash B, and Rinse bottles with Axiom™ Wash Buffer A, Axiom™ Wash Buffer B, and Axiom™ Water, respectively.
9. Empty the Waste bottle.
10. Press the blue confirmation button on the GeneTitan™ MC Instrument to continue.
    A fluidics check is run (~1 minute).
11. Open the trash bin and empty, then press the blue confirmation button to continue.
    If already empty, the trash bin remains locked and the **Status** pane reads "Trash bin is empty".
12. Remove used trays and plates when drawers open, then press the blue confirmation button to continue.
    If there are no consumables to remove, the **Status** pane reads "Drawers are empty".

Denature the hybridization-ready samples on the Hyb-Ready Denaturation Plate

1. Ensure that the thermal cycler is powered on and that the **PharmacoFocus Denature** protocol with the heated lid option is selected.
2. Open the lid of the thermal cycler, then place the sealed Hyb-Ready Denaturation Plate on the thermal cycler.
   Check the integrity of the seal as evaporation during denaturation can negatively impact assay performance.
3. Close the lid.
   For thermal cyclers with variable lid tension, follow manufacturer's instructions for adjusting lid tension.
4. Start the **PharmacoFocus Denature** protocol.
5. After the **PharmacoFocus Denature** protocol is complete, remove the plate from the thermal cycler, then place on the preheated metal chamber.

IMPORTANT! Avoid leaving denatured samples at room temperature for any length of time. When you are ready to transfer the denatured samples to the hybridization tray at the end of the **PharmacoFocus Denature** protocol, place the plate on a 96-well metal chamber preheated at 48°C to minimize sample cooling.

Prepare the hybridization tray and load into the GeneTitan™ MC Instrument

For more information, see Appendix A, "Recommended techniques for GeneTitan™ MC Instrument operation".

CAUTION! Perform the following steps under a fume hood.

1. Remove the 384-Layout GeneTitan™ Hybridization Tray from packaging.
2. Label the hybridization tray.

   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 F1.
3. After the **PharmacoFocus Denature** protocol has completed, remove the Hyb-Ready Denaturation Plate from the thermal cycler, then place it into a 96-well metal chamber that has been prewarmed in an oven at 48°C.
4. Move the warm 96-well metal chamber containing the Hyb-Ready Denaturation Plate to a fume hood.
5. Remove the seal from Hyb-Ready Denaturation Plate, then discard.
6. Place the hybridization tray under the fume hood, then remove the hybridization tray cover.
7. Obtain a P200 12-channel pipette and set it at 35 µL.

8. Slowly transfer the denatured samples from the wells of the 96-array format Hyb-Ready Denaturation Plate into the corresponding wells in quadrant 1 of the 384-Layout GeneTitan™ Hybridization Tray, using the guidance for plate format switching listed in the following table.

96-array format Hyb-Ready Denaturation Plate	384-Layout GeneTitan™ Hybridization Tray
Row A	Row A, odd wells
Row B	Row C, odd wells
Row C	Row E, odd wells
Row D	Row G, odd wells
Row E	Row I, odd wells
Row F	Row K, odd wells
Row G	Row M, odd wells
Row H	Row O, odd wells

   - 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 well occurs when the array plate is stacked together with the hybridization tray by the GeneTitan™ MC Instrument.

9. Load the array plate and hybridization tray into the GeneTitan™ MC Instrument.

   IMPORTANT! After the GeneTitan™ MC Instrument has stacked the array plate and hybridization tray, the instrument extends the drawer. The sandwich of the array plate and hybridization tray must be manually clamped and inspected before the array processing can begin. Carefully review and execute the array plate/hybridization tray clamping procedure steps.

   For more information, see “Load an array plate and hybridization tray into the GeneTitan™ MC Instrument” on page 119.

Hybridization continues on the GeneTitan™ MC Instrument for 23.5 to 24 hours before loading the Ligation/Staining/Stabilization reagent trays into the instrument occurs. Near the end of the 23.5- to 24-hour hybridization period in the GeneTitan™ MC Instrument, see “Stage 5: Prepare the GeneTitan™ reagents and trays” on page 95.

Plate format switching

Plate format switching is performed during the hybridization transfer step. The denatured, hybridization-ready samples are transferred from a 96-well PCR plate (Hyb-Ready Denaturation Plate) to a 384-Layout GeneTitan™ Hybridization Tray. The samples must only be transferred to the quadrant 1 wells of the hybridization tray. The following figure illustrates the hybridization transfer step when the switch from 96-well format to 384-well format occurs.

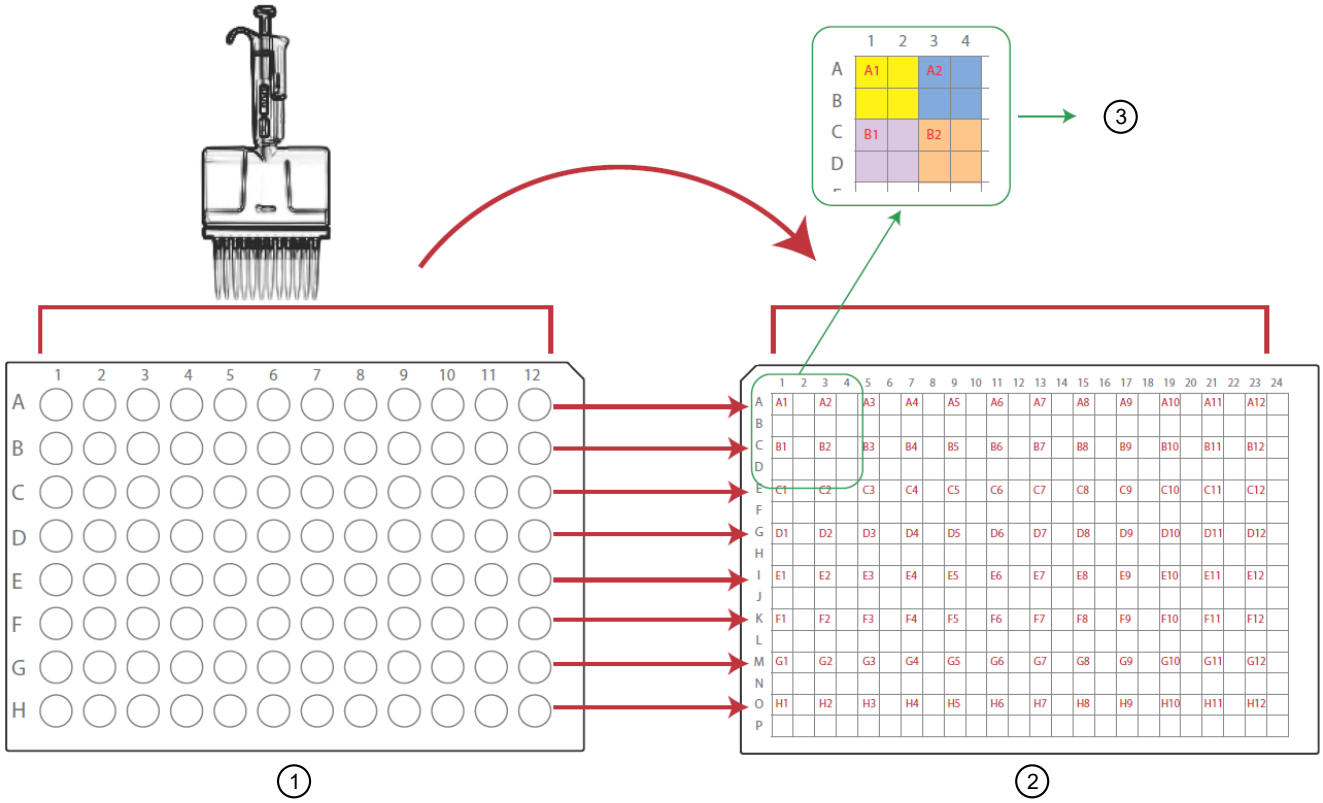


Figure 12 Plate format switching during hybridization transfer.

(1) 96-format Hyb-Ready Denaturation Plate

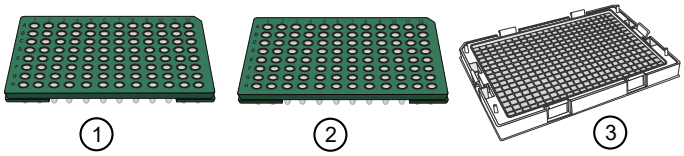
(2) 384-Layout GeneTitan™ Hybridization Tray

(3) Quadrants explained: A 384-format plate consists of 96 four-well sections (96 x 4 = 384). Each of the 4 well in a section is a "quadrant". In the example shown, wells A1, A3, C1, and C3 of the of the 384-format plate are all designated as Quadrant 1 (Q1) wells within their respective 4-well section.

The red well numbers in the 384-Layout GeneTitan™ Hybridization Tray image represent the corresponding well transferred from the 96-well Hyb-Ready Denaturation Plate (all in Quadrant 1—the upper left quadrant of a section).

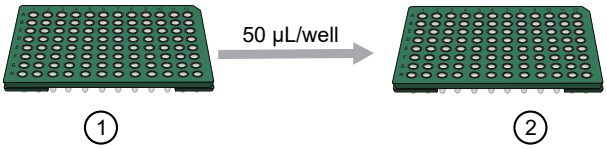
Workflow for Stage 4: Transfer, denature, then hybridize the denatured samples

Labware required



1. Hyb-Ready Plate with samples
2. Hyb-Ready Denaturation Plate, 96-well PCR plate compatible with thermal cycler to be used for denaturation
3. 384-Layout GeneTitan™ Hybridization Tray

Transfer samples from the Hyb-Ready Plate to the Hyb-Ready Denaturation Plate.

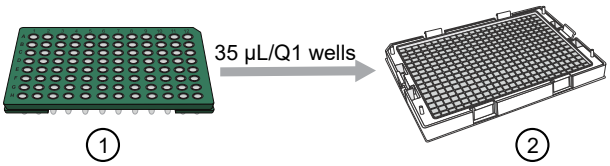


1. Hyb-Ready Plate
2. Hyb-Ready Denaturation Plate with 50 µL/well hybridization-ready samples

Denature the hybridization-ready samples on the Hyb-Ready Denaturation Plate

1. 10 minutes at 95°C.
2. 3 minutes at 48°C.

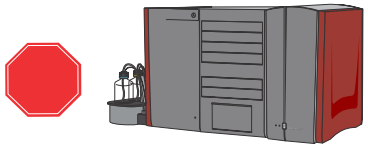
Prepare the hybridization tray



1. Denatured hybridization-ready samples on the Hyb-Ready Denaturation Plate. Keep samples on a 48°C heat 96-well metal chamber.
2. Hybridization tray with samples in Q1. If needed, pierce bubbles.

Process the hybridization tray in the GeneTitan™ MC Instrument.

Complete the manual clamping and verification procedure of the array plate-hybridization tray stack.



Stage 5: Prepare the GeneTitan™ reagents and trays

Begin this stage when hybridization in the GeneTitan™ MC Instrument is near completion (1.5 hours before completion), so that the reagent trays can be loaded for the GeneTitan™ array processing steps.

IMPORTANT! The reagent trays prepared in this stage are for the continued processing of an Axiom™ PharmacoFocus™ Mini 96-Array Plate that:

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

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

Time required

Activity	Time
Prepare reagents (thaw and organize)	~30 minutes
Hands-on time for GeneTitan™ reagent preparation	~50 minutes
GeneTitan™ MC Instrument loading	~10 minutes
Total	**~90 minutes**

Materials, labware, and reagents required

Equipment and labware required for Stage 5

Quantity	Item
Instruments	
1	GeneTitan™ MC Instrument
1	Vortexer
As required	Laboratory tissue
1	Marker, fine point, permanent
1	Cooler for enzyme
1	Mini microcentrifuge (microcentrifuge with microtube rotor)
1	Pipet-Aid™ Pipette Controller

(continued)

Quantity	Item
1 each	Rainin™ pipettes • P200 single-channel pipette • P1000 single-channel pipette • P200 multichannel pipette
Consumables	
As required	Pipette tips (for Rainin™ pipettes listed above)
1	Pipette, serological: 5 x 1/10 mL
As required	Aluminum foil (optional)
5	Matrix™ Reagent Reservoirs, 25 mL
4	15-mL conical tube
As indicated	Items required from the Axiom™ 384HT GeneTitan™ High Volume Consumables Kit (Cat. No. 902629) • Two 384-Layout GeneTitan™ Stain Tray (Stain 1) • One 384-Layout Axiom™ Stain 2 Tray • One 384-Layout Axiom™ Stabilization Tray • One 384-Layout Axiom™ Ligation Tray • One 384-Layout GeneTitan™ Scan Tray • Six 384-Layout GeneTitan™ Scan and Stain Tray Cover

Reagents required and reagent handling for Stage 5

Prepare reagents according to the following table.

Module	Qty	Reagent and cap color	Thaw, then place on ice	Place on ice	Place at room temperature
Module 4-1 –20°C	1	Axiom™ Ligate Buffer[1]			✓
	1	Axiom™ Ligate Enzyme	⊗ Do not thaw. Keep at –20°C until ready to use.		
	1	Axiom™ Ligate Soln 1	✓		
	1	Axiom™ Probe Mix 1	✓		
	1	Axiom™ Stain Buffer	✓		
	1	Axiom™ Stabilize Soln	✓		
Module 4-2 2°C to 8°C	1	Axiom™ Ligate Soln 2			✓
	1	Axiom™ Probe Mix 2[2]		✓	
	1	Axiom™ Wash A[1]			✓
	1	Axiom™ Stain 1-A[2]		✓	
	1	Axiom™ Stain 1-B[2]		✓	
	1	Axiom™ Stain 2-A[2]		✓	
	1	Axiom™ Stain 2-B[2]		✓	
	1	Axiom™ Stabilize Diluent[1]		✓	
	1	Axiom™ Water			✓
	1	Axiom™ Hold Buffer[2]			✓
Estimated reagent thawing time is ~30 minutes.					

[1] Check for precipitate. If precipitate is present, repeat the vortex and centrifuge step.

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

Notes on handling reagents with precipitates

Prepare Axiom™ Wash A

During storage of the Axiom™ Wash A (in Module 4-2 stored at 4°C), precipitation in the form of clear crystals can sometimes occur. Therefore, use the following procedure 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. Use the following procedure to resuspend any precipitate before use.

---

1. Vortex 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 Axiom™ Stabilize Diluent

During storage of the Axiom™ Stabilize Diluent (in Module 4-2 stored at 4°C), precipitation in the form of clear crystals can sometimes occur. Therefore, use the following procedure to ensure that any precipitate is returned to solution prior to use.

The presence of some precipitate is okay and does not adversely impact assay performance. Use the following procedure to resuspend any precipitate before use.

1. Vortex, then centrifuge briefly.
2. Examine the reagent for precipitate.
3. If precipitate is observed, warm the tube to room temperature, vortex, then centrifuge again.

Prepare Axiom™ Ligate Buffer

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

The presence of some precipitate is okay and does not adversely impact assay performance. Use the following instructions to resuspend most precipitate before use.

1. Vortex for 30 seconds.
2. Examine the buffer for precipitate.
3. If precipitate is still present, warm the bottle with your hands, then vortex again for 30 seconds.

Prepare the remaining reagents

1. Leave the Axiom™ Ligate Enzyme at –20°C until ready to use.
2. Thaw the following reagents from the Module 4-1 on the benchtop at room temperature. Vortex and centrifuge them briefly, then place them on ice.
   - Axiom™ Ligate Soln 1
   - Axiom™ Probe Mix 1
   - Axiom™ Stabilize Soln
   - Axiom™ Stain Buffer
3. Prepare the remaining reagents from Module 4-2 as follows.
   a. Gently flick each tube 2 to 3 times to mix, then centrifuge them.
   b. Place the reagents on ice, except for the following. Leave them on the benchtop at room temperature.
      - Axiom™ Hold Buffer
      - Axiom™ Ligate Soln 2
      - Axiom™ Water
      - Axiom™ Wash A

Label master mix tubes and reagent reservoirs

1. Label the side of each conical tube as indicated in the following table.

Tube size	Label	Contents
15 mL	S1	Stain 1 Master Mix
15 mL	S2	Stain 2 Master Mix
15 mL	Stbl	Stabilization Master Mix
15 mL	Lig	Ligation Master Mix

2. Place the 4 tubes on ice.
3. Label the 5 Matrix™ Reagent Reservoirs (25 mL) as indicated in the following table.

Label	Reservoir size	Contents
S1	25 mL	Stain 1 Master Mix
S2	25 mL	Stain 2 Master Mix
Stbl	25 mL	Stabilization Master Mix
Lig	25 mL	Ligation Master Mix
Hold	25 mL	Axiom™ Hold Buffer

Prepare the stain, ligation, and stabilization master mixes

Prepare the Stain 1 Master Mix

1. Use the appropriate serological and single-channel pipettes to add reagents to the 15-mL tube labeled “S1” in the following order. This recipe provides enough for both S1 reagent trays.

Reagent and cap color	Per array	Master mix 96+
Axiom™ Wash A	76.8 µL	8.8 mL
Axiom™ Stain Buffer	1.6 µL	184 µL
Axiom™ Stain 1-A	0.8 µL	92 µL
Axiom™ Stain 1-B	0.8 µL	92 µL
Total	**80 µL (40 µL x 2)**	**9.2 mL**

2. Gently invert the tube 10 times to mix. Do not vortex.

3. Place the tube on ice and protect it from direct light. For example, cover it with aluminum foil or the ice bucket lid.

Prepare the Stain 2 Master Mix

1. Use the appropriate serological and single-channel pipettes to add reagents to the 15-mL tube labeled “S2” in the following order.

Reagent and cap color	Per array	Master mix 96+
Axiom™ Wash A	38.4 µL	4.6 mL
Axiom™ Stain Buffer	0.8 µL	96 µL
Axiom™ Stain 2-A	0.4 µL	48 µL
Axiom™ Stain 2-B	0.4 µL	48 µL
Total	**40 µL**	**4.8 mL**

2. Gently invert the S2 tube 10 times to mix. Do not vortex.

3. Place on ice, then protect from direct light. For example, cover it with aluminum foil or the ice bucket lid.

Prepare the Stabilization Master Mix

1. Use the appropriate serological and single-channel pipettes to add reagents to the 15-mL tube labeled “Stbl” in the following order.

Reagent and cap color	Per array	Master mix 96+
Axiom™ Water	35.5 µL	4.3 mL
Axiom™ Stabilize Diluent	4.0 µL	480 µL
Axiom™ Stabilize Soln	0.5 µL	60 µL
Total	**40 µL**	**4.8 mL**

2. Vortex the Stbl tube at high speed for 3 seconds.

3. Place it on ice.

Prepare the Ligation Master Mix—part 1

The Ligation Master Mix is prepared in 2 parts.

1. Place the 15-mL conical tube labeled "Lig" on ice.

2. Use the appropriate serological and single-channel pipettes to add reagents to the Lig tube in the following order.

Reagent and cap color	Per array	Master mix 96+
Axiom™ Ligate Buffer	25.2 µL	3.1 mL
Axiom™ Ligate Soln 1	5.0 µL	624 µL
Axiom™ Ligate Soln 2	1.2 µL	150 µL
Total	**31.4 µL**	**3.9 mL**

3. Mix well by vortexing the Lig tube for 3 seconds.

4. Place the tube back on ice.

Prepare the Ligation Master Mix—part 2

The Ligation Master Mix is prepared in 2 parts.

1. Remove the Axiom™ Ligate Enzyme from the –20°C freezer. Place it in a cooler chilled to –20°C.

2. Use the appropriate single-channel pipettes to add reagents to the 15-mL tube labeled “Lig” in the following order.

   Note: Gently flick the Axiom™ Ligate Enzyme tube 2-3 times, then centrifuge it briefly immediately before adding the enzyme to the master mix.

Reagent and cap color	Per array	Master mix 96+
Ligation Master Mix from part 1 preparation	31.4 µL	3.9 mL
Axiom™ Probe Mix 1	4.0 µL	499 µL
Axiom™ Probe Mix 2	4.0 µL	499 µL
Axiom™ Ligate Enzyme	0.6 µL	75 µL
Total	**40 µL**	**5.0 mL**

3. Gently invert the Lig tube 10 times to mix. Do not vortex.

4. Place the tube on ice and protect it from direct light. For example, cover it with aluminum foil or the ice bucket lid.

Aliquot master mixes and Axiom™ Hold Buffer into trays

Label the GeneTitan™ reagent trays

When preparing the reagent trays to be loaded onto the GeneTitan™ MC Instrument, it is helpful 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. Do *not* write on any other side, because the writing 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. If needed, review reagent tray handling techniques before continuing. See Appendix A, "Recommended techniques for GeneTitan™ MC Instrument operation".

1. Gather the scan tray and the stain trays and covers from the Axiom™ 384HT GeneTitan™ High Volume Consumables Kit.

2. Obtain the 384-layout stain trays and label each specific tray as indicated in the following table.

   When preparing the hybridization and reagent trays to be loaded onto the GeneTitan™ MC Instrument, the front of each tray must be marked in a way that identifies its contents.

Stain tray type	Label color	Label the tray
384-Layout GeneTitan™ Stain Tray (Stain 1-1)	White	Stain 1-1
384-Layout GeneTitan™ Stain Tray (Stain 1-2)	White	Stain 1-2
384-Layout Axiom™ Stain 2 Tray	Blue	Stain 2
384-Layout Axiom™ Ligation Tray	Yellow	Lig
384-Layout Axiom™ Stabilization Tray	Green	Stbl

About aliquoting reagents to GeneTitan™ trays

IMPORTANT! Always aliquot reagents to the bottom of the reagent 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.

Place trays on the benchtop to pipet. If the trays will not be used immediately, protect them from light by covering with foil or placing them in a cabinet.

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.

Scan tray: It is important to fill all 96 wells with Axiom™ Hold Buffer. The scan tray has an open-bottom design, so it is very important that all 96 wells of the scan tray receive 170 µL of Axiom™ Hold Buffer.

Stain trays: Fill only quadrant 1 of the stain trays with ligation, staining, and stabilization reagents.

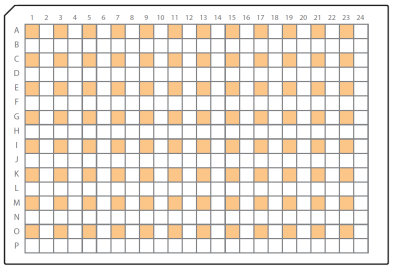
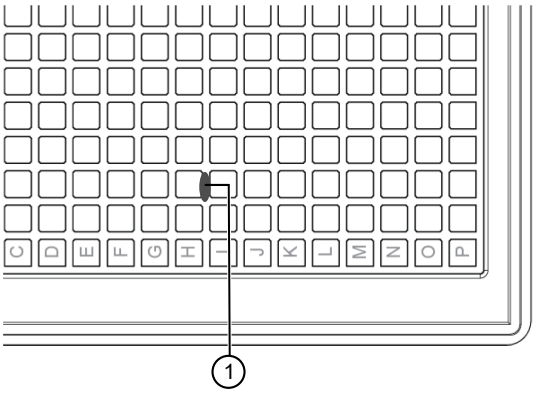


Figure 13 Quadrant 1 wells of a 384-layout Axiom™ stain tray.

Aliquot the Stain 1 Master Mix

1. Place the reagent reservoir labeled “S1” on the benchtop at room temperature.
2. Pour the S1 Master Mix into the S1 reagent reservoir.
3. Load a P200 12-channel pipette with 12 new pipette tips, then aliquot 40 µL per Q1 well to both trays labeled “S1”.
   - Dispense to the first stop only to avoid creating bubbles.
   - It is not necessary to change pipette tips between additions of the Stain 1 Master Mix.
4. Visually inspect the S1 trays.
   a. If bubbles are present, puncture them with a pipette tip.
   b. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.



(1) Example of a droplet of liquid that has splashed onto the well divider of a stain tray during reagent aliquoting. Ensure that no droplets of liquid rest on the well dividers.

IMPORTANT! 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.

5. Place covers on the S1 trays. Orient the covers correctly on the trays with the notched corners together.
6. Protect the trays from light if not immediately loading onto the GeneTitan™ MC Instrument.

Aliquot the Stain 2 Master Mix

1. Place the reagent reservoir labeled “S2” on the benchtop at room temperature.
2. Pour the S2 Master Mix into the S2 reagent reservoir.
3. Load a P200 12-channel pipette with 12 new pipette tips, then aliquot 40 µL per Q1 well to the tray labeled “S2”.
   - Dispense to the first stop only to avoid creating bubbles.
   - It is not necessary to change pipette tips between additions of the Stain 2 Master Mix.
4. Visually inspect the S2 tray.
   a. If bubbles are present, puncture them with a pipette tip.
   b. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place a cover on the S2 tray. Orient the cover correctly on the tray with the notched corners together.
6. Protect the tray from light if not immediately loading onto the GeneTitan™ MC Instrument.

Aliquot the Stabilization Master Mix

1. Place the reagent reservoir labeled “Stbl” on the benchtop at room temperature.
2. Pour the Stabilization Master Mix into the Stbl reagent reservoir labeled “Stbl”.
3. Load a P200 12-channel pipette with 12 new pipette tips, then aliquot 40 µL per Q1 well to the tray labeled “Stbl”.
   - Dispense to the first stop only to avoid creating bubbles.
   - It is not necessary to change pipette tips between additions of the Stabilization Master Mix.
4. Visually inspect the Stbl tray.
   a. If bubbles are present, puncture them with a pipette tip.
   b. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place a cover on the tray. Orient the cover correctly on the tray with the notched corners together.

Aliquot the Ligation Master Mix

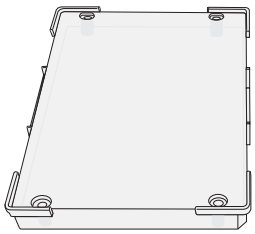
1. Place the reagent reservoir labeled “Lig” on the benchtop at room temperature.
2. Pour the Ligation Master Mix into the Lig reagent reservoir labeled “Lig”.

3. Load a P200 12-channel pipette with 12 new pipette tips, then aliquot 40 µL per Q1 well to the tray labeled “Lig”.
   - Dispense to the first stop only to avoid creating bubbles.
   - It is not necessary to change pipette tips between additions of the Ligation Master Mix.
4. Visually inspect the Lig tray.
   a. If bubbles are present, puncture them with a pipette tip.
   b. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place a cover on the Lig tray. Orient the cover correctly on the tray with the notched corners together.
6. Protect the tray from light if not immediately loading onto the GeneTitan™ MC Instrument.

Aliquot the Axiom™ Hold Buffer to the scan tray

CAUTION!
- Do not remove the scan tray from its protective blue base until loading it onto the GeneTitan™ MC Instrument.
- To avoid scratching, do not touch the bottom of the tray with pipette tips.
- Dispense Axiom™ Hold Buffer to only the first stop.

1. Ensure that the Axiom™ Hold Buffer has equilibrated to room temperature.
2. Place the reagent reservoir labeled “Hold” on the benchtop at room temperature.
3. Vortex the Axiom™ Hold Buffer, then pour it into the Hold reservoir.
4. Remove the scan tray from its pouch.
5. Remove the scan tray cover but leave the scan tray on its protective blue base.
6. Place the cover upside down to prevent dust or static from collecting on the bottom of the cover.



7. Use a 12-channel P200 pipette with new tips to dispense 170 µL of Axiom™ Hold Buffer to *each* of the 96 Q1 wells of the 384-Layout GeneTitan™ Stain Tray.
   - Dispense to the first stop and avoid touching the bottom of the tray.
   - Avoid touching the bottom of the tray with the pipette tips.
   - It is not necessary to change pipette tips between additions of the Axiom™ Hold Buffer.
8. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.

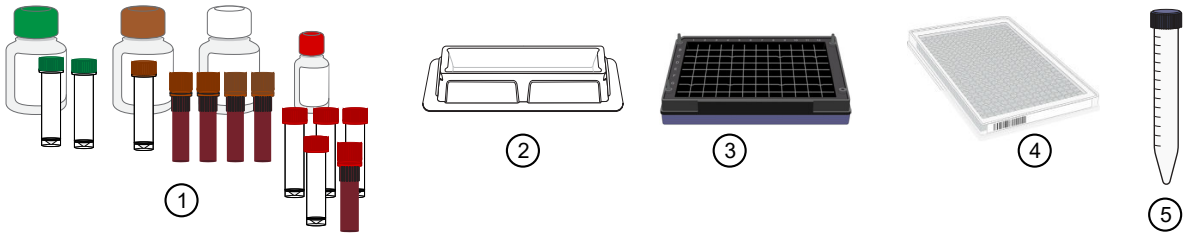
9. Cover the tray by orienting the notched corner of the scan tray cover over the notched edge of the tray with the flat side of the cover against the scan tray.

IMPORTANT! The scan tray has an open-bottom design, so it is important that all 96-wells of the scan tray receive 170 µL of Axiom™ Hold Buffer.

If needed, review instructions for loading reagent trays. See "Stage 3: Ligate, wash, stain, and scan" on page 126.

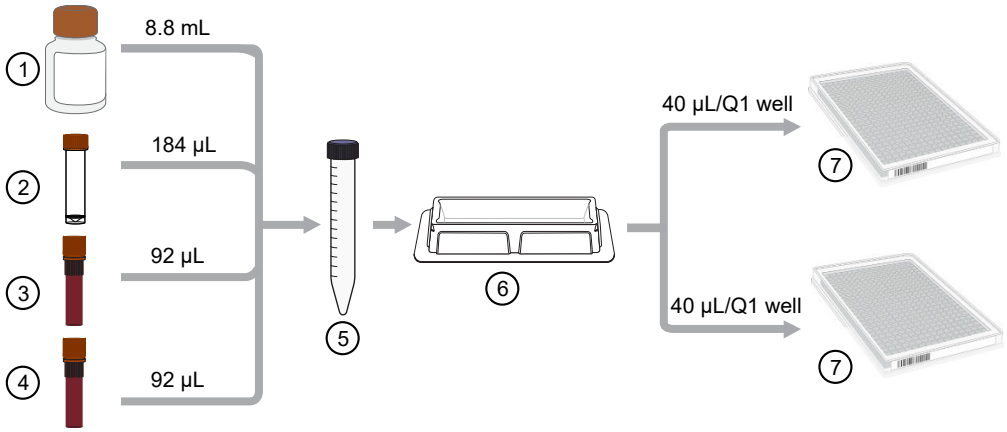
Workflow for Stage 5: Prepare GeneTitan™ reagents and trays

Materials required



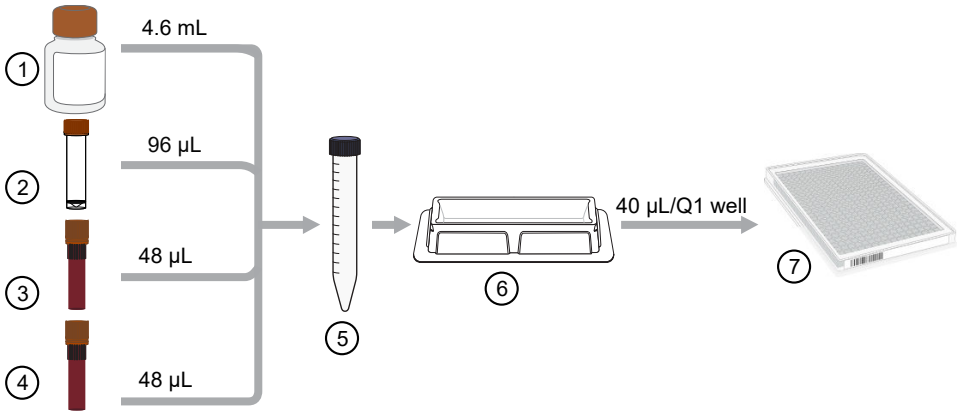
1. Module 4-1 and Module 4-2 reagents
2. Quantity = 5
3. 384-Layout GeneTitan™ Scan Tray, quantity = 1
4. 384 Layout stain trays:
   - 384-Layout GeneTitan™ Stain Tray (Stain 1), quantity = 2
   - 384-Layout Axiom™ Stain 2 Tray, quantity = 1
   - 384-Layout Axiom™ Stabilization Tray, quantity = 1
   - 384-Layout Axiom™ Ligation Tray, quantity = 1
5. Quantity = 4

Prepare two Stain 1 trays.



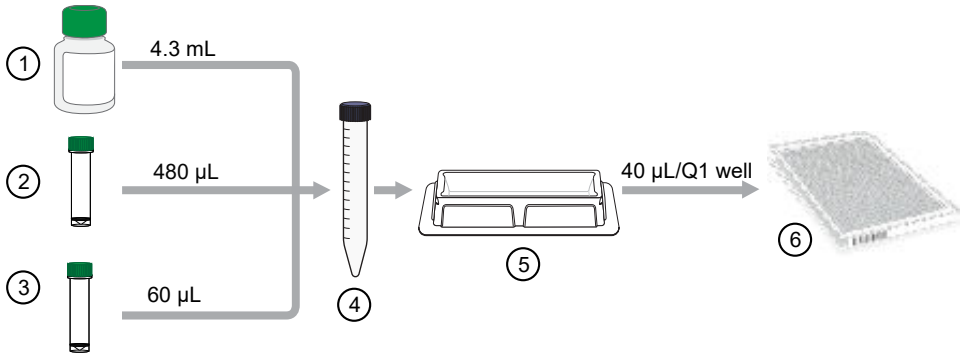
① Axiom™ Wash A
② Axiom™ Stain Buffer
③ Axiom™ Stain 1-A
④ Axiom™ Stain 1-B
⑤ Invert the tube 10 times to mix. Do not vortex.
⑥ Pour into a reagent reservoir.
⑦ Trays labeled “S1”. Ensure that there are no droplets on well dividers.

Prepare Stain 2 tray.



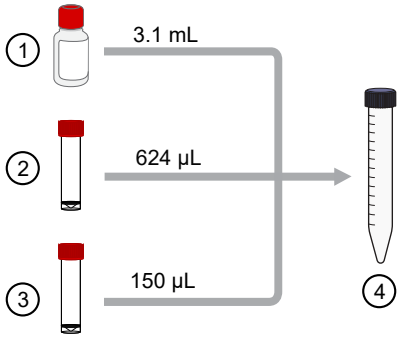
① Axiom™ Wash A
② Axiom™ Stain Buffer
③ Axiom™ Stain 2-A
④ Axiom™ Stain 2-B
⑤ Invert the tube 10 times to mix. Do not vortex.
⑥ Pour into a reagent reservoir.
⑦ Tray labeled “S2”. Ensure that there are no droplets on well dividers.

Prepare the stabilization tray.



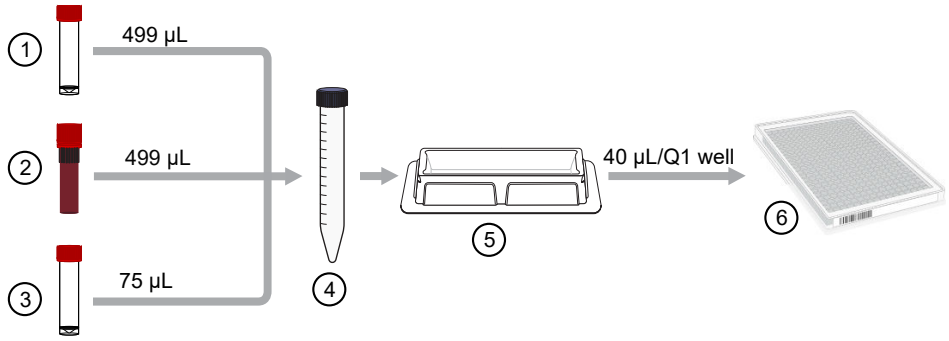
① Axiom™ Water
② Axiom™ Stabilize Diluent
③ Axiom™ Stabilize Soln
④ Vortex to mix.
⑤ Pour into a reagent reservoir.
⑥ Tray labeled “Stbl”. Ensure that there are no droplets on well dividers.

Prepare the Ligation Master Mix, part 1.



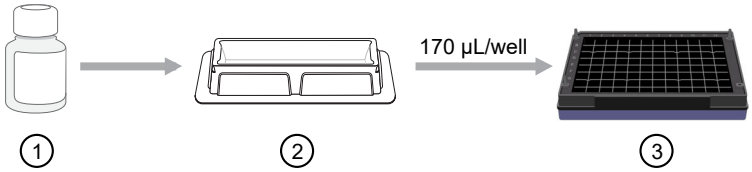
① Axiom™ Ligate Buffer
② Axiom™ Ligate Soln 1
③ Axiom™ Ligate Soln 2
④ Ligation Master Mix tube. Vortex to mix.

Prepare the Ligation Master Mix, part 2, then prepare the ligation tray.



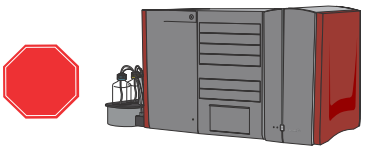
1. Axiom™ Probe Mix 1
2. Axiom™ Probe Mix 2
3. Axiom™ Ligate Enzyme
4. Add reagents to the Ligation Master Mix tube from Part 1. Invert the tube 10X to mix. Do not vortex.
5. Pour into a reagent reservoir.
6. Tray labeled “Lig”. Confirm that there are no droplets on well dividers.

Aliquot the Axiom™ Hold Buffer to the scan tray.



1. Axiom™ Hold Buffer
2. Pour into a reagent reservoir.
3. Confirm that there are no droplets on well dividers.

Process array plates on the GeneTitan™ MC Instrument.



5 Process array plates with the GeneTitan™ Multi-Channel Instrument

IMPORTANT! For optimal GeneTitan™ MC Instrument performance, ensure that the maximum relative humidity is 80% for temperatures up to 75.2°F (24°C), with a minimum humidity of 30 ±7% relative humidity. Operating outside the working environment specifications leads to higher static levels, and results in the evaporation of reagents from stain trays.

IMPORTANT! Review Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation” for details on array processing setup options and consumable handling.

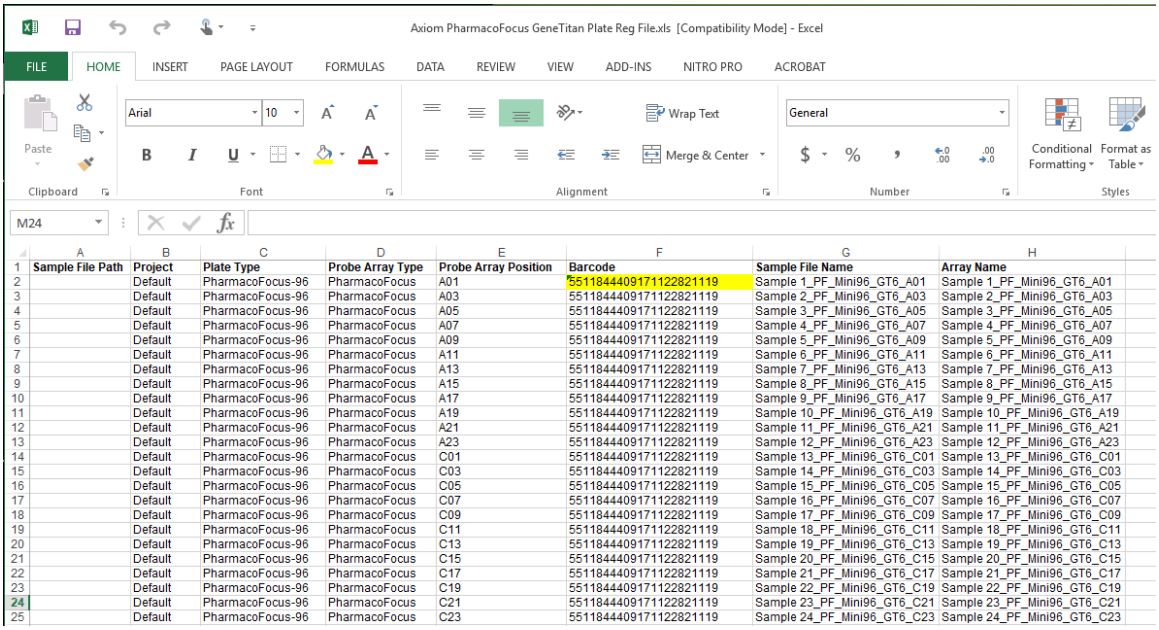
Stage 1: Create and upload a GeneTitan™ Array Plate Registration file

A batch registration file must be created and uploaded with GeneChip™ Command Console™ (GCC) software before you start "Stage 2: Hybridize plates in the GeneTitan™ MC Instrument" on page 114. This file contains information critical for data file generation during scanning, and for tracking the experimental results for each sample loaded onto an array plate. This file can be created at any time before loading the array plate and hybridization tray onto the GeneTitan™ MC Instrument.

Note: When creating the GeneTitan™ Array Plate Registration file, you can scan the barcode of the hybridization tray to implement sample traceability. If you do not upload the sample file names before scanning the array plate barcode, the software assigns names to the samples.

The array plate barcode is scanned when you are ready to load the array plate and samples onto the GeneTitan™ MC Instrument for processing.

1. If you have already created, then saved a batch registration file but have not yet uploaded the file to GCC, open the file, then go to step 6.
2. From the **Launcher** window, open **GCC Portal** ▸ **Samples** ▸ **GeneTitan™ Array Plate Registration**.
3. In the GeneTitan™ Array Plate Registration window, from the **GeneTitan Array Plate Type** list, select the plate type to be processed.
4. Click **Download**.
5. In the **Samples** tab of the GeneTitan™ Array Plate Registration file, enter a unique name for each sample (**Sample File Name**) and any additional information.
   Additional information on the GeneTitan™ Array Plate Registration file is in the *GeneChip™ Command Console™ User Guide* (Pub. No. 702569).
6. Scan the array plate barcode into the yellow **Barcode** field, column **F**. See Figure 14.
7. Scan the barcode of the hybridization tray if the array plate registration file template includes a column for the hybridization tray barcode.
8. Save the file.
   By default, the file is saved in the `Applied Biosystems Download` folder.
9. Return to the GCC Portal GeneTitan™ Array Plate Registration page.
   a. Click **Browse**, navigate to the array plate registration file, then click **Open**.
   b. Under **Step 3**, click **Upload**, wait for the information to load, then click **Save** found at the bottom of the next window that is displayed.



Axiom PharmacoFocus GeneTitan Plate Reg File.xls [Compatibility Mode] - Excel

	A	B	C	D	E	F	G	H
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array Position	Barcode	Sample File Name	Array Name
2		Default	PharmacoFocus-96	PharmacoFocus	A01	5511844409171122821119	Sample 1_PF_Mini96_GT6_A01	Sample 1_PF_Mini96_GT6_A01
3		Default	PharmacoFocus-96	PharmacoFocus	A03	5511844409171122821119	Sample 2_PF_Mini96_GT6_A03	Sample 2_PF_Mini96_GT6_A03
4		Default	PharmacoFocus-96	PharmacoFocus	A05	5511844409171122821119	Sample 3_PF_Mini96_GT6_A05	Sample 3_PF_Mini96_GT6_A05
5		Default	PharmacoFocus-96	PharmacoFocus	A07	5511844409171122821119	Sample 4_PF_Mini96_GT6_A07	Sample 4_PF_Mini96_GT6_A07
6		Default	PharmacoFocus-96	PharmacoFocus	A09	5511844409171122821119	Sample 5_PF_Mini96_GT6_A09	Sample 5_PF_Mini96_GT6_A09
7		Default	PharmacoFocus-96	PharmacoFocus	A11	5511844409171122821119	Sample 6_PF_Mini96_GT6_A11	Sample 6_PF_Mini96_GT6_A11
8		Default	PharmacoFocus-96	PharmacoFocus	A13	5511844409171122821119	Sample 7_PF_Mini96_GT6_A13	Sample 7_PF_Mini96_GT6_A13
9		Default	PharmacoFocus-96	PharmacoFocus	A15	5511844409171122821119	Sample 8_PF_Mini96_GT6_A15	Sample 8_PF_Mini96_GT6_A15
10		Default	PharmacoFocus-96	PharmacoFocus	A17	5511844409171122821119	Sample 9_PF_Mini96_GT6_A17	Sample 9_PF_Mini96_GT6_A17
11		Default	PharmacoFocus-96	PharmacoFocus	A19	5511844409171122821119	Sample 10_PF_Mini96_GT6_A19	Sample 10_PF_Mini96_GT6_A19
12		Default	PharmacoFocus-96	PharmacoFocus	A21	5511844409171122821119	Sample 11_PF_Mini96_GT6_A21	Sample 11_PF_Mini96_GT6_A21
13		Default	PharmacoFocus-96	PharmacoFocus	A23	5511844409171122821119	Sample 12_PF_Mini96_GT6_A23	Sample 12_PF_Mini96_GT6_A23
14		Default	PharmacoFocus-96	PharmacoFocus	C01	5511844409171122821119	Sample 13_PF_Mini96_GT6_C01	Sample 13_PF_Mini96_GT6_C01
15		Default	PharmacoFocus-96	PharmacoFocus	C03	5511844409171122821119	Sample 14_PF_Mini96_GT6_C03	Sample 14_PF_Mini96_GT6_C03
16		Default	PharmacoFocus-96	PharmacoFocus	C05	5511844409171122821119	Sample 15_PF_Mini96_GT6_C05	Sample 15_PF_Mini96_GT6_C05
17		Default	PharmacoFocus-96	PharmacoFocus	C07	5511844409171122821119	Sample 16_PF_Mini96_GT6_C07	Sample 16_PF_Mini96_GT6_C07
18		Default	PharmacoFocus-96	PharmacoFocus	C09	5511844409171122821119	Sample 17_PF_Mini96_GT6_C09	Sample 17_PF_Mini96_GT6_C09
19		Default	PharmacoFocus-96	PharmacoFocus	C11	5511844409171122821119	Sample 18_PF_Mini96_GT6_C11	Sample 18_PF_Mini96_GT6_C11
20		Default	PharmacoFocus-96	PharmacoFocus	C13	5511844409171122821119	Sample 19_PF_Mini96_GT6_C13	Sample 19_PF_Mini96_GT6_C13
21		Default	PharmacoFocus-96	PharmacoFocus	C15	5511844409171122821119	Sample 20_PF_Mini96_GT6_C15	Sample 20_PF_Mini96_GT6_C15
22		Default	PharmacoFocus-96	PharmacoFocus	C17	5511844409171122821119	Sample 21_PF_Mini96_GT6_C17	Sample 21_PF_Mini96_GT6_C17
23		Default	PharmacoFocus-96	PharmacoFocus	C19	5511844409171122821119	Sample 22_PF_Mini96_GT6_C19	Sample 22_PF_Mini96_GT6_C19
24		Default	PharmacoFocus-96	PharmacoFocus	C21	5511844409171122821119	Sample 23_PF_Mini96_GT6_C21	Sample 23_PF_Mini96_GT6_C21
25		Default	PharmacoFocus-96	PharmacoFocus	C23	5511844409171122821119	Sample 24_PF_Mini96_GT6_C23	Sample 24_PF_Mini96_GT6_C23

Figure 14 Example of a GeneTitan™ Array Plate Registration file.

Stage 2: Hybridize plates in the GeneTitan™ MC Instrument

Materials, labware, and reagents required

Reagents required

The following reagents from the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit are required for the hybridization step.

Reagent	Module
Axiom™ Wash Buffer A (both bottles, 1 L)	Module 3, Room temperature Part No. 901472
Axiom™ Wash Buffer B	
Axiom™ Water	

Materials required

- Hybridization tray containing denatured samples.

  Note: The denatured samples must be transferred to the hybridization tray only after the GeneTitan™ MC Instrument is ready for loading, described in the "Load an array plate and hybridization tray into the GeneTitan™ MC Instrument" on page 119.

- An Axiom™ PharmacoFocus™ Mini 96-Array Plate is required for this step. Before inserting this plate into the GeneTitan™ MC Instrument for hybridization, the array plate must be at room temperature.

Warm array plate to room temperature

The array plate must be at room temperature before setting up hybridization on the GeneTitan™ MC Instrument.

1. Remove the array plate packaging from the 4°C refrigerated storage.
2. Open the array plate box, then remove the pouch containing the array plate and protective base. Do not open the pouch.
3. Equilibrate the unopened pouch on the bench for at least 25 minutes.
4. At the end of the array warm-up time, open the pouch, then scan the array plate barcode into the batch registration file.

   See "Stage 1: Create and upload a GeneTitan™ Array Plate Registration file" on page 112.

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

Set up the GeneTitan™ MC Instrument

1. From the GCC **Launcher**, select **GCC GeneTitan Control**.

   The system initializes. After initialization, the **System Status** tab is selected and shows the status of the hybridization oven at the bottom of the **Log** panel. The status reads *<Time of day> System Ready*.

Note: The instrument control software displays a warning message when a problem is detected during the fluid dispense operations. The filters in the GeneTitan™ Wash A, Wash B, and Rinse bottles must be replaced if the software displays such a warning.

IMPORTANT! Do not close the scanner application by right-clicking and selecting the **Close** option. This method causes the scanner application to exit abnormally and delays processing of the next plate. The correct way to close the application is described in "Shut down the GeneTitan™ MC Instrument" on page 133.

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2. Click the **System Setup** tab to continue the array plate process.

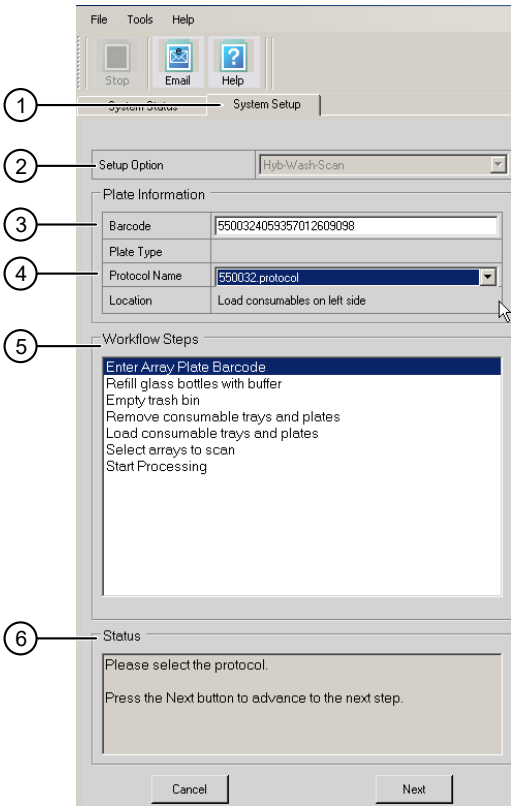

Figure 15 System Setup tab.

① **System Setup** tab.
② **Setup Option**: Access a dropdown list of the various options available for processing Axiom™ array plates.
③ **Barcode**: The array plate barcode. The barcode can be scanned or entered manually.
④ **Protocol Name**: The dropdown list of protocols that are displayed is based on the first 6 digits of the array plate barcode. Only the protocols that are valid for the array plate type that is loaded are displayed.
⑤ **Workflow Steps**: This field displays an overview of the user actions that are required to process an array plate that is based on the **Setup Option** selected.
⑥ **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, click the **Next** button or to press the blinking blue confirmation button on the GeneTitan™ MC Instrument to continue.

3. From the **Setup Option** list, select **Hyb-Wash-Scan**.
Other options available are described under "Setup options for array plate processing" on page 176.

4. Click **Next**.

Note: A message is displayed when there is insufficient disk space. Delete or move DAT files to another location to free up sufficient disk space for the data that are generated. One mini 96-array plate requires ~7 GB.

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5. Scan or manually enter the array plate barcode, then click **Next**.

   The first 6 characters of the barcode identify the type of plate being loaded, the protocol that GeneTitan™ MC Instrument uses to process the plate, and the imaging device parameters required for the plate.

   Note: If an error message is displayed after entering the array plate barcode, do the following:
   - Ensure that the library files for the type of array plate that you are using are correctly installed.
   - Library files must be installed before 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.
   - Try manually entering the array plate barcode.

   ---

6. Select a protocol from the **Protocol Name** list, then click **Next**.

7. Refill the bottles with Module 3 reagents.

   - Wash A: fill with Axiom™ Wash Buffer A—keep at 2 L full.
   - Wash B: fill with Axiom™ Wash Buffer B—use all 600 mL of Wash B from the reagent kit per array plate. Fill to 1-L mark when processing 2 plates on the same day.
   - Rinse: fill with Axiom™ Water—keep at 1 L full.

   IMPORTANT! Always ensure that the GeneTitan™ bottles containing Wash A and Rinse are above the 50% mark when setting up the system to process an array plate.

   All 600 mL of the Wash Buffer B from the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit must be emptied into the GeneTitan™ Wash B bottle when setting up the system.

   - Using all of the Wash B contents from the reagent kit ensures that the GeneTitan™ Wash B bottle is filled to more than the minimum requisite 35% of bottle volume.
   - If you intend to load 2 array plates on the same day, fill the Wash B bottle to the 1-L mark (use both bottles from the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit).

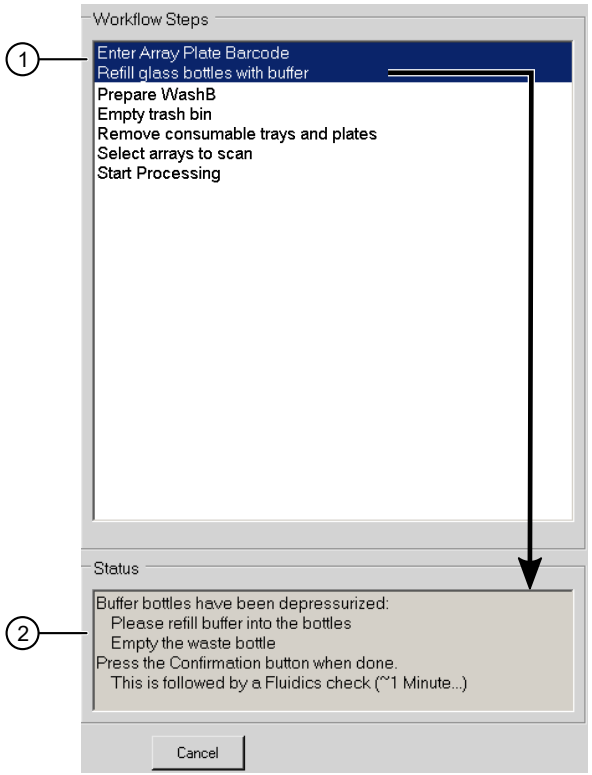
   Do not overfill the bottles.

   - The maximum volume for the Wash B and Rinse bottles is 1 L. Fill to the 1-L mark only.
   - The maximum volume for the Wash A bottle is 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. However, even if you fill the bottle now, the instrument cannot complete the step that was in progress.

8. Empty the waste bottle.

9. Press the blue confirmation button on the GeneTitan™ MC Instrument to continue.
   A fluidics check is run (~1 minute).



① **Workflow Step**
② Specific instructions for the current workflow step are displayed in the **Status** pane.

10. Empty the trash bin.
    a. Open the trash bin and empty.
    b. If already empty, the trash bin remains locked and the **Status** pane reads "Trash bin is empty".
    c. Press the blue confirmation button on the GeneTitan™ MC Instrument to continue.
11. Remove consumable trays and plates.
    a. Remove used trays and plates when drawers open.
    b. If no consumables are present to remove, the **Status** window reads "Drawers are empty".
    c. Press the blue confirmation button on the GeneTitan™ MC Instrument to continue.
12. Continue to “Load an array plate and hybridization tray into the GeneTitan™ MC Instrument” on page 119 when prompted by the GCC software.

Load an array plate and hybridization tray into the GeneTitan™ MC Instrument

The steps in "Set up the GeneTitan™ MC Instrument" on page 115 must be completed before starting the following procedure.

1. When drawer 6 opens, load the array plate and hybridization tray in the following manner:
   a. Examine the wells of the hybridization tray for bubbles, then 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 array plate are clamped. Bubbles under an array can result in black spots on the array image.

   b. Load the uncovered hybridization tray on the right side of the drawer.

   c. Remove the array plate and protective blue base from its package. For more information on the array packaging, see "Array plate packaging" on page 168.

      To avoid dust or other damage, leave the array plate packaged until ready to load onto the GeneTitan™ MC Instrument. The array plate must be loaded on its protective blue base. The white plastic shipping cover on top of the array plate *should not* be loaded in the GeneTitan™ MC Instrument.

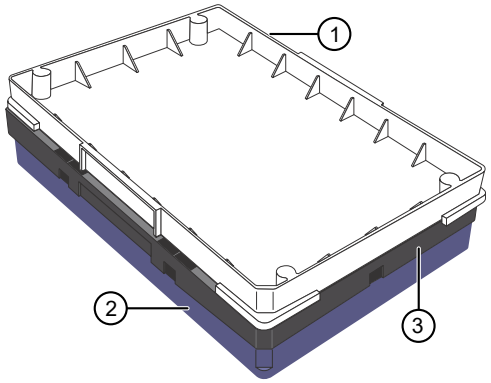


Figure 16 Array plate components, as shipped.

1. Shipping cover to be discarded
2. Array plate protective base
3. 384HT array plate

d. Load the array plate with the protective blue base on the left side of the drawer.

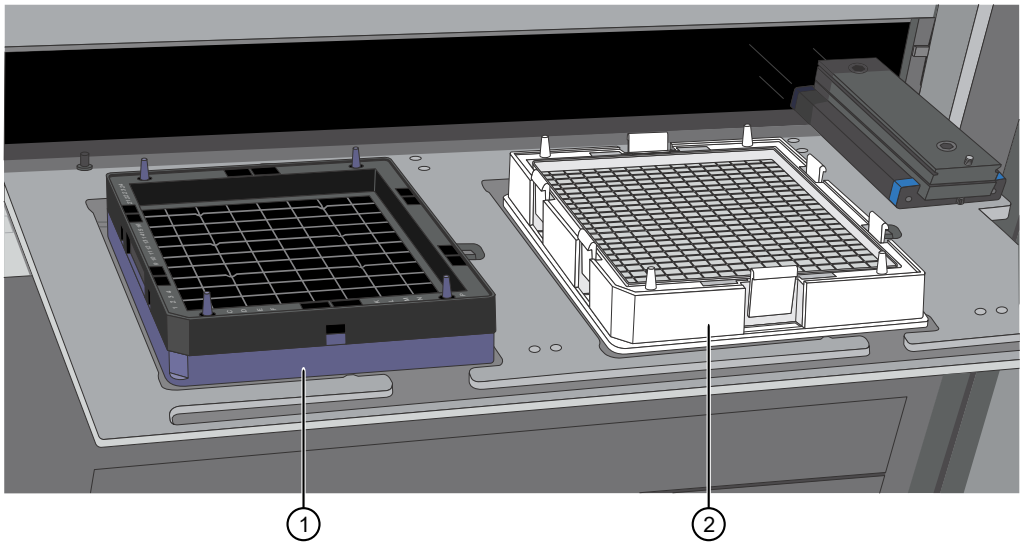


Figure 17 Array plate on protective blue base and the hybridization tray properly loaded into drawer 6.

1. Array plate on protective base
2. Hybridization tray

IMPORTANT! Do not install a 3-plate stack of trays. Ensure that you have removed the white plastic shipping cover.

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.

An error message is displayed if the instrument has difficulty reading the barcode on the plate. Plate barcodes must face the internal barcode reader in the back of the drawer. Improper tray positioning can crash the GeneTitan™ MC Instrument, result in substantial instrument damage, and loss of samples.

e. Press the blue confirmation button on the GeneTitan™ MC Instrument to continue.

Note: When an array plate is loaded on the left side of the drawer, the internal barcode reader reads the barcode of the array plate. The barcode is compared with the barcode and the plate type that is specified in the **Barcode** and **Plate Type** fields that were selected during the **Setup**. 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 out the tray and prompts you to load the correct plate with the proper orientation into the instrument.

If an error occurs, check the loading of the array plate and click **OK** to retry. Alternatively, click **Skip** if the instrument continues to have problems after ensuring that the trays have been loaded in the proper orientation.

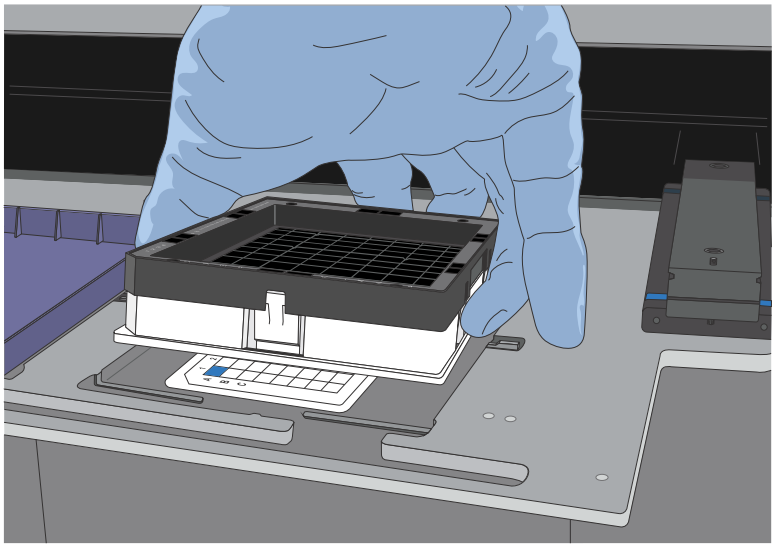
2. Select the arrays to scan. By default, all arrays are selected.

3. Click **Next**, then click **OK** in the **Start Processing** dialog box to start processing the samples.
   The GeneTitan™ MC Instrument places the array plate on top of the hybridization tray (now called the plate stack). The GCC software starts the process for placing the array plate onto the hybridization tray. A **Clamping in Progress** dialog appears.

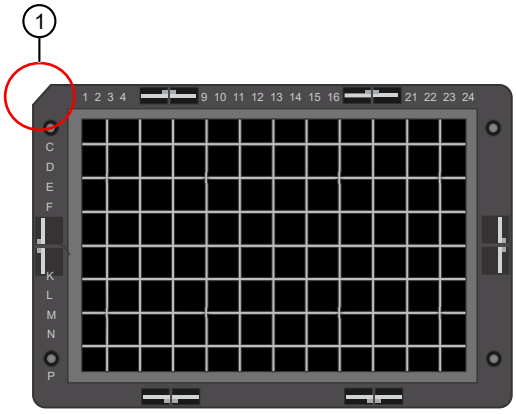
4. Press **OK**, then wait for the drawer to open completely before retrieving the array plate and hybridization tray combination for manual clamping and inspection.
   After clamping is complete in the instrument, drawer 6 opens and the **Ensure Clamping** dialog appears. Do not click **OK** yet. The sandwich of the array plate and hybridization tray must be manually clamped and inspected before the array processing can start.

CAUTION! At this stage, the array plate does not latch securely to the hybridization tray. Always grip plate stack from the hybridization tray (lower part) of the plate stack. *Do not* grip only the array plate to remove the plate stack from the drawer of the GeneTitan™ MC Instrument.

5. Complete the following steps to clamp the array plate manually to the hybridization tray.
   a. Grip the body of the hybridization tray by hand then remove the plate stack from drawer 6 right location of the GeneTitan™ MC Instrument.

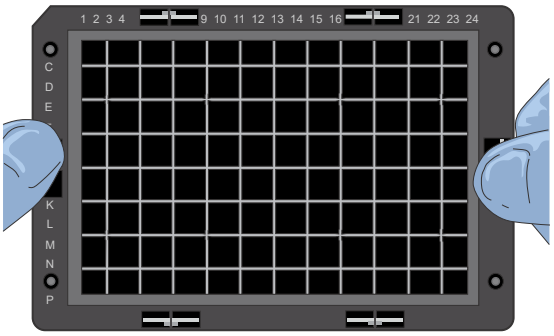


b. Place the plate stack on a flat surface of the table or the lab bench. Position the plate stack to match the orientation as shown in the picture.



1 Chamfer/notched corner

c. Position the left and right thumb fingers on the location indicated in the picture. Press the array plate downward until the clicking sound is detected.



d. With the plate stack resting on a flat surface, rotate the stack 90° clockwise. Position the left and right thumbs on the locations that are indicated in the picture. Press the array plate downward until the clicking sound is detected.

6. Ensure the clamping of the plate stack to check that the array plate is securely fastened to the hybridization tray. Using your thumbs, press the array plate downward following the positions that are specified in Figure 18. No clicking sound indicates proper clamping.

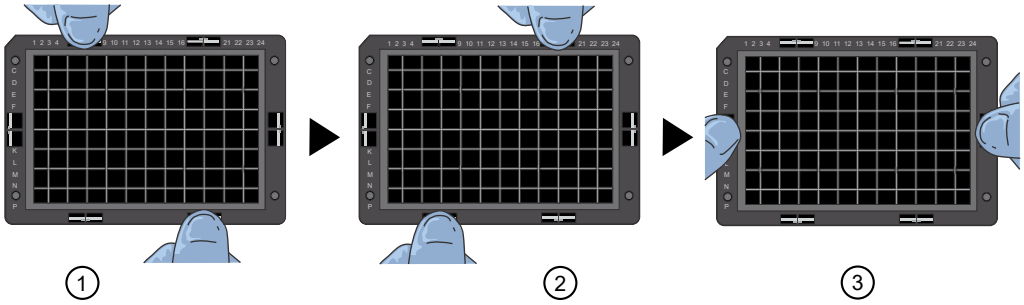


Figure 18 Clamping verification steps.

7. Inspect the array plate for bubbles.
   a. **Keeping the plate stack level**, inspect the bottom for bubbles under the arrays—*do not* tilt or invert the plates.
   b. If bubbles are present, gently tap the plate until the bubbles move out from under the arrays—*do not* unclamp the plate stack.
8. Return the plate stack to the drawer with the notched corner facing you, then press the blue confirmation button on the GeneTitan™ Instrument to proceed.
9. A message is displayed if plate orientation is not correct or if the hybridization tray barcode cannot be read. If this message appears, complete one or both of the following actions.
   - Check the loading of the array plate and click **OK**.
   - Click **Skip** if the instrument continues to have problems reading the barcode and after ensuring that the correct trays have been placed in the proper orientation.
10. Continue to "Load a second array plate and hybridization tray" on page 124.

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

When a second array plate and hybridization tray can be loaded

After processing starts on the first plate stack, you have a specific length of time during which you can load another array plate and hybridization tray. This length of time is displayed in the **Work Flow** window above the **Hybridization Oven Status** pane. You cannot load another hybridization tray before or after this time.

IMPORTANT! The next array plate and hybridization tray must be loaded during the time frame of that is displayed above the **Hybridization Oven Status** pane. You cannot load another hybridization tray before or after this time. You are required to wait until the current process is finished which results in disruption of the multiplate workflow.

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

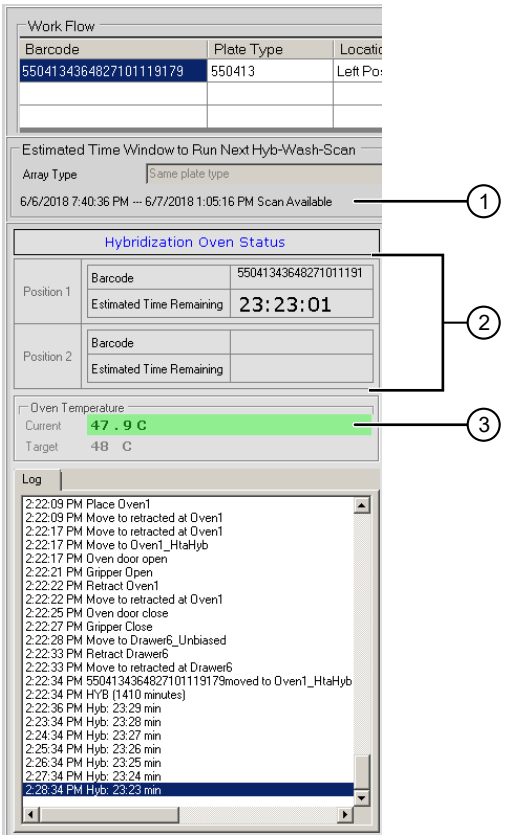


Figure 19 When to load a second array plate and hybridization tray based on oven status information.

① This pane displays the amount of time during which another array plate and hybridization tray can be loaded. Additional plates cannot be loaded before or after this time as the instrument is operating. In this figure, the system is currently available.

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

Position 1—left side of the oven

Position 2—right side of the oven

③ Green indicates that the current oven temperature is in the target temperature range. Yellow indicates that the oven temperature is outside of the target temperature range.

Load a second array plate and hybridization tray

1. Select the **System Setup** tab.

2. Load an array plate and hybridization tray in the same manner as the previous plate and tray were loaded.

   a. Scan or manually enter the array plate barcode, then click **Next**.

   b. Load the array plate with the blue protective base and the hybridization tray without the cover, then press the blue confirmation button.

c. Select the arrays to scan, then click **Next**.

d. Ensure that the plates are clamped securely when prompted, then press the blue confirmation button.

e. Click **OK** when prompted to resume plate processing.

3. Select the **System Status** tab to view the status of the array plates in the **Work Flow** pane.

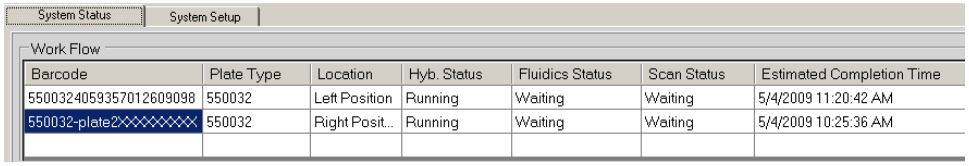


Figure 20 Example of the Work Flow pane when 2 plates are loaded and are in the hybridization oven.

Queue a second plate for scanning

Use the **Scan** option in the **System Setup** tab to start a second scan workflow. The software automatically moves the second plate into the scanner when the first plate has completed scanning.

GeneChip™ Command Console™ v4.3 or later is required for this procedure.

1. Start the first scan workflow in the GeneTitan™ MC Instrument. Wait until the first plate is loaded into the imaging device and scanning starts.

2. Go to the **System Setup** tab, then select **Scan** from the **Setup Option** dropdown list. The **Setup Option** dropdown list is active only after the first plate starts scanning.

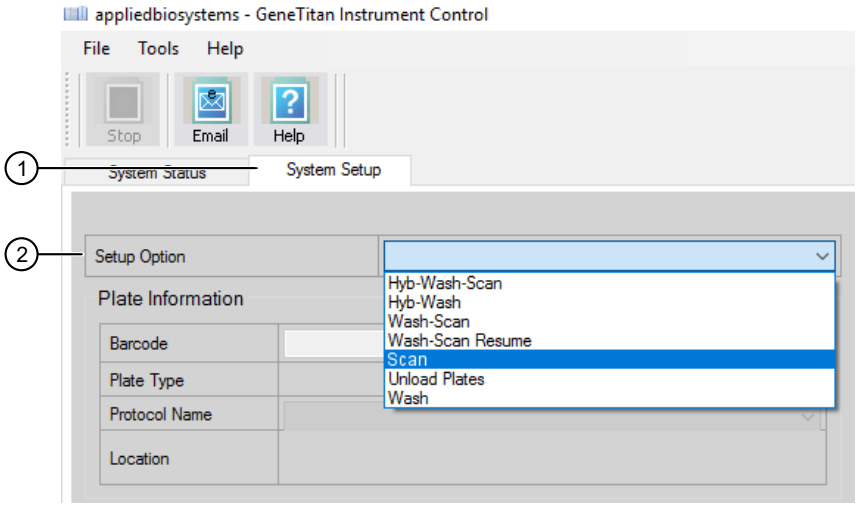


Figure 21 Scan setup option to process a second array plate.

① **System Setup** tab ② **Scan Option** dropdown list

3. Click **Next** in the lower left section of the window under the **Status** pane.

4. Scan or manually enter the array plate barcode, then click **Next**.

5. Follow the instructions in the **Status** pane and empty the trash bin if needed, then press the blue confirmation button on the instrument 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 combination in drawer 2 of the GeneTitan™ MC Instrument, on the left or right side, as instructed in the **Status** pane. Ensure that the array plate/scan tray combination is loaded in the correct orientation in the drawer. If needed, see Figure 29 for further information on the proper alignment and loading of plates, covers, and trays in the GeneTitan™ MC Instrument.
8. When ready, press the blue confirmation button on the instrument.
9. Select the arrays to scan in the **Array Selection** section in the upper right corner of the window, then click **Next**.
10. In the **Start Processing** confirmation message, click **OK** to continue.
    The second queued plate runs after the first scan finishes and the scanner is available.

Stage 3: Ligate, wash, stain, and scan

The GeneTitan™ tray loading process

When hybridization of an array plate is complete, a message window appears to alert you to resume the workflow setup. Press **OK** to return to the **System Setup** tab.

This message window prompt to continue into the reagent load step occurs when hybridization is complete. **Estimated Time Remaining** displayed in the **Hybridization Oven Status** pane can display a time remaining of 0 to 30 minutes.

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

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

Note: The time estimate that is 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 the message window prompt to resume the reagent loading step is displayed, there is no need to wait for the estimated time to count down to zero.

Load trays in GeneTitan™ Instrument

1. Follow the prompts in the **Status** pane.
   a. Refill the reagent bottles, if needed.
      - Wash Bottle A—2 L
      - Wash Bottle B—Fill to 1-L mark only
      - Rinse—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 although the base is empty.

   d. Press the blue confirmation button on the GeneTitan™ Instrument to continue.

2. Load consumable trays and plates in the following sequence. Follow the prompts in the **Status** pane.

IMPORTANT! After trays are loaded onto the drawer, examine each cover for droplets of liquid. Liquid on the cover can result in a capillary phenomenon. As a result, the tray can stick to the cover and be lifted out of place inside the GeneTitan™ Instrument. If liquid is present on the cover, remove the tray, clean the cover and top of the tray with a laboratory tissue, and reload the tray.

CAUTION! Orient trays as indicated by the guide inside the drawer. Improper orientation can cause the run to fail. If needed, see Appendix A, "Recommended techniques for GeneTitan™ MC Instrument operation" for a review of proper loading techniques.

   a. When drawer 2 opens:
      - Left side: Scan tray with cover. Remove the protective blue base from the scan tray immediately before loading. Do not load the protective blue base.
      - When complete, press the blue confirmation button on the GeneTitan™ Instrument to continue.

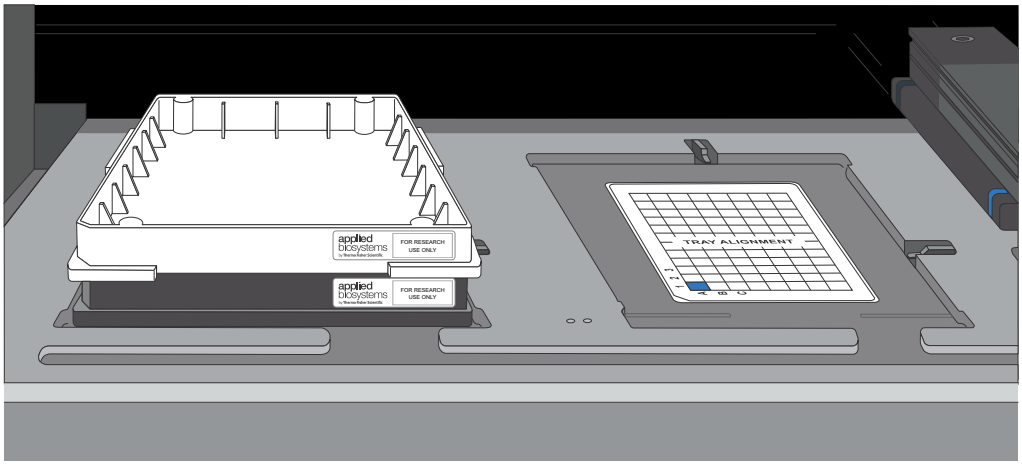


Figure 22 Drawer 2: Scan tray with cover on left side.

b. When drawer 3 opens:

- Left side: Stain 1 tray (white label) with cover.
- Right side: Ligation tray (yellow label) with cover.
- Press the blue confirmation button on the GeneTitan™ Instrument to continue.

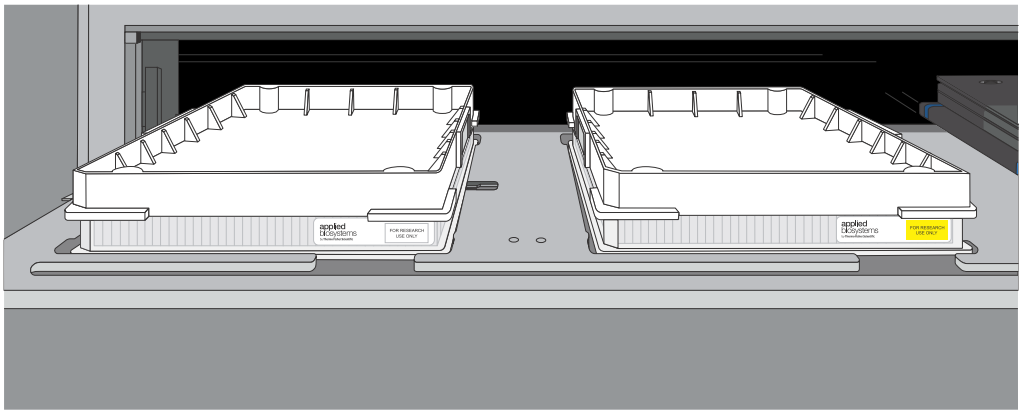


Figure 23 Drawer 3 trays.

- Left side: Stain 1 tray with cover (white label).
- Right side: Ligation tray with cover (yellow label).

c. When drawer 4 opens:

- Left side: Stain 2 tray (blue label) with cover.
- Right side: Stabilization tray (green label) with Axiom™ Stabilize Soln and cover.
- Press the blue confirmation button on the GeneTitan™ Instrument to continue.

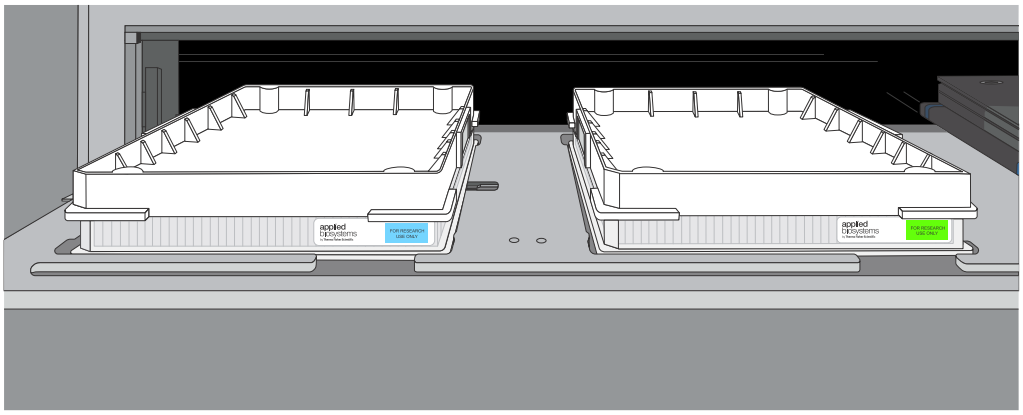


Figure 24 Drawer 4 trays.

- Left side: Stain 2 tray with cover (blue label).
- Right side: Stabilization tray with Axiom™ Stabilize Soln (green label).

d. When drawer 5 opens:
   - Left side: Stain 1 tray (white label) with cover.
   - Press the blue confirmation button on the GeneTitan™ Instrument to continue.

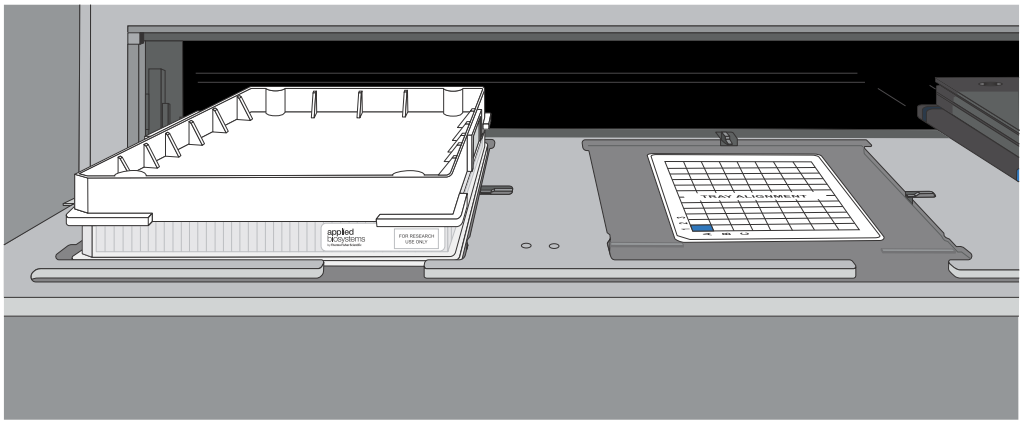


Figure 25 Drawer 5: Stain 1 tray with cover (white label) on left side.

3. At the **WorkFlow Option** window prompt, click **Yes** to load another array plate and hybridization tray.

4. In **Setup Option**, select **Setup Another Run**, then click **Next**.

5. Scan or manually enter the array plate barcode, then click **Next**.

6. Select a protocol, then click **Next**.

7. When drawer 6 opens:
   a. Remove the blue cover from the previous array plate.
   b. Load a new array plate and new blue base on the left side of the drawer, then load a new hybridization tray on the right side of the drawer.
   c. Press the confirmation button.
   d. Click **OK** to continue.
   e. When drawer 6 opens, ensure that the plate stack is securely clamped. For clamping procedure review, see step 5 on page 121.
   f. Press the blue confirmation button.

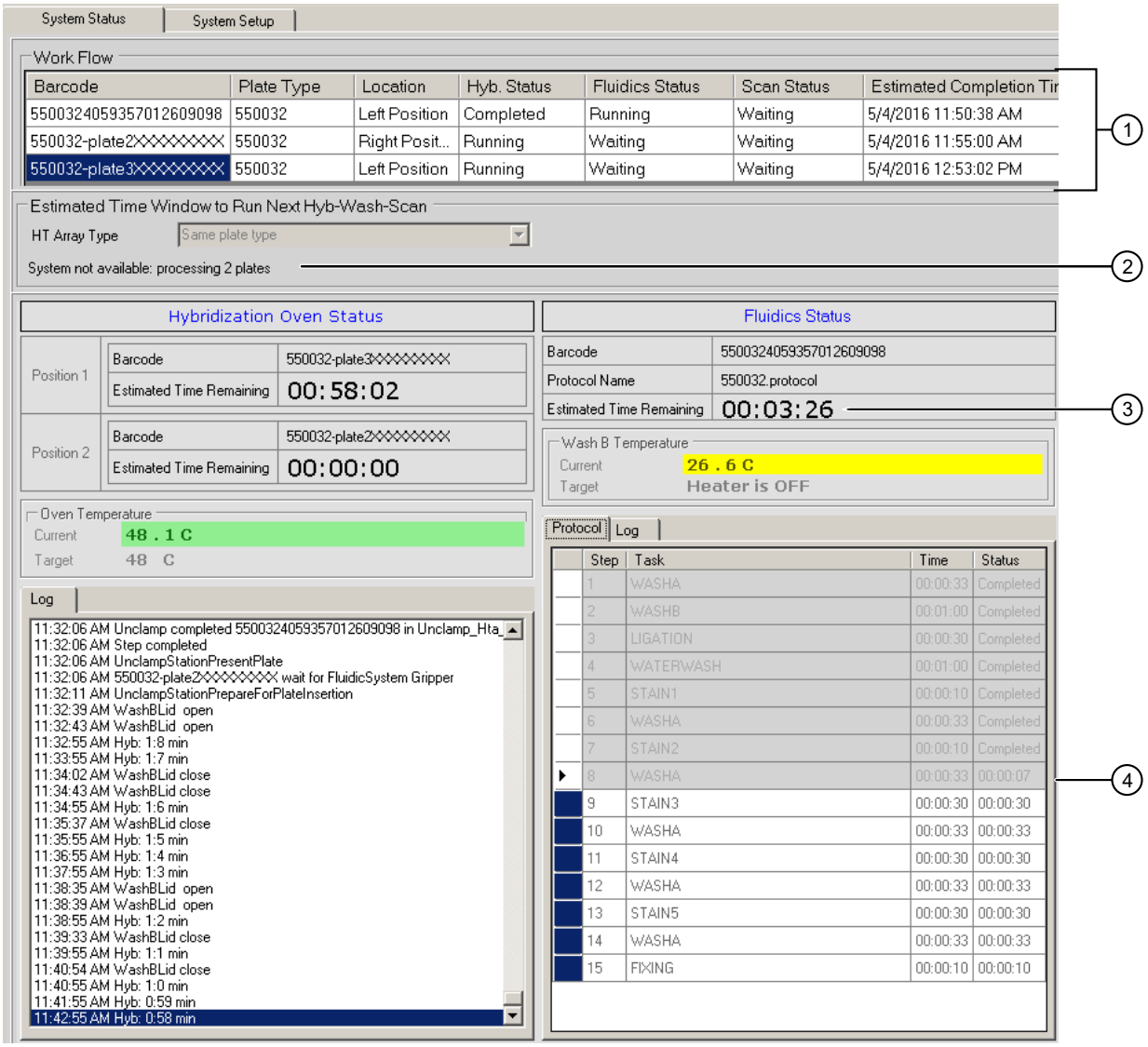
GeneTitan™ MC Instrument internal array plate activity

The following is a description of array plate movements in the GeneTitan™ MC Instrument when a multiplate workflow is performed.

1. The plate stack, which has finished hybridization, is temporarily moved from the hybridization oven to drawer 1.
2. The new plate stack in drawer 6 is moved to the hybridization oven.
3. The plate stack temporarily in drawer 1 (step 1) is moved to the unclamping station where it is unclamped and then 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 stabilization tray cover must be in the trash.

The following figure is an example of how the **System Status Workflow** window appears when 3 array plates are being processed.



① **Work Flow** pane displays the number of array plates being processed and where they are in the instrument. In this example, 3 array plates are being processed: 2 are in the hybridization oven and 1 is in fluidics.

② The status that is displayed indicates that another (fourth) plate cannot be added to the hybridization oven because both oven slots are currently in use.

③ **Estimated Time Remaining** is displayed for the current process. If needed the **Estimated Time Remaining** for fluidics is adjusted. Changes can be due to process interruptions such as a drawer being opened.

④ The step that is currently executing in fluidics.

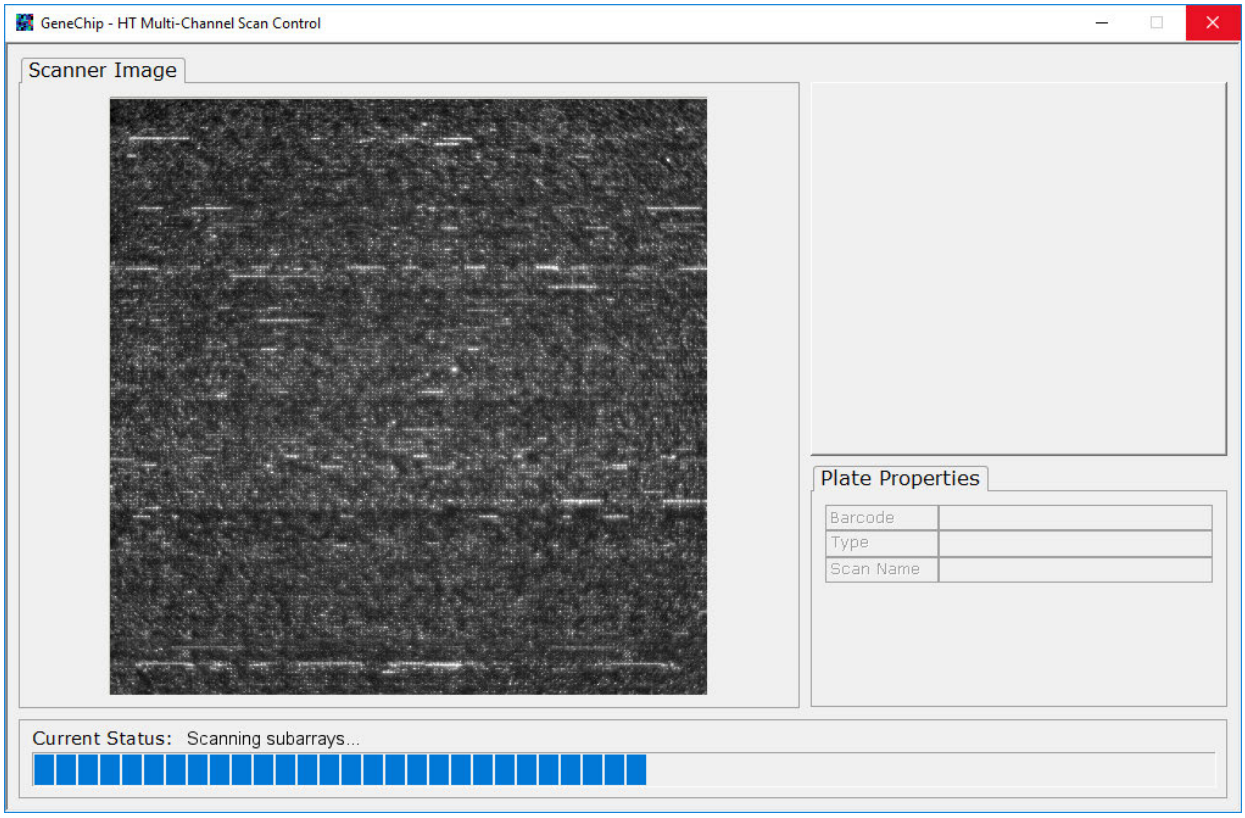
Continue the scan workflow

After a plate has completed the fluidics stage of the workflow, the GeneTitan™ Instrument moves the plate to the imaging device.

When the scanning process starts, a Scan Control window displaying the scan image appears. This window must remain open while the array plate is being scanned.

CAUTION! The **Scan Control** window must remain open while the array plate is being scanned. If the window is closed, the scanning process halts. If needed, this window can be minimized without interference to the imaging.

Do not manually, or through the GCC transfer utility, move data that are associated with the plate that is being processed/scanned. Transferring data dramatically slows scanning and can cause the computer to freeze.



Shut down the GeneTitan™ MC Instrument

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

1. From the **System Setup** window, open the **Setup Options** dropdown list, then select **Unload Plates**.
2. Unload all the consumables as prompted.
3. Power off the GeneTitan™ MC Instrument by opening **Tools** ▸ **Shutdown**.
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 Three-plate workflow for Axiom™ PharmacoFocus™ Mini 96-Array Plates using an overnight DNA precipitation step

When using the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow, 1 to 2 people can process up to 3 Axiom™ PharmacoFocus™ Mini 96-Array Plates in one 40-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.

This chapter assumes user familiarity with all procedures for target preparation and array processing steps as described in this user guide.

Overview of the 3-plate workflow with overnight precipitation

The following figure and table describe the timing and duration of the hands-on processing necessary for performing the 3-plate workflow.

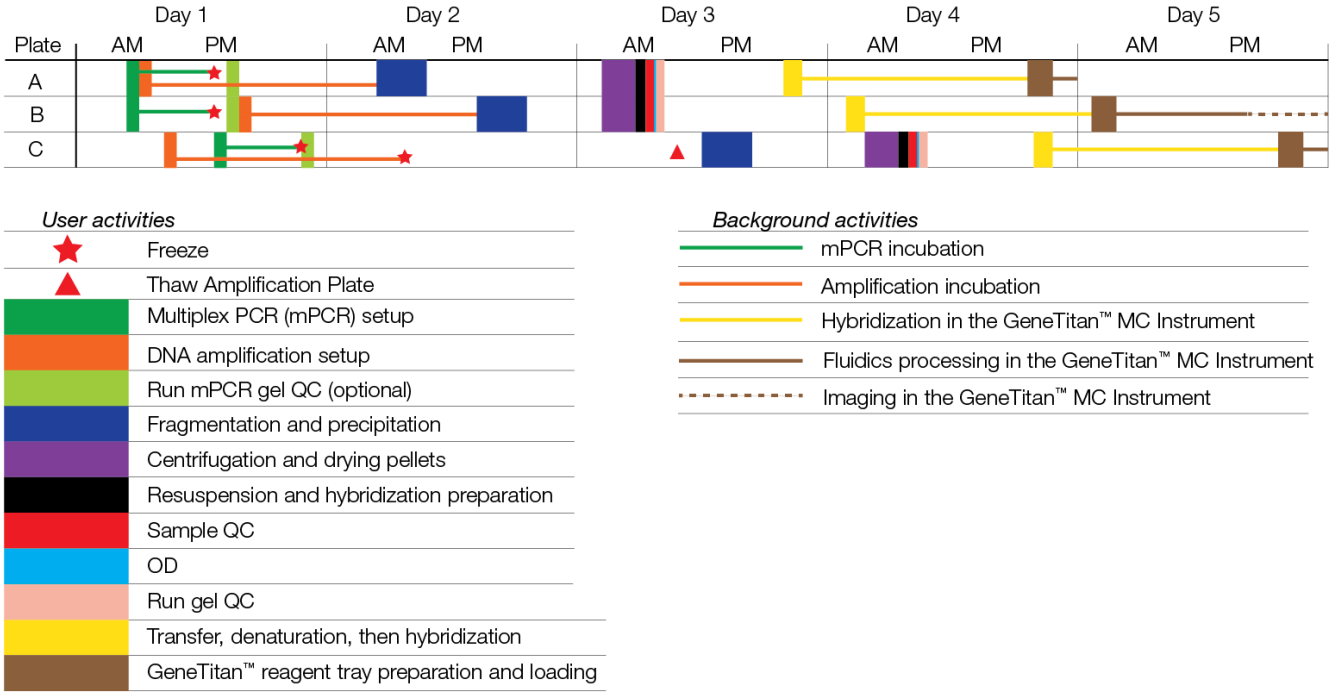


Figure 26 Full week activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow.

The 3 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 that is described in this chapter. On day 1, it is recommended that one person be responsible for setting up the whole-genome amplification for all 3 plates, and that another person take care of setting up and running mPCR for all 3 plates. It is also helpful to have 2 people working together on day 3 and day 4.

Table 4 Daily steps for Axiom™ PharmacoFocus™ Assay 3-plate workflow with overnight precipitation.

Day	Activities	Plates
1	• Amplify 3 plates of genomic DNA. • mPCR setup and incubation for 3 plates. • mPCR gel QC for 3 plates (optional). • Freeze the mPCR Product Plates until needed for mPCR spike-in step during fragmentation.	• A, B, C • A, B, C • A, B, C • A, B, C
2	• Fragment and precipitate 2 plates amplified on day 1. • Freeze 1 plate of amplified DNA for fragmentation on day 3.	• A, B • C
3	• Fragment and precipitate 1 plate of amplified DNA. • Centrifuge, dry, resuspend, prepare Hyb-Ready Plate, sample QC (OD and gel QC run) 2 plates precipitated on day 2. • Transfer, denature, then begin hybridization for 1 plate on the GeneTitan™ MC Instrument. • Freeze and store 1 Hyb-Ready Plate.	• C • A, B • A • B
4	• Centrifuge, dry, resuspend, prepare Hyb-Ready Plate, sample QC (OD and gel QC run) 1 plate precipitated on day 3. • Transfer, denature, then begin hybridization for 2 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

IMPORTANT! The timing of these steps is critical because of constraints on both the target preparation performed on the laboratory bench, and the array processing performed using the GeneTitan™ MC Instrument.

See:
- "Time required for the stages of manual target preparation" on page 137.
- "Timing considerations for GeneTitan™ array processing" on page 138.

Time required for the stages of 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 then stored.

Manual target preparation stage	Hands-on time	Total preparation time[1]	Incubation/ hybridization/ processing
Stage 1A: Perform multiplex PCR (mPCR)	30 minutes	60 minutes	~3.5 hours
Stage 1B: Amplify the genomic DNA	30 minutes	90 minutes	23 ±1 hour
Stage 2: Fragment and precipitate the DNA	2 hours	2 hours	Overnight precipitation
Stage 3A: Centrifuge the Precipitation Plate and dry the DNA pellet	30 minutes	80 minutes	N/A
Stage 3B: Resuspend the pellets and prepare for hybridization	25 minutes	25 minutes	N/A
Stage 3C: Perform quantification and fragmentation QC checks	45 minutes	45 minutes	N/A
Stage 4: Transfer, denature, then hybridize the denatured samples	25 minutes	50 minutes	23.5–24 hours hybridization
Stage 5: Prepare the GeneTitan™ reagents and trays	60 minutes	90 minutes	Additional time for processing mini-96 arrays: ~6.5 hours

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

Timing considerations for GeneTitan™ array processing

IMPORTANT! Maintaining consistent timing during GeneTitan™ MC Instrument setup is critical to containing the user interventions of the 3-plate workflow within a work day. After one process starts late, there is little opportunity to catch up until the end of the workflow.

The hybridization time for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow on the GeneTitan™ MC Instrument is 23.5–24 hours. This timing provides a 30-minute window during which you are prompted by the instrument control software to load the reagents required for washing and staining.

The following table lists and describes the time required to process array plates on the GeneTitan™ MC Instrument.

Steps on the GeneTitan™ MC Instrument	Time required
Hybridization of 2 plates in 1 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]	~1.5 hours for mini 96-array format

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

Change oven temperatures for the 3-plate workflow

Multiple ovens are required for manual target preparation. If you are running the 3-plate/week workflow, 3 ovens are recommended. The following table lists and describes the different oven temperatures required 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] Required for preheating of the 96-well metal chamber for hybridization transfer.

Though only 2 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. The following table provides a list of suggested settings for 3 ovens when performing the 3-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 the 3-plate workflow

At least 2 thermal cyclers are required for the 3-plate workflow. Multiplex PCR runs for all 3 plates are performed on day 1, and each run is approximately 3.5 hours. See “Thermal cycler recommendations and protocols” on page 28.

Thaw the frozen plate of amplified DNA

One plate (C) in the workflow was frozen at the end of the 23-hour DNA amplification stage on day 2. This plate must be thawed before performing the fragmentation step on day 3 using the following instructions.

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

   For example, pour ultra-pure water into a small tray. Place the frozen plate on the water in the tray.

2. Leave the plate in the water bath for ~1 hour until all wells have thawed, especially the middle wells.

3. Centrifuge at 1,000 rpm for 30 seconds to bring down any droplets or condensation that is generated from thawing the plate.

4. Avoid cross-contamination of wells during vortexing.
   a. Remove the seal, then blot the top of the plate with a laboratory tissue.

   b. Tightly reseal the plate with a fresh seal.

   c. Vortex the plate for 30 seconds to mix.

5. Centrifuge at 1,000 rpm for 30 seconds.

Target preparation and array processing for the 3-plate workflow using an overnight precipitation step

Note: The day 1 schedule is written for 2 people working together. One person sets up the whole-genome amplification for all 3 plates, and the other person sets up and runs the mPCR reactions for all 3 plates. When the mPCR run is complete, the mPCR QC gel can be run (optional), then the plates are frozen until needed.
The genomic DNA sample for amplification must be properly prepared. See Chapter 2, “Prepare genomic DNA”.

Day 1 activities

- Start whole-genome amplification of the 3 plates; each plate must incubate 23 ±1 hour after amplification begins.
- All amplifications must be set up on day 1 to allow for a 23 ±1-hour amplification incubation for each plate and to minimize movement between preamplification and post-amplification areas.
- Begin thawing the amplification reagents, particularly the Axiom™ 2.0 Amp Soln, and Axiom™ Water, 60 minutes before the start of each reaction.
- Set up and run the multiplex PCR for the 3 mPCR Reaction Plates. Two thermal cyclers are required.
- mPCR QC gels (optional) can be run for plate A, plate B, and plate C.
- Freeze all 3 mPCR Product Plates until needed for mPCR spike-in during fragmentation.

IMPORTANT! Amplification preparation must take place in an Amplification Staging Room or dedicated area such as a biosafety hood with dedicated pipettes, tips, vortex, etc. See "Preamplification/amplification staging area" on page 27.

Table 5 Day 1 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using overnight precipitation

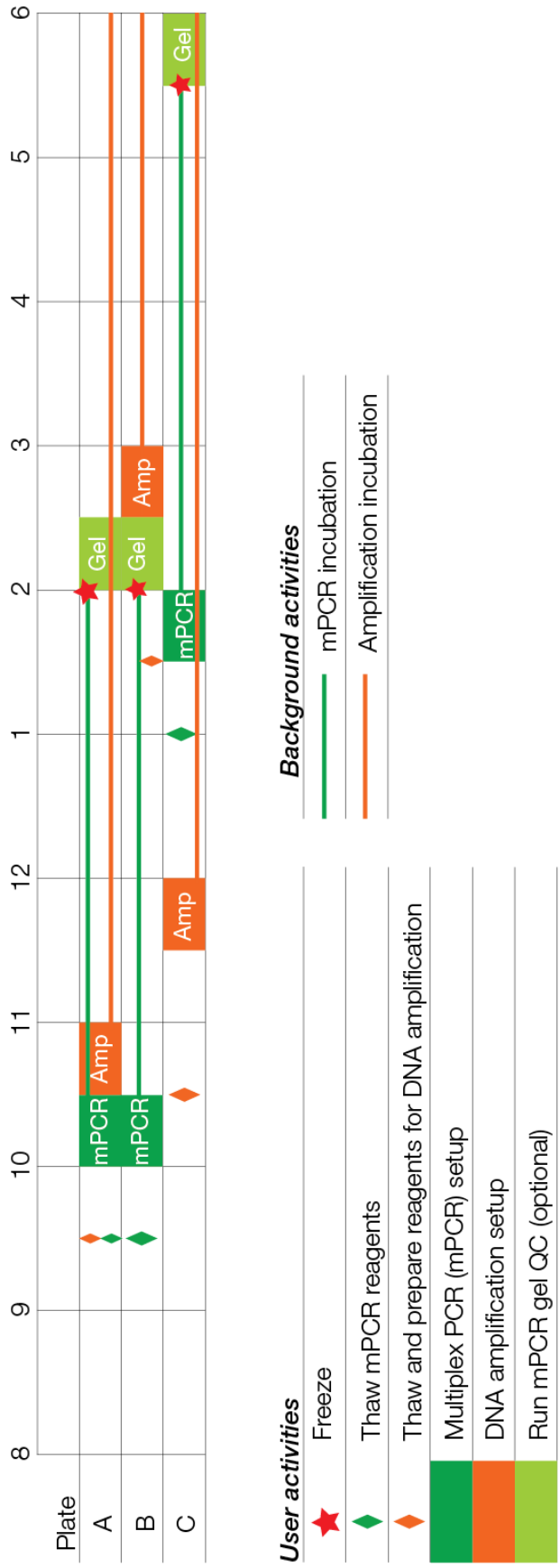
Activity	Plate	Approximate times[1]		Hands-on time
		Start time	End time	
mPCR setup	A, B	9:30 a.m.	10:30 a.m.	30 minutes
DNA amplification	A	9:30 a.m.	11:00 a.m.	30 minutes
DNA amplification	C	10:30 a.m.	12:00 p.m.	30 minutes
mPCR setup	C	1:00 p.m.	2:00 p.m.	30 minutes
DNA amplification	B	1:30 p.m.	3:00 p.m.	30 minutes
mPCR QC gel (optional)	A, 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 Product 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 the start of thawing reagents.

If needed, see:

- "Stage 1A: Perform multiplex PCR (mPCR)" on page 44.
- "Stage 1B: Amplify the genomic DNA" on page 51.

Day 1 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with overnight precipitation



Day 2 activities

- Table 6 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 6 Day 2 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using overnight precipitation.

Activity	Plate	Approximate times[1]	
		Start time	End time
Thaw mPCR Product Plate	A	9:30 a.m.	10:00 a.m.
Fragment and precipitate	A	10:00 a.m.	12:00 p.m.
Freeze (–20°C) Amplification Sample Plate	C	11:00 a.m.	—
Thaw mPCR Product Plate	B	1:30 p.m.	2:00 p.m.
Fragment and precipitate	B	2:00 p.m.	4:00 p.m.

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

Day 2 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with overnight precipitation

User activities	
	Thaw mPCR Product Plate
	Freeze
	Prepare reagents for fragmentation
	Fragmentation and precipitation

Background activities	
	Amplification incubation

Day 3 activities

- Centrifuge, dry, resuspend, and QC plates A and B.
- Thaw plate C. See “Thaw the frozen plate of amplified DNA” on page 140.
- Fragment (including the 65°C stop amplification reaction step) and precipitate plate C.
- Transfer the samples from Hyb-Ready Plate A to Hyb-Ready Denaturation Plate A
- Perform denaturation on Hyb-Ready Denaturation Plate A.
- Transfer Hyb-Ready Denaturation Plate A samples to hybridization tray A.
- Load hybridization tray A and array plate into GeneTitan™ MC Instrument, then begin hybridization.

WARNING! The hybridization tray preparation must take place under a running fume hood.

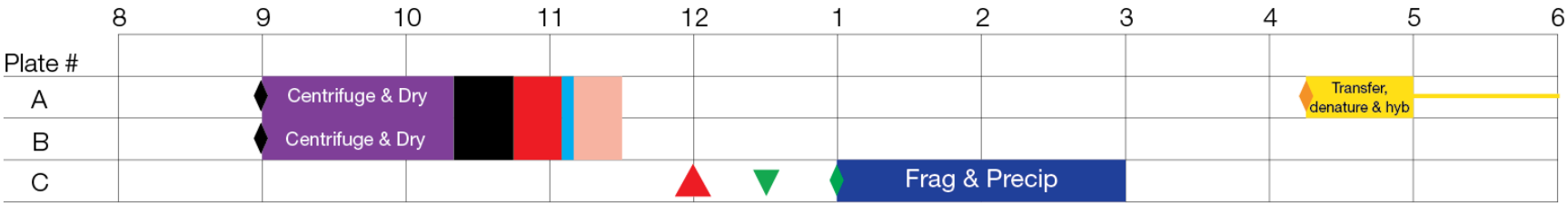
IMPORTANT! Amplified plates that are frozen must be thawed, then thoroughly mixed. See “Thaw the frozen plate of amplified DNA” on page 140.

Table 7 Day 3 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using overnight precipitation.

Activity	Plate	Approximate times[1]	
		Start time	End time
Centrifuge and dry	A, B	9:00 a.m.	10:20 a.m.
Resuspension and hybridization preparation	A, B	10:20 a.m.	10:45 a.m.
Sample QC	A, B	10:45 a.m.	11:05 a.m.
Sample quantification (OD)[2]	A, B	11:05 a.m.	11:10 a.m.
Fragmentation gel QC run	A, B	11:05 a.m.	11:30 a.m.
Store the Hyb-Ready Plate at –20°C	B	11:10 a.m.	—
Thaw plate C	C	12:00 p.m.	1:00 p.m.
Thaw the mPCR Product Plate	C	12:30 p.m.	1:00 p.m.
Fragment and precipitate	C	1:00 p.m.	3:00 p.m.
Transfer, denaturation, then hybridization[3]	A	4:15 p.m.	5:05 p.m.

[1] Approximate start time indicates the start of thawing reagents.
[2] Sample quantification runs concurrently with the fragmentation gel QC run. Load the Gel QC Plate first, then read the OD QC Plate.
[3] Setup = 50 minutes, hybridization = 23.5–24 hours.

Day 3 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with overnight precipitation



User activities

▼	Thaw mPCR Product Plate
◆	Prepare reagents for resuspension and hybridization prep
◆	Prepare reagents for fragmentation
◆	Warm array plate to room temperature
▲	Thaw Amplification Plate
	Fragmentation and precipitation
	Centrifugation and drying pellets
	Resuspension and hybridization preparation
	Sample QC
	Sample quantitation—OD
	Fragmentation gel QC run
	Transfer, denaturation, then hybridization

Background activities

	Hybridization in the GeneTitan™ MC Instrument

Day 4 activities

- Transfer and denaturation of samples. Load array plate and hybridization tray in the GeneTitan™ MC Instrument for plates B and C.
- Centrifuge, dry, resuspend, then QC plate C.
- Prepare GeneTitan™ reagent trays, then load reagent trays for plate A.

WARNING! The hybridization tray preparation must take place under a running fume hood.

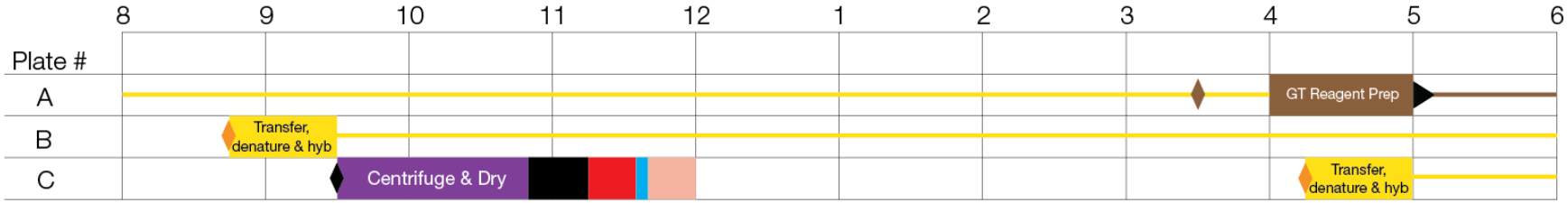
IMPORTANT! The GeneTitan™ reagent trays for array processing cannot be loaded until the array plate has finished hybridization, and they should not be prepared more than 1.5 hours before hybridization finishes. The GeneTitan™ reagent trays cannot be prepared ahead of time and stored.

Table 8 Day 4 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using overnight precipitation.

Activity	Plate	Approximate times[1]	
		Start time	End time
Transfer, denaturation, then hybridization[2]	B	8:45 a.m.	9:35 a.m.
Centrifuge and dry	C	9:30 a.m.	10:50 a.m.
Resuspension and hybridization preparation	C	10:50 a.m.	11:15 a.m.
Sample QC	C	11:15 a.m.	11:35 a.m.
Sample quantification (OD)[3]	C	11:35 a.m.	11:40 a.m.
Fragmentation gel QC run	C	11:35 a.m.	12:00 p.m.
GeneTitan™ reagent preparation and loading	A	3:30 p.m.	5:00 p.m.
Transfer, denaturation, then hybridization[2]	C	4:15 p.m.	5:05 p.m.

[1] Approximate start time indicates the start of thawing reagents.
[2] Setup = 50 minutes, hybridization = 23.5–24 hours.
[3] Sample quantification runs concurrently with the fragmentation gel QC run. Load the Gel QC Plate first, then read the OD QC Plate.

Day 4 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with overnight precipitation



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
	Transfer, denaturation, then hybridization

Background activities

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

Day 5 activities

- 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 9 Day 5 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using overnight precipitation.

Activity	Plate	Approximate times[1]	
		Start time	End time
GeneTitan™ reagent preparation and loading	B	8:00 a.m.	9:30 a.m.
GeneTitan™ reagent preparation and loading	C	3:30 p.m.	5:00 p.m.

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

Day 5 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with overnight precipitation

User activities

♦	Thaw reagents for GeneTitan™ reagent tray preparation
▶	Load array plate into GTMC, begin wash-scan
▬	GeneTitan™ reagent tray preparation and loading

Background activities

─	Hybridization in the GeneTitan™ MC Instrument
─	Fluidics processing in the GeneTitan™ MC Instrument
- - -	Imaging in the GeneTitan™ MC Instrument

7 Three-plate workflow for Axiom™ PharmacoFocus™ Mini 96-Array Plates using a 3-hour DNA precipitation step

The Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using the 3-hour DNA precipitation step allows faster assay turnaround time. Using this accelerated workflow, plate 1 CEL files are available within 72 hours, and CEL files for all 3 plates are available by day 5. One to 2 people can process 3 Axiom™ PharmacoFocus™ Mini 96-Array Plates in 1 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 9- to 10-hour workdays.

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

This chapter assumes user familiarity with all procedures for target preparation and array processing steps as described in this user guide.

Overview of the 3-plate workflow with 3-hour precipitation

The following figures and table describe the timing and duration of the hands-on processing necessary for performing the 3-plate workflow.

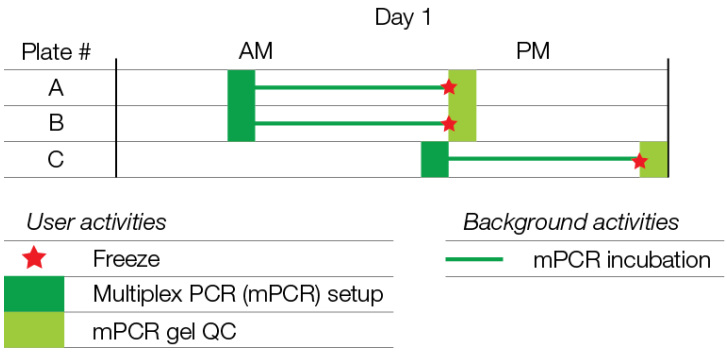


Figure 27 mPCR activities for Axiom™ PharmacoFocus™ Assay 3-plate workflow using 3-hour precipitation.

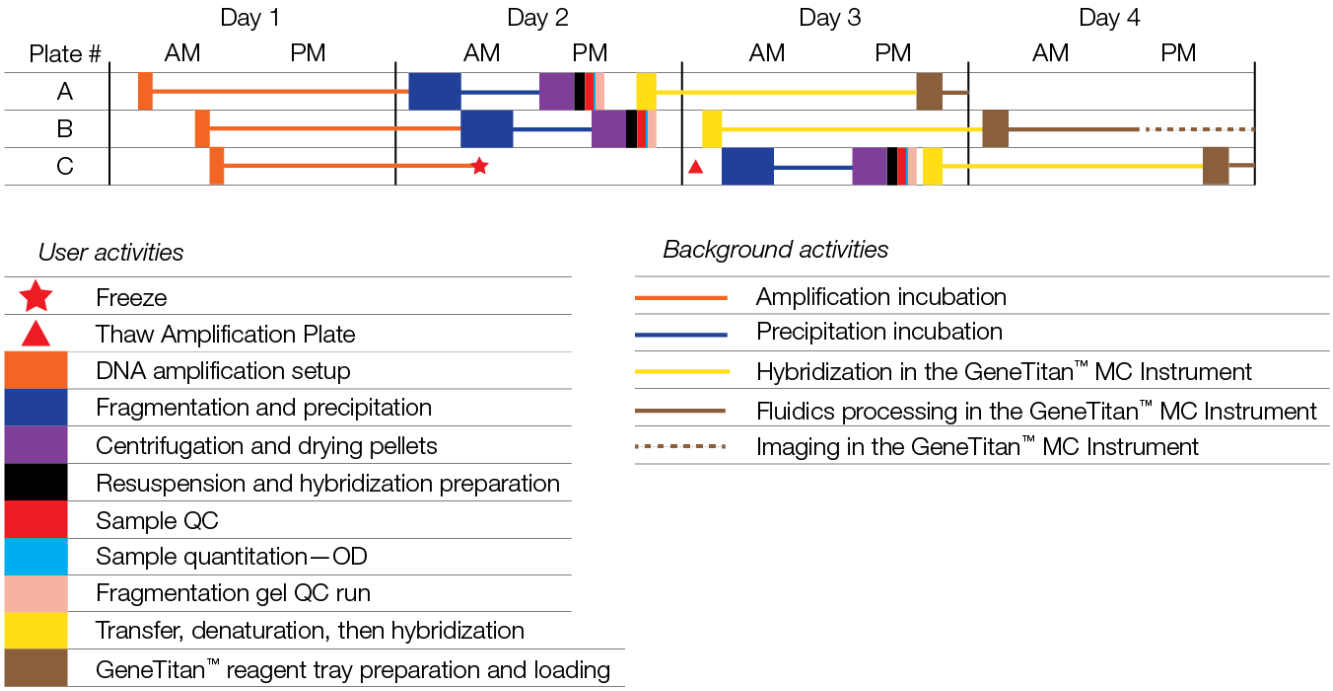


Figure 28 Full week activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow.

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

Table 10 Daily steps for Axiom™ PharmacoFocus™ Assay 3-plate workflow with 3-hour precipitation.

Day	Activities	Plates
1	• Amplify 3 plates of genomic DNA. • mPCR setup and incubation for 3 plates. • mPCR gel QC for 3 plates (optional). • Freeze mPCR Product Plates until needed for mPCR spike-in step during fragmentation.	• A, B, C • A, B, C • A, B, C • A, B, C
2	• Fragment and precipitate 2 plates amplified on day 1. • Freeze 1 plate of amplified DNA for fragmentation on day 3. • Centrifuge, dry, resuspend, prepare Hyb-Ready Plate, sample QC (OD and gel QC run) 2 plates. • Freeze and store 1 Hyb-Ready Plate. • Transfer, denature, then begin hybridization for 1 plate on the GeneTitan™ MC Instrument.	• A, B • C • A, B • B • A
3	• Transfer, denature, then begin hybridization for 1 plate on the GeneTitan™ MC Instrument. • Fragment and precipitate 1 plate of amplified DNA. • Centrifuge, dry, resuspend, prepare Hyb-Ready Plate, sample QC (OD and gel QC run) 1 plate. • GeneTitan™ reagent trays preparation and loading.	• B, C • C • C • A
4	GeneTitan™ reagent trays preparation and loading for 2 plates.	B, C

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

See:

- "Time required for stages of manual target preparation" on page 154.
- "Timing considerations for GeneTitan™ array processing" on page 154.

Time required for stages of 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 then stored.

Manual target preparation stage	Hands-on time	Total preparation time[1]	Incubation/ hybridization/ processing
Stage 1A: Perform multiplex PCR (mPCR)	30 minutes	60 minutes	~3.5 hours
Stage 1B: Amplify the genomic DNA	30 minutes	90 minutes	23 ±1 hour
Stage 2: Fragment and precipitate the DNA	2 hours	2 hours	3-hour precipitation
Stage 3A: Centrifuge the Precipitation Plate and dry the DNA pellet	30 minutes	80 minutes	N/A
Stage 3B: Resuspend the pellets and prepare for hybridization	25 minutes	25 minutes	N/A
Stage 3C: Perform quantification and fragmentation QC checks	45 minutes	45 minutes	N/A
Stage 4: Transfer, denature, then hybridize the denatured samples	25 minutes	50 minutes	23.5–24 hours hybridization
Stage 5: Prepare the GeneTitan™ reagents and trays	60 minutes	90 minutes	Additional time for processing mini 96 arrays: 6.5 hours

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

Timing considerations for GeneTitan™ array processing

IMPORTANT! Maintaining consistent timing during GeneTitan™ MC Instrument setup is critical to containing the user interventions of the 3-plate workflow within a work day. After one process starts late, there is little opportunity to catch up until the end of the workflow.

The hybridization time for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow on the GeneTitan™ MC Instrument is 23.5–24 hours. This timing provides a 30-minute window during which you are prompted by the instrument control software to load the reagents required for washing and staining.

The following table lists and describes the time required to process array plates on the GeneTitan™ MC Instrument.

Steps on the GeneTitan™ MC Instrument	Time required
Hybridization of 2 plates in 1 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

(continued)

Steps on the GeneTitan™ MC Instrument	Time required
Fluidics	~5 hours each plate
Imaging[1]	~1.5 hours for Mini 96-array format

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

Change oven temperatures for the 3-plate workflow

Multiple ovens are required for manual target preparation. If you are running the 3-plate/week workflow, 3 ovens are recommended. The following table lists the different oven temperatures required 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] Required for preheating of the 96-well metal chamber for hybridization transfer.

The following table lists suggested settings for 3 ovens when performing the 3-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	N/A	N/A	N/A

Thermal cycler requirements for the 3-plate workflow

At least 2 thermal cyclers are required for the 3-plate workflow. Multiplex PCR runs for all 3 plates are performed on day 1, and each run is approximately 3.5 hours. See “Thermal cycler recommendations and protocols” on page 28.

Thaw the frozen plate of amplified DNA

One plate (C) in the workflow was frozen at the end of the 23-hour DNA amplification stage on day 2. This plate must be thawed before performing the fragmentation step on day 3 using the following instructions.

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

   For example, pour ultra-pure water into a small tray. Place the frozen plate on the water in the tray.

2. Leave the plate in the water bath for ~1 hour until all wells have thawed, especially the middle wells.

3. Centrifuge at 1,000 rpm for 30 seconds to bring down any droplets or condensation that is generated from thawing the plate.

4. Avoid cross-contamination of wells during vortexing.
   a. Remove the seal, then blot the top of the plate with a laboratory tissue.

   b. Tightly reseal the plate with a fresh seal.

   c. Vortex the plate for 30 seconds to mix.

5. Centrifuge at 1,000 rpm for 30 seconds.

Target preparation and array processing for the 3-plate workflow using a 3-hour precipitation step

Note: The day 1 schedule is written for 2 people working together. One person sets up the whole-genome amplification for all 3 plates, and the other person sets up and runs the mPCR reactions for all 3 plates. When the mPCR run is complete, the mPCR QC gel can be run (optional), then the plates are frozen until needed.
The genomic DNA sample for amplification must be properly prepared. See Chapter 2, “Prepare genomic DNA”.

Day 1 activities

- Start whole-genome amplification of the 3 plates; each plate must incubate 23 ±1 hours after amplification begins.
- All amplifications must be set up on day 1 to allow for a 23 ±1-hour amplification incubation for each plate and to minimize movement between preamplification and post-amplification areas.
- Begin thawing the amplification reagents, particularly the Axiom™ 2.0 Amp Soln, and Axiom™ Water, 60 minutes before the start of each reaction.
- Set up and run the multiplex PCR for the 3 mPCR Reaction Plates. Two thermal cyclers are required.
- mPCR gel QC (optional) can be run for plate A, plate B, and plate C.
- Freeze all 3 mPCR Product Plates until needed for mPCR spike-in during fragmentation.

IMPORTANT! Amplification preparation must take place in an Amplification Staging Room or dedicated area such as a biosafety hood with dedicated pipettes, tips, vortex, etc. See "Preamplification/amplification staging area" on page 27.

Table 11 Day 1 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using 3-hour precipitation.

Activity	Plate	Approximate times[1]		Hands-on time
		Start time	End time	
mPCR setup	A, 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 gel QC (optional)	A, B	1:00 p.m.	2:00 p.m.	30 minutes
mPCR gel QC (optional)	C	4:30 p.m.	5:30 p.m.	30 minutes
Freeze mPCR Product 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 the start of thawing reagents.

If needed, see:

- "Stage 1A: Perform multiplex PCR (mPCR)" on page 44.
- "Stage 1B: Amplify the genomic DNA" on page 51.

Day 1 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with 3-hour precipitation

User activities

- Thaw mPCR reagents
- Thaw and prepare reagents for DNA amplification
- Freeze
- Multiplex PCR (mPCR) setup
- Prepare samples for mPCR QC gel run (optional)
- Run mPCR gel QC (optional)
- DNA amplification setup

Background activities

- mPCR incubation
- Amplification incubation

Day 2 activities

- It is recommended that 2 people work together for day 2 activities.
- Table 12 shows the steps that are 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 3 hours.
- Centrifuge, dry, resuspend, and QC plates A and B.
- Perform transfer, denaturation, then 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 12 Day 2 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using 3-hour precipitation.

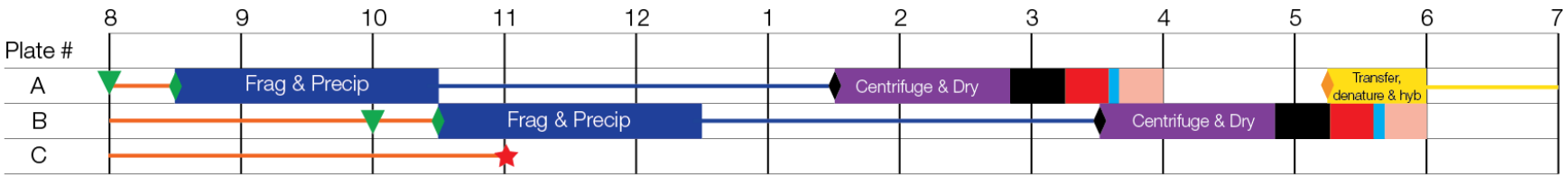
Activity	Plate	Approximate times[1]	
		Start time	End time
Thaw the mPCR Product Plate	A	8:00 a.m.	8:30 a.m.
Fragment and precipitate	A	8:30 a.m.	10:30 a.m.
Thaw the mPCR Product Plate	B	10:00 a.m.	10:30 a.m.
Incubate the Precipitation Plate at –20°C	A	10:30 a.m.	1:30 p.m.
Fragment and precipitate	B	10:30 a.m.	12:30 p.m.
Freeze the (–20°C) Amplification Plate	C	11:00 a.m.	—
Incubate the Precipitation Plate at –20°C	B	12:30 p.m.	3:30 p.m.
Centrifuge and dry	A	1:30 p.m.	2:50 p.m.
Resuspension and hybridization preparation	A	2:50 p.m.	3:15 p.m.
Sample QC	A	3:15 p.m.	3:35 p.m.
Centrifuge and dry	B	3:30 p.m.	4:50 p.m.
Sample quantification (OD)[2]	A	3:35 p.m.	3:40 p.m.
Fragmentation gel QC run	A	3:35 p.m.	4:00 p.m.
Resuspension and hybridization preparation	B	4:50 p.m.	5:15 p.m.
Sample QC	B	5:15 p.m.	5:35 p.m.

Table 12 Day 2 activities for the Axiom PharmacoFocus Assay Mini 96-Array Format Manual Workflow using 3-hour precipitation. *(continued)*

Activity	Plate	Approximate times[1]	
		Start time	End time
Transfer, denaturation, then hybridization[3]	A	5:15 p.m.	6:05 p.m.
Sample quantification[2]	B	5:35 p.m.	5:40 p.m.
Fragmentation gel QC run	B	5:35 p.m.	6:00 p.m.
Store the Hyb-Ready Plate at –20°C	B	5:40 p.m.	—

[1] Approximate start time indicates the start of thawing reagents.
[2] Sample quantification runs concurrently with frag gel QC run. Load Gel QC Plate first, then read the OD QC Plate.
[3] Setup = 50 minutes, hybridization = 23.5–24 hours.

Day 2 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with 3-hour precipitation



User activities

▼	Thaw mPCR Product Plate
♦	Prepare reagents for fragmentation
♦	Prepare reagents for resuspension and hybridization prep
★	Freeze
♦	Warm array plate to room temperature

Background activities

—	Amplification incubation
—	Precipitation incubation
—	Hybridization in the GeneTitan™ MC Instrument

User activities

	Fragmentation and precipitation
	Centrifugation and drying pellets
	Resuspension and hybridization preparation
	Sample QC
	Sample quantitation—OD
	Fragmentation gel QC run
	Transfer, denaturation, then hybridization

Day 3 activities

- It is recommended that 2 people work together for the day 3 activities.
- Perform transfer, denaturation, then hybridization of plate B.
- Thaw plate C. See “Thaw the frozen plate of amplified DNA” on page 140.
- Fragment (including the 65°C stop amplification reaction step) and precipitate plate C.
- Centrifuge, dry, resuspend, and QC plate C.
- Prepare the GeneTitan™ reagent trays for plate A, then load.
- Perform transfer, denaturation, then hybridization for plate C.

WARNING! The hybridization tray preparation must take place under a running fume hood.

IMPORTANT! Amplified plates that are frozen must be thawed, then thoroughly mixed. See “Thaw the frozen plate of amplified DNA” on page 140.

Table 13 Day 3 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using 3-hour precipitation.

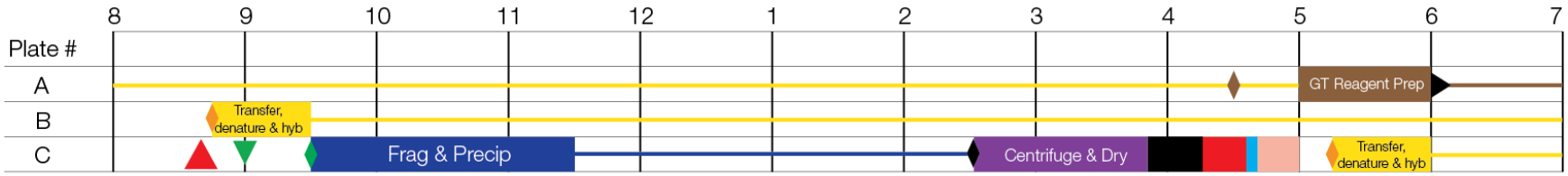
Activity	Plate	Approximate times[1]	
		Start time	End time
Transfer, denaturation, then hybridization	B	8:45 a.m.	9:35 a.m.
Thaw the Amplification Plate	C	8:30 a.m.	9:30 a.m.
Thaw the mPCR Product Plate	C	9:00 a.m.	9:30 a.m.
Fragment and precipitate	C	9:30 a.m.	11:30 a.m.
Incubate the Precipitation Plate at –20°C	C	11:30 a.m.	2:30 p.m.
Centrifuge and dry	C	2:30 p.m.	3:50 p.m.
Resuspension and hybridization preparation	C	3:50 p.m.	4:15 p.m.
Sample QC	C	4:15 p.m.	4:35 p.m.
GeneTitan™ reagent tray preparation and loading	A	4:30 p.m.	6:00 p.m.
Sample quantification (OD)[2]	C	4:35 p.m.	4:40 p.m.

Table 13 Day 3 activities for the Axiom PharmacoFocus Assay Mini 96-Array Format Manual Workflow using 3-hour precipitation. *(continued)*

Activity	Plate	Approximate times[1]	
		Start time	End time
Fragmentation gel QC run	C	4:35 p.m.	5:00 p.m.
Transfer, denaturation, then hybridization[3]	C	5:15 p.m.	6:05 p.m.

[1] Approximate start time indicates the start of thawing reagents.
[2] Sample quantification runs concurrently with the fragmentation gel QC run. Load the Gel QC Plate first, then read the OD QC Plate.
[3] Setup = 50 minutes, hybridization = 23.5–24 hours.

Day 3 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with 3-hour precipitation



User activities

▲	Thaw Amplification Plate
▼	Thaw mPCR Product Plate
◆	Warm array plate to room temperature
◆	Prepare reagents for fragmentation
◆	Prepare reagents for resuspension and hybridization prep
◆	Thaw reagents for GeneTitan™ reagent tray preparation
▶	Load array plate into GTMC, begin wash-scan
	Fragmentation and precipitation
	Centrifugation and drying pellets
	Resuspension and hybridization preparation
	Sample QC
	Sample quantitation - OD
	Fragmentation gel QC run
	Transfer, denaturation, then hybridization
	GeneTitan™ reagent tray preparation and loading

Background activities

	Precipitation incubation
	Hybridization in the GeneTitan™ MC Instrument
	Fluidics processing in the GeneTitan™ MC Instrument

Day 4 activities

- Prepare GeneTitan™ reagent trays, then load reagent trays 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 14 Day 4 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow using 3-hour precipitation.

Activity	Plate	Approximate times[1]	
		Start time	End time
GeneTitan™ reagent preparation and loading	B	8:00 a.m.	9:30 a.m.
GeneTitan™ reagent preparation and loading	C	4:30 p.m.	6:00 p.m.

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

Day 4 activities for the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow 3-plate workflow with 3-hour precipitation

User activities

- Thaw reagents for GeneTitan™ reagent tray prep
- Load array plate into GTMC, begin wash-scan
- GeneTitan™ reagent tray preparation and loading

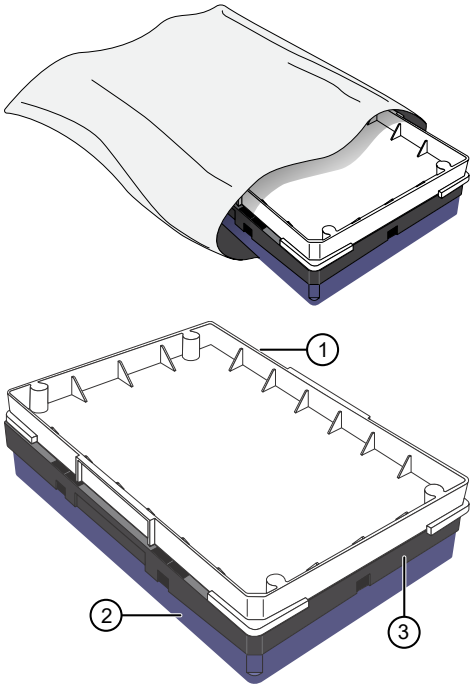
Background activities

- Hybridization in the GeneTitan™ MC Instrument
- Fluidics processing in the GeneTitan™ MC Instrument
- Imaging in the GeneTitan™ MC Instrument

A Recommended techniques for GeneTitan™ MC Instrument operation

This chapter describes the recommended techniques and procedures to follow when using the GeneTitan™ Multi-Channel (MC) Instrument for the fluidics processing and array scanning steps of the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow. Being familiar with these techniques helps to ensure the success of the assay. Detailed safety information and instruction on using the GeneTitan™ MC Instrument is in the *GeneTitan™ Multi-Channel Instrument User Guide* (Pub. No. 08-0308) and the *GeneChip™ Command Console™ User Guide* (Pub. No. 702569).

Array plate packaging

Item	Image	Details
Axiom™ PharmacoFocus™ Mini 96-Array Plate, Cat. No. 952380, or Axiom™ PharmacoFocus™ Mini 96-Array Plate Core, Cat. No. 952424	 ① Shipping cover (to be discarded) ② Array plate protective base ③ Array plate	The array plate package includes the following: • White plastic cover: The function of the white plastic cover for the array plate is to protect the array plate during transport. You can discard this after removing the array plate. • Array plate: The array plate must be protected at all times from damage or exposure to dust. The array plate must be in the blue array plate protective base at all times. • Protective base: The blue array plate protective base in the package must be used to protect the array plate from damage. • Desiccant pack: The desiccant pack can be discarded after the array plate is removed from the pouch.

Proper tray alignment and placement

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 Instrument drawer.

CAUTION! Be careful not to damage the consumables or bend the blue scan tray protective base cover posts or scan tray posts.

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

IMPORTANT! The drawer bed is *not* notched, so it is mechanically possible to load the tray in the wrong orientation. Be careful to avoid this mistake.

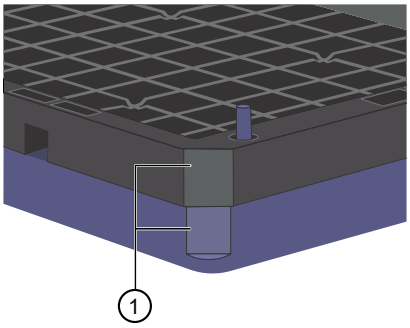


Figure 29 Notched corners aligned.

(1) The notched corner of array plate that is aligned with the notched corner of blue protective base.

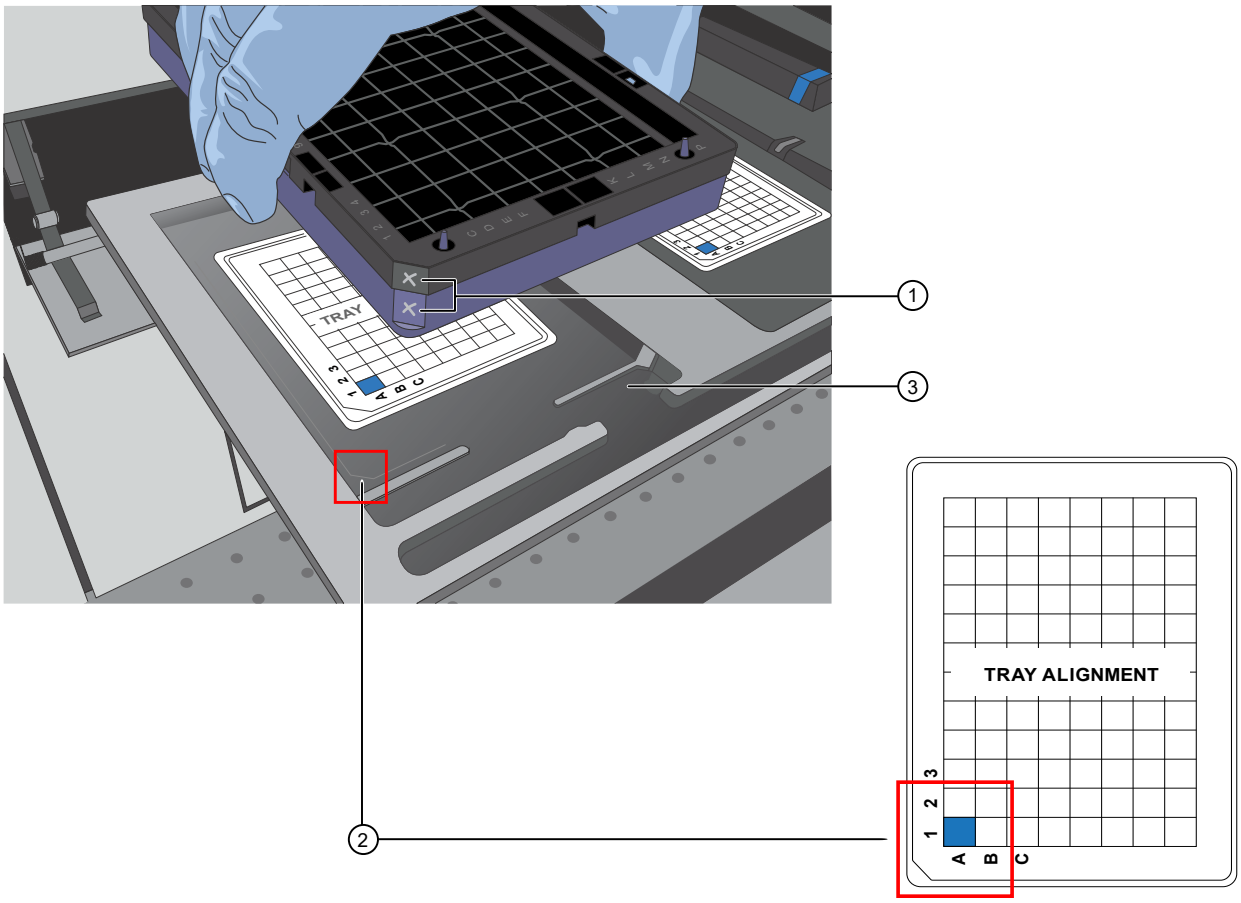


Figure 30 Notched corners marked and aligned with tray alignment guide.

(1) The notched corners of array plate and base that are marked with permanent marker.
(2) The notched corner of all plates, bases, and covers and must be seated in the front left corner of the drawer, as indicated in the Tray Alignment guide.
(3) Plates and trays must be seated in this groove.

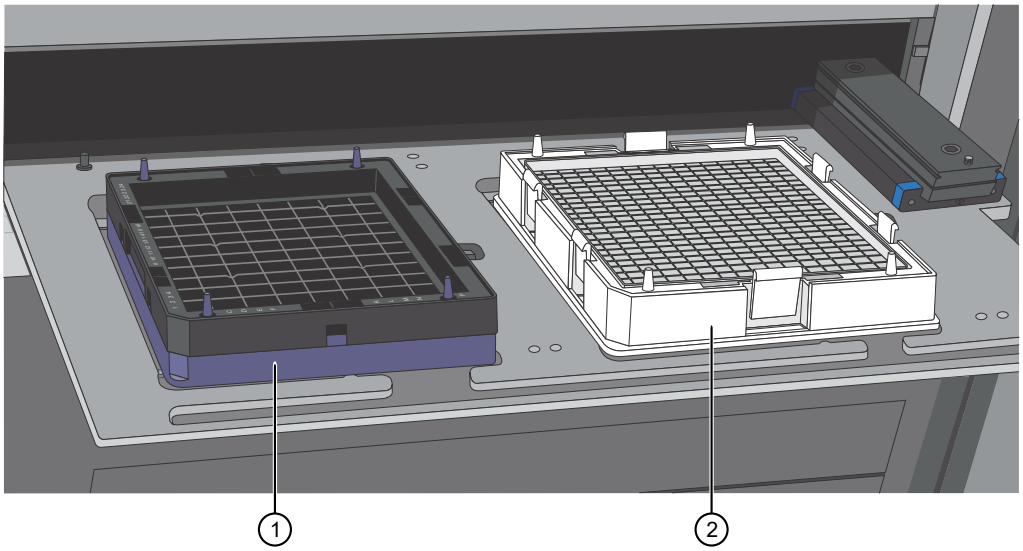


Figure 31 Array plate with blue protective base and the hybridization tray aligned properly loaded into drawer 6.

① Array plate on blue protective base
② Hybridization tray

Scan tray

The scan tray must be loaded into the GeneTitan™ Instrument with the scan tray cover only.

Do not load the scan tray while still on the protective base.

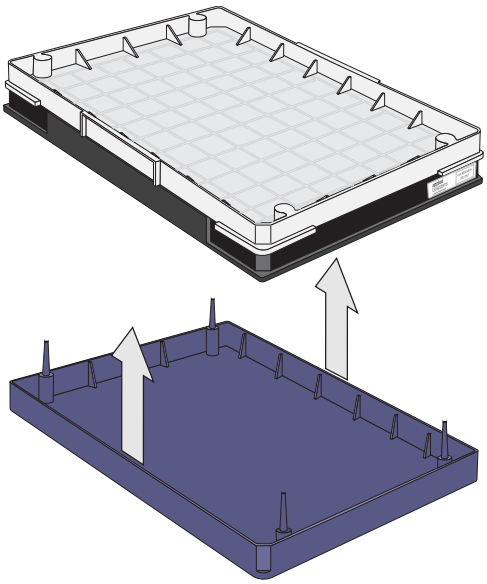


Figure 32 Remove the scan tray and cover from the base before loading in the GeneTitan™ Instrument.

Proper orientation of consumables

It is important that consumables be oriented properly when loaded into/onto the GeneTitan™ MC Instrument. The barcodes face into the instrument.

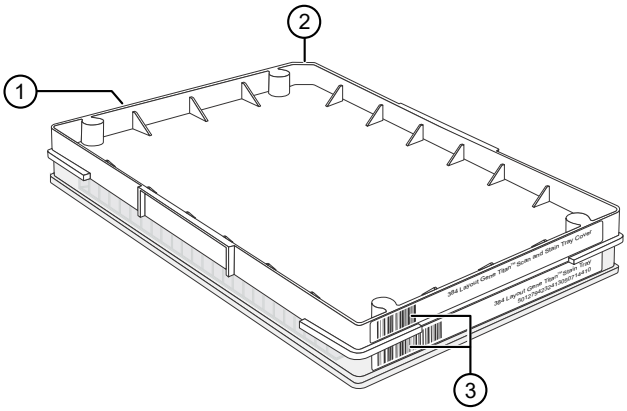


Figure 33 Example shows consumables that must be rotated and loaded on the drawer so that the barcodes face into the instrument.

1. Front of instrument (facing you).
2. Notched corners. The notched corners face out and left.
3. Barcodes. The barcodes face to the rear of the instrument where scanning by the internal barcode reader takes place.

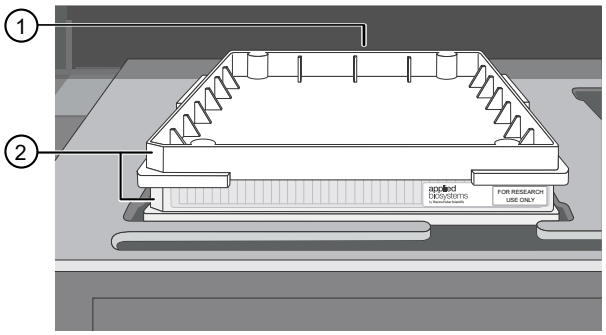


Figure 34 Example of properly loaded GeneTitan™ tray consumables.

A GeneTitan™ stain tray and the stain tray cover are shown in this example.

1. Barcodes face the rear of the instrument.
2. Notches face out and left. "For Research Use Only" faces out.

Drawer tabs in the GeneTitan™ MC Instrument

The GeneTitan™ MC Instrument drawers have tabs, or fingers, that restrain the consumable. The fingers retract when the drawer is open and extend when the drawer closes. When you load the plates or trays, ensure that the fingers are retracted and place trays onto the instrument drawers only after the drawer is fully extended. Ensure that the tray is not resting on the fingers. Notify your field service engineer if the fingers do not retract automatically.

IMPORTANT! Do not place the consumables on top of the drawer fingers—this position prevents the instrument from functioning correctly.

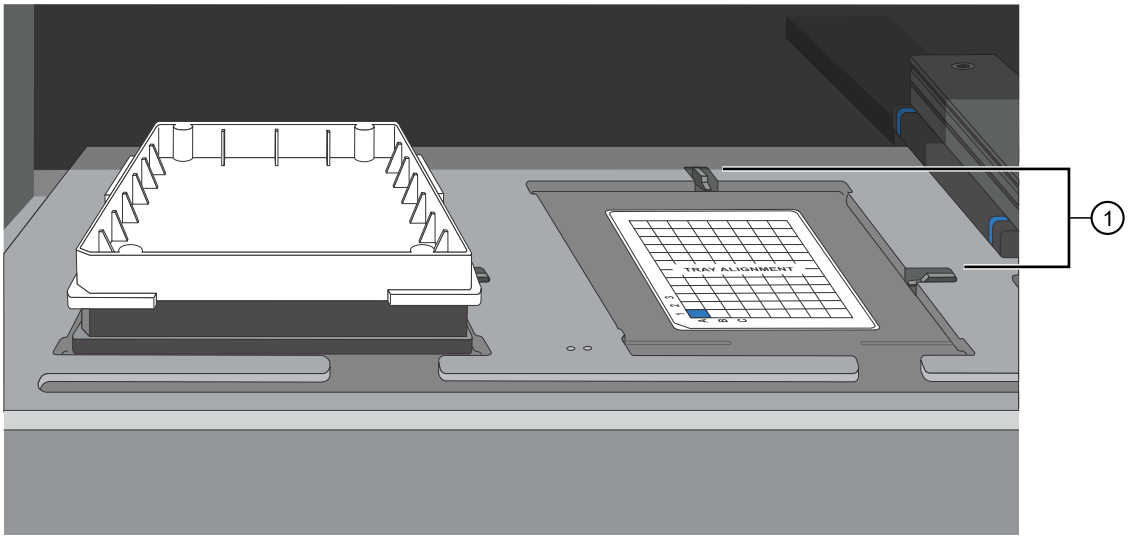


Figure 35 Location of drawer tabs, or fingers.

(1) Drawer tabs, or fingers, in the GeneTitan™ MC Instrument.

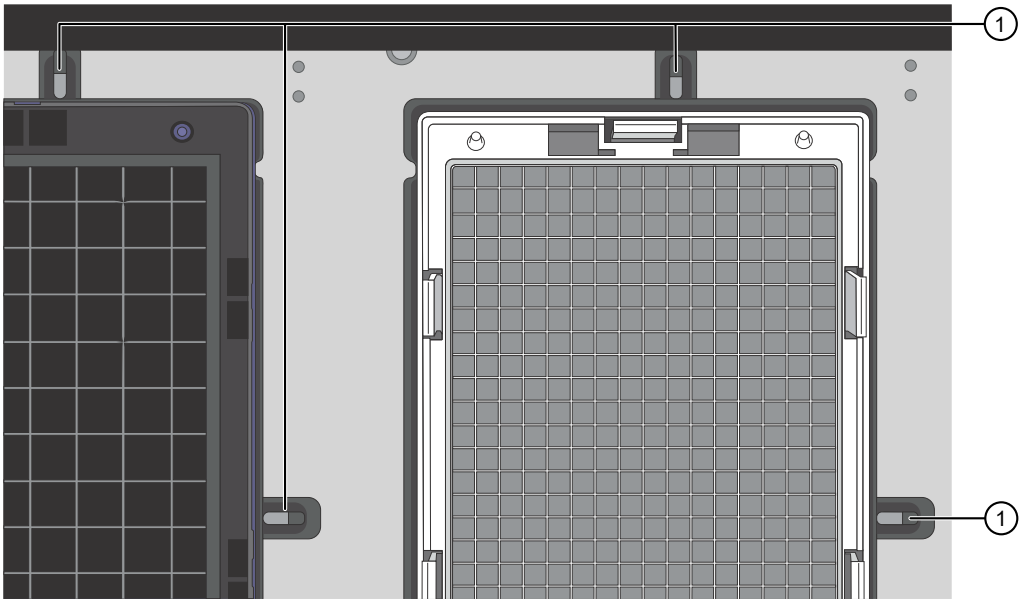


Figure 36 Place trays on the drawer when the tabs, or fingers, are retracted.

① Fingers retracted

Stain trays and covers

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

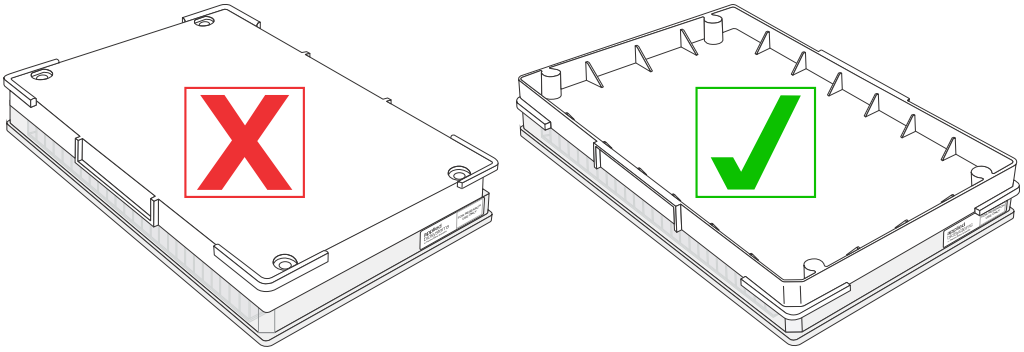


Figure 37 Placement of covers on trays.

Label GeneTitan™ hybridization and reagent trays

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan™ MC Instrument, it is helpful 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, because writing can interfere with sensors inside the GeneTitan™ MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan™ MC Instrument, you can also mark the notched corner of the trays and lids.

Label a GeneTitan™ 384 Layout Hybridization Tray

Label a GeneTitan™ 384 Layout Hybridization Tray on the front part of the short side of the tray, next to the notch at the left, as shown in the following image. The proper section for labeling is nearest to the notched corner, corresponding to the A1 through F1 wells.

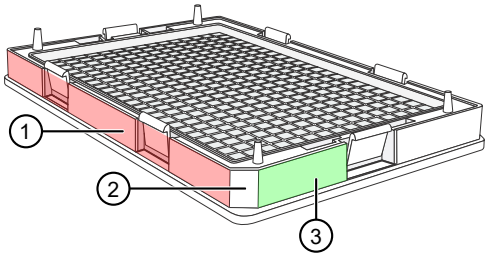


Figure 38 Correct area to label a GeneTitan™ 384 Layout Hybridization Tray.

1. Do not label hybridization tray on the long side.
2. Notched corner of the hybridization tray faces the front.
3. Label the hybridization tray here.

CAUTION! Writing on the wrong side of the hybridization tray can interfere with the operation of the sensors in the GeneTitan™ MC Instrument.

Label a GeneTitan™ 384 Layout Reagent Tray

You can label a GeneTitan™ 384 Layout Reagent Tray on the left side of the front of the tray as shown in the following image. The correct side is nearest to the notched corner, corresponding to the A1 through F1 wells.

IMPORTANT! This procedure is for noncolor-coded reagent trays. It is not necessary to label color-coded reagent trays.

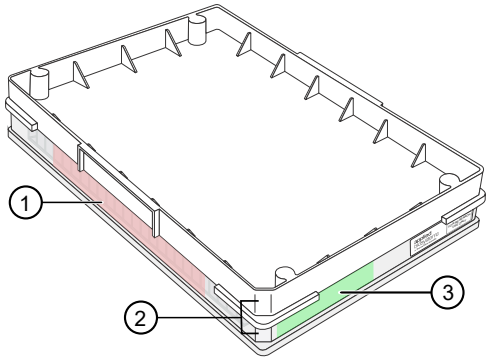
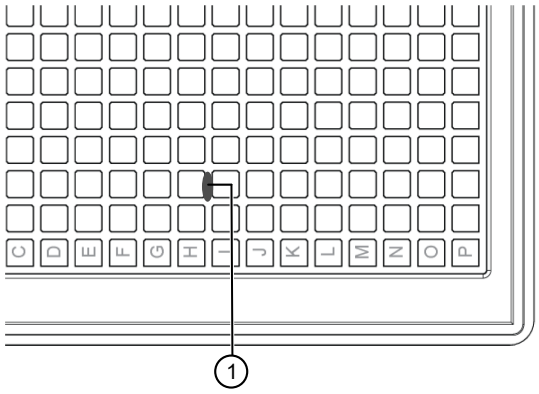


Figure 39 Correct area to label a GeneTitan™ 384 Layout Reagent Tray.

1. Do not label the reagent tray on the long side.
2. Notched corners of the reagent tray and cover must align and face the front.
3. Label the reagent tray here.

Guidelines for aliquoting reagents to GeneTitan™ trays

Droplets near or on the top of the well dividers can cause the cover to stick to the tray during GeneTitan™ MC Instrument processing.



1. Example of a droplet of liquid that has splashed onto the well divider of a stain tray during reagent aliquoting. Ensure that no droplets of liquid are on the top of the well dividers. Blot with a laboratory tissue to remove.

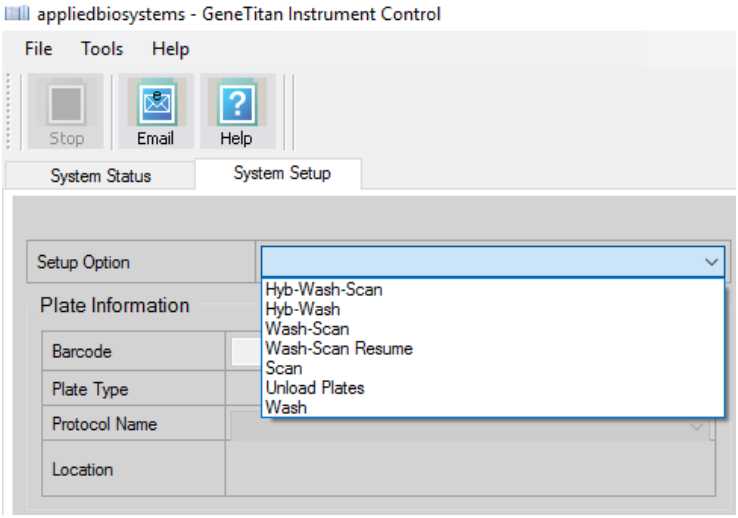
- If the trays are not being used immediately, protect them from light by covering with foil or placing in a cabinet.
- After aliquoting the Ligation, Stain 1, Stain 2, and Stabilization Master Mix reagents to the trays, it is not necessary to spread the reagent to each corner of the well. The reagent spreads evenly when the array plate is inserted into the reagent tray during processing with the GeneTitan™ MC Instrument.

Setup options for array plate processing

There are 3 steps performed by the GeneTitan™ MC Instrument for array plate processing:

- Hybridization
- Wash and Stain
- Imaging (Scan)

The GeneChip™ Command Console™ software provides options to perform all these steps, or only some of the steps. This section describes the **System Setup** options.



Hyb-Wash-Scan

The **Hyb-Wash-Scan** setup option enables you to hybridize, wash-ligate-stain-stabilize, and scan an array plate on the GeneTitan™ MC Instrument.

- **Hyb**: The array plate is moved to the hybridization oven inside the instrument. Each denatured sample in the hybridization tray is hybridized to an array on the array plate.
  - Time that is required for 96 samples = 23.5 hours
- **Wash**: Samples on arrays are ligated, washed, stained, and stabilized.
  - Time that is required for 96 samples = ~5 hours
- **Scan**: The array plate is moved to the imaging device in the GeneTitan™ MC Instrument and each array is scanned.
  - Time that is required for 96 samples = ~1.5 hours

Hyb-Wash

When the **Hyb-Wash** setup option is selected, 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.

1. If the array plate cannot be scanned immediately after the **Hyb-Wash** process is complete, store the array plate following these steps:
   a. Wrap the array plate (in the scan tray with black protective base) in aluminum foil to protect from light.

      No lid is required. Do not tilt or invert the plate stack. If tilted or inverted, the Hold Buffer spills out of the tray. To prevent liquid spillage, keep the plate level when handling it. Do not touch the bottom optical surface of the scan tray.

   b. Store at 4°C.

   c. Scan the array plate in 3 days or less.

2. When ready to scan, prepare the array plate following these steps:
   a. Protect the plate from light.

   b. Bring the plate to room temperature for approximately 50 minutes.

   c. Remove the aluminum foil, then load the plate onto the GeneTitan™ MC Instrument.

Wash-Scan

Note: The **Wash-Scan** option is available in GCC version 6.1 or later.

Use the **Wash-Scan** option if:

- The array plate was hybridized in an oven separate from the GeneTitan™ MC Instrument.
- To bypass the hybridization step and perform only the wash/stain and scan steps.

Note: If the **Wash-Scan** option is selected, it usually takes 25–30 minutes to warm up the Wash B.

Wash-Scan Resume

Use the **Wash-Scan Resume** option if fluidics processing has been interrupted (for example, a power failure at your facility). This allows you to resume an interrupted workflow at any point in the **Wash** stage.

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 array plate from the scan tray then place the array in its blue protective base.

The step at which the run was aborted is identified by:

- Viewing the **System Status** window if you are aborting the last plate through the fluidics system.
- Starting the **Resume** process.

Select **Wash/Scan Resume** from the **System Setup** tab, then follow the prompts to unload and reload all drawers.

The trays are loaded. It is up to you to determine whether to load fresh reagents or reuse the trays already in the GeneTitan™ Multi-Channel (MC) Instrument. Base your decision on the step where the problem occurred.

To help ensure that the samples are processed correctly, we recommend that you:

- Load new stain trays with fresh reagents.
- Load a new scan tray.

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

Resume step

For **Resume**, select the step at which to resume plate processing. You can select any step that has not yet been started.

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

Scan

Use the **Scan** option for the following circumstances.

- To rescan an entire array plate or specific arrays on a plate that failed to scan for reasons such as bubbles or gridding failure.
- To scan a plate that has already been hybridized, stained and washed on a different GeneTitan™ MC Instrument.

Unload Plates

Use the **Unload Plates** option to unload plates and trays from the instrument when processing is complete or has been aborted.

Wash

The **Wash** workflow enables you to bypass the scan step, performing only wash and stain.

Note: When the **Wash** option is selected, allow 25–30 minutes to warm up the Wash B.

IMPORTANT! After the **Wash** workflow is complete, scan the array plate as soon as possible. Array plate data can be affected when the plate is not scanned immediately after washing.

When to abort a process

If needed, the processing of array plates can be aborted.

If a plate is in the fluidics station, the abort process can take up to 3 minutes. The status window displays “AbortRequested” and then changes to “Aborted”.

A clamped array plate/hybridization tray stack that is aborted while it is in the oven or in drawer 6 is moved to drawer 1.

To retrieve the array plate and related consumables after the instrument aborts a process, take the following actions as needed.

- Use the **Unload Plates** option.
- Start another run. That forces the system to unload the aborted plates.

An instrument-initiated abort can occur for the following reasons.

- The plates are improperly placed.
- The uninterruptible power supply (UPS) detects a long power interruption, draining the UPS to 75% power.
- The equipment malfunctions.

When the system aborts the processing, follow the instructions that are displayed in the user interface.

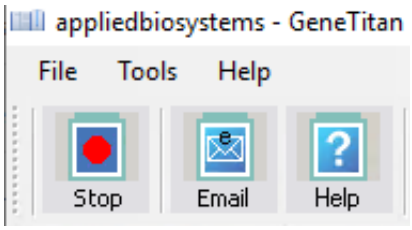
The operator can initiate an abort on 1 plate and the gripper can continue to process other plates in the instrument.

Abort a process

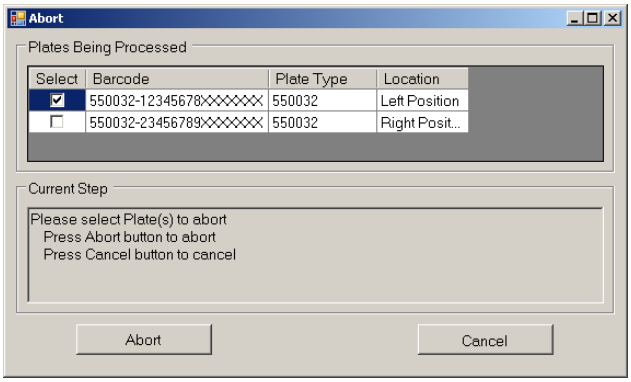
Use the following procedure to abort a process underway in the GeneTitan™ MC Instrument.

Note: If reagents are loading, do not use this method. Instead, click **Cancel** in the reagent load step.

1. Click **Stop** in the upper left corner of the **Instrument Control** window.



2. In the **Abort** dialog box, select the array plate to abort, then click **Abort**.



3. In the confirmation box, click **Yes**.

4. Wait until the status of the array plate in the **Work Flow** pane in the **Instrument Control** display changes from "AbortRequested" to "Aborted".

Note: If reagents are loading, abort the plate by clicking **Cancel** 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.

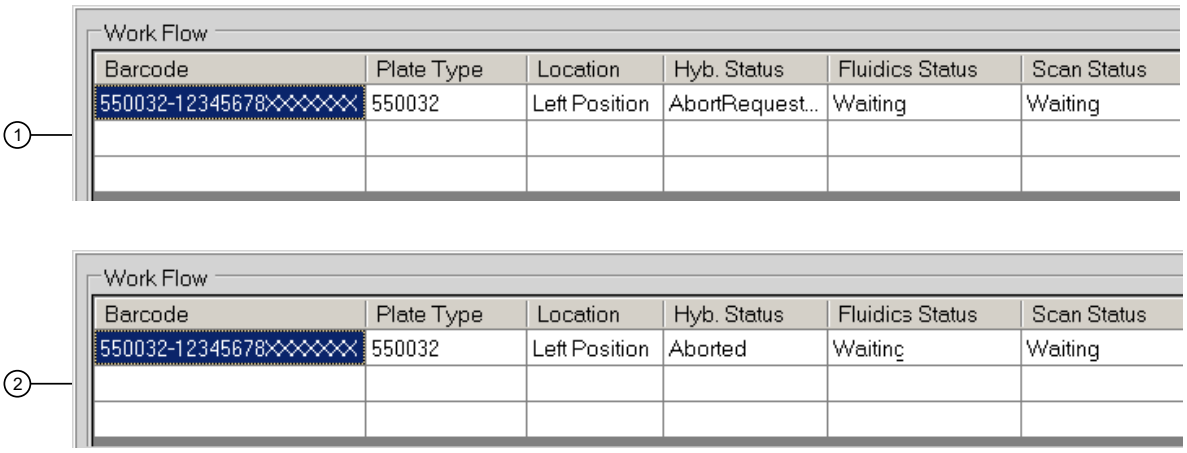


Figure 40 The Work Flow pane.

① Shows that the abort has been requested.
② Shows that the abort has been completed.

5. After the abort process is completed, take one of the following actions to retrieve the array plate and related consumables.

- In the **Setup Option** dropdown list, select **Unload Plates**.
- Start to load a new array plate.

Email notifications from the GeneTitan™ MC Instrument

You can configure the GeneChip™ Command Console™ software to send email notifications about the GeneTitan™ MC Instrument status. It is critical that you know when the instrument requires attention for sample handling or troubleshooting. Rapid notification can lessen the risk of sample loss.

The system can notify you when a process starts, completes, aborts, or encounters an error.

For instructions on setting up notifications, see the *GeneChip™ Command Console™ User Guide* (Pub. No. 702569).

GeneTitan™ MC Instrument lamp

The GeneTitan™ MC Instrument uses a xenon arc lamp system that is warranted to provide 500 hours of illumination for imaging the array at 2 wavelengths. The xenon lamp has a limited lifetime and must 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™ Multi-Channel Instrument User Guide* (Pub. No. 08-0308).

See the user guide for the Lambda LS and Smart controller system. Never manually switch the lamp and the controller on or off. The GeneTitan™ 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 2 hours before it is automatically switched off if there are no more plates being transferred from the fluidics to the imaging station. This automatic switching is by design and is intended behavior. Do not try to save the lamp life by powering off the switch on the lamp.

Note: The power switch on the shutter box must always be ON. The OPEN/CLOSE switch on the shutter box must always be at the AUTO position.

B

Register samples in GeneChip™ Command Console™

GeneTitan™ Array Plate Registration file

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

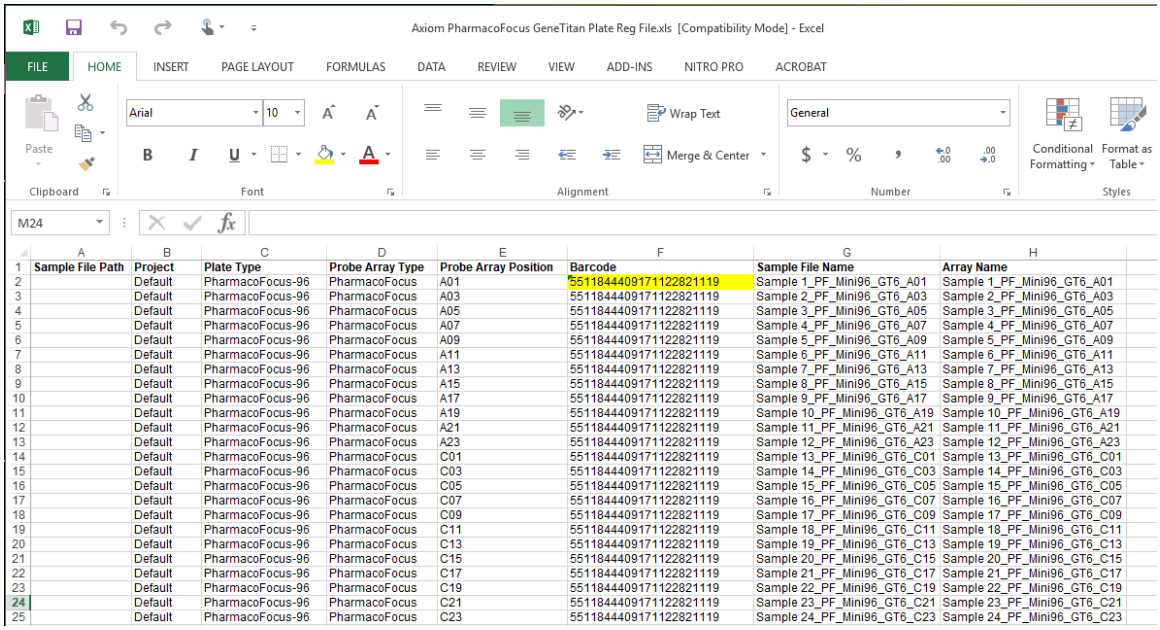
Note: The GeneTitan™ Array Plate Registration file uses the *.xls Microsoft™ Excel™ file extension. Do not use the*.xlsx file extension.

Create a GeneTitan™ Array Plate Registration file

1. In GCC Portal, click **Samples** ▸ **GeneTitan Array Plate Registration**.

2. Create a new template in GCC that includes fields required for sample traceability.
3. Select the array plate to be processed on the GeneTitan™ MC Instrument.
4. Select the newly created template that contains the fields that are required for traceability.

5. Select the **GeneTitan Array Plate Type** from the dropdown list.
6. Select the project where the sample registration data and all associated data files are saved.
7. Click **Download**.
8. Click the Microsoft™ Excel™ icon to open the spreadsheet.



	A	B	C	D	E	F	G	H
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array Position	Barcode	Sample File Name	Array Name
2		Default	PharmacoFocus-96	PharmacoFocus	A01	551184440917112282119	Sample 1_PF_Mini96_GT6_A01	Sample 1_PF_Mini96_GT6_A01
3		Default	PharmacoFocus-96	PharmacoFocus	A03	551184440917112282119	Sample 2_PF_Mini96_GT6_A03	Sample 2_PF_Mini96_GT6_A03
4		Default	PharmacoFocus-96	PharmacoFocus	A05	551184440917112282119	Sample 3_PF_Mini96_GT6_A05	Sample 3_PF_Mini96_GT6_A05
5		Default	PharmacoFocus-96	PharmacoFocus	A07	551184440917112282119	Sample 4_PF_Mini96_GT6_A07	Sample 4_PF_Mini96_GT6_A07
6		Default	PharmacoFocus-96	PharmacoFocus	A09	551184440917112282119	Sample 5_PF_Mini96_GT6_A09	Sample 5_PF_Mini96_GT6_A09
7		Default	PharmacoFocus-96	PharmacoFocus	A11	551184440917112282119	Sample 6_PF_Mini96_GT6_A11	Sample 6_PF_Mini96_GT6_A11
8		Default	PharmacoFocus-96	PharmacoFocus	A13	551184440917112282119	Sample 7_PF_Mini96_GT6_A13	Sample 7_PF_Mini96_GT6_A13
9		Default	PharmacoFocus-96	PharmacoFocus	A15	551184440917112282119	Sample 8_PF_Mini96_GT6_A15	Sample 8_PF_Mini96_GT6_A15
10		Default	PharmacoFocus-96	PharmacoFocus	A17	551184440917112282119	Sample 9_PF_Mini96_GT6_A17	Sample 9_PF_Mini96_GT6_A17
11		Default	PharmacoFocus-96	PharmacoFocus	A19	551184440917112282119	Sample 10_PF_Mini96_GT6_A19	Sample 10_PF_Mini96_GT6_A19
12		Default	PharmacoFocus-96	PharmacoFocus	A21	551184440917112282119	Sample 11_PF_Mini96_GT6_A21	Sample 11_PF_Mini96_GT6_A21
13		Default	PharmacoFocus-96	PharmacoFocus	A23	551184440917112282119	Sample 12_PF_Mini96_GT6_A23	Sample 12_PF_Mini96_GT6_A23
14		Default	PharmacoFocus-96	PharmacoFocus	C01	551184440917112282119	Sample 13_PF_Mini96_GT6_C01	Sample 13_PF_Mini96_GT6_C01
15		Default	PharmacoFocus-96	PharmacoFocus	C03	551184440917112282119	Sample 14_PF_Mini96_GT6_C03	Sample 14_PF_Mini96_GT6_C03
16		Default	PharmacoFocus-96	PharmacoFocus	C05	551184440917112282119	Sample 15_PF_Mini96_GT6_C05	Sample 15_PF_Mini96_GT6_C05
17		Default	PharmacoFocus-96	PharmacoFocus	C07	551184440917112282119	Sample 16_PF_Mini96_GT6_C07	Sample 16_PF_Mini96_GT6_C07
18		Default	PharmacoFocus-96	PharmacoFocus	C09	551184440917112282119	Sample 17_PF_Mini96_GT6_C09	Sample 17_PF_Mini96_GT6_C09
19		Default	PharmacoFocus-96	PharmacoFocus	C11	551184440917112282119	Sample 18_PF_Mini96_GT6_C11	Sample 18_PF_Mini96_GT6_C11
20		Default	PharmacoFocus-96	PharmacoFocus	C13	551184440917112282119	Sample 19_PF_Mini96_GT6_C13	Sample 19_PF_Mini96_GT6_C13
21		Default	PharmacoFocus-96	PharmacoFocus	C15	551184440917112282119	Sample 20_PF_Mini96_GT6_C15	Sample 20_PF_Mini96_GT6_C15
22		Default	PharmacoFocus-96	PharmacoFocus	C17	551184440917112282119	Sample 21_PF_Mini96_GT6_C17	Sample 21_PF_Mini96_GT6_C17
23		Default	PharmacoFocus-96	PharmacoFocus	C19	551184440917112282119	Sample 22_PF_Mini96_GT6_C19	Sample 22_PF_Mini96_GT6_C19
24		Default	PharmacoFocus-96	PharmacoFocus	C21	551184440917112282119	Sample 23_PF_Mini96_GT6_C21	Sample 23_PF_Mini96_GT6_C21
25		Default	PharmacoFocus-96	PharmacoFocus	C23	551184440917112282119	Sample 24_PF_Mini96_GT6_C23	Sample 24_PF_Mini96_GT6_C23

9. Enter a unique name for each sample in the **Sample File Name** column and any additional information, such as array plate barcode.

Note: The array plate's barcode can be scanned into the **Barcode** field. The barcode is stored in the sample file for each array.

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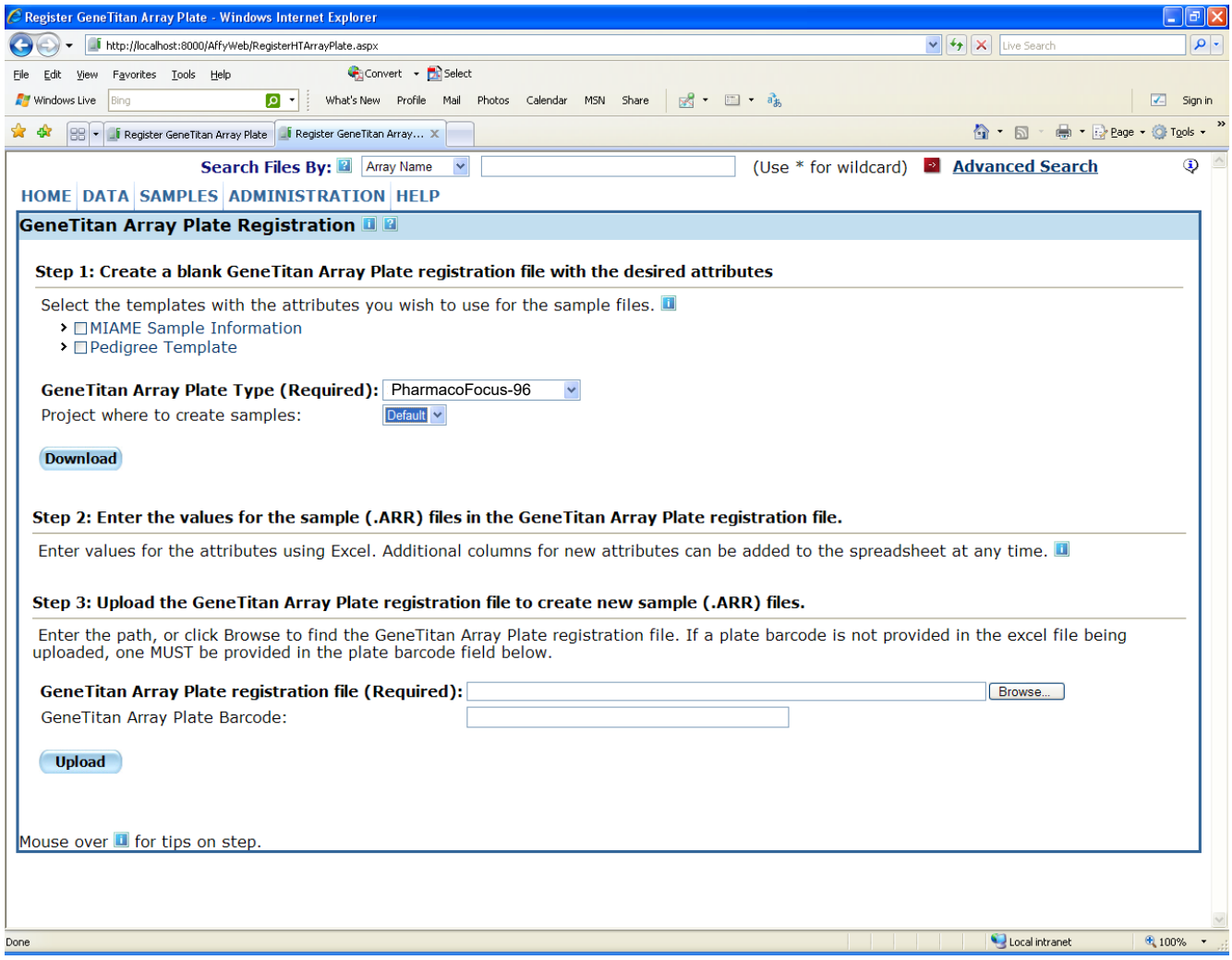
10. Complete one of the following:
    - If you are ready to load the array plate onto the GeneTitan™ MC Instrument, scan the array plate barcode into column F, then proceed to step 11.
    - If you are not ready to load the array plate onto the GeneTitan™ MC Instrument, proceed to step 11.
11. Follow these steps to save the file:
    a. Click **File ▸ Save As**.
    b. Enter a name for the array plate registration file.
    c. Click **Save**.
12. Follow these steps when you are ready to load the array plate onto the GeneTitan™ MC Instrument.
    a. Click **Browse**, navigate to the GeneTitan™ Array Plate Registration file, then click **Open**.

b. Scan the array plate barcode, if it has not already been scanned, and save the registration file.

c. Click **Upload**, wait for the information to load, then click **Save** found at the bottom of the next window that is displayed.



C mPCR quality control gel protocol

The mPCR quality control gel protocol 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.

Materials required

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Equipment	
Gel imager	**MLS**
Pipette, multichannel and single channel	**MLS**
Plate centrifuge	**MLS**
Vortexer	**MLS**
E-Gel™ products and reagents	
Mother E-Base™ Device	EBM03
Daughter E-Base™ Device	EBD03
E-Gel™ *48* Agarose Gels, 2%	G800802
TrackIt™ Cyan/Orange Loading Buffer	10482028
Low EDTA TE Buffer (10 mM Tris-HCL PH 8.0, 0.1 mM EDTA)	75793
50 bp DNA Ladder	New England BioLabs™ N3236S
Gel diluent [1]	

(continued)

Item	Source
Consumables	
Adhesive film—use one of the following • MicroAmp™ Clear Adhesive Film • Microseal™ 'B' PCR Plate Sealing Film	 • 4306311 • Bio-Rad™ MSB1001
Pipette tips (same brand as pipette)	MLS
96-well PCR plate	MLS
1.7-mL microcentrifuge tube	MLS

[1] See "Prepare the gel diluent" on page 187.

Prepare the gel diluent

Dilute the TrackIt™ Cyan/Orange Loading Buffer

The following procedure prepares a 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer.

1. Add 500 µL of TrackIt™ Cyan/Orange Loading Buffer to 49.5-mL nuclease-free water. Total volume 50 mL.
2. Mix well.
3. Store at room temperature.

Prepare the 50 bp DNA Ladder

The following steps prepare a 250-fold dilution of the New England BioLabs™ 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 100-fold diluted TrackIt™ Cyan/Orange Loading Buffer.
2. Vortex the tube to mix well. Briefly centrifuge to get the droplets down.

Prepare mPCR samples for gel analysis

1. If frozen, thaw the mPCR Product Plate on benchtop at room temperature.
2. Ensure that the plate seal is affixed tightly, vortex the plate, then centrifuge briefly.
3. Prepare the mPCR samples for gel analysis by diluting samples of the mPCR Product Plate 120-fold.
   a. Prepare the mPCR Dilution Plate (12-fold dilution from mPCR Product Plate).
      1. Obtain a 96-well PCR plate, then aliquot 22 µL of low EDTA TE buffer per well.
      2. Transfer 2 µL of the mPCR product to the appropriate wells of the 96-well PCR plate with TE buffer.
      3. Seal the plate, vortex, then centrifuge briefly.
   b. Prepare the mPCR Gel QC Plate (10-fold dilution from the mPCR Dilution Plate).
      1. Obtain a 96-well PCR plate, then aliquot 18 µL of gel diluent (TrackIt™ Cyan/Orange Loading Buffer diluted 100-fold).
      2. Transfer 2 µL of the mPCR dilution plate to the appropriate wells of the 96-well plate with gel diluent.
      3. Seal, vortex, then centrifuge briefly.
      4. Proceed immediately to the next step. (See “Run the mPCR QC gel” on page 188).

Run the mPCR QC gel

This protocol is based on running QC gels for 96 samples. Two E-Gel™ *48* Agarose Gels, 2% are needed.

1. Tightly seal the mPCR Gel QC Plate.
2. Vortex the plate for 3 seconds. Briefly centrifuge to get the droplets down.
3. Power on one or more E-Base™ devices.
4. Push the **Power/Prg** button on each to ensure the gel base is in **EG** mode, not EP mode.
5. Remove 2 combs from the gel.
6. Place the E-Gel™ *48* Agarose Gel into an E-Base™ unit.
7. Load 15 µL from each well of the mPCR Gel QC Plate into its designated wells.
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.

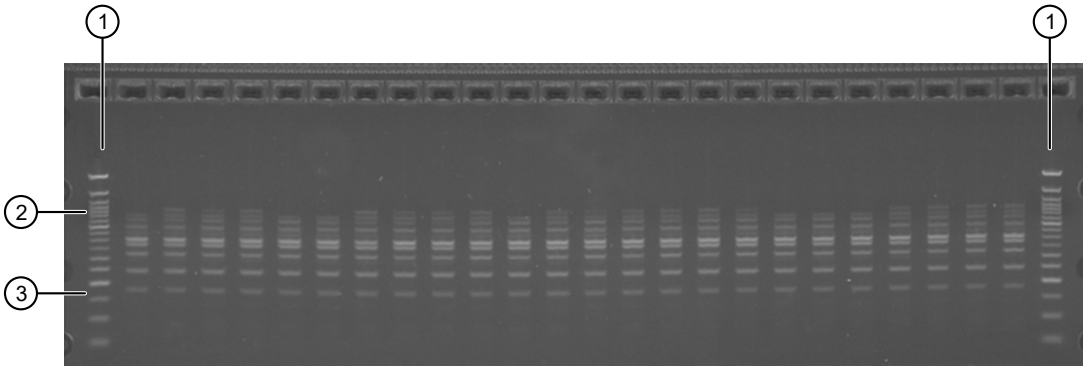


Figure 41 Example of a typical mPCR QC E-gel. All samples were amplified and show multiple DNA bands that fall between 150 bp and 700 bp.

(1) 50-bp ladder
(2) 700 bp
(3) 150 bp

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.

D Fragmentation quality control gel protocol

Equipment required

"MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Gel Imager	**MLS**
Pipette, multichannel or single channel P20	**MLS**
Plate centrifuge	**MLS**
Vortexer	**MLS**

E-Gel™ and reagents required

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Mother E-Base™ Device	EBM03
Daughter E-Base™ Device	EBD03
E-Gel™ *48* Agarose Gels, 4%	G800804
Applied Biosystems™ 25 bp DNA Ladder, or a similar product prepared as instructed by the manufacturer	931343
TrackIt™ Cyan/Orange Loading Buffer	10482028
UltraPure™ DNase/RNase-Free Distilled Water	10977023

Consumables required

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Adhesive film—use one of the following: • MicroAmp™ Clear Adhesive Film • Microseal™ 'B' PCR Plate Sealing Film	 • 4306311 • Bio-Rad™ MSB1001
Pipette tips	Same brand as pipettor

Prepare the gel diluent

Dilute the TrackIt™ Cyan/Orange Loading Buffer

A 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer can be used in "Stage 3C: Perform quantification and fragmentation QC checks" on page 79.

1. Add 500 µL of TrackIt™ Cyan/Orange Loading Buffer to 49.5-mL nuclease-free water. Total volume 50 mL.
2. Mix well.
3. Store at room temperature.

Dilute the 25 bp DNA Ladder

Applied Biosystems™ 25 bp DNA Ladder, Cat. No. 931343, is required for this procedure.

1. Add 25 µL of the 25 bp DNA Ladder to 125 µL of UltraPure™ DNase/RNase-Free Distilled Water.
2. Mix well.
3. Store at 4°C until use.

Run the fragmentation QC gel

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

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

1. Tightly seal the Gel QC Plate.
2. Vortex the center of the plate for 3 seconds. Centrifuge at 1,000 rpm for 30 seconds to get the droplets down.
3. Connect an E-Base™ device to an electrical outlet.
4. Push the **Power/Prg** button on each to ensure the gel base is in **EG** mode, not EP mode.
5. Take the gel out of the pouch, then remove the combs.
6. Place the E-Gel™ *48* Agarose Gel into the base unit.
7. Load 15 µL from each well of the Gel QC Plate onto the gel.
8. Load 15 µL of diluted 25 bp DNA Ladder into the marker wells (M).
9. Load 15 µL nuclease-free water into any unused wells.
10. Run the gels for 19 minutes.
11. Capture a gel image.

Fragmentation QC gel images should look similar to the following gel image.

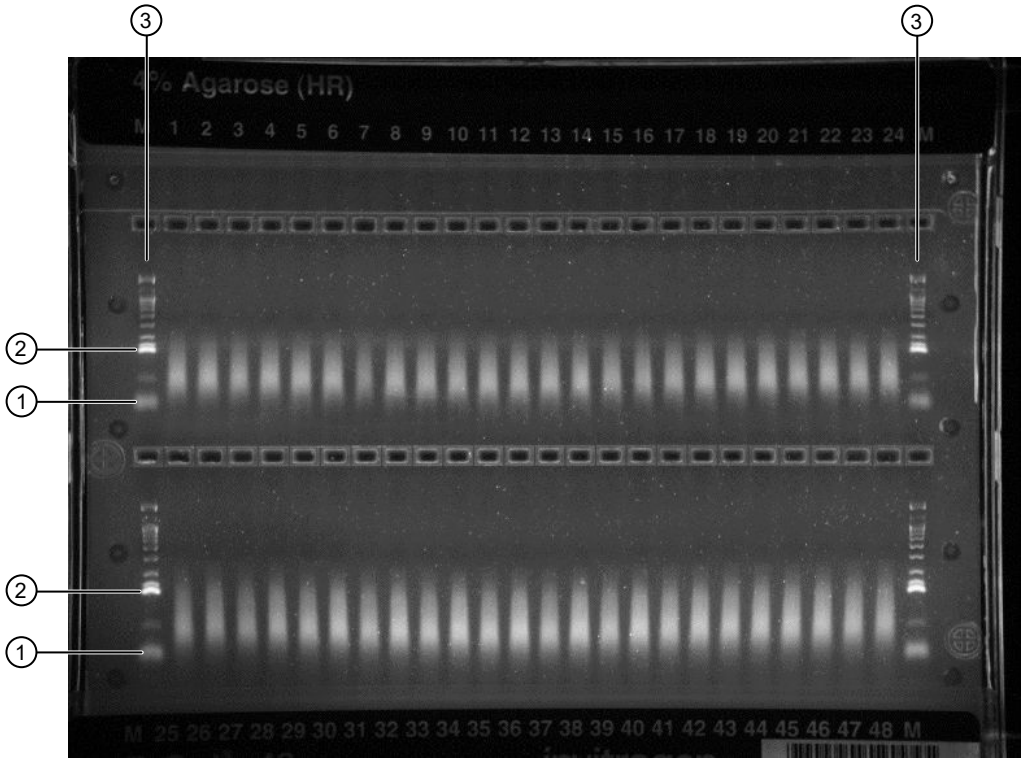


Figure 42 Fragments fall between 125 bp and 25 bp on a successful gel image.

① 25 bp
② 125 bp
③ 25 bp DNA Ladder

Sample quantification after resuspension

Equipment required

Spectrophotometer

We recommend that you use one of the following spectrophotometers, or the equivalent.

Item	Source
Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer	51119600
SpectraMax® Plus 384 Microplate Reader	Molecular Devices® PLUS 384
DTX 880 Multimode Detector with genomic filter slide	Beckman Coulter™ Detector 987921 Filter slide A30184

Quantify the diluted samples

During target preparation, 2 plates of diluted samples are prepared: 1 for OD quantification and 1 for a QC gel to check the fragmentation reaction.

For OD quantification, readings must be taken at wavelengths of 260 nm, 280 nm, and 320 nm. See “Plate reader guidelines for sample quantification” on page 195.

OD yield evaluation guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow. If the median yield for the plate is <1,000 µg DNA per sample:

- Pause the protocol.
- Evaluate all steps that are performed to that point to determine the possible source of the low yields.

This DNA yield corresponds to an A_{260} value of approximately 0.49 and an A_{260}-A_{320} value of approximately 0.50.

Plate reader guidelines for sample quantification

When performing sample quantification, the plate reader must 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 OD_{260}
- C = the observed OD_{320} (an estimate of a blank reading)
- D = 120 (the net dilution factor when preparing the OD sample plate)
- V = 115 (the volume of the sample in µL after the resuspension step)
- E = 0.05 (the extinction coefficient of duplex DNA at 260 nm)
- P = the optical path length for the plate type and plate reader used.

If your plate reader does not record the OD_{320}, the OD_{260} of a blank solution of water only must be used for the parameter "C".

Troubleshooting

GeneTitan™ Instrument support files for troubleshooting

Log files

The different GeneChip™ Command Console™ (GCC) components generate log files. The logs provide a record of the tasks performed by the different components, such as the migration tools and installer. These log files provide useful information for troubleshooting problems. Sometimes these files are required by your field application scientist (FAS), field service engineer (FSE), or the Thermo Fisher Scientific call center to help with troubleshooting.

GeneChip™ Command Console™ log files

The following files are generated by the GeneTitan™ MC Instrument. All the GCC log files are from the following path: `C:\Command_Console\Logs`.

Log file type	Description
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.

Other GeneChip™ Command Console™ files

The following GCC files and requests are sometimes used by FAS or FSE for troubleshooting.

- Library files (*.PARAMS, *.MASTER, *.WORKFLOW, *.SMD, *.MEDIA) in `C:\Command_Console\Library`, excluding the large analysis library files (CDF, PSI, GRC).
- Provide a list of all sub folders and their contents under the library files folder that is in `C:\Command_Console\Library`. Ensure that there are no duplicate library files, as these files can cause problems
- GCC system configuration file that is found at `C:\Command_Console\Configuration\Calvin.System.config`.
- Pending job order files that are in `C:\Command_Console\Jobs`
- Other GCC related information, such as
  - The number of files under `C:\Command_Console\Data`, including sub directory.
  - If the system is a networked system or a stand-alone system.
  - Other applications that are installed on the system, such as antivirus application, Microsoft™ Office™ , and Internet Explorer® versions.

GCC log files for GeneTitan™ MC Instrument systems

Log files for the GeneTitan™ MC Instrument control processes are placed in subdirectories of the `C:\Command_Console\Logs\` folder. Thermo Fisher Scientific sometimes requests the following files for troubleshooting.

GeneTitan™ MC Instrument fluidics

- `C:\Command_Console\Logs\96F\`
  - Subdirectories are named by date (for example, Log7-29-2016)

    Collect all dated directories and contents from the time the GeneTitan™ application was started, not just from the date of the event. Some logging goes into files from the date the application started so these files can be critical for troubleshooting.

    All the log directories from the date the run was started to the date of the event are essential.
- `C:\Command_Console\Logs\96F\FluidicErrorLog` - all files in this directory.

GeneTitan™ MC Instrument imaging device

- `C:\Affymetrix\GeneChipHTScanControlMC\Log` - collect all dated directories and contents from the time the GeneTitan™ application was started.
- `C:\Affymetrix\GeneChipHTScanControlMC\RunLog` - collect all dated directories and contents from the time the GeneTitan™ application was started.

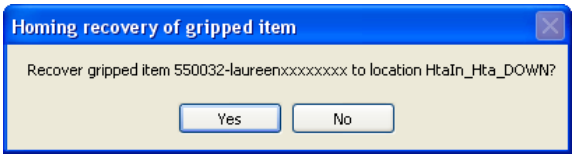
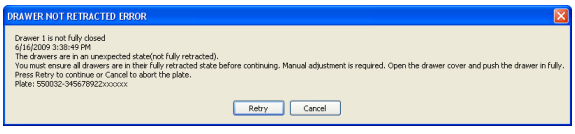
GeneTitan™ MC Instrument

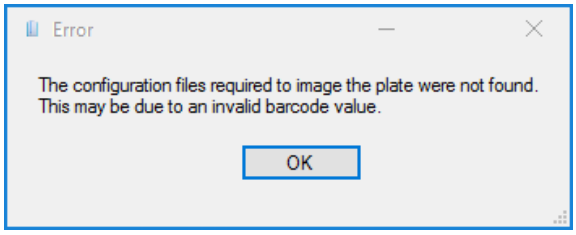
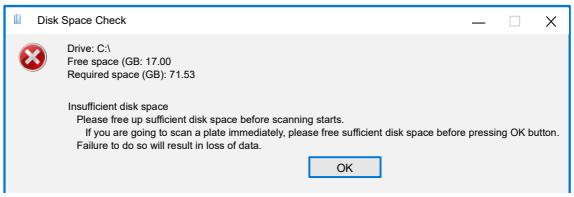
This section provides instructions on how to identify and solve simple problems with the GeneTitan™ MC Instrument. If a problem or error occurs that is not listed in this chapter, contact Thermo Fisher Scientific Technical Support for help.

For software errors that do not involve hardware crashes, the most common solution is to close or exit the application, then restart it. If the same error occurs, close the application and power off the computer, then restart. If the error still occurs, power off the GeneTitan™ MC Instrument, then restart.

Observation	Possible cause	Recommended action
Plate trapped in the GeneTitan™ MC Instrument	• Plate (or plate with lid) not properly loaded in drawer. • Notched edge of lid and plate not aligned. • Gripper failed to retrieve plate. • System requires adjustment.	1. Restart the GeneTitan™ MC Instrument by unplugging and reconnecting power cord. 2. Run the **Unload Plates** setup option. 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 the hybridization station: finish hybridization off line. • Plate in the scanner: rescan using **Scan Only** function. • Plate in fluidics: use **Wash/Scan Resume** to resume the fluidics process. **IMPORTANT!** Do not manually, or through the GCC transfer utility, move any data associated with the current plate that is being processed/scanned.

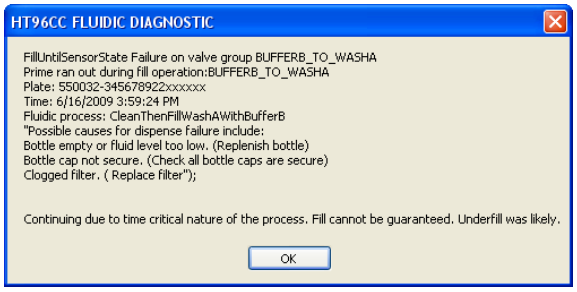
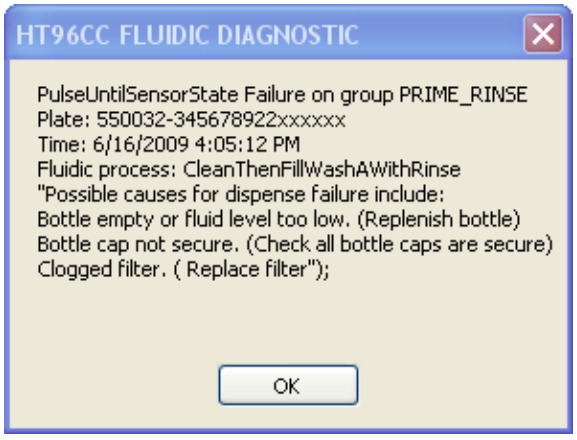
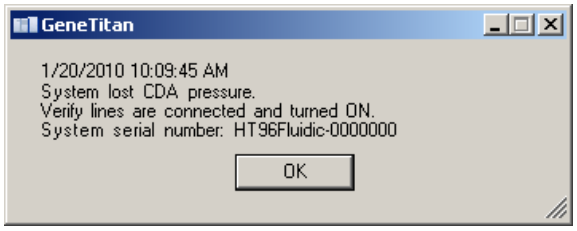
Observation	Possible cause	Recommended action
Hybridization aborted	• System-initiated abort: power loss. • User-initiated abort: – User error – Other	If the array plate and hybridization tray are still clamped, contact your local field service engineer with information on the workstation model.
		If the plate stack is moved to drawer 1: 1. Remove the plate stack and finish hybridization offline. 2. Return the hybridized array plate stack to the GeneTitan™ MC Instrument and finish processing using the **Wash/Scan** process.
Fluidics aborted	• System-initiated abort: power loss. • User-initiated abort: incorrect protocol selected.	Follow the recommendations and instructions under "Wash-Scan Resume" on page 177.

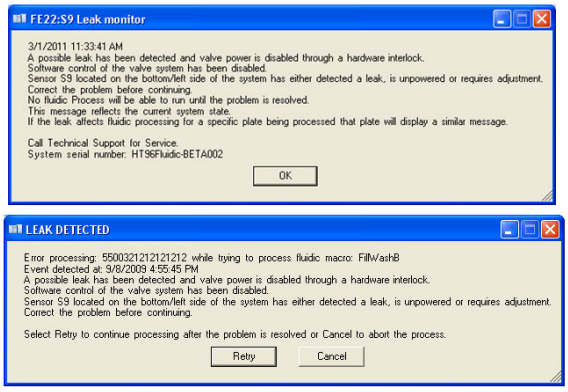
Observation	Possible cause	Recommended action
Homing recovery of gripped item 	Indicates that an item is in the gripper, and normal startup of the GeneTitan™ MC Instrument is not possible. The item must be removed from the instrument before you can start processing array plates.	Recommendation: click **Yes**. If you click **No**, nothing occurs. Homing will not complete and you will not be able to use the system. The item that is 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 Hyb Tray and Array Plate Drawer(n)L/R_Hta_DOWN where n is the drawer number and L or R to indicate the left or right side. The _Hta_ (second term) indicates that 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
Drawer not retracted error 	The drawer that is listed in the message is not fully closed.	Manually push the drawer back into the instrument until it is fully closed. There are 2 stop positions with audible clicks. Push until you hear the second click and the drawer is fully seated.

Observation	Possible cause	Recommended action
Array registration error message 	The protocol file for the array plate barcode could not be found.	Check that the array plate barcode has been entered correctly.
		Ensure that the library files required for the type of array plate you are using have been installed, and are installed in the correct directory.
		Restart the GeneTitan™ Instrument control software after library files have been installed.
Insufficient disk space notice  This message appears when you first initialize the software and instrument, or when you select arrays for imaging.	There is not sufficient memory on the computer hard drive to save the data from an array plate.	Free up sufficient disk space before starting imaging with the GeneTitan™ MC Instrument.

GeneTitan™ Instrument fluidic diagnostic messages

Observation	Possible cause	Recommended action
Failed prime  If this message is displayed during a water wash step, array processing has been compromised. If this message is displayed during cleanup, array processing is okay, but cleanup will not be complete.	The fluid level is either too low or the bottle is empty.	Always ensure that the GeneTitan™ bottles containing Axiom™ Wash Buffer A and Axiom™ Water are above the 50% mark when setting up the system to process an array plate.
		We recommend that all 600 mL of the Axiom™ Wash Buffer B from the Axiom™ PharmacoFocus™ Assay Mini 96 Reagent Kit be emptied into the GeneTitan™ Wash B bottle when setting up the system to process a plate. Using all 600 mL of the Axiom™ Wash Buffer B ensures that the bottle is filled to more than the requisite 35% of Wash B bottle volume.

Observation	Possible cause	Recommended action
Fluidics diagnostic dispense error  BUFFER*x* = Buffer bottle A, B, or Rinse. WASH*x* = Wash A or B reservoir in the fluidics station.  	Reagent bottle is empty or too low.	Replenish fluid level in the Rinse or Wash Bottle B to the 1-L mark. Do not overfill. **IMPORTANT!** 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 when a plate is running is to ensure that bottle caps are secure.
		Replenish fluid level in Wash Bottle A to 2 L.
	GeneTitan™ reagent bottle cap is loose.	Fasten the bottle cap.
	The GeneTitan™ reagent bottle filter is clogged.	Replace the filter. See “Bottle filter replacement” on page 207.
Loss in CDA pressure  	The instrument experienced a loss in Clean Dry Air (CDA) pressure.	Ensure that all lines are connected and turned on.
		Ensure that the facility CDA or the portable CDA compressor is in working condition. See the *GeneTitan™ Multi-Channel Instrument Site Preparation Guide* (Pub. No. 08-0305) for the portable compressor model that has been verified with the GeneTitan™ MC Instrument.
		Contact your local field application specialist and notify the engineer about the error message.

Observation	Possible cause	Recommended action
Leak detected Leak checks are performed at application startup and any time a fluidic process, such as priming, filling, draining, is performed. Leak detection is a hard-wired sensor that shuts off fluid flow without software control. Leaks are normally confined to the drip pan found inside the system. 	• System malfunction. • The GCC application being manually closed using Windows™ Task Manager during a fill operation resulting in an application exit without stopping flow.	Contact Thermo Fisher Scientific support. The system cannot be used for fluidic processing until the problem is resolved.
Filter change required error message The software displays warning messages for the filter in each reagent bottle when it detects a problem or shows a trend of increased fill times during fluid fill operations. When an error is detected, a message box is displayed along with the information on the specific operation (dispense-related check or fill-related check).	One or more reagent bottle filters are clogged or worn out.	Change all 3 reagent bottle filters, even if only 1 is reported as problematic. See “Bottle filter replacement” on page 207.

GeneTitan™ Multi-Channel Instrument care

Overview

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

- The GeneTitan™ Multi-Channel (MC) Instrument must be positioned on a sturdy level bench away from extremes in temperature and away from moving air.
- Always run a Shutdown protocol when the instrument is off or unused overnight or longer to prevent salt crystals from forming in the fluidics system.
- Always use deionized (DI) water to prevent contamination of the lines. Swap out old buffers with freshly prepared buffer at each system startup.

IMPORTANT! Before performing maintenance power off the instrument to avoid injury if an electrical malfunction occurs.

Maintenance

The GeneTitan™ family of instruments requires 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 must be performed regularly to ensure that the imaging device remains in working order.

Monthly

Wipe down the outer surface of the imaging device with a dry cloth.

Every 6 months

1. Replace the cooling fan air filters at the rear of the instrument.
2. Replace the Micropore™ filters in the Wash A, Wash B, and Rinse bottles. If you run 4-8 plates/week, then replace the Micropore™ filters more frequently.

Outer enclosure fan filters

Cleaning schedule

The GeneTitan™ fan filter cartridge must be cleaned at least every 90 days of service. Note that in some service locations, the presence of excessive dust or particulate matter can require cleaning the cartridge more often than 90 days.

A plugged filter cartridge can cause excessive temperatures in the machine that can cause unwanted evaporation of GeneTitan™ reagents.

Figure 43 GeneTitan™ fan filter cartridge.

Clean the GeneTitan™ MC Instrument fan filter

Contact your field service engineer for GeneTitan™ fan filter ordering information when new filters are required.

Number of filters that are required per GeneTitan™ MC Instrument: 3

1. Slide the filter cartridge from the fan filter cartridge at the rear of the GeneTitan™ MC Instrument.
2. Submerse the filter in clean DI water. Rinse, then agitate gently to dislodge material.
3. Remove from water and dry with clean compressed air or towels.
4. When the filter cartridge is dry to the touch, reinstall the cartridge in the GeneTitan™ MC Instrument.

Bottle filter replacement

The bottles that are used in GeneTitan™ MC Instrument contain a filter to remove particulates that can exist in the buffers and DI water. The filters in the 3 GeneTitan™ fluidics bottles (Wash A, Wash B, and Rinse) must be replaced when the filters are clogged.

When the instrument detects an increase in the amount of time that is required to perform the fill operations, a **Filter Change Required** message window opens. The message window provides information on fluid dispense errors that were detected for any of the bottles during a dispense operation. All 3 filters must be changed when a warning is displayed for any of the 3 filters.

Note: The reagent bottles are depressurized when this warning message is displayed. It is safe to change the filters in all 3 fluidic bottles when this message is displayed.

After changing the filters in all 3 bottles using the procedure that is described in this section, press the **Yes** button to continue. If you select to ignore the error message, press the **No** button. This warning message is displayed each time GeneChip™ Command Console™ instrument control software is launched. You can also experience data quality problems when particulate matter is not trapped by the filters because they are clogged.

We recommend having 3 spare filters on hand in the event the filters must be replaced.

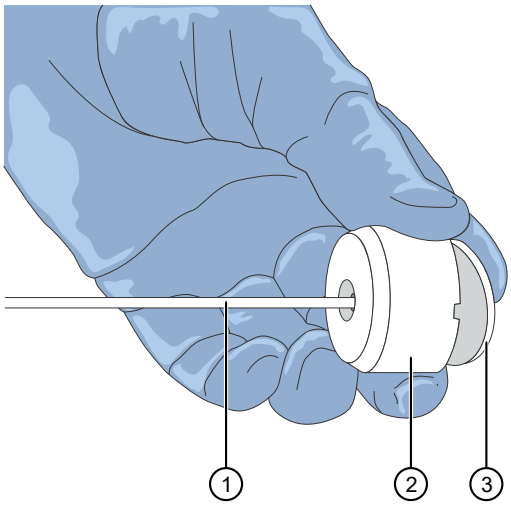


Figure 44 Components of the reagent buffer supply line and filter.

1. Buffer supply line
2. Filter holder
3. Filter

Remove and inspect the reagent bottle filters

1. Loosen, then remove the cap on the bottle.
2. Carefully remove the filter from the end of the filter body (see Figure 44).

3. Visually inspect the filter. If one of the filters appears to have a concentration of dirt or contaminate in it, discard it. Replace the filter in all 3 reagent bottles with a new one.
4. Replace the cap on the reagent bottle when finished.

Replace fluidics bottle filter

GeneTitan™ Fluidics Bottle Filter part details:

Thermo Fisher Scientific Cat. No. 01-0671

1. Loosen the reagent bottle cap, then remove the draw tube.
2. Carefully remove the filter from the end of the filter body.
3. Insert a new filter into the end of the filter holder.
4. Replace the cap on the reagent bottle, then tighten it.
5. Repeat these steps for each bottle.

IMPORTANT! Replace 1 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.

Xenon lamp replacement in the GeneTitan™ MC Instrument

This section applies to the GeneTitan™ MC Instrument.

After the normal life expectancy of the lamp has expired, the software application alerts you to the requirement to replace the lamp. The lamp replacement procedure is simple but good health and safety precautions must be followed.

CAUTION! 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 Device Status** pane displays lamp life and imaging device status notices for the GeneTitan™ MC Instrument.

In normal operation, the pane displays the hours of life that is left in the lamp.

Imaging Device Status	
Barcode	
Estimated Time Remaining	
Lamp Life Remaining	163 hours

A red or yellow notice is displayed when the lamp life is getting short.

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

A red notice is also displayed when the imaging device is offline.

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

Note: The 300-watt xenon lamp in the GeneTitan™ MC Instrument is warranted for 500 hours. The instructions to remove and replace the lamp are found in “Remove the xenon lamp” on page 210, and “Replace the xenon lamp” on page 211. After changing the lamp, you must manually reset the lamp life clock.

Remove the xenon lamp

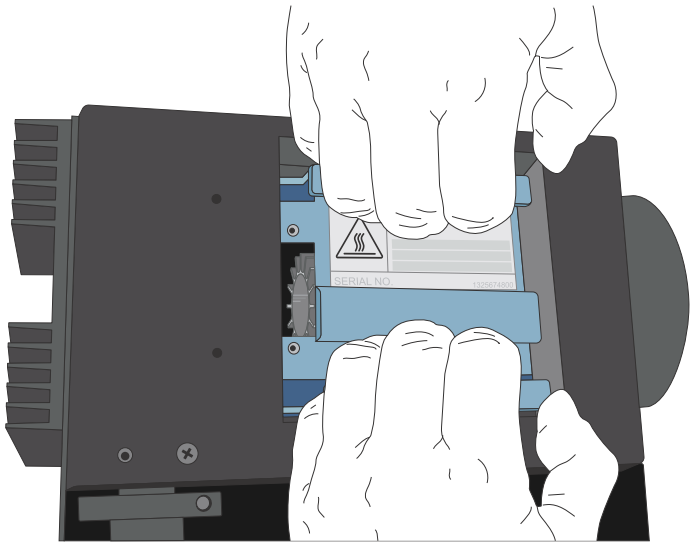
WARNING! Power off the lamp using the switch in the rear of the unit and then disconnect the power cord. Allow to cool before attempting to replace the lamp.

1. Unscrew the 4 retaining bolts with your fingers.

① Remove these 4 bolts.

2. Remove, then set aside the warning cover to reveal the xenon lamp that is contained inside.

3. Place a hand on each side of the blue plastic flange, then lift out the lamp in a vertical motion. Both hands must be used to remove the lamp. Apply equal pressure on each side of the lamp and gently lift.

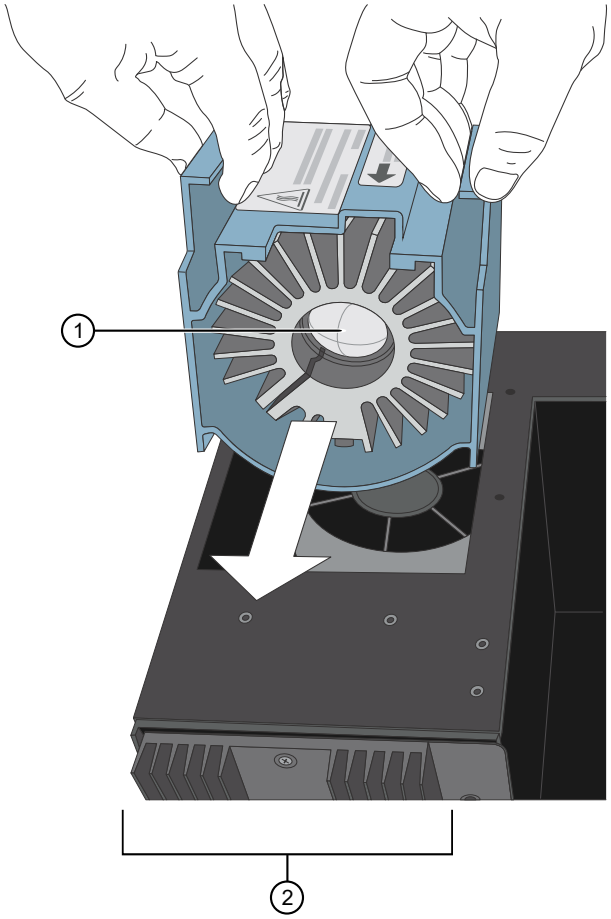


Replace the xenon lamp

A new Cermax™ Xenon Arc Lamp (Cat. No. 01-0740) is required for this procedure.

IMPORTANT! 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 rear heat sink on the unit, and then vertically lower the lamp to install.



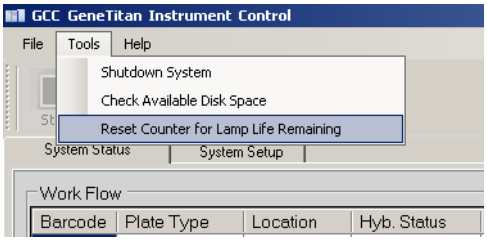
① Xenon bulb faces away from the fan and towards the heat sink.
② Heat sink on the Lambda LS unit.

2. Replace the warning cover, then hand tighten the bolts.

Reset the lamp life counter

Using the **GCC GeneTitan Instrument Control** module accessed from the **Launcher** window, you must alert the software that the lamp has been replaced so that the hours of the lamp counter are reset. This menu option is only available when the system is not processing any plates.

1. Select **Tools** ▸ **Reset Counter for Lamp Life Remaining**.



2. Click **Yes** in the message window to reset the counter.

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, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

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. 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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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.

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! **HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! **4L Reagent and Waste Bottle Safety.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also 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:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.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

Document	Publication number	Description
Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Site Preparation Guide	MAN0019173	Provides guidance on reagents, instruments, and supplies required to run the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow.
Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow Quick Reference	MAN0019174	An abbreviated reference for the target preparation step of the Axiom™ PharmacoFocus™ Assay Mini 96-Array Format Manual Workflow. This document is for experienced users.
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 benchtop instrument. This document detailing the use, care, and maintenance for the GeneTitan™ MC.
GeneTitan™ Multi-Channel Instrument Site Preparation Guide	08-0305	Provides guidance on creating and maintaining the proper environment required for the GeneTitan™ MC Instrument.
Software and analysis		
GeneChip™ Command Console™ User Guide	702569	This user guide provides instructions on using Applied Biosystems™ GeneChip™ Command Console™ software (GCC) used to control GeneChip™ instrument systems. GeneChip™ Command Console™ software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip™ arrays.

(continued)

Document	Publication number	Description
Axiom™ Analysis Suite User Guide	703307	Axiom™ Analysis Suite advances genotyping data analysis with a single-source software package to enable complete genotyping analysis of all Axiom™ arrays. This document provides instructions on using the software to automate the Best Practices Workflow to deliver accurate results in a single step for export in PLINK, VCF, or TXT formats.
Axiom™ Genotyping Solution Data Analysis User Guide	MAN0018363	This guide provides information and instructions for analyzing Axiom™ genotyping array data. It includes the use of Axiom™ Analysis Suite, Applied Biosystems™ Array Power Tools and SNPolisher R package to perform quality control analysis (QC) for samples and plates, SNP filtering before downstream analysis, and advanced genotyping methods.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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