

Axiom™ 2.0 Assay Mini 96-Array Format

USER GUIDE

Manual Protocol

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The Axiom™ Mini 96 Genotyping Solution

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About the Axiom™ Mini 96 Genotyping Solution

The Axiom™ Mini 96 Genotyping Solution is a genotyping technology platform that includes a manual assay and new array configuration. This solution has applications in applied agriculture research and human disease research. The Axiom Mini 96 solution offers the capability to genotype approximately 50,000 variants from diploid species or 32,000 variants from polyploid species in combination with a processing throughput of greater than 3,000 samples per week. The Axiom™ Mini 96-array layout retains full compatibility with the currently existing Axiom instrumentation platform and downstream data analysis. The Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol uses the Axiom™ 2.0 Assay Mini 96 Reagent Kit.

For agriculture applications, the Axiom Mini 96 Genotyping Solution is capable of genotyping samples using DNA extracted from leaves and seeds. The use of DNA microarrays for easy, cost-effective genotyping of single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms (indels) plays an important role in genotype-trait association studies and marker-assisted selection in both plant and animal breeding programs.

For human disease research applications, Thermo Fisher Scientific conducted an empirical screen of genomic content from dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). The screen included markers from HapMap and the 1000 Genomes Project as well as other sources, using HapMap phase 3 samples and/ or the original 270 HapMap samples. All of this information has gone into creating a proprietary Thermo Fisher Scientific database of validated markers that can be interrogated using the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol.

The Axiom Mini 96 Solution is ideal for screening of large numbers of samples in molecular breeding programs where turn-around time, accuracy and ease-of-use are all important. The Axiom Mini 96 Genotyping Solution enables manual preparation of DNA target preparation, DNA amplification and enzymatic fragmentation of post-amplification products for instances when immediate processing of samples is required. Following target preparation, arrays are processed using GeneTitan™ Multi-Channel (MC) Instrument. The Axiom Mini 96 solution also offers traceability of samples through the use of barcoded consumables.

The Axiom 2.0 Assay interrogates biallelic SNPs and simple indels in a single, fully automated assay protocol. Starting with genomic DNA, the samples are processed by performing a manual target preparation protocol followed by automated processing of the array plates in the GeneTitan MC Instrument.

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

Cel files generated by the GeneTitan MC Instrument are processed using the Axiom™ Genotyping Algorithm version 1 (Axiom GT1) available through Applied Biosystems Microarray Power Tools or Axiom™ Analysis Suite v2.0 or later.

Assay features

The *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol User Guide* provides instructions for manual target preparation and processing of Axiom Mini 96-array format plates on the GTMC.

Target preparation for the Axiom 2.0 Mini 96 manual protocol is done in 96-array format. The hyb ready target is then 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 hyb ready samples after thermal denaturation from a 96-array format PCR plate to a 384-array format Hyb Tray.

[Figure 1](#) illustrates the new Mini 96-array format plate layout. The arrays are glued only to the quadrant 1 positions of a 384 plate. Quadrant 1 refers to the odd column well positions in rows A/C/E/G/I/K/M/O.



① Position A1

Figure 1 Axiom Mini 96-Array Format Plate (bottom view)

This user guide covers the use of new GeneTitan™ MC consumables and Applied Biosystems™ GeneChip™ Command Console™ v 4.3 or higher (AGCC) for the preparation of the Axiom 2.0 stain reagents. Each tray has a unique part number and barcode that offers traceability. The new trays have the following labels and barcodes:

Stain 1 Tray Cat. No. 501279		384 Layout GeneTitan® Stain Tray 5012791234567070914587		FOR RESEARCH USE ONLY
Stain 2 Tray Cat. No. 501394		384 Layout Axiom® Stain2 Tray 5013941234567070914598		FOR RESEARCH USE ONLY
Ligation Tray Cat. No. 501398		384 Layout Axiom® Ligation Tray 5013981234567070914606		FOR RESEARCH USE ONLY
Stabilizing Tray Cat. No. 501396		384 Layout Axiom® Stab. Tray 5013961234567070914599		FOR RESEARCH USE ONLY

The unique barcodes, in conjunction with the Applied Biosystems GeneChip Command Console software, prevents users from making errors when placing the trays in the GeneTitan MC Instrument during Stage 3 of the array processing with the GeneTitan MC Instrument ("Stage 3: Ligate, Wash, Stain and Scan" on page 124).

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 Axiom assay on the GeneTitan MC Instrument.

After the trays have been prepared, the user must ensure 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 Instrument will not proceed with the processing of the trays. Refer to "Load trays onto the GeneTitan™ MC Instrument" on page 126 for detailed instruction.

Applied Biosystems GeneChip Command Console v 4.3 or higher also offers the facility for queuing a second plate for scanning before the scan of the first plate is complete. The software automatically moves the second plate into the scanner when the first plate has completed scanning. Refer to "Queuing a second plate for scanning" on page 120 for instructions.

Overview of the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol

Running the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol requires the following sets of steps:

1. Genomic DNA Prep: Resulting in samples that meet requirements in [Chapter 2, "Genomic DNA preparation and requirements" on page 13](#)
2. Target Preparation of the samples:
 - See [Chapter 4, "Axiom™ 2.0 Assay for Mini 96-Array manual target preparation" on page 44](#)
3. Array Processing, done with:
 - GeneTitan MC Instrument
 - GeneTitan Instrument Control software
 - Applied Biosystems GeneChip Command Console v 4.3 or higher softwareSee [Chapter 5, "Array processing with the GeneTitan™ MC Instrument" on page 96](#).

A list of the required equipment and supplies for running the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol can be found in the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435.

Running multiple plate workflows

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

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

These limitations require careful timing. For detailed information, please refer to [Chapter 6, "Processing three Axiom™ array plates per week" on page 136](#).



Genomic DNA preparation and requirements

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

For this protocol, you will use the Axiom™ 2.0 Assay Mini 96 Reagent Kit, Cat. No. 903013 (sufficient for two Mini 96-array format plates). These kits contain a tube labeled Genomic DNA (Part No. 900421). The use of at least one positive control DNA sample on each plate is recommended. For human samples, the Reference Genomic DNA provided in Module 1 can serve as the control. For plant or animal samples, use a genomic DNA sample that meets the recommended nucleic acid purity and concentration specifications and is from the same species that is represented on the array. Ideally this control sample has also demonstrated passing genotyping performance when used in the Axiom Genotyping Solution. If no DNA control for your specific sample type is available, then the Axiom Reference Genomic DNA can serve as positive control for the target preparation portion of the assay. The size and purity of sample gDNA can be compared with those of the control DNA to assess sample quality. The control DNA should also be used routinely as an experimental positive control and for troubleshooting purposes.

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

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 above requirements.

- Blood
- Saliva
- Cell line
- WGA pre-amplified DNA: Genomic DNA amplified with the REPLI-g® Kit (a whole genome amplification kit; QIAGEN, Cat. No. 150025) has been tested successfully with the Axiom 2.0 Genome-Wide Human Reagent Kit Assay. The REPLI-g Kit was used to amplify 20 ng genomic DNA, and the resulting yields were quantitated by a PicoGreen® assay. The amplified products (either 100 or 200 ng amplified DNA as required according to the Axiom array type) were used (without purification) as the input DNA sample in the subsequent Axiom™ 2.0 assay steps. The stability of this amplified product to storage and repeated cycles of freeze/thaw have not been evaluated by Thermo Fisher Scientific.

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

The following sources of animal gDNA have been successfully tested in the laboratories at Thermo Fisher Scientific for DNA that meets the requirements below:

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

The following sources of plant gDNA have been successfully tested in the laboratories at Thermo Fisher Scientific for DNA that meets the requirements below:

- Seeds
- Leaves

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

General requirements

- Starting DNA must be double-stranded for the purpose of accurate concentration determination.
- DNA must be of high purity.
DNA should be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (i.e., EDTA). The gDNA extraction/ purification method should render DNA that is generally salt-free because high concentrations of particular salts can also inhibit enzyme reactions. DNA purity is indicated by OD₂₆₀/OD₂₈₀ and OD₂₆₀/OD₂₃₀ ratios. The OD₂₆₀/OD₂₈₀ ratio should be between 1.8 and 2.0 and the OD₂₆₀/OD₂₃₀ ratio should be greater than 1.5. We recommend that DNA samples that do not meet these criteria be cleaned up as described under ["Genomic DNA cleanup" on page 17](#).
- DNA must not be degraded.
The approximate average size of gDNA may be assessed on a 1% agarose gel using an appropriate size standard control. Approximately 90% of the DNA must be greater than 10 Kb in size. Control DNA can be run on the same gel for side-by-side comparison.

Special requirements

Pre-amplification area

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

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

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

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

Equipment and reagents recommended

Table 1 E-Gel® and reagents required

Item	Supplier	Cat. No.
Mother E-Base™ Device	Thermo Fisher Scientific	EB-M03
Daughter E-Base™ Device (optional for running multiple gels in parallel)		EB-D03
E-Gel® 48 1% agarose gels		G8008-01
RediLoad™		750026
E-Gel® 96 High Range DNA Marker		12352-019

Guidelines for preparing the genomic DNA plate for gel analysis

- Loading a DNA mass of 10 ng to 20 ng per well is recommended. If lower amounts are loaded, omission of the loading dye is recommended in order to improve visualization. Loading ≥ 25 ng gDNA per well can improve the image.
- Add 3 μL of 0.1X of *RediLoad* (*RediLoad* 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 H_2O (for example, if the volume of genomic DNA is 5 μL , add 3 μL of *RediLoad*, and bring to 20 μL total by adding 12 μL of H_2O).
- Seal, vortex and spin.

To run a 48 lane 1% agarose E-Gel:

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

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

[Figure 2](#) shows gel images of intact gDNA (that is suitable for use in the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol) and degraded gDNA samples. Customers whose gDNA is degraded (similar to the image in [Figure 2](#)) should perform a test experiment to investigate the performance of their samples in the Axiom genotyping assay prior to beginning any large scale genotyping projects.

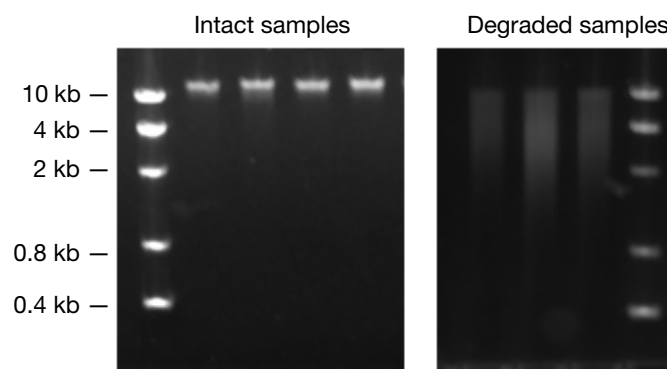


Figure 2 Gel images showing intact gDNA and degraded gDNA

Genomic DNA extraction/purification methods

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

Genomic DNA cleanup

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

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

(See the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435 for reagents, equipment, labware and consumables for Axiom 2.0 Assay Mini 96-Array Format Manual Protocol).

Genomic DNA preparation

This step needs to be done before proceeding with the DNA amplification stage for manual target preparation.

The genomic DNA (gDNA) you will process using the Axiom 2.0 Assay should meet the general requirements listed earlier in this chapter. The amount of gDNA depends on which Axiom array will be used in the downstream protocol. The table below shows the sample input requirements for Axiom 2.0 Assay Mini 96-Array Format Manual Protocol.

Table 2 Input requirements for Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

Sample type	Volume per well (μL)	Input mass per well (ng)	gDNA concentration (ng/μL)
Human	8.7	100	11.5
Diploid plants and animals	8.7	150	17.2
Polyploid plants and animals	8.7	200	23

To prepare gDNA:

"1: Thaw samples and control"

"2: Quantitate and dilute gDNA"

"3: Aliquot the diluted samples and the control"

"4: Freeze or proceed"

"5: Create a Batch Registration file"

Duration

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

Equipment, consumables and reagents required

Equipment and consumables

The equipment and consumables listed in [Table 3](#) are required for this stage.

Table 3 Equipment and consumables required for genomic DNA preparation

Quantity	Item
As required	Adhesive seals for plates
1	Ice bucket, filled with ice
1 each	Pipettes: • Single-channel P10 or P20 • Optional: multi-channel P10 or P20
As required	Pipette tips
1	Plate, deep well: Eppendorf 96 Deep-well Plate, 2000 μL
1	Plate centrifuge
1	Plate spectrophotometer (required only if no OD measurements available for samples)
1	Vortexer

Reagents

The reagents listed in Table 4 are required for this stage.

Table 4 Reagents required for genomic DNA preparation

Reagent	Supplier	Cat. No.
From the Axiom™ 2.0 Assay Mini 96 Reagent Kit		903013
<ul style="list-style-type: none"> Axiom Reference Genomic DNA (use as a positive control if genotyping Human samples) Located in Module 1, -20°C 	Thermo Fisher Scientific	Part No. 900421
User-supplied		
<ul style="list-style-type: none"> Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) 	Thermo Fisher Scientific	75793
<ul style="list-style-type: none"> Positive control gDNA (if genotyping non-Human samples) 		

1: Thaw samples and control

Thaw the components listed below to room temperature:

- gDNA samples
- gDNA positive control sample. For human studies, use Axiom Reference Genomic DNA (from the Axiom 2.0 Assay Mini 96 Reagent Kit)

To thaw, either:

- Place items on benchtop for one hour
- Thaw in a water bath:
 - Fill a small plastic dish with Millipore water. Do not immerse the sample plate or tube when placing it in the bath.
 - Thaw the sealed sample plate and Reference sample for a half-hour.
 - Remove the sample plate and/or sample tube from the water bath and wipe-dry using lab wipes. Ensure the outside is completely dry before opening the sample plate or tube to minimize any contamination, which can lead to reaction failure.

2: Quantitate and dilute gDNA

To quantitate and dilute the gDNA:

- Gently vortex (50% maximum) and spin the gDNA and control DNA.
- Recommendation:* quantitate each sample (e.g., using the Quant-iT™ PicoGreen® dsDNA Kit).
- Using reduced EDTA TE buffer, dilute each sample to a concentration of:
 - 11.5 ng/μL for Human DNA samples
 - 17.2 ng/μL for diploid plant and animal DNA samples
 - 23 ng/μL for polyploid plant and animal DNA samples
- Seal, vortex and spin.

3: Aliquot the diluted samples and the control

Next, the samples and control are placed in an Eppendorf 96 Deep-well Plate, 2000 μ L for target preparation.

Aliquot diluted samples and reference genomic DNA to the deep-well plate as follows:

1. 8.7 μ L of each diluted gDNA sample (this should be the equivalent of 100-200 ng of gDNA, as required by the sample type).
2. 8.7 μ L of the control DNA.

We recommend including at least one positive control on each plate.

3. Seal and spin.

4: Freeze or proceed

At this point you can:

- Store the sample plate at -20°C , or
- Proceed to DNA amplification (see [Chapter 4, "Axiom™ 2.0 Assay for Mini 96-Array manual target preparation" on page 44](#)).

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

5: Create a Batch Registration file

IMPORTANT! It is very important to create and upload a GeneTitan™ Array Plate Registration file with your sample information prior to loading the array plate and hybridization tray into the GeneTitan™ MC Instrument. We recommend that you create (but not upload) this file at the same time you prepare your plate of genomic DNA. When your samples are ready for hybridization, you will scan the array plate barcode and upload the file to Applied Biosystems GeneChip Command Console v4.3 or higher.

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

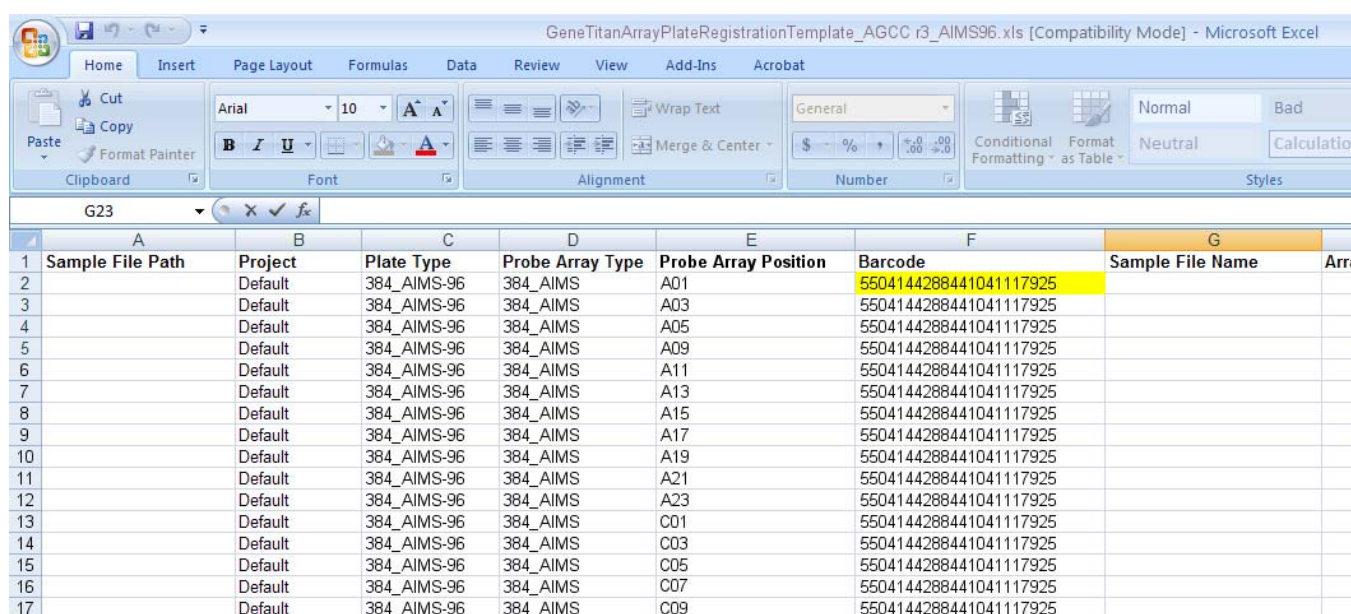
- Data file generation during imaging.
- Tracking the experimental results for each sample loaded onto an array plate.

See also [Figure 3](#) for a screen shot showing an example of a batch registration file.

To create a Batch Registration file:

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

The array plate barcode is scanned when you are ready to load the array plate and samples onto the GeneTitan MC Instrument for processing. Please refer to [Chapter 5, "Stage 1: Create and upload Batch Registration file" on page 106](#) for information on scanning the barcodes.



	A	B	C	D	E	F	G	H
	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array Position	Barcode	Sample File Name	Arr
1		Default	384_AIMS-96	384_AIMS	A01	5504144288441041117925		
2		Default	384_AIMS-96	384_AIMS	A03	5504144288441041117925		
3		Default	384_AIMS-96	384_AIMS	A05	5504144288441041117925		
4		Default	384_AIMS-96	384_AIMS	A09	5504144288441041117925		
5		Default	384_AIMS-96	384_AIMS	A11	5504144288441041117925		
6		Default	384_AIMS-96	384_AIMS	A13	5504144288441041117925		
7		Default	384_AIMS-96	384_AIMS	A15	5504144288441041117925		
8		Default	384_AIMS-96	384_AIMS	A17	5504144288441041117925		
9		Default	384_AIMS-96	384_AIMS	A19	5504144288441041117925		
10		Default	384_AIMS-96	384_AIMS	A21	5504144288441041117925		
11		Default	384_AIMS-96	384_AIMS	A23	5504144288441041117925		
12		Default	384_AIMS-96	384_AIMS	C01	5504144288441041117925		
13		Default	384_AIMS-96	384_AIMS	C03	5504144288441041117925		
14		Default	384_AIMS-96	384_AIMS	C05	5504144288441041117925		
15		Default	384_AIMS-96	384_AIMS	C07	5504144288441041117925		
16		Default	384_AIMS-96	384_AIMS	C09	5504144288441041117925		
17		Default	384_AIMS-96	384_AIMS				

Figure 3 Example of a Batch Registration file

3

Preparation before you start

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Introduction

This manual assay format allows the user to run the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol twice using one Axiom™ 2.0 Assay Mini 96 Reagent Kit (Cat. No. 903013). 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 2.0 Assay Mini 96-Array Format Manual Protocol.

One key item this manual assay format 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 2.0 Assay Mini 96-Array Format Manual Protocol Manual Target Preparation is in the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435.

Axiom™ 2.0 Assay Mini 96 Reagent Kit, arrays, and GeneTitan™ consumables required

The table below lists the Axiom 2.0 Assay Mini 96 reagents, manual target prep consumables, and GeneTitan consumables required to process two Axiom Mini 96-array format plates.

Table 5 Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol: arrays, reagents, and GeneTitan consumables required

Cat. No.	Description	Quantity
Available upon design	Axiom™ Mini 96-Array Format Plate	2
902629	Axiom™ 384HT High Volume Consumables Kit (each kit is sufficient for processing 5 Mini 96-Array format plates)	1
903014	Axiom™ Mini 96 Consumables Kit for QC (each kit is sufficient for 10 runs)	1
902986	Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (each kit is sufficient for preparing four Mini 96 target preps)	1
903013	Axiom™ 2.0 Assay Mini 96 Reagent Kit ¹	1

¹ Please refer to [Table 15 on page 43](#) for a complete description of the kit components.

Requirements and recommendations

This section describes requirements and recommendations for facilities and equipment needed to perform the Axiom™ 2.0 Assay for Mini 96-Array Format Manual Protocol.

Room temperature

When referred to in the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol, room temperature is 18 to 25°C.

Special requirements

Amplification staging area

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

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

Fume hood

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

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

Control recommendations

A negative control is not required for this assay.

We recommend including one positive control with every set of samples processed. A positive control (Axiom Reference Genomic DNA 103) is included in the Axiom 2.0 Mini 96 Reagent Kit for human genotyping array designs.

Plate requirements and recommendations

The plates listed below on [Table 6](#) are required for performing manual target preparation. These plates are available in the Axiom 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (Cat. No. 902986) and Axiom Mini 96 Consumables Kit for QC (Cat. No. 903014), or purchased individually through the manufacturer or distributor. Refer to the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435, for more information.

Table 6 Sample plates required for Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

✓	Plate description	Manufacturer/ distributor	Cat. No.
<input type="checkbox"/>	Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate	Bio-Rad	HSS-9641
<input type="checkbox"/>	Eppendorf 96 Deep-well Plate, 2000 µL	Eppendorf	951033481
<input type="checkbox"/>	Bio-Rad Hard-Shell® Low-Profile 96-Well Skirted PCR Plate	Bio-Rad	HSP-9631
<input type="checkbox"/>	Greiner Bio-One 96 Well UV-Star® Microplate	E&K Scientific	25801

Thermal cycler recommendations

The following thermal cyclers are recommended:

- Bio-Rad PTC-200, or
- Bio-Rad DNA Engine Tetrad 2 #PTC-0240, or
- Applied Biosystems 9700 (with gold, silver, or aluminum block), or
- Applied Biosystems 2720

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

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

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

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



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

Thermal cycler consumables

Table 7 provides details into the consumables to be used with each thermal cycler.

Table 7 Thermal cycler consumables for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

Thermal cycler model	PCR plate type	Seal ¹
Bio-Rad PTC-200	<ul style="list-style-type: none"> Bio-Rad Hard-Shell® Low-Profile 96-Well Skirted PCR Plate (Bio-Rad Cat. No. HSP-9631) Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate (Bio-Rad Cat. No. HSS-9641)² 	MicroAmp™ Clear Adhesive Film from Thermo Fisher Scientific (Cat. No. 4306311)
Applied Biosystems 9700	<ul style="list-style-type: none"> Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate (Bio-Rad Cat. No. HSS-9641)² 	MicroAmp™ Clear Adhesive Film from Thermo Fisher (Cat. No. 4306311)
Applied Biosystems 2720	<ul style="list-style-type: none"> Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate (Bio-Rad Cat. No. HSS-9641)² 	MicroAmp™ Clear Adhesive Film from Thermo Fisher (Cat. No. 4306311)
Bio-Rad Tetrad® 2 PTC-0240	<ul style="list-style-type: none"> Bio-Rad Hard-Shell® Low-Profile 96-Well Skirted PCR Plate (Bio-Rad Cat. No. HSP-9631) Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plate (Bio-Rad Cat. No. HSS-9641)² 	MicroAmp™ Clear Adhesive Film from Thermo Fisher (Cat. No. 4306311)

¹ Microseal “B” film from Bio-Rad (Cat. No. MSB-1001) may be used in place of MicroAmp Clear Adhesive Film for the Bio-Rad and Applied Biosystems thermal cyclers.

² Included in the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (Cat. No. 902986)

Oven recommendations

The following ovens are recommended:

- BINDER ED 56 Drying and Heating Chamber. Refer to the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435, for ordering information.
- Applied Biosystems GeneChip Hyb Oven 645

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

 - If using an Applied Biosystems GeneChip Hyb Oven, set the rotation speed to 15 RPM to aid in even heat distribution.
 - For either Applied Biosystems GeneChip Hyb Oven, plates are placed in the bottom of the oven. To avoid interfering with the rotation apparatus, do not stack plates in the oven.

Plate centrifuge

One plate centrifuge is required for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol. Refer to the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435, for an appropriate plate centrifuge that can be used with the Axiom Genotyping Solution. When centrifuging and drying pellets as instructed under "[Stage 3A: Centrifuge precipitation plate and dry the DNA pellet](#)" on [page 64](#), the centrifuge must be able to spin down plates at:

- Rcf: 3200 xg (4000 RPM for the Eppendorf 5810R with the rotor configuration described in the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435).
- Temperature: 4°C and room temperature.

In addition, the bottom of the rotor buckets should be soft rubber to ensure that the deep-well plates do not crack. Do not use buckets where the plates sit directly on a metal or hard plastic bottom.

Plate shakers

We recommend using one of the following shakers listed in [Table 8](#).

Table 8 Plate shakers

Shaker	Supplier	Cat. No.
Thermo Scientific™ Compact Digital Microplate Shaker	Thermo Scientific	88880023
Jitterbug™	Boekel Scientific	Model 130 000

Equipment care and calibration

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

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

Procedures

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

Seal, vortex, and spin

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

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

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

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

- To remove droplets prior to sealing, overlay a sheet of Kimwipe across the top of the plate and gently pat down to dry.
- Lift the sheet off the plate and discard. Confirm the top of the plate is dry and seal the plate as usual.

• Vortex

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

– Plates:

- For deep well plates (such as Eppendorf 96 Deep-well Plate, 2000 µL plate), vortex at max speed for 5 seconds in each sector for a total of 5 sectors (Figure 4).
- For PCR plates (such as Bio-Rad Hard-Shell or semi-skirted plates, vortex at max speed for 2 seconds in each sector for a total of 5 sectors (Figure 4).

– Reagent Vials: 3 times at max speed for, 1 sec each time.

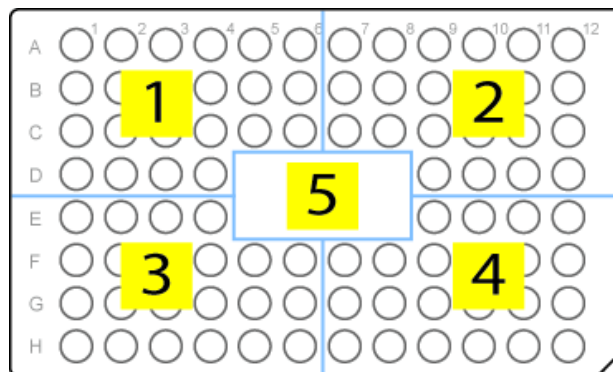


Figure 4 Vortexing plates

- **Spin:** When instructed to spin plates or reagent vials, follow these guidelines unless otherwise instructed (for example, when centrifuging and drying pellets, see [Step 2](#) in the section "Stage 3A: Centrifuge precipitation plate and dry the DNA pellet" on page 64).
 - **Plates:**
 - Spin at 1000 rpm for 30 sec at room temperature.
 - Do not spin for more than 1 min.
 - **Reagent Vials:** 3 sec

Sample quantitation

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

Please refer to [Chapter 2, "Genomic DNA preparation and requirements"](#) on page 13 for more information.

About the reagents and master mix preparation

Axiom 2.0 Assay Mini 96 Reagent Kit components

Each Axiom 2.0 Assay Mini 96 Reagent Kit (Cat. No. 903013) is sufficient to run two Mini 96-array format plates. Refer to [Table 15](#) for a full description of the kit.

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

IMPORTANT! The Axiom 2.0 Assay for Mini 96 is compatible only with reagents from an Axiom Reagent Kit. These reagents are not interchangeable with reagents from other Applied Biosystems reagent kits, such as SNP 6.0, DMET Plus, etc.

Reagents from other suppliers

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

Master mix preparation

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

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

When using reagents at the lab bench

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

Pipettes and pipetting

To efficiently process samples:

- Use a pipette of appropriate size for the volume of liquid being transferred (Table 8).

Table 9 Recommended pipette sizes

Pipette size	Recommended volume range
Single channel and multi-channel P20	2-20 μ L
Single channel and multi-channel P200	20-200 μ L
Single channel and multi-channel P1200	200-1000 μ L

- We recommend the use of Rainin pipettes and tips. Thermo Fisher Scientific has only verified the use of Rainin multi-channel pipettes in this assay. The use of other pipettes may impact the timing of the protocol and may adversely impact the assay. Pipette substitution may violate the terms of the Axiom 2.0 Assay Mini 96-Array Manual Protocol and array replacement policy.
- Always use pipettes that have been calibrated.
- It is essential that you be proficient with the use of single- and multi-channel pipettes. To familiarize yourself with the use of multi-channel pipettes, we strongly recommend practicing several times before processing actual samples. Use water and reagent reservoirs to get a feel for aspirating and dispensing solutions to multiple wells simultaneously.

Single-channel pipettes and serological pipettes

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

- Use single channel pipettes for volumes less than or equal to 2 mL. For volumes between 1 and 2 mL, add the reagent in two portions with a fresh tip for each portion.
- Use serological pipette for volumes >2 mL.

Multi-channel pipettes

Use 8 or 12-channel pipettes when working to add master mix or to transfer samples to plates and GeneTitan trays.

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

Matrix™ 25 mL
reagent reservoirs

The Axiom 2.0 Assay Mini 96-Array Format Manual Protocol requires the use of disposable reservoirs with a “trough within a trough” design. This special design maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent.



Figure 5 Dispense reagents from Matrix™ 25 mL reagent reservoirs

Note: During the precipitation step, the Precipitation Master Mix working volume exceeds the reservoir capacity. The reservoir must be filled twice.

Freeze-thaw
instructions

The Axiom 2.0 Assay Mini 96 Reagent Kit is sufficient to run two Mini 96-array format plates. Excess volume of the Axiom 2.0 Assay Mini 96 Reagent Kit after the first use may be stored in a freezer at -25°C to -15°C or a refrigerator at 2°C to 8°C to be used for a second experiment for up to 60 days after initial use (Table 10). Thermo Fisher Scientific recommends that reagents not exceed three freeze-thaw cycles. Please monitor the freeze-thaw cycles of the reagents by following the guidelines below.

Mark reagent pouches, tubes and bottles to track use

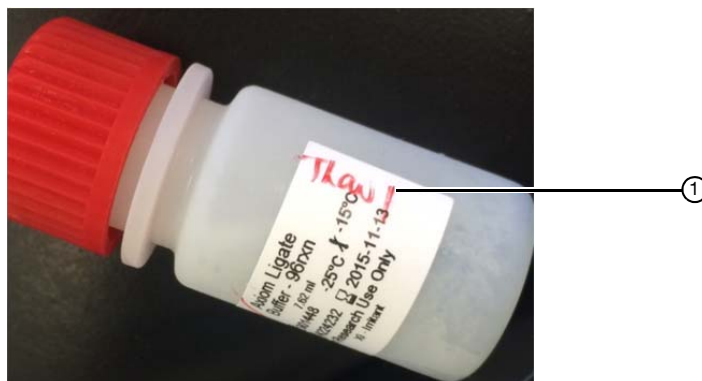
To keep track of usage, we recommend that users mark the pouch while the reagents are thawing.

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

	<u>Date Thawed</u>	<u>Notes</u>
1 <input checked="" type="checkbox"/>	2/9/15	Expt. 1, TY
2 <input type="checkbox"/>		

Figure 6 Example of labeling a reagent pouch

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



① Thaw tally mark

Figure 7 Example of a properly marked reagent bottle that has been thawed once.

- After the experiment, place all tubes and bottles back in the appropriate pouch and place at proper storage temperature. See [Table 10](#).

Table 10 Reagent storage temperature

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

Equipment, consumables, labware, and reagents required

Equipment required

Thermal cycler

Refer to "[Thermal cycler recommendations](#)" on page 24.

Oven

Refer to "[Oven recommendations](#)" on page 25.

Plate centrifuge

Refer to "[Plate centrifuge](#)" on page 26.

Plate shaker

Refer to "[Plate shakers](#)" on page 26.

Consumables required

Table 11 Consumables required for Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol




Labware	Supplier and Cat. No.	Image
<p>Eppendorf 96 Deep-well Plate, 2000 µL</p> <p>Eppendorf Deep-well Plate 96/ 2000 µL, wells clear, PCR clean, border blue</p>	<p>Part of the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (Kit Cat. No. 902986, Plate Part No. 203079).</p> <p>Alternate Supplier: Eppendorf, Cat. No. 951033481 Cat. No. 0030501349</p>	
<p>Matrix™ Reagent Reservoirs, 25 mL</p> <p>Thermo Scientific™ Matrix™ Reagent Reservoirs, 25 mL</p>	<p>Part of the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (Kit Cat. No. 902986, Part No. 203077).</p> <p>Alternate Supplier: Thermo Scientific Cat. No. 8093-11</p>	
<p>Bio-Rad Hard-Shell 96-well plate</p> <p>Bio-Rad Hard-Shell® Low-Profile 96-Well Skirted PCR Plates</p>	<p>Part of the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (Kit Cat. No. 902986, Plate Part No. 203015).</p> <p>Alternate Supplier: Bio-Rad, Cat. No. HSP-9631</p>	

Table 11 Consumables required for Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol (Continued)

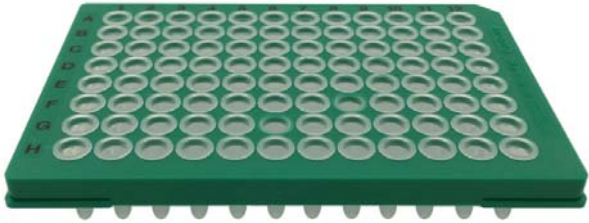
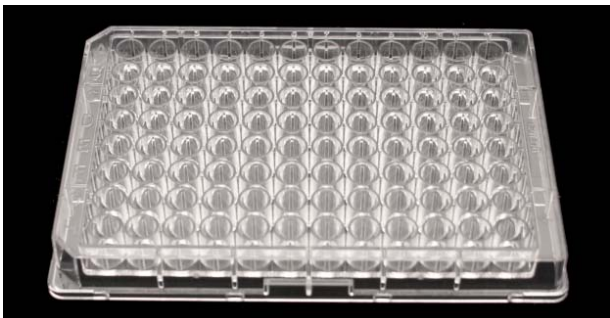




Labware	Supplier and Cat. No.	Image
96 Half-Skirt Plate Bio-Rad Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plates	Part of the Axiom™ 2.0 Assay Mini 96 Manual Target Preparation Consumables Kit (Kit Cat. No. 902986, Plate Part No. 203009). Alternate Supplier: Bio-Rad, Cat. No. HSS-9641	
96 Well UV Plate Greiner UV-Star® 96 well plates	Part of the Axiom™ Mini 96 Consumables Kit for QC (Kit Cat. No. 903014, Plate Part No. 202609). Alternate Suppliers: Fisher Scientific, E&K Scientific, Greiner Bio-One Cat. No. 655801	
50 mL conical-bottom centrifuge tubes, polypropylene	Various	
15 mL conical-bottom centrifuge tubes, polypropylene	Various	

Table 11 Consumables required for Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol (Continued)

Labware	Supplier and Cat. No.	Image
96-well block Cooling Chamber for 0.2 mL tubes, 96 holes (4 for 1.5 mL & 6 for 0.5 mL tubes), Dim.: 6 1/8"L x 3 1/8"W x 1" H	Diversified Biotech Cat. No. CHAM-1000	
96-well PCR racks	Various	

GeneTitan™ MC Instrument consumables

All consumables for the GeneTitan MC Instrument are provided by Thermo Fisher Scientific. The following table provides guidance on the consumables that are shipped with the array plate. Consult [Chapter 5, "Before using the GeneTitan™ Multi-Channel Instrument" on page 96](#) for information on aligning and loading trays into the GeneTitan MC Instrument.

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

Table 12 Axiom™ 384HT High Volume Consumables Kit, Cat. No. 902629¹

Qty	Item
10	384 Layout GeneTitan™ Stain Tray (Stain 1)
5	384 Layout Axiom™ Stain2 Tray
5	384 Layout Axiom™ Stab. Tray
5	384 Layout Axiom™ Ligation Tray
5	384 Layout GeneTitan™ Hyb Tray
5	384 Layout GeneTitan™ Scan Tray
25	384 Layout GeneTitan™ Scan and Stain Tray Cover

¹ Each Axiom™ 384HT High Volume Consumable Kit is sufficient to process 5 Axiom Mini 96-array format plates. These trays are required for processing Axiom Mini 96-array format plates on the GeneTitan™ Multi-Channel Instrument. Please use the new Axiom 384HT High Volume Consumable Kit when running the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol.

Table 13 Axiom™ Mini 96-Array Format Plate

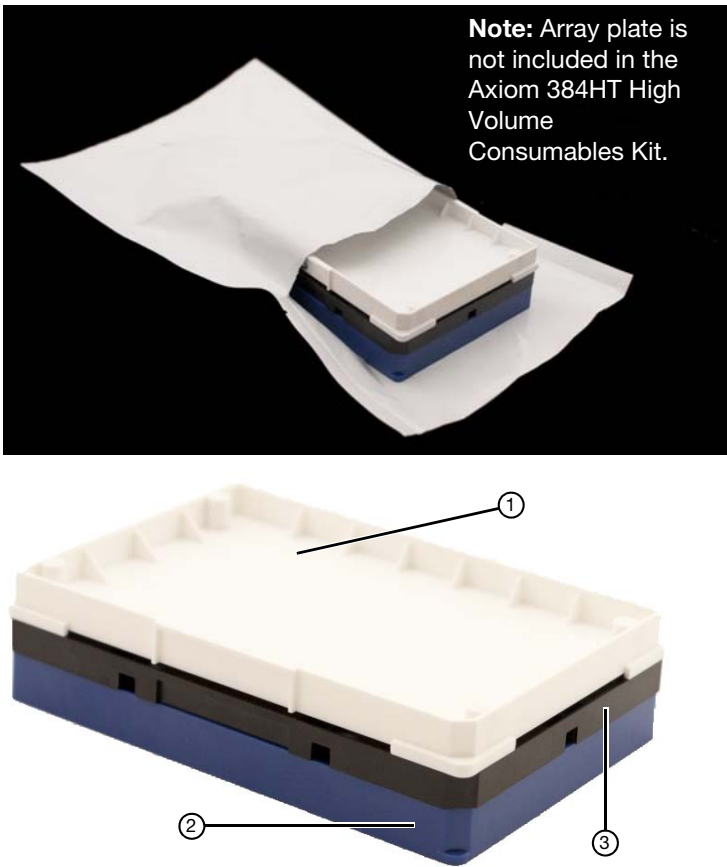
Item	Cat. No.	Labware image	Information
Axiom Mini 96-Array Format Plate, various designs	Varies, depending on array design. (All array plates have the Part No. 202091 etched on the plastic)	 <p>Note: Array plate is not included in the Axiom 384HT High Volume Consumables Kit.</p> <p>① Shipping cover (to be discarded) ② Array plate protective base ③ Array plate</p>	<p>The Axiom array plate shipping package includes the following:</p> <ul style="list-style-type: none"> • 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. • The array plate must be protected at all times from damage or exposure to dust. The array plate must be in the blue array plate protective base at all times. • The blue array plate protective base in the package must be used to protect the array plate from damage.

Table 14 Axiom™ GeneTitan™ MC Instrument consumables Axiom™ 384HT High Volume Consumables Kit, Cat. No. 902629

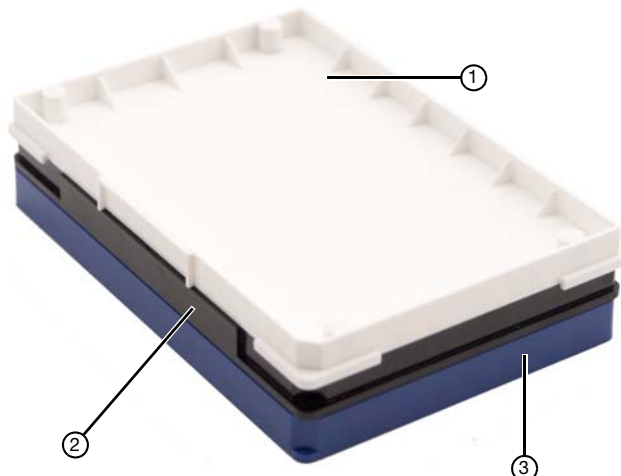
Item	Cat. No.	Labware image	Information
384HT GeneTitan Scan Tray and Cover¹	902279/501280	 <p>① Barcoded scan tray cover ② GeneTitan™ scan tray ③ Scan tray protective base</p>	<p>The Axiom scan tray shipping package includes the following:</p> <ul style="list-style-type: none"> • The GeneTitan scan tray includes a scan tray cover. The tray cover should be used to cover the scan tray before placing the tray in the GeneTitan MC Instrument. • The scan tray must be protected at all times from damage or exposure to dust. The scan tray must be in the blue plate cover at all times 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.

Table 14 Axiom™ GeneTitan™ MC Instrument consumables Axiom™ 384HT High Volume Consumables Kit, Cat. No. 902629 (Continued)



Item	Cat. No.	Labware image	Information
Blue Scan Tray Protective Base	Cat. No. 202096		<ul style="list-style-type: none"> 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 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.
Scan tray with cover¹	Scan Tray 501280 Cover 501315		<ul style="list-style-type: none"> The GeneTitan scan tray must be loaded with the scan tray cover into the GeneTitan MC Instrument. Do not load the scan tray with the protective base.

Table 14 Axiom™ GeneTitan™ MC Instrument consumables Axiom™ 384HT High Volume Consumables Kit, Cat. No. 902629 (Continued)





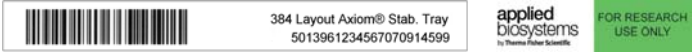


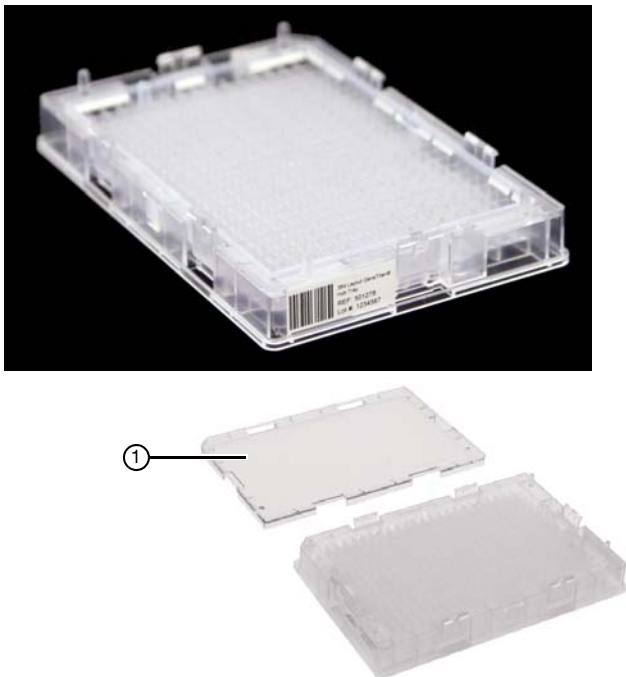
Item	Cat. No.	Labware image	Information
GeneTitan Stain Trays¹	501279 - Stain 1 501394 - Stain 2 501398 - Ligation 501396 - Stab		<ul style="list-style-type: none"> The GeneTitan stain trays are packaged in zip-top bags to keep them free of dust. Each GeneTitan stain tray is uniquely barcoded. <p>IMPORTANT: Each GeneTitan 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 may result in assay failure. When transferring the trays to the GeneTitan 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 Instrument will not proceed with the processing of the trays.</p>
		Stain 1 Tray 	
		Stain 2 Tray 	
		Ligation Tray 	
		Stabilizing Tray 	
GeneTitan Scan and Stain Tray cover¹	501315		<ul style="list-style-type: none"> The GeneTitan scan and stain tray covers are provided to prevent any evaporation of the stains in stain trays and the array holding buffer in the scan tray. The GeneTitan scan and stain tray covers are barcoded.

Table 14 Axiom™ GeneTitan™ MC Instrument consumables Axiom™ 384HT High Volume Consumables Kit, Cat. No. 902629 (Continued)

Item	Cat. No.	Labware image	Information
GeneTitan stain tray cover, shown on top of the stain tray¹	Cover 501315		<ul style="list-style-type: none"> The GeneTitan stain trays must be placed in the GeneTitan MC Instrument with the GeneTitan stain tray cover.
384 Hyb Tray	501278	 <p>① Discard hyb tray cover</p>	<ul style="list-style-type: none"> The 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 which should be discarded prior to use. 384 hyb tray cover, Part No. 203006

¹ **Note:** After aliquoting the appropriate solution to each tray type, the tray should be loaded into the GeneTitan™ MC Instrument with the barcode facing away from the operator, i.e., barcode should be on the back side.

Proper tray alignment and loading

Proper alignment and loading of trays and covers is critical when using the GeneTitan MC Instrument. Each tray and cover has one notched corner. The notched corner of the tray and its corresponding cover or protective base must be in vertical alignment with each other. Consult [Chapter 5, "Before using the GeneTitan™ Multi-Channel Instrument" on page 96](#) for important information on aligning trays and loading them into the GeneTitan MC Instrument.

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



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

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

Correct placement of cover on stain tray.



Incorrect placement of cover on stain tray.



Figure 8 Placement of covers on trays

Labeling GeneTitan™ hybridization and reagent trays

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

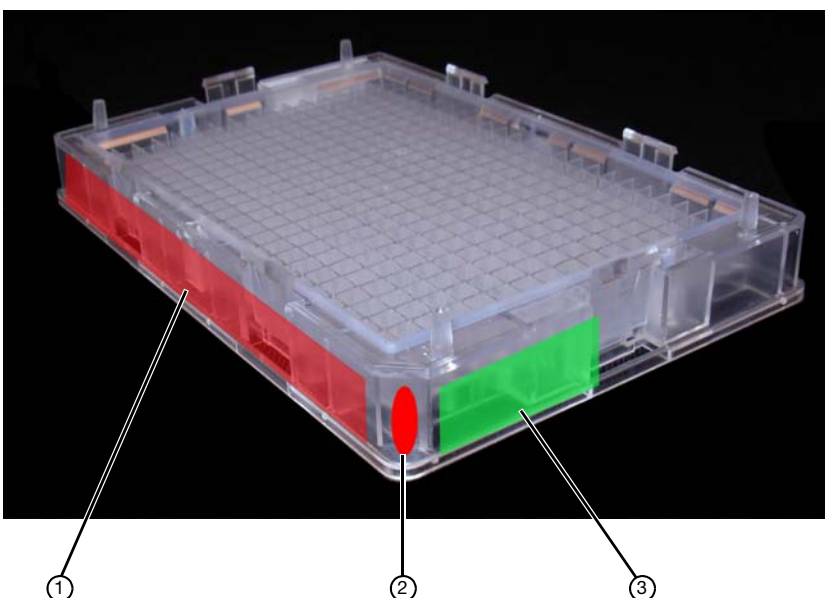
IMPORTANT! It is critical that you write only on the proper locations of the proper sides of hyb and stain trays. **Do NOT** write in any other location, as this can interfere with sensors inside the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain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

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

- ["Labeling for hyb trays"](#), below
- ["Labeling for stain trays" on page 42](#)

Labeling for hyb trays

You may label the hyb tray on the front part of the **short side of the tray, next to the notch at the left**, as shown in [Figure 9](#). The proper section for labeling is closest to the notched corner, corresponding to the A1 and F1 wells.



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

Figure 9 Labeling GeneTitan™ hyb trays

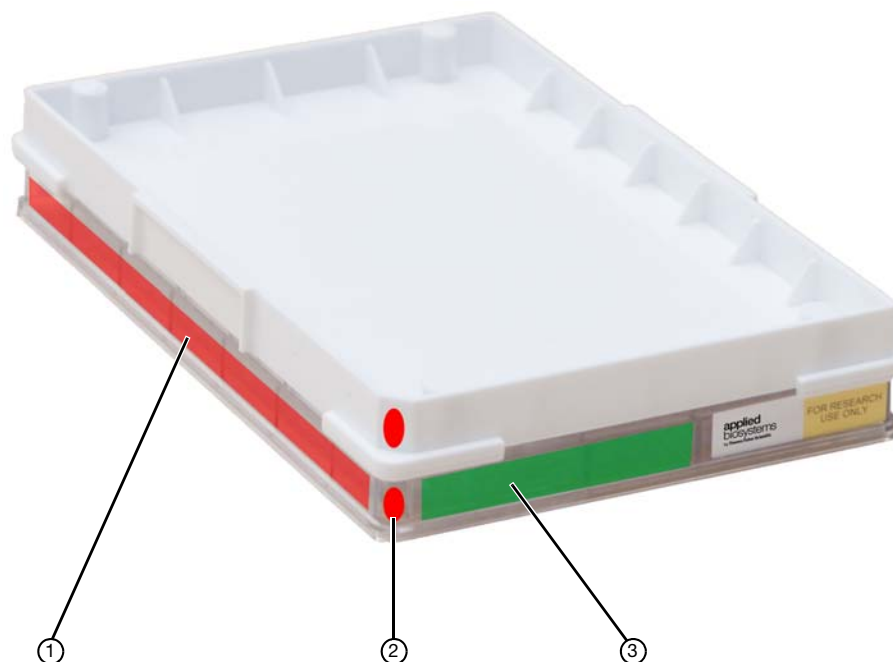


CAUTION! Writing on the wrong side of the hyb tray may interfere with the operation of sensors in the GeneTitan MC Instrument.

Labeling for stain trays

You may label the stain trays on the **left side of the front of the tray** as shown in [Figure 10](#). The correct side is closest to the notched corner, corresponding to the A1 through F1 wells.

IMPORTANT! Do not confuse hyb trays with stain trays.



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

Figure 10 Labeling GeneTitan™ stain tray (stain tray shown with lid)

Reagents for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol

The Axiom™ 2.0 Assay for Mini 96-Array Format Manual Protocol uses the Axiom 2.0 Assay Mini 96 Reagent Kit (Cat. No. 903013). Kits consist of 4 modules for different stages of the assay with some modules having both 4°C and –20°C pouches. There are specific instructions for which reagents are needed and how to treat them within each stage.

Table 15 Axiom™ 2.0 Assay Mini 96 Reagent Kit, Cat. No. 903013 (sufficient for processing two Axiom Mini 96-array format plates)¹

Module	Components	Storage
Module 1: Part No. 901711	<ul style="list-style-type: none"> • Axiom 2.0 Denat Soln 10X • Axiom 2.0 Neutral Soln • Axiom Water • Axiom Reference gDNA 103 • Axiom 2.0 Amp Soln • Axiom 2.0 Amp Enzyme 	–25°C to –15°C
Module 2—Pouch 1 of 2: Part No. 901528	<ul style="list-style-type: none"> • Axiom Frag Enzyme • Axiom 10X Frag Buffer • Axiom Precip Soln 2 • Axiom Hyb Buffer • Axiom Hyb Soln 1 	–25°C to –15°C
Module 2—Pouch 2 of 2: Part No. 901529	<ul style="list-style-type: none"> • Axiom Frag Diluent • Axiom Frag Rxn Stop • Axiom Precip Soln 1 • Axiom Resusp Buffer • Axiom Hyb Soln 2 	2°C to 8°C
Module 3	<ul style="list-style-type: none"> • Axiom Wash Buffer A: Part No. 901446 (4 bottles per kit) • Axiom Wash Buffer B: Part No. 901447 (2 bottles per kit) • Axiom Water: Part No. 901578 (2 bottles per kit) 	room temperature 15°C to 30°C
Module 4—Pouch 1 of 2: Part No. 901278	<ul style="list-style-type: none"> • Axiom Ligase Buffer • Axiom Ligase Enzyme • Axiom Ligase Soln 1 • Axiom Probe Mix 1 • Axiom Stain Buffer • Axiom Stabilize Soln 	–25°C to –15°C
Module 4—Pouch 2 of 2: Part No. 901276	<ul style="list-style-type: none"> • Axiom Ligase Soln 2 • Axiom Probe Mix 2 • Axiom Wash A • Axiom Stain 1-A • Axiom Stain 1-B • Axiom Stain 2-A • Axiom Stain 2-B • Axiom Stabilize Diluent • Axiom Water • Axiom Hold Buffer 	2°C to 8°C
Axiom Hold Buffer: Part No. 903012	<ul style="list-style-type: none"> • Axiom Hold Buffer (1 bottle) 	2°C to 8°C

¹ Axiom™ 2.0 Assay Mini 96 Reagent Kit only states Axiom 2.0 on Mod 1. Do not use reagents from DMET™ Plus Solution, CytoScan™ Reagent Kit or any expression reagent kits.

Axiom™ 2.0 Assay for Mini 96-Array manual target preparation

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Introduction

Manual target preparation for the Axiom Mini 96-array enables you to process 96 samples at a time without the use of automation equipment. The protocol is performed in two parts:

- Part 1: Manual Target Preparation, as described in this chapter
- Part 2: Array Processing is performed on the GeneTitan™ Multi-Channel (MC) Instrument

Array handling and processing protocols require the use of a GeneTitan MC Instrument, as described in [Chapter 5, "Array processing with the GeneTitan™ MC Instrument"](#) on page 96.

IMPORTANT! Read all the instructions in [Chapter 3, "Preparation before you start"](#) on page 22, before performing manual target preparation.

A list of all equipment and resources required for the Axiom 2.0 Assay for Mini 96-Array Format Manual Protocol is in the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435.

Using the manual target preparation protocol, a single operator can process three gDNA and array plates a week during a forty-hour work week for a total of 288 arrays. See [Chapter 6, "Processing three Axiom™ array plates per week"](#) on page 136 for further information.

Stage 1: DNA amplification

IMPORTANT! Before proceeding to DNA amplification, perform the gDNA preparation described in [Chapter 2, "Genomic DNA preparation and requirements" on page 13](#).

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

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

"1: Prepare for DNA amplification" on page 47

"2: Prepare the Denaturation Master Mix" on page 48

"3: Add Denaturation Master Mix to samples" on page 49

"4: Add Neutralization Solution to samples" on page 49

"5: Prepare the Amplification Master Mix" on page 50

"6: Add Amplification Master Mix to samples" on page 50

"7: Store remaining reagents" on page 51

"8: Freeze or proceed" on page 51

IMPORTANT! Amplification preparation should take place in an a dedicated area such as a biosafety hood with dedicated pipettes, tips, vortex, etc. See ["Amplification staging area" on page 23](#) for more information.

Duration

For 96 samples:

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

Input required

The gDNA Sample Plate: an Eppendorf 96 Deep-well Plate, 2000 µL with 8.7 µL of each gDNA diluted to a concentration of 11.5 ng/µL, 17.2 ng/µL, or 23 ng/µL as required according to the sample type.

See ["Genomic DNA preparation" on page 18](#) for more information.

Equipment, consumables and reagents required

Equipment and consumables

The equipment and consumables listed in [Table 16](#) are required for this stage.

Table 16 Equipment and consumables required for Stage 1: DNA amplification

Quantity	Item
As required	Adhesive seals for 96-well plate - MicroAmp Clear adhesive film
1	Cooler, chilled to -20°C
1	Microcentrifuge tube holder
1	15 mL tube holder
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1 each	Rainin Pipettes: <ul style="list-style-type: none"> • Single-channel P200 • Single-channel P1000 • Multi-channel P20 • Multi-channel P200 • Multi-channel P1200
As needed	Pipette tips
As needed	Pipette, serological <ul style="list-style-type: none"> • 5 x 1/10 mL • 10 x 1/10 mL
1	Pipet aid
1	Plate centrifuge, at room temperature
1	Oven, set at 37°C
2	15 mL conical tube
1	Vortexer
1	Timer
3	Matrix™ 25 mL Reagent Reservoir Cat. No. 8093-11



Reagents required

Table 17 Reagents required for Stage 1: DNA amplification

From the Axiom™ 2.0 Assay Mini 96 Reagent Kit	Module
Axiom 2.0 Denat Soln 10X	Module 1, -20°C Part No. 901711
Axiom 2.0 Neutral Soln	
Axiom 2.0 Amp Soln	
Axiom Water	
Axiom 2.0 Amp Enzyme	

1: Prepare for DNA amplification

To prepare for DNA amplification

- Set an incubator/oven temperature at 37°C.
We recommend using one of these ovens:
 - BINDER ED 56
 - Applied Biosystems GeneChip™ 645 Hybridization Oven (turn rotation on to 15 rpm)
- Set the centrifuge temp to room temperature.
- Thaw and prepare the reagents and sample plate.

To thaw and prepare the reagents:

- Thaw the gDNA sample plate on the benchtop at room temperature and pulse-spin to get all the droplets down.

IMPORTANT!

- gDNA samples must be brought to room temperature before proceeding with denaturation.
 - gDNA samples must be 8.7 µL volume of each gDNA at a concentration of 11.5 ng/µL, 17.2 ng/µL, or 23 ng/µL, depending on the sample type, in an Eppendorf 96 Deep-well Plate, 2000 µL (see ["Genomic DNA preparation" on page 18](#)).
- Thaw the following reagents in a small water bath on the benchtop at room temperature (small water bath: small tray or container, such as a pipet tip box, filled with fresh filtered water):
 - Axiom 2.0 Denat Soln 10X
 - Axiom 2.0 Neutral Soln
 - Axiom 2.0 Amp Soln
 - Axiom Water
 - Leave the Axiom 2.0 Amp Enzyme in the cooler in the freezer until ready to use.
 - Vortex all reagents (except Axiom 2.0 Amp Enzyme), then place at room temperature.
 - Axiom 2.0 Amp Soln: Vortex for 30 sec to thoroughly mix.
 - Axiom 2.0 Neutral Soln: Vortex for 30 sec to thoroughly mix.
 - Axiom 2.0 Denat Soln 10X: Vortex and pulse-spin before use.

- Axiom 2.0 Amp Enzyme: Gently invert and flick the tube 3 times to mix and pulse-spin just before use.

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

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

Table 18 Labeling tubes

Label	Tube size	Temperature	Contents
• <i>D MM</i>	15 mL	Leave at room temperature	Denaturation Master Mix
• <i>Amp MM</i>	15 mL	Leave at room temperature	Amplification Master Mix

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

Table 19 Labeling reagent reservoirs for DNA amplification

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

2: Prepare the Denaturation Master Mix

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

1. Per [Table 20](#), dilute the appropriate volume of Axiom 2.0 Denat Soln 10X using the Axiom Water.

Table 20 Preparing Denaturation Master Mix (*D MM*)

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked <i>D MM</i> , add:		
Axiom Water	7.8 µL	2.2 mL
Axiom 2.0 Denat Soln 10X	0.9 µL	244 µL
Total volume	8.7 µL	2.4 mL

2. Vortex and leave at room temperature.

3: Add Denaturation Master Mix to samples

To add the Denaturation Master Mix to your samples (carry out the following steps at room temperature):

1. Ensure the gDNA samples in the Sample Plate are fully thawed. Pulse-spin the plate to get all the droplets down.
Remember: Samples must be at room temperature for this step.
2. Gently pipet or pour the Denaturation Master Mix into the reagent reservoir marked *D MM*.
3. Carefully remove the seal from the Sample Plate and discard the seal.
4. Using a P20 12-channel pipette, add **8.7 µL of Denaturation Master Mix** to each sample (total volume: 17.4 µL/well).
 - Pipet directly into the liquid of each well. Do not mix by pipetting up and down.
 - Change tips between each addition.
 - This plate is now known as the **Denaturation Plate**.
5. Seal and vortex the Denaturation Plate. Start the timer for a **10 minute incubation** after vortexing.
6. Pulse-spin the Denaturation Plate to 1000 rpm at room temperature.
Note: The quick spin time is included in the 10 minute incubation.
7. Visually examine the volume in each well.
 - a. Keep a record of any wells that visually appear to have a particularly low or high volume; these samples may need to be repeated.
 - b. Do **NOT** stop to measure volumes; proceed without delay.
8. Complete the **10 minute incubation** on the benchtop at room temperature.
While completing the incubation at room temperature, prepare the Neutralization Soln as described in [Step 1 on page 49](#).
9. After incubation **immediately** add the Neutralization Soln as described in ["4: Add Neutralization Solution to samples" on page 49](#).

4: Add Neutralization Solution to samples

To add the Neutralization Master Mix to your samples (carry out the following steps at room temperature):

1. Measure **7.5 mL of Axiom 2.0 Neutral Soln** and slowly pipet the reagent into the reagent reservoir marked *N Soln*.
2. Carefully remove the seal from the Denaturation Plate and discard the seal.
3. Using a P200 12-channel pipette, add **56.6 µL of Axiom 2.0 Neutral Soln** to each sample (total volume: 74 µL/well).
 - Pipet down the wall of each well. Change tips between each addition.
 - The plate is now known as the **Neutralization Plate**.
4. Seal, vortex, and pulse-spin the Neutralization Plate.
5. Visually examine the volume in each well (should be ~74 µL/well) and:
 - a. Keep a record of any wells that visually appear to have a particularly low or high volume; these samples may need to be repeated.
 - b. Do **NOT** stop to measure volumes.
6. Proceed immediately to ["5: Prepare the Amplification Master Mix" on page 50](#).

5: Prepare the Amplification Master Mix

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

1. Per [Table 21](#), pipet the appropriate amount of Axiom 2.0 Amp Soln into the tube labeled *Amp MM* at room temperature.

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

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

Table 21 Amplification Master Mix (*Amp MM*)

Reagent	per sample	Master mix 96+
To the 15 mL tube marked <i>Amp MM</i> , add:		
Axiom 2.0 Amp Soln	97.9 µL	12.0 mL
Axiom 2.0 Amp Enzyme	2.2 µL	267 µL
Total volume	100.1 µL	12.3 mL

2. Remove the Axiom 2.0 Amp Enzyme from the freezer and place in a portable cooler at -20°C.
 - a. Invert and flick the Axiom 2.0 Amp Enzyme tube three times, then pulse-spin.
 - b. Per [Table 21 on page 50](#), add the appropriate amount of Axiom 2.0 Amp Enzyme to the tube labeled *Amp MM*.
 - c. Vortex the Amplification Master Mix well, invert the tube 2 times, and then vortex again.

6: Add Amplification Master Mix to samples

1. *Slowly* pour the Amplification Master Mix to the reagent reservoir labeled *Amp MM*.
2. Carefully remove the seal from the Neutralization Plate and discard the seal.
3. Using a P200 12-channel pipette, *slowly* add **100.1 µL of Amplification Master Mix** to each sample of the Neutralization Plate.
 - Pipet down the wall of the well (total volume: 174.1 µL/well). Do not mix by pipetting up and down.
 - Change tips between each addition.

Note: After adding the Amplification Master Mix, the plate is now known as the Amplification Plate.
4. Seal tightly, vortex twice, and spin the Amplification Plate for one minute at 1000 rpm (as described in "[Seal, vortex, and spin](#)" on page 27).
5. Place the sealed Amplification Plate in an oven set at 37°C and leave **undisturbed for 23 ±1 hr**.

Note: If using a GeneChip™ Hybridization Oven, place the plate on the bottom of the oven. Plates do not rotate. Set the rotor for 15 rpm speed. See "[Oven recommendations](#)" on page 25 for more information.

7: Store remaining reagents

Store remaining Module 1 reagents for future use. Follow guidelines presented in the section "[Freeze-thaw instructions](#)" on page 30.

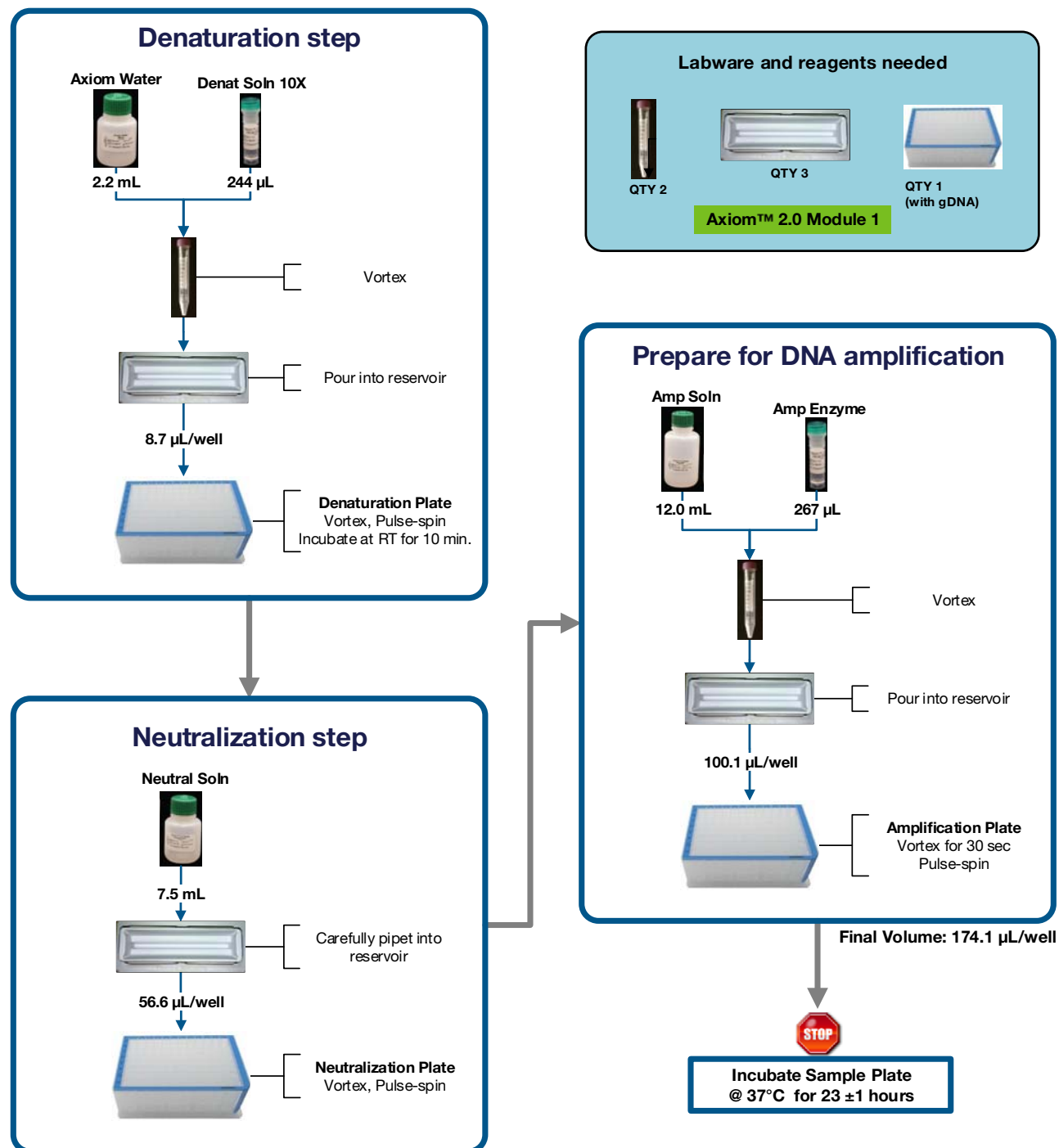
8: Freeze or proceed

After the incubation finishes, you can either:

- Proceed to "[Stage 2: Fragmentation and Precipitation](#)" on page 53.
- Store the Amplification Plate at –20°C.

Note: If freezing, do not perform the stop amplification reaction step before you store the Amplification Plate at –20°C. The Stop Amplification Reaction step will be performed after thawing the frozen plate, as described in "[1: Prepare for fragmentation and precipitation](#)" on page 54.

Stage 1: DNA amplification



Stage 2: Fragmentation and Precipitation

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

- "1: Prepare for fragmentation and precipitation" on page 54
- "2: Incubate samples in preheated ovens" on page 56
- "3: Prepare the Fragmentation Master Mix" on page 57
- "4: Add the Fragmentation Master Mix to samples" on page 57
- "5: Add the Stop Solution to the samples" on page 58
- "6: Prepare the Precipitation Master Mix" on page 58
- "7: Freeze the Precipitation Plate overnight" on page 59
- "8: Store remaining reagents" on page 59

Duration Total time: approximately 2 hours.

Input required Amplification Plate from "Stage 1: DNA amplification" on page 45.

Equipment, consumables and reagents required **Equipment and consumables**
The equipment and consumables listed in Table 22 are required for this stage.

Table 22 Equipment and consumables required for Stage 2: Fragmentation and Precipitation

Quantity	Item
As required	Adhesive seals for 96-well plates
1	Freezer set to –20°C (Designate a shelf where the precipitation plates can be left undisturbed)
1	Cooler, chilled to –20°C
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1 each	Rainin pipettes: <ul style="list-style-type: none"> • Single channel P1000 • Single channel P200 • Multi-channel P20 • Multi-channel P200 • Multi-channel P1200
As needed	Pipette tips for pipettes listed above
1	Pipet-Aid®
As needed	Pipette, serological <ul style="list-style-type: none"> • 5 x 1/10 mL • 10 x 1/10 mL
1	Plate centrifuge set at room temp
1	Mini microcentrifuge (microfuge with microtube rotor)
2-3	Ovens (see "Oven recommendations" on page 25): <ul style="list-style-type: none"> • One oven set at 37°C • One oven set to 65°C

Table 22 Equipment and consumables required for Stage 2: Fragmentation and Precipitation (Continued)

Quantity	Item
1	15 mL conical tube
1	50 mL conical tube
1	50 mL conical tube holder
3	Matrix™ 25 mL Reagent Reservoir Cat. No. 8093-11
1	Vortexer

Reagents required

Table 23 Reagents required for Stage 2: Fragmentation and Precipitation

Reagent	Module
From the Axiom™ 2.0 Assay Mini 96 Reagent Kit	
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 - Refer to the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide, Pub. No. 703435	
Isopropanol (2-Propanol), 99.5%	

1: Prepare for fragmentation and precipitation

Set ovens and centrifuge

- Set the incubators/ovens.
 - If you are running one plate per week, you will need to set two incubators/ovens as follows, preferably the night before:
 - One oven set at 37°C. Use an oven that can sustain a constant temperature of 37°C and has a temperature accuracy of ±1°C.
 - One oven set at 65°C.
 - If you are running the three plate per week manual target preparation workflow, three ovens are recommended. See [Chapter 6, "Processing three Axiom™ array plates per week" on page 136](#) for further information.
- Set the centrifuge temp to room temperature.

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

Thaw and prepare the amplified DNA samples and reagents

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

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

To thaw and prepare the fragmentation reagents:

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

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

To thaw and prepare the precipitation reagents:

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

Label tubes and reagent reservoirs

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

Table 24 Label conical tubes

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

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

Table 25 Label reagent reservoirs for Fragmentation and Precipitation

Label	Temperature	Contents
• <i>Frg MM</i>	Leave reservoir at room temperature	Fragmentation Master Mix
• <i>Stop</i>	Leave reservoir at room temperature	Frag Rxn Stop
• <i>Precip MM</i>	Leave reservoir at room temperature	Precipitation Master Mix

2: Incubate samples in preheated ovens

Note: OPTIONAL: Remove samples for quantifying amplification yield by the PicoGreen Assay.

1. Carefully remove the seal from the Amplification Plate and discard the seal.
2. Transfer 4 µL of samples from each well to a 96 well PCR plate such as a Bio-Rad Hard-Shell 96-well plate, HSP-9631 and set aside for later quantitation (e.g., using the Quant-iT™ PicoGreen® dsDNA Kit from Thermo Fisher Scientific).
3. Reseal the Amplification Plate and proceed to "[Stop the DNA amplification reaction](#)".

Stop the DNA amplification reaction

1. Place the Amplification Plate in the 65°C oven:
 - If proceeding directly from the end of "[Stage 1: DNA amplification](#)" on [page 51](#), transfer the Amplification Plate from the 37°C oven to the 65°C oven. Ensure the seal is still securely attached to the plate to minimize evaporation.
 - If working with a thawed plate, change the seal, vortex, and pulse-spin the Amplification Plate as instructed in "[Thaw and prepare the amplified DNA samples and reagents](#)" on [page 55](#) before placing it in the 65°C oven.
2. Incubate for 20 minutes.

Prepare for fragmentation

1. Transfer the Amplification Plate from the 65°C oven to the 37°C oven.
 - Press on the seal, if needed.
2. Incubate for 45 minutes.

3: Prepare the Fragmentation Master Mix

To prepare the Fragmentation Master Mix:

1. Start making the Fragmentation Master Mix when there is still five minutes to the finish of the 37°C **incubation**, using the values in the table below. Transfer the Axiom Frag Enzyme to a –20°C portable cooler. Keep in cooler until ready to use.

Table 26 Axiom Fragmentation Master Mix

Reagent	per sample	Master mix 96+
To the 15 mL tube marked <i>Frg MM</i> , add:		
Axiom 10X Frag Buffer	19.9 µL	3.4 mL
Axiom Frag Diluent	4.5 µL	766 µL
Axiom Frag Enzyme	0.4 µL	74 µL
Total volume	24.8 µL	4.2 mL

- Add the reagents from [Table 26](#) to the *Frg MM* tube in the order shown, using appropriate single channel pipettes.
- Just before the end of the **45 minute 37°C incubation**, flick the Axiom Frag Enzyme tube 2 to 3 times, and spin.
- Add the Axiom Frag Enzyme to the *Frg MM* tube at the end of the **45 minute 37°C incubation**.

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

2. Vortex twice and place on ice.
3. Slowly pour the Fragmentation Master Mix in the reagent reservoir labeled *Frg MM* placed at room temperature.

4: Add the Fragmentation Master Mix to samples

IMPORTANT! Work quickly to perform this set of steps to minimize the time that the Fragmentation Plate is out of the 37°C oven.

1. Carefully remove the Amplification Plate from the 37°C oven and place on the bench top at room temperature.
Do not place the Amplification Plate on ice.
2. Carefully remove the seal from the Amplification Plate and discard the seal.
3. Pipetting directly into the liquid of each well, use a P200 12-channel pipette to add **24.8 µL of Fragmentation Master Mix** to each reaction. Do not mix by pipetting up and down.
 - Change tips after each addition.
 - After adding the Fragmentation Master Mix to the plate, the plate is now known as the **Fragmentation Plate**.
4. Seal the Fragmentation Plate and vortex twice.
5. Start the timer for **30 min**.

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

6. Pulse-spin the Fragmentation Plate to 1000 rpm in the plate centrifuge at room temperature.
7. Quickly transfer plate to **37°C oven and incubate for 30 min.**



CAUTION! Be watchful for the end of the thirty minute incubation period. **Fragmentation is an exact 30 minute incubation step.** Longer and shorter incubation times may lead to poor performance of the assay.

Prepare the Stop solution a few minutes before the end of the **30 minute incubation** period, as described in "[5: Add the Stop Solution to the samples](#)", below.

5: Add the Stop Solution to the samples

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

1. A few minutes before the end of the 30 minute incubation period, **measure 2.0 mL of the Axiom Frag Rxn Stop solution** and transfer into the reagent reservoir labeled *Stop*.
2. Remove the Fragmentation Plate from the oven and place on the bench top at room temperature.
3. At the **end of the 30 minute fragmentation incubation period**, carefully remove the seal from the Fragmentation Plate and discard the seal.
4. Using a P20 12-channel pipette, end the fragmentation reaction by adding **8.3 µL of Stop Solution** to each reaction. Do not mix by pipetting up and down.
 - Pipet directly into the liquid of each well.
 - Change tips after each addition.
 - Proceed immediately to the next step.
5. Seal and vortex and do a quick spin at 1000 rpm.
6. Leave the Fragmentation Plate on the benchtop while you prepare the Precipitation Master Mix.

6: Prepare the Precipitation Master Mix

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

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

Table 27 Precipitation Master Mix

Reagent	per sample	Master mix 96+
To the 50 mL tube marked <i>Precip MM</i> , add:		
Axiom Precip Soln 1	103.5 µL	11.2 mL
Axiom Precip Soln 2	0.9 µL	94.1 µL
Isopropanol	261 µL	28.2 mL
Total volume	365.4 µL	39.5 mL

Note: Use a 10 mL serological pipette to pipet Axiom Precip Soln 1.

2. Vortex the *Precip MM* tube and place on benchtop at room temperature.

3. Pour approximately half of the Precipitation Master Mix into the reagent reservoir labeled *Precip MM*.

Note: The total volume of the Precipitation Master Mix exceeds the reservoir capacity (25 mL). Pour approximately half of the Precipitation Master Mix and re-fill the reservoir with the rest of the Precipitation Master Mix after the first half has been exhausted.

4. Carefully remove the seal from the Fragmentation Plate and discard the seal.
5. Using a P1200 12-channel pipette, add **365.4 µL of Precipitation Master Mix** to each sample. Mix well by pipetting up and down 6-7 times within the solution. Observe the solution while it is within the tips—it should look homogeneous after pipetting 5-7 times. If not, repeat mixing a few more times until the solution looks homogeneous.
 - **Do not vortex the plate after isopropanol addition to avoid cross-contamination of the samples.**
 - Change tips after each addition.

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

6. Blot the top of the plate with a Kimwipe and seal tightly with a Microamp seal.

7: Freeze the Precipitation Plate overnight

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

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

8: Store remaining reagents

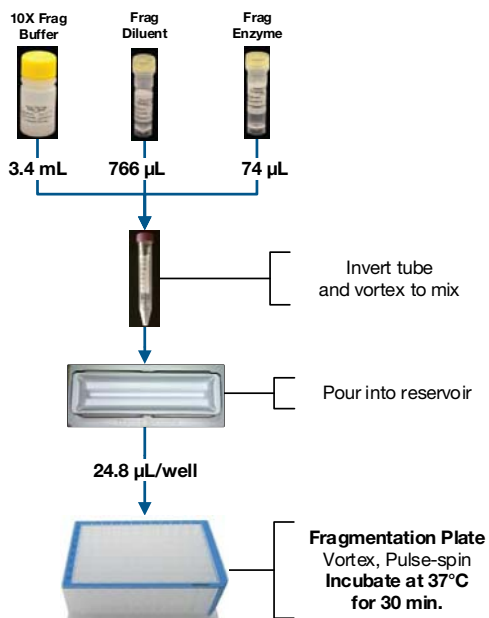
Store remaining Module 2-1 and Module 2-2 reagents for future use. Follow guidelines presented in the section "[Freeze-thaw instructions](#)" on page 30.

Stage 2: Fragmentation and precipitation

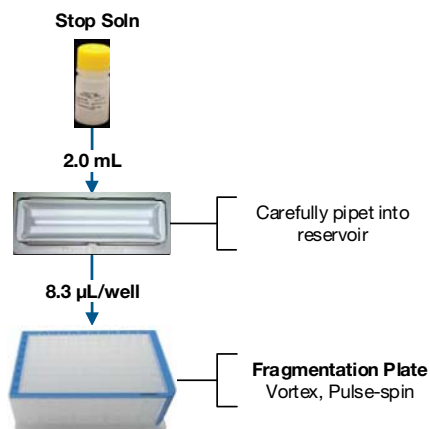
Incubate samples in pre-heated ovens

- 1) 20 min. @ 65°C
- 2) 45 min. @ 37°C

Fragmentation step



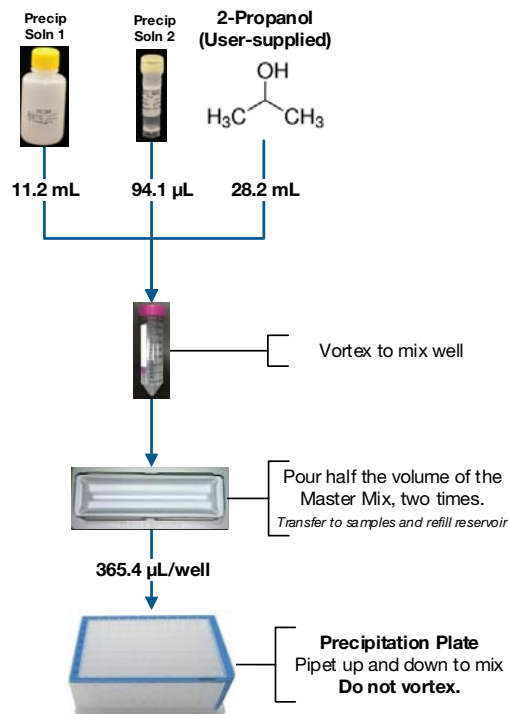
Stop fragmentation reaction



Labware and reagents needed



Precipitation step



Final Volume: 572.6 µL/well

Incubate Precipitation Plate @ -20°C overnight

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

This stage requires the following sets of steps:

"Stage 3A: Centrifuge precipitation plate and dry the DNA pellet" on page 64

"Stage 3B: Resuspension and hybridization preparation" on page 65

"1: Prepare for resuspension and hybridization preparation" on page 65

"2: Prepare DNA pellets and warm the reagents" on page 65

"3: Thaw and prepare the reagents" on page 65

"4: Label tubes and reservoirs" on page 66

"6: Add hybridization cocktail to DNA pellets" on page 67

"7: Resuspension of DNA pellets" on page 67

"5: Prepare the hybridization cocktail" on page 66

"8: Prepare the Hyb Ready Sample Plate" on page 67

"10: Freeze or proceed" on page 67

"Stage 3C: Sample QC" on page 68

"1: Prepare for sample QC" on page 68

"2: Perform QC checks" on page 69

"3: Freeze or proceed" on page 69



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

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

Duration

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

Total: 2.5 hr

Input required

Precipitation Plate from "Stage 2: Fragmentation and Precipitation" on page 53.

Equipment, consumables, and reagents required

The equipment and consumables listed in [Table 28](#) are required for this stage.

Table 28 Equipment and consumables required for Stage 3: Drying, Resuspension and Sample QC

Quantity	Item
As required	Adhesive seals for 96-well plates
1	Marker, fine point, permanent
1 each	Rainin pipettes: <ul style="list-style-type: none"> • Single channel P20 • Single channel P-100 • Multi-channel P20 • Multi-channel P-200
As needed	Pipette tips for pipettes listed above
2	Bio-Rad Hard-Shell 96-well plate, Bio-Rad Cat. No. HSP-9631 or any 96-well PCR plate for making the dilutions: <ul style="list-style-type: none"> • Dilution QC Plate • Gel QC Plate
1	Bio-Rad Hard-Shell 96-well plate, Bio-Rad Cat. No. HSP-9631 or 96 Half-Skirt Plate, Bio-Rad Cat. No. HSS-9641 <ul style="list-style-type: none"> • Hyb Ready Plate
1	96-Well PCR Racks, if needed (to hold the 96 Half-Skirt Plate)
1	96 Well UV Plate (Greiner UV-Star® 96 well plate) <ul style="list-style-type: none"> • OD plate
1	Oven set at 37°C
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Fume hood
1	Plate centrifuge set at 4°C
1	15 mL conical tube
1	5 mL serological pipette
1	Pipet aid
1	Shaker, either: <ul style="list-style-type: none"> • Thermo Scientific™ Compact Digital Microplate Shaker • Jitterbug
1	Vortexer
4	Matrix™ 25 mL Reagent Reservoir Cat. No. 8093-11

Reagents required


Table 29 Reagents required for Stage 3: Drying, Resuspension and QC

Reagent	Module
From the Axiom™ 2.0 Assay Mini 96 Reagent Kit	
Axiom Hyb Buffer	Module 2-1, -20°C Part No. 901528
Axiom Hyb Soln 1	
Axiom Resusp Buffer	Module 2-2, 2-8°C Part No. 901529
Axiom Hyb Soln 2	
Other reagents required for QC steps (optional)	
Gel Diluent, 15 mL of 1000-fold dilution of TrackIt™ Cyan/Orange Loading Buffer (see Appendix B, "Fragmentation quality control gel protocol" on page 159 for dilution instructions.)	
15-fold dilution of TrackIt™ 25 bp DNA Ladder (Cat. No. 10488-022)	
Nuclease-free water, ultrapure MB Grade (Cat. No. 71786; for OD and Dilution QC Plate preparation)	

Gels and related materials required

At the end of this stage, verifying the fragmentation reaction is highly recommended. See [Appendix B, "Fragmentation quality control gel protocol" on page 159](#) for the required gel and related materials.

Stage 3A: Centrifuge precipitation plate and dry the DNA pellet

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


To centrifuge and dry the DNA pellets:

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

Use an oven that can sustain a constant temperature of 37°C and has a temperature accuracy of $\pm 1^\circ\text{C}$ (we recommend the BINDER ED 56). If using an Applied Biosystems GeneChip Hyb Oven, set the rotation speed to 15 rpm to distribute heat.

2. Transfer the Precipitation Plate from the -20°C freezer to a pre-chilled centrifuge. Centrifuge the plate **for 40 min at 4°C** at 3200 $\times g$ (4000 RPM for the Eppendorf 5810R centrifuge with the rotor configuration described in the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435).

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

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

3. Immediately after the 40 min centrifugation period, empty the liquid from each plate as follows:
 - a. Carefully remove the seal from the Precipitation Plate and discard the seal.
 - b. Invert plate over a clean waste container to allow the liquid to drain. Collect liquid and dispose of liquid according to local, state, and federal regulations.
 - c. While still inverted, gently press the plate on a pile of Kimwipes on a bench and allow them to drain for 5 min. Transfer the plate to a new pile of Kimwipes twice during the 5 min period.

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

- Tightly seal the plate upon completion

NOTE: If using an Applied Biosystems 645 oven:

- Place the plate on the bottom of the oven. Plates do not rotate.
- Turn off the rotor during the 20 min drying time.

5. Do one of the following:

- Proceed directly to ["Stage 3B: Resuspension and hybridization preparation" on page 65](#), even if some droplets of liquid remain. Leave the sample plate at room temperature. It is helpful to begin preparing reagents for Stage 3B while centrifuging and drying pellets.
- Store the plate for resuspension later in the same day:
 - Tightly seal the plate.
 - If resuspension will be carried within 4 hours, keep the plate at room temperature.
 - If resuspension will be carried out in more than 4 hours, store the plate in a refrigerator (2-8°C).

- Store the plate for resuspension on another day:
 - Tightly seal the plate.
 - Store the plate at -20°C.

Stage 3B: Resuspension and hybridization preparation

1: Prepare for resuspension and hybridization preparation

To prepare for Resuspension and hybridization

1. Set the centrifuge to room temperature.

2: Prepare DNA pellets and warm the reagents

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, resuspension buffer, and hybridization buffer to room temperature (18-25°C) is very critical for the success of the Axiom 2.0 Mini 96 Assay. When any of these are cooler than room temperature, pellets may not resuspend completely. This may result in compromised assay performance. Please note following guidelines on how to work with plates with fresh, cold, or frozen pellets:

Pellets

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

Resuspension and hybridization reagents

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

3: Thaw and prepare the reagents

To thaw and prepare the reagents:

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

4: Label tubes and reservoirs

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

Table 30 Label tube

Label	Tube size	Temperature	Contents
• <i>Hyb C</i>	15 mL	Room temperature in fume hood	Hybridization cocktail

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

Table 31 Label reagent reservoirs for Resuspension and hybridization preparation

Label	Temperature	Contents
• <i>Hyb C</i>	Room temperature in fume hood	Hybridization cocktail

5: Prepare the hybridization cocktail

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

Note: Make sure all reagents have equilibrated to room temperature before preparing the Hybridization Cocktail.



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

1. Prepare the Hybridization Cocktail in the *Hyb C* 15 mL tube.
 - a. Add the reagents in [Table 32](#) to the *Hyb C* tube in the order shown.

Table 32 Hybridization cocktail

Reagent	per sample	Master mix 96+
To the 15 mL tube labeled <i>Hyb C</i> , add:		
Axiom Resuspension Buffer	15.2 µL	1.99 mL
Axiom Hyb Buffer	30.7 µL	4.0 mL
Axiom Hyb Soln 1	0.22 µL	28.4 µL
Axiom Hyb Soln 2	3.9 µL	511 µL
Total volume	50 µL	6.5 mL

- b. Vortex twice to mix.

6: Add hybridization cocktail to DNA pellets

To resuspend the pellets (carry out the following steps at room temperature):

1. Pour the Hyb Cocktail into the reagent reservoir labeled *Hyb C*.
2. Carefully remove the seal from the Precipitation Plate and discard the seal.
3. Using a P200 12-channel pipette, transfer **50 µL of Hyb Cocktail** to each well of the Precipitation Plate. Avoid touching the pellets with the pipette tips.
 - Change pipette tips after each addition.
 - After adding Hybridization Cocktail, the plate is known as the Resuspension Plate.
4. Seal the Resuspension Plate.
5. Briefly spin down the plate in a room temperature centrifuge for 30 seconds.

7: Resuspension of DNA pellets

1. Place the sealed Resuspension Plate on one of the following shakers:
 - Thermo Scientific™ Compact Digital Microplate Shaker: at **speed 900 rpm for 15 min**
 - Jitterbug: at **speed 7 for 15 min**
2. Inspect the Resuspension Plate from the bottom. If the pellets are not dissolved, repeat [Step 1](#).
3. Pulse-spin plate to 1000 rpm.

8: Prepare the Hyb Ready Sample Plate

To prepare the Hyb Ready Sample Plate

1. Choose a 96-well plate that will be compatible with the thermo cycler model that will be used for sample denaturation. See [Table 7 on page 25, "Thermal cycler consumables for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol"](#).
Note: The Axiom™ Mini 96 Consumables Kit includes the 96 Half-Skirt Plate as the Hyb Ready Sample Plate.
2. Label the 96-well PCR plate as Hyb Ready [Sample ID].
3. Set a P200 12-channel pipette to 55 µL (this is slightly higher than the volume of the sample in each well of the Resuspension Plate).
4. Using the P200 pipette, **transfer the entire contents of each well** in the Resuspension Plate to the labeled Hyb Ready Plate.
 - Change pipette tips after each transfer.
5. Seal and pulse-spin.

9: Store remaining reagents

Store remaining Module 2-1 and Module 2-2 reagents for future use. Follow the guidelines presented in the section "[Freeze-thaw instructions](#)" on page 30.

10: Freeze or proceed

At this point you can:

- Proceed to "[Stage 3C: Sample QC](#)" (highly recommended), below; or
- Proceed to "[Stage 4: Denaturation and hybridization](#)"; or
- Store the Hyb Ready samples at -20°C.

Stage 3C: Sample QC

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

1: Prepare for sample QC

To prepare for Sample QC:

Prepare the reagents

Obtain the reagents for Sample QC:

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

The Gel Diluent is a 1000-fold dilution of the TrackIt Cyan/Orange Loading Buffer as described in "[Diluting the TrackIt™ Cyan/Orange Loading Buffer and 25 bp Ladder](#)" on page 160.

3. 90 µL of a 15-fold dilution of TrackIt™ 25 bp DNA Ladder as described in "[Diluting the TrackIt™ Cyan/Orange Loading Buffer and 25 bp Ladder](#)" on page 160.
4. One E-Gel 48 Agarose Gel, 4% Agarose

Label reservoirs

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

Table 33 Label reagent reservoirs for QC

Label	Temperature	Contents
• <i>H₂O</i>	Leave reservoir at room temperature	Nuclease-free Water
• <i>Gel Dil</i>	Leave reservoir at room temperature	Gel Diluent

Prepare Sample QC plates

1. Label two Bio-Rad Hard-Shell 96-well plates, or any 96-well PCR plate for making the dilutions:
 - Label one plate as *Dil QC*
 - Label the second plate as *Gel QC*
2. Obtain one 96 well UV plate. This will be referred to as OD Plate.

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

2: Perform QC checks

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

1. Prepare Dilution QC Plate and OD Plate.
Pour 15 mL nuclease-free water into the reagent reservoir labeled *H2O*.
The water will be used to make the Dilution QC Plate and the OD Plate.
 - a. Add 33 µL nuclease-free water to each well of the *Dil QC* Plate.
 - b. Add 90 µL nuclease-free water to each well of the OD Plate.
2. Prepare the Dilution QC Plate:
 - a. Transfer **3 µL of the Hyb Ready sample** from each well of the *Hyb Ready Plate* to the corresponding well of the *Dil QC* Plate. Change pipette tips after each transfer.
 - b. Seal, vortex, and pulse-spin.
3. Prepare OD Plate:
 - a. Carefully remove the seal from the Dilution QC Plate and discard the seal.
 - b. Transfer **10 µL of each *Dil QC* sample** to the to the corresponding wells of the 96 well UV plate. Change pipette tips after each transfer.
 - c. Mix by pipetting up and down.
 - Change pipette tips after each addition.
 - Final sample mass dilution is 120-fold.

See [Appendix C, "Sample quantitation after resuspension"](#) on page 162 for more information on performing the Sample Quantitation.

4. Prepare *Gel QC* Plate:
 - a. Pour 15 mL of Gel Diluent into the reagent reservoir labeled *Gel Dil*.
 - b. Add **120 µL of Gel Diluent** to each well of the *Gel QC* Plate.
 - c. Transfer **3 µL of each *Dil QC* sample** to the corresponding wells of the *Gel QC* Plate. Change pipette tips after each transfer.
 - d. Seal, vortex, and pulse-spin the plate.
5. Run gel as described in [Appendix B, "Fragmentation quality control gel protocol"](#) on page 159.

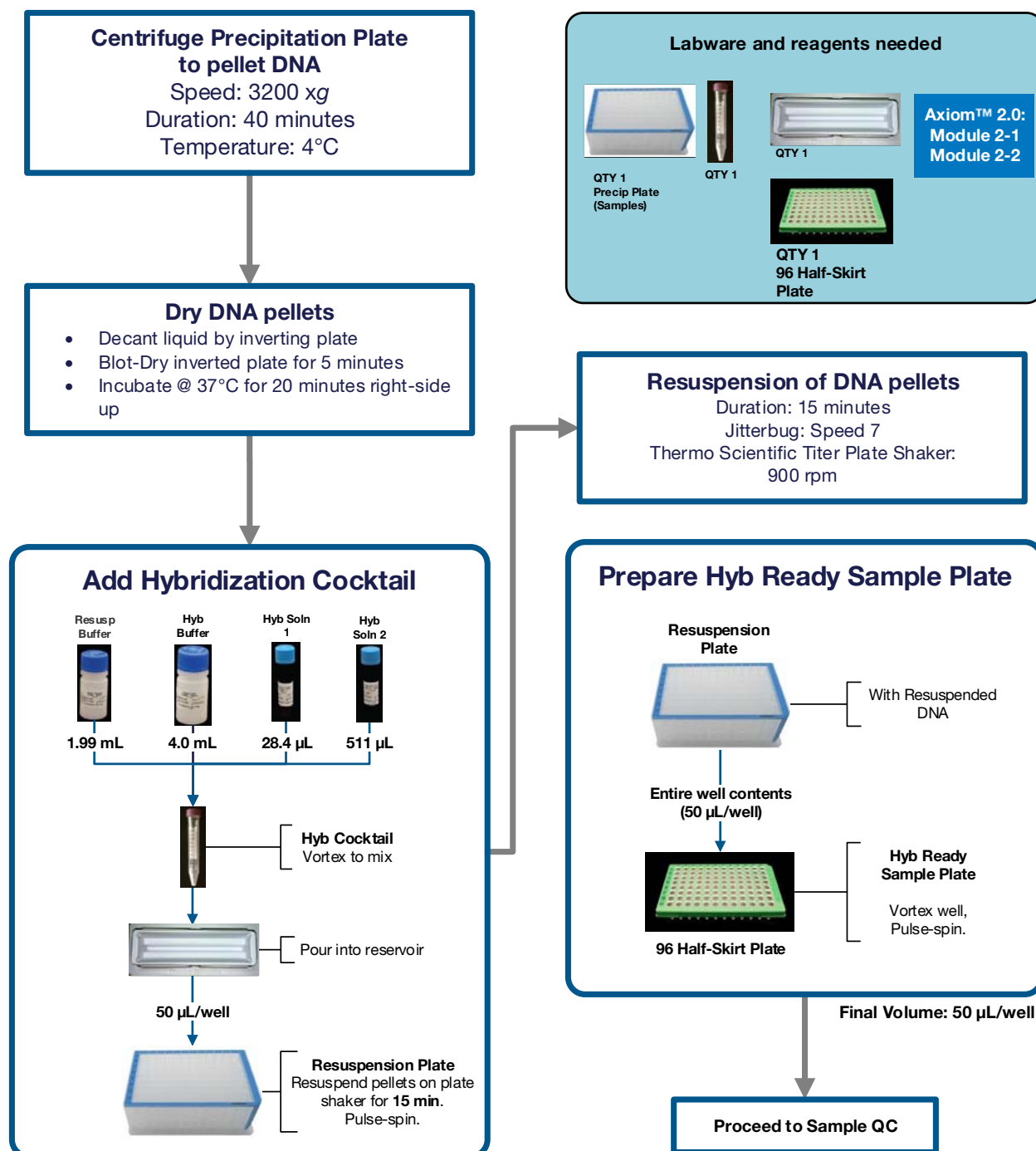
After the QC checks, the Dilution QC Plate, OD plate, and remaining *Gel QC* samples can be discarded once satisfactory results from the gel and OD 260 readings have been obtained.

3: Freeze or proceed

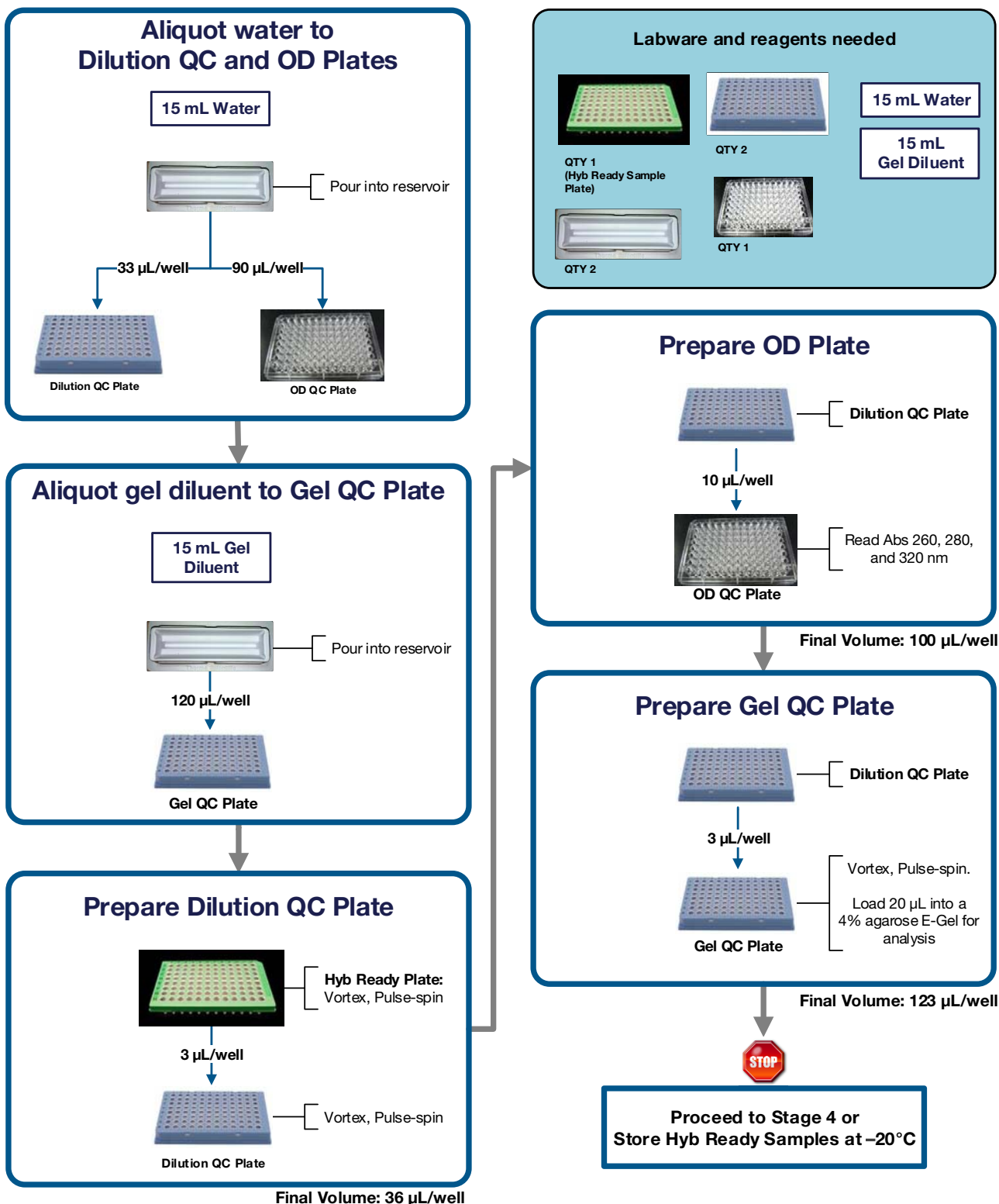
At this point you can:

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

Stage 3B: Resuspension and hybridization preparation



Stage 3C: Sample QC



Stage 4: Denaturation and hybridization

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

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

This stage requires the following sets of steps:

["1: Prepare for Denaturation and hybridization" on page 74](#)

["2: Prepare hyb ready samples stored at –20°C" on page 74](#)

["3: Prepare the GeneTitan™ MC Instrument" on page 74](#)

["4: Denature the Hyb Ready Sample Plate" on page 75](#)

["5: Prepare hybridization tray and load into GeneTitan™ MC Instrument" on page 76](#)

To perform Stage 4:

- If the Hyb Ready Plate was stored at –20°C, go to ["2: Prepare hyb ready samples stored at –20°C" on page 74](#).
- If you are proceeding directly from the end of Stage 3 [on page 69](#), go to ["4: Denature the Hyb Ready Sample Plate" on page 75](#).



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

Duration

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

Required input from previous stage

- Hyb Ready Sample Plate

Equipment, consumables, and reagents required


The following thermal cyclers are recommended:

- Bio-Rad PTC-200, or
- Bio-Rad DNA Engine Tetrad 2 #PTC-0240, or
- Applied Biosystems 9700, or
- Applied Biosystems 2720

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

The thermal cycler needs to be programmed with the **Axiom 2.0 Denature** protocol (see ["Thermal cycler recommendations" on page 24](#)).

Table 34 Equipment required for Stage 4: Denaturation and Hybridization

Quantity	Equipment	
1	GeneTitan MC Instrument	
1	Rainin P200 12-channel Pipette	
As needed	Pipette tips	
1	Thermal Cycler	Appropriate thermal cycler, programmed with the Axiom 2.0 Denature protocol (see " Thermal cycler recommendations " on page 24).
1	96 well metal chamber warmed in a 48°C oven ¹	

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

Table 35 Consumables required for Stage 4: Denaturation and hybridization

Quantity	Consumable	Supplier and Cat. No.
1	• One Axiom myDesign Genotyping Mini 96-Array Format Plate in a protective base	Thermo Fisher Scientific various Cat. No.s
1	• 384 Hyb Tray ¹	Part No. 501278

¹ The Consumables for the GeneTitan MC Instrument are packaged separately from the Axiom array plates. The 384 Hyb Tray, along with other GeneTitan MC consumables, are included in the Axiom™ 384HT High Volume Consumables Kit (Cat. No. 902629).

Table 36 Reagents required from the Axiom™ 2.0 Assay Mini 96 Reagent Kit

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

1: Prepare for Denaturation and hybridization

To prepare for Denaturation and hybridization

1. Preheat the 96-well metal chamber in a 48°C oven.
2. **Allow array plate to equilibrate to room temperature for a minimum of 25 minutes.**
 - a. Leave the array plate in the pouch at room temperature, for a minimum of 25 minutes, before opening and loading on the GeneTitan MC Instrument to allow the plate to come to room temperature.
 - b. At the end of the array warm up time, open the pouch and scan the array plate barcode into the
 - c. Batch Registration file as described in [Appendix D, "Registering samples in GeneChip™ Command Console™" on page 170.](#)



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

3. Power up the thermal cycler and prepare for the **Axiom 2.0 Denature** protocol to run with the heated lid option selected.

2: Prepare hyb ready samples stored at –20°C

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

1. Warm up the Hyb Ready Plate at room temperature for 5 minutes. It is not necessary to equilibrate the plate for longer duration.
2. Make sure the Hyb Ready Plate is sealed well.

If the plate is not sealed well:

- a. Spin the plate and carefully remove the old seal.
- b. If there is condensation on the top of the plate, blot dry gently with a Kimwipe.
- c. Use a fresh seal and tightly reseal the plate.
3. Vortex the Hyb Ready Plate briefly, then spin at 1000 rpm for 30 seconds.
4. Place the Hyb Ready Plate at room temperature.

3: Prepare the GeneTitan™ MC Instrument

Before you denature your Hyb Ready samples, ensure that the GeneTitan MC Instrument is ready for use by following the instructions given in [Chapter 5, "Stage 2: Hybridization" on page 107](#) and [Appendix D, "Registering samples in GeneChip™ Command Console™" on page 170.](#)

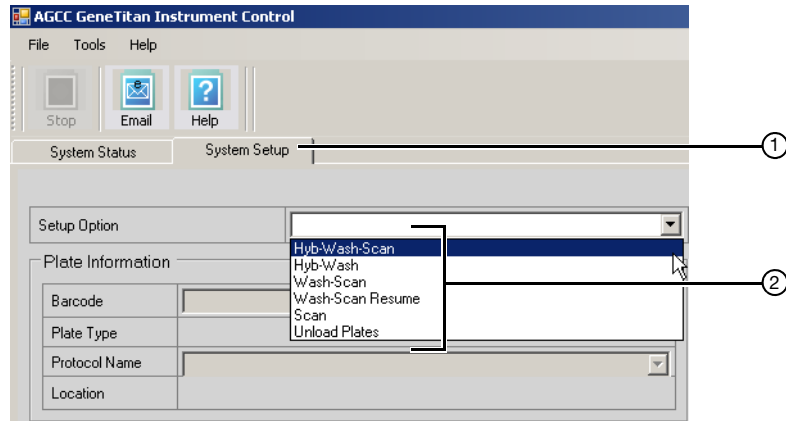
A brief summary of the steps which need to be performed is:

1. Prepare the reagents from Module 3 as described in [Table 37](#):

Table 37 Reagents from Module 3

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

2. Launch AGCC and select **AGCC GeneTitan Control**.
3. Upload your sample registration file now.
If you do not upload your samples before scanning the array plate barcode, the software will assign names to your sample.
Note: When creating the sample registration file, you have the ability to add the barcode of the hybridization tray as a sample file attribute, to enable traceability in the system. Refer to the AGCC User Guide, Chapter 4, for details about adding attributes to your sample files.
4. Select the **System Setup** tab (Figure 11).



- ① System Setup tab
- ② Setup Options

Figure 11 Setup options for processing array plates

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

4: Denature the Hyb Ready Sample Plate

1. Make sure the thermal cycler is powered on and the **Axiom 2.0 Denature** protocol with the heated lid option has been selected.
2. Open the lid of the thermal cycler and place the sealed Hyb Ready Plate on the thermal cycler. Check the integrity of the seal as evaporation during denaturation can negatively impact assay performance.
3. Close the lid. For thermal cyclers with variable lid tension (such as the Bio-Rad PTC-200 or the Bio-Rad DNA Engine Tetrad 2 #PTC-0240) follow manufacturer's instructions for adjusting lid tension.
4. Start the **Axiom 2.0 Denature** protocol, described on "[Thermal cycler recommendations](#)" on page 24).

5: Prepare hybridization tray and load into GeneTitan™ MC Instrument

Plate Format Switching during Hyb Transfer Step: During this step, you will be switching plate formats. The denatured Hyb Ready samples will be transferred from a 96-format PCR plate to a 384-format Hyb Tray. The samples must only be transferred to the Quadrant 1 wells of the 384-format Hyb Tray. [Figure 12](#) illustrates the hyb transfer step when the switch from 96-format to 384-format occurs.

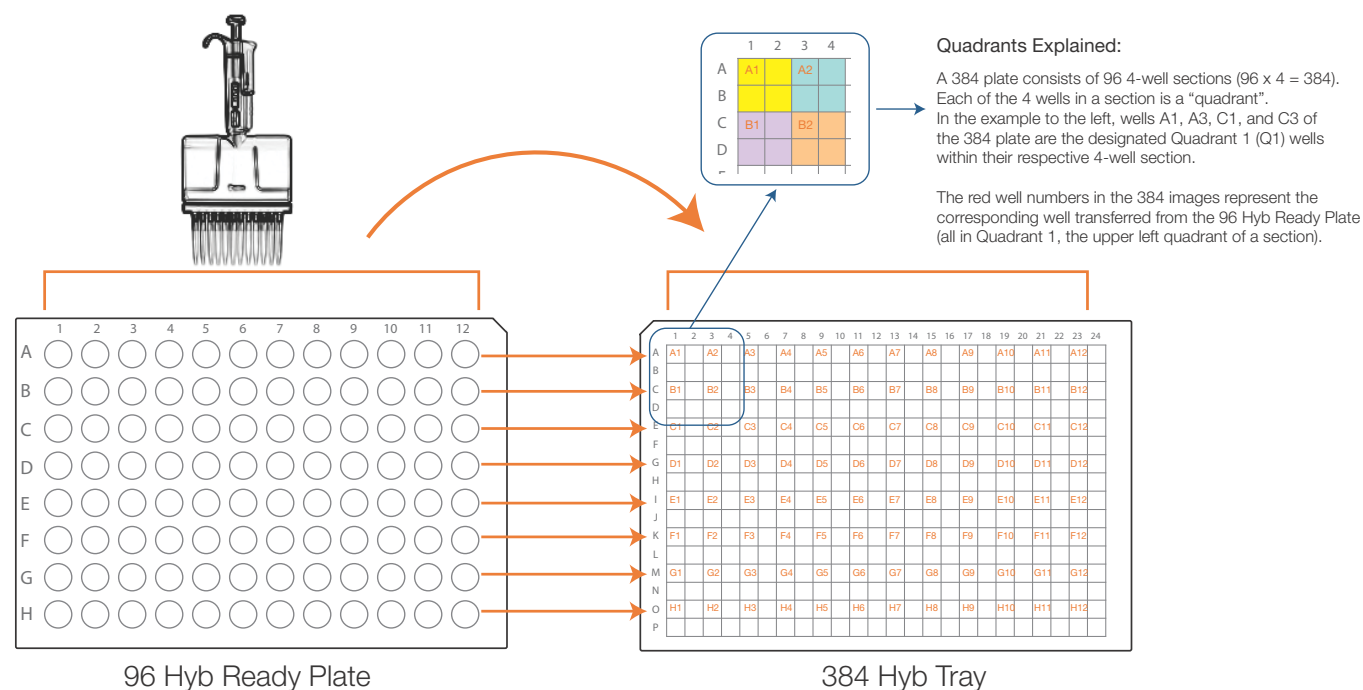


Figure 12 Plate format switching during hyb transfer



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

1. After the **Axiom 2.0 Denature** protocol has completed, remove the Hyb Ready Plate from the thermal cycler and place into a 96-well block that has been pre-warmed in an oven at 48°C.
2. Move the warm 96-well block containing the denatured Hyb Ready Plate to a fume hood.
3. Remove seal from Hyb Ready Plate and discard.
4. Remove the 384 Hyb Tray from packaging.
5. Label the hyb tray. See the note below and [Figure 9 on page 41](#) for more information.

IMPORTANT!

- It is critical that you write only on the proper location of the hyb tray (on the edge in front of wells A1 and F1) as illustrated in [Figure 9 on page 41](#). Do **NOT** write on any other side, as this can interfere with sensors inside the GeneTitan MC Instrument and result in experiment failure.
- Be sure to remove the hyb tray cover before transferring the denatured samples.

IMPORTANT! Do not confuse hyb trays with stain trays.

6. Place the hyb tray under the fume hood. Remove the hyb tray cover.
7. Obtain a P200 12-channel pipette and set it at **35 µL**. Slowly transfer the denatured samples from the 96-well Hyb Ready Plate into the corresponding quadrant 1 wells of the 384 Hyb Tray as instructed below in [Table 38](#) Plate Format Switching Guidance.

Table 38 Plate format switching guidance: Transfer denatured samples from a 96-well format PCR plate to wells in quadrant 1 of a 384-well format hyb tray.

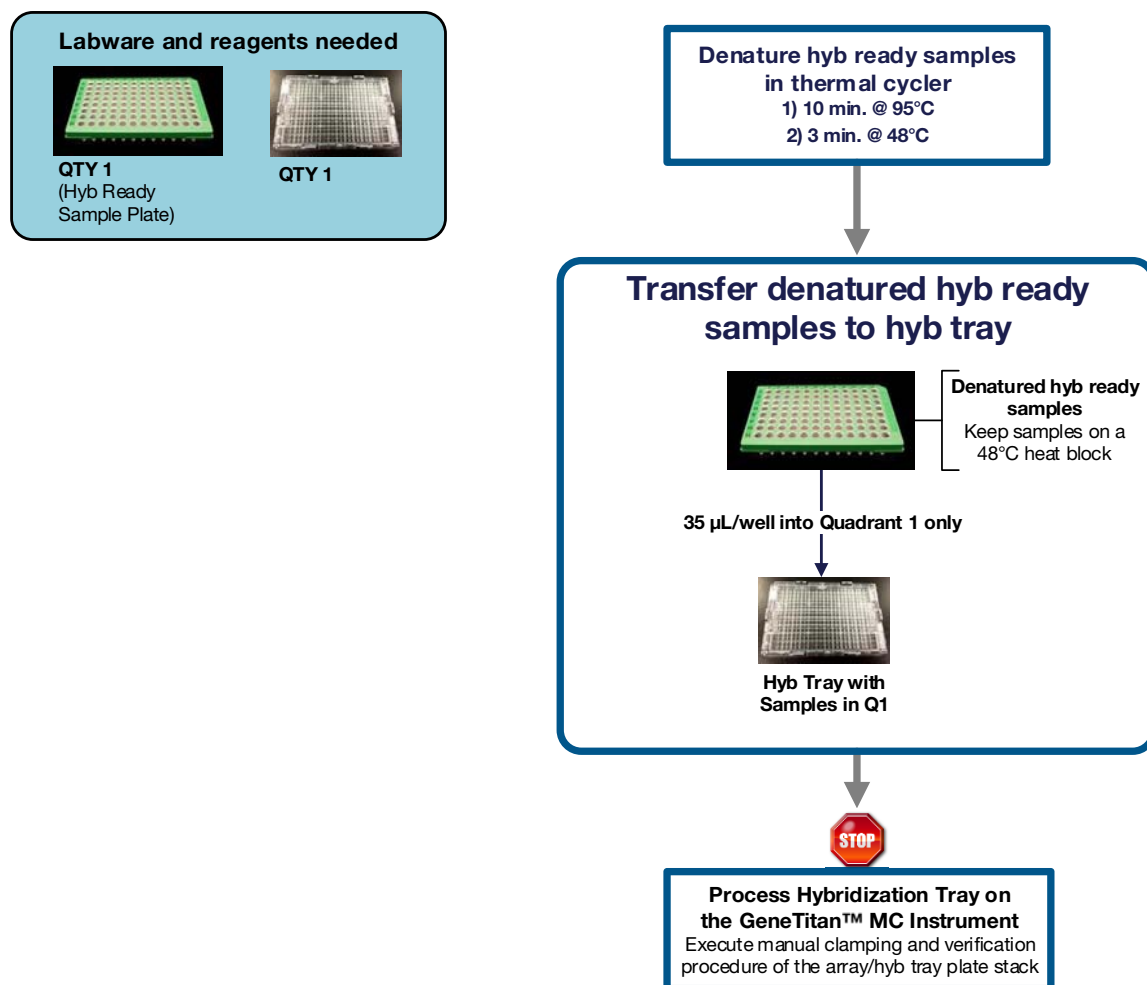
96-well format Hyb Ready PCR Plate	384-well format hyb tray
Row A	Row A, odd wells
Row B	Row C, odd wells
Row C	Row E, odd wells
Row D	Row G, odd wells
Row E	Row I, odd wells
Row F	Row K, odd wells
Row G	Row M, odd wells
Row H	Row O, odd wells

- 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 hyb tray. Puncture any air bubbles that you see using a clean pipette tip.
 - There is no need to spread the sample around the bottom of the hyb tray wells. Sample distribution across the well will occur when the array plate is stacked together with the hyb tray by the GeneTitan MC Instrument.
8. Load the array plate and hyb tray into the GeneTitan MC Instrument (see "[Load Axiom™ array plate and hyb tray onto the GeneTitan™ MC Instrument](#)" on page 112).

IMPORTANT! The sandwich of the array plate and hybridization tray needs to be manually clamped and inspected before the array processing can begin. Carefully review and execute the array plate/hyb tray clamping procedure steps as detailed in [Figure 37, "Array Plate/Hyb Tray Clamping Procedure"](#) on page 116.

Hybridization will continue on the GeneTitan MC Instrument for 23.5-24 hours before you can load the Ligation/Staining/Stabilization reagent trays into the GeneTitan MC Instrument. Near the end of the 23.5 to 24 hour hybridization period in the GeneTitan MC, proceed to "[Stage 5: GeneTitan™ reagent preparation](#)".

Stage 4: Denaturation and hybridization



Stage 5: GeneTitan™ reagent preparation

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

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

IMPORTANT! The reagent trays prepared in this step, "Stage 5: GeneTitan™ reagent preparation" are for the continued processing of an Axiom array plate that:

- Has completed the hybridization stage.
- Is ready for transfer to the fluidics area.

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

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

"1: Prepare for GeneTitan™ reagent preparation" on page 82

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

"3: Aliquot master mixes and Axiom Hold Buffer into trays" on page 87

"4: Store remaining reagents" on page 92

The reagents and trays required are as follows:

Table 39 Reagent trays required for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol on the GeneTitan™ MC Instrument

Type of tray	Part No.	Quantity	Tray designation	Master mix/reagent
384 Layout GeneTitan Stain Tray	501279	2	S1	Stain 1 Master Mix
384 Layout Axiom™ Stain2 Tray	501394	1	S2	Stain 2 Master Mix
384 Layout Axiom™ Stab Tray	501396	1	Stbl	Stabilization Master Mix
384 Layout Axiom™ Ligation Tray	501398	1	Lig	Ligation Master Mix
Scan Tray	902279	1	Scan Tray	Hold Buffer

Equipment, consumables, and reagents required

Table 40 Equipment required for Stage 5: Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan™ MC Instrument

Quantity	Equipment
1	GeneTitan MC Instrument
1	Ice bucket with ice
As Needed	Kimwipes
As Needed	Markers
1	Cooler for enzyme
1	Microcentrifuge
1	Pipet-Aid
1 each	Rainin pipettes: single channel <ul style="list-style-type: none"> • P200 • P1000 Rainin pipettes: 12-channel: <ul style="list-style-type: none"> • P200
1	Vortexer

Table 41 Consumables required for Stage 5: Manually Preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan™ MC Instrument

Quantity	Consumable	Cat. No.
As required	Aluminum foil (optional)	
1 kit ¹ includes:	Axiom™ 384HT High Volume Consumables Kit (Sufficient for 5 x Mini 96-Array Format Plates)	902629
10	• 384 Layout GeneTitan™ Stain Tray (Stain 1)	
5	• 384 Layout Axiom™ Stain2 Tray	
5	• 384 Layout Axiom™ Stab. Tray	
5	• 384 Layout Axiom™ Ligation Tray	
5	• 384 Layout GeneTitan™ Hyb Tray	
5	• 384 Layout GeneTitan™ Scan Tray	
25	• 384 Layout GeneTitan™ Scan and Stain Tray Cover	
1	Pipette, serological: 5 x 1/10 mL	
As required for pipettes listed in Table 40	Pipette tips	
5	Matrix™ 25 mL reagent reservoir	8093-11
4	15 mL conical tube	

¹ Each Axiom™ 384HT High Volume Consumable Kit is sufficient to process five Axiom Mini 96- or 384-array format plates. These trays are required for processing Axiom 384 array plates on the GeneTitan™ Multi-Channel Instrument.

Reagents required

Table 42 Axiom 2.0 reagents required for Stain and Ligation stage (for processing two Mini 96-array format plates)

Reagent	Module
Axiom Ligate Buffer	Module 4-1, -20°C Part No. 901278
Axiom Ligate Enzyme	
Axiom Ligate Soln 1	
Axiom Probe Mix 1	
Axiom Stain Buffer	
Axiom Stabilize Soln	
Axiom Ligate Soln 2	Module 4-2, 2-8°C Part No. 901276
Axiom Probe Mix 2 ¹	
Axiom Wash A	
Axiom Stain 1-A ¹	
Axiom Stain 1-B ¹	
Axiom Stain 2-A ¹	
Axiom Stain 2-B ¹	
Axiom Stabilize Diluent	
Axiom Water	
Axiom Hold Buffer ¹	
Additional Axiom Hold Buffer (1 bottle) (For processing the second Axiom Mini 96-array format plate)	2°C to 8°C Part No. 903012

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

1: Prepare for GeneTitan™ reagent preparation

Thaw and prepare the reagents

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

Table 43 Reagents required for GeneTitan™ MC Instrument reagent tray preparation

Module	Reagent	Thaw on benchtop, then place on ice	Place on ice	Place on benchtop at room temperature
Module 4-1 –20°C	Axiom Ligase Buffer ¹	✓ for 30 min		
	Axiom Ligase Enzyme	Keep at –20°C until ready to use		
	Axiom Ligase Soln 1	✓		
	Axiom Probe Mix 1	✓		
	Axiom Stain Buffer	✓		
	Axiom Stabilize Soln	✓		
Module 4-2 2 to 8°C	Axiom Ligase Soln 2			✓ for 30 to 40 min
	Axiom Probe Mix 2 ²		✓	
	Axiom Wash A			✓ for 30 min
	Axiom Stain 1-A ²		✓	
	Axiom Stain 1-B ²		✓	
	Axiom Stain 2-A ²		✓	
	Axiom Stain 2-B ²		✓	
	Axiom Stabilize Diluent		✓	
	Axiom Water			✓
	Axiom Hold Buffer ¹ (1 bottle required) ³			✓

¹ This bottle can also be thawed in a dish with room temperature Millipore water.

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

³ Axiom Hold Buffer for preparing the Scan Tray for the second plate are provided in Cat. No. 903012.

Preparing Axiom Wash A and Axiom Stabilize Diluent

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

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

To prepare the Axiom Wash A:

1. Vortex the bottle for 30 sec.
2. Place on the benchtop at room temperature for 30 min.
3. Examine the reagent for precipitate (look into the top of the bottle).
4. If precipitate is still present, vortex again for 30 sec.
5. Leave on the benchtop until ready to use.

To prepare the Stabilize Diluent:

If crystals are observed in the Axiom Stabilize Diluent:

1. Vortex and spin.
2. Look for precipitate.

If any:

- Warm tube to room temperature and vortex again.

Preparing Axiom Ligase Buffer

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

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

To prepare the Axiom Ligase Buffer:

1. Place on the benchtop at room temperature for 30 min. This bottle can also be thawed in a dish with room temperature Millipore water.
2. Examine the buffer for precipitate (look into the top of the bottle).
3. If precipitate is present, vortex the bottle for 30 sec.
4. Re-examine the buffer for precipitate.
5. If precipitate is still present, warm the bottle with your hands and vortex again for 30 sec.
6. If precipitate is still present after hand warming proceed with the protocol below.
7. Leave the Axiom Ligase Buffer on the benchtop until ready to use.

Prepare the remaining reagents

To prepare the remaining reagents for GeneTitan MC Instrument Plate Preparation:

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

Label the Master Mix tubes

1. Mark the side of each tube with one of designations shown in [Table 44](#).

Table 44 Labeling master mix tubes for stain, ligation, and stabilization reagents

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

Label the reagent reservoirs

1. Label five Matrix 25 mL reagent reservoirs (Cat. No. 8093-110) as indicated in the table below.

Table 45 Labeling reagent reservoirs

Reservoir designation	Contents
<i>S1</i>	• Stain 1 Master Mix
<i>S2</i>	• Stain 2 Master Mix
<i>Stbl</i>	• Stabilization Master Mix
<i>Lig</i>	• Ligation Master Mix
<i>Hold</i>	• Axiom Hold Buffer

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

Prepare Stain 1 Master Mix

To prepare the Stain 1 Master Mix:

1. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *S1* in the order shown in [Table 46](#). This recipe will provide enough for both *S1* reagent trays.

Table 46 Stain 1 Master Mix

Reagent	per array	Master mix 96+
To the tube marked <i>S1</i> , add:		
• Axiom Wash A	67.2 µL	7.8 mL
• Axiom Stain Buffer	1.4 µL	163 µL
• Axiom Stain 1-A	0.7 µL	81 µL
• Axiom Stain 1-B	0.7 µL	81 µL
Total	70 µL (35 µL x 2)	8.1 mL

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

Prepare Stain 2 Master Mix

To prepare the Stain 2 Master Mix:

1. Use appropriate single-channel pipettes to add reagents to the 15 mL tube labeled *S2* in the order shown in [Table 47](#).

Table 47 Stain 2 Master Mix

Reagent	per array	Master mix 96+
To the tube marked <i>S2</i> , add:		
• Axiom Wash A	33.6 µL	4.3 mL
• Axiom Stain Buffer	0.70 µL	90 µL
• Axiom Stain 2-A	0.35 µL	45 µL
• Axiom Stain 2-B	0.35 µL	45 µL
Total	35 µL	4.5 mL

2. Gently invert the *S2* tube 10 times to mix. Do not vortex.
3. Place on ice and protect from direct light (e.g., cover with aluminum foil or ice bucket lid).

Prepare Stabilization Master Mix

To prepare the Stabilization Master Mix:

1. Use appropriate single-channel pipettes to add reagents to the 15 mL tube labeled *Stbl* in the order shown in [Table 48](#).

Table 48 Stabilization Master Mix

Reagent	per array	Master mix 96+
To the tube marked <i>Stbl</i> , add:		
• Axiom Water	31.1 µL	4.0 mL
• Axiom Stabilize Diluent	3.5 µL	451 µL
• Axiom Stabilize Soln	0.4 µL	56 µL
Total	35 µL	4.5 mL

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

Prepare Ligation Master Mix

The Ligation Master Mix is prepared in two stages.

Ligation Master Mix Stage 1, begin preparing the Ligation Master Mix:

1. Place the 15 mL conical tube marked *Lig* on ice.
2. Use appropriate single-channel pipettes to add reagents to the 15 mL tube labeled *Lig* in the order shown in [Table 49](#).

Table 49 Ligation Master Mix preparation: Stage 1

Reagent	per array	Master mix 96+
To the tube marked <i>Lig</i> , add:		
• Axiom Ligate Buffer	22.1 µL	2.9 mL
• Axiom Ligate Soln 1	4.4 µL	575 µL
• Axiom Ligate Soln 2	1.1 µL	138 µL
Subtotal	27.5 µL	3.6 mL

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

Ligation Master Mix Stage2, finish preparing the Ligation Master Mix:

1. Remove the Axiom Ligase Enzyme from the –20°C freezer and place in a cooler chilled to –20°C.
2. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Lig* in the order shown in [Table 50](#).

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

Table 50 Ligation Master Mix preparation: Stage 2

Reagent	per array	Master mix 96+
• Ligation Master Mix from Stage 1	27.5 µL	3.6 mL
• Axiom Probe Mix 1	3.5 µL	460 µL
• Axiom Probe Mix 2	3.5 µL	460 µL
• Axiom Ligase Enzyme	0.53 µL	69 µL
Total	35 µL	4.6 mL

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

3: Aliquot master mixes and Axiom Hold Buffer into trays

Label the trays

1. Gather the scan tray and the stain trays and covers from the Axiom™ 384 HT High Volume GeneTitan™ Consumables Kit.
2. When preparing the hybridization and reagent trays to be loaded onto the GeneTitan MC Instrument, you will need to mark the front of each tray in a way that identifies its contents. Obtain the stain trays and label each specific tray as listed on [Table 51](#):

IMPORTANT! It is critical that you write only on the proper location of the stain/reagent trays (on the edge in front of wells A1 to F1) as illustrated in [Figure 10 on page 42](#). Do **NOT** write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and lids.

Table 51

Stain tray type	Label color	Label the tray
384 Layout GeneTitan™ Stain Tray, Part No. 501279	White	<i>S1-1</i>
384 Layout GeneTitan™ Stain Tray, Part No. 501279	White	<i>S1-2</i>
384 Layout Axiom™ Stain2 Tray, Part No. 501394	Blue	<i>S2</i>
384 Layout Axiom™ Ligation Tray, Part No. 501398	Yellow	<i>Lig</i>
384 Layout Axiom™ Stabilization Tray, Part No. 501396	Green	<i>Stbl</i>

About aliquoting reagents to trays

Stain Trays: Only fill Quadrant 1 of the stain trays with ligation, staining, and stabilization reagents.

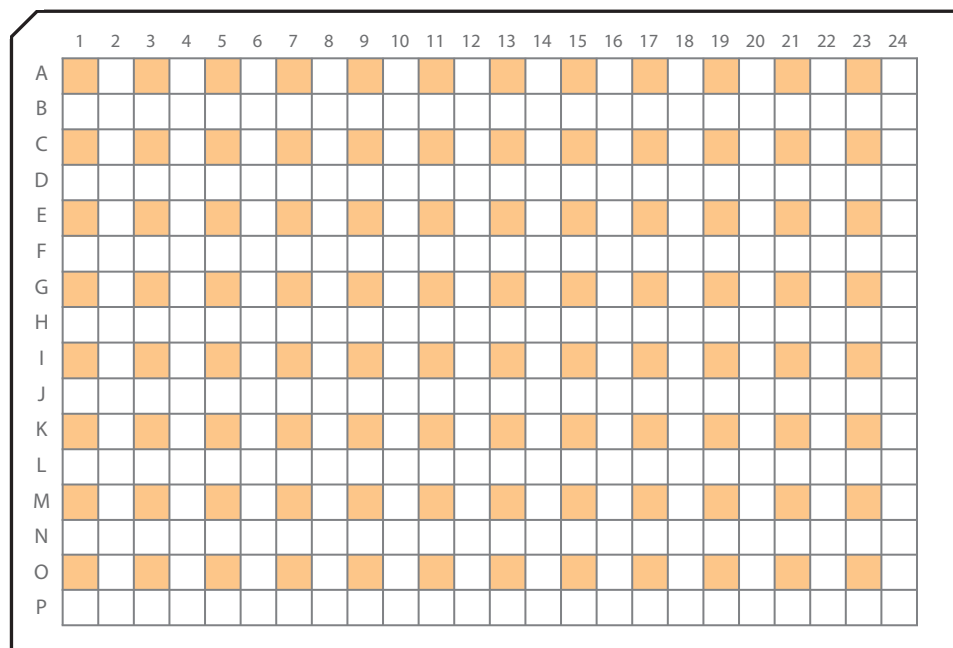


Figure 13 Quadrant 1 wells of a 384 stain tray

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

IMPORTANT! Immediately load the reagent trays onto the GeneTitan MC Instrument.

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

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

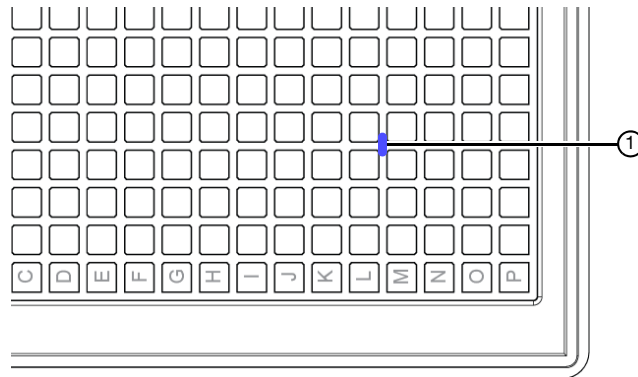
Stain 1 Master Mix

To Aliquot the Stain 1 Master Mix:

1. Pour the S1 Master Mix into the reagent reservoir marked *S1*, placed on the bench top at room temperature.
2. Load a P200 12-channel pipette with 12 new pipette tips and aliquot **35 µL per Q1 well** to both S1 trays. Dispense to the first stop only to avoid creating bubbles.
 You do not need to change pipette tips between additions of the Stain 1 Master Mix.

3. If:

- Bubbles are present, puncture them with a pipette tip.
- Droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove. (Figure 14).



① Example of a droplet of liquid that has splashed onto the well divider of a stain tray during reagent aliquoting.

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

Figure 14 Well dividers in stain trays (partial tray view)

4. Place covers on the S1 trays. Orient cover correctly on the tray with the notched corners together (Figure 15).



① Notched corners of stain tray and lid.
Notched corners should face the front.

Figure 15 Placing cover on stain tray

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

Stain 2 Master Mix

To aliquot the Stain 2 Master Mix:

1. Carefully pipet or pour the Stain 2 Master Mix into the reagent reservoir marked *S2*, placed on the bench top at room temperature.
2. Load a P200 12-channel pipette with 8 new pipette tips and aliquot **35 µL per Q1 well** to the *S2* tray. Dispense to the first stop only to avoid creating bubbles.
You do not need to change pipette tips between additions of the Stain 2 Master Mix.
3. If:
 - Bubbles are present, puncture them with a pipette tip.
 - Droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove.
4. Place a cover on the *S2* tray. Orient the cover correctly on the tray with the notched corners together (Figure 15).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Stabilization Master Mix

To aliquot the Stabilization Master Mix:

1. Carefully pipet or pour the Stabilization Master Mix into the reagent reservoir marked *Stbl*, placed on the bench top at room temperature.
2. Load a P200 12-channel pipette with 12 new pipette tips and aliquot **35 µL per Q1 well** to the *Stbl* tray. Dispense to the first stop only to avoid creating bubbles.
You do not need to change pipette tips between additions of the Stabilization Master Mix.
3. If:
 - Bubbles are present, puncture them with a pipette tip.
 - Droplets of liquid splashed onto the well dividers, blot the top of the tray with a Kimwipe.
4. Place a cover on the tray. Orient cover correctly on the tray with the notched corners together (Figure 15).

Ligation Master Mix

To aliquot the Ligation Master Mix:

1. Carefully pipet or pour the Ligation Master Mix into the reagent reservoir marked *Lig*, placed on the bench top at room temperature.
2. Load a P200 12-channel pipette with 12 new pipette tips and aliquot **35 µL per Q1 well** to the *Lig* tray. Dispense to the first stop only to avoid creating bubbles.
You do not need to change pipette tips between additions of the Ligation Master Mix.
3. If:
 - Bubbles are present, puncture them with a pipette tip.
 - Droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove.

4. Place a cover on the tray. Orient cover correctly on the tray with the notched corners together (Figure 15).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Axiom Hold Buffer

To aliquot the Axiom Hold Buffer to the scan tray:

1. Ensure that the Axiom Hold Buffer has equilibrated to room temperature. Vortex and then pour the Axiom Hold Buffer into the reagent reservoir labeled *Hold*, placed on the bench top at room temperature.
2. Remove the scan tray from its pouch.
3. Remove the scan tray cover, but leave the scan tray on its protective black base.
4. Place the cover as shown in Figure 17 on page 92 to prevent dust or static from accumulating on the bottom of the cover.
 - Use a 12-channel P200 pipette with new pipette tips to aliquot **170 µL to EACH of the 96 wells** of the 384 Layout GeneTitan Scan Tray. Dispense to the first stop and avoid touching the bottom of the tray.
 - You do not need to change pipette tips between additions of the Hold buffer.
5. If droplets of liquid splashed onto the well dividers, place a Kimwipe on top of the tray to blot and remove.
6. Cover the tray by orienting the notched corner of the scan tray cover over the notched edge of the tray and the flat side of the cover against the scan tray (Figure 16).

IMPORTANT! 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.



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

See "Stage 3: Ligate, Wash, Stain and Scan" on page 124 for instructions on loading the reagent trays.

Always leave the scan tray in its protective blue base.

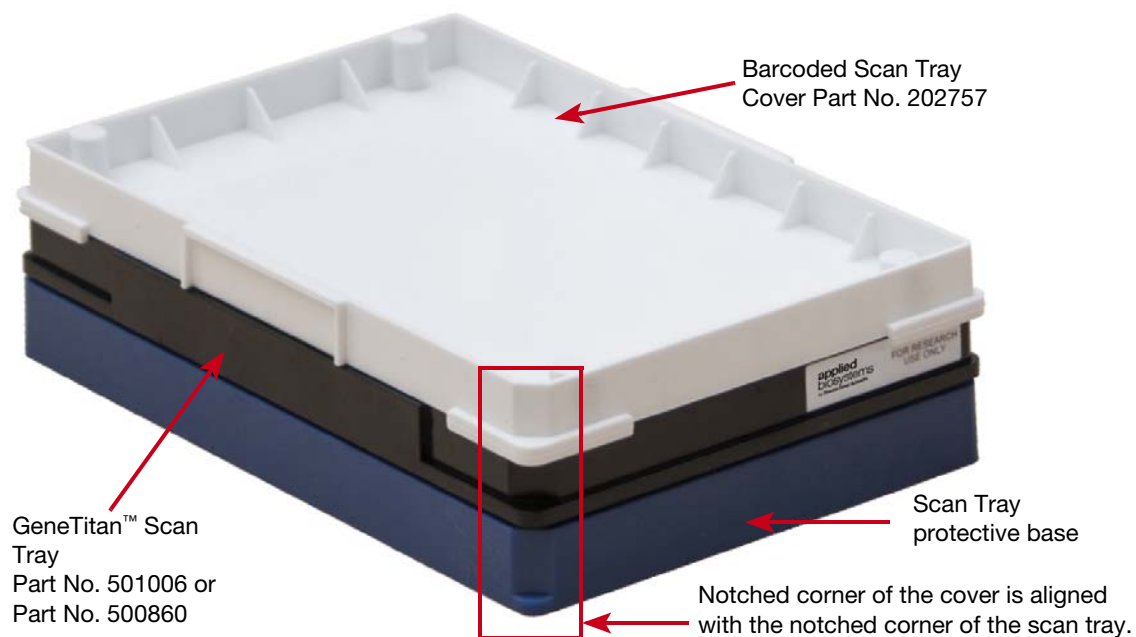


Figure 16 Scan tray with cover on the blue base

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

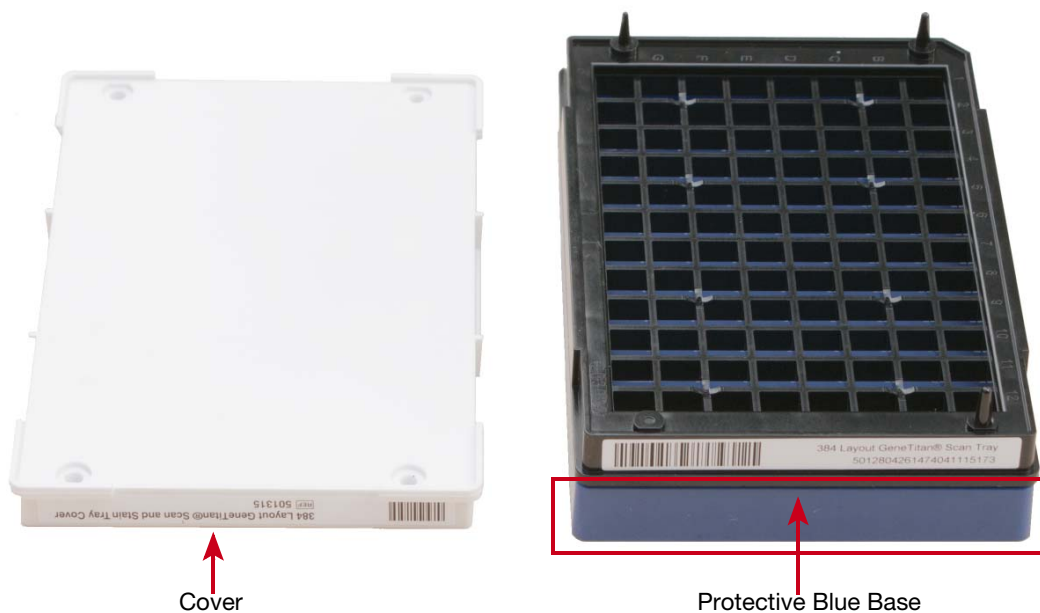
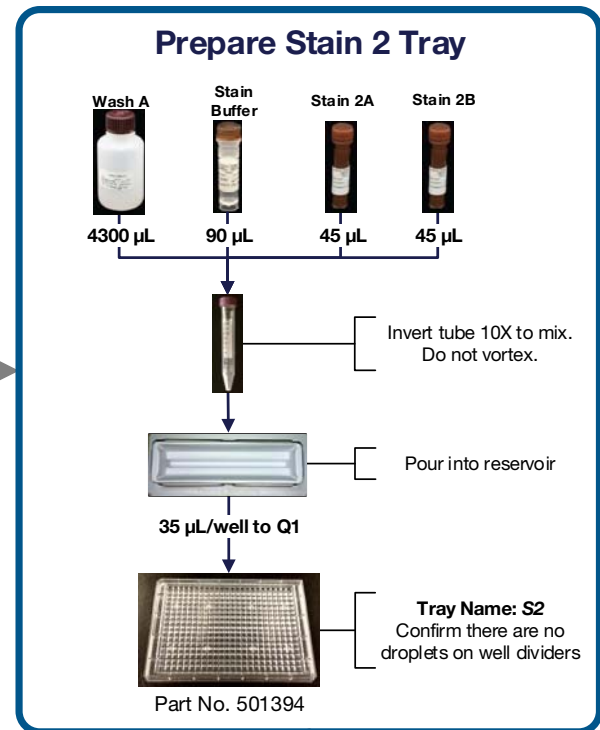
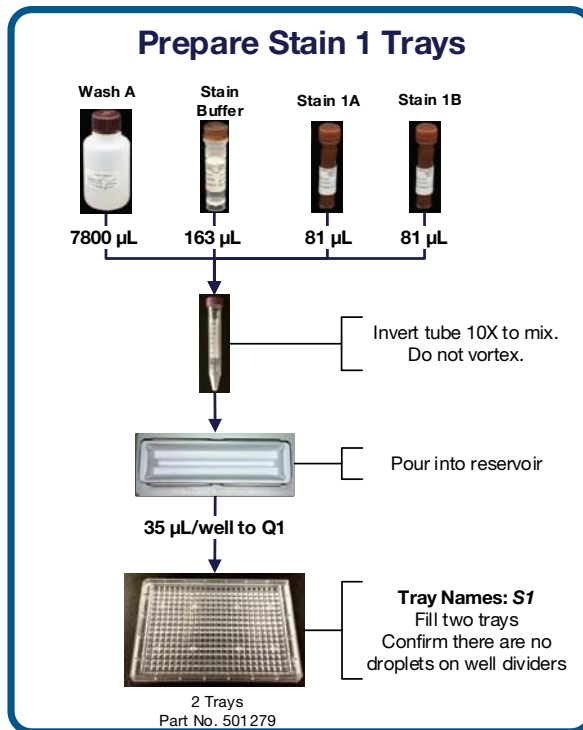
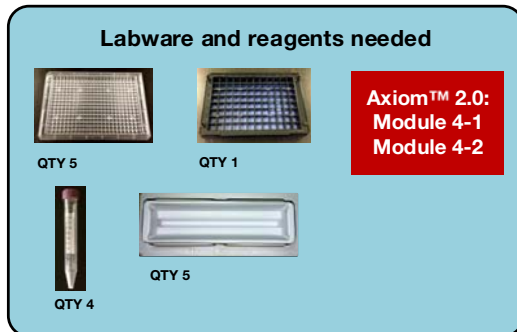


Figure 17 Loading the scan tray with axiom hold buffer

4: Store remaining reagents

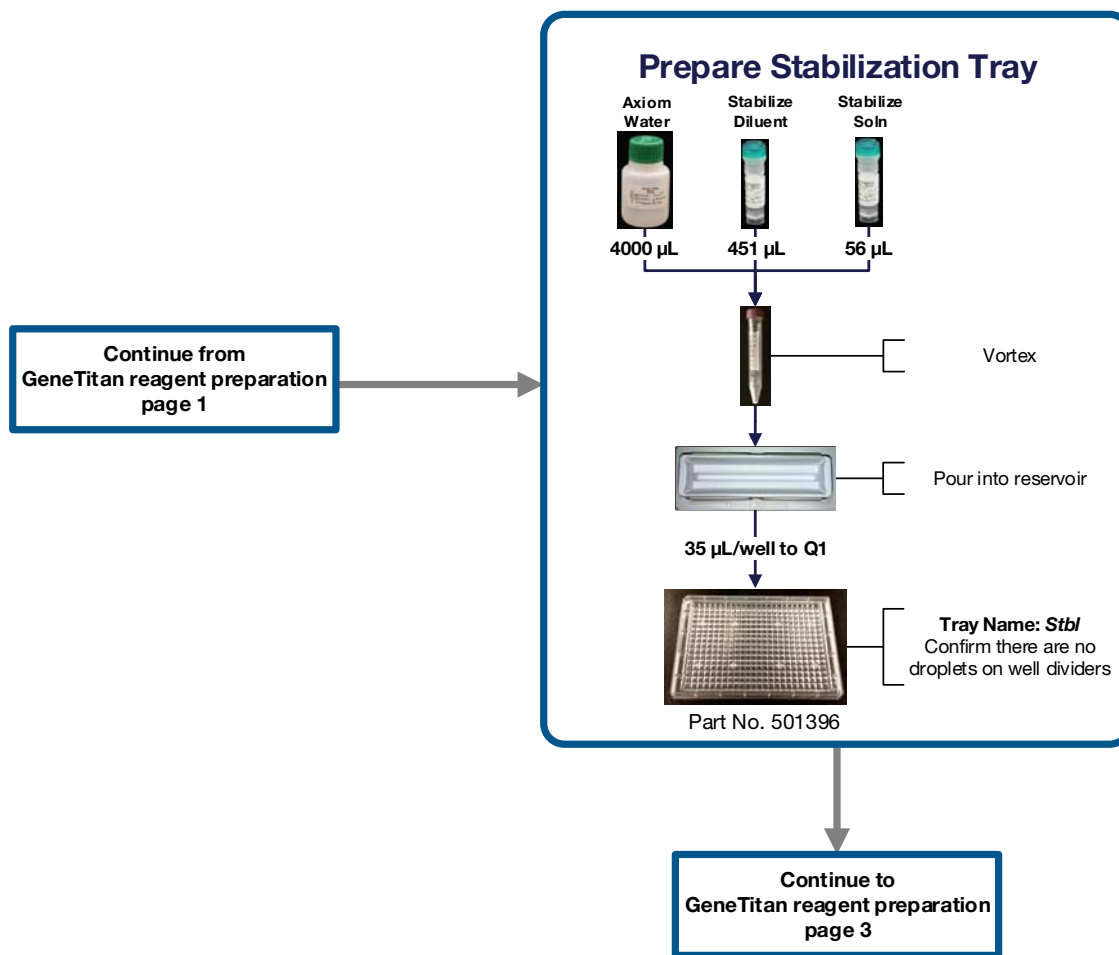
Store remaining Module 4-1 and Module 4-2 reagents for future use. Follow the guidelines presented in the section "[Freeze-thaw instructions](#)" on page 30.

Stage 5: GeneTitan reagent preparation

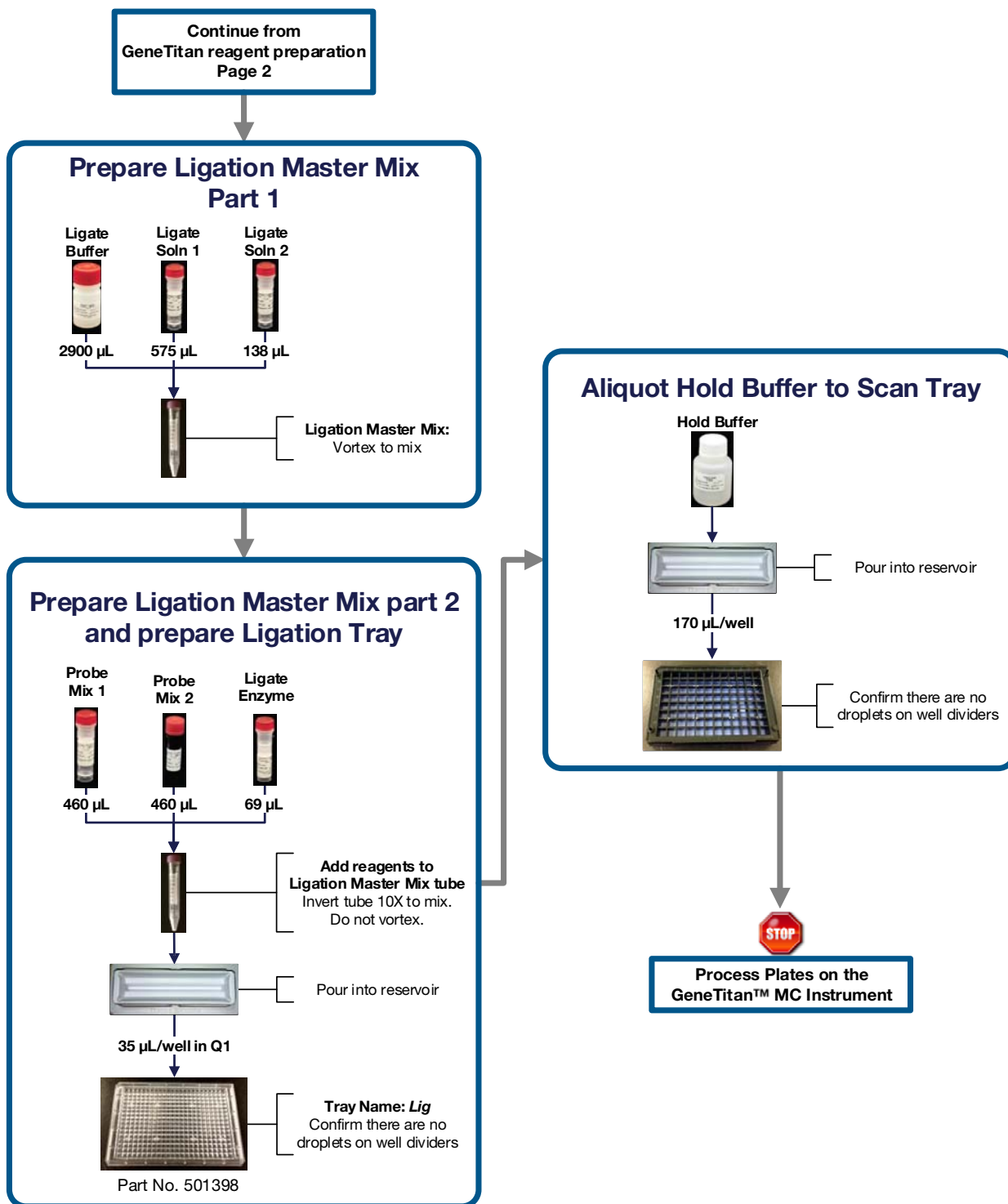


Continue to
GeneTitan reagent preparation
page 2

Stage 5: GeneTitan reagent preparation



Stage 5: GeneTitan reagent preparation



Array processing with the GeneTitan™ MC Instrument

■ Before using the GeneTitan™ Multi-Channel Instrument	96
■ Stage 1: Create and upload Batch Registration file.	106
■ Stage 2: Hybridization	107
■ Stage 3: Ligate, Wash, Stain and Scan.	124

The Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol is designed for processing 96 samples at a time on 96 arrays simultaneously. The protocol is performed in two sets of steps:

- **Target Preparation:** See [Chapter 4, "Axiom™ 2.0 Assay for Mini 96-Array manual target preparation"](#) on page 44.
- **Array Processing:** performed on the GeneTitan™ Multi-Channel (MC) Instrument.

This chapter includes instructions for Part 2: Array Processing.


Before using the GeneTitan™ Multi-Channel Instrument

Proper tray alignment and loading

Proper alignment and loading of a tray and its cover is critical when using the GeneTitan Multi-Channel (MC) MC Instrument. Each tray and cover has one notched corner. The notched corner of the tray and its corresponding cover or protective base must be in vertical alignment with each other, and placed in position A1 per the Tray Alignment guide inside each GeneTitan MC Instrument drawer ([Figure 18](#) and [Figure 19 on page 98](#)).

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

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

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

Note: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and Rinse bottles should be replaced if the software displays such a warning.

IMPORTANT! Remove the plastic protective shipping tray cover.

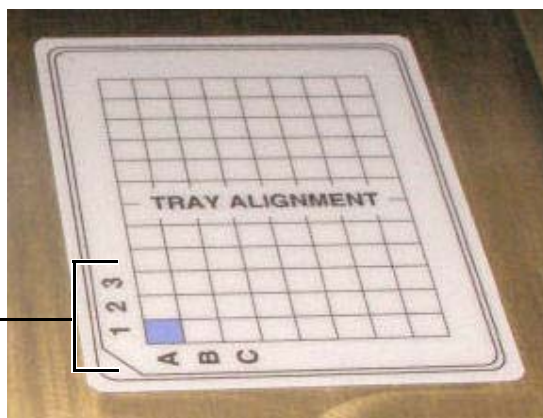
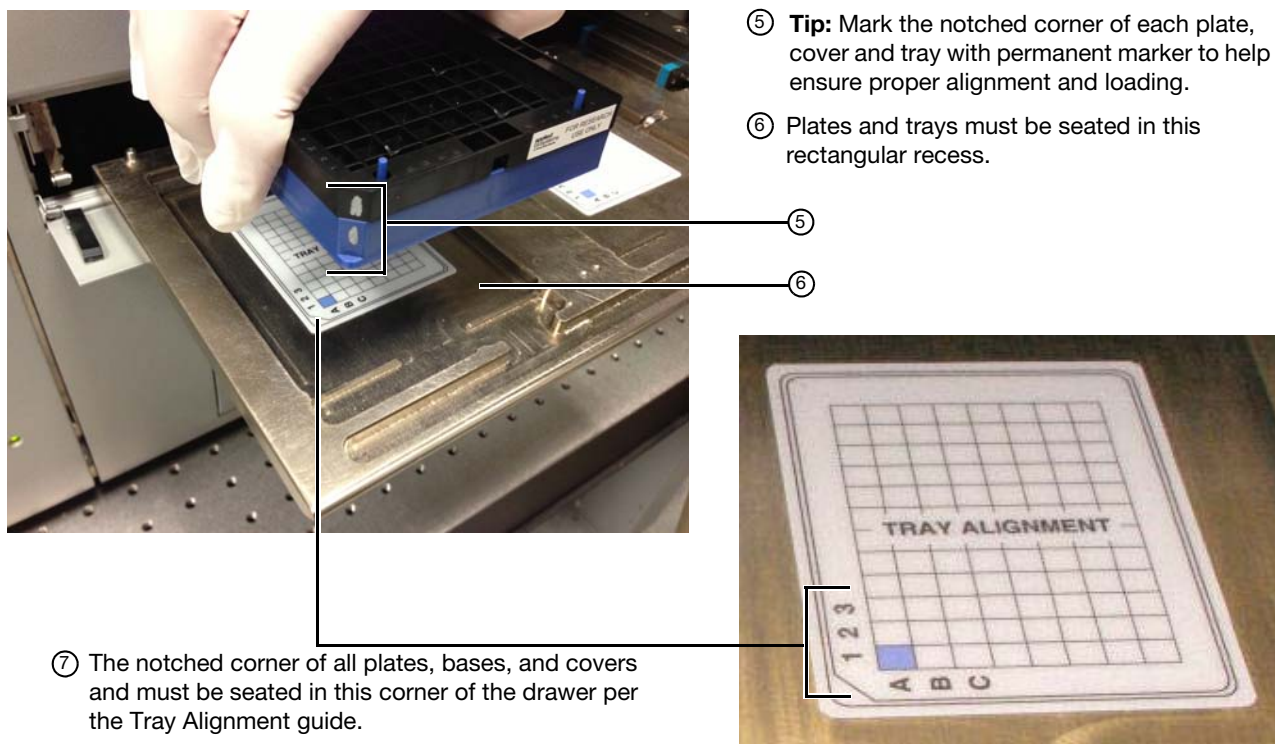
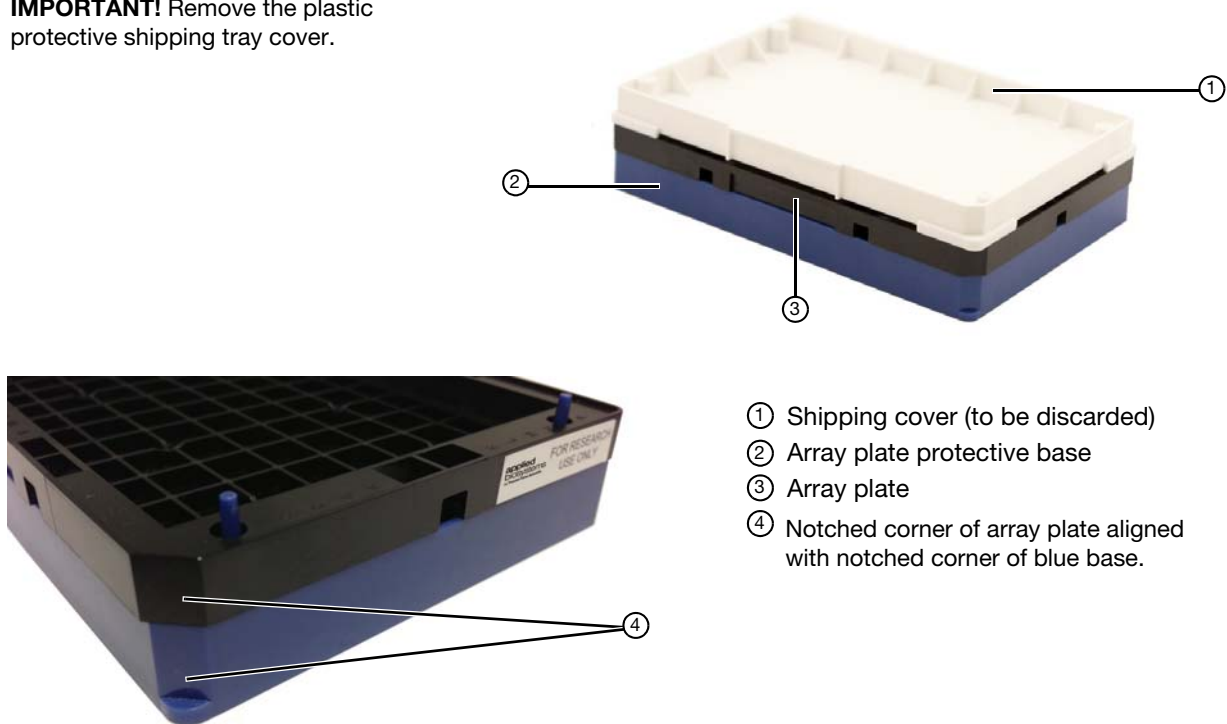
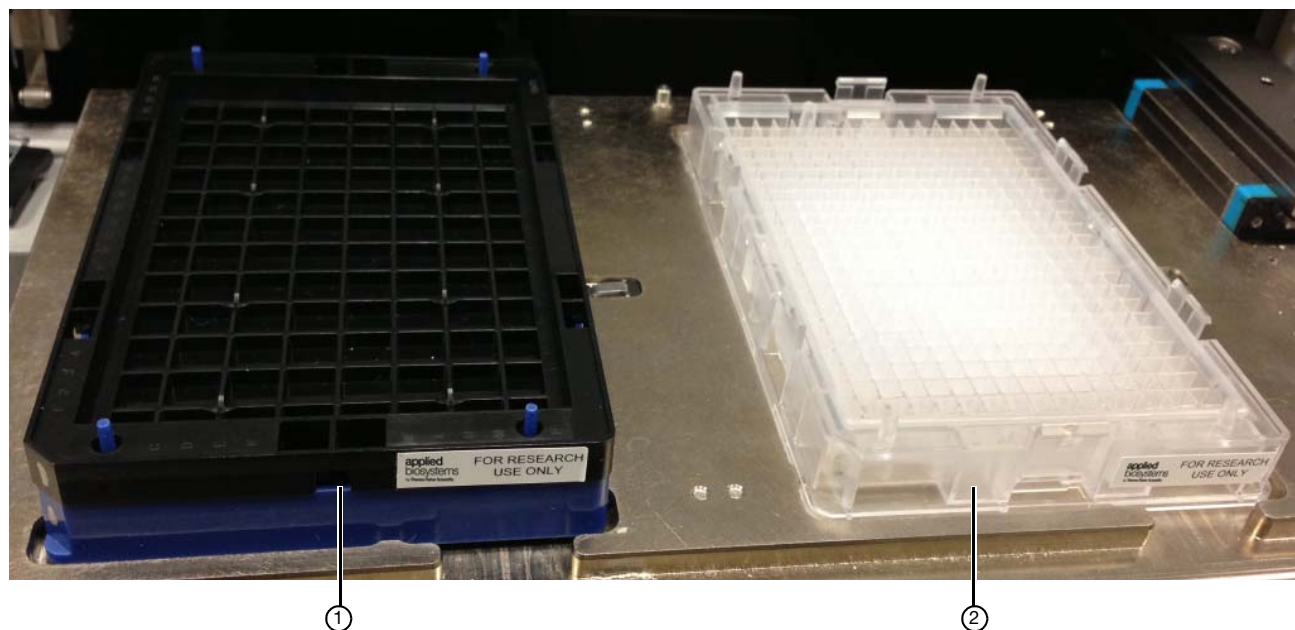


Figure 18 Proper alignment and loading of plates, covers and trays in the GeneTitan™ MC Instrument



- ① Array Plate with Protective Blue Base
- ② Hyb Tray

Figure 19 Array plate with protective blue base and the hyb tray aligned and properly loaded into drawer 6

IMPORTANT! When you install the consumables, ensure that the fingers are retracted ([Figure 20](#)). Do not lay the consumables on top of the drawer fingers - this indicates that the instrument is not functioning correctly. Please notify your Field Service Engineer if the fingers do not retract automatically. You should place the trays into the instrument drawers when a drawer is fully extended by the instrument. The fingers are retracted when the drawer is open and are extended when the drawer is closed in order to restrain the consumable.

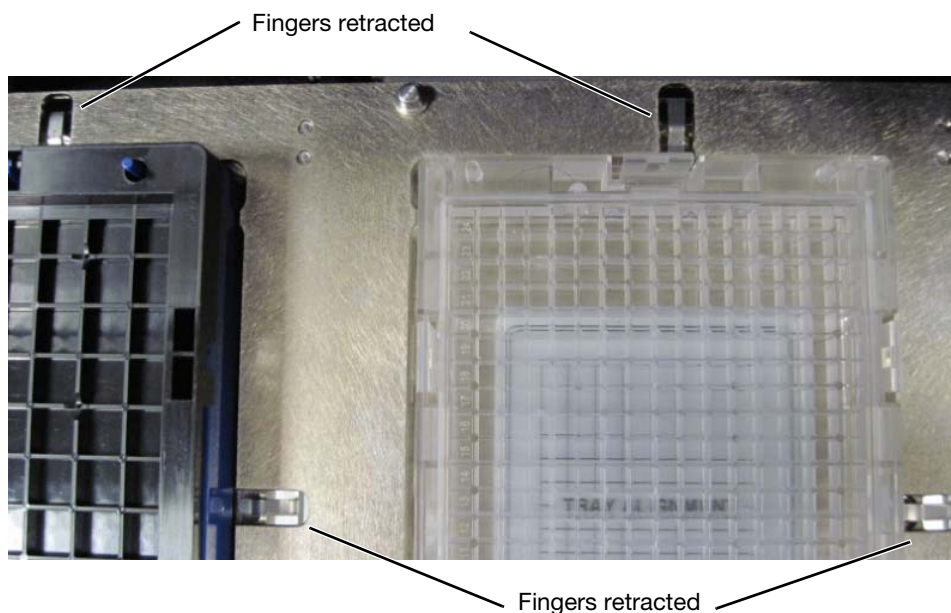


Figure 20 Fingers retracted

Stain trays and covers

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

Correct placement of cover on stain tray.



Incorrect placement of cover on stain tray.



Figure 21 Placement of covers on trays

Labeling GeneTitan hybridization and reagent trays

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

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

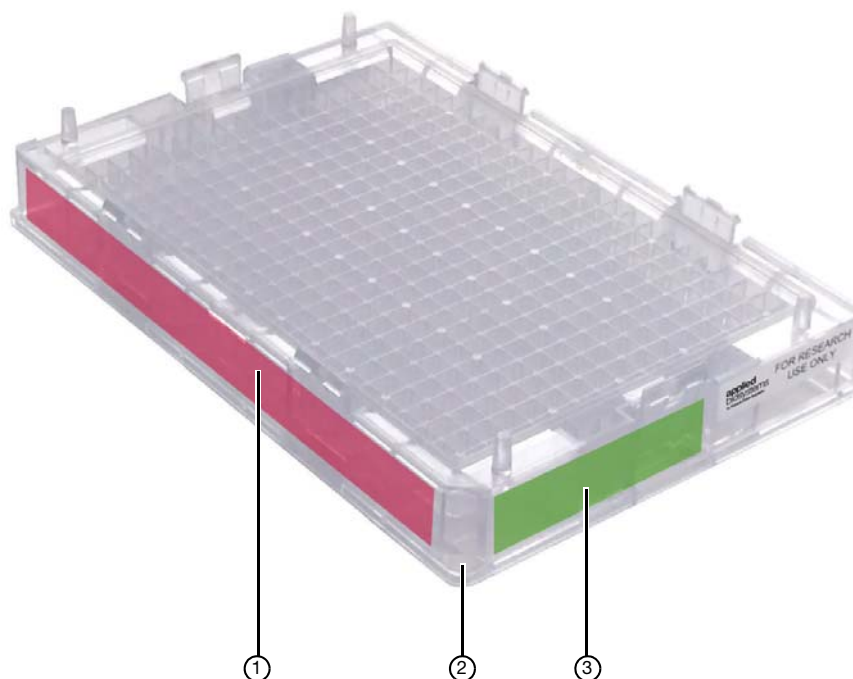
Proper labeling for hyb trays and reagent trays is described in:

- "Labeling for hyb trays", on page 100
- "Labeling for stain trays" on page 100

IMPORTANT! Do not confuse hyb trays with stain trays.

Labeling for hyb trays

You may label the hyb tray on the front part of the **short side of the tray, next to the notch at the left**, as shown in [Figure 22](#). The proper section for labeling is closest to the notched corner, corresponding to the A1 and F1 wells.



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

Figure 22 Labeling hyb trays



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

Labeling for stain trays

You may label the stain trays on the **left side of the front of the tray** as shown in [Figure 23](#). The correct side is closest to the notched corner, corresponding to the A1 through F1 wells.



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

Figure 23 Labeling stain tray (stain tray shown with lid)

Email and telephone notifications from the GeneTitan™ MC Instrument

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

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

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

- Starts
- Completes
- Aborts
- Encounters an error

GeneTitan™ MC Instrument lamp

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

The GeneTitan Instrument Control software provides a timer that indicates the remaining useful life of the bulb and notifies you when it requires replacement. It is important to adhere to the warnings specified in the *GeneTitan™ Multi-Channel Instrument User Guide*, Pub. No. 08-0308.

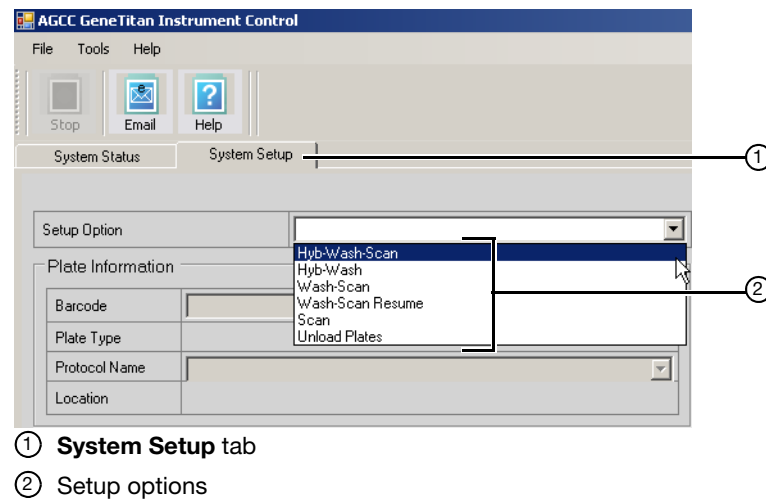
Refer to the *GeneTitan™ MC Instrument User Guide* for the Lambda LS and Smart controller system. The Lamp and the controller should NEVER be switched ON or OFF manually. The GeneTitan MC Instrument control software manages the lamp activity and will switch the lamp ON and OFF as required. It takes 10 minutes to warm-up the lamp. In idle mode the lamp will remain ON for 2 hours before it is automatically

switched OFF and if there are no more plates being transferred from the fluidics to the imaging station. This is by design and is intended behavior. Please do not try to save the lamp life by turning OFF the switch on the lamp.

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

Setup options for array plate processing

The processes (setup options) available for processing array plates are shown in [Figure 24](#). A brief description of each option is given below.



① **System Setup** tab

② Setup options

Figure 24 Setup options for processing array plates

Hyb-Wash-Scan

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

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

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

Note: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and Rinse bottles should be replaced if the software displays such a warning.
- *Scan*: The array plate is moved to the imaging device in the GeneTitan MC Instrument and each array is scanned.
 - Duration for 96 samples = ~1.5 hr

Hyb-Wash

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

If the array plate cannot be scanned immediately after the Hyb-Wash process is complete:

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

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

When ready to scan the array plate:

1. Keeping the plate protected from light, bring the plate to room temperature for ~50 min.
2. Remove the aluminum foil and load onto the GeneTitan MC Instrument.

Wash-Scan

Use this option if:

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

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

Wash-Scan Resume

Use this option if:

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

Scan

Use this option:

- To rescan an entire array plate or specific arrays on a plate that failed to scan for reasons such as bubbles or gridding failure.
- If you have hybridized and performed the fluidics processes on a different GeneTitan MC Instrument than the one you will currently use for the scan, or at a different time.
- If you want to queue a second plate for scanning. Using the Scan option allows you to start a second scan workflow while another scan workflow is already running. See ["Queuing a second plate for scanning" on page 120](#).

Unload plates

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

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

Aborting a process

If necessary, you can abort the processing of one or more array plates. Instructions and an example are shown below in [Figure 25](#).

If the instrument aborts a process, you can retrieve the array plate and related consumables as described in [Figure 25](#). An instrument-initiated abort may occur:

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

To abort array plate processing:

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

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

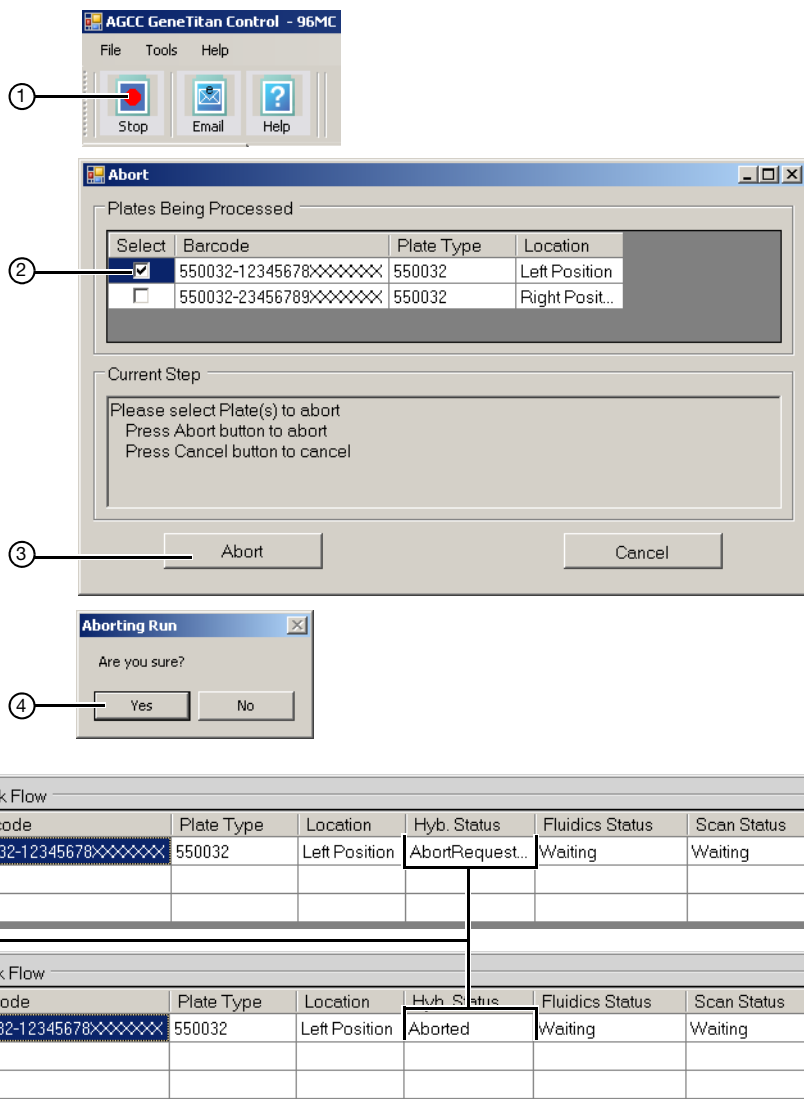


Figure 25 Manually aborting an array plate

Stage 1: Create and upload Batch Registration file

You must create and upload a Batch Registration file in the AGCC software before you begin "[Stage 2: Hybridization](#)" on page 107 (example shown in [Figure 26](#)). This file contains information critical for:

- Data file generation during scanning
 - Tracking the experimental results for each sample loaded onto an array plate
1. If you have not already created a batch registration file, create one now. (See [Appendix D, "Registering samples in GeneChip™ Command Console™"](#) on page 170 for detailed instructions.)
 2. In AGCC, select the array plate format (384 samples) and open a GeneTitan batch registration file template.
 3. Scan the array plate barcode into the yellow barcode field, column F.
 4. Enter a unique name for each sample and any additional information.
 5. Scan the barcode of the hybridization tray if your batch registration file template includes a column for the hybridization tray barcode.
 6. Save the file.
 7. Upload the file.

Note: When creating the sample registration file, you have the ability to scan the barcode of the hybridization tray to implement sample traceability. If you do not upload your samples before scanning the array plate barcode, the software will assign names to your sample.

IMPORTANT! It is very important to create and upload a batch registration file with your sample information prior to starting "[Stage 2: Hybridization](#)" on page 107.

	A	B	C	D	E	F	G
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array Position	Barcode	Sample File Name
2		Default	384_AIMS-96	384_AIMS	A01	5504144288441041117925	
3		Default	384_AIMS-96	384_AIMS	A03	5504144288441041117925	
4		Default	384_AIMS-96	384_AIMS	A05	5504144288441041117925	
5		Default	384_AIMS-96	384_AIMS	A09	5504144288441041117925	
6		Default	384_AIMS-96	384_AIMS	A11	5504144288441041117925	
7		Default	384_AIMS-96	384_AIMS	A13	5504144288441041117925	
8		Default	384_AIMS-96	384_AIMS	A15	5504144288441041117925	
9		Default	384_AIMS-96	384_AIMS	A17	5504144288441041117925	
10		Default	384_AIMS-96	384_AIMS	A19	5504144288441041117925	
11		Default	384_AIMS-96	384_AIMS	A21	5504144288441041117925	
12		Default	384_AIMS-96	384_AIMS	A23	5504144288441041117925	
13		Default	384_AIMS-96	384_AIMS	C01	5504144288441041117925	
14		Default	384_AIMS-96	384_AIMS	C03	5504144288441041117925	
15		Default	384_AIMS-96	384_AIMS	C05	5504144288441041117925	
16		Default	384_AIMS-96	384_AIMS	C07	5504144288441041117925	
17		Default	384_AIMS-96	384_AIMS	C09	5504144288441041117925	

Figure 26 Example of a Batch Registration file for an array plate

Stage 2: Hybridization

Reagents required

Reagents required

Table 52 Reagents required from the Axiom™ 2.0 Assay Mini 96 Reagent Kit

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

- An Axiom Mini 96-Array Format Plate is required for this step. Prior to inserting this plate into the GeneTitan MC Instrument for hybridization, the array plate should be brought to room temperature as described below:

1. Warm up the array plate on the benchtop before setting up hybridization on the GeneTitan MC Instrument.
 - a. Remove the array plate box from the 4°C refrigerator where it is stored.
 - b. Open the box and remove the pouch containing the array plate and protective base.



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

- c. Leave the array plate in the pouch, unopened but placed on the bench for a minimum of 25 minutes before opening and loading on the GeneTitan MC Instrument to allow the plate to come to room temperature.
 - d. At the end of the array warm up time, open the pouch and scan the array plate barcode into the Batch Registration file (see "[Stage 1: Create and upload Batch Registration file](#)" on page 106).
- A hybridization tray containing denatured samples is also required for this step. The denatured samples should be transferred to the hyb tray only after the GeneTitan MC Instrument is ready for loading the hyb tray in the "[Load Axiom™ array plate and hyb tray onto the GeneTitan™ MC Instrument](#)" section on page 112.

Setup the instrument

To setup the instrument:

1. Launch AGCC Launcher and select **AGCC GeneTitan Control** (Figure 27).

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

Note: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and Rinse bottles should be replaced if the software displays such a warning.

IMPORTANT! Please do not close the scanner application by right-clicking on it and choosing the **Close** option. This will cause the scanner application to exit abnormally and cause undue delay in processing the next plate. The correct way to close the application is described in "Shutting down the GeneTitan™ MC Instrument" on page 135.

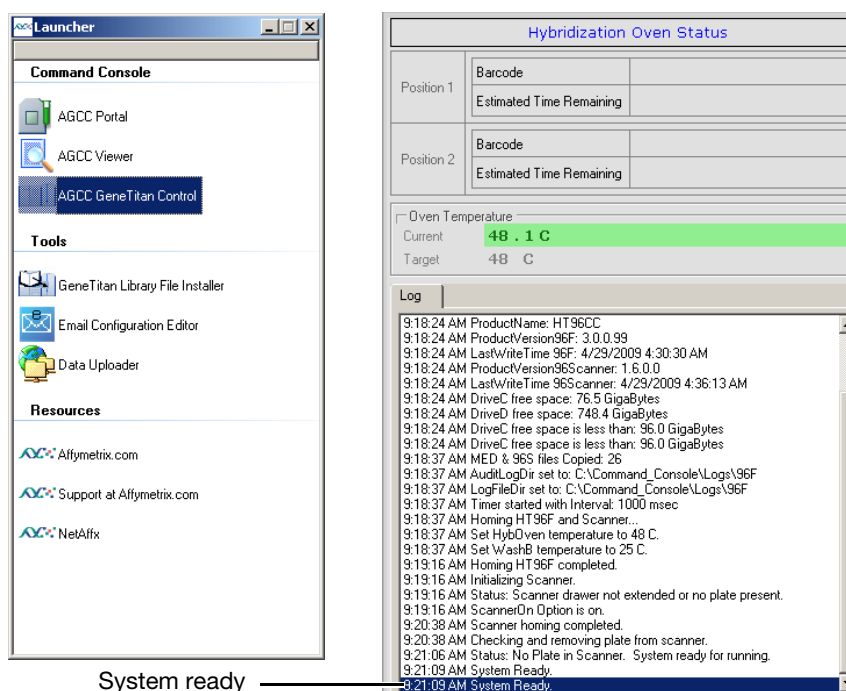


Figure 27 Launching AGCC and initializing the GeneTitan™ MC Instrument

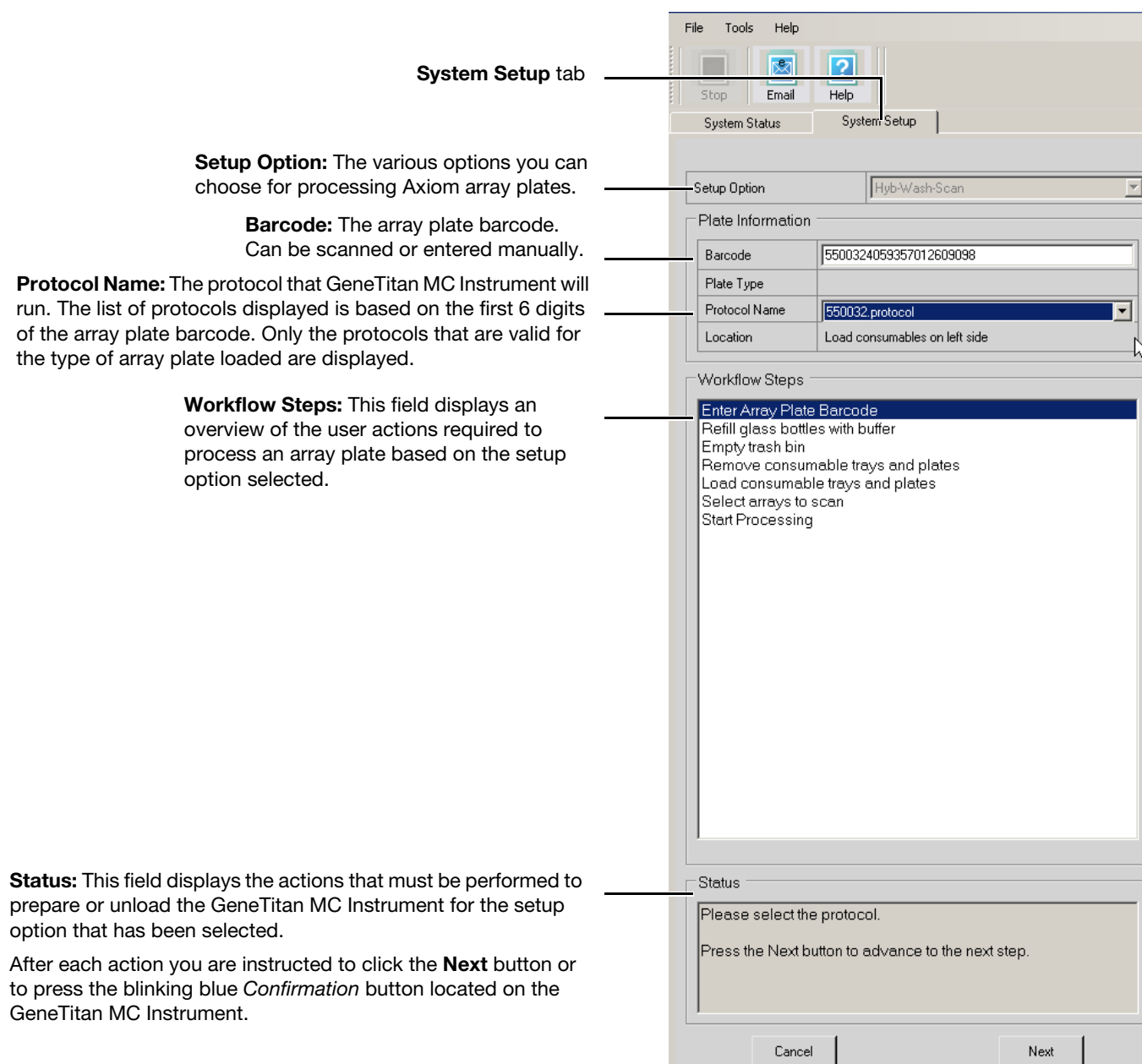


Figure 28 System Setup tab and the information displayed in this pane

2. Select the **System Setup** tab (Figure 28).
3. Configure the software as follows:
 - e. Setup Option: **Hyb-Wash-Scan**
Other options available are described under "Setup options for array plate processing" on page 102.

f. Click **Next**.

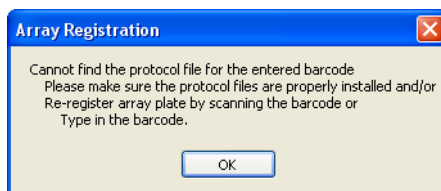
Note: If there is not enough disk space, a message is displayed.

- Delete or move .dat files to another location to free up enough disk space for the data that will be generated by eight Axiom array plates.
- One Axiom Mini 96-array format plate requires ~7 GB

g. Plate Information:

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

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



If this error message is displayed:

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

Figure 29 Barcode Error Message

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

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

4. Complete the remaining workflow steps as follows:

a. **Refill bottles with buffer** (Figure 30 on page 111)

Fill these bottles:

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

IMPORTANT!

- Always ensure that the GeneTitan bottles containing Wash A and Rinse are above the 50% mark when setting up the system to process an Axiom™ 2.0 Assay Mini 96 Reagent Kit should be emptied into the GeneTitan Wash B bottle when setting up the system to process a plate. This ensures that the GeneTitan Wash B bottle is filled to more than the requisite 35% of Wash B bottle volume. Also, do not overfill the bottles. Fill Wash Buffer B and Rinse bottles to the 1L mark only. Wash A keep at 2L. We strongly recommend refilling these bottles every time you are prompted to do so. If the volume in any of these bottles becomes too low during a run, a message is displayed. However, even if you fill the bottle at this time, the instrument may not be able to successfully complete the step that was in progress.
- Wash B: If you intend to load two array plates on the same day, fill the Wash B bottle to the 1L mark (use both bottles from the Axiom™ 2.0 Assay Mini 96 Reagent Kit).

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

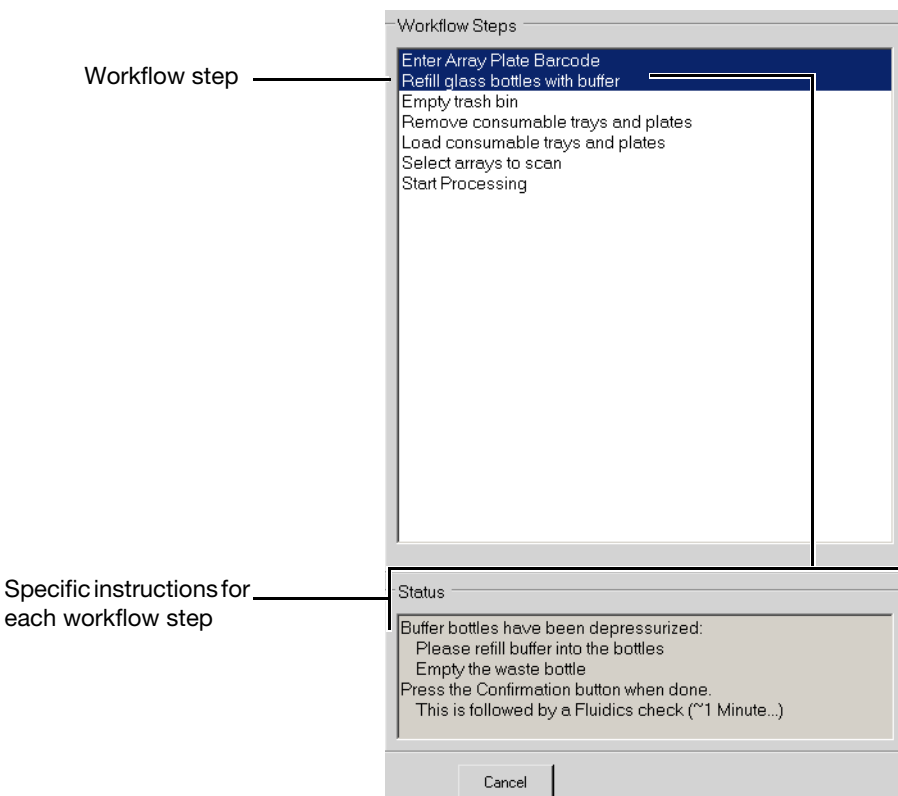


Figure 30 Example of the remaining workflow steps

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

- e. **Remove consumable trays and plates**
 - Remove used trays and plates when drawers open.
 - If no consumables to remove, the Status window reads “Drawers are empty.”
 - Press the **Confirmation** button to continue.
- f. Continue to "[Load Axiom™ array plate and hyb tray onto the GeneTitan™ MC Instrument](#)" on page 112.

Procedure to clamp a Mini 96-array format plate to hybridization tray

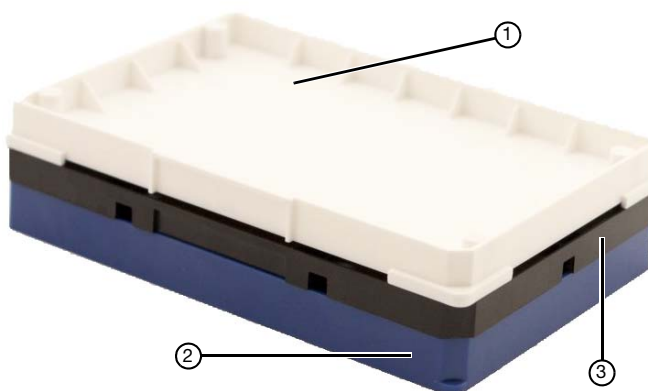
Note: Follow the procedure in [Chapter 5, "Stage 2: Hybridization"](#) of this manual to initiate the hybridization step. Once the AGCC software prompts the user to load the array plate and hybridization tray onto GTMC, follow the procedure below to complete this task.

Load Axiom™ array plate and hyb tray onto the GeneTitan™ MC Instrument

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

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

- b. Load the uncovered hyb tray on the right side of the drawer ([Figure 32](#)).
- c. Remove the array plate and protective blue base from its package.
 To avoid dust or other damage, leave the array plate packaged until ready to load onto the GeneTitan MC Instrument. The array plate must be loaded on its protective blue base, as shown in [Figure 32](#) below. The white plastic cover on top of the array plate SHOULD NOT be loaded in the GeneTitan MC Instrument ([Figure 31](#)).
- d. Load the array plate **with the protective blue base** on the left side of the drawer ([Figure 32](#)).



- ① Shipping cover (to be discarded)
- ② Array plate protective base
- ③ Array plate

Figure 31 Array plate packaging

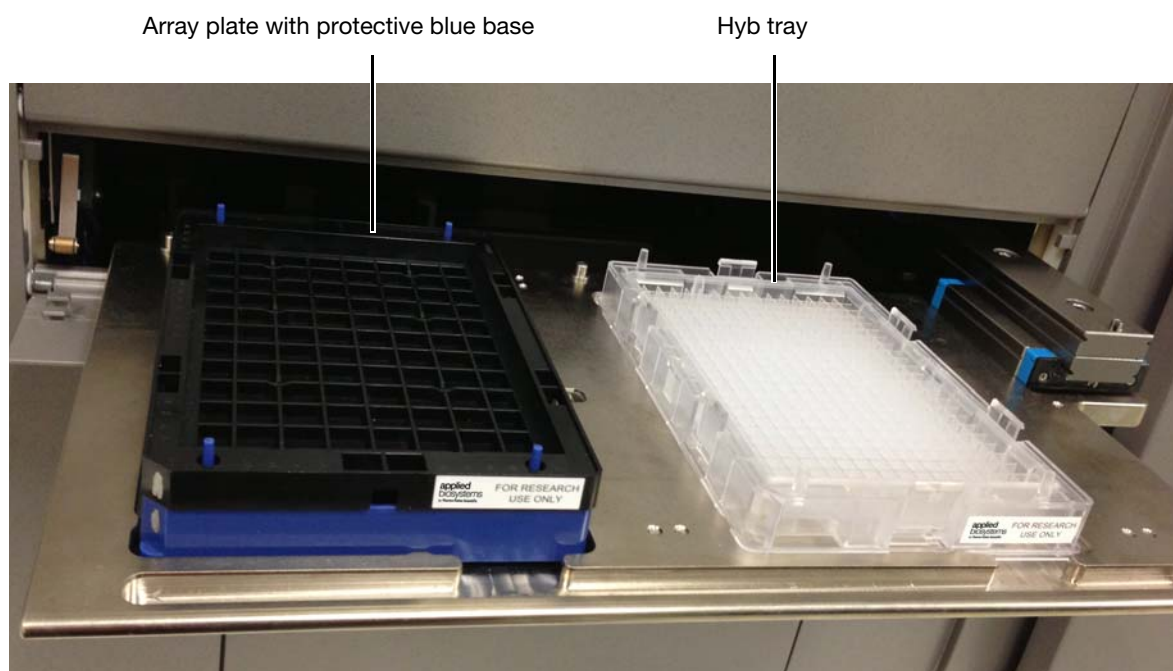


Figure 32 Array plate with protective blue base and the hyb tray properly loaded into drawer 6

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



CAUTION! The notched corner of each plate, cover and tray must be aligned as indicated by the Tray Alignment guide in the drawer.

The error message shown in [Figure 33](#) may be displayed. Plate barcodes must face the internal barcode reader (back of the drawer). Improper tray positioning can cause the GeneTitan MC Instrument to crash, and can result in substantial damage to the instrument and loss of samples.

- e. Press the **Confirmation** button on the GeneTitan MC Instrument.

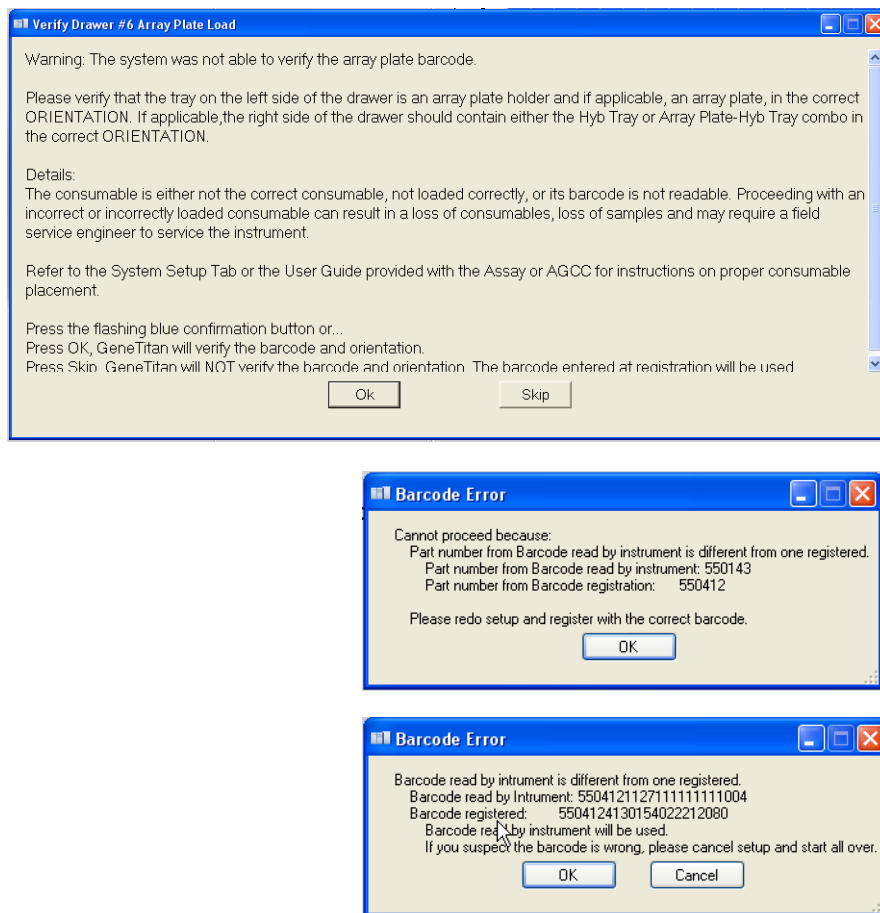
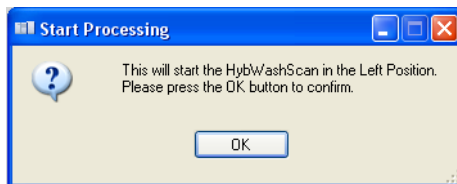


Figure 33 Barcode error messages

When you load the array plate on the left side of the drawer, the internal bar code reader reads the barcode of the array plate and compares it with the barcode and the plate type specified in the **Barcode** and **Plate Type** fields on the Setup page. If the information is correct, the application allows you to proceed to the next step. If the instrument is unable to read the barcode, it will push the tray out and will prompt ([Figure 33](#)) you to load the correct plate with the proper orientation into the instrument ([Figure 32](#)).

- Check the loading of the array plate and click **OK** to retry; or
 - Click **Skip** if the instrument has problems reading the barcode and after verifying that the trays have been placed in the proper orientation.
- f. Select the arrays to scan. By default, all arrays are selected.

- Click **Next**, then click **OK** in the Start Processing dialog box to begin processing the samples (Figure 34).



Click **OK** to confirm that you wish to proceed with hybridization.

The plate stack is in the left position (the left side of the drawer).

Figure 34 Click OK to Start Processing the First Array Plate and Hyb Tray

The array plate is placed on top of the hyb tray (now referred to as the plate stack). The software starts the process for placing the array plate on to the hybridization tray. Press **OK** on the dialog shown in Figure 35 and wait for the drawer to open completely before retrieving the array plate and hybridization tray combo for manual clamping and inspection. The sandwich of the array plate and hybridization tray needs to be manually clamped and inspected before the array processing can begin. Once clamping is complete the dialog shown in Figure 36 on will be displayed. If you do not press OK in Figure 35 the dialog box will go away without intervention and Figure 36 will be displayed.

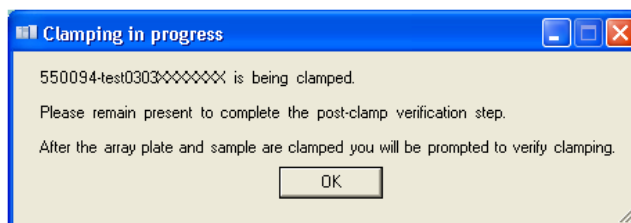


Figure 35

- When drawer 6 opens and the prompt in Figure 36 is displayed:

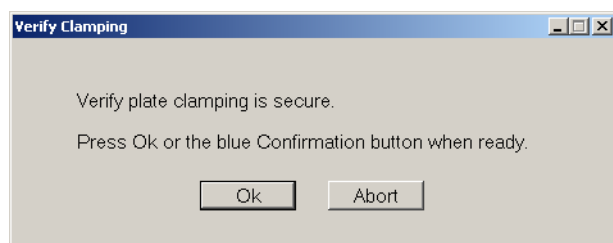
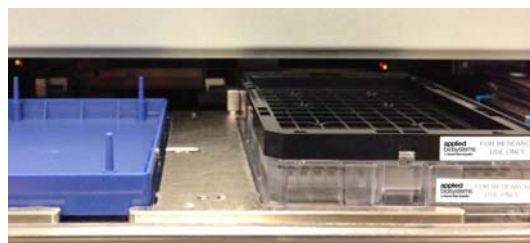
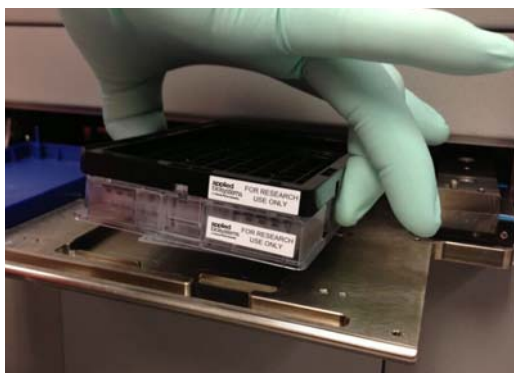


Figure 36



CAUTION! At this stage, the array plate does not latch securely to the hyb tray. DO NOT grip only the array plate to remove the plate stack from the drawer of the GeneTitan MC Instrument.

4. Follow the sequence of events in [Figure 37](#) to clamp the array plate securely to the hyb tray.



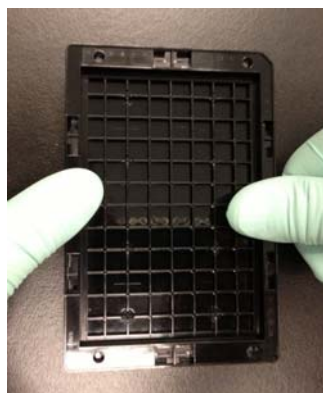
- 1 Grip the **body** of the hyb tray by hand then remove the plate stack from drawer 6 right location of the GeneTitan MC Instrument.



- 2 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.



- 3 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 and stopped.



- 4 While resting on the flat surface, rotate the plate stack 90° clockwise direction. 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 and stopped.

Figure 37 Array Plate/Hyb Tray Clamping Procedure

- g. Verify the plate stack to ensure the array plate is securely clamped to the hybridization tray. Press the array plate downward following the positions specified in Figure 38. No clicking sound indicates proper clamping.

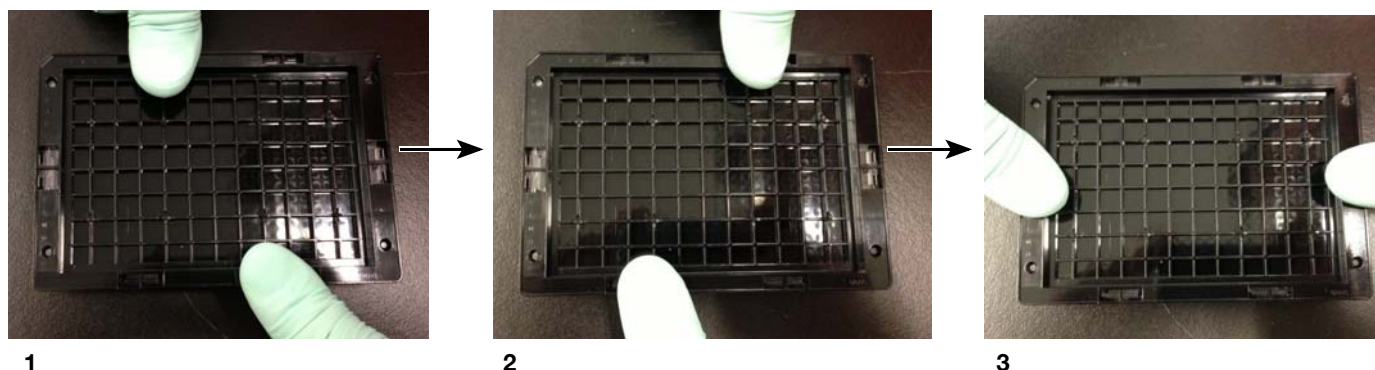


Figure 38 Clamping verification procedure

- h. **Keeping the plate level**, inspect the bottom of the plate stack for bubbles under the arrays—**do NOT invert the plates**.
- i. If bubbles are present, gently tap the plate until the bubbles move out from under the arrays—**do NOT unclamp the plate stack**.
- j. Return the plate stack to the drawer with the notched corner facing you, and press the **Confirmation** button to proceed.

The message in Figure 39 may be displayed again if plate orientation is incorrect or if the hybridization tray barcode cannot be read.

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

- k. Click **OK** to proceed.

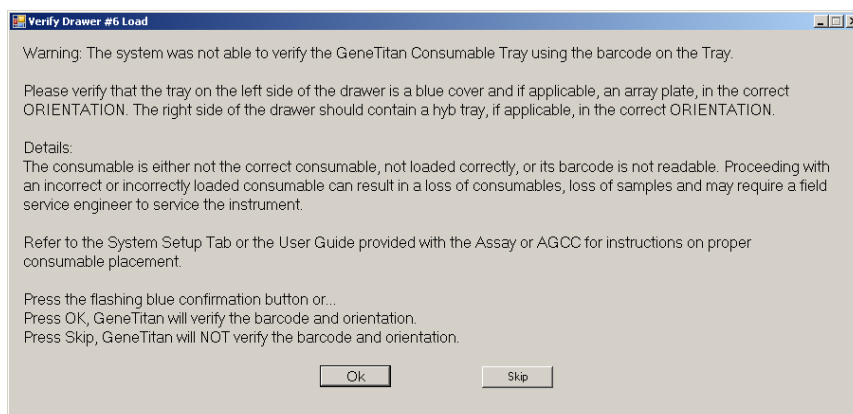


Figure 39 Verification message

5. Proceed to "Load a second Axiom™ array plate and hybridization tray onto the GeneTitan™ MC Instrument" on page 118.

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

When you can load a second array plate and hyb tray

Once processing begins, you have a specific period of time during which you can load another Axiom array plate and hyb tray. This period of time is displayed above the Hyb Oven Status pane (Figure 40). You cannot load another hyb tray before or after this period of time.

IMPORTANT! You must load the next array plate and hyb tray during the period of time displayed above the Hyb Oven Status. You cannot load another hyb tray before or after this period of time. You will have to wait until the current process is finished which will result in disruption of the eight plate workflow and fewer than eight plates processed per week.

Note: While the first plate is in the oven, you can load another plate if the time spacing requirement is met. This is to ensure that the second plate does not have to wait for system resources in its workflow. The time spacing is roughly equal to the longer of the scan time of the first plate (up to ~7.5 hrs.).

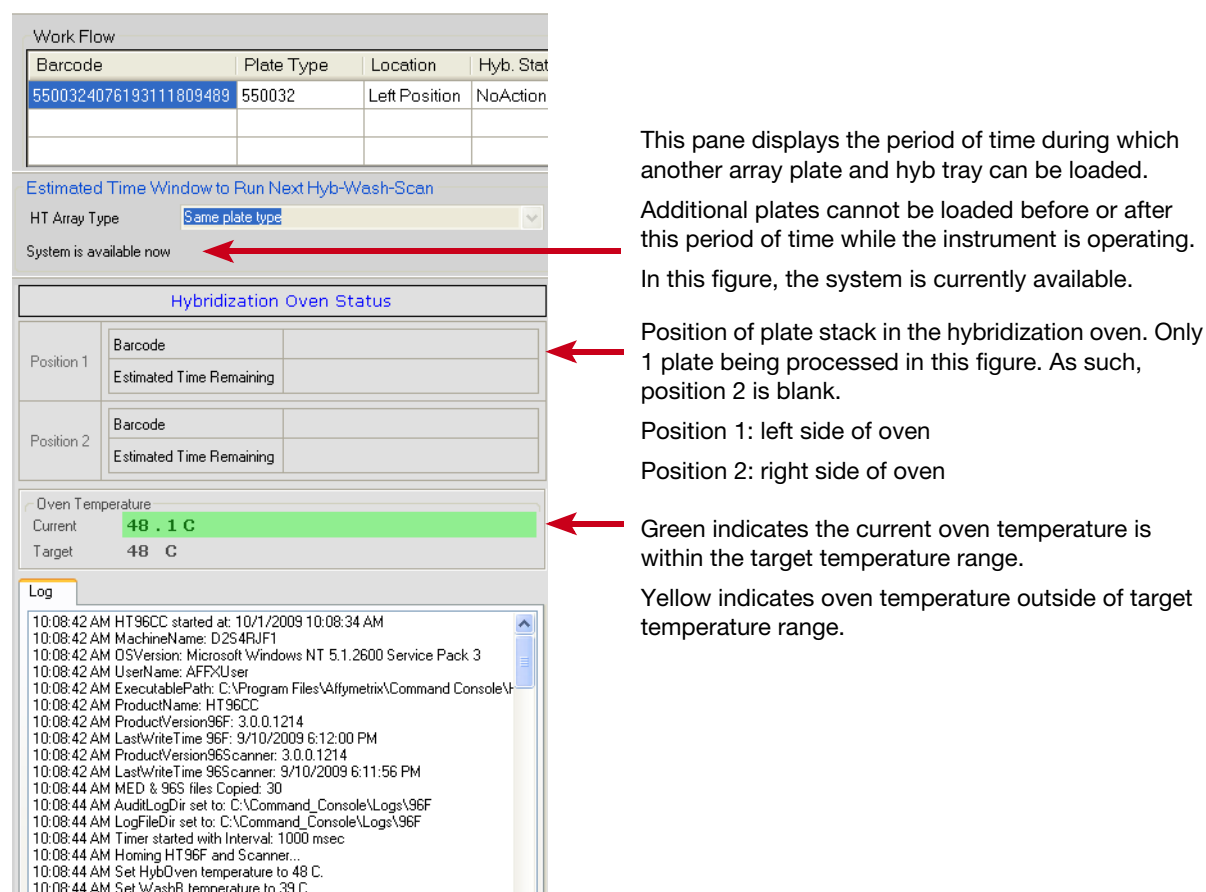


Figure 40 Loading a Second hyb tray based on hybridization oven status information

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

- b. Load the Axiom array plate **with the blue base** and the hyb tray **without the cover**, then press the Confirmation button.
- c. Select the arrays to scan, then click **Next**.
- d. Ensure that the plates are clamped securely when prompted, then press the **Confirmation** button.
- e. Click **OK** when prompted to resume plate processing (Figure 41).

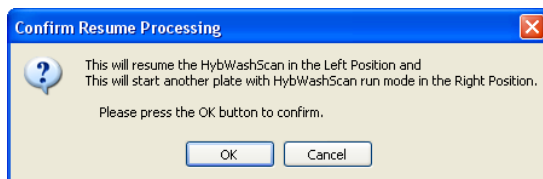


Figure 41 Confirm resume processing prompt

Select the System Status tab to view Axiom array plate status in the WorkFlow window (Figure 42).

System Status

System Setup

Work Flow

Barcode	Plate Type	Location	Hyb. Status	Fluidics Status	Scan Status	Estimated Completion Time
5500324059357012609098	550032	Left Position	Running	Waiting	Waiting	5/4/2009 11:20:42 AM
550032-plate2XXXXXX	550032	Right Posit...	Running	Waiting	Waiting	5/4/2009 10:25:36 AM

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

Figure 42 Example of the workflow window when two plates are loaded and are in the hybridization oven

Queuing a second plate for scanning

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

1. Start the first Scan workflow in the GeneTitan Instrument. Wait until the first plate is loaded into the imaging device and starts scanning.
2. Go to the **System Setup** tab and select **Scan** from **Setup Option** drop-down list (Figure 43).
 The Setup Option drop-down list is active only after the first plate begins scanning.

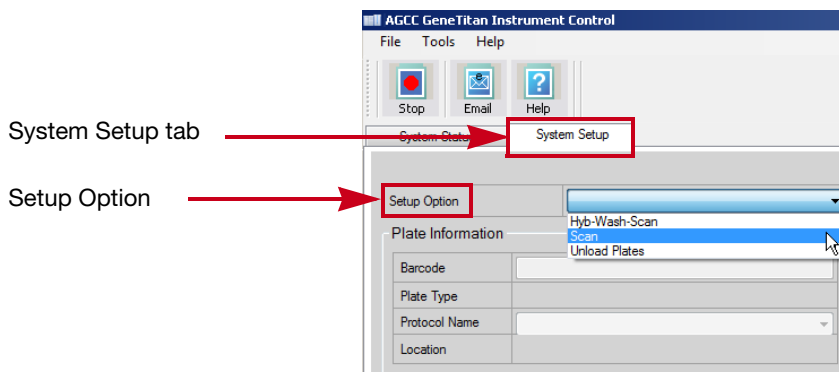


Figure 43 Scan setup option for processing a second array plate

3. Click **Next** in the lower left section of the window under the Status box.
4. Scan or manually enter the Axiom array plate barcode, then click **Next**.
5. Following the instructions in the Status box, empty the trash bin if necessary and then press the GeneTitan Confirmation button to continue.
6. Place the array plate on top of a scan tray in the correct orientation such that notched corner of the array plate and scan tray are aligned.
7. Load the array plate/scan tray combo in drawer 2 of the GeneTitan Instrument, on the left or right side, as instructed in the Status box.
 - Be sure to load the array plate/scan tray combo in the correct orientation in the drawer. If necessary, refer to [Figure 18 on page 97](#) for further information on the proper alignment and loading of plates, covers and trays in the GeneTitan™ MC Instrument.
8. Press the GeneTitan Confirmation button when ready.
9. Select the arrays to scan in the Array Selection section in the upper right corner of the window, then click **Next**.
10. A Start Processing confirmation message appears (Figure 44). Click **OK** to continue.

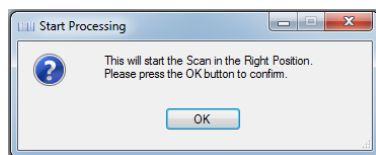


Figure 44 Start scan confirmation message

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

Status window prompts and actions required

As a part of normal GeneTitan MC Instrument operations you may see the following status prompts. [Table 53](#), [Table 54](#) [Table 55](#) and explains the necessary actions required. [Table 56](#) and [Table 57](#) explain possible barcode error messages and the necessary action required.

Table 53 Refilling buffer bottles and emptying the waste bottle

Status window prompt	Action required	Receptacle – reagent
Buffer bottles have been depressurized. Please refill buffer into the bottles. Empty the waste bottle.	<ul style="list-style-type: none"> • Replenish the fluid in Wash Bottles A and B, and the Rinse bottle¹. • Empty the Waste Bottle. • Press the <i>Confirmation</i> button to continue. 	<ul style="list-style-type: none"> • Wash Bottle A: fill with Axiom Wash Buffer A up to 2L. • Wash Bottle B: fill with Axiom Wash Buffer B to the 1L mark. • Rinse: fill with Axiom Water to the 1L mark. <p>Do not overfill these bottles.</p>

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

Table 54 Emptying the trash bin

Status window prompt	Action required	Receptacle – Reagent
Empty trash bin	<ul style="list-style-type: none"> • Open and empty the trash bin. • Press the <i>Confirmation</i> button to continue. <p>NOTE: If the trash bin is empty, you will not be able to open it. Continue the process by pressing the blue confirmation button</p>	—

Table 55 Selecting which arrays to scan

Status window prompt	Action required	Reagent and receptacle
Select arrays to scan	<ul style="list-style-type: none"> • Accept the default (all arrays selected) if appropriate. Otherwise, select the arrays to be scanned. • Click Next, then click OK to start processing. 	—

Table 56 Loading the Array Plate and Hyb Tray; Barcode Error Messages

Status window prompt	Action required	Reagent – receptacle
Load array plate tray on [Left/Right] side of drawer. Load hyb tray without cover on [Left/Right] side of drawer.	Load the array plate with the blue base and the hyb tray in drawer 6. <ul style="list-style-type: none"> • IMPORTANT: The blue base must remain in “left side HTA in” even when empty. • IMPORTANT: The trays must be positioned correctly. If the trays are placed incorrectly, the software will display an error dialog box indicating the barcode could not be read. • Press the Confirmation button to continue. 	<ul style="list-style-type: none"> • Hyb Tray loaded with denatured samples.
<p>Text version of the error message</p> <p>WARNING: The system was not able to verify the array plate barcode.</p> <p>Please verify that the tray on the left side of the drawer has a blue protective base and if applicable, an array plate, in the correct ORIENTATION. The right side of the drawer should contain a hyb tray, if applicable, in the correct ORIENTATION.</p> <p>Details:</p> <ul style="list-style-type: none"> • The consumable is either not the correct consumable, not loaded correctly, or its barcode is not readable. Proceeding with an incorrect or incorrectly loaded consumable can result in a loss of consumables, loss of samples and may require a field service engineer to service the instrument. • Refer to the System Setup tab or the user guide provided with the assay or AGCC for instructions on proper consumable placement. • Press the flashing blue confirmation button or... <ul style="list-style-type: none"> – Press OK, the GeneTitan MC Instrument will verify the barcode and orientation. – Press Skip, the GeneTitan MC Instrument will NOT verify the barcode and orientation. The barcode entered at registration will be used. 		<p>These messages are displayed if:</p> <ul style="list-style-type: none"> • A plate has been loaded improperly. • The bar code is missing or obscured

Table 57 Loading the Scan tray and stain tray; barcode error messages

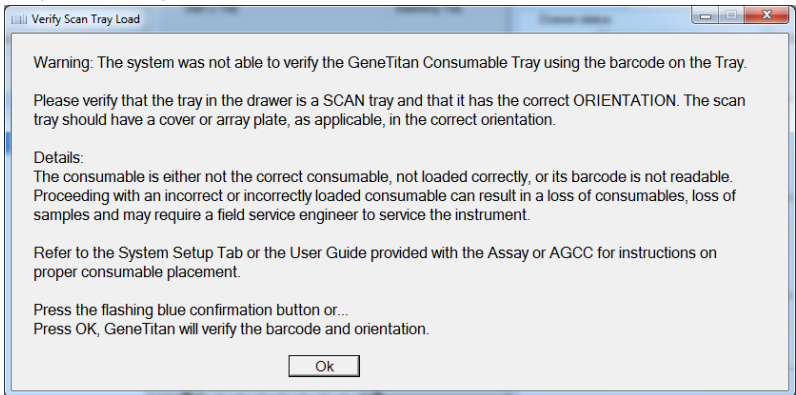
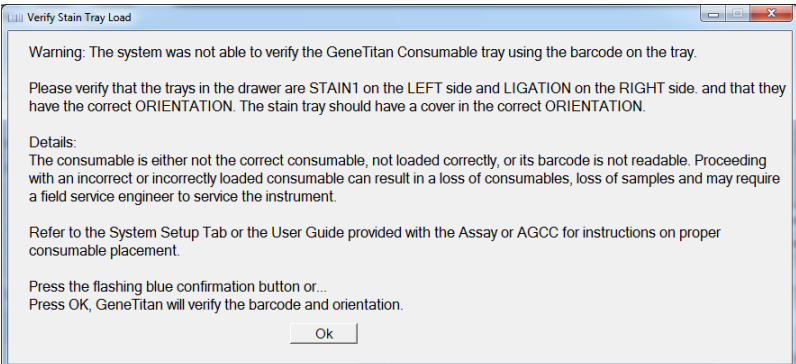
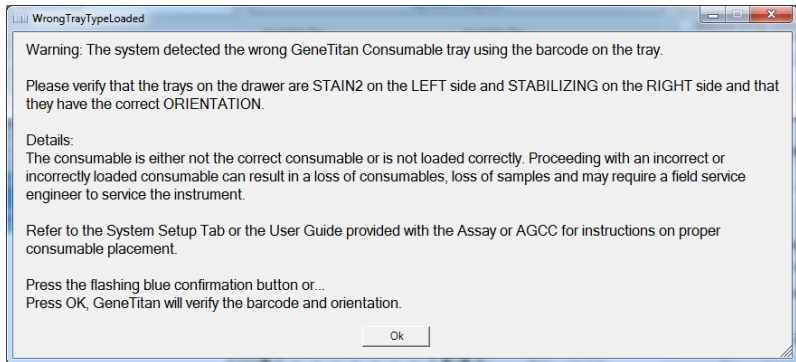
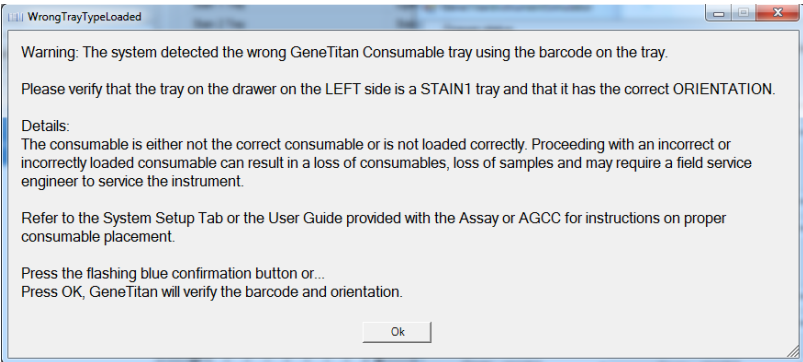
Error message	Action required
<p>Verify Scan Tray Load</p>  <p>Warning: The system was not able to verify the GeneTitan Consumable Tray using the barcode on the Tray.</p> <p>Please verify that the tray in the drawer is a SCAN tray and that it has the correct ORIENTATION. The scan tray should have a cover or array plate, as applicable, in the correct orientation.</p> <p>Details: The consumable is either not the correct consumable, not loaded correctly, or its barcode is not readable. Proceeding with an incorrect or incorrectly loaded consumable can result in a loss of consumables, loss of samples and may require a field service engineer to service the instrument.</p> <p>Refer to the System Setup Tab or the User Guide provided with the Assay or AGCC for instructions on proper consumable placement.</p> <p>Press the flashing blue confirmation button or... Press OK, GeneTitan will verify the barcode and orientation.</p> <p>Ok</p>	<p>The system was not able to verify that GeneTitan Consumable tray using the barcode on the tray.</p> <ul style="list-style-type: none"> • Verify that the tray in the drawer is a Scan Tray • Verify that the Scan Tray is placed in the drawer in the correct orientation • The Scan Tray should have a cover or Array Plate, as applicable, in the correct orientation <p>NOTE: When a tray has been correctly loaded but the system is unable to read the barcode, a Skip button is present in the error message allowing you the option to proceed.</p>
<p>Wrong Stain Trays - Drawer 3</p>  <p>Warning: The system was not able to verify the GeneTitan Consumable tray using the barcode on the tray.</p> <p>Please verify that the trays in the drawer are STAIN1 on the LEFT side and LIGATION on the RIGHT side. and that they have the correct ORIENTATION. The stain tray should have a cover in the correct ORIENTATION.</p> <p>Details: The consumable is either not the correct consumable, not loaded correctly, or its barcode is not readable. Proceeding with an incorrect or incorrectly loaded consumable can result in a loss of consumables, loss of samples and may require a field service engineer to service the instrument.</p> <p>Refer to the System Setup Tab or the User Guide provided with the Assay or AGCC for instructions on proper consumable placement.</p> <p>Press the flashing blue confirmation button or... Press OK, GeneTitan will verify the barcode and orientation.</p> <p>Ok</p>	<p>The system was not able to verify that GeneTitan Consumable tray using the barcode on the tray.</p> <ul style="list-style-type: none"> • Verify that the trays in drawer 3 are: <ul style="list-style-type: none"> – STAIN 1 on the Left, and – LIGATION on the Right • Verify that the trays are placed in the drawer in the correct orientation • Verify that the trays have covers and that the covers are on the trays in the correct orientation <p>Note: When a tray has been correctly loaded but the system is unable to read the barcode, a Skip button is present in the error message allowing you the option to proceed.</p>

Table 57 Loading the Scan tray and stain tray; barcode error messages

Error message	Action required
<p>Wrong Stain Trays - Drawer 4</p> 	<p>The system detected the wrong GeneTitan Consumable Tray using the barcode on the tray.</p> <ul style="list-style-type: none"> • Verify that the trays in drawer 4 are: <ul style="list-style-type: none"> – STAIN 2 on the Left, and – STABILIZING on the Right • Verify that the trays are placed in the drawer in the correct orientation <p>Note: When a tray has been correctly loaded but the system is unable to read the barcode, a Skip button is present in the error message allowing you the option to proceed.</p>
<p>Wrong Stain Tray - Drawer 5</p> 	<p>The system detected the wrong GeneTitan Consumable Tray using the barcode on the tray.</p> <ul style="list-style-type: none"> • Verify that the tray in drawer 5 is: <ul style="list-style-type: none"> – STAIN1 on the Left • Verify that the tray is placed in the drawer in the correct orientation <p>Note: When a tray has been correctly loaded but the system is unable to read the barcode, a Skip button is present in the error message allowing you the option to proceed.</p>

Stage 3: Ligate, Wash, Stain and Scan

Equipment,
consumables, and
reagents required

Scan tray with Axiom Hold Buffer

- Cover the tray by orienting the notched corner of the cover over the notched edge of the tray and leave on the benchtop (no need to protect from light; [Figure 45](#)).



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

Always leave the scan tray in its protective blue base.

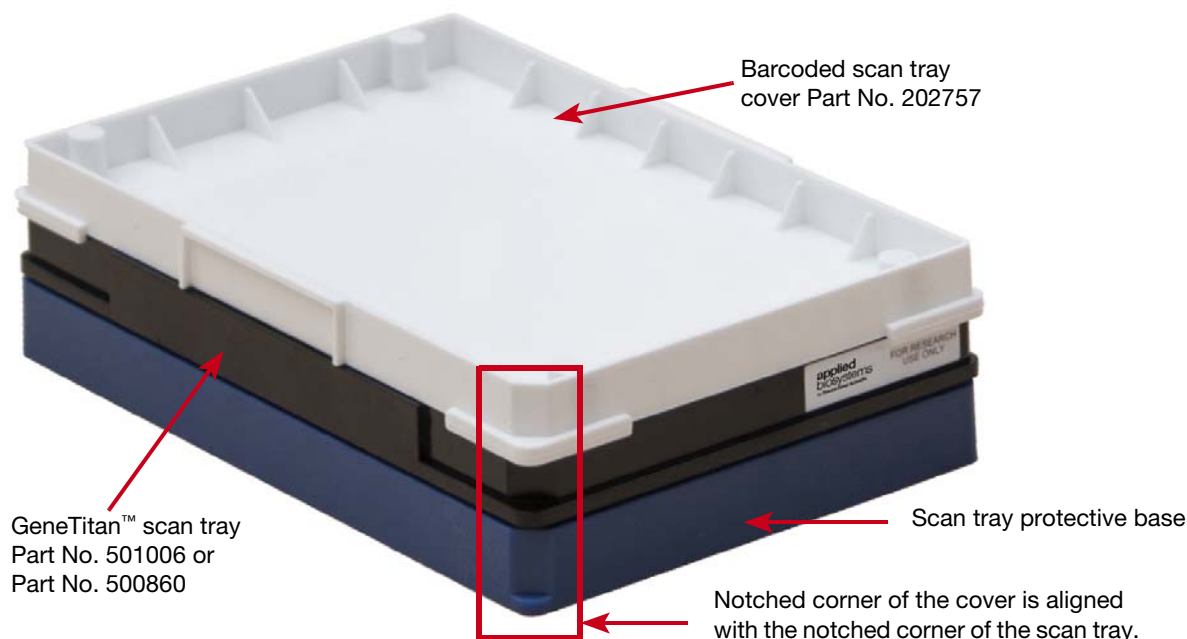


Figure 45 The scan tray with cover on the blue base.

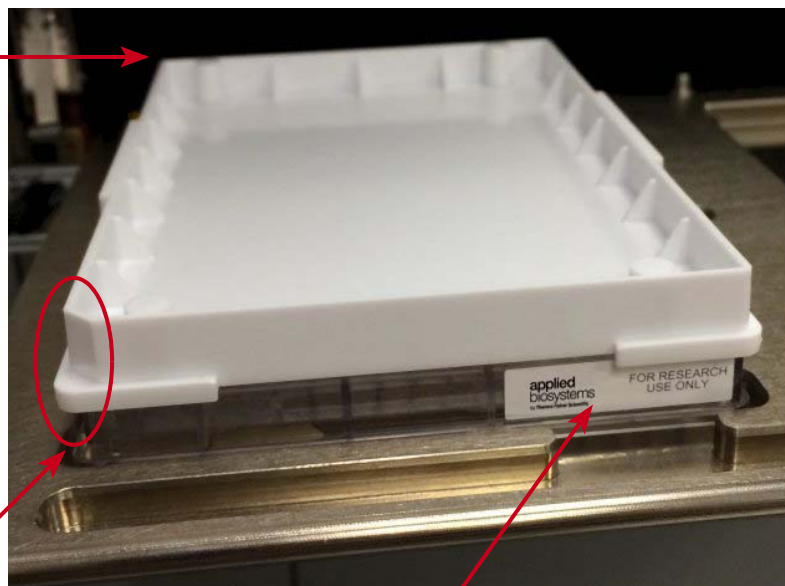
Proper installation of the GeneTitan™ tray consumables

It is very important that you load the GeneTitan tray consumables in the proper orientation. The barcode faces into the instrument (refer to [Figure 46](#) and [Figure 47](#)).



Figure 46 You must rotate and load the trays so that the barcode faces into the instrument

Barcode faces
in and back.



Notch faces out and left.

The Applied Biosystems logo and
“For Research Use Only” faces out

Figure 47 The proper loading of the GeneTitan™ tray consumables is shown (the image shows the stain tray and the stain tray cover as an example).

Note: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and Rinse bottles should be replaced if the software displays such a warning.

Load trays onto the GeneTitan™ MC Instrument

To load trays onto the GeneTitan MC Instrument:

When hybridization of an Axiom array plate has finished, a message (Figure 48) will alert you to resume the workflow setup. Press **OK** and the software takes you directly back to the System Setup tab.

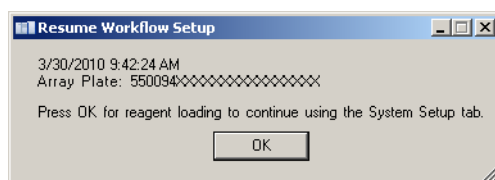


Figure 48 The Resume Workflow Setup message

This prompt to continue into reagent load (Figure 48) occurs when the hyb is complete. “Estimated Time Remaining” displayed under “Hybridization Oven Status” may display a time remaining of 0 to 30 minutes when the prompt occurs.

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

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

Note: The time estimate displayed on some systems may lag due to high CPU utilization. The GeneTitan MC Instrument allows the workflow to synchronize with the system clock to compensate for this situation during the final half hour of the hyb

time estimate. When this prompt to resume reagent loading is displayed to the user there is no need to wait for the estimated time to count down to zero.

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

1. Follow the prompts in the Status window.
 - a. Wash Bottles A and B, and the Rinse Bottle—refill as necessary (the system will prime itself again); Waste bottle—empty if necessary.
Wash Bottle A—2L. Wash Bottle B and Rinse Bottle—fill to 1L mark only.
 - b. Empty the trash bin.
 - c. Remove consumable trays and plates as instructed, except for the blue base.
Leave the blue array plate base in drawer 6 even though the base is empty.
2. Load consumable trays and plates as follows:
 - a. Follow the prompts in the Status window (load sequence and prompts in [Table 58](#)).
 - b. Once loaded, examine each cover for droplets of liquid.
 - c. If any liquid is present, remove the tray, clean the cover and top of the tray with Kimwipes, and reload the tray.



CAUTION!

- Orient trays as indicated by the guide inside the drawer ([Figure 50 on page 130](#)). Improper orientation may cause the run to fail.
 - Remove the protective blue base from the scan tray immediately prior to loading ([Figure 49 on page 129](#)).
 - Examine each cover for droplets of liquid after loading. Liquid on the cover can result in capillary phenomenon. As a result, the tray may stick to the cover and be lifted out of place inside the instrument.
-

Table 58 Sequence for loading the trays with reagents

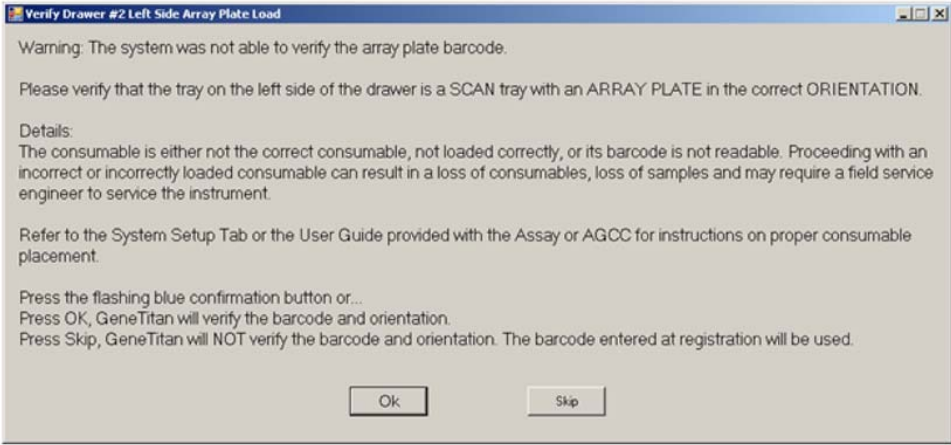
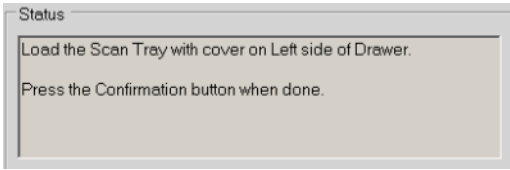
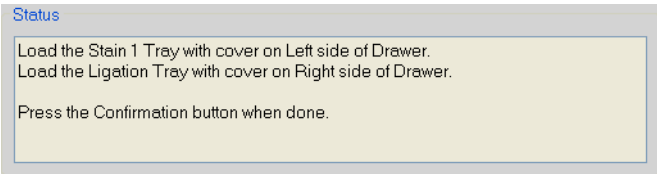
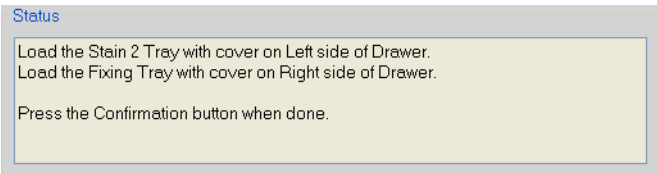
Loading Sequence by Drawer Number	Left	Right
	<p>NOTE: If the software is unable to verify the barcode on the scan tray and the scan tray cover, the software will display the following error message.</p> 	
2	<p>Scan Tray with cover—do not load the protective blue base (left side of drawer as indicated in Status window) Figure 49 on page 129</p> 	
3	<p>Stain Tray with Stain 1</p>	<p>Ligation Tray</p> <p>Figure 51 on page 130</p> 
4	<p>Stain Tray with Stain 2</p>	<p>Stabilization Tray with Stabilization Reagent</p> <p>Figure 52 on page 131</p> 

Table 58 Sequence for loading the trays with reagents (Continued)

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

Figure 53 on page 131

Status

Load the Stain 1 Tray with cover on Left side of Drawer.

Press the Confirmation button when done.

Scan tray with cover loaded in drawer 2.



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



Figure 49 Scan tray loaded in drawer 2

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

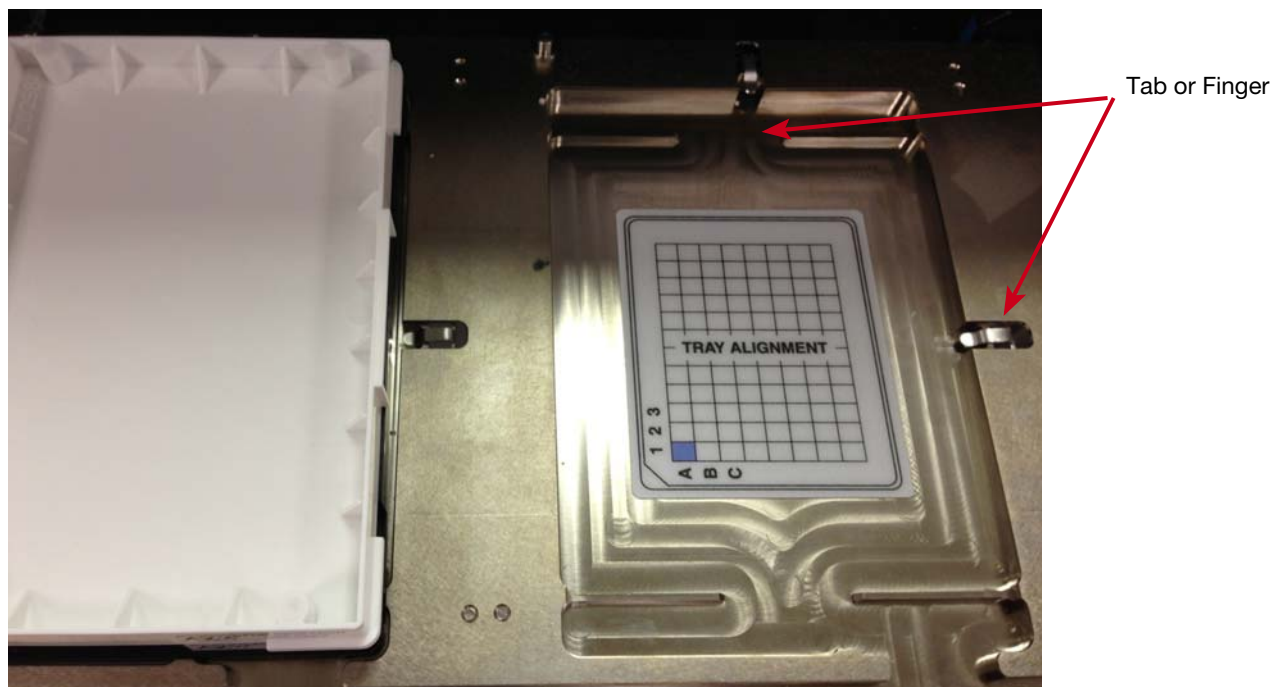


Figure 50 Loading the plates or trays

Drawer 3

Stain 1 Tray (left, white label)
and
Ligation Tray (right, yellow label)



Figure 51 Stain 1 tray and ligation tray loaded in drawer 3

Drawer 4

Stain 2 Tray (left, blue label)
and
Stbl Tray (right, green label)



Figure 52 Stain 2 tray and stabilization tray loaded in drawer 4

Drawer 5

Stain 1 Tray (left, white label)



Figure 53 Stain 1 tray loaded in drawer 5

3. At the prompt shown in [Figure 54](#), click **Yes** to load another Axiom array plate and hyb tray.

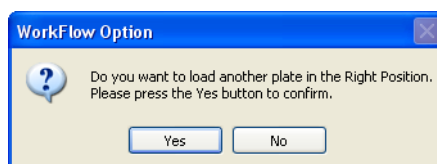


Figure 54 Prompt asking to load another plate. Right or left position determined by the position of Axiom™ array plates already in the GeneTitan™ MC Instrument.

4. Follow the prompts and:
 - a. Setup Option: select **Setup Another Run**, then click **Next**.
 - b. Scan or manually enter the Axiom array plate barcode, then click **Next**.
 - c. Select a protocol, then click **Next**.
 - d. When drawer 6 opens:
 - Remove the blue cover from the previous Axiom array plate.
 - Load a new Axiom array plate and new blue base on the left; load a new hyb tray on the right.
 - Press the **Confirmation** button.
 - e. Click **OK** when prompted ([Figure 55](#)).



Figure 55 Confirm Resume Processing message

- f. When drawer 6 opens, confirm that the plate stack is securely clamped by following the procedure in [Figure 37](#), then press the **Confirmation** button.

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

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

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

[Figure 56](#) is an example of how the System Status Workflow window will appear when three Axiom array plates are being processed.

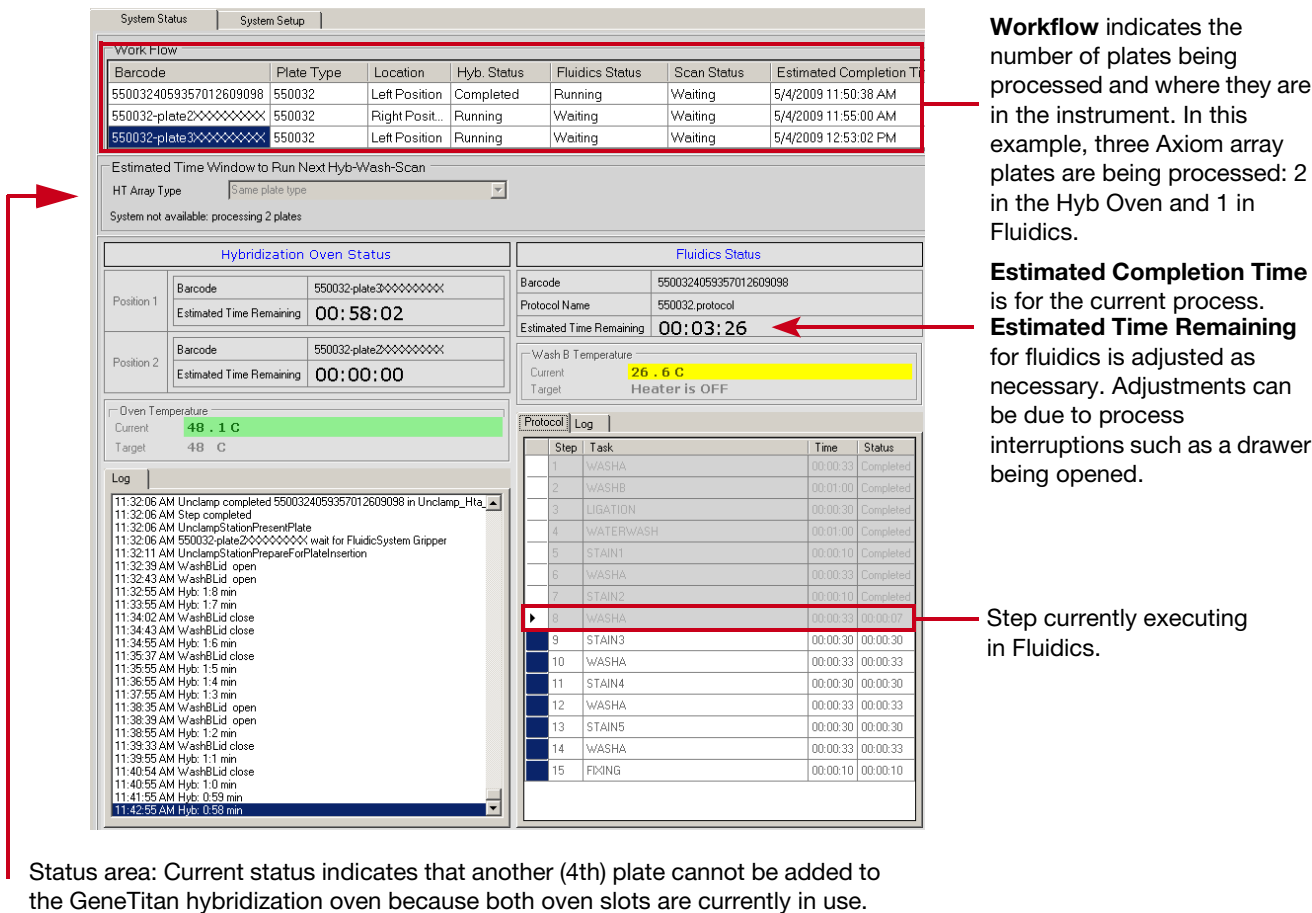


Figure 56 Example of the System Status window—three Axiom™ array plates are being processed

Continuing the workflow

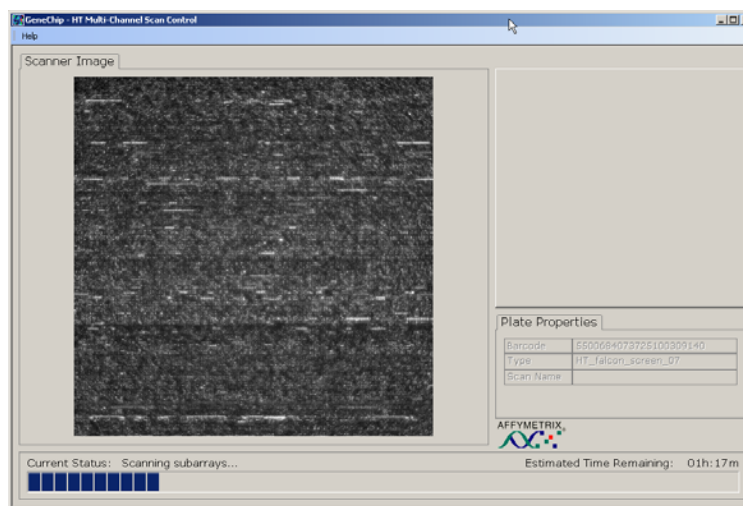
Once a plate has gone through the fluidics stage of the process, it is moved to the imaging device.

When the scanning process begins, the window shown in [Figure 57](#) is displayed. This window must remain open while Axiom array plates are being scanned.



CAUTION!

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



This window must remain open while scanning is in progress.

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

Figure 57 Scan Control window

Shutting down the GeneTitan™ MC Instrument

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



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

To shutdown the GeneTitan MC Instrument:

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

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

6

Processing three Axiom™ array plates per week

The manual target preparation workflow for three plate per week is described in the following sections:

- Overview of the three-plate workflow for manual target preparation. 137
- Thawing frozen plates of amplified DNA. 140
- Manual target preparation and array processing 141

When using the Axiom™ 2.0 Assay for Mini 96-Array Format Manual Protocol, one person can process up to three Axiom™ Mini 96-array format plates in one forty-hour work week.

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

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

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

- Chapter 4, "Axiom™ 2.0 Assay for Mini 96-Array manual target preparation" on page 44
- Chapter 5, "Array processing with the GeneTitan™ MC Instrument" on page 96

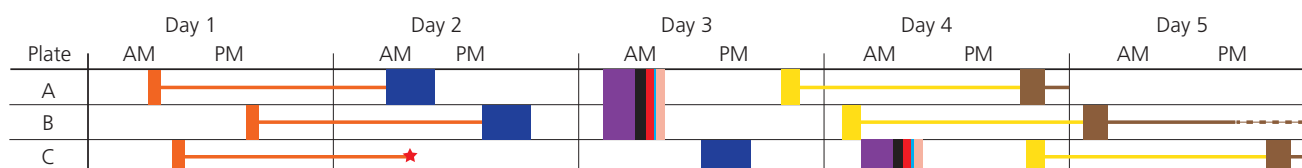
Overview of the three-plate workflow for manual target preparation

Table 59 displays the timing and duration of the hands-on processing necessary for performing the three plate workflow by one person.

Table 59 Daily steps for manual target preparation workflow

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

Full Week Activities for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol three plate workflow



User activities

★	Freeze
	Hybridization Setup (Denature & Transfer to Hyb Tray)
	DNA Amplification Setup
	GeneTitan® Reagent Tray Prep & Loading
	Fragmentation & Precipitation
	Off-deck Centrifugation & Drying Pellets
	Resuspension and Hybridization Preparation
	Sample QC
	OD
	Run Gel QC

Background activities

	Amplification incubation
	Hybridization in the GeneTitan™ MC Instrument
	Fluidics processing in the GeneTitan™ MC Instrument
	Imaging in the GeneTitan™ MC Instrument

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

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

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

These constraints are described in more detail in:

- ["Timing issues for manual target preparation" on page 138](#)
- ["Timing Issues for GeneTitan™ MC array processing" on page 139](#)

Timing issues for manual target preparation

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

Table 60 Time required for manual target preparation

Manual preparation	Hands-on time required	Total prep time ¹	Incubation/ hybridization/ processing
"Stage 1: DNA amplification"	0.5 hr	1.5 hr	23 ±1 hr
"Stage 2: Fragmentation and Precipitation"	2 hr	2 hr	Overnight Precipitation
"Stage 3: Centrifuge and Drying, Resuspension and Hybridization Preparation, and Sample QC"			
• "Stage 3A: Centrifuge precipitation plate and dry the DNA pellet"	30 min	1 hr 20 min	N/A
• "Stage 3B: Resuspension and hybridization preparation"	25 min	25 min	N/A
• "Stage 3C: Sample QC"	45 min	45 min	N/A
"Stage 4: Denaturation and hybridization"	25 min	45	23.5 - 24 hr hybridization
"Stage 5: GeneTitan™ reagent preparation"	1 hr	1.5 hr	Additional time for processing: 96 arrays: 12.5 hr

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

Timing Issues for GeneTitan™ MC array processing

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

The hybridization time for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol on the GeneTitan MC Instrument is 23.5 to 24 hr (Table 61). This provides a 30 min window during which you are prompted by the instrument control software to load the reagents required for washing and staining.

Table 61 Time required for array plate processing on the GeneTitan MC Instrument

Steps on the GeneTitan MC Instrument	Time required
Hybridization of two plates in one day <ul style="list-style-type: none"> • First plate loaded at 9:30 a.m. • Second plate loaded at 5:00 p.m. 	23.5 hr each plate
Loading Reagent Trays	15 min
Fluidics	5 hr each plate
Imaging ¹	up to 7.5 hr depending on array format

¹ For labs that run several array plate formats, imaging times may vary.

Changing oven temperatures for the three plate workflow

Multiple ovens are required for manual target preparation. If you are running the three plate/week workflow, three ovens are recommended. Table 62 lists the different temperatures required for each step. Though only two ovens are strictly required, we recommend maintaining separate 37°C ovens for the amplification and fragmentation stages to avoid confusion of plates and to minimize excess opening and closing of oven doors during incubation periods. Table 63 provides a list of suggested settings for three ovens when performing the three plate/week workflow.

Table 62 Oven temperatures needed for each step of the workflow

Workflow step	Oven temp
Amplification	37°C
Stopping Amplification	65°C
Pre-Fragmentation Incubation	37°C
Fragmentation Incubation	37°C
Drying	37°C
Hybridization ¹	48°C

¹ For preheating of the 96-well metal chamber for hyb transfer

Table 63 Suggested settings for ovens when performing three plate/week manual target preparation workflow

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

Thawing frozen plates of amplified DNA

To thaw frozen plates of amplified DNA:

- Place the deep well plate in a small water bath.
For example, pour Millipore water into a small tray. Place the frozen plate in the water in the tray.
- Leave the plate in the water bath for ~50 min until all wells have thawed.
- Spin down at 1000 rpm for 30 sec.
- To avoid cross-contamination of wells during vortexing:
 - Remove the seal and blot the top of the plate with a Kimwipe.
 - Tightly reseal the plate with a fresh seal.
- Vortex the plate for 30 sec to thoroughly mix (refer to guidelines described in ["Seal, vortex, and spin" on page 27](#)).
- Spin at 1000 rpm for 30 sec.

Manual target preparation and array processing

Day 1

- On this day you start amplification of the three plates: each plate must incubate 23 ± 1 hours after amplification begins.
- All amplifications should be set up on Day 1 to allow for a 23 ± 1 hr amplification incubation for each plate and to minimize movement between pre-amplification and post-amplification areas.
- Begin thawing the amplification reagents, particularly the Axiom 2.0 Amp Soln, 60 min prior to the start of each reaction.

IMPORTANT! Amplification preparation should take place in an Amplification Staging Room or dedicated area such as biosafety hood with dedicated pipettes, tips, vortex, etc. See "[Amplification staging area](#)" on page 23 for more information.

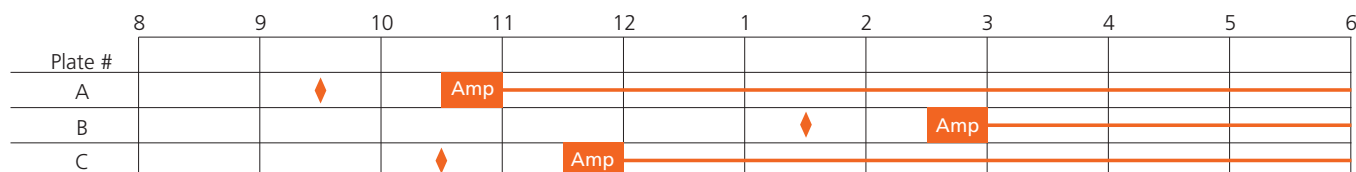
Table 64 Day 1 activities for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

Activity	Plate	Approximate times ¹		Duration
		Start time	End time	
DNA amplification	A	9:30 a.m.	11:00 a.m.	30 min
DNA amplification	C	10:30 a.m.	12:00 p.m.	30 min
DNA amplification	B	1:30 p.m.	3:00 p.m.	30 min

¹ Approximate start time indicates start of thawing of reagents.

See "[Stage 1: DNA amplification](#)" on page 45 for more information on the protocol.

Day 1 Activities for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol three plate workflow



User activities

	Thaw and prepare reagents for DNA amplification
	DNA amplification setup

Background activities

	Amplification incubation
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Day 2

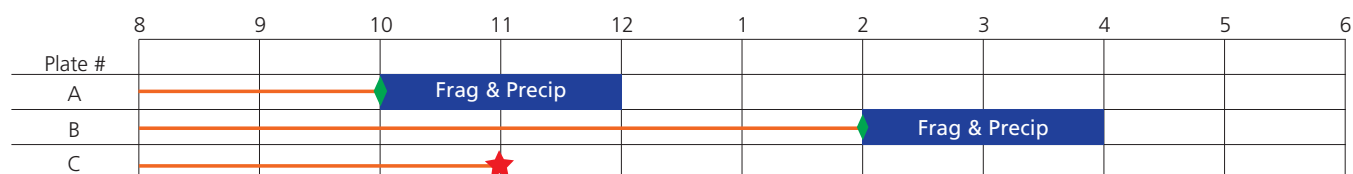
- Table 65 shows the steps that need to be performed on the second day.
- Plates A and B are fragmented and precipitated on Day 2 without freezing to preserve a 23 hr amplification incubation.
- Precipitation is carried out at –20°C overnight.

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

Table 65 Day 2 activities for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

Activity	Plate	Approximate times		Duration
		Start time	End time	
Fragment and Precipitate	A	10:00 a.m.	12:00 p.m.	2 hours
Freeze (–20°C)	C	11:00 a.m.	—	Overnight
Fragment and Precipitate	B	2:00 p.m.	4:00 p.m.	2 hours

Day 2 Activities for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol three plate workflow



User activities

★	Freeze
◆	Prepare reagents for fragmentation
	Fragmentation & Precipitation

Background activities

	Amplification incubation
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Day 3

- Centrifuge, dry, resuspend and QC Plates A and B.
- Thaw Plate C (see ["Thawing frozen plates of amplified DNA" on page 140](#)).
- Fragment (including the 65°C Stop Amplification Reaction step) and precipitate Plate C.
- Perform Denaturation on Plate A.
- Transfer Plate A samples to Hyb Tray A
- Load Hyb Tray A and array plate into GeneTitan MC Instrument and begin hybridization.



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

IMPORTANT! Amplified plates that are frozen must be thawed and thoroughly mixed by following the procedure under ["Thawing frozen plates of amplified DNA" on page 140](#).

Table 66 Day 3 activities for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

Activity	Plate	Approximate times		Duration
		Start time	End time	
Centrifuge and Dry	A, B	9:00 a.m.	10:20 a.m.	1 hour 20 min
Resuspension and Hyb Preparation	A, B	10:20 a.m.	10:45 a.m.	25 min
Sample QC	A, B	10:45 a.m.	11:05 a.m.	20 min
Sample Quantitation (OD) ¹	A, B	11:05 a.m.	11:10 a.m.	5 min
Frag Gel QC Run	A, B	11:05 a.m.	11:30 a.m.	25 min
Thaw Plate C	C	12:00 p.m.	1:00 p.m.	1 hour
Fragment and Precipitate	C	1:00 p.m.	3:00 p.m.	2 Hours
Denature and Hybridization	A	4:15 p.m.	5:00 p.m.	45 min setup, 23.5 to 24 hours Hyb

¹ Sample Quantitation runs concurrently with Frag Gel QC Run. Load the Gel QC Plate first, then read the OD QC Plate.

Day 3 Activities for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol three plate workflow

	8	9	10	11	12	1	2	3	4	5	6
Plate #											
A		Centrifuge & Dry							Denature & Hyb		
B		Centrifuge & Dry									
C						Frag & Precip					

User activities

◆	Prepare reagents for Resuspension and Hyb Prep
◆	Prepare reagents for Fragmentation
◆	Warm array plate to room temperature
▲	Thaw DNA amplification plate
	Fragmentation & Precipitation
	Centrifugation & Drying Pellets
	Resuspension and Hybridization Preparation
	Sample QC
	Sample Quantitation - OD
	Fragmentation Gel QC Run
	Denature & Hybridization

Background activities

	Hybridization in the GeneTitan™ MC Instrument
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Day 4

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



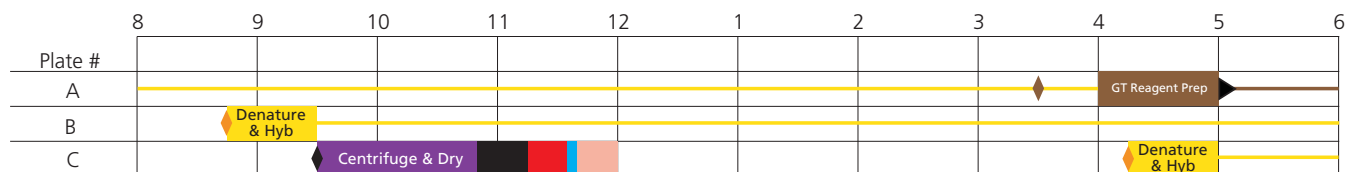
WARNING! The Hybridization Tray preparation should take place under a running fume hood.

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

Table 67 Day 4 activities for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

Activity	Plate	Approximate times		Duration
		Start time	End time	
Denature and Hybridization	B	8:45 a.m.	9:30 a.m.	45 min setup, 23.5 to 24 hours Hyb
Centrifuge and Dry	C	9:30 a.m.	10:50 a.m.	1 hour 20 min
Resuspension and Hyb Preparation	C	10:50 a.m.	11:15 a.m.	25 min
Sample QC	C	11:15 a.m.	11:35 a.m.	20 min
Sample Quantitation (OD) ¹	C	11:35 a.m.	11:40 a.m.	5 min
Fragmentation Gel QC Run	C	11:35 a.m.	12:00 p.m.	25 min
GeneTitan Reagent Prep and Loading	A	3:30 p.m.	5:00 p.m.	1 hour
Denature and Hybridization	C	4:15 p.m.	5:00 p.m.	45 min setup, 23.5 to 24 hours Hyb

¹ Sample Quantitation runs concurrently with Frag Gel QC Run. Load the Gel QC Plate first, then read the OD QC Plate.

Day 4 Activities for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol three plate workflow

User activities

◆	Warm array plate to room temperature
◆	Prepare reagents for Resuspension and Hyb Prep
◆	Prepare reagents for GeneTitan Reagent Preparation
◆	Warm array plate to room temperature
▶	Load array plate into GTMC, begin Wash-Scan
■	Centrifugation & Drying Pellets
■	Resuspension and Hybridization Preparation
■	Sample QC
■	Sample Quantitation - OD
■	Fragmentation Gel QC Run
■	GeneTitan™ Reagent Tray Prep & Loading
■	Hybridization Setup (Denature & Transfer to Hyb Tray)

Background activities

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

Day 5

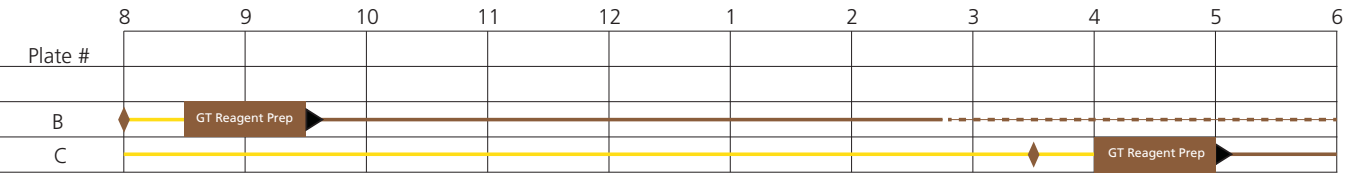
- GeneTitan reagents preparation and loading for Plates B and C.

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

Table 68 Day 5 activities for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol

Activity	Plate	Approximate times		Duration
		Start time	End time	
GeneTitan Reagent Tray Prep and Loading	B	8:00 a.m.	9:30 a.m.	1 hour
GeneTitan Reagent Tray Prep and Loading	C	3:30 p.m.	5:00 p.m.	1 hour

Day 5 Activities for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol three plate workflow



User Activities	
	Thaw reagents for GeneTitan™ Reagent Tray Prep
	Load array plate into GTMC, begin Wash-Scan
	GeneTitan Reagent Tray Prep & Loading

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

GeneTitan™ Multi-Channel Instrument

Refer to the *GeneTitan™ Multi-Channel Instrument User Guide*, Pub. No. 08-0306 for further troubleshooting information.

Table 69 GeneTitan™ MC Instrument troubleshooting guidelines for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol

Problem	Possible causes	Possible actions
Plate trapped in GeneTitan Multi-Channel Instrument.	<ul style="list-style-type: none"> • 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. 	<ol style="list-style-type: none"> 1. Restart the GeneTitan Multi-Channel Instrument. 2. Run the setup option <i>Unload Plates</i>. 3. If the plate remains trapped in the instrument, call Thermo Fisher Scientific support.
Computer frozen.	<ul style="list-style-type: none"> • Too many processes running. • Attempting to transfer data while an array plate is being scanned (imaged). 	<p>Restart the computer and unload all of the plates.</p> <ul style="list-style-type: none"> • Plates in Hyb station: finish hybridization off-line. • Plate in Scanner: rescan using Scan Only function • Plate in Fluidics: use Wash/Scan Resume to resume the fluidics process. <p>Do not manually, or through the AGCC transfer utility, move any data associated with the current plate that is being processed/scanned.</p>
Hybridization aborted: <ul style="list-style-type: none"> • System-initiated abort • User-initiated abort 	<p>System-initiated abort:</p> <ul style="list-style-type: none"> • Power loss <p>User-initiated abort:</p> <ul style="list-style-type: none"> • User error • Other 	<p>Array plate and hyb tray are still clamped:</p> <ul style="list-style-type: none"> • Contact your local field service engineer with information on the workstation model. • The plate stack is moved to drawer 1. • Remove the plate stack and finish hybridization offline. • Return the hybridized array plate stack to the GeneTitan Multi-Channel Instrument and finish processing using the Wash/Scan process.

Table 69 GeneTitan™ MC Instrument troubleshooting guidelines for the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol (Continued)

Problem	Possible causes	Possible actions
FAILED messages	See "GeneTitan™ MC Instrument messages that appear when the instrument has a fluidics problem" on page 150	
FLUIDIC DIAGNOSTIC messages	See "Fluidic diagnostic messages" on page 150.	
Fluidics aborted: • System-initiated abort • User-initiated abort	System-initiated abort: • Power loss User-initiated abort: • Incorrect protocol selected	Follow the recommendations and instructions under "Wash/Scan Resume" on page 155.

Miscellaneous Messages

Table 70 Miscellaneous messages and recommended actions

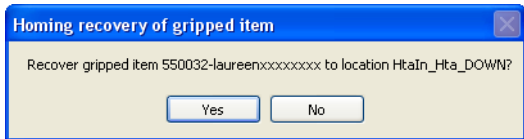
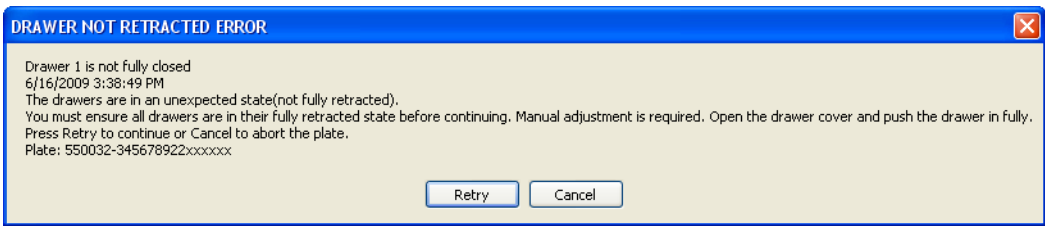
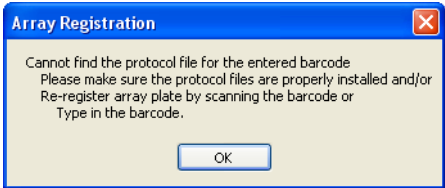
Message and recommended action	
 <p>Indicates that an item is in the gripper, and normal startup of the GeneTitan Multi-Channel Instrument is not possible. The item must be removed from the instrument before you can begin processing array plates.</p>	<p>Recommendation: click Yes.</p> <p>If you click No, nothing will occur. Homing will not complete and you not be able to use the system. The item held by the gripper will be moved to either:</p> <ul style="list-style-type: none"> • Drawer 2—plates and trays • Trash Bin—covers <p>The drawer names will reflect the location (left or Right) and the drawer number (1 through 6). Examples:</p> <p>Drawer2L_Hta_DOWN = Scan tray on left side of drawer 2</p> <p>HtaHyb = Clamped Hyb Tray and Array Plate</p> <p>Drawer(n)L/R_Hta_DOWN where n is the drawer number and L or R to indicate the left or right side. The _Hta_ (second term) indicates the item held. An example is drawer1R_HtaHyb_DOWN indicating it is an array plate with a hyb tray or Drawer2L_ScanHta_Pk_DOWN indicating it is an array plate with a scan tray</p>

Table 70 Miscellaneous messages and recommended actions

Message and recommended action	
 <p>The drawer listed in the message is not fully closed. Manually push the drawer back into the instrument until it is fully closed. There are two stop positions with audible clicks; push until you hear the second click and the drawer is fully seated.</p>	
	<ul style="list-style-type: none"> • 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.

Fluidic diagnostic messages

Table 71 GeneTitan™ MC Instrument messages that appear when the instrument has a fluidics problem

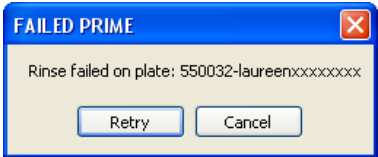
Problem and possible causes	
 <p>Rinse bottle—fluid level too low or bottle empty.</p>	<p>If this message is displayed:</p> <ul style="list-style-type: none"> • during a water wash step, array processing has been compromised. • during cleanup, array processing is OK, but cleanup will not be complete. <p>Always ensure that the GeneTitan bottles containing Wash A and Rinse are above the 50% mark when setting up the system to process an Axiom array plate.</p> <p>All 600 mL of the Wash Buffer B from the Axiom™ 2.0 Mini-96 Reagent Kit should be emptied into the GeneTitan Wash B bottle when setting up the system to process a plate. This ensures that the GeneTitan Wash B bottle is filled to more than the requisite 35% of Wash B bottle volume.</p>

Table 71 GeneTitan™ MC Instrument messages that appear when the instrument has a fluidics problem

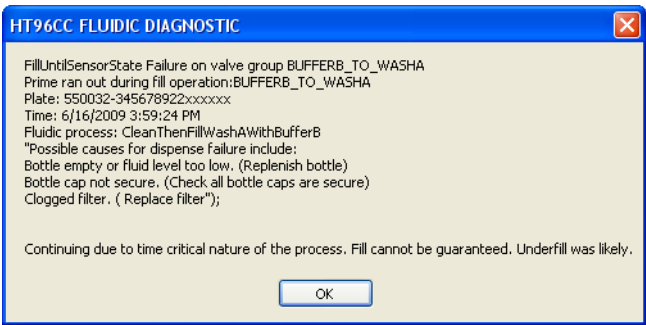
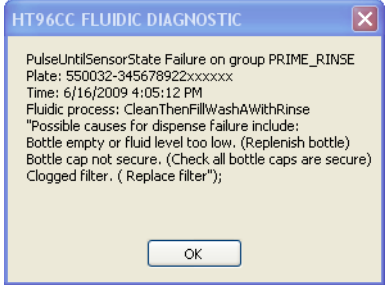
Problem and possible causes	
	<p>About this message:</p> <ul style="list-style-type: none"> • BUFFERX = Buffer bottle A, B or Rinse • WASHX = Wash A or B reservoir in the fluidics station. <p>Recommended actions:</p> <ul style="list-style-type: none"> • Replenish fluid level in the Rinse or Wash Bottle B to the 1L mark. Do not overfill. <ul style="list-style-type: none"> – Only replenish bottles when prompted by the UI. Replenishing during fluidic processing may cause system malfunction including overflowing inside the system and more problems. The only thing to do while a plate is running is to make sure bottle caps are secure. • Replenish fluid level in Wash Bottle A to 2L. • Secure the bottle cap. • Replace the filter <p>Instructions for filter replacement in the <i>GeneTitan™ Multi-Channel Instrument User Guide</i>, Pub. No. 08-0306.</p> <p>If the problem persists, call Thermo Fisher Scientific support.</p>
	<p>The typical cause is an unsecure bottle cap.</p> <p>If the failure is detected during priming, the instrument will pause and wait for the problem to be corrected.</p> <p>If the failure is detected during another process, and if the cause is a clogged filter, wait until the end of the run to replace the filter.</p> <p>Instructions for filter replacement in the <i>GeneTitan™ Multi-Channel Instrument User Guide</i>, Pub. No. 08-0306.</p>

Table 71 GeneTitan™ MC Instrument messages that appear when the instrument has a fluidics problem

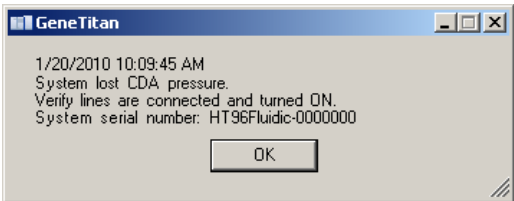
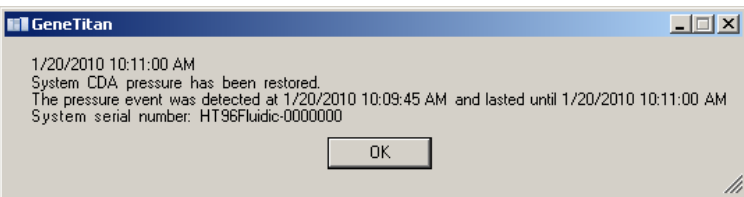
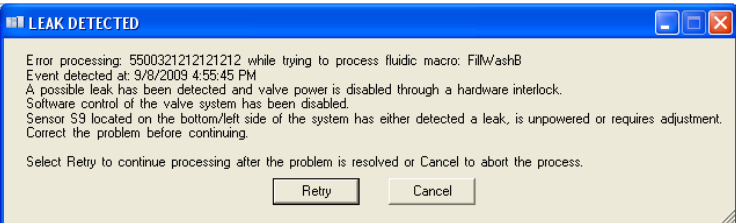

Problem and possible causes	
<p>When the instrument experiences a loss in Clean Dry Air (CDA) pressure, the software will display the warning message.</p>  <p>When the pressure is detected again, a dialog message confirming the availability of CDA pressure is displayed.</p> 	<p>Possible Causes</p> <p>Please verify that the facility CDA or the portable CDA compressor is in working condition. Refer to the GeneTitan MC Instrument Site Preparation Guide for the portable compressor model that has been validated with the GeneTitan MC Instrument. Contact your local field application specialist and notify the engineer about the error message.</p>
<p>Leak Detected</p> <p>Leak checks are performed at application startup and any time a fluidic process (priming filling draining etc.) is performed. The leak detection is a hard-wired sensor which will shut off fluid flow without software control. Leaks are normally confined to the drip pan located inside the system.</p>  	<p>Causes:</p> <ul style="list-style-type: none"> • System malfunction • User killing the application using task manager during a fill operation resulting in application exit without stopping flow. <p>Solution:</p> <p>Contact Thermo Fisher Scientific field support. The system cannot be used for any fluidic processing until this is resolved.</p>

Table 71 GeneTitan™ MC Instrument messages that appear when the instrument has a fluidics problem

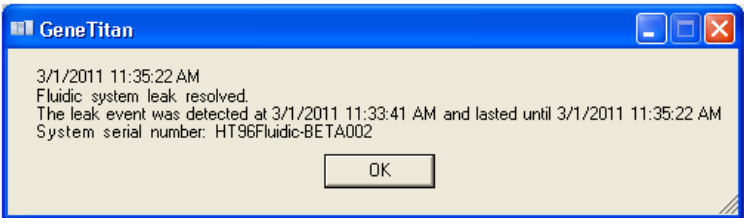
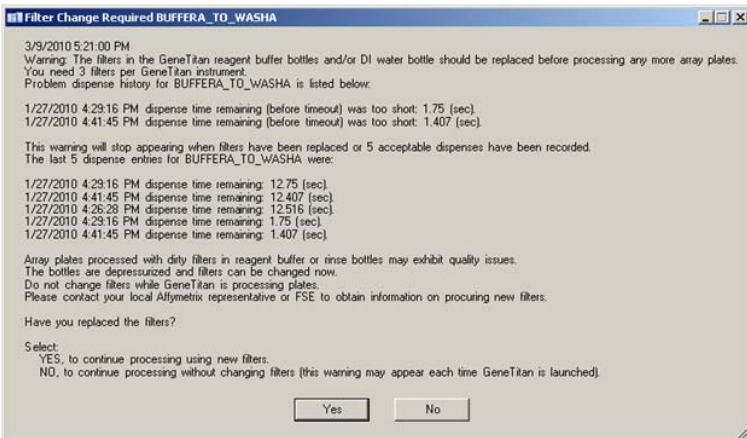
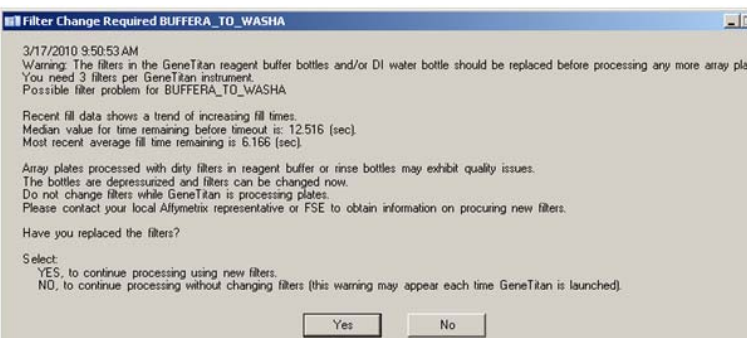
Problem and possible causes	
<p>Leak Resolved</p> 	<p>This message is displayed when the leak is resolved (meaning the sensor LED is again lit up). If the original leak detected message was not acknowledged it will be automatically removed from the GUI and replaced by the following message. It will remain displayed until another leak is detected or the user acknowledges it by clicking OK. To resolve this issue complete the following tasks:</p> <ul style="list-style-type: none"> • Verify all internal and external tubing is connected and clean • Verify wash reservoirs are clean • Verify all bottle caps are secure and that no bottle cap is crimping a supply line. • Verify vacuum is working properly • Do not refill bottles or empty waste except when prompted to by the GeneTitan application. • Contact your facility group to ensure CDA is supplied to your GeneTitan system. <p>Contact Thermo Fisher Scientific Field Service to have the sensor adjusted or replaced if the problem persists even after correcting for the usual causes outlined above.</p>

Table 71 GeneTitan™ MC Instrument messages that appear when the instrument has a fluidics problem

Problem and possible causes	
<p>Filter Error Message: Dispense related check</p>  <p>Filter Error Message: Fill related check</p> 	<p>The filters in the GeneTitan fluidics bottles (Wash A, Wash B, and Rinse) need to be replaced when the filters are worn out. The software displays warning message boxes for the filter in each reagent bottle when it detects a problem or shows a trend of increased fill times during fluid fill operations.</p> <p>If an error is detected as described above, then a message box titled “Filter Change Required” is displayed along with the information on the specific dispense operation. You should change all three filters when a warning is displayed for any one of the three filters.</p>

Wash/Scan Resume

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

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

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

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

To help ensure that the samples are processed correctly, we recommend that you:

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

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

Resume step

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

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

Aborting a run

- Abort can take up to three minutes if a plate is in the Fluidics station. Status window Abort Requested changes to Abort Completed.
- Clamped Array-Plate-Hyb Tray stack that is aborted from the oven or from drawerIN (drawer 6) is moved to drawer 1.
- Proceed as follows:
 - Use the Unload Plates option to remove the aborted plate(s).
 - Start another run which will force an unload of the aborted plate(s)

System-initiated

- Power interruption
- Plate loaded incorrectly
- Equipment malfunction

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

User-initiated

Can abort processing of individual array plates.

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



Safety


For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.



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

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

Chemical safety

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


- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "[Documentation and support](#)" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) 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.

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

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

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

Biological hazard safety

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

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

Precautions

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



Fragmentation quality control gel protocol

Protocol for running a fragmentation quality control gel

Equipment required

Table 72 Equipment required

Item	Supplier	Cat. No.
Gel Imager	Your choice	—
Pipette, multi- or single-channel P20	Your choice	—
Plate centrifuge	Your choice	—
Vortexer	Your choice	—

E-Gels and reagents

Table 73 E-Gel and reagents required

Item	Supplier	Cat. No.
Mother E-Base™ Device	Thermo Fisher Scientific	EB-M03
Daughter E-Base™ Device (optional for running multiple gels in parallel)		EB-D03
E-Gel® 48 4% agarose gels		G8008-04
TrackIt™ 25 bp DNA Ladder		10488-022
TrackIt™ Cyan/Orange Loading Buffer		10482-028
Nuclease-free Water	Your choice	—

Consumables

Table 74 Gel and reagents required

Item	Supplier	Cat. No.
Adhesive film – use one of the following:		
• MicroAmp® Clear Adhesive Film	• Thermo Fisher Scientific	• 4306311
• Microseal® 'B' Film	• Bio-Rad	• MSB1001
Pipette Tips	Same brand as pipette	—

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

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

To dilute the TrackIt Cyan/Orange Loading Buffer:

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

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

To dilute the TrackIt 25bp Ladder (Cat. No. 10488-022, Thermo Fisher Scientific):

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

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

**Fragmentation QC
gel protocol**

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

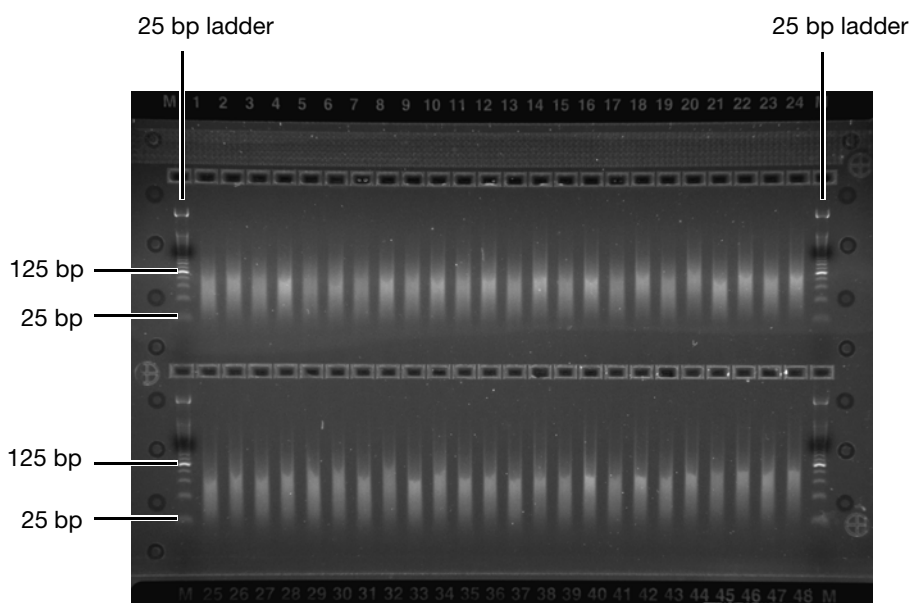
- Row A, C, E, G, or
- Row B, D, F, H

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

To run a fragmentation QC gel:

1. Tightly seal the Gel QC Plate produced during "Stage 3C: Sample QC".
2. Vortex the plate for 1 sec each corner and 1 sec in the center at the maximum setting; spin at 1000 rpm for 30 sec.
3. Connect an E-Base™ device(s) to an electrical outlet.
4. Push the Power/Prg button on each to ensure the program is in EG mode (not EP mode).
5. Take the gel out of the pouch and remove the combs.
6. Place the E-Gel® 48 gel into an E-Base unit.
7. Load 20 µL from each well of the Gel QC plate onto the gels.
8. Load 15 µL of diluted TrackIt 25 bp ladder into the marker wells (M).
9. Load 20 µL nuclease-free water into any unused wells.
10. Run the gels for 22 min.
11. Image the gel.

Fragmentation QC gel images should look similar to the gel shown in [Figure 58](#).



Fragments should fall between 125 bp and 25 bp.

Figure 58 Example of a typical fragmentation QC e-gel



Sample quantitation after resuspension

■	Protocol for sample quantitation after resuspension	162
■	Suggested protocol for OD quantitation using the DTX 880	164
■	Performing Sample Quantitation on a plate reader other than the DTX880 . .	169

Protocol for sample quantitation after resuspension

Equipment required The following equipment is required for this protocol.

Table 75 Equipment required for sample quantitation after resuspension

Quantity	Item
1	DTX 880 Multimode Detector with Genomic Filter Slide

Quantitate the diluted samples

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

For OD quantitation, readings should be taken at wavelengths of 260, 280, and 320 nm. See "[Suggested protocol for OD quantitation using the DTX 880](#)" on page 164 for more information.

To quantitate the diluted samples prepared for OD quantitation:

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

When the protocol is finished running, a list of results is displayed. If you used the formula provided in this appendix, two XML files are generated ([Figure 59](#)). Open the ResultData file with Microsoft® Excel® to view and assess the OD readings. RawData file information is included in the ResultData file.

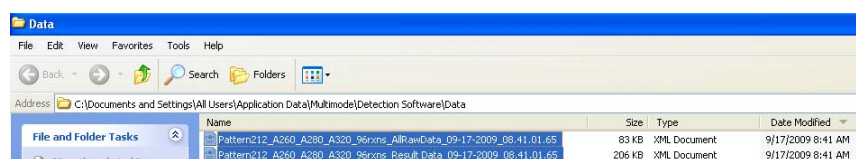


Figure 59 List of files that are generated post DTX-880 scan



Assess the OD readings

If using the formula provided in this appendix, the raw data is included in the final Result Data file. [Figure 60](#) is an example of a Result Data file. Your OD readings should be similar to those displayed below.

	A	B	C	D	E	F	G	H
	Well	Layout	REDUCTION_A1 - Abs260	REDUCTION_A2 - Abs280 ()	REDUCTION_A3 - Abs320 ()	REDUCTION_A4 - Purity ()	REDUCTION_A5 - Concentration	REDUCTION_A6 - Mass/rxn (ug)
6								
7	A1	S1	0.665	0.3471	0.0333	1.9159	13.0697	653.4828
8	A2	S2	0.6194	0.3253	0.0335	1.9041	12.1221	606.1034
9	A3	S3	0.6465	0.3156	0.0331	2.0485	12.691	634.5517
10	A4	S4	0.6011	0.3106	0.0343	1.9353	11.7269	586.3448
11	A5	S5	0.624	0.3207	0.0341	1.9457	12.2048	610.2414
12	A6	S6	0.5698	0.3123	0.0357	1.8245	11.0503	552.5172
13	A7	S7	0.6103	0.3269	0.0343	1.8669	11.9172	595.8621
14	A8	S8	0.5979	0.3135	0.0329	1.9072	11.6897	584.4828
15	A9	S9	0.601	0.3189	0.0338	1.8846	11.7352	586.7586
16	A10	S10	0.6893	0.3636	0.034	1.8958	13.5579	677.8966
17	A11	S11	0.6494	0.3461	0.0343	1.8763	12.7262	636.3103
18	A12	S12	0.6766	0.3587	0.0331	1.8863	13.3138	665.6897
19	B1	S13	0.6103	0.3167	0.0339	1.9271	11.9255	596.2759
20	B2	S14	0.6663	0.3221	0.0338	2.0686	13.0862	654.3103
21	B3	S15	0.5896	0.3102	0.0343	1.9007	11.489	574.4483
22	B4	S16	0.5771	0.3096	0.0358	1.864	11.1993	559.9655
23	B5	S17	0.5824	0.3099	0.0343	1.8793	11.34	567
24	B6	S18	0.5934	0.3217	0.0357	1.8446	11.5386	576.931
25	B7	S19	0.589	0.3223	0.0346	1.8275	11.4703	573.5172
26	B8	S20	0.6577	0.345	0.0342	1.9064	12.9	645
27	B9	S21	0.6513	0.3457	0.0363	1.884	12.7241	636.2069
28	B10	S22	0.6398	0.3489	0.0334	1.8338	12.5462	627.3103
29	B11	S23	0.6155	0.3502	0.0345	1.7576	12.0207	601.0345
30	B12	S24	0.6461	0.3435	0.0334	1.8809	12.6766	633.8276
31	C1	S25	0.5668	0.3134	0.0343	1.8086	11.0172	550.8621
32	C2	S26	0.5919	0.317	0.033	1.8672	11.5634	578.1724
33	C3	S27	0.6093	0.3362	0.0327	1.8123	11.9297	596.4828

Figure 60 Example of result data file with acceptable OD readings

OD yield assessment guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the Axiom 2.0 Mini 96 target prep samples. If the median yield for the plate is <525 µg DNA:

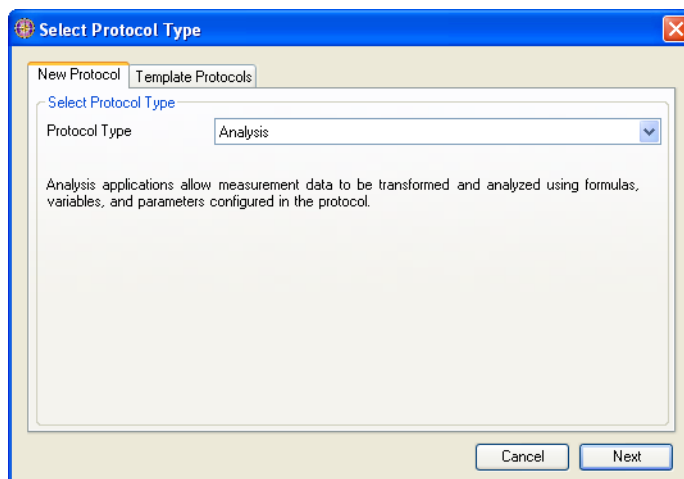
- Pause the protocol.
- Assess each of the steps performed to that point to determine the possible source of the low yields.

This DNA yield corresponds to an A_{260} value of approximately 0.59 and an A_{260} - A_{320} value of approximately 0.50.

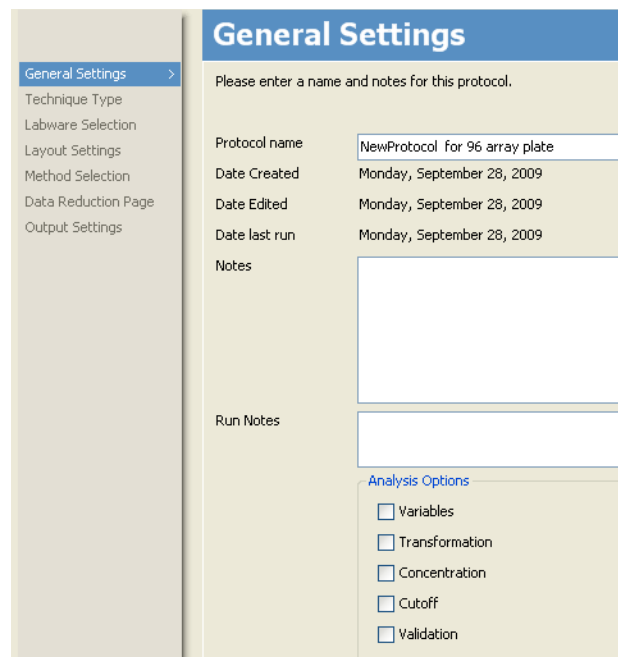
Suggested protocol for OD quantitation using the DTX 880

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

Protocol Type: Analysis



General Settings: enter a name for the protocol





Technique Type: select Absorbance

Technique Type

Select the desired technique type from the list below.

Technique Type

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

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

Labware Selection

Select the desired labware type from the list below.

Type of Labware	Name
	Standard 96
	Standard 384
	Standard 1536
	x_DTX_Abs_Greiner 384 V15 clear std
	x_Abs_Greiner 96 V15 clear std
	x_Abs_Greiner 96 UV clear std

Layout Settings: as appropriate for 96-array format plates

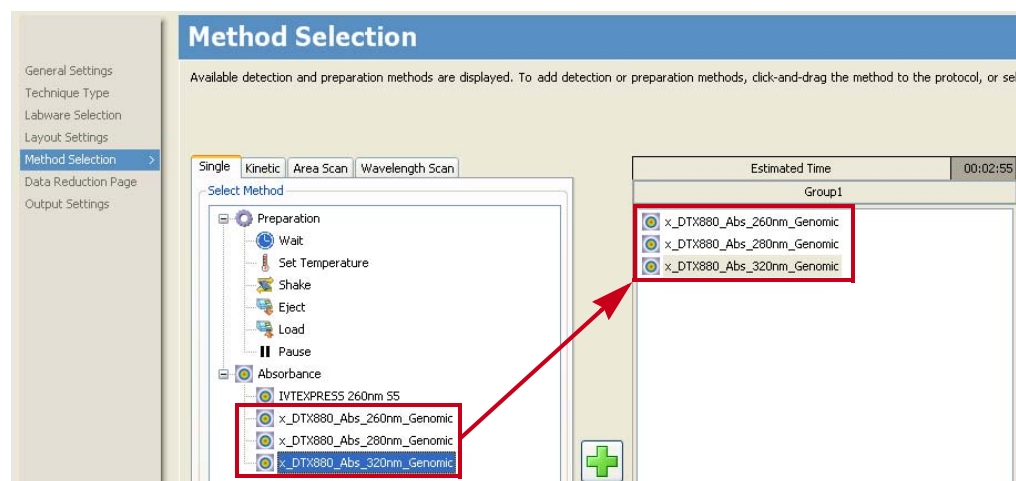
Layout Settings

Type: Filling: ☐ Vertical ☒ Horizontal Flow: ☐ Constant ☒ Incremental Replicates: Number ☐ Vertical ☒ Horizontal

Index:

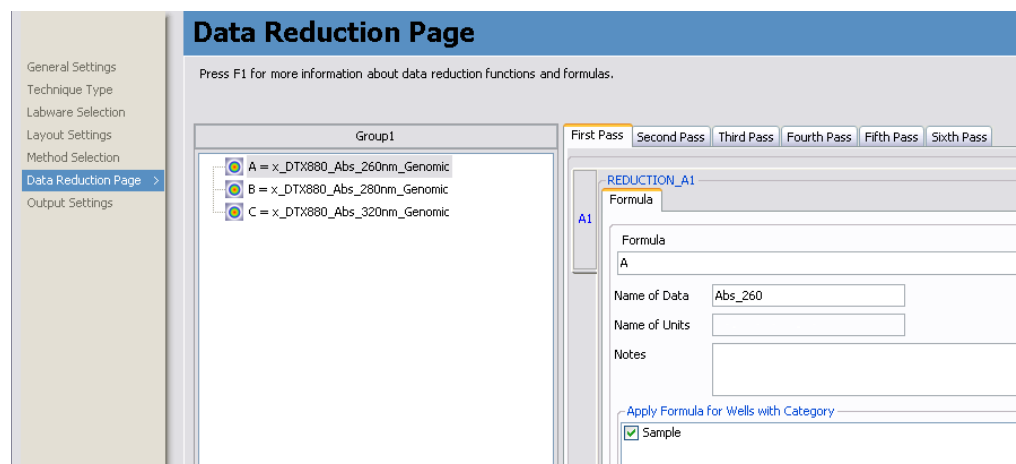
	1	2	3	4	5	6	7	8
A	S1	S2	S3	S4	S5	S6	S7	S8
B	S13	S14	S15	S16	S17	S18	S19	S20
C	S25	S26	S27	S28	S29	S30	S31	S32

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



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

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





Data Reduction Page

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

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

Group1

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

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

REDUCTION_A2

A2

Formula

B

Name of Data Abs_280

Name of Units

Notes

Apply Formula for Wells with Category

☒ Sample

Data Reduction Page

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

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

Group1

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

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

REDUCTION_A3

A3

Formula

C

Name of Data Abs_320

Name of Units

Notes

Apply Formula for Wells with Category

☒ Sample

Data Reduction Page

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

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

Group1

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

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

REDUCTION_A4

A4

Formula

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

Name of Data Concentration

Name of Units ug/L

Notes

0.29 = Pathlength

120 = Dilution Factor

0.05 = DNA extinction coefficient

Apply Formula for Wells with Category

☒ Sample

Data Reduction Page

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

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

Group1

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

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

REDUCTION_A5

Formula

A/B

Name of Data Purity

Name of Units

Notes

Apply Formula for Wells with Category

☒ Sample

Data Reduction Page

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

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

Group1

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

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

REDUCTION_A6

Formula

$((A-C)/0.29)*300$

Name of Data Mass/rxn

Name of Units ug

Notes

300 = 120 x 0.05 x 50
50 = volume per reaction of resuspended pellet

Apply Formula for Wells with Category

☒ Sample

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

Output Settings

Select data output and printer options.

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

Perform after completing measurement(s)

- ☒ Export to Microsoft® Excel (Old Format, Version < 3.2)
- ☒ Define User Defined Excel Export
- ☒ Show Result Viewer
- ☐ Create .XML and .dat data files
- ☐ Automatic Print out after measurement.

Save the protocol.



Performing Sample Quantitation on a plate reader other than the DTX880

Your plate reader should be calibrated to ensure accurate readings.

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

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

Where:

A = the observed OD₂₆₀

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

D = 120 (the net dilution factor when preparing the OD Sample plate as described in the Automated Target Preparation Protocol)

V = 50 (the volume of the sample in µL after the resuspension step)

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

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

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

The optical path length is dependent on the type of plate and may depend on the spectrophotometer used. Check your manufacturer's recommendations for the path length for your instrument and plate type or for recommendations on how to measure this quantity. The SpectraMax Plus384, described as an alternative spectrophotometer in the *Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide*, Pub. No. 703435, can employ an automated path length detection system. Consult this instrument's user guide for more information.

The resulting yield calculations can be compared against the typical yields shown in column H of [Figure 60 on page 163](#) and against ["OD yield assessment guidelines" on page 163](#).



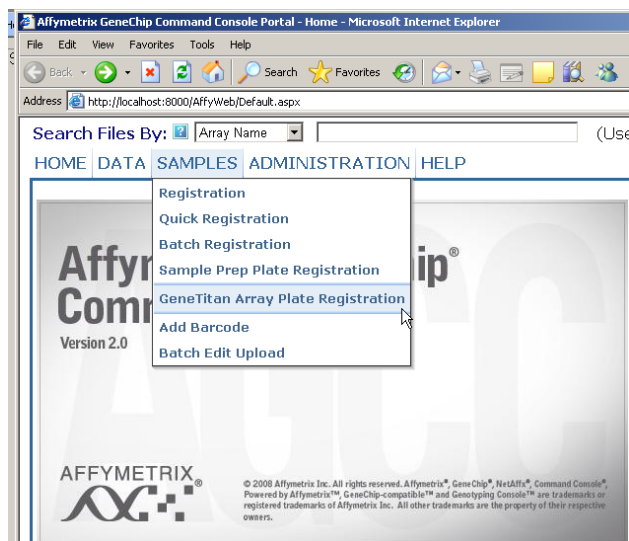
Registering samples in GeneChip™ Command Console™

Creating a GeneTitan™ Array Plate Registration file

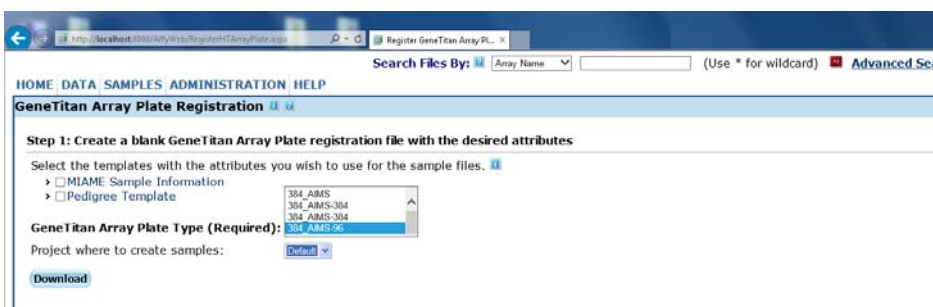
A GeneTitan Array Plate Registration file is a Microsoft® Excel® spreadsheet that includes information on the samples you are processing on a single array plate. This information includes the array plate format, the array plate barcode, and sample file names so that you can track the samples that are loaded onto a particular array plate. The version of Microsoft Excel must be 1997-2000 (file extension is .xls; not .xlsx).

To create a GeneTitan Array Plate Registration file:

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



2. Create a new template in AGCC that includes fields that will achieve sample traceability
3. Select the array plate to be processed on the GeneTitan MC Instrument.
 - a. Select the newly created template that contains the fields required for sample traceability.
 - b. Select the array plate type.
 - c. Select the project where sample registration and all associated data files will be saved.
 - d. Click **Download**.



4. Complete the registration file as follows:

- Click the Microsoft Excel box on the bottom bar of the monitor to open the Excel spreadsheet.
- Enter a unique name for each sample (Sample File Name) and any additional information you would like to include, such as hybridization tray barcode.

Note: Tip: The AGCC template created in [Step 2](#) must have a field that reads Hyb Tray Barcode. The Excel file that will be downloaded will have a column header that reads, “**Hyb Tray Barcode:*:Text**”. The Barcode of the hybridization tray can be scanned into the “Hyb Tray Barcode” text field. This barcode will be stored in to the sample file for each array.

- Do 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 and proceed to the next step.
 - If you are not ready to load the array plate onto the GeneTitan MC Instrument, proceed directly to the next step.

GeneTitanArrayPlateRegistrationTemplate_AGCC r3_AIMS96.xls [Compatibility Mode] - Microsoft Excel							
G23							
	A	B	C	D	E	F	G
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array Position	Barcode	Sample File Name
2		Default	384_AIMS-96	384_AIMS	A01	5504144288441041117925	
3		Default	384_AIMS-96	384_AIMS	A03	5504144288441041117925	
4		Default	384_AIMS-96	384_AIMS	A05	5504144288441041117925	
5		Default	384_AIMS-96	384_AIMS	A09	5504144288441041117925	
6		Default	384_AIMS-96	384_AIMS	A11	5504144288441041117925	
7		Default	384_AIMS-96	384_AIMS	A13	5504144288441041117925	
8		Default	384_AIMS-96	384_AIMS	A15	5504144288441041117925	
9		Default	384_AIMS-96	384_AIMS	A17	5504144288441041117925	
10		Default	384_AIMS-96	384_AIMS	A19	5504144288441041117925	
11		Default	384_AIMS-96	384_AIMS	A21	5504144288441041117925	
12		Default	384_AIMS-96	384_AIMS	A23	5504144288441041117925	
13		Default	384_AIMS-96	384_AIMS	C01	5504144288441041117925	
14		Default	384_AIMS-96	384_AIMS	C03	5504144288441041117925	
15		Default	384_AIMS-96	384_AIMS	C05	5504144288441041117925	
16		Default	384_AIMS-96	384_AIMS	C07	5504144288441041117925	
17		Default	384_AIMS-96	384_AIMS	C09	5504144288441041117925	

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

Register GeneTitan Array Plate - Windows Internet Explorer

http://localhost:8000/AffyWeb/RegisterHTArrayPlate.aspx

File Edit View Favorites Tools Help

Search Files By: Array Name (Use * for wildcard) Advanced Search

HOME DATA SAMPLES ADMINISTRATION HELP

GeneTitan Array Plate Registration

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

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

☐ MIAME Sample Information

☐ Pedigree Template

GeneTitan Array Plate Type (Required): 384_AIMS-96

Project where to create samples:

Download

Step 2: Enter the values for the sample (.ARR) files in the GeneTitan Array Plate registration file.

Enter values for the attributes using Excel. Additional columns for new attributes can be added to the spreadsheet at any time.

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

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

GeneTitan Array Plate registration file (Required): C:\Documents and Settings\AFFXUser\Desktop\Affymetrix_Downloads\GeneTitanArrayPlateR Browse

GeneTitan Array Plate Barcode:

Upload

Mouse over for tips on step.

HOME DATA SAMPLES ADMINISTRATION HELP

Confirm GeneTitan Arrays Plate Sample Registration

Registered GeneTitan Array Plate Samples successfully.



GeneTitan™ Multi-Channel Instrument care

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■	Troubleshooting	183

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

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

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

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

Cleaning and maintenance

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

The following tasks should be performed regularly to ensure the imaging device remains in working order.

Monthly

Wipe down the outer surface of the imaging device with a dry cloth.

Every six months

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

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

Servicing the outer enclosure fan filters

Cleaning schedule

The GeneTitan fan filter cartridge (Figure 61) should be cleaned at least every 90 days of service. Note that in some service locations, the presence of excessive dust or particulate matter may necessitate cleaning the cartridge more often than 90 days.

A plugged filter cartridge can cause excessive temperatures within the machine that can cause unwanted evaporation of GeneTitan reagents.

Part details for GeneTitan fan filter:

Thermo Fisher Scientific Cat. No. 01-0669

Number of filters required per GeneTitan Instrument: 3

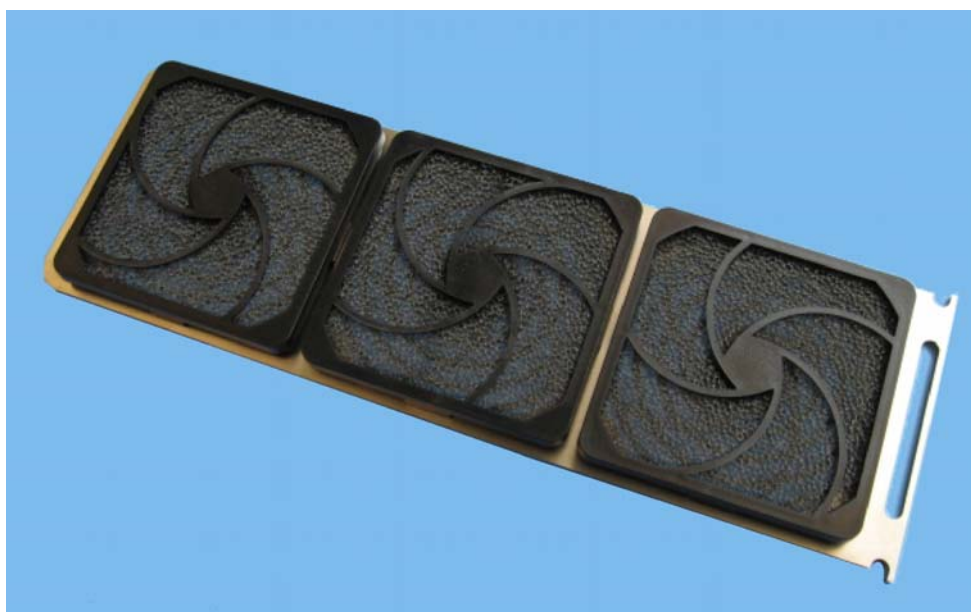


Figure 61 The GeneTitan™ filter cartridge

Cleaning procedure

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

Replacing the bottle filters

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

The message boxes displayed in [Figure 62](#) will provide information on fluid dispense errors that were detected by the instrument for any of the bottles or when the instrument detects an increase in the amount of time that is required to perform the fill operations.

If an error is detected as described above, then a message box titled “Filter Change Required” is displayed ([Figure 62](#)) along with the information on the specific dispense operation. You should change all three filters when a warning is displayed for any one of the three filters.

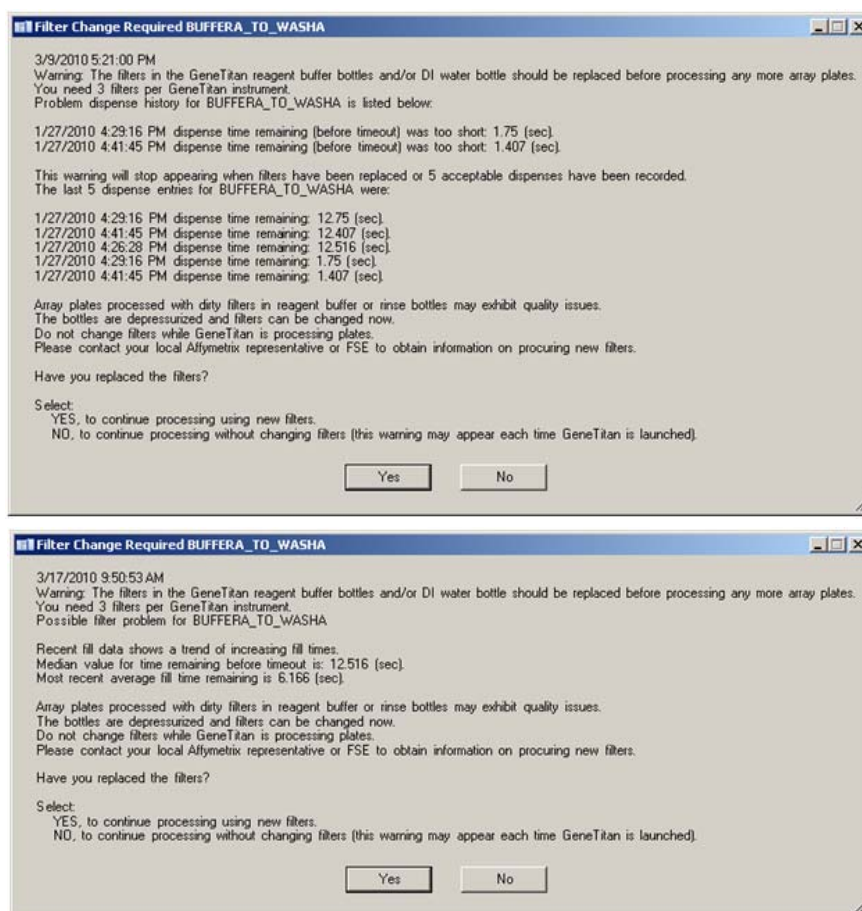


Figure 62 Filter Change Required Messages

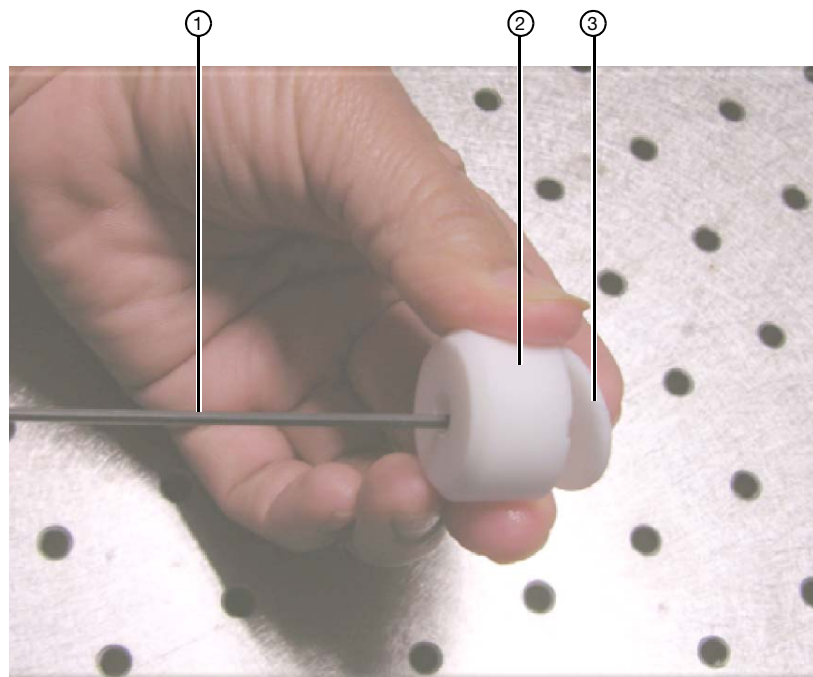
Note: The reagent bottles are depressurized when this warning message is displayed. It is safe to change the filters in all three fluidic bottles when this message is displayed.

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

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

GeneTitan reagent bottle filters part details:

Thermo Fisher Scientific Cat. No. 01-0671



- ① Buffer supply line
- ② Filter holder
- ③ Filter

Figure 63 Replacing the filter

Removing and inspecting the filter

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

Replacing the filter

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

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

Replacing the Xenon lamp in the GeneTitan™ MC Instrument

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

Thermo Fisher Scientific GeneTitan xenon lamp Cat. No. 01-0740

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

Lamp life/imaging device status notices

The Imaging Status pane displays lamp life and Imaging Device status notices for the GeneTitan MC Instrument.

In normal operation, the pane displays the hours of life left in the lamp (Figure 64):

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

Figure 64 Lamp life above tolerance

It displays a red or yellow notice when the lamp life is getting short (Figure 65):

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

Figure 65 Lamp life above tolerance

It also displays a red notice when the Imaging Device is offline (Figure 66):

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

Figure 66 Imaging device offline

Note: The 300 watt xenon lamp in the GeneTitan MC Instrument is warranted for 500 hours. The instructions to replace the lamp are available on the following page. After changing the lamp, it is necessary to reset the lamp life clock manually.



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

Removing the xenon lamp

1. Unscrew the four retaining bolts. They should be finger tight ([Figure 67](#)).



Figure 67 Unscrewing the bolts

2. Remove and set aside the warning cover to reveal the xenon lamp contained within.
3. Place each hand on each side of the blue plastic flange and lift out the lamp in a vertical motion ([Figure 68](#)). You must use both hands to remove the lamp successfully. Apply equal pressure on each side of the lamp and gently lift.

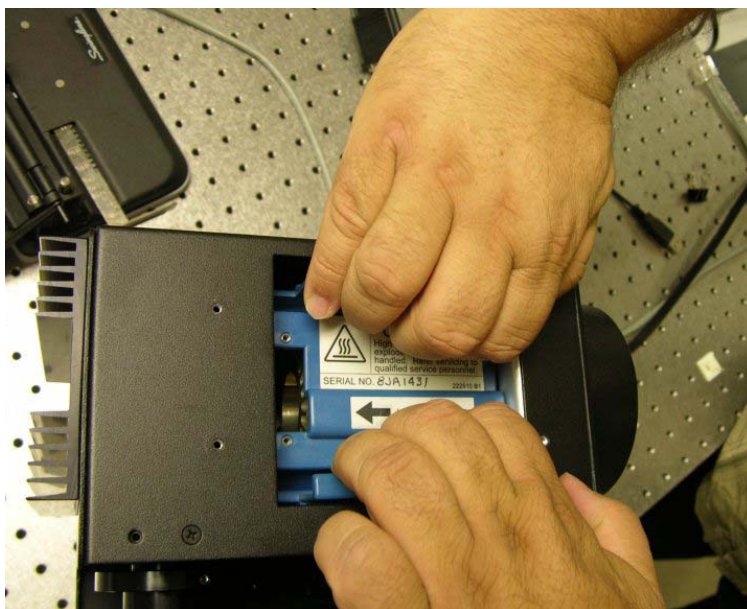


Figure 68 Lifting out the lamp

Replacing the lamp

 **CAUTION!** Ensure that you install the lamp in the correct orientation.

1. Hold the lamp by the blue plastic flanges. Ensure that the lamp bulb faces inward toward the reflecting mirror ([Figure 69](#)) and vertically insert the lamp ([Figure 70](#)).
2. Replace the warning cover and hand tighten the bolts ([Figure 67](#)).

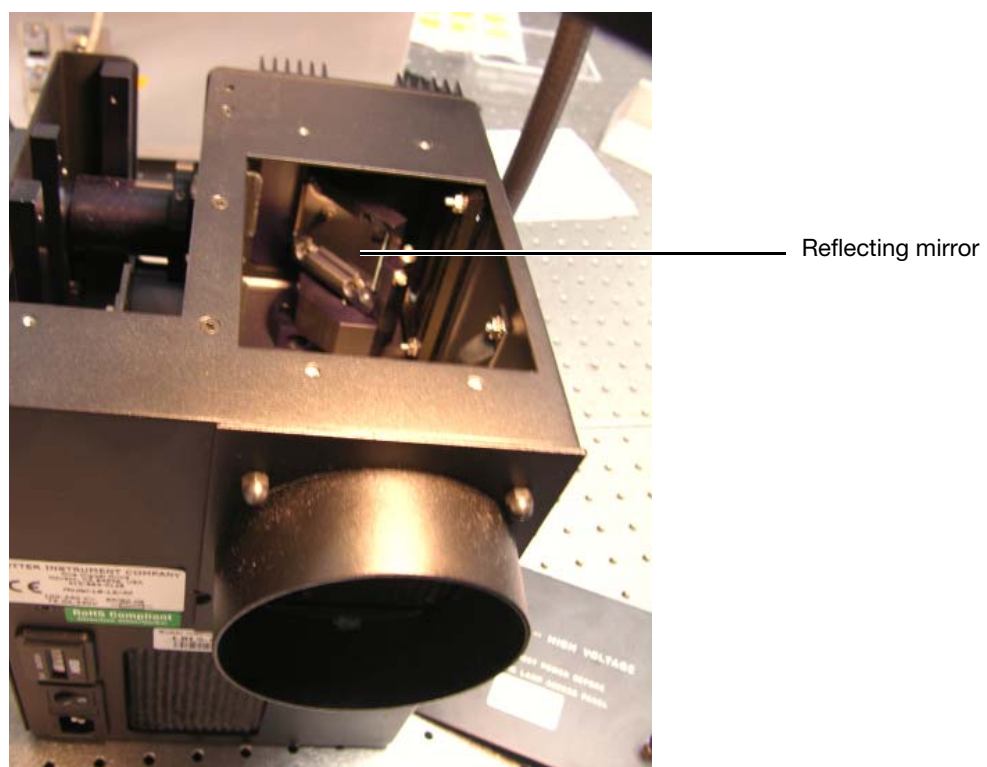


Figure 69 The reflecting mirror

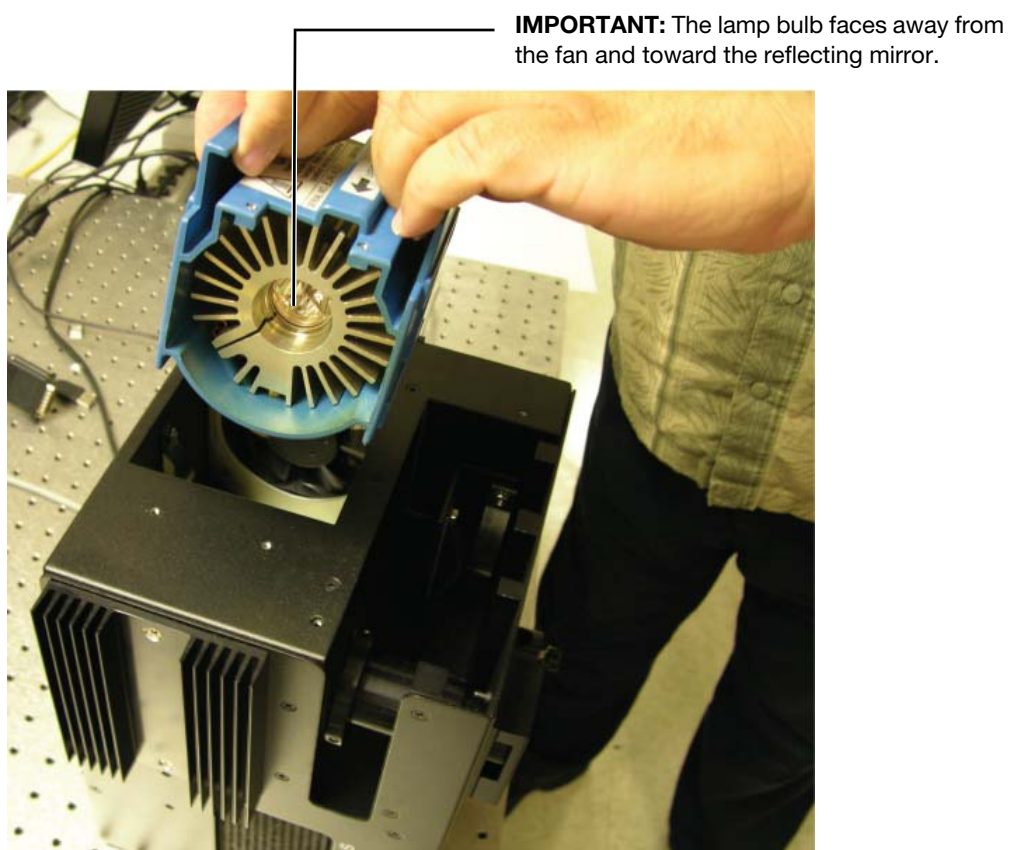


Figure 70 Inserting the lamp



Resetting the lamp counter

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

1. On the software application click **Tools** → **Reset Counter for Life Remaining** (Figure 71).

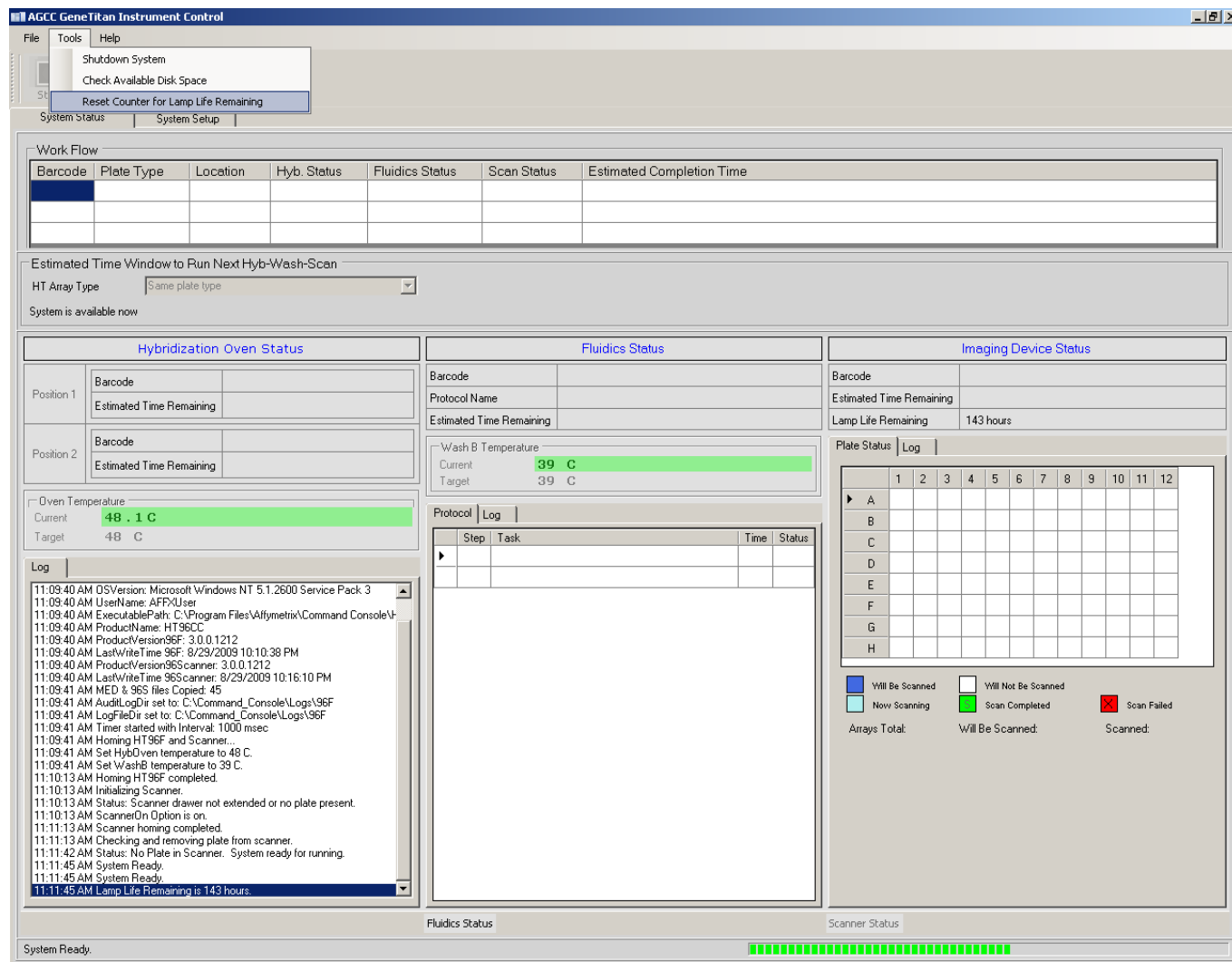


Figure 71 Inserting the lamp

2. The software will display a message that asks you to confirm the lamp life counter is being reset as a result of lamp replacement (Figure 72).

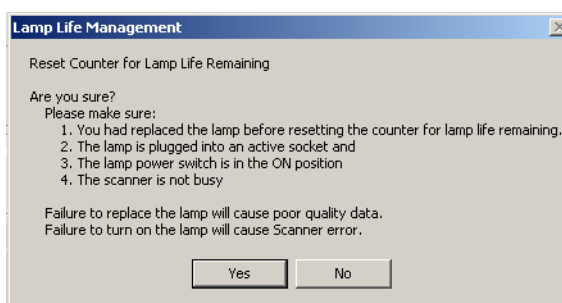


Figure 72 Are you sure?

3. Click **Yes** if you want to reset the counter. The software will display a message that confirms that the software has reset the counter (Figure 73).

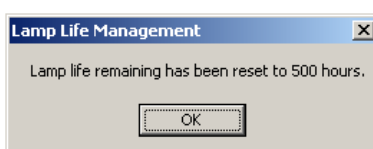


Figure 73 The counter is reset.

Troubleshooting

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

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

Log files

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

AGCC log files

The following files are generated by the GeneTitan Instruments. All the AGCC log files are from the following path: C:\Command_Console\Logs. The different log files include:

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

Other AGCC files

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

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

AGCC 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 may need the following files for troubleshooting:

GeneTitan MC Instrument fluidics

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

GeneTitan MC Instrument imaging device

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

Problems and solutions

This section provides instructions on how to identify and solve problems with the unit. If problems arise with the instruments use the following tables to locate the description that matches the problem. If you cannot find a solution call Thermo Fisher Scientific technical support for assistance.

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

Insufficient disk space notice

If there is not enough memory on the computer's drives to save the data from an array plate, a notice appears (Figure 74) when:

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

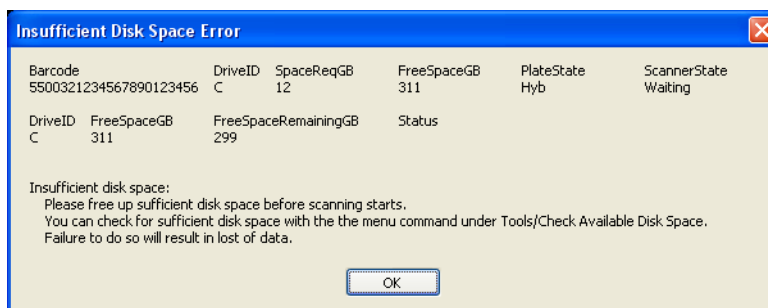


Figure 74 Insufficient disk space notice

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

Documentation and support

Related documentation

Table 76 Documentation related to the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol

Document	Publication number	Description
<i>Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol Site Preparation Guide</i>	703435	Provides guidance on reagents, instruments, and supplies required to run the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol.
<i>Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol QRC</i>	703436	An abbreviated reference for the Axiom 2.0 Assay Mini 96-Array Format Manual Protocol target preparation intended for experienced users.
<i>GeneTitan™ MC Protocol for Axiom™ 384HT Array Plate Processing QRC</i>	703164	
<i>GeneTitan™ Multi-Channel Instrument User Guide</i>	08-0308	The GeneTitan Multi-Channel (MC) Instrument automates array processing from target hybridization to data generation by combining a hybridization oven, fluidics processing, and state-of-the art imaging device into a single bench-top instrument. This document detailing the use, care, and maintenance for the GeneTitan MC Instrument.
<i>GeneTitan™ Multi-Channel Instrument Site Preparation Guide</i>	08-0305	Provides guidance on creating and maintaining the proper environment required for the GeneTitan Multi-Channel Instrument.
Data analysis and software		
<i>Axiom™ Genotyping Solution Data Analysis Guide</i>	702961	This guide provides information and instructions for analyzing Axiom genotyping array data. It includes the use of Axiom™ Analysis Suite, Applied Biosystems Microarray Power Tools (formerly APT) and SNPolarizer R package to perform quality control analysis (QC) for samples and plates, SNP filtering prior to downstream analysis, and advanced genotyping methods.

Table 76 Documentation related to the Axiom™ 2.0 Assay Mini 96-Array Format Manual Protocol

Document	Publication number	Description
<i>Applied Biosystems™ GeneChip™ Command Console™ Software User Guide</i>	702569	This user guide provides instructions on using Applied Biosystems GeneChip Command Console Software (formerly AGCC) used to control GeneChip instrument systems. Command Console Software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip Arrays.
<i>Axiom™ Analysis Suite User Guide</i>	703307	This user guide provides instructions on using Axiom™ Analysis Suite—a single-source software package to enable complete genotyping analysis of all Axiom arrays.

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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.

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References

- Manolio T.A. and Collins F.S.: **The HapMap and Genome-Wide Association Studies in Diagnosis and Therapy**. *Annu Rev Medicine* 2009, **60**:443–56
- Klein RJ, Zeiss C, Chew EY, *et al.*: **Complement factor H polymorphism in age-related macular degeneration**. *Science* 2005, **308**:385–89
- Hindorff LA, Junkins HA, Mehta JP, and Manolio TA.: **A Catalog of Published Genome-Wide Association Studies**. Available at: www.genome.gov/gwastudies. Accessed 09/28/2009.www.genome.gov/gwastudies. Accessed 09/28/2009.
- Paux, E., *et al.*, **Sequence-based marker development in wheat: Advances and applications to breeding**. *Biotechnology Advances*, Corrected Proof, online 1 October 2011. doi:10.1016/j.biotechadv.2011.09.015
- Rincon, G., *et al.* **Hot topic: Performance of bovine high-density genotyping platforms**. *Holsteins and Jerseys Journal of Dairy Science* 2011, **94**: 6116-6121

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