Axiom™ 2.0 Assay 96-Array Format Manual Workflow

USER GUIDE

for use with:
Axiom™ Arrays
Axiom™ 2.0 Reagent Kit

Catalog Numbers 901758
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Revision D00

ThermoFisher SCIENTIFIC

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Products: Axiom™ 2.0 Reagent Kit

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Products: Axiom™ Array Plates

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

The information in this guide is subject to change without notice.

Revision history

Table 1 Revision history of Pub. No. MAN0018000.

Revision	Date	Description
D00	22 April 2024	Added an “Important” note on page 24 explaining the two 96-array plate configurations.
C.0	10 November 2023	Changed to Multiskan™ SkyHigh Microplate Spectrophotometer.
B.0	28 September 2023	Added information for GeneTitan MC Fast Scan Instrument.

A.0	19 May 2022	• Initial release in Thermo Fisher Scientific document control system. Supersedes legacy Affymetrix publication number 702990.
		• Add the following product options:
		  • MicroAmp EnduraPlate Optical 96-Well Clear Reaction Plates.
		  • Bio-Rad HSP9631, HSP9601, HSS9601, and HSS9641 PCR plates.
		  • Eppendorf DeepWell Plate 96 for microbiome assay use.
		  • ProFlex 96 well PCR System thermal cycler.
		  • Genomic DNA Standard (Ref 103) positive control, available for purchase separately.
		• Added information for the microbiome assay.
		• Updated the gDNA input requirements and added the microbiome DNA input requirements.

Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Corporate entity

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Contents

1 About the Axiom™ 2.0 Assay

NOTE: In this chapter and throughout the document, the instructions and consumables for the GeneTitan™ MC Instrument are applicable to the GeneTitan™ MC Fast Scan Instrument.

The Axiom™ Solution consists of a technology platform that includes assay biochemistry, automated and manual target preparation options, multiple array plate formats, and array processing on the GeneTitan™ MC or GeneTitan™ MC Fast Scan Instrument. This solution has applications in human genetics research, basic and applied agriculture research, and microbiome research.

Axiom™ myDesign™ Genotyping Arrays allow you to select your own content, enabling you to include markers relevant for your specific study. Markers for myDesign arrays can be chosen from many sources including, but not limited to, SNPs from the Axiom™ Genomic Database; sequencing initiatives; or your own personally selected variants. From whole-genome to targeted variant studies, Axiom myDesign Arrays enable rapid advances in genetics research.

The Axiom Genotyping Solution is a product line that provides catalog arrays with potential benefits such as:

- Optimized arrays for high coverage, cost-effective genetics and microbiome studies
- Automated and manual target preparation which includes methods for DNA amplification, fragmentation, purification and resuspension of the target in hybridization cocktail.
- Hands-free processing of array plates on the GeneTitan™ MC or GeneTitan™ MC Fast Scan Instrument
- Automated software packages for stream-lined analysis.

References

1. Klein RJ, Zeiss C, Chew EY, et al.: **Complement factor H polymorphism in age-related macular degeneratio**n. *Science* 2005, 3**08**:385–89
2. 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.
3. Fairley S, et al.: **The International Genome Sample Resource (IGSR) collection of open human genomic variation resources**. Nucleic Acids Research, Volume 48, Issue D1, 08 January 2020, Pages D941–D947.

Overview of the Axiom 2.0 Assay Manual Workflow Assay

Running the Axiom 2.0 Assay requires the following sets of steps:

1. Genomic DNA Preparation--Resulting in samples that meet requirements spelled out in Chapter 2, "Genomic DNA preparation and requirements" on page 11.
2. Target Preparation of the samples (see Chapter 4, "Manual target preparation" on page 40).
3. Array Processing, done with
   - GeneTitan™ MC or GeneTitan™ MC Fast Scan Instrument
   - GeneTitan Instrument Control software
   - GCC Portal software

   See Chapter 5, "Array processing with the GeneTitan™ MC Instrument" on page 85.

A list of the required equipment and supplies for running the Axiom 2.0 Assay manual target preparation can be found in the *Axiom™ 2.0 Assay Manual Workflow Site Preparation Guide*, Pub. No. MAN0018132.

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 forty-hour week. The timing of steps is critical, whether using automated target preparation or manual target preparation because of the following constraints:

- Incubation after DNA Amplification is 23 hours, ± 1 hour.
- Hybridization in the GeneTitan Instrument is 23.5 hours.
- Reagent trays for wash/stain/imaging must be prepared as hybridization finishes
- Limits to when a second hybridization tray and array plate can be loaded into the GeneTitan™ MC or GeneTitan™ MC Fast Scan Instrument.

These limitations require careful timing.

The details are covered in Chapter 6, "Process 3 Axiom™ array plates per week" on page 122.

2 Genomic DNA preparation and requirements

IMPORTANT! Note for Axiom™ Microbiome Array users: For guidance on DNA sample preparation and requirements for the Axiom Microbiome Array, see Section 1, Chapter 2 "DNA Preparation and Requirements" of the *Axiom™ Microbiome Solution User Guide* (Pub. No. 703408).

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 can affect the enzymatic reactions to be performed.

For this protocol, you will use the Axiom™ 2.0 Reagent Kit (Cat. No. 901758). Axiom™ Genomic DNA Standard (Ref. 103) (Cat. No. 951957) is available for purchase separately. This DNA meets the requirements outlined below, and is included for use as a control. The size and purity of sample gDNA can be compared with those of the control DNA to assess sample quality. The control DNA should also be used routinely as an experimental positive control and for troubleshooting purposes.

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

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

Sources of genomic DNA

The following sources of human gDNA have been successfully tested in the 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 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 bovine 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 and meet the requirements:

- Seeds
- Leaves

The following sources of microbial gDNA and cDNA from RNA viruses have been successfully tested and meet the requirements:

- Stool

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

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

General requirements

- Starting DNA must be double-stranded for the purpose of accurate concentration determination.
- DNA must be of high purity.
  DNA should be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (i.e., EDTA). The gDNA extraction/ purification method should render DNA that is generally salt-free because high concentrations of particular salts can also inhibit enzyme reactions. DNA purity is indicated by OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios. The OD_{260}/OD_{280} ratio should be between 1.8 and 2.0 and the OD_{260}/OD_{230} ratio should be greater than 1.5. We recommend that DNA samples that do not meet these criteria be cleaned up as described under "Genomic DNA cleanup" on page 14.
- DNA must not be degraded.
  The approximate average size of gDNA can be assessed on a 1% agarose gel using an appropriate size standard control. Approximately 90% of the DNA must be greater than 10 Kb in size. Control DNA can be run on the same gel for side-by-side comparison.

Special requirements

Pre-amplification area

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

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

Ideally, this pre-amplification area would be separate from the amplification staging area described in Chapter 3, on page 32, however these areas can be combined due to space and equipment limitations.

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

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

Equipment and reagents recommended

Table 1 E-Gel™ and reagents required

Item	Cat. No.
E-Gel™ Power Snap Plus Electrophoresis Device	G9110
iBright™ CL750 Imaging System	A44116
E-Gel® *48* 1% agarose gels	G8008-01
*Redi*Load™	750026
E-Gel® 96 High Range DNA Marker	12352-019

Guidelines for preparing the genomic dna plate for gel analysis

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

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

1. Insert the two 48 well 1% Agarose E-Gels into the electrophoresis unit.
2. Remove 2 combs.
3. Load 20 µL from the above plate onto two 48 well 1% agarose E-Gels.
4. Load 15 µL of diluted High Range DNA Marker (1:3 dilution or ~0.34 X from stock) into all marker wells (as needed).
5. Fill all empty wells with water.
6. Run the gel for ~27 minutes.

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 1 shows gel images of intact gDNA (that is suitable for use in the Axiom 2.0 Assay) and degraded gDNA samples. Customers whose gDNA is degraded (similar to the image in Figure 1) should perform a test experiment to investigate the performance of their samples in the Axiom assay before beginning any large scale projects.

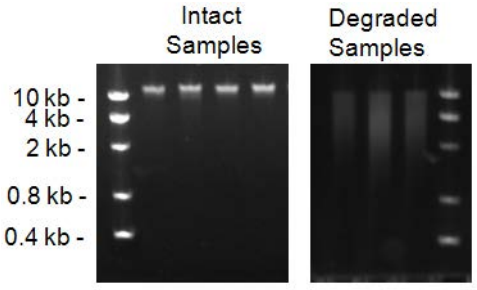


Figure 1 Gel images showing intact gDNA and degraded gDNA.

Genomic DNA extraction/purification methods

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

Genomic DNA cleanup

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

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

Genomic DNA preparation

This step needs to be done before proceeding with the DNA amplification stages.

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. All human Axiom arrays (except the Axiom™ Genome-Wide Pan-African Array Set) require a total of 100 ng. The Axiom Genome-Wide Pan-African Array Set requires a total of 300 ng, or 100 ng per array (there are 3 arrays in the Axiom Genome-Wide Pan-African Array Set). Diploid plants and animals require 150 ng per array and polyploid plants and animals require 200 ng per array. For Axiom Microbiome Arrays, a total of 50 ng of gDNA is required per array.

Table 2 Input requirements for Axiom 2.0 Assay.

Sample type	Volume per well	Input mass per well	gDNA concentration
Human	20 µL	100 ng	5 ng/µL
Diploid plants and animals	20 µL	150 ng	7.5 ng/µL
Polyploid plants and animals	20 µL	200 ng	10 ng/µL
Stool	20 µL	50 ng	2.5 ng/µL

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 GeneTitan Array Plate Registration file"

Note: For detection of RNA viruses, RNA must be reverse transcribed to yield input amenable to Axiom target preparation using the protocol outlined in Section 1, Chapter 3 of the *Axiom™ Microbiome Solution User Guide* (Pub. No. 703408).

Duration

Thirty to 60 minutes for reagents to thaw and 30 minutes for setup.

Equipment, consumables and reagents required

Equipment and consumables

The equipment and consumables listed in Table 3 are required for this stage.

Table 3 Equipment and consumables required for "Genomic DNA preparation".

Quantity	Item
As required	Adhesive seals for plates
1	Ice bucket, filled with ice
1 each	Pipettes: • Single channel P10 or P20 • Optional: multichannel P10 or P20
As required	Pipette tips
1	Deepwell plate: • For Axiom Genotyping: ABgene 96 Square Well Storage; Cat. No. AB-0932 or Eppendorf 96 Deepwell Plate, 2,000 µL; Eppendorf, Cat. No. 951033481. • For Axiom Microbiome: Eppendorf 96 Deepwell Plate, 2,000 µL; Eppendorf, Cat. No. 951033481.[1]
1	Plate centrifuge
1	Plate spectrophotometer (required only if no OD measurements available for samples)
1	Vortexer

[1] This is the only plate supported for Axiom microbiome use. Using a different plate type can result in assay failure.

Reagents

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

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

Table 4 Reagents required for "Genomic DNA preparation".

Reagent	Source
Genomic DNA Standard (includes Axiom™ Genomic DNA Standard (Ref.103) for use as a positive control), –20°C	951957
Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	MLS
Positive control gDNA (if genotyping non-Human samples)	
No template control (if assaying microbial samples)	

1. Thaw samples and control

Thaw the components listed below to room temperature:

- gDNA samples
- gDNA positive control sample. For human and microbiome studies, use Axiom Reference Genomic DNA 103.

To thaw, either:

- Place items on benchtop for 1 hour
- Thaw in a water bath:
  a. Fill a small plastic dish with ultra-pure water. Do not overfill as the level of the water should not overflow when the sample tubes or plates are placed in the bath.
  b. Thaw the sealed sample plate and reference sample for 30 minutes.
  c. Remove the sample plate and/or sample tube from the water bath, then wipe dry using laboratory tissues. Ensure that 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

Note: Do *not* dilute the Reference Genomic DNA 103 control. It is already at a working concentration.

1. Gently vortex (50% maximum) and centrifuge the gDNA and gDNA positive control sample (if not using Axiom Reference Genomic DNA 103).
2. *Recommendation*: quantitate each sample (for example, using the Quant-iT™ PicoGreen® dsDNA Kit).
3. Using reduced EDTA TE buffer, dilute each sample to a concentration of:
   - 5 ng/µL for human DNA samples
   - 7.5 ng/µL for diploid plant and animal DNA samples
   - 10 ng/µL for polyploid plant and animal DNA samples
   - 2.5 ng/µL for stool samples
4. Seal, vortex and centrifuge.

3. Aliquot the diluted samples and the control

Next, the samples and control are placed in the following deep well plate for target preparation:

Axiom genotyping

For Axiom genotyping arrays, use the ABgene 96 Square Well Storage Plate, Cat. No. AB-0932 or Eppendorf 96 Deepwell Plate, 2,000 µL; Eppendorf, Cat. No. 951033481.

Aliquot diluted samples and control gDNA into the ABgene 96 Square Well Storage Deepwell Plate:

1. Aliquot 20 µL of each diluted gDNA sample (this should be the equivalent of 100 to 200 ng of gDNA, as required by the sample type) reserving at least 1 empty well if planning to include a positive control.

   Note: Thermo Fisher Scientific recommends including at least 1 positive control on each plate.
2. If including a positive control, aliquot 20 µL of gDNA control to the empty well reserved in Step 1.
3. Seal and centrifuge.

Note: For samples to be processed on the Axiom Genome-Wide Pan-African Array Set, 3 identical deep well plates of 100 ng gDNA per well should be made

Axiom Microbiome

- For Axiom™ Microbiome Arrays, use the Eppendorf™ 96 Deepwell Plate, 2,000 µL; Cat. No. 951033481. This plate is the only plate supported for Axiom microbiome use. Using a different plate type may result in assay failure.

Aliquot diluted samples and controls into the Eppendorf Deepwell Plate for 96-format manual target preparation

1. Aliquot 20 µL of each diluted gDNA sample. This should be the equivalent of 50 ng of gDNA, as required.
2. Aliquot the controls:
   - Positive control: Aliquot 20 µL of the Reference Genomic DNA 103 control into its designated well.
   - Negative control: Aliquot 20 µL of no template control (elution buffer or reduced EDTA TE buffer) into its designated well.
3. When including cDNA templates (see Section 1, Chapter 3, "cDNA Synthesis for RNA Samples" in the *Axiom™ Microbiome Solution User Guide*, Pub. No. 703408) first transfer 2.5 µL of Reduced EDTA TE Buffer to the sample plate. Then add 17.5 µL of cDNA template generated.
4. Seal and centrifuge.

   Note: Thermo Fisher Scientific requires including Axiom Reference Genomic DNA 103 as a positive control and the use of a no template control on each plate.

4. Freeze or proceed

At this point you can:

- Store the sample plate at –20°C, or
- Proceed to DNA Amplification for Manual Target Preparation. See Chapter 4, "Manual target preparation" on page 40.

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

5. Create a GeneTitan Array Plate Registration file

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

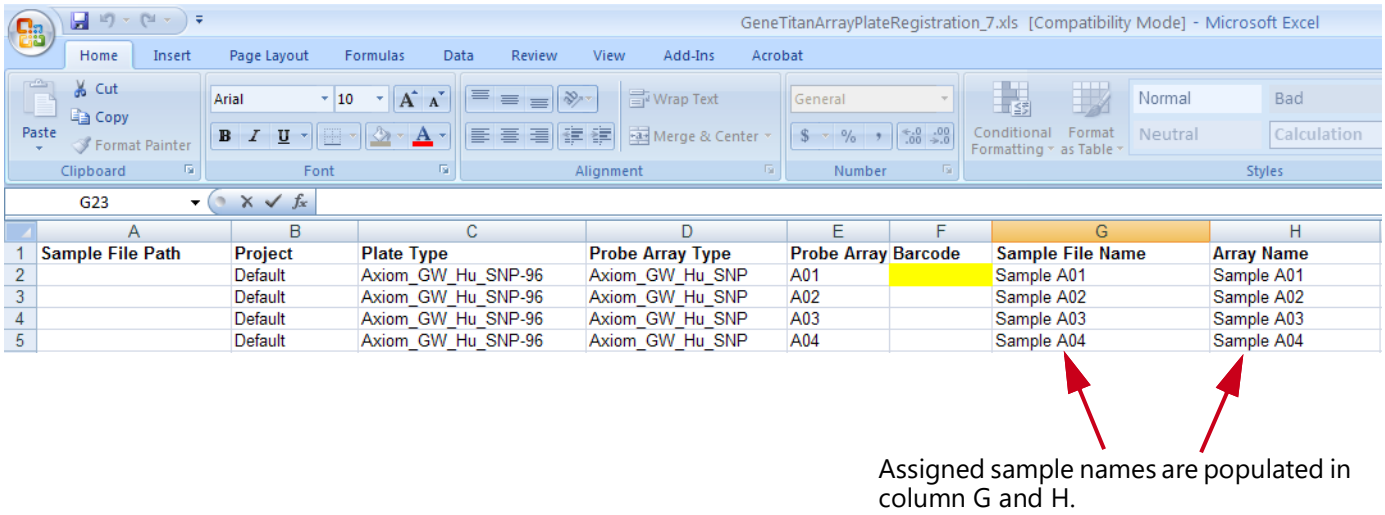
GeneTitan Array Plate Registration files contain information that is critical for:

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

Detailed instructions for creating this file are located in Appendix C, "Registering samples in GeneChip™ Command Console™" on page 152. See also Figure 2 for a screen shot showing an example of a GeneTitan Array Plate Registration file.

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

The array plate barcode will not be scanned until you are ready to load the array plate and samples onto the GeneTitan MC Instrument for processing.



	A	B	C	D	E	F	G	H
1	**Sample File Path**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array**	**Barcode**	**Sample File Name**	**Array Name**
2		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A01		Sample A01	Sample A01
3		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A02		Sample A02	Sample A02
4		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A03		Sample A03	Sample A03
5		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A04		Sample A04	Sample A04

Figure 2 Example of a GeneTitan Array Plate Registration file.

3 Preparation before you start

This section provides information on procedures that are performed multiple times during manual target preparation and on steps that are critical to the success of the manual target preparation. It is essential that you familiarize yourself with the information in this section before running the manual target preparation for Axiom™ 2.0 Assay.

A list of all equipment and resources required for the Axiom 2.0 Assay with manual target preparation is in the *Axiom™ 2.0 Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018132.

Required materials

Axiom 2.0 Reagent Kit, arrays, and GeneTitan consumables required

The following table lists the Axiom™ 2.0 reagents and GeneTitan™ consumables required to process 1 Axiom™ 96-array format plate.

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

Table 5 Axiom™ 2.0 Assay 96-array format–arrays, reagents, and GeneTitan consumables required.

Quantity	Description	Source
1	Axiom™ 96-array format plates	Available upon design
1	Axiom™ GeneTitan™ MC Consumables	901606
1	Axiom™ 2.0 Reagent Kit	901758

Consumables required

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

Table 6 Consumables required for Axiom™ 2.0 Assay 96-Array Format Manual Workflow.

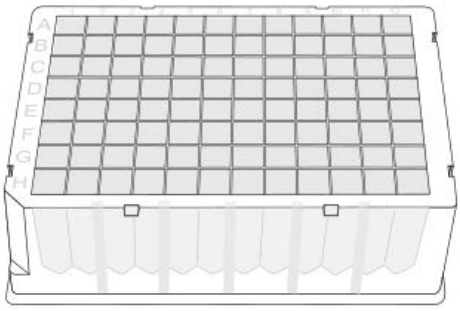
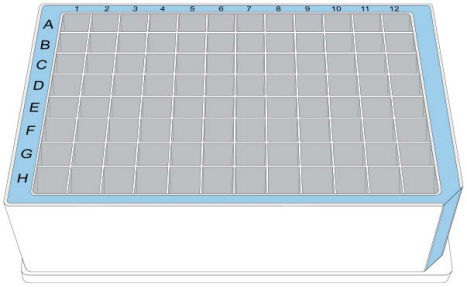
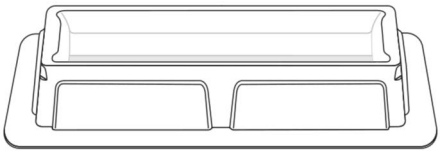
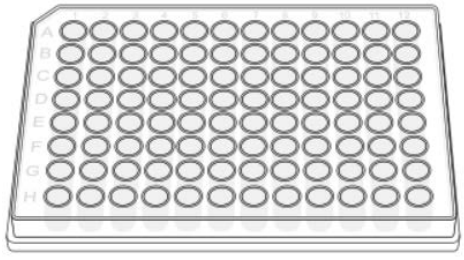
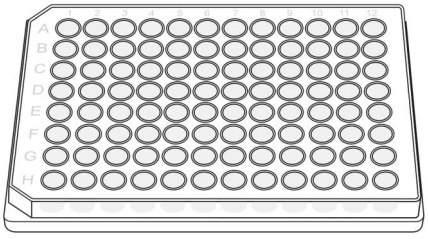
Labware	Source	Labware image
Deepwell plate for Axiom™ genotyping arrays: ABgene™ 96 Square Well Storage Plate, 2.2 mL ABgene™ Storage Plate, 96-well, 2.2 mL, square well, conical	Fisher Scientific™, AB-0932	
Deepwell plate for Axiom™ microbiome arrays: Eppendorf™ 96 Deepwell Plate, 2,000 µL Deepwell Plate 96/2,000 µL, wells clear, 2,000 µL, PCR clean, border blue. NOTE: The Eppendorf™ 96 Deepwell Plate is the only plate supported for Axiom™ microbiome use. Using a different plate type may result in assay failure.	Eppendorf™, 951033481	
25 mL Reagent Reservoir	Fisher Scientific™, 809311	
OD Plate, option 1 Corning™ UV-Transparent Microplates	Axiom™ 96-format Consumables Kit for QC, 902909. Also available from Fisher Scientific™, 07-200-623	
OD Plate, option 2 Greiner Bio-One UV-Star™ 96-well UV Spectroscopy Microplate	Fisher Scientific™, 07-000-407	

Table 6 Consumables required for Axiom™ 2.0 Assay 96-Array Format Manual Workflow. (Continued)

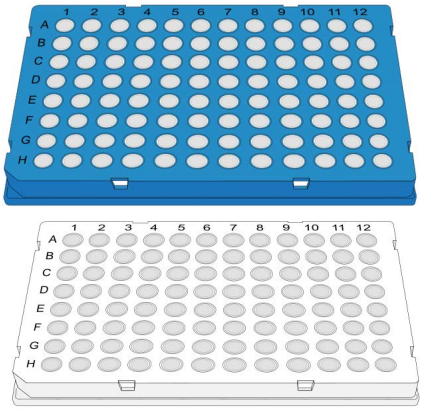
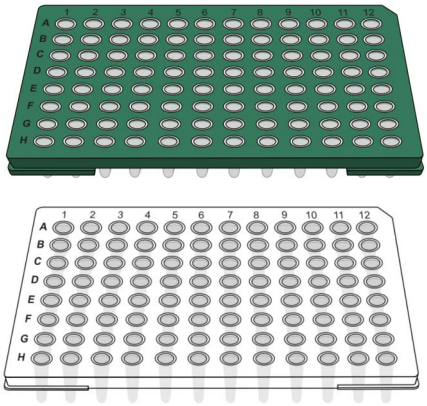
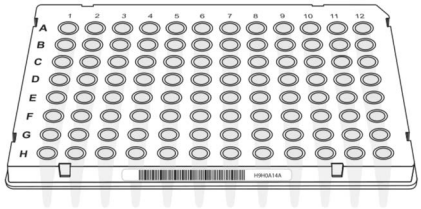
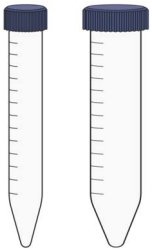
Labware	Source	Labware image
Bio-Rad™ 96-well Low-Profile Full-Skirted PCR Plate Bio-Rad™ Hard-Shell® Low-Profile 96-Well Skirted PCR Plates	Bio-Rad Laboratories™, HSP9631(blue) or HSP9601(white)	
Bio-Rad™ 96-well High Profile Semi-Skirted PCR Plate Bio-Rad™ Hard-Shell® High-Profile 96-Well Semi-Skirted PCR Plates Note: See Table 10 for the PCR plate type recommended for your specific thermal cycler model.	BioRad Laboratories™, HSS9641(green) or HSS9601 (clear)	
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate, Cat. No. 4483354 or 4483352	4483354 or 4483352	
1.7 mL Microcentrifuge Tubes, DNAse and RNAse-free	Common labware - order through your preferred labware supplier	

Table 6 Consumables required for Axiom™ 2.0 Assay 96-Array Format Manual Workflow. (Continued)

Labware	Source	Labware image
15 mL and 50 mL Conical-bottom Centrifuge Tubes, Polypropylene	MLS	
MicroAmp™ Clear Adhesive Film	4306311	
Serological pipettes, following sizes: • 5 mL • 10 mL • 25 mL • 50 mL	MLS	
Pipette tips: 20 µL, 200 µL, 1,000 µL	MLS	
GeneTitan Zerostat Antistatic Gun and Ion-Indicator Cap	74-0014	
96-well Block Cooling Chamber for 0.2 mL tubes, 96 holes (4 for 1.5 mL & 6 for 0.5 mL tubes), Dim.: 6 1/8”L x 3 1/8”W x 1” H	Diversified Biotech™, R-1007-1	

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.

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

Table 7 Axiom GeneTitan Tray Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606)

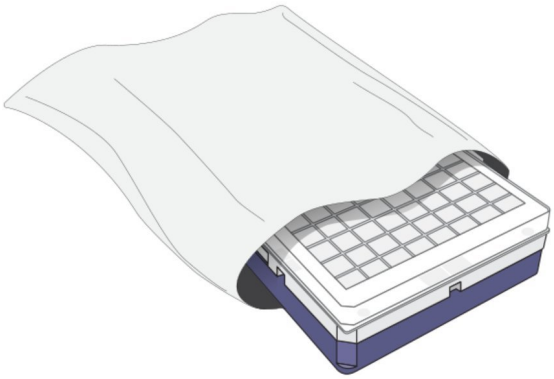
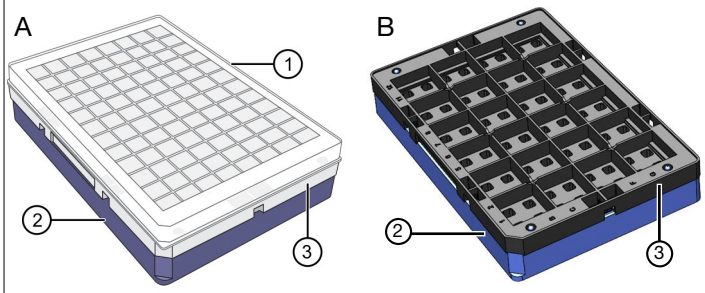
Item	Part Number	Labware Image	Information
Axiom 96-array format plate, various designs	Varies, depending on array design	Note: Array plate is not included in the Axiom GeneTitan Consumables Kit.   ① Clear tray shipping cover (to be discarded) ② Array plate protective base ③ Array plate **IMPORTANT!** The 96-format array plate is shipped in 1 of 2 different configurations; A or B, above. Configuration A includes a clear shipping cover and a clear array plate. Configuration B does not include a cover and has a black array plate. Both configurations are acceptable.	96-array format plate: • Array Plate A (lower left image) is comprised of 3 parts: clear plastic cover, array plate, and blue array plate protective base. The clear plastic cover for the array plate protects the array plate during transport. Discard after opening pouch. • Array Plate B (lower right image) is comprised of 2 parts: array plate, and blue array plate protective base. • Array plates must always be kept in the blue array plate protective base at all times. The blue array plate protective base in the package holds the array and protects it from damage.

Table 7 Axiom GeneTitan Tray Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606)

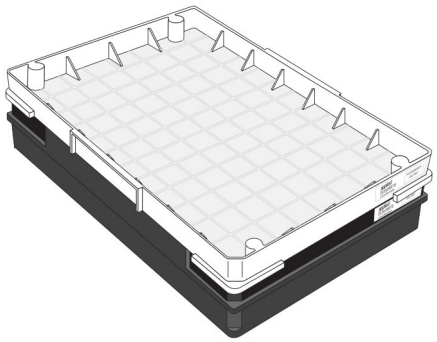
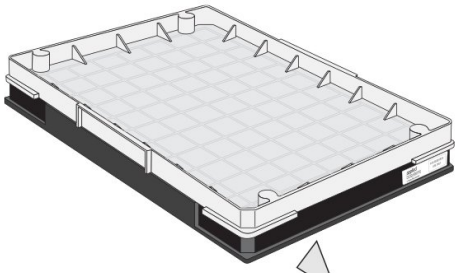
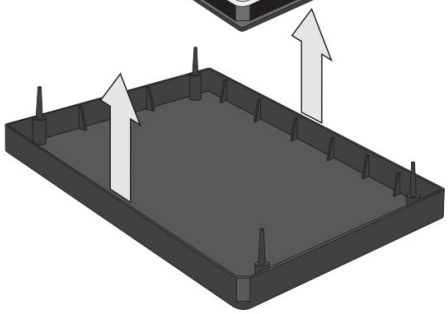
Item	Part Number	Labware Image	Information
Scan tray	900746 Box 501006 Pouch	  • The scan tray must be loaded into the GeneTitan Instrument with the scan tray cover only.  • Do not load the scan tray with the protective base still on.	96-format scan tray: • Comprised of 3 parts: scan tray, black protective base, and a scan tray cover. • The black scan tray protective base in the package protects the glass bottom of the scan tray from damage before it is loaded into the GeneTitan MC Instrument. • The scan tray cover protects the contents in the scan tray and must be deionized before used. See Appendix D, "Deionization procedure for GeneTitan trays and covers" on page 155. • Remove the black scan tray protective base before loading the scan tray with the scan tray cover into the GeneTitan MC Instrument.

Table 7 Axiom GeneTitan Tray Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606)

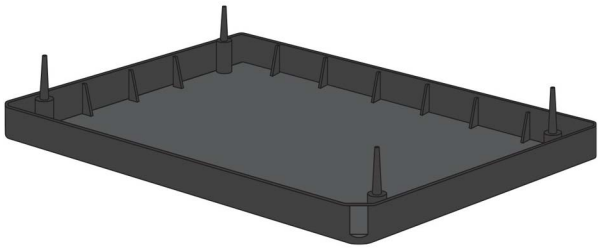
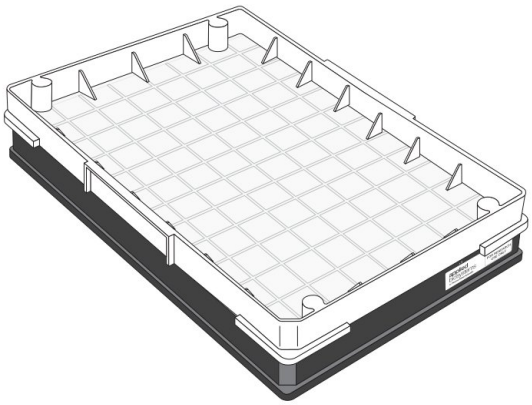
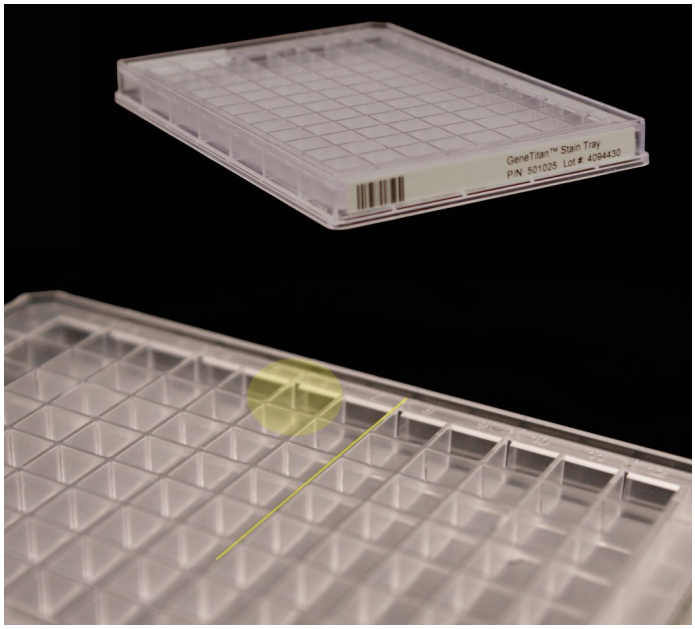
Item	Part Number	Labware Image	Information
Black scan tray protective base, shown without the scan tray with cover			• The black scan tray protective base in the package is used to protect the bottom of the scan tray glass from damage. The black scan tray protective base is distinct from the blue array plate protective base and must not be used with the array plate. • Remove and set aside the protective base from the scan tray before loading.
Scan tray with cover, shown without the black protective base			• The GeneTitan scan tray must be loaded with the scan tray cover into the GeneTitan MC Instrument. • **Do not load the scan tray with the protective base**.
GeneTitan™ 5 Stain Trays Kit	4249910 Kit 501025 Tray		• The GeneTitan™ Stain Tray Kit comes with 5 stain trays packaged in zip-top bags to keep them free of dust. • The GeneTitan stain trays are barcoded and the trays have separator walls that are flush with the frame of the stain tray, as shown by the yellow line and the yellow oval in the lower photo.

Table 7 Axiom GeneTitan Tray Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606)

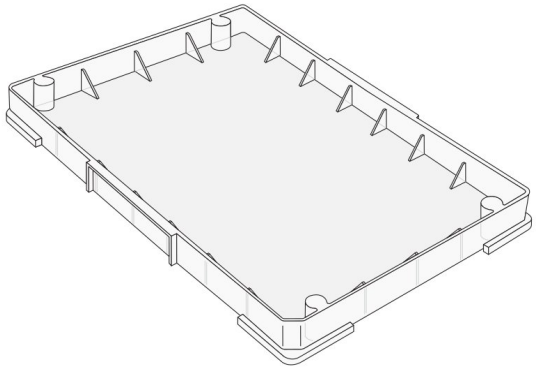
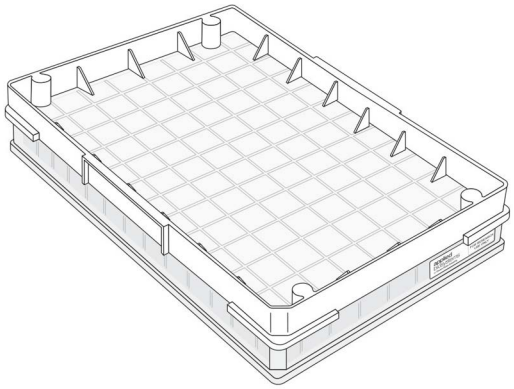
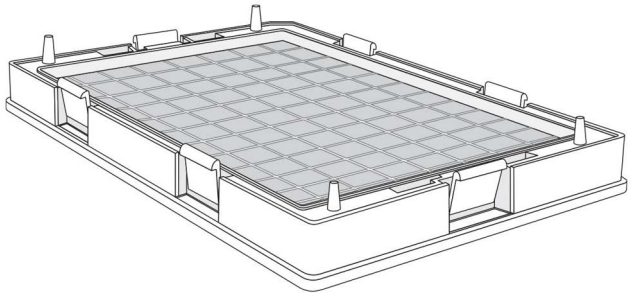
Item	Part Number	Labware Image	Information
GeneTitan Scan and Stain Tray cover	202757		• The GeneTitan scan and stain tray covers are provided to prevent any evaporation of the stains in stain trays and the array holding buffer in the scan tray • All Stain and scan trays must be placed in the GeneTitan MC Instrument with the GeneTitan stain tray cover. • All tray covers must be de-ionized to remove static electricity before placing the cover on the tray. • See Appendix D, "Deionization procedure for GeneTitan trays and covers" on page 155 for the anti-static procedure.
GeneTitan Stain Tray shown with the Stain Tray cover	Tray 501025 Cover 202757		• The GeneTitan stain trays must be placed in the GeneTitan MC Instrument with the GeneTitan stain tray cover. • It is important to remove the static electricity on the cover and the stain tray before loading the tray into the GeneTitan MC Instrument. • See Appendix D, "Deionization procedure for GeneTitan trays and covers" on page 155 for the anti-static procedure.

Table 7 Axiom GeneTitan Tray Consumables (from the Axiom™ GeneTitan™ Consumables Kit, Cat. No. 901606)

Item	Part Number	Labware Image	Information
GeneTitan Hybridization Tray	900747		After aliquoting the denatured hybridization-ready samples into the hybridization tray, the tray should be immediately loaded into the GeneTitan MC Instrument with the barcode facing away from the operator. The barcode should be on the back side.

Reagent kits

The Axiom™ 2.0 Assay 96-Array Format Manual Workflow requires 1 Axiom 2.0 Reagent Kit (Cat. No. 901758) to process samples for one 96-array plate.

Table 8 Axiom™ 2.0 Reagent Kit, Cat. No. 901758 (Sufficient for one 96-array format array plate).

Component	Contents	Storage
Module 1: Part No. 901711	• Axiom 2.0 Denat Soln 10X • Axiom 2.0 Neutral Soln • Axiom Water • Axiom 2.0 Amp Soln • Axiom 2.0 Amp Enzyme	–25°C to –15°C
Module 2—Pouch 1 of 2: Part No. 901528	• Axiom Frag Enzyme • Axiom 10X Frag Buffer • Axiom Precip Soln 2 • Axiom Hyb Buffer • Axiom Hyb Soln 1	–25°C to –15°C
Module 2—Pouch 2 of 2: Part No. 901529	• Axiom Frag Diluent • Axiom Frag Rxn Stop • Axiom Precip Soln 1 • Axiom Resusp Buffer • Axiom Hyb Soln 2	2°C to 8°C
Module 3	• Axiom Wash Buffer A: PN 901446 (2 bottles per kit) • Axiom Wash Buffer B: PN 901447 (1 bottle per kit) • Axiom Water: PN 901578 (1 bottle per kit)	Room temperature
Module 4—Pouch 1 of 2: Part No. 901278	• Axiom Ligate Buffer • Axiom Ligate Enzyme • Axiom Ligate Soln 1 • Axiom Probe Mix 1 • Axiom Stain Buffer • Axiom Stabilize Soln	–25°C to –15°C
Module 4—Pouch 2 of 2: Part No. 901276	• Axiom Ligate Soln 2 • Axiom Probe Mix 2 • Axiom Wash A • Axiom Stain 1-A • Axiom Stain 1-B • Axiom Stain 2-A • Axiom Stain 2-B • Axiom Stabilize Diluent • Axiom Water • Axiom Hold Buffer	2°C to 8°C

Equipment required

Thermal cycler recommendations

The following thermal cyclers are recommended:

- Applied Biosystems™ Proflex PCR System, or
- Applied Biosystems™ 9700 (with gold, sliver, or aluminum block), or
- Applied Biosystems™ 2720, or
- Bio-Rad™ PTC-200, or
- Bio-Rad™ DNA Engine Tetrad 2 PTC-0240

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

We have verified the performance of this assay using the following thermal cyclers: ProFlex PCR System, 9700 (with a gold, silver or aluminum block), 2720, PTC-200, and Tetrad 2 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 minutes
2. 48°C 3 minutes
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 follow the manufacturer's instructions for adjusting lid tension.

Oven recommendations

The following ovens are recommended:

- Thermo Scientific™ Heratherm™ Advanced Protocol Microbiological Incubator.
- BINDER™ ED 56 Drying and Heating Chamber, or the BINDER™ BD 56 Standard-Incubator with natural convection
  See the *Axiom™ 2.0 Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018132, for vendor information.
- GeneChip Hybridization 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 contact your Field Service Engineer (FSE) or Technical Support regarding the compatibility of this oven with the Axiom 2.0 Assay.

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

Multiple ovens are required for manual target preparation. The exact number depends upon whether you are running only a single sample plate and array plate through the

workflow, or if you are trying to run the 3 plate/week manual target preparation workflow.

- If you are running individual plates, you will need 2 ovens for the workflow.
- If you are running the 3 plate /week workflow, 3 ovens are recommended.

See "Changing oven temperatures for the 3-plate Workflow" on page 125 of Chapter 6, "Process 3 Axiom™ array plates per week" for more information.

Plate centrifuge

One plate centrifuge is required for the Axiom™ 2.0 Assay 96-Array Format Assay. See the *Axiom™ 2.0 Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018132, for an appropriate plate centrifuge that can be used with the Axiom™ Solution. When centrifuging and drying pellets as instructed under "Stage 3: Centrifuge and dry pellets, resuspension and hybridization preparation, and sample QC" on page 56, the centrifuge must be able to centrifuge plates at:

- Rcf: 3,200 x *g* (4,000 rpm for the Eppendorf 5810R with the rotor configuration described in the *Axiom™ 2.0 Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018132).
- Temperature: 4°C and room temperature.

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

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

Plate shakers

We recommend using one of the shakers listed the following table.

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

Table 9 Shakers.

Shaker	Source
Thermo Scientific Digital Microplate Shaker	88882005 or 88882006
Thermo Scientific Compact Digital Microplate Shaker	88880023
Jitterbug™	Boekel Scientific, Model 130 000

Requirements and recommendations

This section describes requirements and recommendations for facilities and equipment needed to perform the Axiom 2.0 Assay with manual target preparation.

Room temperature

When referred to in the Axiom 2.0 Assay, room temperature is 18–25°C.

Special requirements

Amplification staging area

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

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

Fume hood

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

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

Control recommendations

For Axiom™ genotyping arrays:

We recommend including 1 positive control with every set of samples processed. Axiom™ Reference Genomic DNA 103 (Cat. No. 951957) is a positive control for human array designs. If genotyping nonhuman samples, you must provide your own positive control gDNA. See Table 2 on page 15 for gDNA input requirements. A negative control is not required for this assay.

For Axiom™ Microbiome arrays:

Two controls are required:

- Positive control: The Axiom™ Reference Genomic DNA 103 must be included in each array plate to provide both assay QC and array QC metrics.
- Negative control or no template control (NTC): It is necessary to run 1 NTC reaction. It is recommended that the user run the same buffer used for elution during their gDNA extraction. Alternatively, reduced EDTA TE buffer can be used as a negative control.

For detailed guidance on DNA sample preparation and requirements for the Axiom™ microbiome array, see Section 1, Chapter 2 “DNA Preparation and Requirements” of the *Axiom™ Microbiome Solution User Guide* (Pub. No. 703408).

Plate requirements and recommendations for target preparation

The following types of plates are required for performing manual target preparation. See the *Axiom™ 2.0 Assay 96_Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018132, for vendor information.

- Deepwell plate:
  - Axiom™ Genotyping Assay: ABgene™ 96 Square Well Storage Plate, 2.2 mL or Eppendorf 96 Deepwell Plate, 2,000 µL; Eppendorf, Cat. No. 951033481.
  - Axiom™ Microbiome Assay: Eppendorf™ 96 Deepwell Plate, 2,000 µL

    Note: The Eppendorf™ plate is the only plate supported for Axiom™ microbiome use. Using a different plate type may result in assay failure.
- Bio-Rad™ Hard-Shell 96-Well PCR Plate, High-Profile, Semi-Skirted (Cat. No. HSS9601 or HSS9641, or MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate, Cat. No. 4483354 or 4483352) for the following thermal cyclers:
  - Applied Biosystems™ GeneAmp PCR System 9700 (with gold-plated or silver block)
  - Applied Biosystems™ ProFlex PCR System
- Bio-Rad™ Hard-Shell 96-Well PCR Plate Low-Profile, Full-Skirted (Cat. No. HSP9631 or HSP9601) or Bio-Rad Hard-Shell 96-Well PCR Plate, High-Profile, Semi-Skirted (Cat. No. HSS9601 or HSS9641, or MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate, Cat. No. 4483354 or 4483352) for the following thermal cyclers:
  - Bio-Rad™ PTC-200 or
  - Bio-Rad™ DNA Engine Tetrad 2 PTC-0240
- Corning™ UV-Transparent Microplate or Greiner Bio-One™ UV-Star™ 96-Well UV Spectroscopy Microplate

Thermal cycler consumables

Table 10 provides details into the consumables to be used with each thermal cycler.

Table 10 Thermal cycler consumables for the Axiom™ 2.0 Assay 96-Array Format Manual Workflow.

Model	PCR plate type	Seal[1]
Applied Biosystems ProFlex PCR System	• BioRad Hard-Shell High-Profile 96-Well Semi-Skirted PCR Plate (Cat. No. HSS9601 or HSS9641), or • MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate (Cat. No. 4483354 or 4483352)	Applied Biosystems MicroAmp Clear Adhesive Film (Cat. No. 4306311)
Applied Biosystems 9700	• Bio-Rad Hard-Shell High-Profile 96-Well Semi-Skirted PCR Plate (Cat. No. HSS9601 or HSS9641, or • MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate (Cat. No. 4483354 or 4483352)	Applied Biosystems MicroAmp Clear Adhesive Film (Cat. No. 4306311)
Applied Biosystems 2720	• Bio-Rad Hard-Shell High-Profile 96-Well Semi-Skirted PCR Plate (Cat. No. HSS9601 or HSS9641, or • MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate (Cat. No. 4483354 or 4483352)	Applied Biosystems MicroAmp Clear Adhesive Film (Cat. No. 4306311)
Bio-Rad PTC-200	• Bio-Rad Hard-Shell High-Profile 96-Well Semi-Skirted PCR Plate (Cat. No. HSS9601 or HSS9641, or • MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate (Cat. No. 4483354 or 4483352) • Bio-Rad Hard-Shell Low-Profile Thin-Wall 96-Well Skirted PCR Plate (Cat. No. HSP9601 or HSP9631)	Applied Biosystems MicroAmp Clear Adhesive Film (Cat. No. 4306311)
Bio-Rad Tetrad 2 PTC-0240	• Bio-Rad Hard-Shell High-Profile 96-Well Semi-Skirted PCR Plate (Cat. No. HSS9601 or HSS9641, or • MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate (Cat. No. 4483354 or 4483352) • Bio-Rad Hard-Shell Low-Profile Thin-Wall 96-Well Skirted PCR Plate (Cat. No. HSP9601 or HSP9631)	Applied Biosystems MicroAmp Clear Adhesive Film (Cat. No. 4306311)

[1] Microseal 'B' Film from BioRad (Cat. No. MSB-1001) may be used in place of MicroAmp Clear Adhesive Film for the Applied Biosystems and BioRad thermal cyclers for Axiom™ genotyping arrays only. For Axiom™ microbiome applications, only use the MicroAmp Clear Adhesive Film. The use of the Bio-Rad Microseal 'B' Film has not been validated for Axiom™ microbiome use.

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.

Guidelines for handling plates and reagents

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

Blot-dry

- Before sealing plates, check the top of the plate to ensure that there are no droplets. If droplets are present, blot-dry the top of the plate before sealing to ensure a tight seal.
  - To remove droplets before sealing, overlay a sheet of laboratory tissue across the top of the plate and gently pat down to dry. Discard the tissue after blotting.
  - Ensure that the top of the plate is dry and seal the plate as usual.

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.

Vortex

- Plates:
  - For deep well plates (such as ABgene 2.2 mL square well storage plates, and the Eppendorf 96 Deepwell Plate), vortex 5 seconds in each sector for a total of 5 sectors (Figure 3).
  - For PCR plates (such as Bio-Rad Hard Shell or semi-skirted plates, vortex 2 seconds in each sector for a total of 5 sectors (Figure 3).
- Reagent vials and bottles:
  - Reagent vials with a capacity of less than 2 mL, vortex the vials 3 times, 1 second each time at the maximum setting.
  - Reagent bottles with a capacity larger than 2 mL, vortex the bottle 3 times for a few seconds each time at the maximum setting. If precipitate is present in the bottle, follow the instructions in the reagent handling section for the appropriate stage.

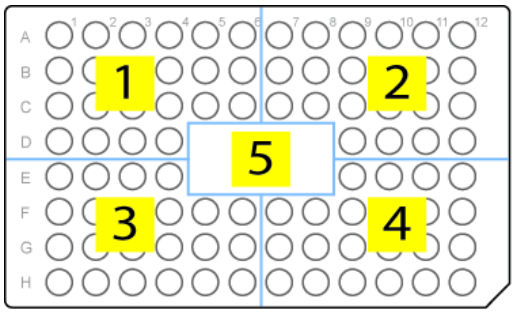


Figure 3 Vortexing plates.

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

Centrifuge

When instructed to perform a brief centrifugation step of plate or reagent vials, follow these guidelines, unless otherwise instructed (for example, during Stage 3A when centrifuging the Precipitation plate to pellet the DNA).

- Plates:
  - Centrifuge plates to 1,000 rpm for 30 seconds at room temperature.
  - Do not centrifuge for more than 1 minute.
- Reagent vials:
  - Briefly centrifuge for 3 seconds on the microcentrifuge.

Sample quantitation

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

See Chapter 2, "Genomic DNA preparation and requirements" on page 11 for more information.

About the reagents and master mix preparation

Axiom 2.0 Reagent Kit components

- Reagent 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 is compatible only with reagents from an Axiom Reagent Kit. These reagents are not interchangeable with reagents from other Applied Biosystems™ reagent kits.

Reagents from other suppliers

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

Master mix preparation

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

Note: The volumes of master mixes prepared are designed to provide consistent handling of reagents and consistent assay results. The percent overage of different master mixes can 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 10).

Table 11 Recommended pipette sizes.

Pipette size	Recommended volume range
Single channel P20 / 12-channel P20	1-20 µL
P50 (optional)	20-50 µL
Single channel P200 / 12-channel P200	20-200 µL
Single channel P1000 / 12-channel P1200	200-1,000 µL

- We recommend the use of Rainin pipettes and tips. Thermo Fisher Scientific has only verified the use of Rainin 12-channel pipettes in this assay. The use of other pipettes (such as other brands or 8-channel pipettes) can impact the timing of the protocol and can adversely impact the assay. Pipette substitution may violate the terms of the Axiom 2.0 Assay and array replacement policy.
- Always use pipettes that have been calibrated.
- It is essential that you be proficient with the use of single- and multichannel pipettes. To familiarize yourself with the use of multichannel pipettes, we strongly recommend practicing several times before processing actual samples. Use water and reagent reservoirs to get a feel for aspirating and dispensing solutions to multiple wells simultaneously.

Single channel pipettes and serological pipettes

Use single channel pipettes for preparing Master Mixes and for puncturing bubbles in GeneTitan trays. The single-channel pipettes 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 2 portions with a fresh tip for each portion.
- Use serological pipette for volumes >2 mL.
- In most cases, 25 or 50 mL serological pipettes will not fit into the mouths of the reagents bottles. Multiple transfers using 5 or 10 mL serological pipettes will need to be performed.

Multichannel pipettes

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

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

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.

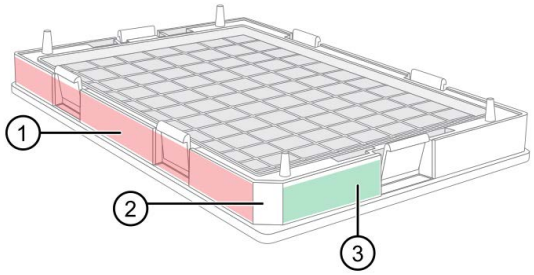
IMPORTANT! It is critical that you write only on the proper locations of the proper sides of hybridization and stain trays. Do **NOT** write in any other location, as this can interfere with sensors inside the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of covers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

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

- "Labeling for hybridization trays", below
- "Labeling for stain trays" on page 39

Labeling for hybridization trays

You can label the hybridization tray on the front part of the **short side of the tray, next to the notch at the left,** as shown in Figure 4. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.



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

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

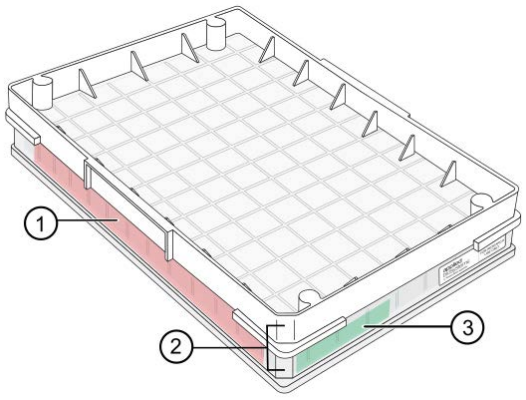
③ Label the hybridization tray in this area.

Figure 4 Labeling GeneTitan Hybridization Trays.

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

Labeling for stain trays

You can label the stain trays on the **left side of the front of the tray** as shown in Figure 5. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.



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

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

③ Label the stain tray here.

Figure 5 Labeling GeneTitan Stain Tray.

See "Stage 5: Manually prepare reagent trays for the GeneTitan MC Instrument" on page 72 for detailed information.

4 Manual target preparation

Target preparation for the Axiom™ 2.0 Assay 96-Array Format Manual Workflow enables you to perform target preparation to process 96 samples at a time. The protocol is performed in 2 parts:

- Part 1: Manual target preparation as described in this chapter.
- Part 2: Array processing is performed on the GeneTitan™ Multi-Channel (MC) Instrument.

Note: Array handling and processing protocols still require the use of a GeneTitan MC Instrument, as described in Chapter 5, "Array processing with the GeneTitan™ MC Instrument" on page 85.

IMPORTANT! Read all the instructions in Chapter 3, "Preparation before you start" on page 20, before performing manual target preparation.

A list of all equipment and resources required for the Axiom 2.0 Assay with manual target preparation is in the *Axiom™ 2.0 Assay Manual Workflow Site Preparation Guide*, Pub. No. MAN0018132.

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

IMPORTANT! Before proceeding to DNA Amplification, perform the gDNA preparation described in Chapter 2, "Genomic DNA preparation and requirements" on page 11.

Using the manual target preparation protocol, a single operator can process 3 gDNA and array plates a week during a forty-hour work week for a total of 288 arrays. See Chapter 6, "Process 3 Axiom™ array plates per week" on page 122 for more information.

Stage 1: Amplify the genomic DNA

IMPORTANT! Before proceeding to DNA Amplification, perform the gDNA preparation described in Chapter 2, "Genomic DNA preparation and requirements" on page 11.

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 43

"2. Prepare the Denaturation Master Mix" on page 45

"3. Add Denaturation Master Mix to samples" on page 45

"4. Add Neutralization Solution to samples" on page 46

"5. Prepare and add the Amplification Master Mix" on page 46

"6. Freeze or proceed" on page 47

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 32 for more information.

Duration

For 96 samples:

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

Input required

The Sample Plate for **genotyping studies** must have 20 µL of each gDNA diluted to a concentration of 5 ng/µL or 10 ng/µL, as required according to the sample type, into an **ABgene 96 square well storage plate, 2.2 mL or Eppendorf 96 Deepwell Plate, 2,000 µL**.

The Sample Plate for **microbiome studies** must have 20 µL of stool gDNA diluted to a concentration of 2.5 ng/µL and/or 17.5 µL cDNA must be diluted with 2.5 µL reduced EDTA TE buffer into the **Eppendorf 96 Deepwell Plate, 2,000 µL**. A no template control (NTC) and Genomic DNA Standard (REF 103) must be plated.

See "Genomic DNA preparation" on page 15 for more information.

IMPORTANT! For detailed guidance on DNA sample preparation and requirements for the Axiom Microbiome Array, see Section 1, Chapter 2 “DNA Preparation and Requirements” of the *Axiom™ Microbiome Solution User Guide* (Pub. No. 703408).

Equipment, consumables and reagents required

Equipment and consumables

The equipment and consumables listed in Table 12 are required for this stage.

Table 12 Equipment and consumables required for Stage 1.

Quantity	Item
As required	Adhesive seals for 96-well plate - Applied Biosystems MicroAmp Clear adhesive film
1	Cooler, chilled to –20°C
1	50 mL tube holder
1	15 mL tube holder
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1 each	Rainin pipettes: • Single-channel P200 • Single-channel P1000 • Multi-channel P20 • Multi-channel P200 • Multi-channel P1200
As needed	Pipette tips
As needed	Pipette, serological • 5 x 1/10 mL • 10 x 1/10 mL
1	Pipet aid
1	Plate centrifuge, at room temperature
1	Oven, set at 37°C
1	50-mL conical tube
1	15-mL conical tube
1	Vortexer
1	Timer
3	25-mL reagent reservoir

Reagents required

Table 13 Reagents required for Stage 1.

Axiom 2.0 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

Perform the initial setup

1. Set an incubator/oven temperature at 37°C.
2. Set the centrifuge temp to room temperature.
3. Thaw and prepare the gDNA Sample plate and reagents.

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

Thaw and prepare the reagents

1. Thaw the gDNA Sample Plate on the benchtop at room temperature. After fully thawed, centrifuge plate to get all droplets down.

IMPORTANT! gDNA samples must be brought to room temperature before proceeding with denaturation.

2. Thaw the Module 1 pouch (Part No. 901711) as described on Table 14 below:

Table 14 Reagent preparation for DNA amplification.

Reagent	Treatment
Axiom 2.0 Denat Soln 10X	• Thaw at room temperature. • Vortex, centrifuge, and keep at room temperature.
Axiom 2.0 Neutral Soln	• Thaw in a small water bath on the benchtop at room temperature. • Vortex for 30 seconds to thoroughly mix. • Keep at room temperature.
Axiom 2.0 Amp Soln	• Thaw in a small water bath on the benchtop at room temperature. • Vortex for 30 seconds to thoroughly mix. • Keep at room temperature.

Table 14 Reagent preparation for DNA amplification.

Reagent	Treatment
Axiom Water	• Thaw in a small water bath on the benchtop at room temperature. • Vortex and keep at room temperature keep at room temperature.
Axiom 2.0 Amp Enzyme	• Leave frozen in a cooler until ready to use. • Gently invert and flick the tube 3 times to mix and centrifuge 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 ultra-pure water. The Axiom 2.0 Amp Soln must be thoroughly mixed before use.

3. Label the 15-mL and 50-mL conical tubes as indicated in the following table:

Label	Tube Size	Temperature	Contents
D MM	15 mL	Leave tube at room temperature	Denaturation Master Mix
Amp MM	50 mL	Leave tube at room temperature	Amplification Master Mix

4. Label three 25-mL reagent reservoirs as indicated in the following table.

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

2. Prepare the Denaturation Master Mix

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

1. Per Table 15 on page 45, dilute the appropriate volume of Axiom 2.0 Denat Soln 10X using the Axiom Water.

Table 15 Preparing Denaturation Master Mix (*D MM*).

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked *D MM*, add:		
Axiom 2.0 Denat Soln 10X	2 µL	400 µL
Axiom Water	18 µL	3.6 mL
Total volume	**20 µL**	**4 mL**

2. Vortex and leave at room temperature.

3. Add Denaturation Master Mix to samples

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

1. Centrifuge the Sample Plate.

   Remember: Samples must be at room temperature for this step.
2. 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 and pipetting directly into the liquid of each well, add **20 µL of Denaturation Master Mix** to each sample of the plate (total volume 40 µL/well).
   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 **10 minute incubation**.
6. Do a quick centrifuge on the Denaturation Plate in a room temperature centrifuge by bringing centrifuge speed to 1,000 rpm (takes ~1 minute).

   Note: The quick centrifuge time is included in the 10 minute incubation.
7. Visually examine the volume in each well (should be 40 µL/well) and:
   a. Keep a record of any wells that visually appear to have a particularly low or high volume; these samples may need to be repeated.
   b. Do **NOT** stop to measure volumes; proceed without delay.
8. Complete the **10 minute incubation** on the benchtop at room temperature.

   While completing the incubation at room temperature, pour the Neutralization Soln into the reagent reservoir as described in Step 1 on page 46.
9. After incubation **immediately** add the Neutralization Soln as described in "4. Add Neutralization Solution to samples" on page 46.

4. Add Neutralization Solution to samples

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

1. Pour the Axiom 2.0 Neutral Soln into the reagent reservoir marked *N Soln*.
2. Carefully remove the seal from the Denaturation Plate and discard the seal.
3. Using a P200 12-channel pipette, pipetting down the wall of each well, add **130 µL of Axiom 2.0 Neutral Soln** to each sample (total volume 170 µL/well). Change tips between each addition.
   The plate is now known as the **Neutralization Plate**.
4. Seal, vortex, and centrifuge the Neutralization Plate.
5. Visually examine the volume in each well (should be ~170 µL/well) and:
   a. Keep a record of any wells that visually appear to have a particularly low or high volume; these samples may need to be repeated.
   b. Do **NOT** stop to measure volumes.
6. Proceed immediately to "5. Prepare and add the Amplification Master Mix" on page 46.

5. Prepare and add the Amplification Master Mix

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

1. Per Table 16, pipette the appropriate amount of Axiom 2.0 Amp Soln into the 50-mL tube labeled *Amp MM* at room temperature.

Note: Use a 10 mL serological pipette to transfer Axiom 2.0 Amp Soln to the tube. The bottles have narrow openings, and a 25 mL pipette will not fit through the mouth of the bottle.

Table 16 Amplification Master Mix (*Amp MM*).

Reagent	Per sample (µL)	Master Mix 96+
To the 50 mL tube marked *Amp MM*, add:		
Axiom 2.0 Amp Soln	225 µL	26.0 mL
Axiom 2.0 Amp Enzyme	5 µL	578 µL
Total volume	**230 µL**	**26.58 mL**

2. Remove the Axiom 2.0 Amp Enzyme from the freezer and place in a portable cooler at –20°C.
   a. Flick the Axiom 2.0 Amp Enzyme tube 3 times, then centrifuge.
   b. Per Table 16 on page 46, 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.
   d. S***lowly*** pour the Amplification Master Mix to the reagent reservoir labeled *Amp MM*.
   e. Carefully remove the seal from the Neutralization plate and discard the seal.

f. Using a P1200 12-channel pipette, ***slowly*** add **230 µL Amplification Master Mix** to each well of the Neutralization plate, pipetting down the wall of the well (there will now be a total volume of 400 µL/well).
Do not mix by pipetting up and down.
Change tips between each addition.

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

g. Seal tightly, vortex twice, and centrifuge the Amplification Plate for 1 minute at 1,000 rpm (as described in "Guidelines for handling plates and reagents" on page 35).

h. Place the sealed amplification plate in an oven set at **37**°C and leave **undisturbed for 23 ± 1 hour**.

Note: If using a GeneChip™ Hybridization Oven, place the plate on the bottom of the oven. Plates do not rotate. Set the rotor for 15 rpm speed. See "Oven recommendations" on page 30 for more information.

6. Freeze or proceed

After the incubation finishes, you can either:

- Proceed to "Stage 2: Fragment and precipitate the DNA" on page 48.
- 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 49.

Stage 2: Fragment and precipitate the DNA

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

"1: Prepare for fragmentation and precipitation" on page 49
"3: Prepare Fragmentation Master Mix" on page 52
"4: Add Fragmentation Master Mix to Samples" on page 52
"5: Add the Stop Solution to the Fragmentation Plate" on page 53
"6: Prepare and add Precipitation Master Mix" on page 54

Duration

Total time: approximately 2 hours.

Input required

Amplification Plate from "Stage 1: Amplify the genomic DNA" on page 41.

Equipment, consumables and reagents required

Equipment and consumables

The equipment and consumables listed in Table 17 are required for this stage.

Table 17 Equipment and Consumables Required for Stage 2.

Quantity	Item
As required	Adhesive seals for 96-well plates
1	Freezer set to –20°C (Designate a shelf where the Precipitation Plates can be left undisturbed)
1	Cooler, chilled to –20°C
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1 each	Rainin Pipettes: • Single channel P1000 • Single channel P200 • Multichannel P20 • Multichannel P200 • Multichannel P1200
As needed	Pipette tips for pipettes listed above
1	Pipet-aid
As needed	Pipette, serological, 10 mL
1	Plate centrifuge set at room temperature
1	Mini microcentrifuge (microfuge with microtube rotor)
2-3	Ovens (see "Oven recommendations" on page 30): • One oven set at 37°C • One oven set to 65°C
1	15-mL conical tube

Table 17 Equipment and Consumables Required for Stage 2.

Quantity	Item
1	50-mL conical tube
1	50-mL conical tube holder
3	25-mL reagent reservoir
1	100-mL reagent reservoir
1	Vortexer

Reagents required

Table 18 Reagents required for Stage 2.

Reagent	Module
From the Axiom 2.0 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: See the *Axiom™ 2.0 Assay Manual Workflow Site Preparation Guide*, Pub. No. MAN0018132	
Isopropanol (2-Propanol), 99.5%	96 samples: 65 mL per array plate

1: Prepare for fragmentation and precipitation

Set ovens and centrifuge

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

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

Thaw and prepare the amplified DNA samples and reagents

If the Amplification Plate is frozen (skip this step if the Amplification Plate was not frozen at the end of the previous stage):

1. Place the deepwell plate in a small water bath.
   For example, pour fresh filtered water into a small tray. Place the frozen plate on the water in the tray.
2. Leave the plate in the water bath for ~50 minutes until all wells have thawed.
3. Centrifuge the plate at 1,000 rpm for 30 seconds.
4. To avoid cross-contamination of wells during vortexing:
   a. Remove the seal and blot the top of the plate with a laboratory tissue.
   b. Tightly re-seal the plate using a fresh seal.
5. Vortex the plate for 30 seconds to thoroughly mix.
6. Centrifuge at 1,000 rpm for 30 seconds.

Thaw and prepare the fragmentation reagents

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

Table 19 Reagent preparation for fragmentation and precipitation.

Reagent	Treatment
Axiom 10X Frag Buffer	• Thaw on the benchtop at room temperature. • When thawed, place on ice. Vortex before use.
Axiom Frag Diluent	Keep in refrigerator or place on ice.
Axiom Frag Enzyme	• Leave at –20°C until ready to use. • Gently flick the tube 3 times to mix and centrifuge before use.
Axiom Frag Rxn Stop	• Warm on benchtop to room temperature. • Vortex before use.
Axiom Precip Soln 1	• Warm on benchtop to room temperature. • Vortex before use.
Axiom Precip Soln 2	• Thaw on the benchtop at room temperature. • Vortex and centrifuge before use.
Isopropanol	Keep at room temperature.

Tip: Keep a labeled balance plate of equal weight ready to minimize any time delay before spinning the Fragmentation Plate during later steps.

Label tube and reagent reservoirs

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

Label	Tube size	Temperature	Contents
Frg MM	15 mL	Place tube on ice	Fragmentation Master Mix
Precip MM	50 mL	Place tube at room temperature	Precipitation Master Mix

2. Label 4 reagent reservoirs as indicated in the following table.

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

2. Incubate samples in preheated ovens

Stop the DNA amplification reaction

1. Place the Amplification Plate in the 65°C oven:
   - If proceeding directly from the end of "Stage 1: Amplify the genomic DNA" on page 47, transfer the Amplification Plate from the 37°C oven to the 65°C oven. Ensure that the seal is still securely attached to the plate to minimize evaporation.
   - If working with a frozen Amplification Plate, follow the guidelines in "Thaw and prepare the amplified DNA samples and reagents" on page 50 before placing it in the 65°C oven.
2. Incubate for 20 minutes.
3. Prepare reagents as detailed in "Thaw and prepare the fragmentation reagents" on page 50 after starting the 65°C incubation of the Amplification Plate.
4. Transfer the Amplification Plate from the 65°C oven to the 37°C oven, then incubate for 45 minutes.

3: Prepare Fragmentation Master Mix

Prepare the Fragmentation Master Mix

1. Start making the Fragmentation Master Mix when there is still 5 minutes to the finish of the **37**°C **incubation**, using the values in the table below.
   Transfer the Axiom Frag Enzyme to a –20°C portable cooler until ready to use.

Table 20 Axiom Fragmentation Master Mix.

Reagent	per sample	Master Mix 96+
To the 15 mL tube marked *Frg MM*, add:		
Axiom 10X Frag Buffer	45.7 µL	6.0 mL
Axiom Frag Diluent	10.3 µL	1.35 mL
Axiom Frag Enzyme	1.0 µL	131.0 µL
Total volume	**57 µL**	**7.48 mL**

   Add the reagents from Table 20 to the *Frg MM* tube in the order shown, using appropriate single channel and serological pipettes.

   Just before the end of the **45 minute 37**°C **incubation**, flick the Axiom Frag Enzyme tube 2 to 3 times, and centrifuge.

   Add the Axiom Frag Enzyme to the Fragmentation Master Mix at the end of the **45 minute 37**°C **incubation.**

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

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

4: Add Fragmentation Master Mix to Samples

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

1. Carefully remove the Amplification Plate from the 37°C oven and place 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. Use a P200 8-channel pipette to add **57 µL of Fragmentation Master Mix** to each reaction.
   - Pipet directly into the liquid of each well.
   - Change tips after each addition.
   - After adding the Fragmentation Master Mix to the plate, the plate is now known as the **Fragmentation Plate**.
4. Seal the Fragmentation Plate and vortex twice.
5. Start the timer for **30 minutes**.
6. Centrifuge the Fragmentation Plate in the plate centrifuge at room temperature by bringing the centrifuge to 1,000 rpm and stopping it.

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

7. Quickly transfer plate to **37°C oven and incubate for 30 minutes**.

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

Prepare the Stop solution a few minutes before the end of the **30 minute incubation** period, as described in "5: Add the Stop Solution to the Fragmentation Plate", below.

5: Add the Stop Solution to the Fragmentation Plate

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, pour the Axiom Frag Rxn Stop solution in the reagent reservoir labelled *Stop*.
2. Remove the Fragmentation Plate from the oven and place on the bench top at room temperature.
3. At the **end of the 30 minute fragmentation incubation period**, carefully remove the seal from the Fragmentation Plate and discard the seal.
4. Using a P20 12-channel pipette, end the fragmentation reaction by adding **19 µL of Stop Solution** to each reaction.
   - Pipette 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 centrifuge at 1,000 rpm.
6. Leave the Fragmentation Plate on the benchtop while you prepare the Precipitation Master Mix.

6: Prepare and add Precipitation Master Mix

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

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

Table 21 Precipitation Master Mix.

Reagent	per sample	Master Mix 96+
To make the *Precip MM*:		
Axiom Precip Soln 1	238 µL	26 mL
Axiom Precip Soln 2	2 µL	218 µL
Total volume	240 µL	**26.22 mL**

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

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

---

6. Blot, seal, vortex, then centrifuge the Precipitation Plate.

7: Prepare and add isopropanol to Precipitation Plate

1. Remove the Precipitation Plate from the centrifuge and place on benchtop at room temperature.
2. Pour isopropanol into the reagent reservoir labeled *ISO*.
3. Carefully remove the seal from the Precipitation Plate and discard the seal.
4. Using a P1200 12-channel pipette, add **600 µL isopropanol** to each sample and mix well by pipetting up and down within the solution to ensure mixing. The solution should look homogenous in the tips after pipetting 5-7 times. If not, repeat mixing a few more times until the solution looks mixed.

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

   Change the tips after each addition.
5. Blot the top of the plate with a laboratory tissue and seal tightly with a Microamp seal.

8: Freeze the Precipitation Plate overnight

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

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

IMPORTANT! The 3-hour DNA precipitation workflow extends the day 2 assay schedule. Approximately 9 hours are required to complete Stage 2 through Stage 4. Review Chapter 3 and Chapter 4 for timing details.

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

Stage 3: Centrifuge and dry pellets, resuspension and hybridization preparation, and sample QC

This stage requires the following sets of steps:

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

Duration

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

Total: 2.5 hours

Input required

Precipitation Plate from "Stage 2: Fragment and precipitate the DNA" on page 48.

Equipment, consumables, and reagents required

The equipment and consumables listed in Table 22 are required for this stage.

Table 22 Equipment and consumables required for Stage 3.

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

Table 22 Equipment and consumables required for Stage 3. (Continued)

Quantity	Item
As needed	Pipette tips for pipettes listed above
2	Bio-Rad Hard Shell 96-well plate, HSP-9601, HSP-9631 or any 96-well PCR plate for making the dilutions: • *Dil QC* Plate • *Gel QCPlate*
1	96-well PCR Plate for making the Hyb-Ready Plate[1]: • MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate • Bio-Rad 96-Well High-Profile Semi-Skirted PCR Plate • Bio-Rad 96-Well Low-Profile Full-Skirted PCR Plate
1	OD plate: Greiner UV-Star® 96 well plates or the Corning™ UV-Transparent Microplate
1	Oven set at 37°C
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Fume hood
1	Plate centrifuge set at 4°C
1	15-mL conical tube
1	10-mL serological pipette
1	Pipet aid
1	Plate shaker, either: • Thermo Scientific™ Compact Digital Microplate Shaker • Thermo Scientific™ Digital Microplate Shaker • Jitterbug
1	Vortexer
As needed	Reagent reservoir, 100 mL sterile multichannel

[1] See Table 10 on page 34 and select a 96-well plate that is compatible with the thermal cycler model to be used for sample denaturation.

Reagents required

Table 23 Reagents required for Stage 3.

Reagent	Module
From the Axiom 2.0 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)	**Source**
Gel Diluent, 14 mL of 100-fold dilution of TrackIt™ Cyan/Orange Loading Buffer (see Appendix A, "Fragmentation quality control gel protocol" on page 141 for dilution instructions.)	
6-fold dilution of Applied Biosystems™ 25 bp DNA Ladder	Cat. No. 931343
UltraPure™ DNase/RNase-Free Distilled Water, 14 mL (for OD and Dilution Plate preparation)	Cat. No. 10977015

Gels and related materials required

At the end of this stage, verifying the fragmentation reaction is highly recommended. See Appendix A, "Fragmentation quality control gel protocol" on page 141 for the required gel and related materials.

Stage 3A: Centrifuge the 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.

1. Turn the oven on and preheat to **37**°C.
2. Unless you plan to store the plate for resuspension later in the same day or another day, you may begin thawing/warming the reagents used in this stage as shown in Table 24 on page 61.
3. Remove the Precipitation Plate from the –20°C freezer and centrifuge the plate **for 40 minutes at 4°C** at 3200 x *g*.

   Note: If you are processing 2 plates at the same time, as in the 3 plate/week manual preparation workflow, you can centrifuge both plates at the same time.

4. Immediately after the 40-minute centrifugation period empty the liquid from the Precipitation Plate as follows:
   a. Carefully remove the seal from the Precipitation Plate and discard the seal.
   b. Invert the plate over a waste container and allow the liquid to drain.

c. While still inverted, gently press the plate on a pile of laboratory tissues on a bench and allow to drain for 5 minutes. Transfer the plate to a new pile of tissues during the 5-minute period.

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

Note: If using a GeneChip 645 oven, turn off the rotor during the 20 minutes drying time.

Tip: If you are proceeding directly to "5: Add resuspension buffer to DNA pellets" on page 61, you can prepare the Hybridization Master Mix at this time (Step 1 on page 62). You should also prepare the consumables detailed in "Stage 3B: Resuspension and hybridization preparation" on page 60.

6. After the 20-minute drying step, remove the plate from the oven, tightly seal the plate, and do one of the following:
   - Proceed directly to "5: Add resuspension buffer to DNA pellets" on page 61, even if some droplets of liquid remain. Leave the Precipitation Plate at room temperature.
     or
   - Store the plate for resuspension later in the same day:
     - If you plan to resuspend the pellets within 4 hours, keep the plate at room temperature.
     - If you plan to resuspend the pellets more than 4 hours later, store the plate in a refrigerator (2–8°C). Note: the refrigerated plate has to equilibrate to room temperature for at least 30 minutes before proceeding with the **Resuspension and Hybridization Preparation** protocol.
   - Store the plate for resuspension on another day:
     - Store the plate at –20°C.

Stage 3B: Resuspension and hybridization preparation

1: Prepare for resuspension and hybridization

Set the centrifuge to room temperature.

2: Prepare DNA pellets and warm the resuspension buffer

IMPORTANT! The plate of pelleted DNA and the Resuspension Buffer must be at room temperature before proceeding with this step.

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

DNA pellet types:

- Fresh pellets: A plate with fresh pellets can be kept at room temperature if proceeding with the **Resuspension and Hybridization Preparation** protocol within 4 hours of completing Stage 3A.
- Cold pellets: A plate with fresh pellets that are not processed within 4 hours of completing Stage 3A can be transferred to a refrigerator (2–8°C) if processed during the same day. 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 hours before proceeding with the **Resuspension and Hybridization Preparation** protocol.

Warm the Resuspension Buffer:

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

3: Thaw and prepare the reagents

1. During the centrifugation time prepare the resuspension and hybridization reagents as shown in Table 24:

Table 24 Reagent preparation for resuspension and hybridization.

Reagent	Treatment
Axiom Resuspension Buffer	Warm to room temperature for at least 1 hour. Vortex before use.
Axiom Hybridization Buffer	Vortex and keep at room temperature
Axiom Hybridization Solution 1	Thaw, vortex, centrifuge and keep at room temperature
Axiom Hybridization Solution 2	Vortex, centrifuge and keep at room temperature

4: Label tubes and reservoirs

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

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

2. Label two 25-mL reagent reservoirs as indicated in the following table.

Label	Temperature	Contents
Resus	Room temperature	Axiom Resusp Buffer
Hyb MM	Room temperature in fume hood	Hybridization Master Mix

5: Add resuspension buffer to DNA pellets

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

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

Perform the following steps at room temperature.

1. Pour Axiom Resusp Buffer in the 25-mL reagent reservoir labeled *Resus*.

Note: If you are processing 2 plates at the same time, as in the 3 plate/week manual preparation workflow, you can add resuspension buffer to both plates at the same time and then place them both in the shaker.

2. Carefully remove the seal from the Precipitation Plate and discard the seal.
3. Using a P200 12-channel pipette, transfer **35 µL Axiom Resusp Buffer** to each well of the Precipitation Plate. Avoid touching the pellets with the pipette tip.
   - Change pipette tips after each addition.
   - After adding Resuspension buffer, the plate is known as the **Resuspension Plate**.
4. Seal the Resuspension Plate.

6: Resuspension of DNA pellets

1. Place the sealed Resuspension Plate on one of the following shakers:
   - Thermo Scientific™ Digital Microplate Shaker: at **speed 900 rpm for 10 minutes**.
   - Thermo Scientific™ Compact Digital Microplate Shaker: at **speed 900 rpm for 10 minutes**.
   - Jitterbug: at **speed 7 for 10 minutes**.

   ⚠ **CAUTION!** It is recommended that the remainder of the steps in this stage be preformed under a fume hood.
2. Inspect the Resuspension Plate from the bottom. If the pellets are not dissolved, repeat Step 1 above.
3. Centrifuge at 1,000 rpm to get the droplets down.

7: Prepare the Hybridization Master Mix

1. While the Resuspension Plate is shaking, prepare the Hybridization Master Mix in the *Hyb MM* 15-mL tube.
   a. Add the reagents in Table 25 to the *Hyb MM* tube in the order shown, using serological and single-channel pipettes as needed.

Table 25 Hybridization Master Mix.

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

   b. Vortex twice to mix.

8: Prepare the Hyb-Ready Plate

1. Choose a 96-well plate that is compatible with the thermal cycler model that is used for sample denaturation. See Table 10 on page 34.
2. Label the 96-well PCR plate as **Hyb Ready [Sample ID]** and keep covered.
3. Pour the Hybridization Master Mix to the reagent reservoir labeled *Hyb MM*.
4. Using a P200 12-channel pipette, add **80 µL of the Hyb Master Mix** to each well of the Hyb Ready Plate.
5. Set a P200 12-channel pipette to **45 µL** (this is slightly higher than the volume of sample in each well of the Resuspension Plate).
6. Using the P200 pipette, transfer the **entire contents of each well** of the Resuspension Plate to the corresponding wells of the labeled Hyb Ready Plate.
   - Change pipette tips after each transfer.
7. Seal, vortex twice, and centrifuge.

9: Freeze or proceed

At this point you can:

- Proceed to "Stage 3C: (recommended) Perform quantitation and fragmentation QC checks" on page 64 (highly recommended); or
- Proceed to "Stage 4: Denature and hybridize the Hyb-Ready Plate"; or
- Store the hybridization-ready samples at –20°C.

Stage 3C: (recommended) Perform quantitation and fragmentation QC checks

Before proceeding to "Stage 4: Denature and hybridize the Hyb-Ready Plate", we highly recommend that you perform quantitation and fragmentation quality control checks.

1: Prepare for sample QC

Prepare the reagents

Obtain the reagents for Sample QC:

- 14 mL of UltraPure™ DNase/RNase-Free Distilled Water for the water reservoir.
- 14 mL of Gel Diluent

  The Gel Diluent is a 100-fold dilution of the TrackIt Cyan/Orange Loading Buffer as described in "Dilute the TrackIt Cyan/Orange Loading Buffer".
- 6-fold dilution of the Applied Biosystems™ 25 bp DNA Ladder, Cat. No. 931343, prepared as described in Appendix A.
- One E-Gel 48 Agarose Gel, 4% Agarose, Cat. No. G8008-04.

Label reservoirs

Label two 25-mL reagent reservoirs as indicated:

- Label 1 reservoir as *Water*.
- Label the second reservoir as *Gel Diluent*.

Table 26 Label reagent reservoirs for QC.

Label	Temperature	Contents
Water	Leave reagent reservoir at room temperature	Nuclease-free water
Gel Diluent	Leave reagent reservoir at room temperature	Gel diluent

Prepare sample QC plates

1. Label two 96-well PCR plates as follows:
   - Label one plate as *Dil QC*.
   - Label the second plate as *Gel QC*.
2. Obtain one Greiner UV-Star® 96-well plate or the Corning™ UV-Transparent Microplate. This is the 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

Perform the following steps at room temperature.

1. Pour 15 mL nuclease-free water into the reagent reservoir labeled *Water*. The water is used to make the QC Dilution 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. Pour 15 mL Gel Diluent into the reagent reservoir labeled *Gel Diluent*. Add **120 µL Gel Diluent** to each well of the *Gel QC* Plate.
3. Prepare the Dilution QC Plate:
   a. Transfer **3 µL of the hybridization-ready sample** from each well of the Hyb-Ready Plate to the corresponding well of the *Dil QC* plate. Change pipette tips after each transfer.
   b. Seal, vortex, then centrifuge the *Dilution QC* plate.
   c. Seal the Hyb-Ready Plate.
4. Prepare the OD Plate:
   a. Carefully remove the seal from the *Dil QC* plate and discard the seal.
   b. Transfer **10 µL of each Dil QC sample** to the to the corresponding wells of the OD 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.
   d. Read immediately or seal the plate for analysis at a later time.

   See Appendix B, "Sample quantitation after resuspension" on page 144 for more information on performing the Sample Quantitation.
5. Prepare the *Gel QC* Plate:
   a. Transfer **3 µL of each Dilution QC sample** to the corresponding wells of the corresponding wells of the Gel QC Plate.
   Change pipette tips after each transfer.
   b. Seal, vortex, and centrifuge the plate.
   c. Run gel as described in Appendix A, "Fragmentation quality control gel protocol" on page 141.

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

3. Freeze or proceed

At this point you can:

- Proceed to "Stage 4: Denature and hybridize the Hyb-Ready Plate", below; or
- Store the Hyb-Ready Plate at –20°C.

Stage 4: Denature and hybridize the Hyb-Ready Plate

You will proceed to Stage 4 in one of 2 ways:

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

This stage requires the following sets of steps:

"1: Prepare for denaturation and hybridization" on page 68

"2: Prepare hybridization-ready samples that are stored at –20°C" on page 68

"3: Prepare the GeneTitan MC Instrument" on page 68

"4: Denature the Hyb-Ready Plate" on page 69

"5: Prepare hybridization tray and load into GeneTitan MC Instrument" on page 70

Perform Stage 4:

If the Hyb-Ready Plate was stored at –20°C, go to "2: Prepare hybridization-ready samples that are stored at –20°C" on page 68.

If you are proceeding directly from the end of "Stage 4: Denature and hybridize the Hyb-Ready Plate" on page 65, go to "3: Prepare the GeneTitan MC Instrument" on page 68.

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

Duration

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

Required input from previous stage

- Hyb-Ready Plate

Equipment, consumables, and reagents required

The following thermal cyclers are recommended:

- Applied Biosystems™ ProFlex™ 96-well PCR System, or
- Applied Biosystems ™9700, or
- Applied Biosystems™ 2720, or
- Bio-Rad PTC-200, or
- Bio-Rad DNA Engine Tetrad 2 PTC-0240

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

The thermocycler needs to be programmed with the “Axiom 2.0 Denature” protocol (see "Thermal cycler recommendations" on page 30).

Table 27 Equipment required for Stage 4.

Equipment		Quantity
GeneTitan MC or GeneTitan MC Fast Scan Instrument		1
Rainin P200 12-channel Pipette		1
Pipette tips		As needed
Thermal Cycler	Appropriate thermal cycler, programmed with the "Axiom 2.0 Denature" protocol (see "Thermal cycler recommendations" on page 30).	1
96 well metal chamber warmed in a 48°C oven[1]		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 28 Consumables required for Stage 4: Denaturation and Hybridization.

Consumable	Source	Quantity
• One of the following Axiom array plates: – One Axiom human or non-human 96-array plate in a protective base, or – One Axiom myDesign Genotyping 96-array plate in a protective base – One Axiom Microbiome 96-array plate in a protective base	Various Cat. No.s	1
• Hybridization Tray[1]	Part No. 500867	1

[1] The Consumables for the GeneTitan MC Instrument are packaged separately from the Axiom array plates. The consumables are available in the Axiom™ GeneTitan Consumables Kit (Cat. No.901606). The hybridization tray is available in the Axiom™ GeneTitan™ Consumables Kit.

Table 29 Reagents required from the Axiom 2.0 Reagent Kit.

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

1: Prepare for denaturation and hybridization

1. Preheat the 96-well metal chamber in a 48°C oven.
2. Allow the array plate to equilibrate to room temperature for at least 25 minutes.
   a. Leave the array plate in the pouch at room temperature for 25 minutes before opening and loading on the GeneTitan MC Instrument to allow the plate to come to room temperature.
   b. At the end of the array warm up time, open the pouch and scan the array plate barcode into the Batch Registration file. (See "Creating a GeneTitan™ Array Plate Registration File" on page 152.)

   ⚠ **WARNING!** Do not remove the array plate from the protective base or touch the surface of any arrays.
3. Power up the thermal cycler, then prepare for the **Axiom 2.0 Denature** program to run with the heated lid option selected.

2: Prepare hybridization-ready samples that are 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 a longer duration.
2. Make sure that the Hyb-Ready Plate is sealed well.

   If the plate is not sealed well:
   a. Centrifuge the plate, then carefully remove the old seal.
   b. If there is condensation on the top of the plate, blot dry gently with a laboratory tissue.
   c. Use a fresh seal to tightly reseal the plate.
3. Vortex the Hyb-Ready Plate briefly, then centrifuge at 1,000 rpm for 30 seconds.
4. Place the Hyb-Ready Plate at room temperature.

3: Prepare the GeneTitan MC Instrument

Before you denature your hybridization-ready samples, ensure that the GeneTitan MC Instrument is ready for use by following the instructions given in Chapter 5, "Stage 2: Hybridization" on page 95 and Appendix C, "Registering samples in GeneChip™ Command Console™" on page 152.

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

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

Table 30 Reagents from Module 3 (Part No. 901472).

Reagent	Treatment
Axiom Wash Buffer A (Part No. 901446)	Invert 2-3X for mixing before filling GeneTitan bottle
Axiom Wash Buffer B (Part No. 901447)	Invert 2-3X for mixing before filling GeneTitan bottle
Axiom Water (Part No. 901578)	Not applicable

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

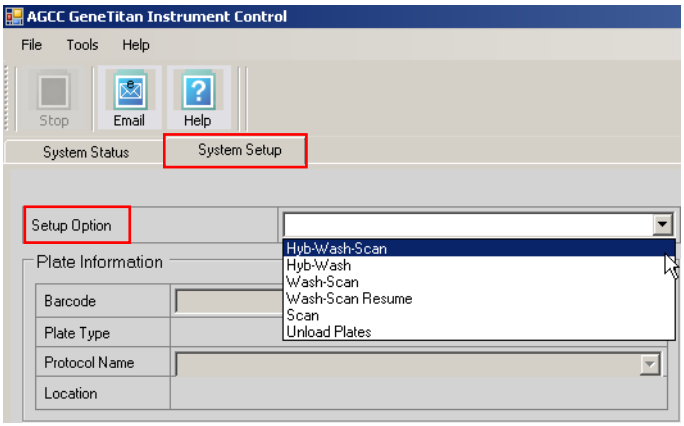


Figure 6 Setup options for processing array plates.

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, then click **Next**.
      - Protocol Name: Select the protocol name,then click **Next**.
6. Fill the Wash A, Wash B, and Rinse bottles when prompted by the user interface.
7. Empty the Waste bottle when prompted by the user interface.

4: Denature the Hyb-Ready Plate

1. Ensure that 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, then 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** program. (See "Thermal cycler recommendations" on page 30).

5: Prepare hybridization tray and load into GeneTitan MC Instrument

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

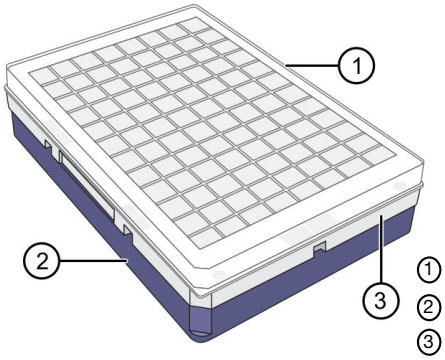
1. After the Axiom 2.0 Denature program has completed, remove the Hyb-Ready Plate from the thermal cycler and place into a 96-well metal chamber that has been pre-warmed in an oven at 48°C.
2. Move the metal chamber containing the denatured Hyb-Ready Plate to a fume hood.
3. Remove Microamp seal from Hyb-Ready Plate and discard.
4. Remove the hybridization tray (from Axiom Array GeneTitan Consumables Kit) from packaging.
5. Label the hybridization tray. See the note below and Figure 4 on page 38 for more information.

IMPORTANT! It is critical that you write only on the proper location of the hybridization tray (on the edge in front of wells A1 and B1) as illustrated in Figure 4 on page 38. Do **NOT** write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure.

IMPORTANT! Do not confuse hybridization trays with stain trays.

6. Place the hybridization tray under the fume hood.
7. Using a P200 12-channel pipette, set at **105 µL**, slowly transfer the denatured samples from the Hyb Ready Plate into the hybridization tray.
   - Dispense to the first stop to avoid creating bubbles.
   - Change pipette tips after each transfer; discard the tip even if it shows some volume left.
8. Ensure that there are no air bubbles present in the hybridization tray. Puncture any air bubbles that you see using a clean pipette tip.
   - There is no need to spread the sample around the bottom of the hybridization tray wells. Sample distribution across the well will occur when the array plate is stacked together with the hybridization tray by the GeneTitan MC Instrument.
9. Load the array plate and hybridization tray into the GeneTitan MC Instrument (see "Load an Axiom Array Plate and Hybridization Tray onto the GeneTitan™ MC Instrument" on page 100).

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



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

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

IMPORTANT! After the GeneTitan MC Instrument has stacked the array plate and hybridization tray, the instrument will extend the drawer. Manually check the stacking by gently pressing the 6 latching points to confirm that the 2 parts are clamped properly, and check underneath the arrays to ensure that there are no bubbles. If bubbles are found, gently tap the plate on top and the bubbles should disappear. Do NOT tip/tilt the array plate/hybridization tray sandwich while inspecting the bottom for bubbles. See Step 3 on page 104 for detailed instructions.

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

You must wait until the hybridization step on the GeneTitan MC Instrument is approximately 1.5 hours from completion (22 hours after the start of hybridization) to begin "Stage 5: Manually prepare reagent trays for the GeneTitan MC Instrument".

Stage 5: Manually prepare reagent trays for the GeneTitan MC Instrument

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: Manually prepare reagent trays for the GeneTitan MC Instrument" are for the continued processing of an Axiom array plate that

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

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

To prepare the reagent trays for the GeneTitan MC Instrument:

"1: Prepare for GeneTitan reagent preparation" on page 75

"2: Prepare the Stain, Ligation and Stabilization Master Mixes" on page 77

"3: Aliquot master mixes and Axiom Hold Buffer into trays" on page 80

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

Table 31 Reagent trays required for the Axiom 2.0 Assay on the GeneTitan MC Instrument.

Type of tray	Number of trays	Tray designation	Master mix/reagent
Stain Tray with cover	2	Stain 1 (or *S1*)	Stain 1 Master Mix
Stain Tray with cover	1	Stain 2 (or *S2*)	Stain 2 Master Mix
Stain Tray with cover	1	*Stbl*	Stabilization Master Mix
Stain Tray with cover	1	*Lig*	Ligation Master Mix
Scan Tray	1	Scan Tray	Hold Buffer

Equipment, consumables and reagents required

Table 32 Equipment required for Stage 5: Manually preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument.

Equipment	Quantity
GeneTitan MC Instrument	1
Ice bucket with ice	1
Microcentrifuge	1
Pipetaid	1
Rainin Pipettes — single channel • P200 • P1000 Rainin Pipettes — 12-channel: • P200	1 each
Vortexer	1

Table 33 Consumables required for Stage 5: Manually preparing Ligation, Staining, and Stabilization Reagent Trays for the GeneTitan MC Instrument.

Consumable	Source	Quantity
Aluminum foil (optional)	MLS	As required
GeneTitan Consumables Kit – Scan Tray and cover – Stain Tray – Covers for trays	Cat. No. 901606	1 kit includes: 1 5 5
Pipette, serological • 5 x 1/10 mL • 10 x 1/10 mL		 1 2
Pipette tips		As required for pipettes listed in Table 32
25-mL reagent reservoir		5
15-mL conical tube		3
50-mL conical tube		1

Reagents required

Axiom Module 4-1 and Module 4-2, are required from the Axiom™ 2.0 Reagent Kit.

- Axiom™ Module 4-1, –20°C, Part No. 901278
- Axiom™ Module 4-2, 2–8°C, Part No. 901276

Thaw and prepare reagents according to the following table:

Table 34 Axiom 2.0 reagents required for stain and ligation stage.

Module	Reagent
Module 4-1, –20°C Part No. 901278	Axiom Ligate Buffer
	Axiom Ligate Enzyme
	Axiom Ligate Soln 1
	Axiom Probe Mix 1
	Axiom Stain Buffer
	Axiom Stabilize Soln
Module 4-2, 2–8°C Part No. 901276	Axiom Ligate Soln 2
	Axiom Probe Mix 2*
	Axiom Wash A
	Axiom Stain 1-A[1]
	Axiom Stain 1-B[1]
	Axiom Stain 2-A[1]
	Axiom Stain 2-B[1]
	Axiom Stabilize Diluent
	Axiom Water
	Axiom Hold Buffer[1]

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

1: Prepare for GeneTitan reagent preparation

Note: Axiom™ Ligate Buffer and Axiom™ Ligate Soln 2 require approximately 30–40 minutes to thaw on the benchtop at room temperature.

Prepare the reagents:

1. Prepare the reagents from Module 4-1 as described in Table 35:

Table 35 Reagents from Module 4-1, –20°C (Part No. 901278).

Reagent	Treatment	Storage before master mix
Axiom Ligate Buffer	Thaw at room temperature[1] 1. Place on bench top at room temperature for 30 minutes. 2. Examine the buffer for precipitate. 3. Vortex the bottle for 30 seconds. 4. Re-examine for precipitate. – If precipitate is still present, warm the bottle with your hands, then vortex again for 30 seconds. – If precipitate is still present after hand-warming, proceed with the protocol. White precipitate is sometimes observed when the Axiom Ligate Buffer is thawed.	Place on ice.
Axiom Ligate Enzyme	Keep at –20°C until ready to use. Just before use: 1. Flick 2 to 3 times to mix. 2. Centrifuge. 3. Place in –20°C portable cooler until use.	Place in –20°C portable cooler.
Axiom Ligate Soln 1	• Thaw at room temperature • Vortex and centrifuge	Place on ice.
Axiom Probe Mix 1	• Thaw at room temperature • Vortex and centrifuge	Place on ice.
Axiom Stain Buffer	• Thaw at room temperature • Vortex and centrifuge	Place on ice.
Axiom Stabilize Soln	• Thaw at room temperature • Vortex and centrifuge	Place on ice.

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

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

2. Prepare the reagents from Module 4-2 as described in Table 36:

Table 36 Reagents from Module 4-2, 2-8°C (Part No. 901276).

Reagent	Treatment	Storage before master mix
Axiom Ligate Soln 2	• Thaw at room temperature • Vortex and centrifuge	Store at room temperature. Do not place on ice.
Axiom Probe Mix 2[1]	• Place on ice • Gently flick each tube 2 to 3 times to mix, then centrifuge.	Place on ice
Axiom Wash A	1. Vortex the bottle for 30 seconds. 2. Place on the benchtop at room temperature for 30 minutes. 3. Examine the reagent for precipitate (look into the top of the bottle). 4. If precipitate is still present, vortex again for 30 seconds.	Place on bench top at room temperature.
Axiom Stain 1-A[1]	• Place on ice • Gently flick each tube 2 to 3 times to mix, then centrifuge.	Place on ice
Axiom Stain 1-B[1]	• Place on ice • Gently flick each tube 2 to 3 times to mix, then centrifuge.	Place on ice
Axiom Stain 2-A[1]	• Place on ice • Gently flick each tube 2 to 3 times to mix, then centrifuge.	Place on ice
Axiom Stain 2-B[1]	• Place on ice • Gently flick each tube 2 to 3 times to mix, then centrifuge.	Place on ice
Axiom Stabilize Diluent	1. Vortex and centrifuge 2. Look for precipitate If any: – Warm tube to room temperature and vortex again.	Place on ice
Axiom Water	N/A[2]	Place on ice
Axiom Hold Buffer[1]	Vortex	Place at room temperature away from light.

[1] These solutions are light sensitive. Keep tubes out of direct light for a prolonged period of time.
[2] Not applicable in this case.

Note: Occasionally, crystals are observed in Axiom Wash A and Axiom Stabilize Diluent upon removal from 2–8°C storage. Before using these solutions, the crystals should be dissolved by warming the solutions to room temperature and then vortexing.

2: Prepare the Stain, Ligation and Stabilization Master Mixes

Label the tubes and reagent reservoirs

Label the tubes:

1. Mark the side of each tube with one of designations shown in Table 37.

Table 37 Labeling master mix tubes for stain, ligation, and stabilization reagents.

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

Note: Use a 5 mL or 10 mL serological pipette to transfer Axiom Wash A, Axiom Water, and Axiom Ligate Buffer. These bottles have narrow openings and a 25 mL serological pipette will not fit.

2. Mark the side of each reagent reservoir with one of the designations shown in Table 38.

Table 38 Labeling reagent reservoirs.

Reagent reservoir designation	Contents
S1	Stain 1 Master Mix
S2	Stain 2 Master Mix
Stbl	Stabilization Master Mix
Lig	Ligation Master Mix
Hold	Axiom Hold Buffer

Prepare Stain 1 Master Mix

1. Use appropriate serological and single-channel pipettes to add reagents to the 50 mL tube labeled *S1* in the order shown in Table 39. This recipe will provide enough for both *S1* reagent trays.

Table 39 Stain 1 Master Mix.

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

2. Gently invert the tube 10 times to mix.
3. Place on ice and protect from direct light (*e.g.,* cover with aluminum foil or ice bucket lid).

Prepare Stain 2 Master Mix

1. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *S2* in the order shown in Table 40.

Table 40 Stain 2 Master Mix.

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

2. Gently invert the *S2 MM* tube 10 times to mix.
3. Place on ice and protect from direct light (*e.g.,* cover with aluminum foil or ice bucket lid).

Prepare Stabilization Master Mix

1. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Stbl* in the order shown in Table 41.

Table 41 Stabilization Master Mix.

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

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

Prepare Ligation Master Mix

The Ligation Master Mix is prepared in 2 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 serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Lig* in the order shown in Table 42.

Table 42 Ligation Master Mix preparation— **Stage 1**

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

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

Ligation Master Mix: Stage 2

To Finish Preparing the Ligation Master Mix:

1. Remove the Axiom Ligation Enzyme from the –20°C freezer and place in a cooler chilled to –20°C.
2. Use appropriate serological and single-channel pipettes to add reagents to the 15 mL tube labeled *Lig* in the order shown in Table 43.

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

Table 43 Ligation Master Mix Preparation—Stage 2.

Reagent	Per array	Master Mix 96+
• Ligation Master Mix from Stage 1	82.42 µL	9.10 mL
• Axiom Probe Mix 1	10.5 µL	1.16 mL
• Axiom Probe Mix 2	10.5 µL	1.16 mL
• Axiom Ligate Enzyme	1.58 µL	174.4 µL
Total	**105 µL**	**11.59 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™ GeneTitan™ Consumables Kit.
2. Label two stain trays *Stain 1* (or *S1-1* and *S1-2*).
3. Label the remaining stain trays:
   - *Stain 2* (or *S2*)
   - *Stbl*
   - *Lig*

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

IMPORTANT! It is critical that you write only on the proper side of the front edge of stain trays, as illustrated in Figure 5 on page 39. The front edge of the tray is the short side with the lettering A through H. Do **NOT** write on any other side, as this can interfere with sensors inside of the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and lids.

IMPORTANT! Do not confuse hybridization trays with stain trays.

Deionize trays and covers

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

See Appendix D, "Deionization procedure for GeneTitan trays and covers" on page 155 for the recommended technique.

About aliquoting reagents to trays

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

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

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

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

Stain 1 Master Mix

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. Using a P200 12-channel pipette with new pipette tips, aliquot **105 µL per well** to both *S1* trays—dispense to the first stop only to avoid creating bubbles.
   You do not need to change pipette tips between additions of the Stain 1 Master Mix.
3. If:
   - Bubbles are present, puncture them with a pipette tip.
   - Droplets of liquid splashed onto the well dividers, place a laboratory tissue on top of the tray to blot and remove. (Figure 7).

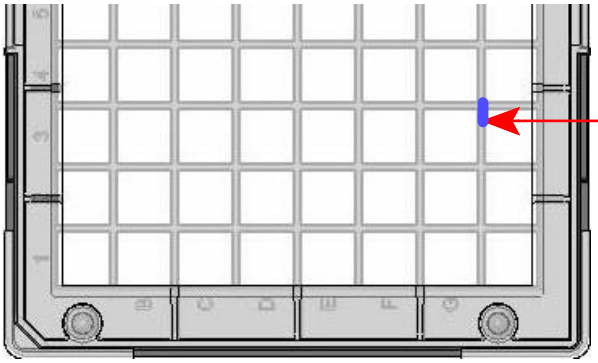


Figure 7 Well Dividers in Trays.

4. Place covers on the *S1* trays. Orient cover correctly on the tray with the notched corners together (Figure 8).

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

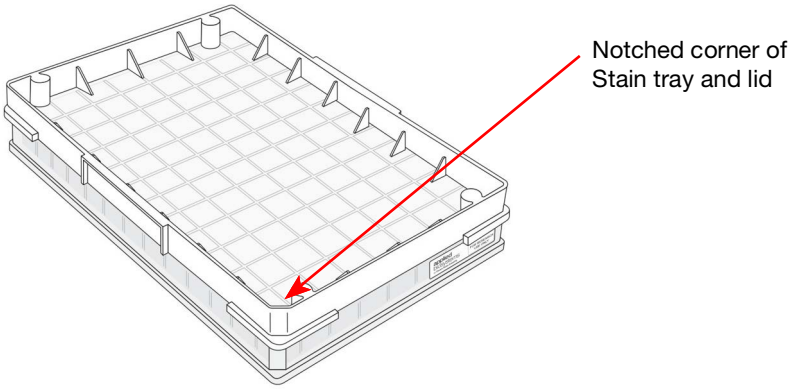


Figure 8 Placing cover on stain tray.

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

Stain 2 Master Mix

Aliquot the Stain 2 Master Mix:

1. Pour the Stain 2 Master Mix into the reagent reservoir marked *S2*, placed on the bench top at room temperature.
2. Using a P200 12-channel pipette with new pipette tips, aliquot **105 µL per well** to the *S2* tray—dispense to the first stop.

   You do not need to change pipette tips between additions of the Stain 2 Master Mix.
3. If:
   - Bubbles are present, puncture them with a pipette tip.
   - Droplets of liquid splashed onto the well dividers, place a laboratory tissue on top of the tray to blot and remove.
4. Place a cover on the *S2* tray. Orient the cover correctly on the tray with the notched corners together (Figure 8).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Stabilization Master Mix

Aliquot the Stabilization Master Mix:

1. Pour the Stabilization Master Mix into the reagent reservoir marked *Stbl*, placed on the bench top at room temperature.
2. Using a 12-channel P200 pipette with new pipette tips, aliquot **105 µL per well** to the *Stbl* tray — dispense to the first stop.

   You do not need to change pipette tips between additions of the Stabilization Master Mix.
3. If:
   - Bubbles are present, puncture them with a pipette tip.
   - Droplets of liquid splashed onto the well dividers, blot the top of the tray with a laboratory tissue.
4. Place a cover on the tray. Orient cover correctly on the tray with the notched corners together (Figure 8).

Ligation Master Mix

Aliquot the Ligation Master Mix:

1. Pour the Ligation Master Mix into the reagent reservoir marked *Lig*, placed on the bench top at room temperature.
2. Using a 12-channel P200 pipette with new pipette tips, aliquot **105 µL per well** to the *Lig* tray — dispense to the first stop.

   You do not need to change pipette tips between additions of the Ligation Master Mix.
3. If:
   - Bubbles are present, puncture them with a pipette tip.
   - Droplets of liquid splashed onto the well dividers, place a laboratory tissue on top of the tray to blot and remove.

4. Place a cover on the tray. Orient cover correctly on the tray with the notched corners together (Figure 8).
5. Protect the tray from light if not immediately loading onto the GeneTitan MC.

Axiom Hold Buffer

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 marked *Hold*, placed on the bench top at room temperature.
2. Remove the scan tray from its pouch.
3. Remove the scan tray cover, but leave the scan tray on its protective black base.
4. Prepare the barcoded scan tray cover (Part No. 202757) that came with the scan tray by completing the deionization procedure described in Appendix D, "Deionization procedure for GeneTitan trays and covers" on page 155. Place the cover to prevent dust or static from accumulating on the bottom of the cover.
5. Use a 12-channel P200 pipette with new pipette tips to aliquot **150 µL to each well** of a scan tray—dispense to the first stop and avoid touching the bottom of the tray.

   You do not need to change pipette tips between additions of the Axiom Hold Buffer.
6. If droplets of liquid splashed onto the well dividers, place a laboratory tissue on top of the tray to blot and remove.
7. Cover the tray by orienting the notched corner of the scan tray cover over the notched edge of the tray and the flat side of the cover against the scan tray (Figure 9).

IMPORTANT! Ensure that the scan tray receives 150 µL of Axiom Hold Buffer per well.

⚠ **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 to avoid generating bubbles.

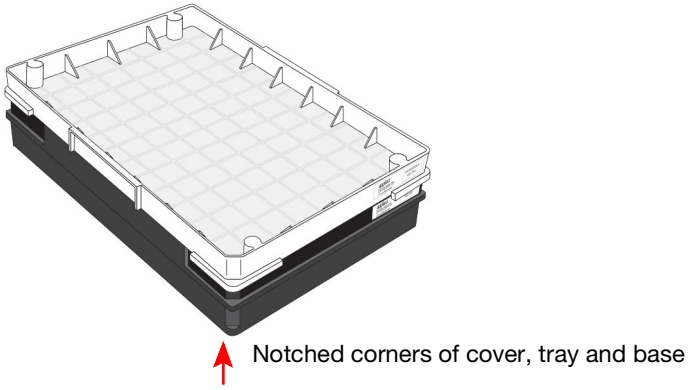


Figure 9 Scan Tray with the clear cover and protective base.

See "Stage 3: Ligate, wash, stain, and scan" on page 111 for instructions on loading the reagent trays.

5 Array processing with the GeneTitan™ MC Instrument

NOTE: In this chapter and throughout the document, the instructions and consumables for the GeneTitan MC Instrument are applicable to the GeneTitan MC Fast Scan Instrument.

The Axiom™ 2.0 Assay is designed for processing 96 samples at a time on Axiom™ Array Plates and custom myDesign™ Array Plates. The protocol is performed in 2 sets of steps:

- Target Preparation, performed on the lab bench without advanced automation. See Chapter 4, "Manual target preparation" on page 40
- Array processing, performed on the GeneTitan Multi-Channel (MC) Instrument

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

Before using the GeneTitan MC Instrument

Proper tray alignment and loading

Proper alignment and loading of plates, covers and trays is critical when using the GeneTitan MC Instrument. Each plate, cover and tray has 1 notched corner. The notched corner of plates, trays, covers and bases must be in vertical alignment with each other, and placed in position A1 per the Tray Alignment guide inside each GeneTitan MC drawer (Figure 10 on page 86 and Figure 11 on page 87).

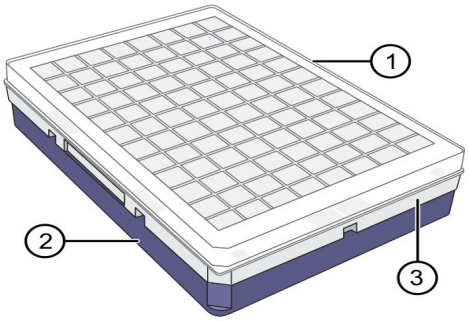
IMPORTANT! When running a multi-plate workflow, you must pay careful attention to the software prompts that tell you which side of the drawer to place or remove a plate/tray.

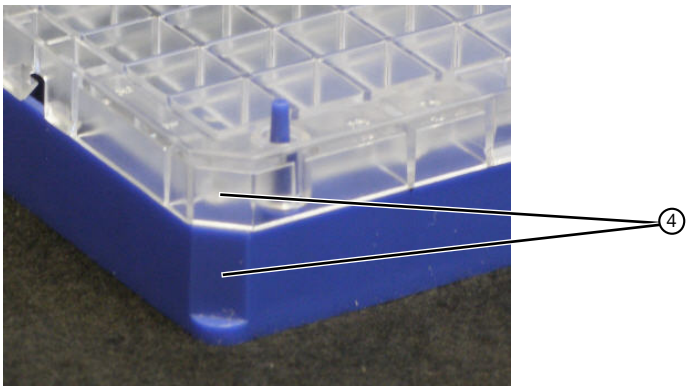
Tip: Mark the notched corner of each plate, cover and tray with permanent marker to help ensure proper alignment and loading onto the GeneTitan MC Instrument.

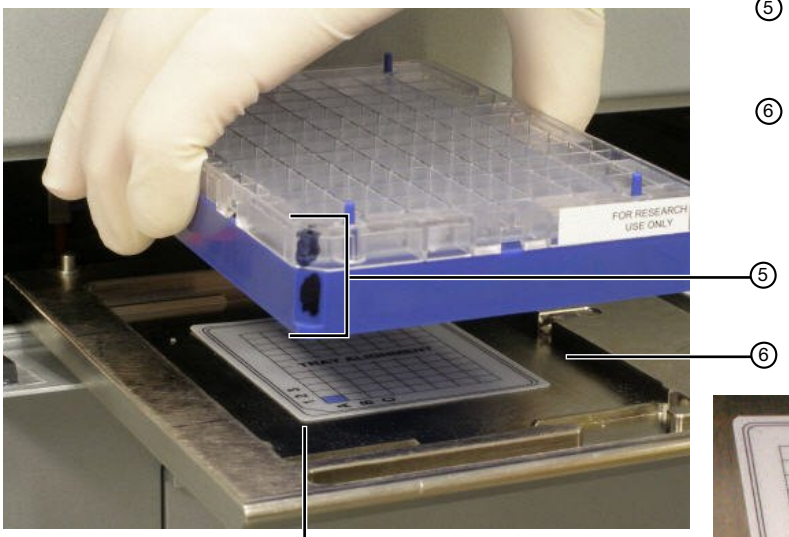
⚠ **CAUTION!** Take care not to damage the consumables or bend the blue cover posts or scan tray posts.

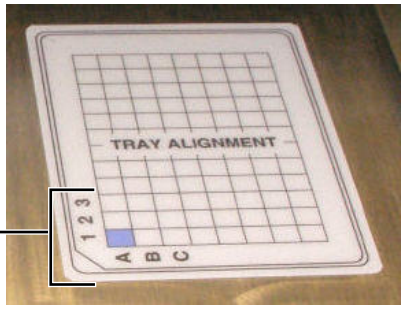
Note: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. See Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 158 for the message displayed to the user and the procedure for replacing the filters.

IMPORTANT! Remove the plastic protective shipping tray cover.









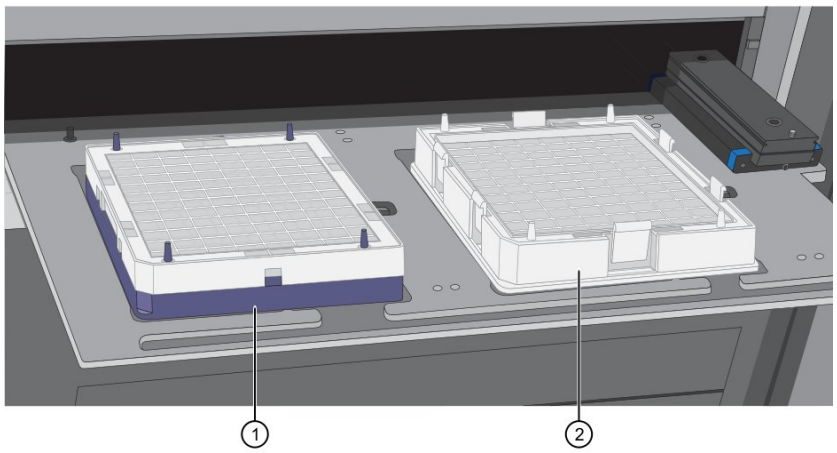
① Clear tray shipping cover (to be discarded)
② Array plate protective base
③ Array plate
④ Notched corner of array plate aligned with notched corner of blue base.

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

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

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

Figure 10 Proper alignment and loading of plates, covers and trays in the GeneTitan MC Instrument.

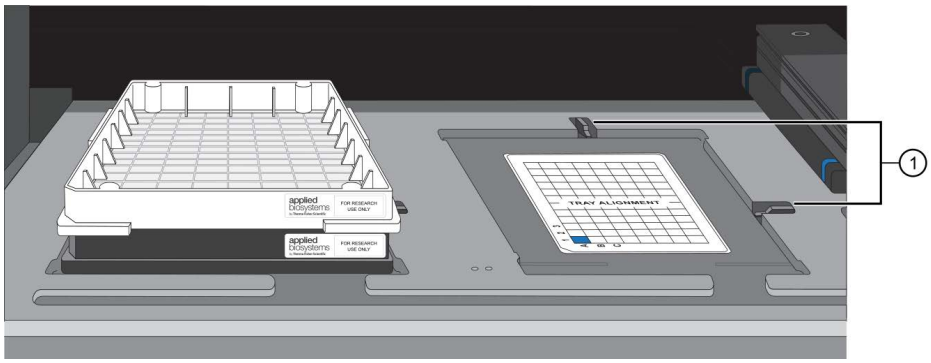


① Array plate with protective blue base

② Hybridization tray

Figure 11 Array plate with protective blue base and the hybridization tray aligned and properly loaded into Drawer 6.

IMPORTANT! When you install the consumables, ensure that the fingers are retracted. Do not lay the consumables on top of the drawer fingers–this indicates that the instrument is not functioning correctly. Notify your field service engineer if the fingers do not retract automatically. You should place the trays into the instrument drawers when a drawer is fully extended by the instrument. The fingers are retracted when the drawer is open and are extended when the drawer is closed in order to restrain the consumable.



① Drawer tab, or “finger” in back and on right.

Figure 12 The location of drawer tabs.

Stain trays and covers

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

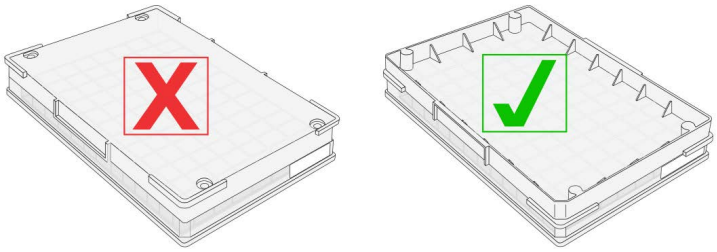


Figure 13 Placement of covers on trays.

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 hybridization and stain trays. Do **NOT** write in any other location, as this can interfere with sensors inside the GeneTitan MC Instrument and result in experiment failure. To ensure proper placement of lids onto stain trays, and trays onto the GeneTitan MC Instrument, you can also mark the notched corner of the trays and lids.

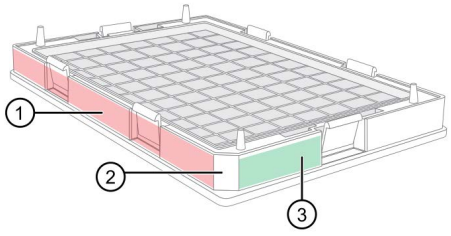
Proper labeling for hybridization trays and reagent trays is described in:

- "Labeling for Hybridization Trays", below
- "Labeling for stain trays" on page 89

IMPORTANT! Do not confuse hybridization trays with stain trays.

Labeling for Hybridization Trays

You can label the hybridization tray on the front part of the **short side of the tray, next to the notch at the left,** as shown in Figure 14. The proper section for labeling is closest to the notched corner, corresponding to the A1 and B1 wells.



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

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

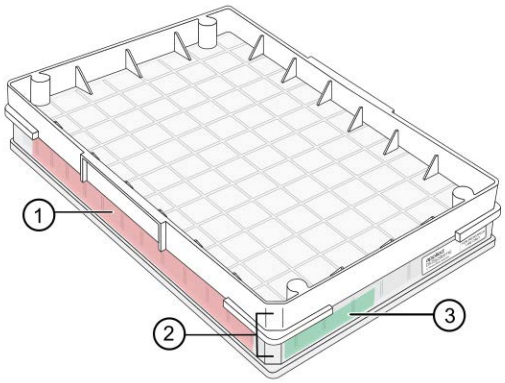
③ Label the hybridization tray in this area.

Figure 14 Labeling GeneTitan Hybridization Trays.

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

Labeling for stain trays

You can label the stain trays on the **left side of the front of the tray** as shown in Figure 15. The correct side is closest to the notched corner, corresponding to the A1 through C1 wells.



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

Figure 15 Labeling GeneTitan stain tray (stain tray shown with lid).

E-mail and Telephone Notifications from the GeneTitan MC Instrument

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

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

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

- Starts
- Completes
- Aborts
- Encounters an error

GeneTitan MC Instrument lamp

The GeneTitan MC Instrument uses a xenon arc lamp system that is warranted for 500 hours to provide illumination for imaging the array at 2 wavelengths. The xenon lamp has a limited lifetime and needs to be replaced at regular intervals.

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

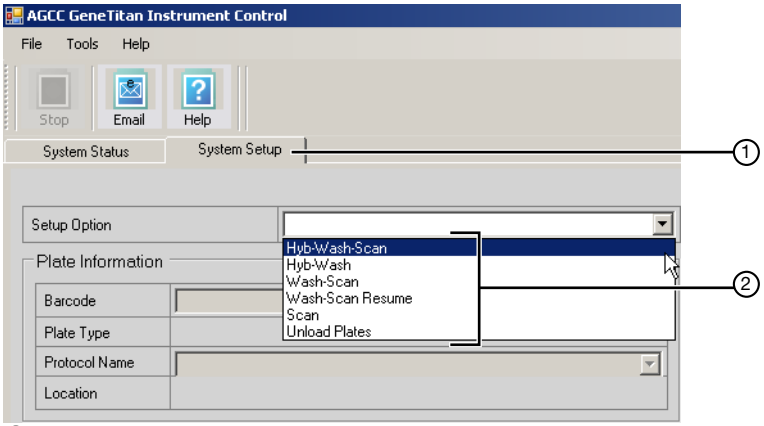
See the *GeneTitan MC Instrument User Guide*, Pub. No. 08-0308, or Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 158 of this user guide for details on replacing the lamp.

See the *GeneTitan MC Instrument User Guide*, Pub. No. 08-0308, for the Lambda LS and Smart controller system. The Lamp and the controller should NEVER be switched ON or OFF manually. The GeneTitan MC Instrument control software manages the lamp activity and will switch the lamp ON and OFF as required. It takes 10 minutes to warm-up the lamp. In idle mode the lamp will remain ON for 2 hours before it is automatically switched OFF and if there are no more plates being transferred from the fluidics to the imaging station. This is by design and intended behavior. 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 16. A brief description of each option is given below.



① **System Setup** tab

② Setup options

Figure 16 Setup options for processing array plates.

Hyb-Wash-Scan

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

IMPORTANT! When running a multi-plate workflow, you must pay careful attention to the software prompts 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 hybridization tray is hybridized to an array on the array plate.
  - Duration for 96 samples = 23.5 hours
- *Wash*: samples on arrays are ligated, washed, stained and fixed.
  - Duration for 96 samples = ~5 hours

Note: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. See Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 158 for the message displayed to the user and the procedure for replacing the filters

- *Scan*: The array plate is moved to the imaging device in the GeneTitan MC Instrument and each array is scanned.
  - Duration for 96 samples = ~3.5—5.5 hours. Scan time varies with instrument type and GCC software version.

Hyb-Wash

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

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

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

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

When Ready to Scan the Array Plate:

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

Wash-Scan

Use this option if:

- The array plate was hybridized 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:

- It was necessary to hybridize the array plate in an oven separate from the GeneTitan MC Instrument.
- 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.
- To scan or re-scan a plate that was hybridized and washed/stained on a different GeneTitan MC Instrument.
- To queue a second plate for scanning. Using the **Scan** option allows you to start a second **Scan** workflow while another **Scan** workflow is already running. See "Queue a second plate for scanning" on page 108.

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 1 or more array plates. Instructions and an example are shown below in Figure 17.

If the instrument aborts a process, you can retrieve the array plate and related consumables as described in Figure 17. An instrument-initiated abort can occur:

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

To abort array plate processing:

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

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

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

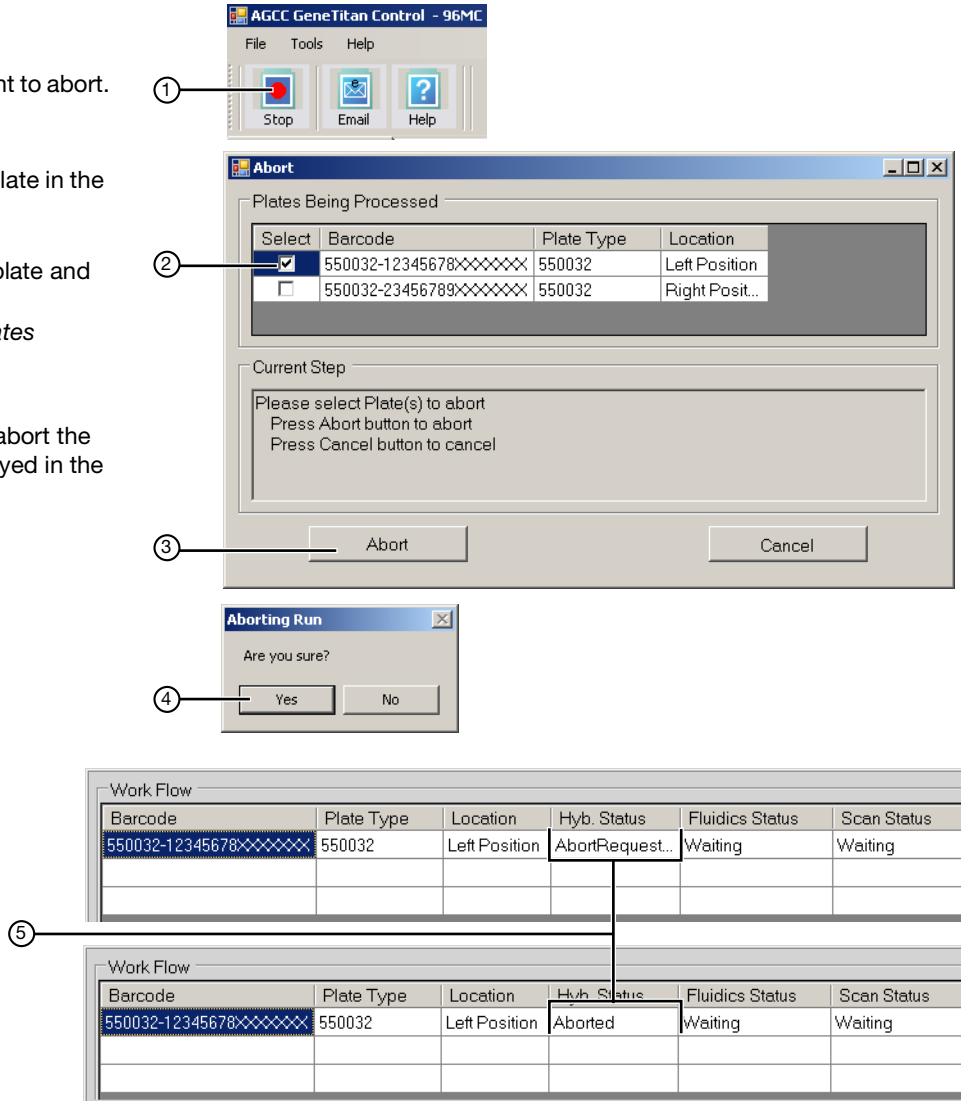


Figure 17 Manually aborting an array plate.

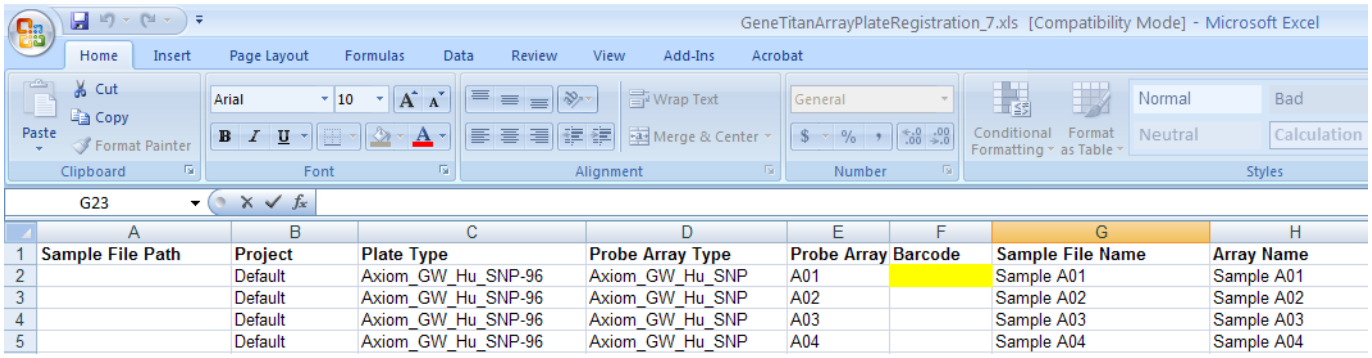
Stage 1: Create and upload a GeneTitan Array Plate Registration File

In the GCC software, you must create and upload a Batch Registration file before you begin "Stage 2: Hybridization" on page 95 (example shown in Figure 18). This file contains information critical for:

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

1. If you have not already created a batch registration file, create one now. (See Appendix C, "Registering samples in GeneChip™ Command Console™" on page 152 for detailed instructions.)
2. In GCC, select the array plate format (96 samples) and open a batch registration file template.
3. Scan the array plate barcode into the yellow barcode field.
4. Enter a unique name for each sample and any additional information.
5. Save the file.
6. Upload the file.

IMPORTANT! It is very important to create and upload a batch registration file with your sample information before starting "Stage 2: Hybridization" on page 95.



	A	B	C	D	E	F	G	H
1	**Sample File Path**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array**	**Barcode**	**Sample File Name**	**Array Name**
2		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A01		Sample A01	Sample A01
3		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A02		Sample A02	Sample A02
4		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A03		Sample A03	Sample A03
5		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A04		Sample A04	Sample A04

Figure 18 Example of a GeneTitan Array Plate Registration File.

Stage 2: Hybridization

Reagents required

Reagents required

Table 44 Reagents Required from the Axiom 2.0 Reagent Kit.

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

- An Axiom 96-array plate is required for this step. Before inserting this plate into the GeneTitan MC Instrument for hybridization, the array plate should be brought to room temperature.
- A hybridization tray containing denatured samples (fromStep 9 on page 70 in Chapter 4) is also required for this step. The denatured samples should be transferred to the hybridization tray only after the GeneTitan MC Instrument is ready for loading the hybridization tray in the "Load trays onto the GeneTitan MC Instrument" on page 113.

Setup the instrument

1. Launch GCC Launcher and select **GCC GeneTitan Control** (Figure 19).

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

Note: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. See Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 158 for the message displayed to the user and the procedure for replacing the filters

IMPORTANT! Do not close the scanner application by right-clicking on it and choosing the “Close” option. This 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 121.

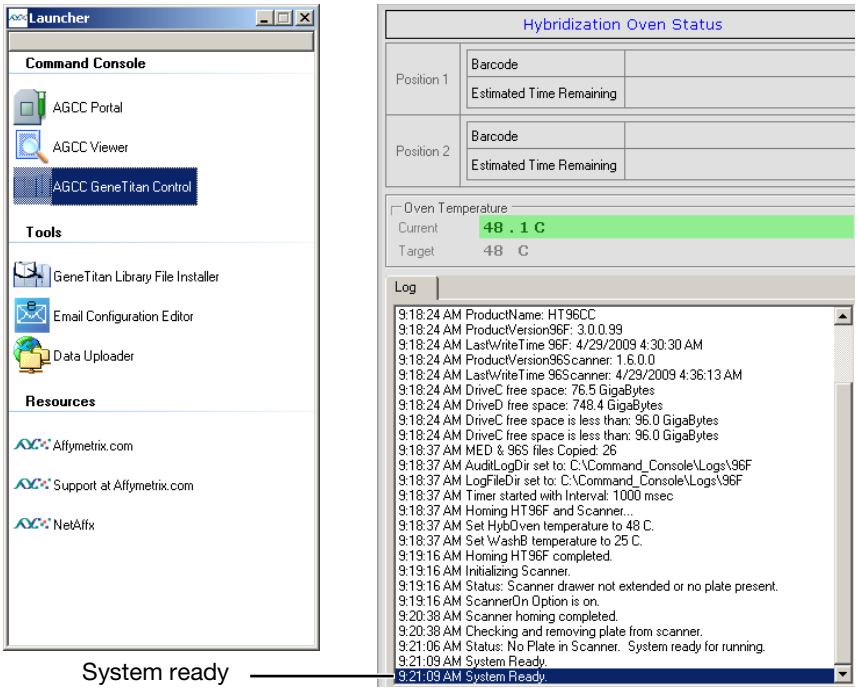


Figure 19 Launching GCC and initializing the GeneTitan MC Instrument.

System Setup tab

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

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

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

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

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

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

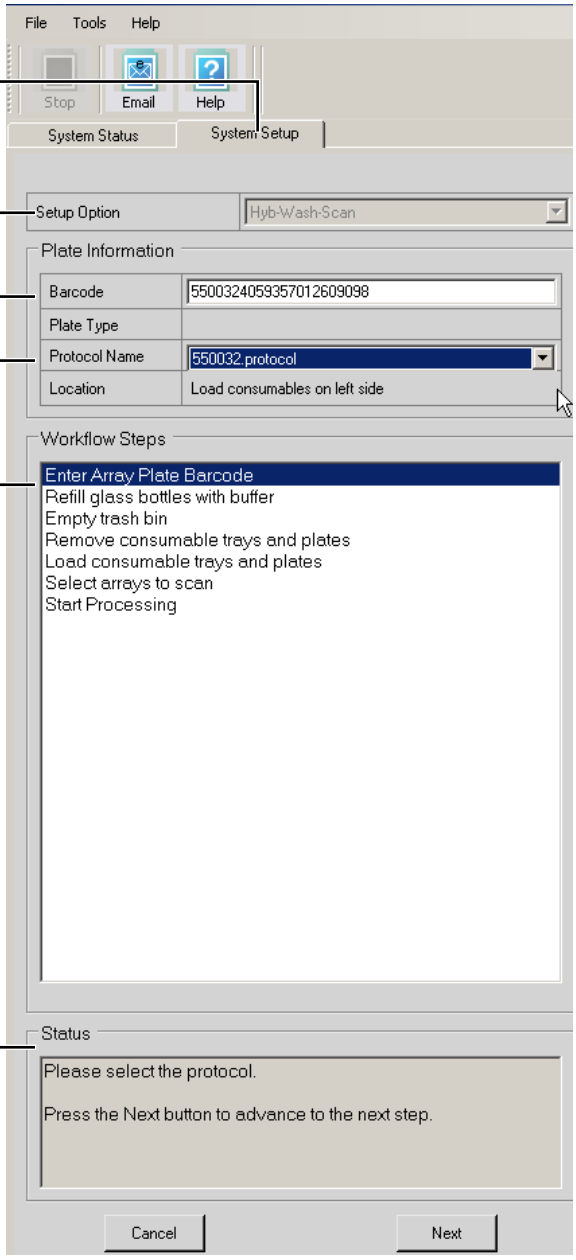


Figure 20 System Setup Tab and the Information Displayed in this Pane

2. Select the **System Setup** tab (Figure 20).
3. Configure the software as follows:
   d. Setup Option: **Hyb-Wash-Scan**

      Other options available are described under "Setup options for array plate processing" on page 90.
   e. 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 8 Axiom array plates.

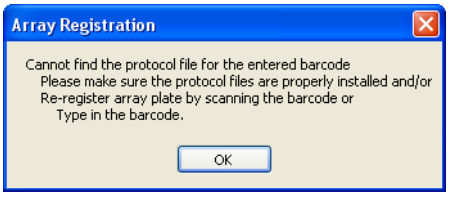
- 96 Axiom array plate requires ~80 GB

f. Plate Information:

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

  The first 6 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.

  550094 <barcode> = Affymetrix 96-array 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 21 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.

  550094.protocol = for Affymetrix 96-array plate barcodes

4. Complete the remaining workflow steps as follows:

   a. **Refill bottles with buffer (**Figure 22 on page 99**).**
   Fill these bottles:

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

IMPORTANT!

- Always ensure that the GeneTitan bottles containing Wash A and Rinse are above the 50% mark when setting up the system to process an Axiom HT array plate. All 600 mL of the Wash buffer B from the Axiom reagent kit should be emptied into the GeneTitan Wash B bottle when setting up the system to process a plate. This ensures that the GeneTitan Wash B bottle is filled to more than the requisite 35% of Wash B bottle volume. Also, do not overfill the bottles. Fill Wash Buffer B and Water bottles to the 1 L mark only. Wash A keep at 2 L. We strongly recommend refilling these bottles every time you are prompted to do so.

  If the volume in any of these bottles becomes too low during a run, a message is displayed (see Chapter 7, "Troubleshooting" on page 132). 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 2 array plates on the same day, fill the Wash B bottle to the 1L mark (use both bottles from the Axiom 2.0 Reagent Kit).

b. Empty the waste bottle.

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

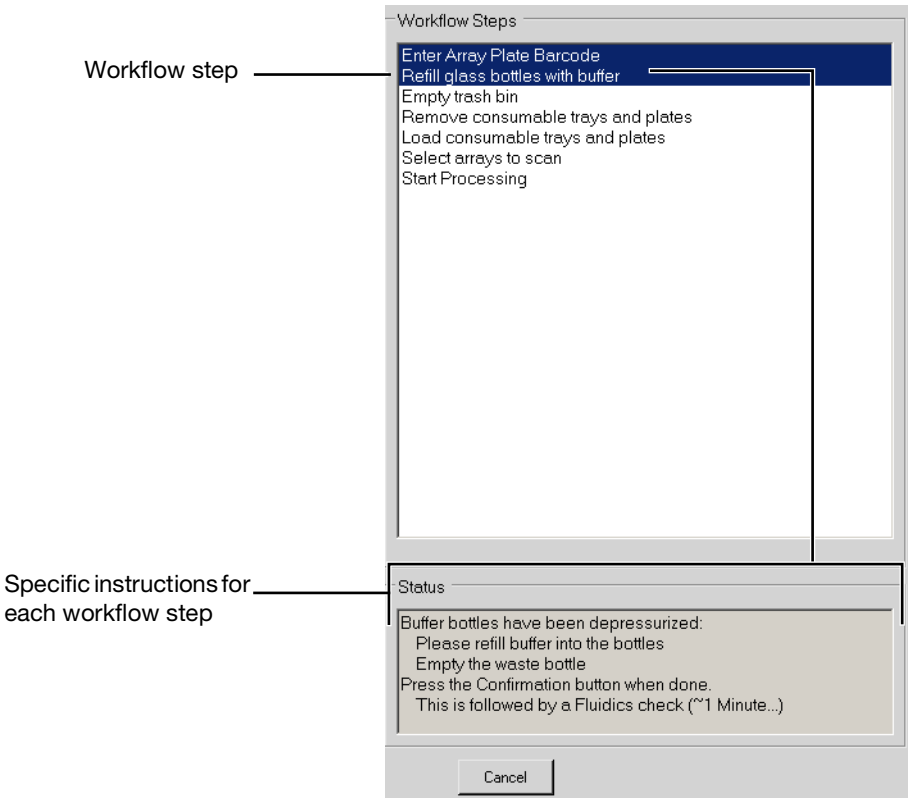


Figure 22 Example of the remaining workflow steps.

d. **Empty trash bin**

- Open the trash bin and empty.

If already empty, the trash bin remains locked and the Status pane reads "Trash bin is empty."

- Press the **Confirmation** button to continue.

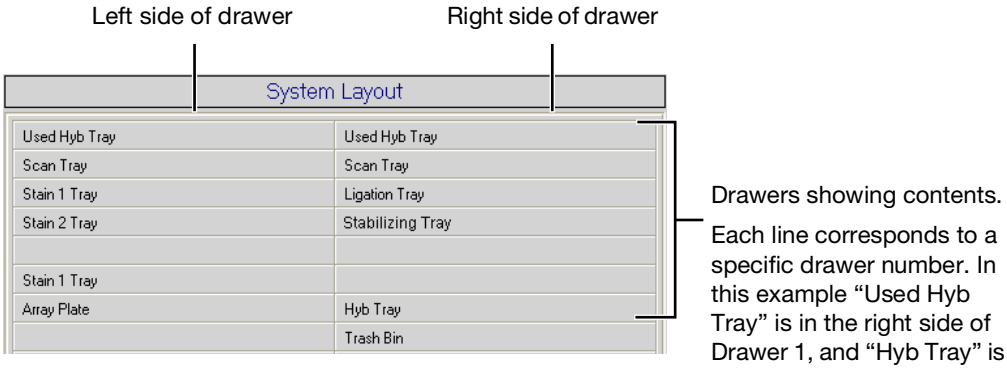
e. **Remove consumable trays and plates**

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

f. Continue to "Load an Axiom Array Plate and Hybridization Tray onto the GeneTitan™ MC Instrument" on page 100.

Load an Axiom Array Plate and Hybridization Tray onto the GeneTitan™ MC Instrument

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



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

Figure 23 System layout—Location of plates inside the GeneTitan MC Instrument

Load an Axiom Array Plate and Hybridization Tray onto GeneTitan MC Instrument:

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

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

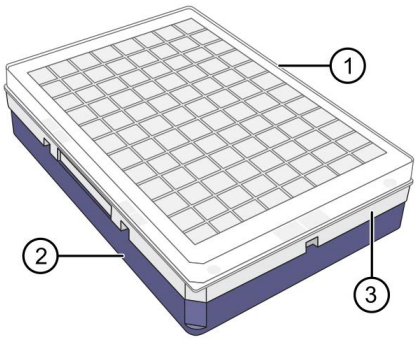
IMPORTANT! Removing bubbles at this step greatly reduces the chance of bubbles under the arrays when the hybridization tray and the Axiom array plates are clamped. Bubbles under an array can result in black spots on the array image.

b. Load the hybridization tray **without the cover** on the right side of the drawer (Figure 25 on page 101).

The array plate must be loaded on its protective blue base, as shown in Figure 25 on page 101 below. The clear plastic cover on top of the array plate SHOULD NOT be loaded in the GeneTitan MC Instrument. See Figure 10 on page 86 for more details on the correct way of loading the array plate.

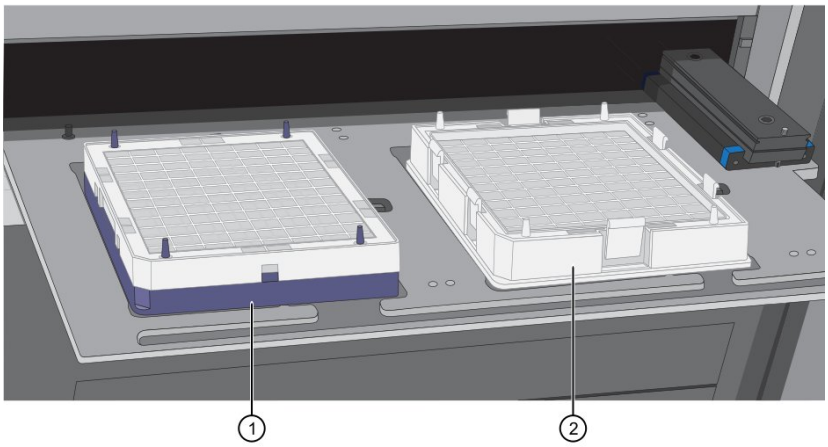
c. Remove the array plate and protective blue base from its package.

To avoid dust or other damage, leave the array plate packaged until ready to load onto the GeneTitan MC Instrument (Figure 24).



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

Figure 24 Array plate packaging.



① Array plate with protective blue base.
② Hybridization tray.

Figure 25 Array plate with protective blue base and the Hybridization Tray properly loaded into Drawer 6.

d. Load the array plate **with the protective blue base** on the left side of the drawer (Figure 25).

> ⚠ **CAUTION!** The notched corner of each plate, cover and tray must be aligned. When loading onto the GeneTitan MC Instrument, the notched edge plates, covers and trays must be aligned as indicated by the Tray Alignment guide in the drawer (Figure 25 on page 101).
>
> The error message may be displayed. Plate barcodes must face the internal barcode reader (back of the drawer). Improper tray positioning can cause the GeneTitan MC Instrument to crash, and can result in substantial damage to the instrument and loss of samples.

e. Press the **Confirmation** button.

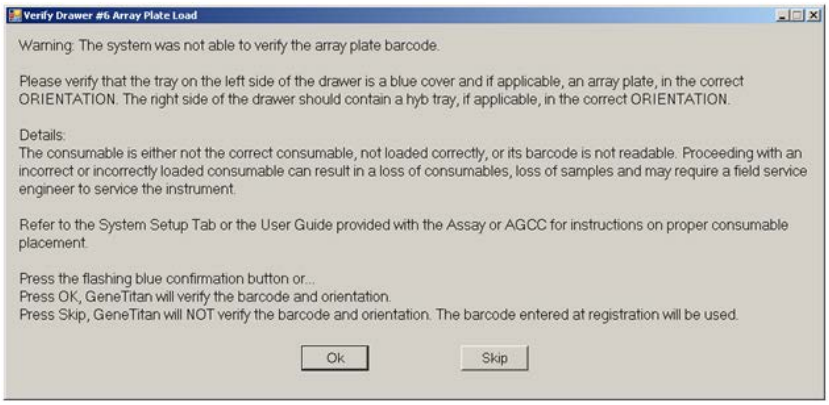
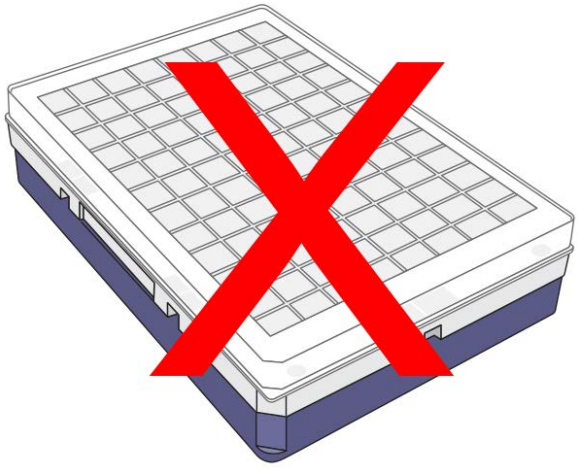


Figure 26 Barcode error message.

When you load the array plate left side of the drawer: The internal bar code reader reads the barcode of the array plate and compares it with the barcode and the plate type specified in the Barcode field and Plate Type field on the Setup page. If the information is correct, the application allows you to proceed to the next step. If the instrument is unable to read the barcode, it will push the tray out and will prompt (Figure 26) you to load the correct plate with the proper orientation into the instrument (Figure 25).

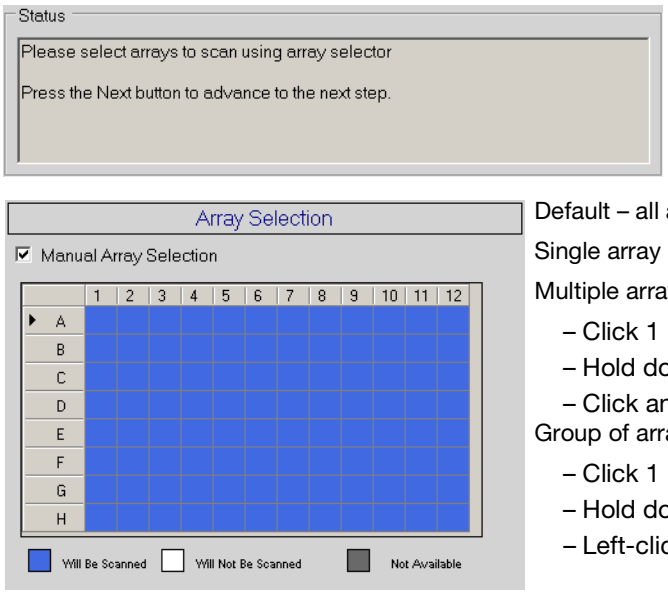
- Click OK to retry and check the loading of the array plate; or
- Click Skip if the instrument has problems reading the barcode and after verifying that the trays have been placed in the proper orientation.

IMPORTANT! Do not install a 3 plate stack of trays. Confirm that you have removed the clear plastic shipping cover as shown in Figure 10 on page 86.



f. Select the arrays to scan (instructions in Figure 27).

   By default, all arrays are selected.



Message in Status window.

Default – all arrays are selected

Single array - click 1 box

Multiple arrays:

- Click 1 box
- Hold down the **Ctrl** key
- Click another box in the same column

Group of arrays:

- Click 1 box
- Hold down the **Shift** key
- Left-click and drag the mouse

Figure 27 Selecting which arrays to scan an array plate.

2. Click **Next**, then click **OK** to begin processing the samples (Figure 28).

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

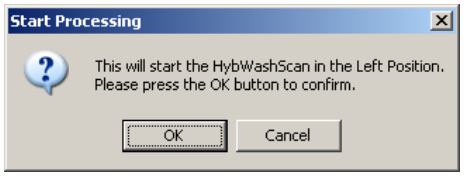


Figure 28 Click OK to start processing the first array plate and hybridization tray.

The software starts the process for clamping the array plate to the hybridization tray. Press **OK** on the dialog shown in Figure 29 and wait for the drawer to open before retrieving the array plate and hybridization tray combo for inspection. The sandwich of the array plate and hybridization tray needs to be manually inspected before the array processing can begin. Once clamping is complete the dialog shown in Figure 30 on page 104 will be displayed. If you do not press OK in Figure 29 the dialog box will go away without intervention and Figure 30 on page 104 will be displayed.

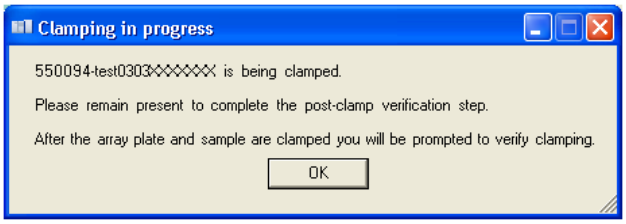
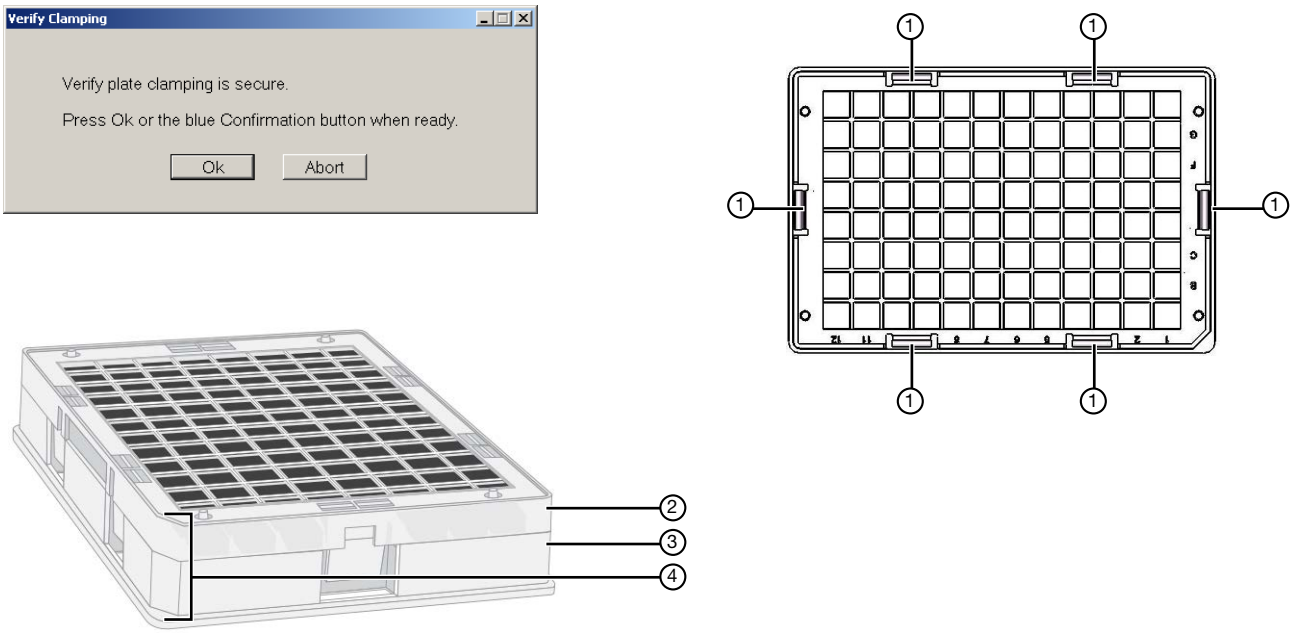


Figure 29 Clamping in progress notification

3. When drawer 6 opens and the prompt in Figure 30 is displayed:



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

Figure 30 Location of camping points on the array plate and hybridization tray.

a. Remove the plate stack and gently press the 2 plates together at each clamping point.

   Listen for a clicking sound which indicates that the plates are now clamped. No clicking sound indicates the plates are already clamped (See Figure 31 for an example of a array plate hybridization tray sandwich).

Figure 31 Array plate and hybridization tray sandwich.

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

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

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

   The message in Figure 32 can be displayed again if plate orientation is incorrect or if the hybridization tray barcode cannot be read. Click **OK** to proceed.

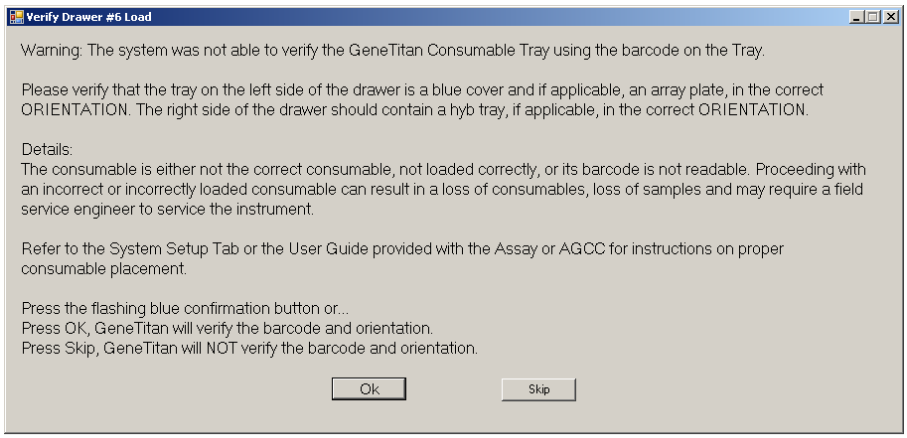


Figure 32 Verification message.

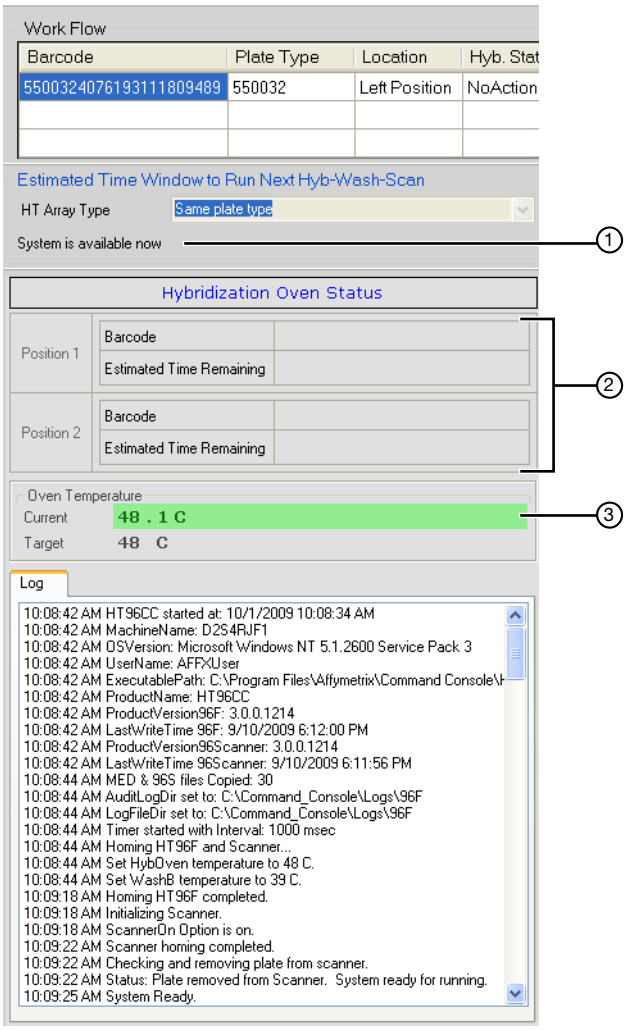
Load a second Axiom Array Plate and Hybridization Tray onto the GeneTitan MC Instrument

When you can load a second array plate and hybridization tray

Once processing begins, you have a specific period of time during which you can load another Axiom array plate and hybridization tray. This period of time is displayed above the Hybridization Oven Status pane (Figure 33). You cannot load another hybridization tray before or after this period of time.

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

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



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

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

In this example the system is currently available.

② Position of plate stack in the hybridization oven.

Position 1 - left side of oven

Position 2 - right side of oven

③ Oven Temperature.

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

Figure 33 Loading a second hybridization tray and hybridization oven status information.

1. Select the System Setup tab.
2. Load an Axiom array plate and hybridization 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 plates **with the blue base** and the hybridization tray **without the cover**, then press the Confirmation button.
   c. Select the arrays to scan, then click **Next**.
   d. Ensure that the plates are clamped securely when prompted, then press the **Confirmation** button.
   e. Click **OK** when prompted to resume plate processing (Figure 34).

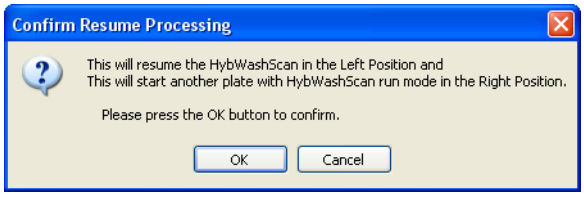
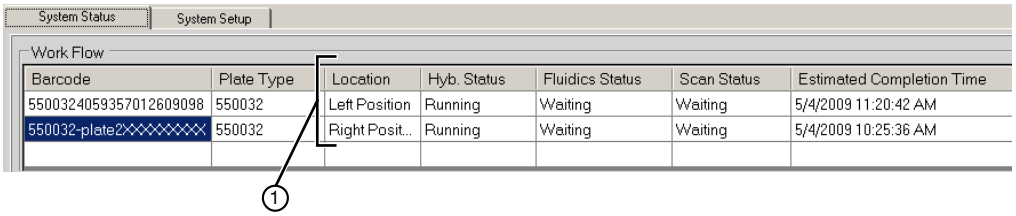


Figure 34 Confirm Resume Processing prompt.

Select the System Status tab to view Axiom array plates status in the WorkFlow window (Figure 35).



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

Figure 35 Example of the workflow window when 2 plates are loaded and are in the hybridization oven.

Queue a second plate for scanning

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

1. Start the first Scan workflow in the GeneTitan Instrument. Wait until the first plate is loaded into the imaging device and starts scanning.
2. Go to the **System Setup** tab and select **Scan** from **Setup Option** drop-down list (Figure 36).
   The Setup Option drop-down list is active only after the first plate begins scanning.

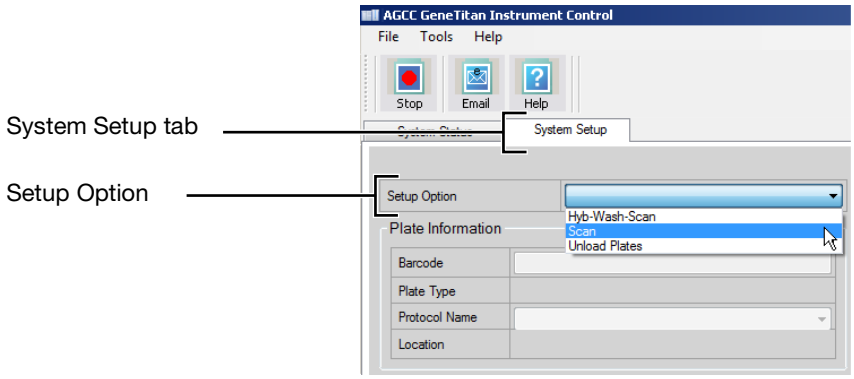


Figure 36 Scan setup option for processing a second array plate

3. Click **Next** in the lower left section of the window under the Status box.
4. Scan or manually enter the array plate barcode, then click **Next**.
5. Following the instructions in the Status box, empty the trash bin if needed and then press the GeneTitan Confirmation button to continue.
6. Place the array plate on top of a scan tray in the correct orientation such that notched corner of the array plate and scan tray are aligned.
7. Load the array plate/scan tray combo in drawer 2 of the GeneTitan Instrument, on the left or right side, as instructed in the Status box.
   - Be sure to load the array plate/scan tray combo in the correct orientation in the drawer. If needed, see Figure 10 on page 86 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 37). Click **OK** to continue.

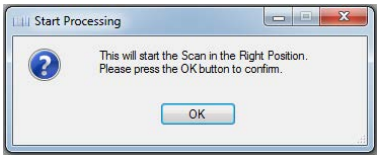


Figure 37 Start scan confirmation message

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

Status window prompts and actions required

Table 45 Refilling buffer bottles and emptying the waste bottle.

Status Window Prompt	Action Required	Receptacle – Reagent
Buffer bottles have been depressurized. Refill buffer into the bottles. Empty the waste bottle.	• ** Replenish the fluid in Wash Bottles A and B, and the Rinse bottle. • Empty the Waste Bottle. • Press the *Confirmation* button to continue.	• Wash Bottle A – fill with Axiom Wash Buffer A up to 2 L. • Wash Bottle B – fill with Axiom Wash Buffer B to the 1-L mark. • Rinse – fill with Axiom Water to the 1-L mark. Do not overfill these bottles.
**** Every time you are prompted to refill the buffer bottles, the system runs a fluidics check (duration ~ 1 minute).**		

Table 46 Emptying the trash bin.

Status window prompt	Action required	Receptacle – reagent
Empty trash bin	• Open and empty the trash bin. • Press the *Confirmation* button to continue. NOTE: If the trash bin is empty, you will not be able to open it. Continue the process by pressing the confirmation button	—

Table 47 Loading the array plate and hybridization tray; barcode error messages.

Status window prompt	Action required	Reagent – receptacle
Load Array Plate Tray on [Left/Right] side of Drawer. Load hybridization tray without cover on [Left/Right] side of Drawer.	Load the array plate with the blue base and the hybridization tray in drawer 6. • **IMPORTANT:** The blue base must remain in "left side HTA in" even when empty. • **IMPORTANT:** The trays must be positioned. 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.	• Hybridization tray loaded with denatured samples.
Text version of the error message Warning: The system was not able to verify the array plate barcode. Verify that the tray on the left side of the drawer is a blue cover and if applicable, an array plate, in the correct ORIENTATION. The right side of the drawer should contain a hybridization tray, if applicable, in the correct ORIENTATION. Details: The consumable is either not the correct consumable, not loaded correctly, or its barcode is not readable. Proceeding with an incorrect or incorrectly loaded consumable can result in a loss of consumables, loss of samples and can require a field service engineer to service the instrument. See the System Setup Tab or the user guide provided with the assay or GCC for instructions on proper consumable placement. Press the flashing confirmation button or... Press OK, The GeneTitan MC Instrument will verify the barcode and orientation. Press Skip, The GeneTitan MC Instrument will NOT verify the barcode and orientation. The barcode entered at registration will be used.		These messages are displayed if: • A plate has been loaded improperly. • The bar code is missing or obscured.

Table 48 Selecting which arrays to scan.

Status window prompt	Action required	Reagent and receptacle
Select arrays to scan	• Accept the default (all arrays selected) if appropriate. Otherwise, select the arrays to be scanned. • Click *Next*, then click *OK* to start processing.	—

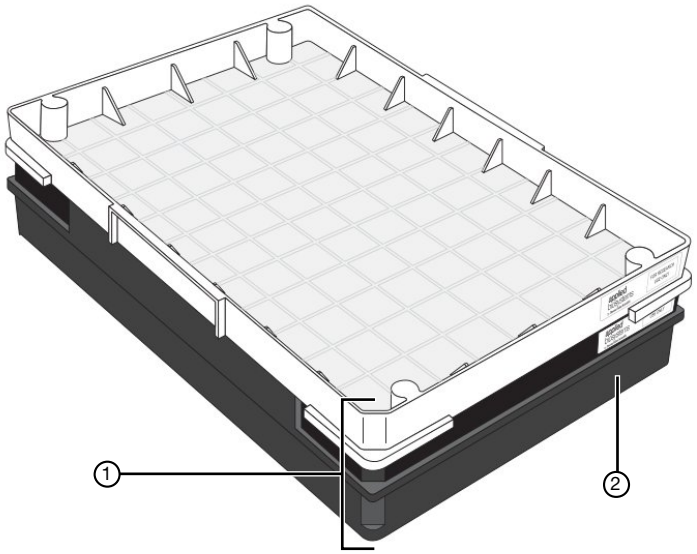
Stage 3: Ligate, wash, stain, and scan

Equipment, consumables, and reagents required

Scan Tray with Axiom Hold Buffer

- Cover the tray by orienting the notched corner of the cover over the notched edge of the tray and leave on the bench top (no need to protect from light; Figure 38).

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

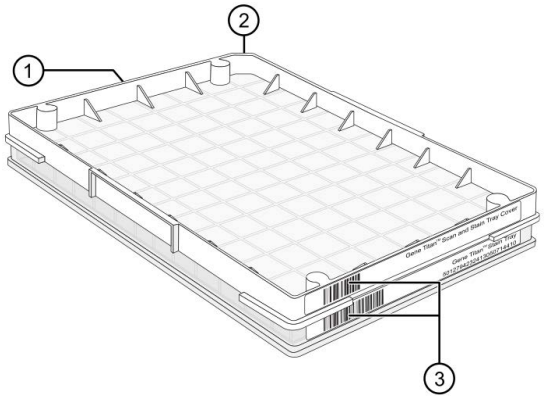


① Notched corner of the cover is aligned with the notched corner of the scan tray.
② Always leave the scan tray in its protective black base.

Figure 38 The scan tray with cover on the black base.

Proper installation of the GeneTitan tray consumables.

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

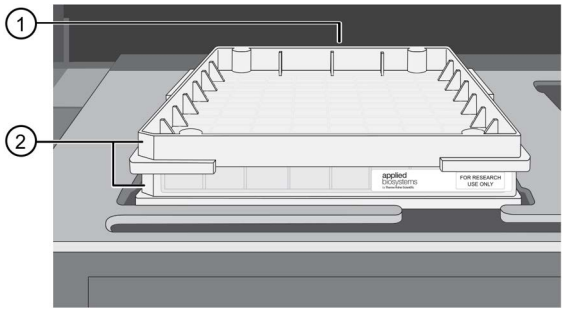


① Front of instrument (facing you).

② Notched corners. The notched corners face out and left.

③ Barcodes. The barcodes face to the rear of the instrument where scanning by the internal barcode reader takes place.

Figure 39 You must rotate and install the trays so that the barcode faces into the instrument.



① Barcodes face the rear of the instrument.

② Notches face out and left. “For Research Use Only” faces out.

Figure 40 The Proper Installation of the GeneTitan Tray Consumables (the image shows the Stain Tray and the Stain Tray cover as an example).

Note: The instrument control software will display a warning if it detects a problem during the fluid dispense operations. The filters in the GeneTitan Wash A, Wash B and DI Water bottles should be replaced if the software displays such a warning. See Appendix E, "GeneTitan™ Multi-Channel Instrument care" on page 158 for the message displayed to the user and the procedure for replacing the filters

Load trays onto the GeneTitan MC Instrument

Load Trays onto the GeneTitan MC Instrument:

When hybridization of an Axiom array plate has finished, a message (Figure 41) will alert you to resume the workflow setup. Press **OK** and the software takes you directly back to the System Setup tab.

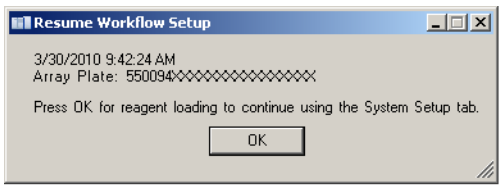


Figure 41 The Resume Workflow Setup Message.

This prompt to continue into reagent load (Figure 41) occurs when the hyb is complete. "Estimated Time Remaining" displayed under "Hybridization Oven Status" can display a time remaining of 0 to 30 minutes when the prompt occurs.

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

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

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

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

1. Follow the prompts in the Status window.
   a. Wash Bottles A and B, and the Rinse Bottle—refill as necessary (the system will prime itself again); Waste bottle—empty if necessary.

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

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

      Leave the blue array plate base in drawer 6 even though the base is empty.
2. Load consumable trays and plates as follows:
   a. Follow the prompts in the Status window (load sequence and prompts in Table 49).
   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 laboratory tissues, and reload the tray.

CAUTION!

- Orient trays as indicated by the guide inside the drawer. Improper orientation can cause the run to fail.
- Remove the protective black base from the scan tray immediately before loading Figure 42 on page 115).
- Examine each cover for droplets of liquid after loading. Liquid on the cover can result in capillary phenomenon. As a result, the tray can stick to the cover and be lifted out of place inside the instrument.

Table 49 Sequence for loading the trays with reagents.

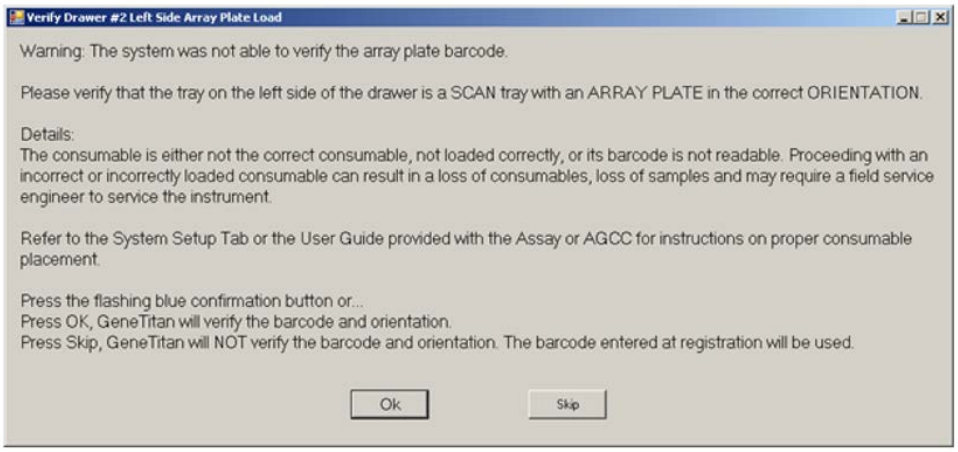
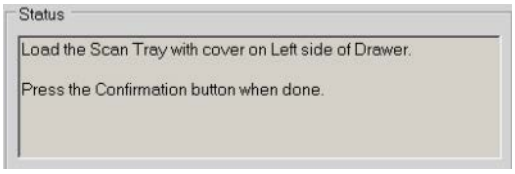
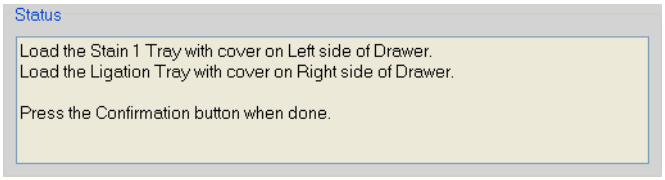
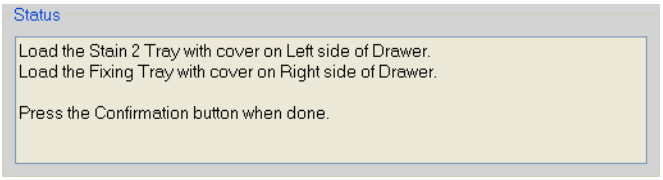
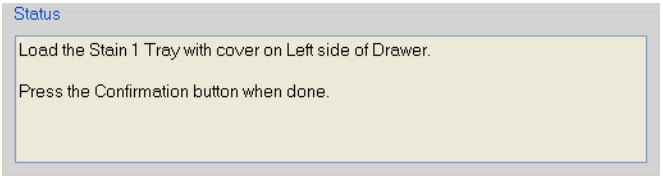
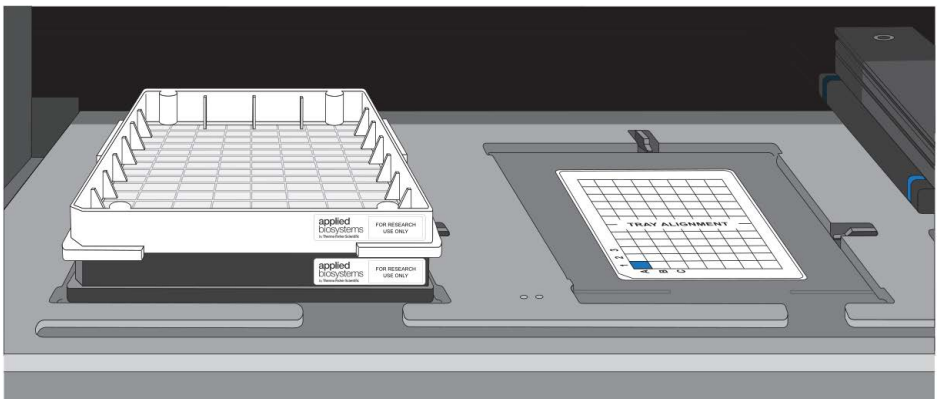
Loading sequence by drawer number	Left	Right
	Note: If the software is unable to verify the barcode on the scan tray and the scan tray cover, the software will display the following error message 	
2	**Scan Tray with cover — do not load the** protective ***black base*** (left side of drawer as indicated in Status window) Figure 42 on page 115 	
3	Stain Tray with Stain 1	Ligation Tray
	Figure 43 on page 116 	

Table 49 Sequence for loading the trays with reagents. (Continued)

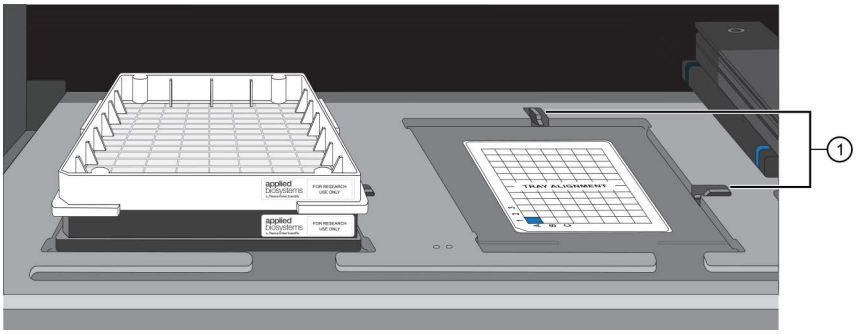
Loading sequence by drawer number	Left	Right
4	Stain Tray with Stain 2	Stbl Tray
	Figure 44 on page 116	
		
5	Stain Tray with Stain 1	Empty
		



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

Figure 42 Scan tray loaded in Drawer 2.

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



① Tab or "finger" in a GeneTitan drawer.

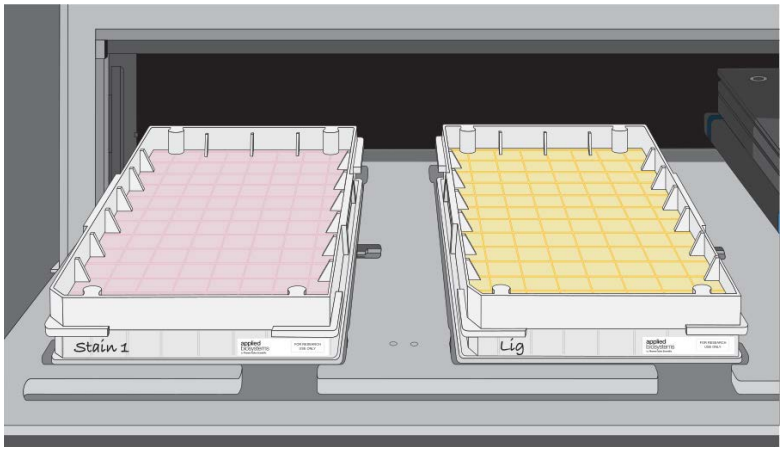


Figure 43 Stain 1 tray and Ligation Tray loaded in Drawer 3.

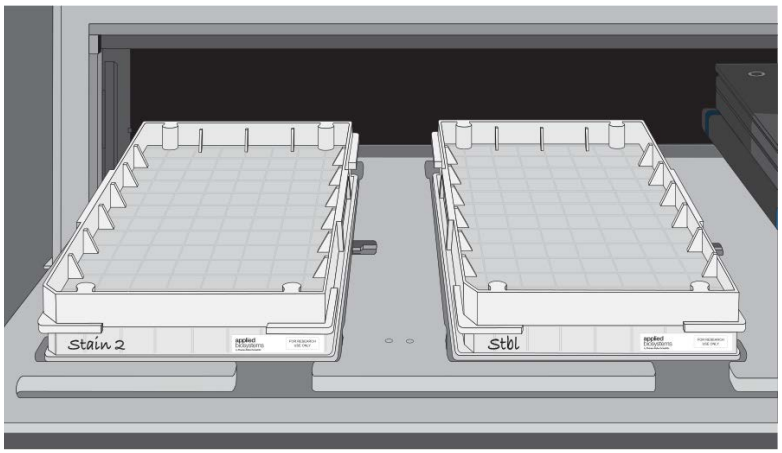


Figure 44 Stain 2 Tray and Stbl Tray loaded in Drawer 4.

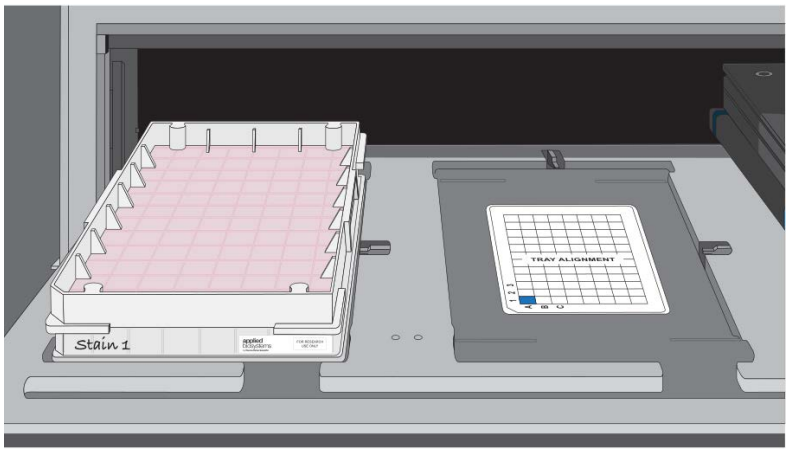


Figure 45 Stain 1 Tray loaded in Drawer 5.

3. At the prompt shown in Figure 46, click *Yes* to load another Axiom array plate and hybridization tray.

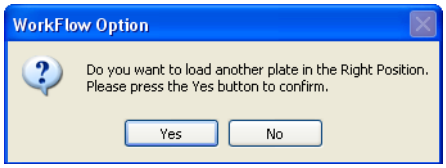


Figure 46 Prompt asking to load another plate. Right or left position determined by the position of Axiom array plates already in the GeneTitan MC Instrument.

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 hybridization tray on the right.
      - Press the **Confirmation** button.
   e. Click **OK** when prompted (Figure 47).

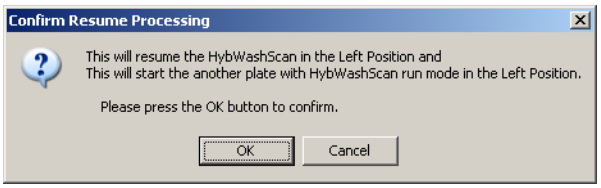


Figure 47 Confirm Resume Processing message.

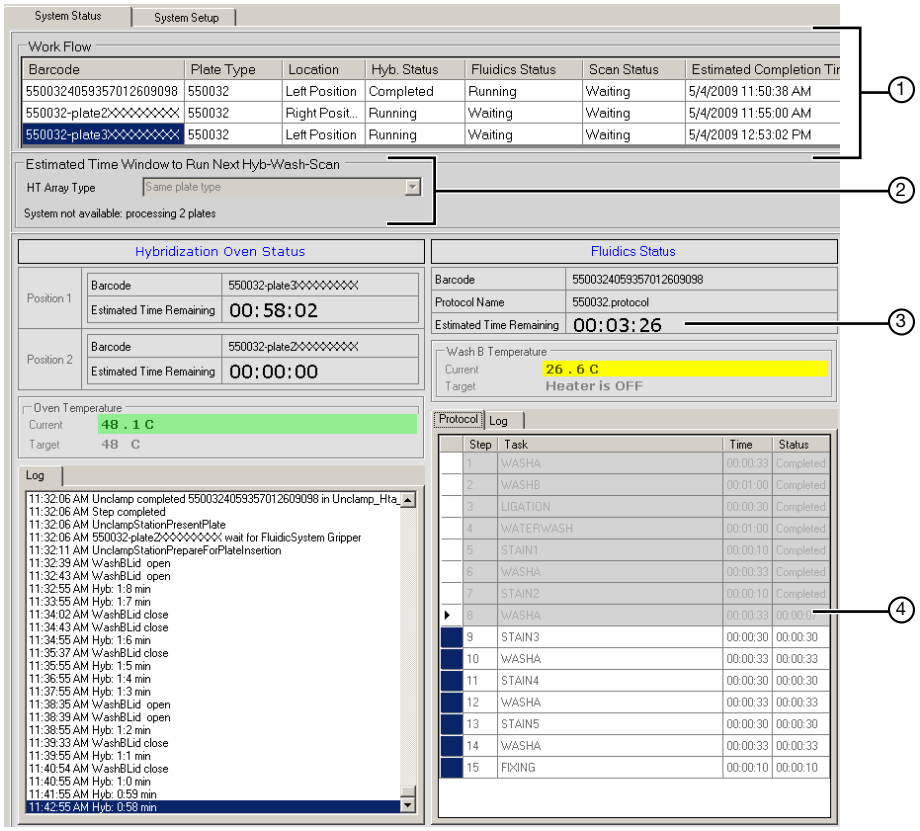
   f. When drawer 6 opens, confirm that the plate stack is securely clamped, then press the **Confirmation** button.

When processing resumes:

1. The plate stack which has finished hybridization is moved from the Hyb oven to drawer 1 temporarily and then moved to the unclamp station after step 2 (it remains clamped).
2. The plate stack in drawer 6 is moved to the Hyb oven.
3. The plate is moved to the unclamped station.
4. The plate stack in the unclamp area is unclamped and moved into the fluidics area.

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

Figure 48 is an example of how the System Status Workflow window will appear when 3 Axiom array plates are being processed.



① **Workflow** indicates the number of plates being processed and where they are in the instrument. In this example, 3 array plates are being processed: 2 in the hybridization oven and 1 in fluidics.
Estimated Completion Time is for the current process.

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

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

④ Step currently executing in fluidics.

Figure 48 Example of the System Status window — 3 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 49 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 GCC transfer utility, move any data associated with the current plate that is being processed/scanned. Transferring data will dramatically slow scanning and can cause the computer to freeze.

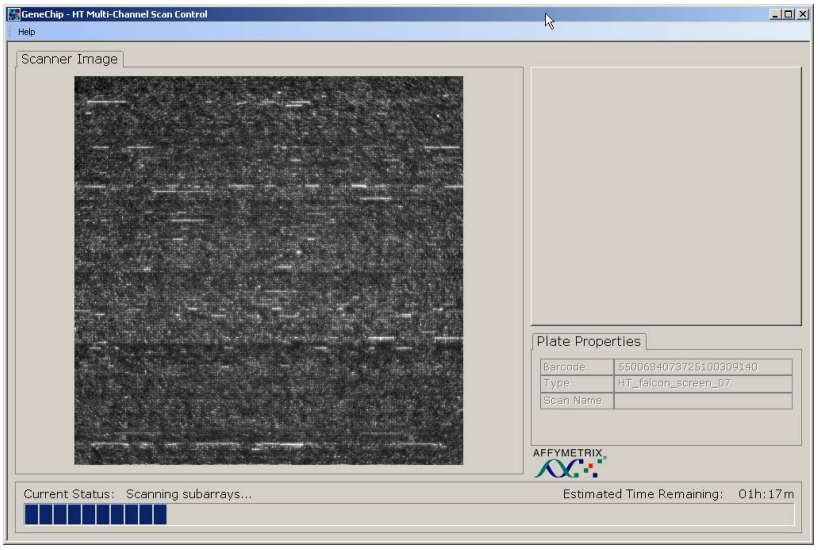


Figure 49 Scan Control window.

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.

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 GCC 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 Process 3 Axiom™ array plates per week

When using the manual target preparation protocol, 1 person can process up to 3 Axiom 96-array plates in one 40-hour work week.

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

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

The 3-plate per week workflow is described in the following sections:

- "Overview of the 3-plate workflow for manual target preparation"
- "Thaw frozen Amplification Plates" on page 126
- "Manual target preparation and array processing" on page 126

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

- Chapter 4, "Manual target preparation" on page 40
- Chapter 5, "Array processing with the GeneTitan™ MC Instrument" on page 85

Overview of the 3-plate workflow for manual target preparation

The table below displays the timing and duration of the hands-on processing necessary for performing the 3 plate workflow by 1 person.

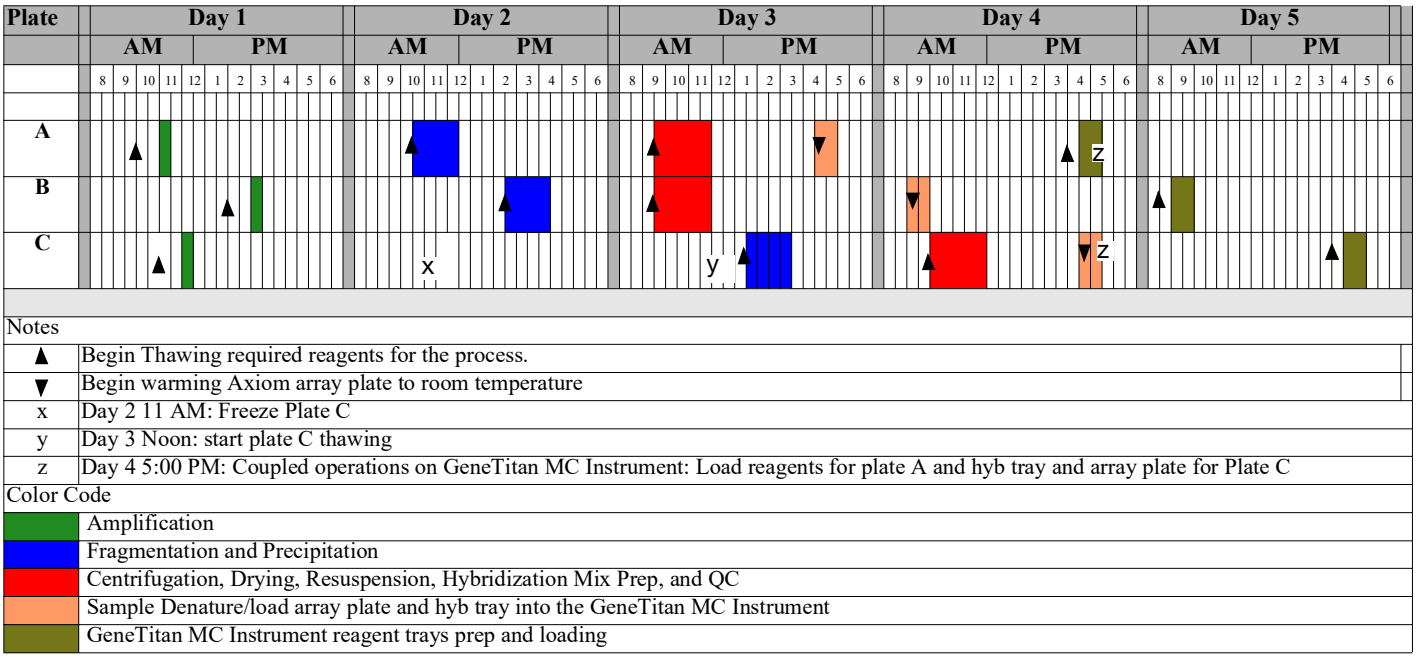


Figure 50 Three plate per week manual target preparation workflow.

The 3 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 3 plates during a 40-hour week, the steps must be performed in the order and with the timing described in this chapter.

Table 50 Daily steps for manual target preparation workflow.

Day	Activities	Plates
1	• Process 3 plates of genomic DNA for whole genome amplification.	A, B, and C
2	• Fragment and precipitate 2 plates amplified on Day 1. • Freeze 1 plate of amplified DNA for fragmentation later in the week.	• A, B • C
3	• Fragment and precipitate 1 plate. • Centrifuge, dry, resuspend and QC 2 plates precipitated on Day 2. • Denature and begin hybridization for 1 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 2 plates on the GeneTitan MC Instrument • GeneTitan reagent trays preparation and loading	• C • B, C • A
5	• GeneTitan reagent trays preparation and loading	• B, C

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 124
- "Timing issues for GeneTitan MC array processing" on page 125

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 51 Time required for manual target preparation.

Manual preparation	Hands-on time required	Total preparation time*	Incubation/hybridization/ processing
DNA Amplification	0.5 hour	1.5 hours	23±1 hour
Fragmentation and Precipitation preparation	2 hours	2 hours	Overnight Precipitation
Resuspension and Hybridization Mix preparation	25 minutes	25 minutes	N/A
Centrifugation/Drying	30 minutes	1 hour 20 minutes	N/A
Frag Gel QC and OD	45 minutes	45 minutes	N/A
Denaturation and hybridization tray/array plate loading on the GeneTitan MC Instrument	25 minutes	45 minutes	23.5–24 hour hybridization
GeneTitan reagent tray preparation and loading on the GeneTitan MC Instrument	1 hour	1.5 hours	Additional time for processing: 96 arrays: ~8.5—10.5 hr.
* 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 3 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 on the GeneTitan MC Instrument is 23.5 to 24 hours (Table 52). This provides a 30-minute window during which you are prompted by the instrument control software to load the reagents required for washing and staining.

Table 52 Time required for array plate processing on the GeneTitan MC Instrument.

Steps on the GeneTitan MC Instrument	Time required
Hybridization of 2 plates in 1 day • First plate loaded at 9:30 a.m. • Second plate loaded at 5:00 p.m.	23.5 hours each plate
Loading reagent trays	15 minutes
Fluidics	5 hours each plate
Imaging	96 arrays: 5.5 hours expected for GeneTitan MC or 3.5 hours expected for GeneTitan MC Fast Scan

Changing oven temperatures for the 3-plate Workflow

Multiple ovens are required for manual target preparation. If you are running the 3 plate/week workflow, 3 ovens are recommended. Table 53 lists the different temperatures required for each step. Though only 2 ovens are strictly required, we recommend maintaining separate 37°C ovens for the amplification and fragmentation stages to avoid confusion of plates and to minimize excess opening and closing of oven doors during incubation periods. Table 54 provides a list of suggested settings for 3 ovens when performing the 3 plate/week workflow.

Table 53 Oven temperatures needed for each step of the workflow.

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

Table 54 Suggested settings for ovens when performing 3-plate/week manual target preparation workflow.

Day of Workflow	Oven 1	Oven 2	Oven 3
Day 1	37°C	N/A	N/A
Day 2	3°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

Thaw frozen Amplification Plates

1. Place the frozen Amplification Plate in a small water bath.

   For example, pour ultra-pure water into a small tray. Place the frozen plate in the water in the tray.
2. Leave the plate in the water bath for ~50 minutes until all wells have thawed.
3. Centrifuge at 1,000 rpm for 30 seconds.
4. To avoid cross-contamination of wells during vortexing:
   a. Remove the seal and blot the top of the plate with a laboratory tissue.
   b. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 seconds to thoroughly mix (see guidelines described in "Guidelines for handling plates and reagents" on page 35).
6. Centrifuge at 1,000 rpm for 30 seconds.

Manual target preparation and array processing

Manual target preparation workflow—Day 1

On this day you start whole genome amplification of the 3 plates: each plate must incubate at 37°C for 23 ±1 hour.

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

Begin thawing the Module 1 reagents, particularly the Axiom 2.0 Amp Soln, 60 minutes before 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 32 for more information.

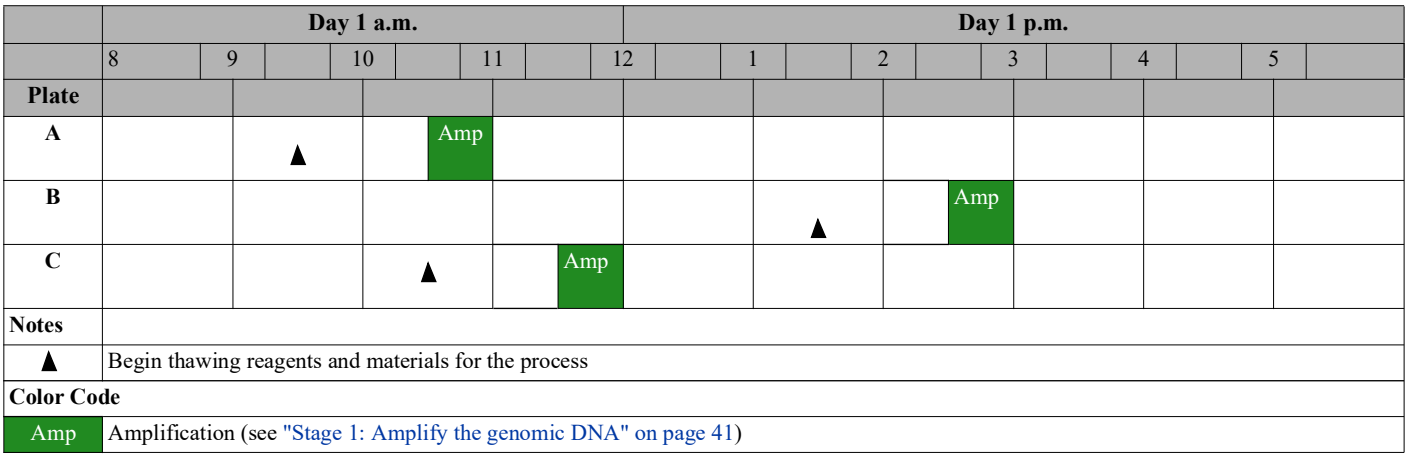


Figure 51 Manual target preparation workflow—Day 1 activities

Table 55 Manual target preparation workflow—Day 1 activities

Activity	Plate ID	Approximate Start Times*
DNA Amplification	A	9:30 a.m.
DNA Amplification	C	10:30 a.m.
DNA Amplification	B	1:30 p.m.
*Approximate start time indicates start of thawing of reagents		

See "Stage 1: Amplify the genomic DNA" on page 41 for more information on the protocol.

Manual target preparation workflow—Day 2

The tables below show the steps that need to be performed on the second day.

Plates A and B are fragmented and precipitated on Day 2 without freezing to preserve a 23 hours amplification incubation.

Precipitation is carried out at –20°C overnight.

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

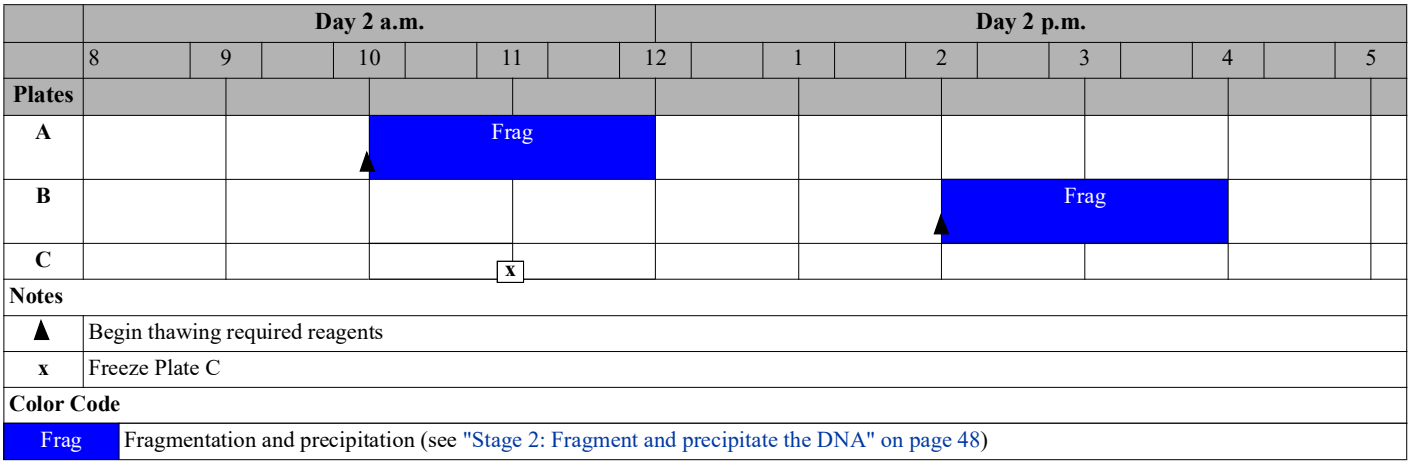


Figure 52 Manual target preparation workflow—Day 2 activities.

Table 56 Manual target preparation workflow—Day 2 activities.

Activity	Plate ID	Approximate start times
Fragment and precipitate	A	10:00 a.m.
Freeze (–20°C)	C	11:00 a.m.
Fragment and precipitate	B	2:00 p.m.

Manual target preparation workflow—Day 3

- Centrifuge, dry, resuspend and QC Plates A and B.
- Thaw Plate C (see "Thaw frozen Amplification Plates" on page 126).
- Fragment (including the 65°C Stop Amplification Reaction step) and precipitate Plate C.
- Perform Denaturation on Plate A.
- Transfer Plate A samples to Hybridization Tray A
- Load Hybridization Tray A and array plate into GeneTitan MC Instrument and begin hybridization.

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

IMPORTANT! Amplification Plates that are frozen must be thawed and thoroughly mixed by following the procedure under "Thaw frozen Amplification Plates" on page 126.

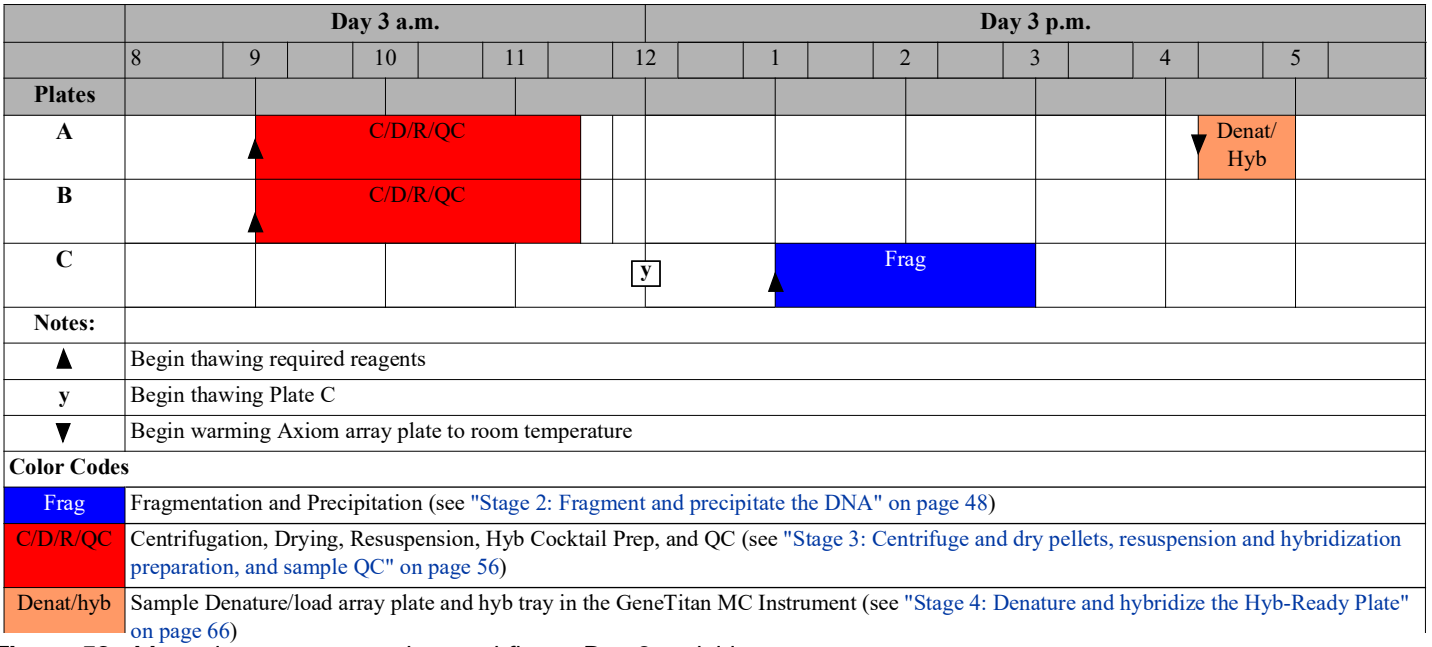


Figure 53 Manual target preparation workflow—Day 3 activities.

Table 57 Manual target preparation workflow—Day 3 activities.

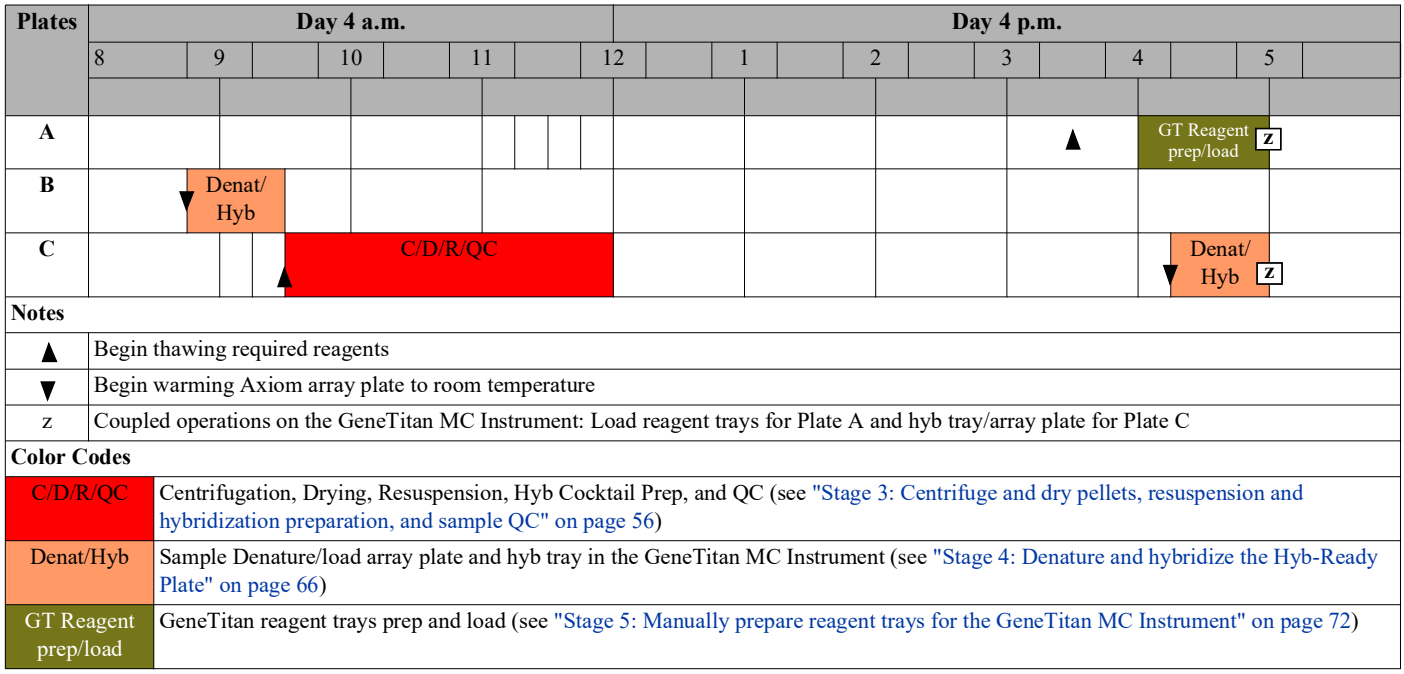
Activity	Plate ID	Approximate start times
Centrifuge/Dry/Resuspend/QC	A, B	9:00 a.m.
Thaw Plate C	C	12:00 a.m.
Fragment and precipitate	C	1:00 p.m.
Denature and Hybridization	A	4:00 p.m.

Manual target preparation workflow—Day 4

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

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

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



Notes	
▲	Begin thawing required reagents
▼	Begin warming Axiom array plate to room temperature
z	Coupled operations on the GeneTitan MC Instrument: Load reagent trays for Plate A and hyb tray/array plate for Plate C

Color Codes	
C/D/R/QC	Centrifugation, Drying, Resuspension, Hyb Cocktail Prep, and QC (see "Stage 3: Centrifuge and dry pellets, resuspension and hybridization preparation, and sample QC" on page 56)
Denat/Hyb	Sample Denature/load array plate and hyb tray in the GeneTitan MC Instrument (see "Stage 4: Denature and hybridize the Hyb-Ready Plate" on page 66)
GT Reagent prep/load	GeneTitan reagent trays prep and load (see "Stage 5: Manually prepare reagent trays for the GeneTitan MC Instrument" on page 72)

Figure 54 Manual target preparation workflow—Day 4 activities.

Table 58 Manual target preparation workflow—Day 4 activities.

Activity	Plate ID	Approximate start times
Denature and Hyb	B	8:45 a.m.
Centrifugation/Drying/Resuspension/QC	C	9:30 a.m.
GeneTitan reagent preparation and loading	A	3:30 p.m.
Denature and Hybridization	C	4:15 p.m.

Manual target preparation workflow—Day 5

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

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

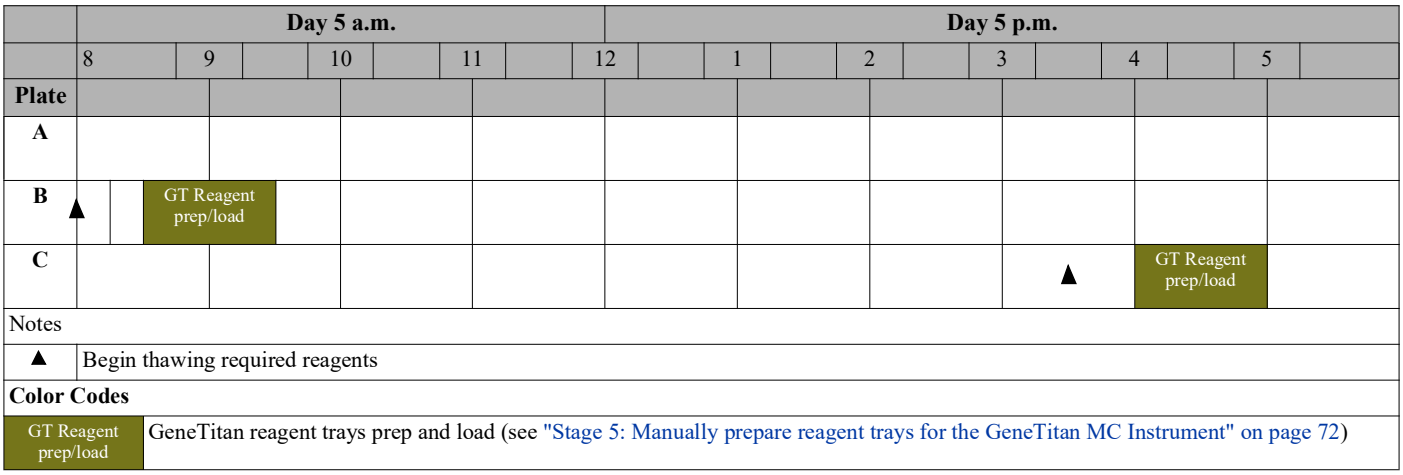


Figure 55 Manual target preparation workflow—Day 5 activities.

Table 59 Manual target preparation workflow—Day 5 activities.

Activity	Plate ID	Approximate start times
GeneTitan reagent tray preparation and loading	B	8:00 a.m.
GeneTitan reagent tray preparation and loading	C	3:30 p.m.

7 Troubleshooting

GeneTitan Multi-Channel Instrument

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

Table 60 GeneTitan Multi-Channel Instrument troubleshooting guidelines for the Axiom™ 2.0 Assay.

Problem	Possible causes	Possible actions
Plate trapped in GeneTitan Multi-Channel Instrument.	• Plate (or plate with lid) not properly loaded in drawer. • Cut edge of lid and plate not aligned. • Gripper failed to retrieve plate. • System requires adjustment.	1. Restart the GeneTitan Multi-Channel Instrument. 2. Run the setup option *Unload Plates* 3. If the plate remains trapped in the instrument, call Thermo Fisher Scientific support.
Computer frozen.	• Too many processes running • Attempting to transfer data while an array plate is being scanned (imaged).	Restart the computer and unload all of the plates. • Plates in Hyb station: finish hybridization off-line. • Plate in Scanner: rescan using Scan Only function • Plate in Fluidics: use Wash/Scan Resume to resume the fluidics process **Do not manually, or through the GCC transfer utility, move any data associated with the current plate that is being processed/ scanned.**
Hybridization aborted: • System-initiated abort • User-initiated abort	System-initiated abort: • Power loss	Array plate and hybridization tray are still clamped: • Contact your local field service engineer with information on the workstation model • The plate stack is moved to drawer 1. • Remove the plate stack and finish hybridization offline. • Return the hybridized array plate to the GeneTitan Multi-Channel Instrument and finish processing using the Wash/Scan process.
FAILED messages	See "Failed messages" on page 135	

Table 60 GeneTitan Multi-Channel Instrument troubleshooting guidelines for the Axiom™ 2.0 Assay.

Problem	Possible causes	Possible actions
FLUIDIC DIAGNOSTIC messages	See "Fluidic diagnostic messages" on page 135.	
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 140.

Miscellaneous messages

Table 61 Miscellaneous messages and recommended actions.

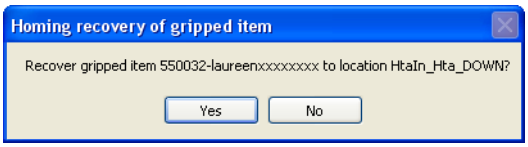
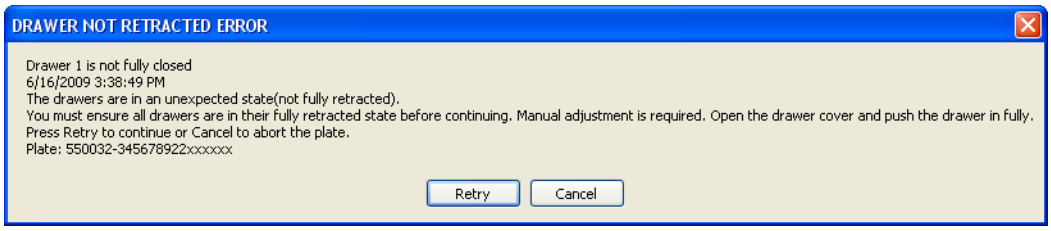
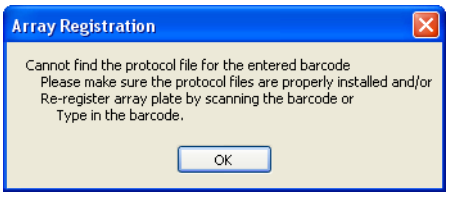
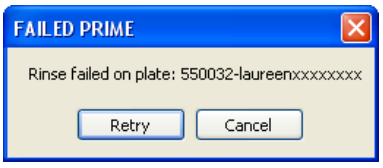
Message and recommended action	
 Indicates that an item is in the gripper, and normal startup of the GeneTitan Multi-Channel Instrument is not possible. The item must be removed from the instrument before you can begin processing array plates.	Recommendation: click Yes. If you click No, nothing will occur. Homing will not complete and you not be able to use the system. The item held by the gripper will be moved to either: • Drawer 2 — plates and trays • Trash Bin — covers The drawer names will reflect the location (left or Right) and the drawer number (1 through 6). Examples: Drawer2L_Hta_DOWN = Scan Tray on left side of drawer 2 HtaHyb = Clamped Hybridization Tray and Array Plate Drawer(n)L/R_Hta_DOWN where n is the drawer number and L or R to indicate the left or right side. The _Hta_ (second term) indicates the item held. An example is drawer1R_HtaHyb_DOWN indicating it is an array plate with a Hybridization Tray or Drawer2L_ScanHta_Pk_DOWN indicating it is an array plate with a scan tray

Table 61 Miscellaneous messages and recommended actions.

Message and recommended action	
 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 2 stop positions with audible clicks; push until you hear the second click and the drawer is fully seated.	
	• Check that the array plate barcode has been entered correctly. • Ensure that the library files required for the type of array plate you are using have been installed, and are installed in the correct directory. • Restart the GeneTitan MC instrument control software after library files have been installed.

Failed messages

Problem and possible causes	
 Rinse bottle — fluid level too low or bottle empty.	If this message is displayed: • during a water wash step, array processing has been compromised. • during cleanup, array processing is OK, but cleanup will not be complete. Always ensure that the GeneTitan bottles containing Wash A and Rinse are above the 50% mark when setting up the system to process an Axiom HT array plate. All 600 mL of the Wash buffer B from the Axiom reagent kit should be emptied into the GeneTitan Wash B bottle when setting up the system to process a plate. This ensures that the GeneTitan Wash B bottle is filled to more than the requisite 35% of Wash B bottle volume.

Fluidic diagnostic messages

Table 62 Problem messages.

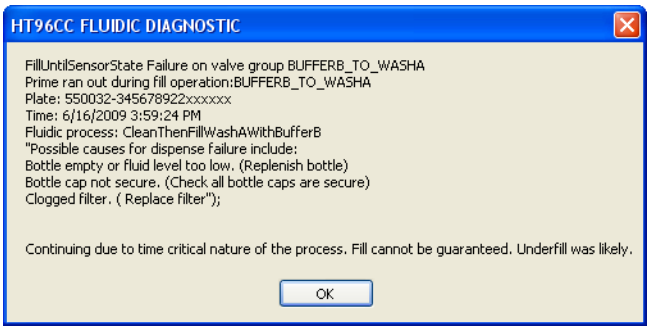
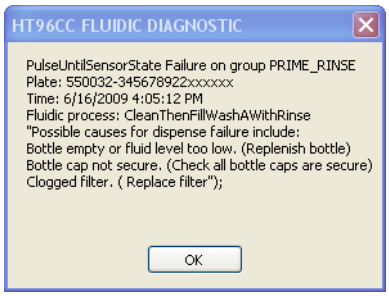
Problem and possible causes	
	About this message: • BUFFERX = Buffer bottle A, B or Rinse • WASHX = Wash A or B reservoir in the fluidics station. Recommended actions: • Replenish fluid level in the Rinse or Wash Bottle B to the 1-L mark. Do not overfill. – Only replenish bottles when prompted by the UI. Replenishing during fluidic processing can cause system malfunction including overflowing inside the system and more problems. The only thing to do while a plate is running is to ensure that bottle caps are secure. • Replenish fluid level in Wash Bottle A to 2 L. • Secure the bottle cap. • Replace the filter Instructions for filter replacement in the *GeneTitan™ Multi-Channel Instrument User Guide*, Pub. No. 08-0306. If the problem persists, call Thermo Fisher Scientific support.
	The typical cause is an unsecure bottle cap. If the failure is detected during priming, the instrument will pause and wait for the problem to be corrected. If the failure is detected during another process, and if the cause is a clogged filter, wait until the end of the run to replace the filter. Instructions for filter replacement in the *GeneTitan™ Multi-Channel Instrument User Guide*, Pub. No. 08-0306.

Table 62 Problem messages. (Continued)

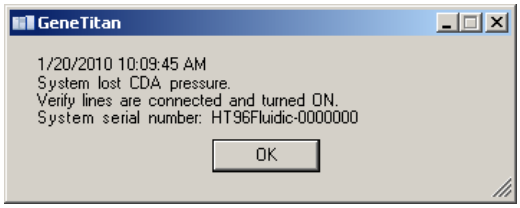
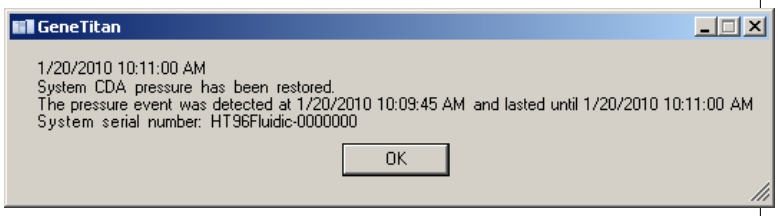
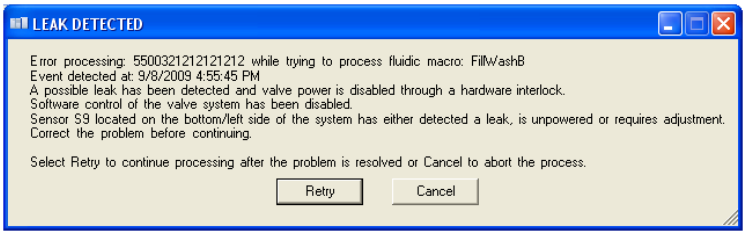
Problem and possible causes	
When the instrument experiences a loss in Clean Dry Air (CDA) pressure, the software will display the warning message.  When the pressure is detected again, a dialog message confirming the availability of CDA pressure is displayed. 	Possible Causes Verify that the facility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

Table 62 Problem messages. (Continued)

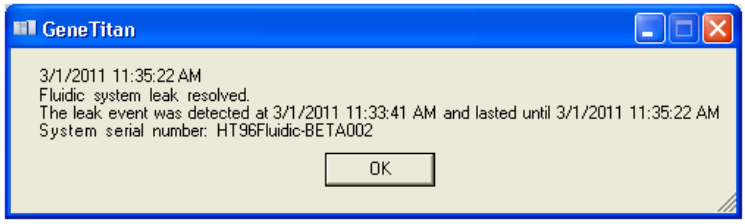
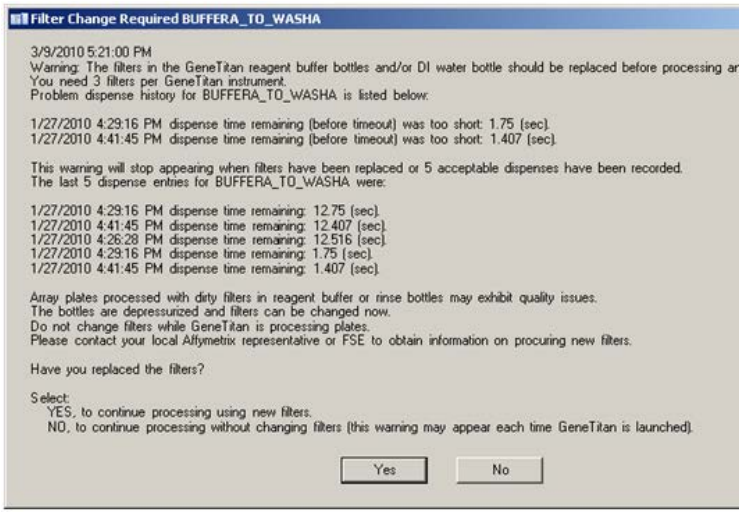
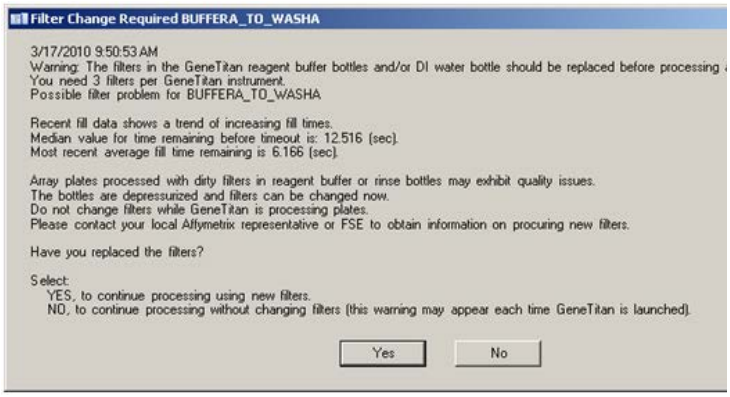
Problem and possible causes	
Leak Resolved 	This message is displayed when the leak is resolved (meaning the sensor LED is again lit up). If the original leak detected message was not acknowledged it will be automatically removed from the GUI and replaced by the following message. It will remain displayed until another leak is detected or the user acknowledges it by pressing OK. To resolve this issue complete the following tasks • Verify all internal and external tubing is connected and clean • Verify wash reservoirs are clean • Verify all bottle caps are secure and that no bottle cap is crimping a supply line. • Verify vacuum is working properly • Do not refill bottles or empty waste except when prompted to by the GeneTitan application. • Contact your facility group to ensure CDA is supplied to your GeneTitan system. Contact 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.

Table 62 Problem messages. (Continued)

Problem and possible causes	
Filter Error Messages   	The filters in the GeneTitan fluidics bottles (Wash A, Wash B and DI Water) need to be replaced when the filters are worn out. The software displays warning message boxes for the filter in each reagent bottle when it detects a problem or shows a trend of increased fill times during fluid fill operations. If an error is detected as described above, then a message box titled “Filter Change Required” is displayed along with the information on the specific dispense operation. You should change all 3 filters when a warning is displayed for any 1 of the 3 filters. See the section "Replacing the filter" on page 161 in Appendix E.

Wash/Scan Resume

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

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

- Viewing the System Status window if you are aborting the last plate through the fluidics system.
- Initiating the resume process.

1. System Setup tab: Select Wash/Scan Resume
2. Follow the prompts to unload and reload all drawers.

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

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

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

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

Resume step

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

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

Aborting a run

- Abort can take up to 3 minutes if a plate is in the Fluidics station. Status window Abort Requested changes to Abort Completed.
- Clamped Array-Plate -Hybridization Tray sandwich aborted from the oven or from drawerIN are moved to drawer 1.
- Proceed as follows:
  - Use the Unload Plates option to remove the aborted plate(s).
  - Start another run which will force an unload of the aborted plate(s)

System-initiated

- Power interruption
- Plate loaded incorrectly
- Equipment malfunction

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

User-initiated

Can abort processing of individual array plates.

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

Fragmentation quality control gel protocol

Fragmentation quality control gel protocol

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

Equipment Required

Table 63 Equipment required.

Item	Source
Gel imager	MLS
Pipette, multi- or single-channel P20	MLS
Plate centrifuge	MLS
Vortex	MLS

E-Gels and Reagents

Table 64 E-Gel and reagents required.

Item	Source
E-Gel™ Power Snap Plus Electrophoresis Device	G9110
iBright™ CL750 Imaging System	A44116
E-Gel® *48* 4% agarose gels	G8008-04
Applied Biosystems™ 25 bp DNA Ladder, or a similar product prepared as instructed by the manufacturer.	931343
TrackIt Cyan/Orange Loading Buffer	10482-028
UltraPure™ DNase/RNase-Free Distilled Water	10977023

Consumables

Table 65 Consumables required.

Item	Source
Adhesive film – use 1 of the following: • MicroAmp Clear Adhesive Film • Microseal 'B' Film	 4306311 Bio-Rad MSB1001
50-mL conical tube	MLS
Pipette tips (Same brand as pipette)	MLS

Dilute the TrackIt Cyan/Orange Loading Buffer

TrackIt Cyan-Orange Loading Buffer, Cat. No. 10482-028, is required for this procedure.

1. Prepare a 100-fold dilution by adding 500 µL of TrackIt Cyan/Orange Loading Buffer to 49.95 mL nuclease-free water.

   Total volume 50 mL.
2. Mix well.
3. Store at room temperature.

Dilute the 25 bp DNA Ladder

Applied Biosystems™ 25 bp DNA Ladder, Cat. No. 931343, is required for this procedure.

1. Prepare a 6-fold dilution by adding 25 µL of 25 bp DNA Ladder to 125 µL of UltraPure DNase/RNase-Free Distilled Water.
2. Mix well.
3. Store at 4°C until use.

Fragmentation QC Gel Protocol

This protocol is based on running QC gels for 96 samples.

To Run a Fragmentation QC Gel:

1. Tightly seal the gel QC plate.
2. Vortex the plate for 1 second each corner and 1 second in the center at the maximum setting, then centrifuge at 675 × g for up to 30 seconds to get the droplets down.
3. Take the gel out of the pouch and remove the combs.
4. Place the E-Gel® 48 gel into an electrophoresis unit.
5. Load 20 µL from each well of the gel QC plate onto the gels.
6. Load 15 µL of diluted TrackIt 25 bp ladder into the marker wells (M).
7. Load 20 µL nuclease-free water into any unused wells.
8. Run the gels for 19 minutes.
9. Take a gel image.

Fragmentation QC gel images should look similar to the gel shown in Figure 56.

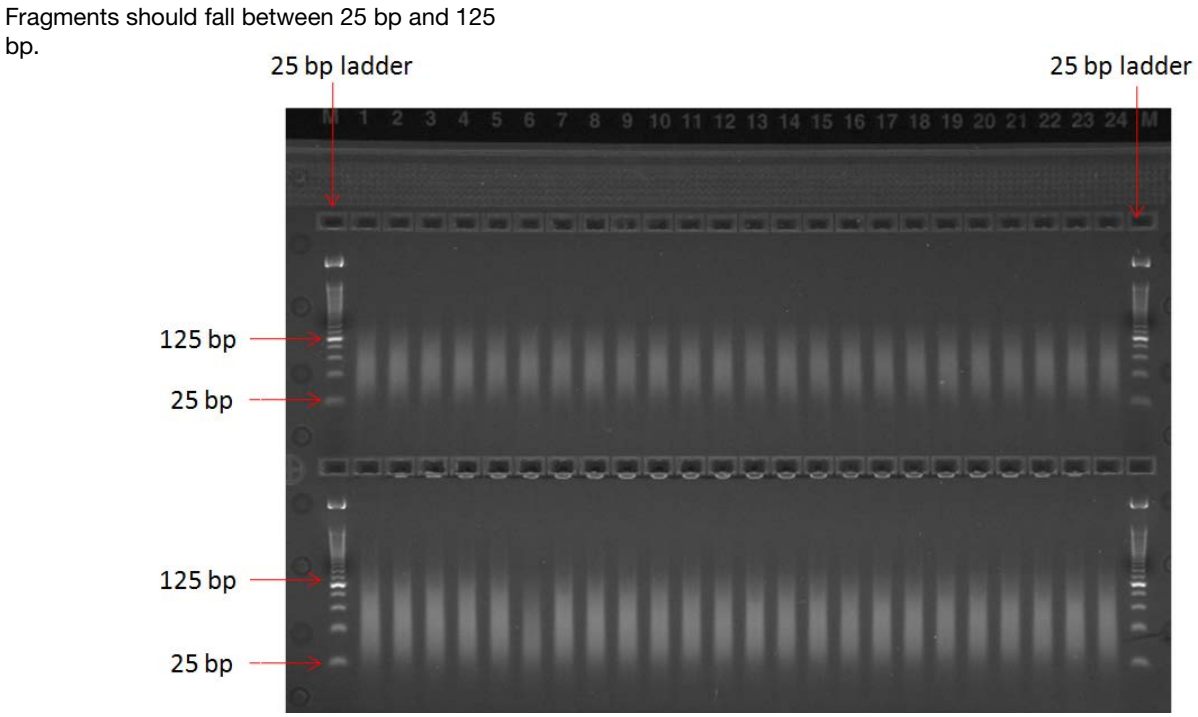


Figure 56 Example of a typical Fragmentation QC E-gel.

IMPORTANT! For Axiom™ Microbiome Array users: The hybridization-ready target derived from cDNA samples may not show fragmentation patterns due to low total mass. To assess general assay performance when cDNA samples are used, ensure that the fragmentation pattern of the Reference Genomic DNA 103 positive control sample has fragments falling between 25 bp and 125 bp.

B

Sample quantitation after resuspension

Equipment required

The following equipment is required for this protocol.
Unless otherwise indicated, all materials are available through thermofisher.com

Table 66 Equipment required for sample quantitation after resuspension.

Quantity	Item	Source
1	Multiskan™ SkyHigh Microplate Spectrophotometer	A51119500C

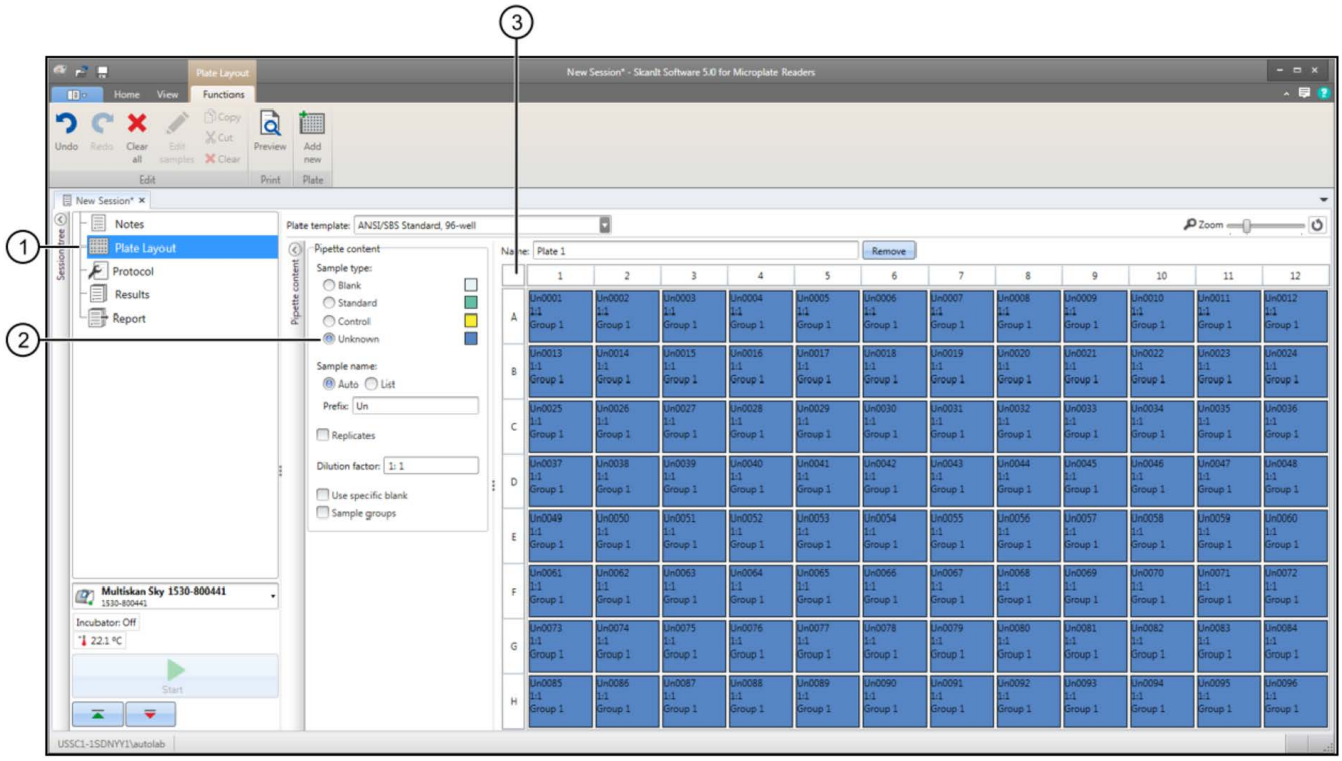
Quantify the diluted samples

During target preparation, 2 plates of diluted samples are prepared: 1 for OD quantification and 1 for a QC gel to check the fragmentation reaction.

For OD quantification, readings must be taken at wavelengths of 260 nm, 280 nm, and 320 nm. See "Plate reader guidelines for sample quantification" on page 151.

Install Axiom™ OD methods on the Multiskan™ Sky Microplate Spectrophotometer

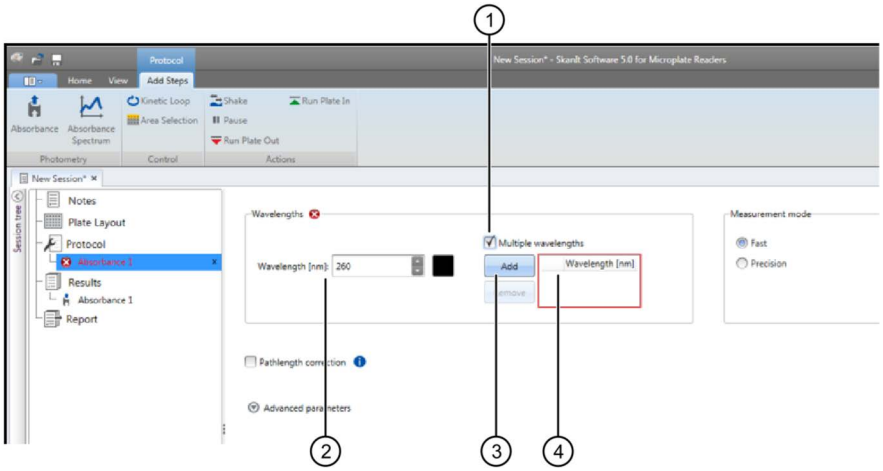
1. Launch the SkanIt™ software, then click **New session**.



① Plate Layout
② Unknown
③ Select small square to define all as unknown.

2. In **New Session** window, click **Plate Layout**, then select **Unknown**.
3. Cick to select the small square above the **A** and to the left of the **1** to assign all the wells as "Unknown".
4. Click **Protocol** in the session tree pane on the left, then click **Absorbance** under the menu bar.

5. Assign 260 nm, 280 nm, and 320-nm wavelengths to be measured.



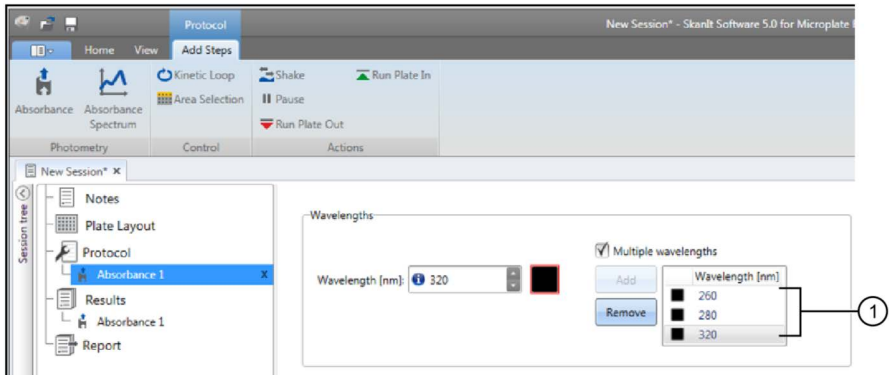
① **Multiple wavelengths** checkbox
② **Wavelength [nm]** field
③ **Add**
④ **Wavelengths [nm]** box

Figure 57 Assign wavelengths.

a. Check the **Multiple wavelengths** checkbox.
b. Enter "260" in the **Wavelength [nm]** field, then click **Add**.

   After clicking **Add**, 260 appears in the **Wavelengths [nm]** box in the middle of the screen.

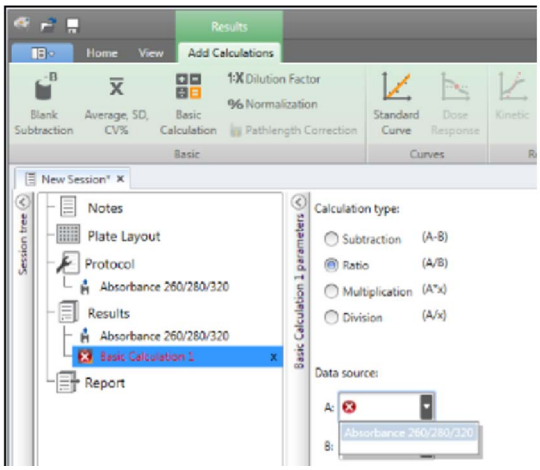
6. Repeat step 5 to add 280 nm and 320-nm wavelengths. When complete, 260, 280, and 320 appears in the **Wavelengths [nm]** box.



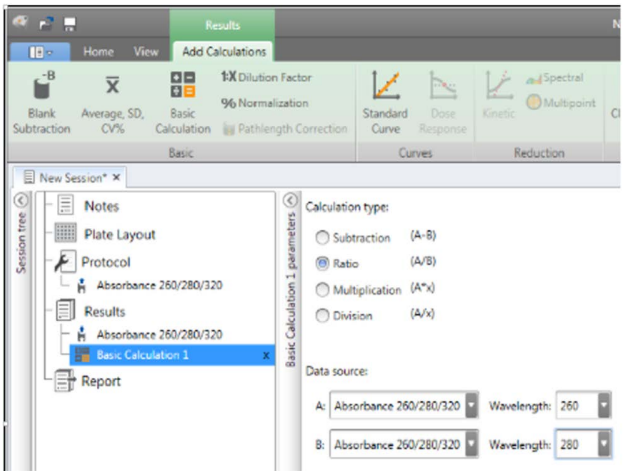
① Wavelengths added appear in **Wavelengths [nm]** box.

7. Click **Absorbance 1** in the session tree pane, then rename it "Absorbance 260/280/320".

8. Add a calculation to the **New Session**.



   a. Under **Results** in the session tree pane, click **Absorance 260/280/320**.
   b. Click **Basic Calculation** to calculate 260 nm/280 nm ratio for each sample, then select **Ratio (A/B)**.
   c. Define the **Data source**:
      - For **A**, select **Absorbance 260/280/320** and for **Wavelength** select **260**.
      - For **B**, select **Absorbance 260/280/320** and for **Wavelength** select **280**.



9. Click **Basic Calculations 1** in the left pane, then rename it "Ratio 260/280".
10. Define the calculation for the DNA yields in each well.
    a. Click the **Custom Formula** button under the menu bar, then click the **Define Variables** button in the middle pane.
    b. In the **Define Variables** window, define a variable that is named "A260".
       1) In the **Variable Name** field, enter "A260".
       2) In the **Source Steps** dropdown list, select **Absorbance 260/280/320**.
       3) For **Wavelengths**, select **260**, then press the **Add** button.

          After clicking the Add button, the new A260 variable and definition move to the right side of the **Define Variables** window.

c. Define a variable named "A320".

1) In the **Variable Name** field, enter "A320".
2) In the **Source Steps** dropdown list, select **Absorbance 260/280/320**.
3) For **Wavelengths**, select 320, then press the **Add** button.
4) After clicking the Add button, the new A320 variable and definition move to the right side of the **Define Variables** window. Click **OK** to close the window and return to the **Custom Formula** screen.

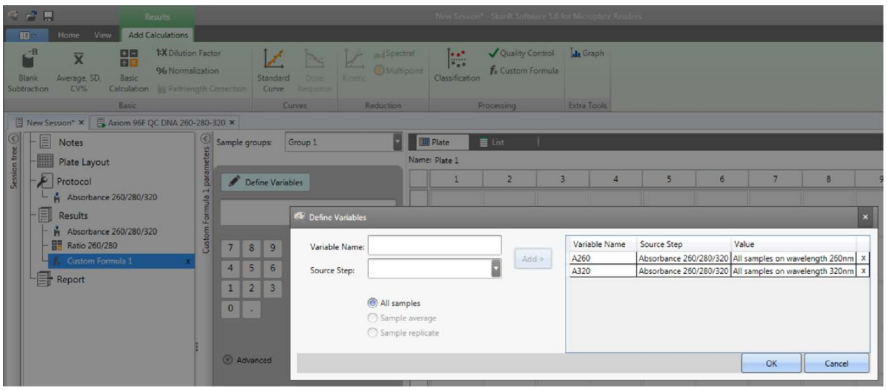
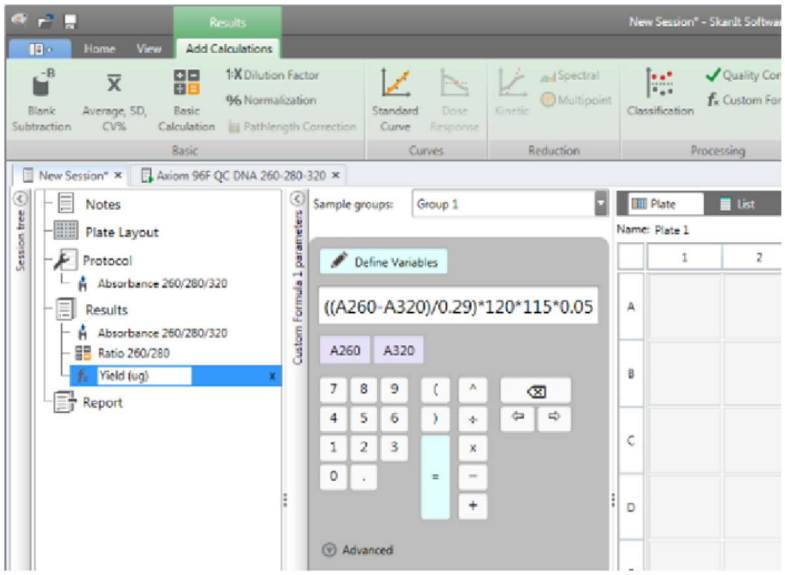


Figure 58 Define Variables window with 2 new variables added.

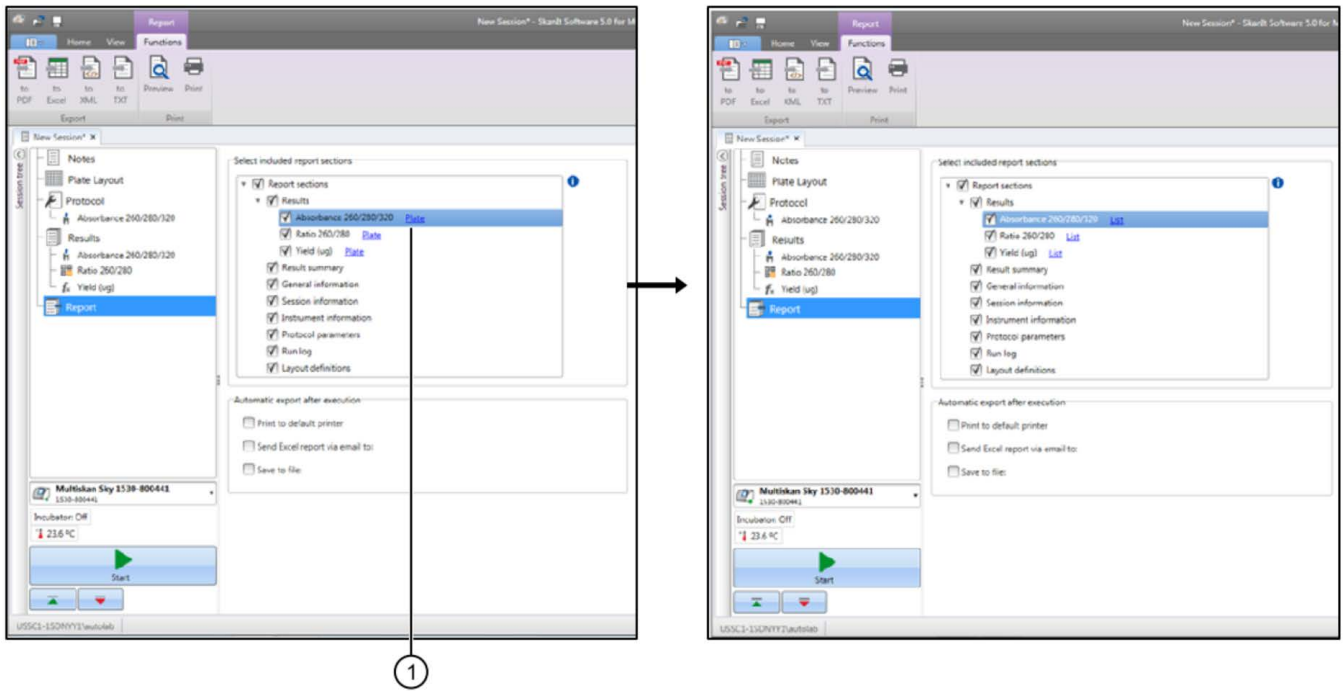
d. In the Custom Formula screen, enter the following DNA yield equation. Use the purple **A260** and **A320** buttons to enter them into the equation.

((A260-A320)/0.29)*120*115*0.05

e. In the left pane, click **Custom Formula 1**, then rename it "Yield (ug)".

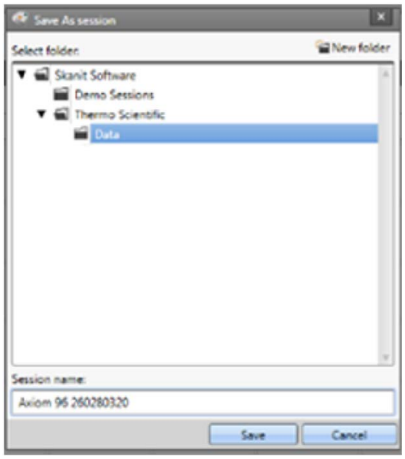


f. Click **Report** in the left pane. Results of the calculation are provided in either Plate or List format. Click **Plate** to change the results to **List** format.



① Click **Plate** to change the results to **List** format, if desired.

11. Click the **Home** tab, then click **Save**.
12. In the **Save As session** window, select or create a folder to save it to, and then enter a **Session name**.



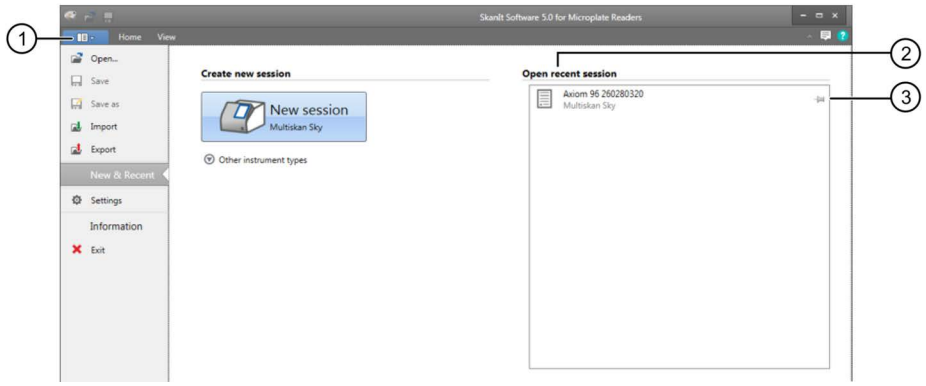
13. Click **Save**.

    The session is available to be used to read the Axiom™ 96-well OD QC Plates.

Use a Multiskan™ Sky session

See the *SkanIt™ Software for Microplate Readers User Manual*, Pub. No. N16243, for further details.

1. In the SkanIt™ software, click the file icon tab to the left of the **Home** tab in the upper left of the window.
2. Open a session using one of the following methods.
   - Click **Open** and navigate to the filepath of the session.
   - Click the session name in the **Open recent session** section on the right side of the window. The session can be pinned to the Open recent session window by clicking on the pin icon on the right.



① File icon tab
② Saved session list
③ Pin

OD yield evaluation guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the Axiom™ 2.0 Assay. If the median yield for the plate is <1,000 µg DNA per sample:

- Pause the protocol.
- Evaluate all steps that are performed to that point to determine the possible source of the low yields.

This DNA yield corresponds to an A_{260} value of approximately 0.49 and an A_{260}-A_{320} value of approximately 0.42.

IMPORTANT! Note for Axiom™ Microbiome Array users: The hybridization-ready target derived from cDNA samples for the Axiom Microbiome Array may be below 1,000 µg per sample. To assess general assay performance when cDNA samples are used, ensure that the OD yield of the Reference Genomic DNA 103 positive control sample is >1,000 µg per sample.

Plate reader guidelines for sample quantification

When performing sample quantification, the plate reader must be calibrated to ensure accurate readings.

The total yield in μg per well can be calculated as:

- (A - C)*D*V*E/P

where:

- A = the observed OD_{260}
- C = the observed OD_{320} (an estimate of a blank reading)
- D = 120 (the net dilution factor when preparing the OD sample plate)
- V = 115 (the volume of the sample in μL after the resuspension step)
- E = 0.05 (the extinction coefficient of duplex DNA at 260 nm)
- P = the optical path length for the plate type and plate reader used.

If your plate reader does not record the OD_{320}, the OD_{260} of a blank solution of water only must be used for the parameter "C".

C 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).

Create a GeneTitan Array Plate Registration File:

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

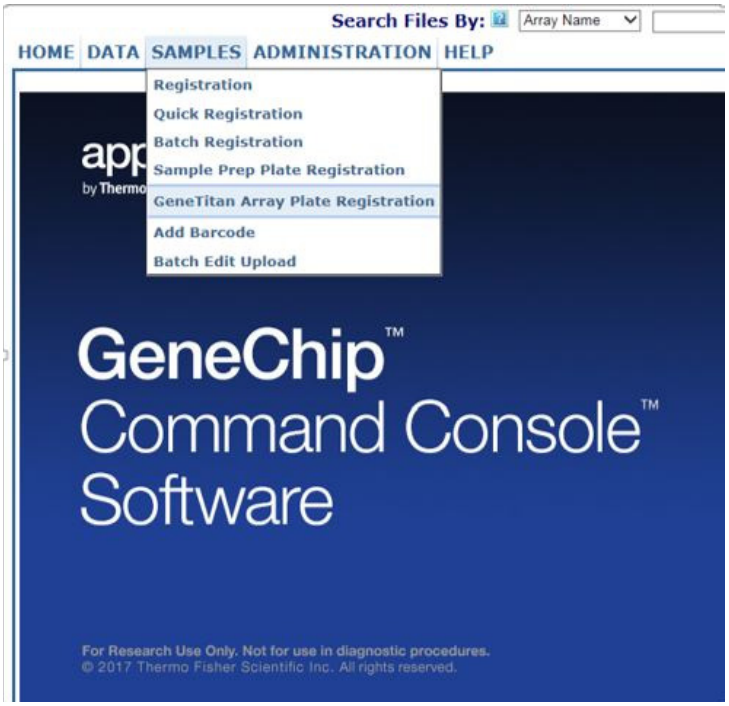


Figure 59 Selecting GeneTitan Array Plate Registration.

2. Step 1 — Figure 60 on page 153:
   a. Select the array plate type.
   b. Click **Download**.

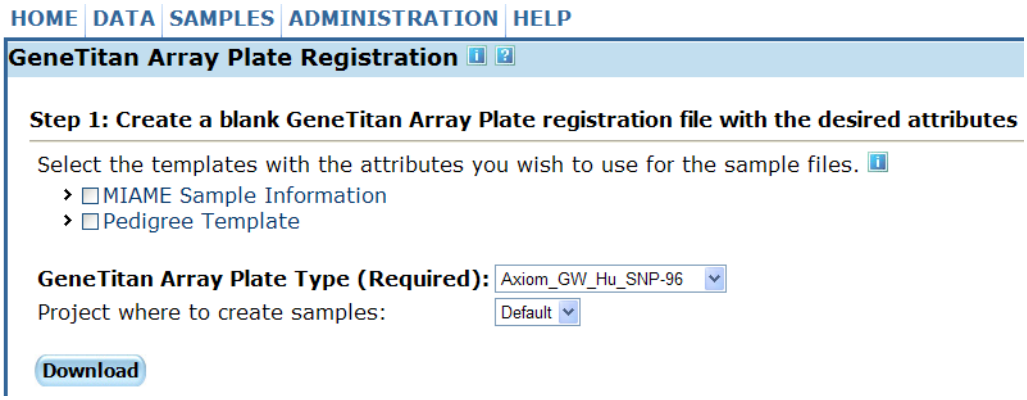
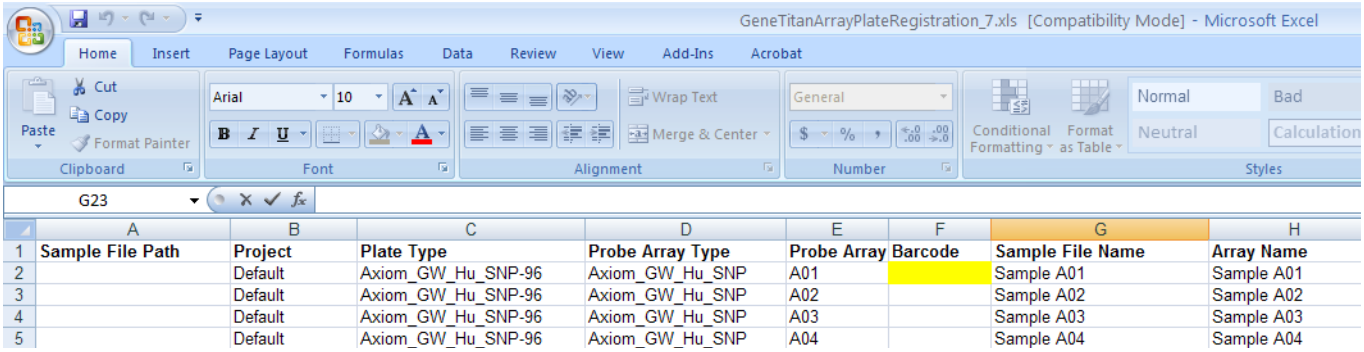


Figure 60 Selecting the type of array plate to be processed.

3. Step 2 — complete the registration file as follows:
   a. Click the Microsoft Excel box on the bottom bar of the monitor to open the Excel spreadsheet.
   b. Enter a unique name for each sample (Sample File Name) and any additional information you would like to include.
   c. Do one of the following:
      - If you are ready to load the array plate onto the GeneTitan MC Instrument, scan the array plate barcode and proceed to the next step.
      - If you are not ready to load the array plate onto the GeneTitan MC Instrument, proceed directly to the next step.



	A	B	C	D	E	F	G	H
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Array	Barcode	Sample File Name	Array Name
2		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A01		Sample A01	Sample A01
3		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A02		Sample A02	Sample A02
4		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A03		Sample A03	Sample A03
5		Default	Axiom_GW_Hu_SNP-96	Axiom_GW_Hu_SNP	A04		Sample A04	Sample A04

Figure 61 Entering sample information into a GeneTitan Array Plate Registration file.

4. Save the file as follows:
   a. Open **File** → **Save As**.
   b. Enter a name for the array plate registration file.
   c. Click **Save**.

   By default, the file is saved in the Affymetrix_Downloads folder.
5. Step 3 — when ready to load the array plate onto the GeneTitan MC Instrument:
   a. Click the **Browse** button, navigate to the file, and click **Open**.
   b. Scan the array plate barcode if not already scanned.

c. Click the **Upload** button (Figure 62), wait for the information to load, then click the **Save** button located at the *bottom* of the next page that is displayed.

   If the samples are successfully registered, the message in Figure 63 is displayed.

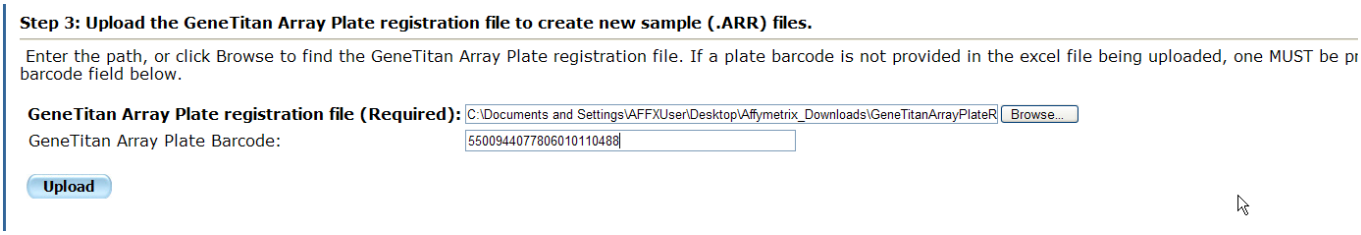


Figure 62 Uploading the array plate registration file to GCC.

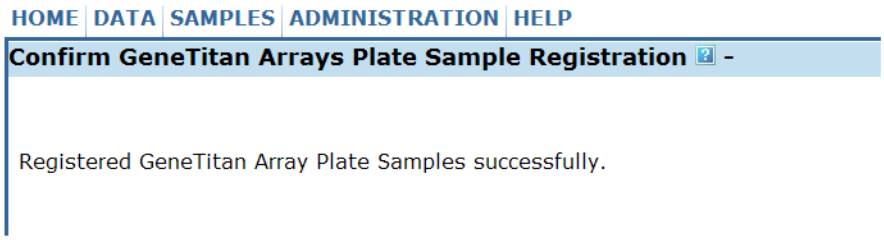


Figure 63 Array plate samples successfully registered.

Deionization procedure for GeneTitan trays and covers

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

IMPORTANT! Except for the Axiom™ array plates, scan tray and the hybridization tray, you must deionize all GeneTitan stain trays, stain tray covers and scan tray cover using an anti-static gun. You must do this before you fill the trays with reagents and before you place the covers on the trays. Deionization removes the static electricity. The presence of static electricity on the underside of the cover can cause the gripper to lift the tray along with the tray cover and can result in an aborted run. See Figure 64, Figure 65 and Figure 66.

Deionize the inner surface of each tray and cover:

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

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

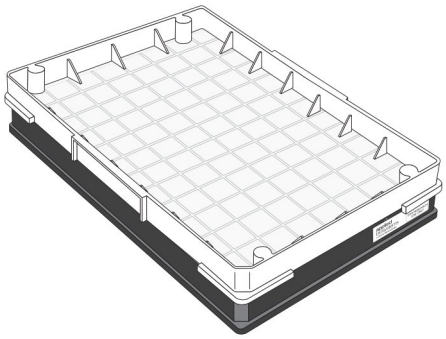


Figure 64 Scan tray with cover. Deionize only the cover.

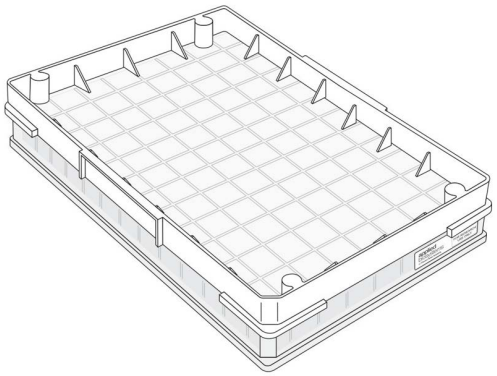


Figure 65 Stain tray with cover. Deionize the cover and the tray.

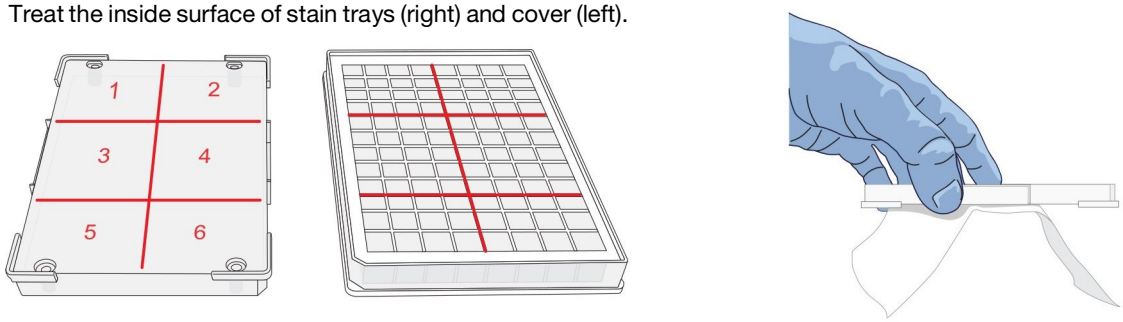
Deionization procedure

The following process provides guidance on how to use the anti-static gun on the stain and scan tray covers only. See Figure 66.

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

1. Treat the plate or cover as if it were divided into 6 sections (see Figure 66), and deionize as follows.
2. Place a laboratory tissue on the benchtop.
3. Place the stain tray on a table top. Use the anti-static gun to aim at the center of each of the 6 sections on a 96-well tray and pull the trigger. Ensure that a stream of ionized particles settles on all wells of the stain tray to dissipate the static electricity. Squeeze and release the trigger slowly 3 times over each section (Squeeze for approximately 2 seconds and release for approximately 2 seconds).
4. Place the stain tray cover with the flat surface facing upward on the tissue.
5. Aim the anti-static gun (Cat. No. 74-0014) approximately one-half inch away from the flat surface and pull the trigger. As you pull the trigger move the gun across the cover so that the stream of ionized particles settles on all areas of the cover and dissipates the static electricity. Squeeze and release the trigger slowly 3 times over each section (squeeze for approximately 2 seconds and release for approximately 2 seconds).
6. Place the treated cover or tray on the laboratory tissue and lift it up (see Figure 66).
7. Do one of the following:
   - If the tissue does not cling to the plastic, proceed with the protocol.
   - If the tissue still clings to the plastic, then perform steps 3 and 4 again. If it continues to cling to the plastic, test the device using the ion-indicator cap to confirm that the unit is still releasing ions. Otherwise, it may be time to replace the unit.

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



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

Figure 66 Removing the static charge from stain trays and covers.

Ion-indicator cap

The ion-indicator cap is a testing device used to verify the release of ions when the anti-static gun is in use (Cat. No. 74-0014, Figure 66).

Testing the Zerostat 3 with the ion-indicator cap

1. Insert the ion-indicator cap into the nose of the Zerostat and then slowly squeeze the release trigger (see Figure 67).

Ion-indicator cap

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

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

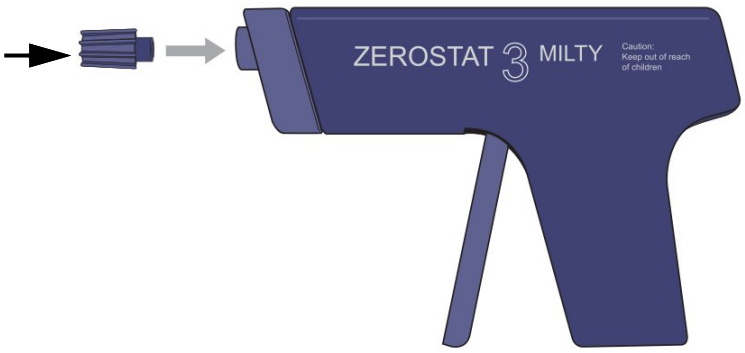


Figure 67 Zerostat 3 anti-static gun (Cat. No. 74-0014) with ion-indicator cap to test functionality.

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

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

GeneTitan™ Multi-Channel Instrument care

This chapter provides instructions on caring for and maintaining the instrument and on troubleshooting if problems arise.

- Always run a Shutdown protocol when the instrument will be off or unused overnight or longer. This will prevent salt crystals from forming within the Fluidics system.
- Always use deionized water to prevent contamination of the lines. 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 can damage the exterior surfaces.

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

Monthly

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

Every 6 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 68) should be cleaned at least every 90 days of service. Note that in some service locations, the presence of excessive dust or particulate matter can necessitate cleaning the cartridge more often than 90 days.

A plugged filter cartridge can cause excessive temperatures within the machine that can cause unwanted evaporation of GeneTitan reagents.

Part details for GeneTitan fan filter:

Thermo Fisher Scientific Cat. No. 01-0669

Number of filters required per GeneTitan instrument: 3

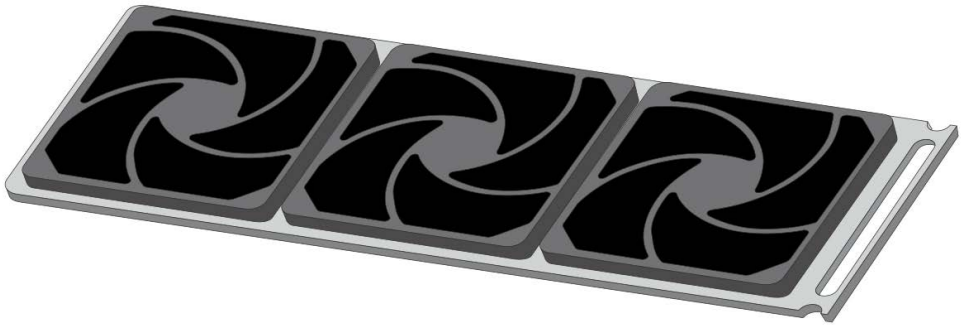


Figure 68 The GeneTitan filter cartridge

Cleaning procedure

1. Slide the filter cartridge from the fan filter cartridge at the rear of the GeneTitan MC Instrument.
2. Submerse in clean DI water. Rinse and agitate gently to dislodge material.
3. Remove from water and dry with clean compressed air or towels.
4. When the filter cartridge is completely dry to the touch, re-install the cartridge.

Replacing the bottle filters

The bottles used in GeneTitan 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 69 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 69) along with the information on the specific dispense operation. You should change all 3 filters when a warning is displayed for any 1 of the 3 filters.

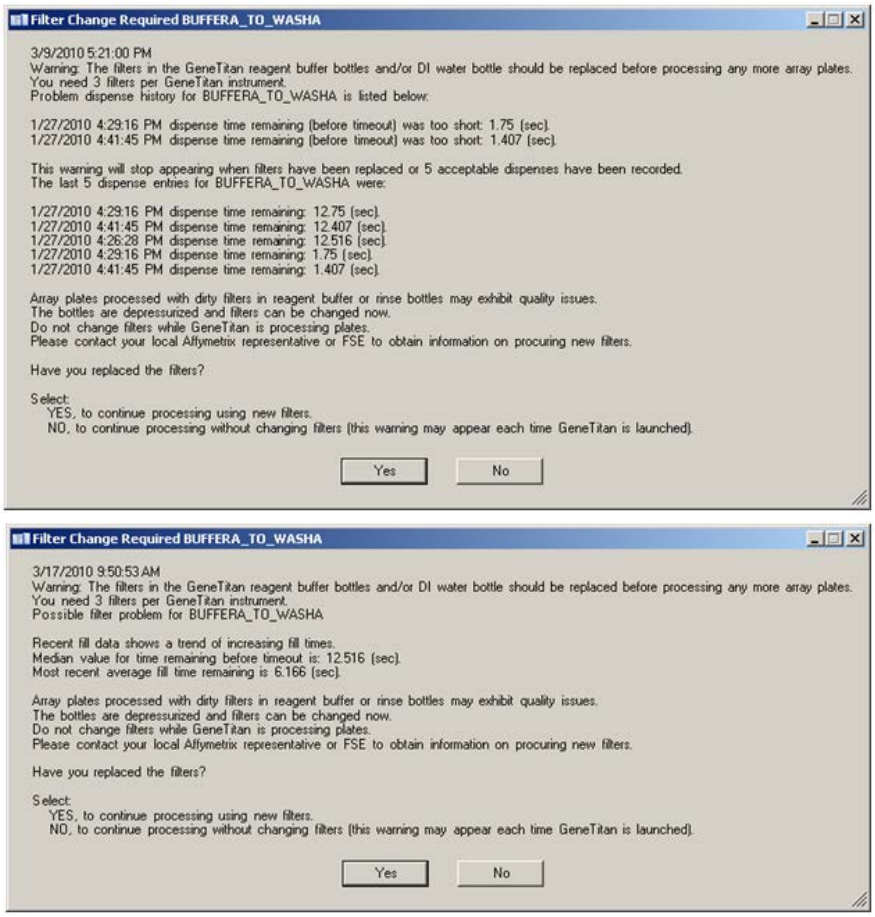


Figure 69 Filter Change Required messages

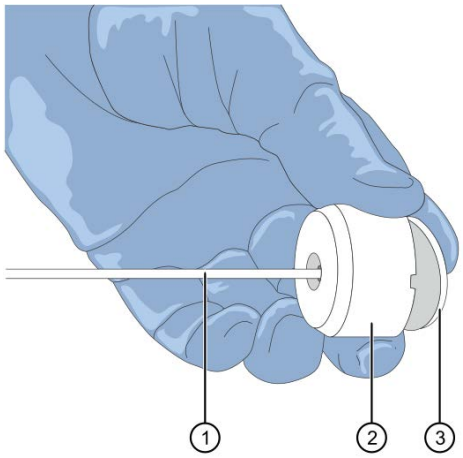
Note: The reagent bottles are depressurized when this warning message is displayed. It is safe to change the filters in all 3 fluidic bottles when this message is displayed.

After changing the filters in all 3 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 GCC instrument control software is launched. You can also experience data quality issues if particulate matter cannot be trapped by the filters because they are clogged.

We recommend that your site keep 3 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 70 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 1 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 1 filter at a time to ensure the correct connection of the buffer supply tube to its respective bottle. The color of the buffer supply tubing matches the bottle color code.

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.

GeneTitan xenon lamp catalog number: Thermo Fisher Scientific 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 71):

Figure 71 Lamp Life above tolerance

It displays a red or yellow notice when the lamp life is getting short (Figure 72):

Figure 72 Lamp Life above tolerance

It also displays a red notice when the Imaging Device is offline (Figure 73):

Figure 73 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 4 retaining bolts. They should be finger tight (Figure 74).

① Unscrew these 4 bolts.

Figure 74 Unscrewing the bolts.

2. Remove and set aside the warning cover to reveal the xeon 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 75). You must use both hands to remove the lamp successfully. Apply equal pressure on each side of the lamp and gently lift.

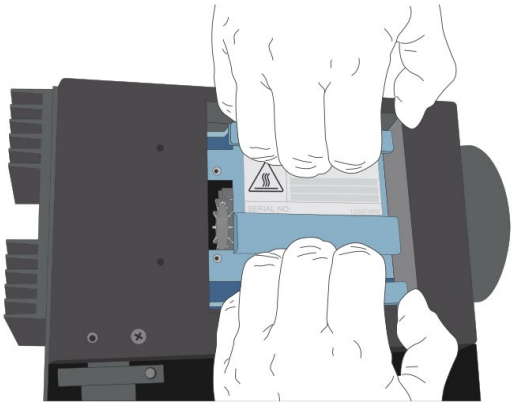
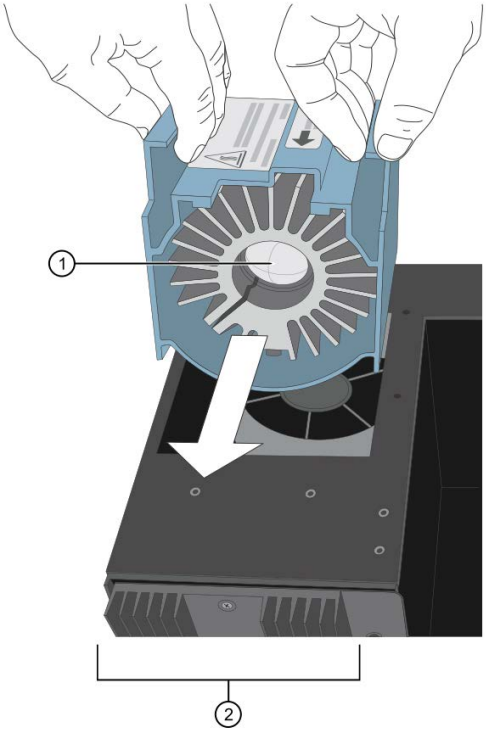


Figure 75 Lifting out the lamp.

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 and vertically insert the lamp (Figure 76).
2. Replace the warning cover and hand tighten the bolts (Figure 74).



① Xenon bulb faces away from the fan and towards the heat sink.
② Heat sink on the Lambda LS unit.

Figure 76 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 77).

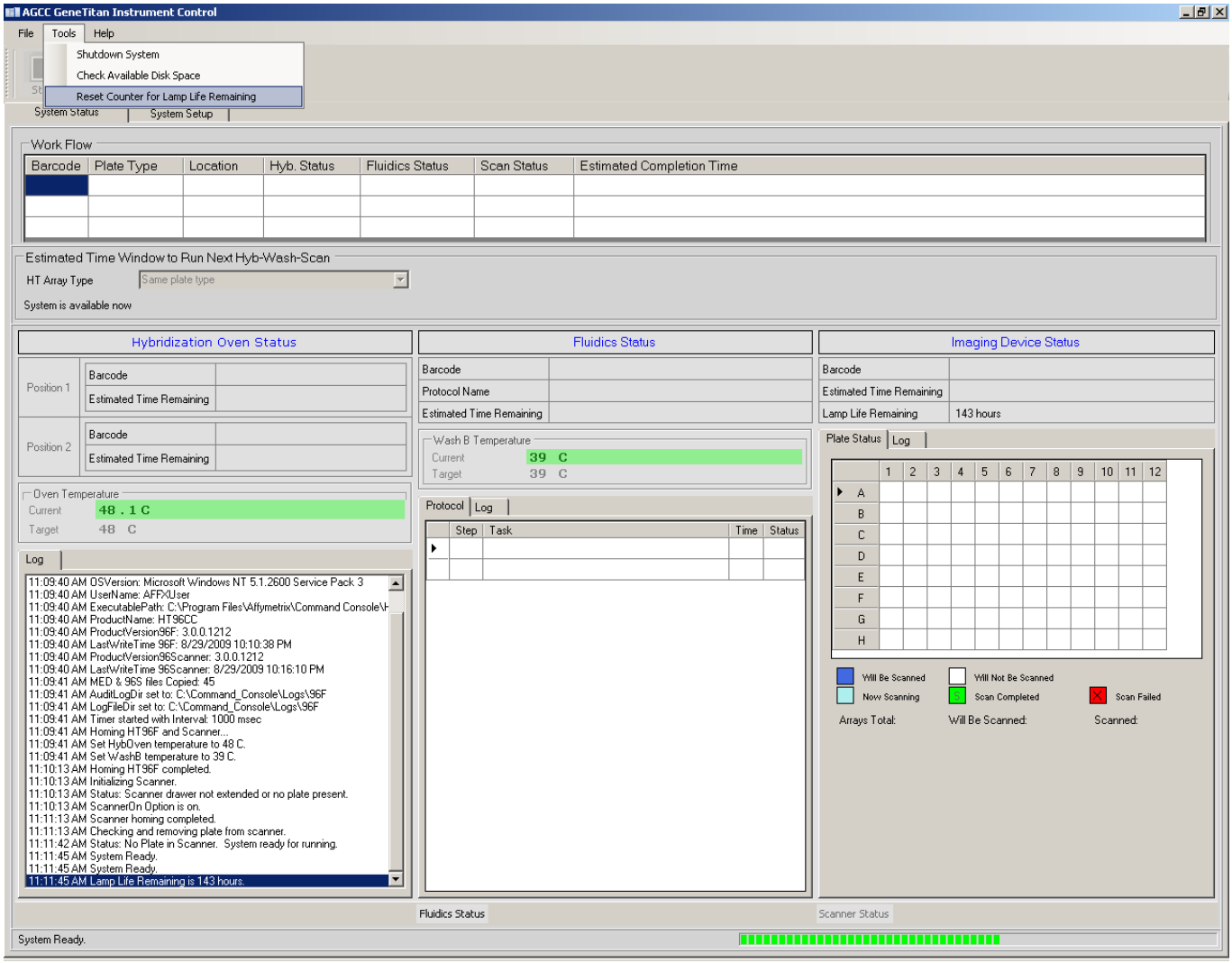


Figure 77 Inserting the lamp

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 78).

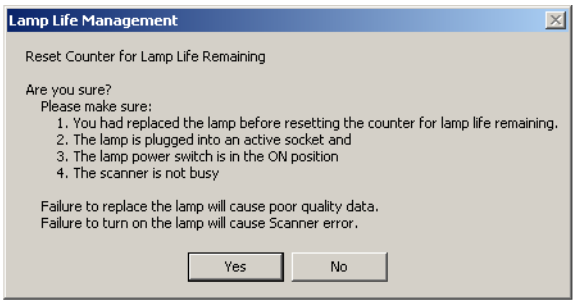


Figure 78 Are you sure?

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 79).

Figure 79 The counter is reset.

Troubleshooting

This section provides instructions on how to identify and solve simple problems with the GeneTitan MC Instrument. If a problem or error occurs that is not listed in this chapter contact Thermo Fisher Scientific Technical Support for assistance.

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

Log files

The log files are produced by different GCC components. The logs provide a record of the tasks performed by different components, such as the migration tools and installer. These log files provide useful information for troubleshooting problems. These files may be requested by your Field Application Scientist (FAS), Field Service Engineer (FSE), or the call center.

GCC log files

The following files are generated by the GeneTitan Instruments. All the GCC 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.
GCC_LibFileImporter. log (with date and time code)	Text file with info on use of the Library File Importer.

Other GCC files

Your FAS and/or FSE 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. GCC system configuration file located at C:\Command_Console\Configuration\Calvin.System.config
4. Pending job order files located in C:\Command_Console\Jobs
5. Other GCC related information, such as:
   a. The number of files under C:\Command_Console\Data, including sub directory.
   b. If the system is a networked system or a standalone system.
   c. Other applications installed on the system, such as antivirus application, MS Office, and Internet Explorer versions.

GCC log files for GeneTitan™ MC Instrument Systems

Log files for the GeneTitan MC Instrument control processes are placed in subdirectories of the C:\Command_Console\Logs\ folder. Thermo Fisher Scientific 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.

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 80) when:

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

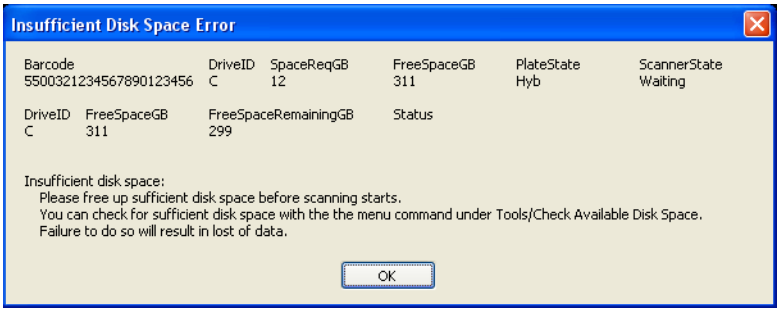


Figure 80 Insufficient disk space notice

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

Safety

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

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

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.

Biological hazard safety

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

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

Documentation and support

Related documentation

Document	Publication number	Description
Axiom™ 2.0 Assay Manual Workflow Site Preparation Guide	MAN0018132	Provides guidance on reagents, instruments, and supplies required to run the Axiom™ 2.0 Assay 96-Array Format Manual Workflow.
Axiom™ 2.0 Manual Target Preparation Protocol Quick Reference	MAN0018119	An abbreviated reference for the target preparation step of the Axiom™ 2.0 Assay 96-Array Format Manual Workflow. This quick reference document is for experienced users.
Axiom™ 2.0 gDNA Sample Preparation Protocol Quick Reference	MAN0017720	An abbreviated reference on preparing the genomic DNA sample.
Axiom™ gDNA Sample Preparation for Genome-Wide BOS 1 Array Plate Quick Reference	702975	An abbreviated reference on preparing the genomic DNA sample for the Genome-Wide BOS 1 Array Plate.
GeneTitan™ MC Protocol for Axiom 2.0 Array Plate Processing Quick Reference	MAN0017718	An abbreviated reference for processing Axiom™ Array Plates with the GeneTitan™ Multi-Channel Instrument.
GeneTitan™ Multi-Channel Instrument User Guide	MAN0027694	The GeneTitan™ Multi-Channel Instrument automates array processing from target hybridization to data generation by combining a hybridization oven, fluidics processing, and state-of-the art imaging device into a single benchtop instrument. This document details the use, care, and maintenance for the GeneTitan™ Multi-Channel Instrument.
GeneTitan™ Multi-Channel Instrument Site Preparation Guide	MAN0025571	Provides guidance on creating and maintaining the proper environment required for the GeneTitan™ Multi- Channel Instrument.
Recommended Alternative Microarray Consumables Quick Reference	MAN0019853	A quick reference document identifying recommended alternative replacement consumables for use in microarray assays.

Document	Publication number	Description
Analysis and software		
Axiom™ Genotyping Solution Data Analysis User Guide	MAN0018363	This guide provides information and instructions for analyzing Axiom™ genotyping array data. It includes the use of Axiom™ Analysis Suite, Applied Biosystems™ Array Power Tools (formerly APT) and SNPolisher™ Package to perform quality control analysis (QC) for samples and plates, SNP filtering before downstream analysis, and advanced genotyping methods.
GeneChip™ Command Console™ (GCC) User Guide	MAN0027771	This user guide provides instructions on using GeneChip™ Command Console™ (GCC) used to control GeneChip™ instrument systems. GeneChip™ Command Console™ software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip™ arrays.
Axiom™ Analysis Suite User Guide	MAN0027928	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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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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