applied biosystems

Axiom™ Propel Fast Wash Workflow, 96-Array Format

USER GUIDE

for use with:
Axiom™ Array Plates
Axiom™ Propel Fast Reagent Kits
Multidrop™ Combi Reagent Dispenser

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ThermoFisher SCIENTIFIC

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

Revision history: MAN0019450 E00 (English)

Revision	Date	Description
E00	10 March 2025	Added notes stating, "If using the Heratherm™ Advanced Protocol Microbiological Incubator, the convection setting should be turned off."
D00	11 April 2024	Added information regarding the two 96-array plate configurations.
C.0	2 November 2023	• Added Axiom™ 96-format Consumables Kit for QC (Windows™ 7) (Cat. No. 902909) as an additional option for UV-plates. • Updated publication numbers in *Related documentation*. • Corrected an error in Table 10. • Added information for the GeneTitan™ MC Fast Scan Instrument.

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

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1 Product information

About the Axiom™ Propel Fast Wash Workflow

The Axiom™ Propel Fast Wash Workflow, 96-Array Format is a workflow for ultra high-throughput microarray genotyping. The workflow includes:

- DNA target preparation using Multidrop™ Combi Reagent Dispenser stations setup for: DNA amplification, fragmentation, purification, and resuspension of the pelleted DNA in hybridization cocktail.
- Array hybridization in an off-line oven.
- Automated array plate processing (ligation, stain, wash, and imaging) in the Applied Biosystems™ GeneTitan™ Multi-Channel (MC) Instrument or the GeneTitan™ MC Fast Scan 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.

- Processing of CEL files generated by the GeneTitan™ MC Instrument, using the Axiom™ Genotyping Algorithm version 1 (Axiom GT1), available through Applied Biosystems™ Analysis Power Tools or Axiom™ Analysis Suite v5.0 or later.

The Axiom™ Propel Fast Wash Workflow includes enhancements to the Axiom™ chemistry improving assay workflow efficiency and flexibility. These enhancements allow a reduced overall fluidics time of ~20% (from 5 hours to 4 hours) in the GeneTitan™ MC Instrument, increasing the daily array plate loading capacity.

The Axiom™ Propel 96F Reagent Kit provides all necessary large-filled reagents for target preparation and GeneTitan™ reagents in volumes that are optimized for processing the modular workflow.

IMPORTANT! The Applied Biosystems™ Axiom™ Propel 96F Reagent Kit is for single use only. This large fill reagent kit is configured to include prime volumes required for use with the Multidrop™ Combi. Discard all excess reagents after use.

About the Axiom™ Genotyping Solution

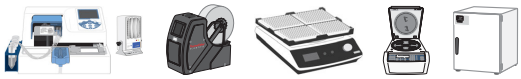
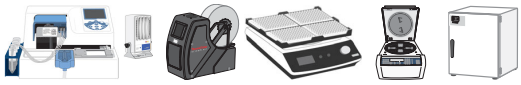
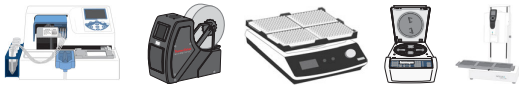
The Axiom™ Propel Fast Wash Workflow, 96-Array Format is part of the Axiom™ Genotyping Solution. The Axiom™ Genotyping Solution is a genotyping microarray platform that includes novel assay biochemistry, array configuration and processing, and automated target preparation on various array plate formats. It offers the capability to genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms (indels) from a variety of species, with a processing throughput of greater than 3,000 samples per week.

High-throughput genotyping through microarray technology has applications in human disease research and basic and applied agriculture research.

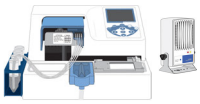
- For human disease research applications, Thermo Fisher Scientific conducted an empirical screen of genomic content from dbSNP (**ncbi.nlm.nih.gov/projects/SNP/**). The screen included markers from HapMap and the 1,000 Genomes Project and other sources, using HapMap phase 3 samples and/or the original 270 HapMap samples. All this information has gone into creating a proprietary database of verified markers that can be interrogated using the Axiom™ Assay.
- For agriculture applications, the Axiom™ Genotyping Solution can genotype samples using DNA extracted from leaves and seeds, playing an important role in genotype-trait association studies and marker-assisted selection in both plant and animal breeding programs.
- For molecular breeding programs, where turn-around time, accuracy, and ease-of-use are all important, the Axiom™ Genotyping Solution is ideal for high-throughput analysis.

The Axiom™ 96-array layout and the Axiom™ 384HT-array layout retain full compatibility with the existing Axiom™ instrumentation platform and downstream data analysis.

Axiom™ Propel Fast Wash Workflow target preparation overview

Assay stage	Instruments required
Stage 1: Amplify the genomic DNA • Three reagent additions with mixing—Denaturation Master Mix, Axiom™ Propel Neutral Solution, Amplification Master Mix • 10-minute denature incubation at room temperature • 22–24 hour amplification incubation at 37°C	
Stage 2: Fragment the DNA • Two reagent additions with mixing—Fragmentation Master Mix, Axiom™ Propel Frag Reaction Stop • 30-minute fragmentation incubation at 37°C	
Stage 3: Precipitate the DNA • Two reagent additions with high-speed mixing—Precipitation Master Mix and isopropanol • Overnight precipitation	
Stage 4: Centrifuge and dry pellets Purify amplified DNA into dried pellets.	
Stage 5: Resuspend the pelleted DNA and prepare for hybridization • Two reagent additions with mixing—Axiom™ Propel Resuspension Buffer, Hybridization Master Mix • 10 minute shaking to resuspend the DNA pellets • Transfer from 96-deepwell plate to half-skirted 96-well PCR plate	
Stage 5A: In-process QC • Three reagent dispenses—Dilution QC Plates, OD QC Plates, Gel QC Plates • Transfer hybridization-ready target from Hyb-Ready Plates to QC plates	
Stage 6: Denature the target and transfer to hybridization tray • Denature target in thermal cycler • Transfer from Hyb-Ready Plates to hybridization tray • Off-line incubation of the array plate/hybridization tray stack at 48°C for 23.5–24 hours	

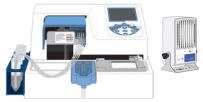
(continued)

Assay stage	Instruments required
Stage 7: Prepare GeneTitan™ reagents Five reagent dispenses—Ligation, Stain 1, Stain 2, Stabilization, Axiom™ Propel Hold Buffer	

Dispense, seal, shake, then centrifuge

For each stage of the Axiom™ Propel Fast Wash Workflow, 96-Array Format conducted at a Multidrop™ Combi Reagent Dispenser, the following steps are typically performed.

- Task name is provided in the table heading.
- Number of plates for the workflow is listed in the first row of the table.
- Each subsequent row in the table lists a step in the task/procedure, with specific details listed. The following image is a general example.

Multidrop™ Combi tasks		
	Plates 1 2 3 4	
①		Dispense • Method: 96-xyz-## • Dispense volume: ## µL
②		Seal • Settings: 150°C, 2.5 seconds
③		Shake • Settings: 1,100 rpm, 30 seconds
④		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 second

GeneTitan™ reagent tray barcodes

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

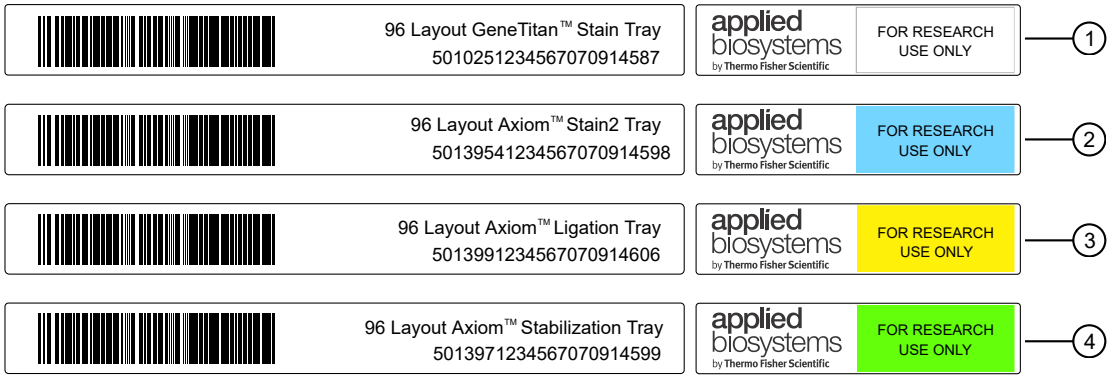


Figure 1 GeneTitan™ reagent tray barcodes and color-coded labels.

① Stain 1 Tray—Part No. 501025
② Stain 2 Tray—Part No. 501395
③ Ligation Tray—Part No. 501399
④ Stabilization Tray—Part No. 501397

The unique barcodes along with the GeneChip™ Command Console™ v4.2 or later software prevents users from making errors when placing the trays in the GeneTitan™ MC Instrument during array processing.

After the trays have been prepared, ensure that the trays are placed in the appropriate drawer location in the GeneTitan™ MC Instrument. Failure to place the proper tray in the correct location results in an error and the GeneTitan™ MC Instrument will not proceed with the processing of the trays. See “Proper tray alignment and placement” on page 214 for detailed instruction.

Overview of the Axiom™ Propel Fast Wash Workflow, 96-Array Format

	Genomic DNA preparation	
Day 1	Chapter 2, Genomic DNA preparation	
	▼	
	Chapter 4, "Target preparation with Multidrop™ Combi Reagent Dispensers for 8 plates"	
Day 1	Stage 1: Amplify the genomic DNA	22–24 hour of Amplification Plate at 37°C.
	▼	Optional stopping point. The post-Amplification Plates can be stored at –20°C for up to 1 week.
Day 2	Stage 2: Fragment the DNA	
	▼	
Day 2	Stage 3: Precipitate the DNA	Overnight precipitation at –20°C.
	▼	
Day 3	Stage 4: Centrifuge and dry DNA pellets	Optional stopping point. The pellets can be stored at –20°C for one day.
	▼	
Day 3	Stage 5: Resuspend the pelleted DNA and prepare for hybridization	Optional stopping point. The hybridization-ready target can be stored at –20°C for up to 2 weeks.
	▼	
Day 3	Stage 6: Denature the target and transfer to hybridization tray	23.5 to 24-hour array hybridization in the offline hybridization oven at 48°C.
	▼	
Day 4	Stage 7: Preparing ligation, stain, stabilization reagent trays, and scan trays for the GeneTitan™ MC Instrument	
	▼	
	Array processing	
Day 4	Chapter 6, "Process array plates with the GeneTitan™ Multi-Channel Instrument" *Array processing is completed with the GeneTitan™ MC Instrument and GeneChip™ Command Console™ software v6.1.1 or later. Scan time varies with instrument type and GCC version.*	Fluidics: ~4 hours Scan times: GTMC = ~5.5 hours GTMC Fast Scan = ~3.5 hours

Multi-plate workflows

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

- Incubation for DNA amplification is 22–24 hours.
- Reagent trays for wash/stain/imaging must be prepared as hybridization finishes.

Contact your local support representative for more information.

2 Genomic DNA preparation

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

Sources of genomic DNA

The following sources of gDNA have been successfully tested in the laboratories at Thermo Fisher Scientific for DNA that meets the requirements for the Axiom™ Assay.

Source	Sample type
Human	• Blood • Saliva • Cell line
Animal[1]	• Blood • Semen • Nasal swabs • Hair bulbs • Ear punch tissue
Plant	• Seeds • Leaves

[1] Success with sample types other than human depend on quality (degree of degradation, level of purity, and so on) and quantity of gDNA extracted.

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

General requirements

- Starting DNA must be double-stranded for accurate concentration determination.
- DNA must be of high purity. DNA must be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (that is, EDTA). The gDNA extraction and purification method must create DNA that is salt-free because high concentrations of particular salts can also inhibit enzyme reactions. Assess DNA purity by measuring the OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios. The OD_{260}/OD_{280} ratio should be between 1.8 and 2.0 and the OD_{260}/OD_{230} ratio should be greater than 1.5. We recommend that DNA samples that do not meet these criteria be cleaned up as described in "Clean up genomic DNA" on page 22.
- DNA must not be degraded. The average size of gDNA can be evaluated on a 1% agarose gel using an appropriate size standard control. Approximately 90% of the DNA must be greater than 10 Kb in size. Control DNA can be run on the same gel for comparison.

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

Special requirements

Preamplification area

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

This preamplification area requires a dedicated set of pipettes and plasticware. If no dedicated area is available, use of a dedicated bench or a dedicated biosafety hood and dedicated pipettes is suggested. If no dedicated bench or biosafety hood is available, a set of dedicated pipettes is recommended.

Ideally, this preamplification area would be separate from the amplification staging area, however, these areas may be combined due to space and equipment limitations.

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

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

Equipment and reagents required

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

Item	Source
E-Gel™ Power Snap Plus Electrophoresis Device	G9110
iBright™ CL750 Imaging System	A44116
Invitrogen™ E-Gel™ *48* Agarose Gels, 1%	G800801
Invitrogen™ *Redi*Load™ Loading Buffer	750026
Invitrogen™ E-Gel™ 96 High Range DNA Marker	12352019

Guidelines for preparing the gDNA Sample Plate for gel analysis

The following guidelines are recommended when preparing the gDNA Sample Plate for gel analysis.

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

Run a 48-lane 1% agarose E-Gel™

1. Insert the E-Gel™ *48* Agarose Gels, 1% into the electrophoresis unit.
2. Remove 2 combs.
3. Load 20 µL of gDNA samples onto the E-Gel™ *48* Agarose Gels, 1%.
4. If needed, load 15 µL of diluted E-Gel™ 96 High Range DNA Marker (1:3 dilution or ~0.34X from stock) into all marker wells.
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 automatically shuts off. The gel is ready for imaging.

E-Gel™ results

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

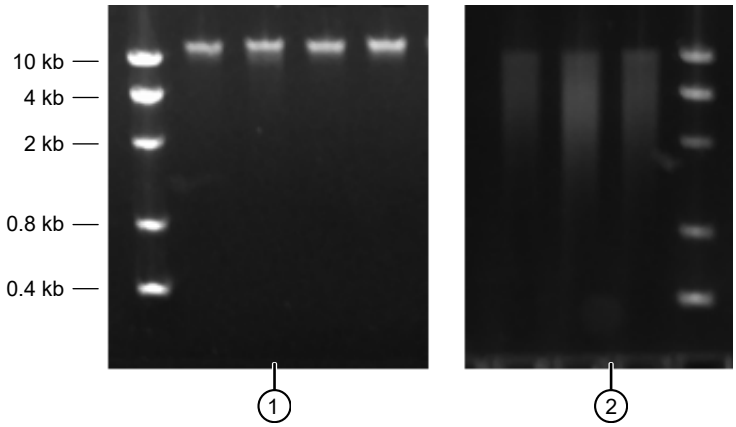


Figure 2 Gel images with intact gDNA and degraded gDNA.

① Intact samples ② Degraded samples

Genomic DNA extraction and purification methods

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

Clean up genomic DNA

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

1. Add 0.5 volumes of 7.5 M NH_4OAc, 2.5 volumes of absolute ethanol (stored at –20°C), to gDNA.
2. Vortex, then incubate at –20°C for 1 hour.
3. Centrifuge at 12,000 × *g* in a microcentrifuge at room temperature for 20 minutes.
4. Remove supernatant, then wash pellet with 80% ethanol.
5. Centrifuge at 12,000 × *g* at room temperature for 5 minutes.
6. Remove the 80% ethanol, then repeat the 80% ethanol wash 1 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 must be done before proceeding with the DNA amplification stages for Axiom™ Propel Fast Wash Workflow target preparation.

The genomic DNA (gDNA) you process using the Axiom™ Assay must meet the general requirements that are listed earlier in this chapter. The amount of gDNA depends on which Axiom™ array is used in the downstream protocol. "Genomic DNA input requirements" on page 23 details the sample input requirements for Axiom™ Propel Fast Wash Workflow, 96-Array Format.

Genomic DNA input requirements

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

Time required

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

Equipment, consumables, and reagents required

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

Equipment and consumables required

Quantity	Item
As required	Adhesive seals for plates
1 each	Pipettes: single channel P10 or P20 Optional: multichannel P10 or P20
As required	Pipette tips
1	Abgene™ 96 Well 2.2 mL Polypropylene Deepwell Storage Plate (AB0932). Referred to as the "96-deepwell plate" in this document.
1	Plate centrifuge
1	Plate spectrophotometer (required only if no OD measurements available for samples)
1	Vortexer

Reagents

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

Reagent	Source
Axiom™ Genomic DNA Standard (Ref 103), –20°C (use as a positive control if genotyping human samples).	951957
Thermo Scientific™ Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA).	Fisher Scientific™, AAJ75793AE
Positive control gDNA (if genotyping nonhuman samples).	
Ultra-pure water, from a purification system or equivalent.	**MLS**

Thaw samples and control

Thaw the following components to room temperature.

- gDNA samples
- gDNA positive control sample. For human studies, use Genomic DNA Standard (Ref 103).

To thaw, either:

- Place items on the bench top for 60 minutes.

- Thaw in a water bath.
  - Fill a small plastic dish with ultra-pure water. Do not overfill to prevent the level of the water overflowing when the sample tubes or plates are placed in the bath.
  - Thaw the sealed gDNA Sample Plate and reference sample for 30 minutes.
  - Wipe off the gDNA Sample Plate after removing from the water bath, and before removing the lid. Wiping off the gDNA Sample Plate minimizes the chances that the water enters the wells, then causes contamination or reaction failure.

Quantify and dilute test sample gDNA

1. Gently vortex (50% maximum), then centrifuge the gDNA and control DNA.
2. *Recommendation*: Quantify each sample (for example, using the Quant-iT™ PicoGreen™ dsDNA Assay 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
4. Seal, vortex, then centrifuge.

Note: Do *not* dilute the Genomic DNA Standard (Ref 103) control.

Aliquot the diluted samples and the control

Aliquot the diluted samples and control gDNA to the 96-deep well plate as follows:

1. Aliquot 20 µL of each diluted gDNA sample to the appropriate well.
   This amount is the equivalent of 100 ng to 200 ng of gDNA, as required by the sample type.
2. Positive control: 20 µL of control gDNA. For genotyping arrays, we recommend including at least one positive gDNA control on each plate. For human array designs, the Genomic DNA Standard (Ref 103) control (Cat. No. 951957) *must* be used.
3. Seal, then centrifuge.

Freeze or proceed

Do one of the following:

- Store the gDNA Sample Plate at –20°C, or
- Proceed to DNA amplification for automated target preparation (see Chapter 4, “Target preparation with Multidrop™ Combi Reagent Dispensers for 8 plates”, or Chapter 5, “Target preparation with Multidrop™ Combi Reagent Dispensers for 4 plates”).

Note: If proceeding immediately to DNA amplification, you can leave the gDNA Sample Plate at room temperature.

GeneTitan™ Array Plate Registration file

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

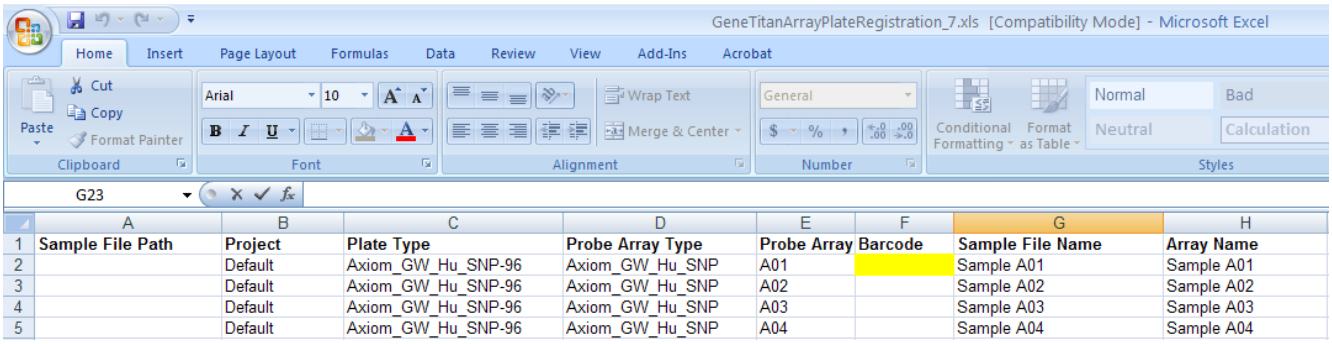
Create and save a GeneTitan™ Array Plate Registration file

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

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

   For more information on the **GeneTitan™ Array Plate Registration** file, see *GeneChip™ Command Console™ User Guide*.
7. Save the file. Do not upload the file at this point.

Details for the array plate and hybridization tray scanning steps, and the GeneTitan™ Array Plate Registration file uploading steps are in Chapter 6, “Process array plates with the GeneTitan™ Multi-Channel Instrument”.



	A	B	C	D	E	F	G	H
1	**Sample File Path**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array**	**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 3 Example of a GeneTitan™ Array Plate Registration file for Axiom™ Array Plate.

3 Set up for the Axiom™ Propel Fast Wash Workflow, 96-Array Format

This chapter contains information describing the procedures, equipment, and materials required for running the Axiom™ Propel Fast Wash Workflow, 96-Array Format. To support operator safety and assay performance, operators must be familiar with this content before starting target preparation. (See Chapter 4, "Target preparation with Multidrop™ Combi Reagent Dispensers for 8 plates", or Chapter 5, "Target preparation with Multidrop™ Combi Reagent Dispensers for 4 plates".) Additional information for the Multidrop™ Combi Reagent Dispenser is in the *Multidrop™ Combi User Manual*. See Appendix H, "Documentation and support" for details.

Required materials

Note: Using equipment, labware, and consumables from sources other than those listed have not been verified with this assay and, therefore, assay performance can not be guaranteed.

Equipment and materials required

The following table lists the equipment, labware, and consumables required for the assay. Exact quantities of equipment, labware, and consumables that are required is dependent on the number of plates that are processed.

Item	Used in stage
Instruments	
Multidrop™ Combi Reagent Dispenser	1, 2, 3, 5, 5A, 7
ALPS™ 3000 Automated Microplate Heat Sealer	1, 2, 3, 5
Compact Digital Microplate Shaker	1, 2, 5
VWR Signature™ High-Speed Microplate Shaker	3
One of the following ovens: • Thermo Scientific™ Heratherm™ Advanced Protocol Microbiological Incubator, capacity 66 L • BINDER™ ED 56 Drying and Heating Chamber • BINDER™ BD 56 Standard-Incubator with natural convection Oven requirements: • Must maintain a constant temperature of 37°C for at least 24 hours with a temperature accuracy of ±1°C (amplification, fragmentation, pellet drying) • Must maintain a constant temperature of 48°C for at least 24 hours with a temperature accuracy of ±1°C (off-line hybridization) • Must maintain a constant temperature of 65°C for at least 24 hours with a temperature accuracy of ±1°C (fragmentation) • If using the Heratherm™ Advanced Protocol Microbiological Incubator, the convection setting should be turned off.	1, 2, 4, 6
Sorvall™ X4R Pro-MD Centrifuge	1, 2, 4, 5, 5A,6
VIAFLO™ 96 Base Unit or VIAFLO™ 384 Base Unit with a 96 Channel Pipetting Head (5–125 µL) and 3-position stage	5, 5A, 6
Thermal cycler, ProFlex™ 96-well PCR System	6
Vortex mixer	1, 2, 5, 5A, 6, 7
Mini centrifuge	2, 7
Freezer, –20°C	3

(continued)

Item	Used in stage
Electronic pipettor	1, 2, 5, 7
P1000 pipette	1, 2, 3, 5, 7
Fume hood	3, 4, 5, 5A, 6
Balance with: • readability of 0.01 g or finer • a maximum capacity of at least 300 g.	Gravimetric checks
Compact in-Tool Ionizing Blower 6432E	1, 2, 3, 5, 5A, 7
GeneTitan™ ZeroStat AntiStatic Gun	7
Digital timer	1, 2
Labware and consumables	
Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]	1, 2, 3, 5, 5A, 7
96-deepwell plate	1[2]
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (half skirt)	5
MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, Blue	5A
Corning™ Clear Polystyrene 96-Well Microplate (used as the base for the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (half skirt))	5, 5A, 6
Greiner Bio-One™ 96-Well UV-Star™ Plate, Flat Bottom, Chimney Style, Clear	5A
Plate Alignment Tool	6
GeneTitan™ consumables	7
50-mL conical tube	Multidrop™ Combi setup procedure, and for master mix preparation
250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)	Master mix preparation
Glass or polypropylene bottle, minimum 1,000 mL capacity (for isopropanol dispensing)	3
Matrix™ Reagent Reservoir, 25 mL	Multidrop™ Combi cleaning procedure
Serological pipettes (5, 10, 25 and 50 mL)	1, 2, 5, 7
P1000 pipette tips	1, 2, 3, 5, 7

(continued)

Item	Used in stage
Integra VIAFLO XYZ GripTips™, 125 µL, 5 XYZ Racks of 384 Tips, Sterile, Filter	5, 5A, 6
Easy Peel Seal (for ALPS™ 3000 Automated Microplate Heat Sealer)	1, 2, 3, 5
MicroAmp™ Clear Adhesive Film	5, 5A
Laboratory tissues	as needed

[1] Each reagent must use a dedicated cassette.
[2] The same 96-deepwell plate is used through Stage 5.

Labware and consumable ordering information

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

Table 1 Labware and consumable images and ordering information.

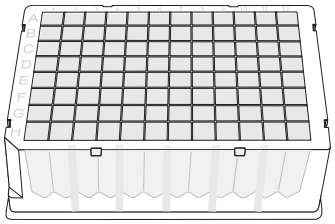
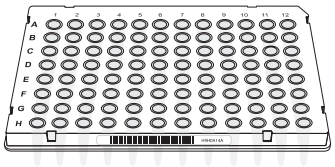
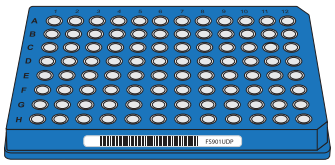
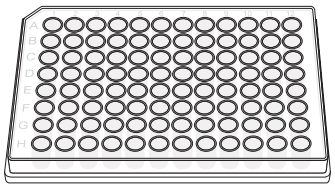
Item	Source	Image
Abgene™ 96 Well 2.2 mL Polypropylene Deepwell Storage Plate, square wells, V-bottom **Note:** This plate is referred to as “96-deepwell plate” throughout this document.	Fisher Scientific™, AB0932	
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (half skirt) **Note:** This plate is referred to as “half-skirted 96-well PCR plate” throughout this document.	4483354	
MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, Blue	A31727	
OD Plate, option 1: Corning™ UV-Transparent Microplate Axiom™ 96-format Consumables Kit for QC, 902909. Also available from Fisher Scientific™, 07-200-623	**OD Plate, option 1:** Corning™ UV-Transparent Microplate	

Table 1 Labware and consumable images and ordering information. *(continued)*

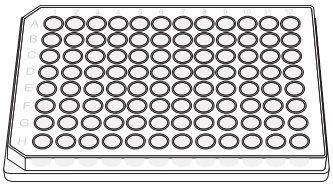
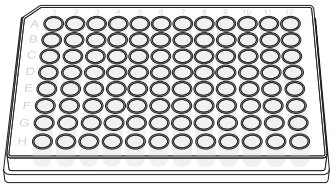
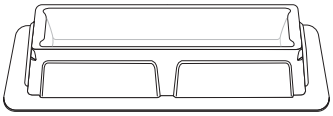
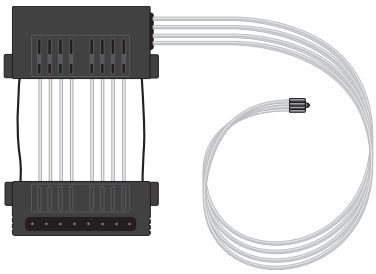
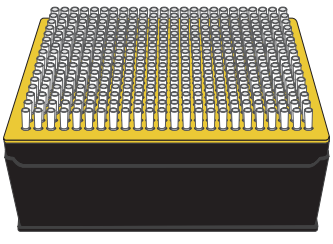
Item	Source	Image
OD Plate, option 2: Greiner Bio-One™ UV-Star™ 96-Well UV Spectroscopy Microplate Fisher Scientific™, 07-000-407	**OD Plate, option 2:** Greiner Bio-One™ UV-Star™ 96-Well UV Spectroscopy Microplate	
Corning™ Clear Polystyrene 96-Well Microplate **Note:** This plate is used as a reusable plate holder for the MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plate on the INTEGRA Biosciences VIAFLO™ stage during Stage 5A: In-process QC to Stage 6: Denature the target and transfer to hybridization tray.	Fisher Scientific™, 07-200-103	
Matrix™ Reagent Reservoir, 25 mL	Fisher Scientific™, 809311	
SMART 2 Standard tube dispensing cassette	N15137, single cassette	
Pipette tips, 1,000 µL	**MLS**	
Serological pipettes, following sizes • 5 mL • 10 mL • 25 mL • 50 mL	**MLS**	
Electronic pipettor (for serological pipettes)	**MLS**	
XYZ GripTips™, 125 µL, 5 XYZ Racks of 384 Tips, Sterile, Filter	INTEGRA Biosciences, 6465	

Table 1 Labware and consumable images and ordering information. *(continued)*

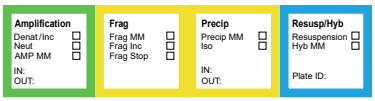
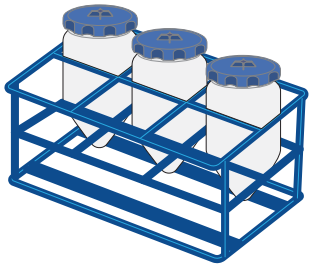
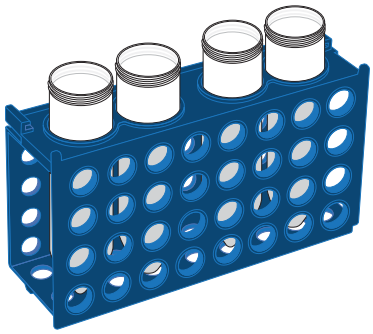
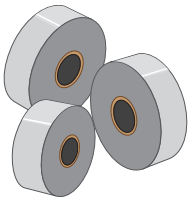
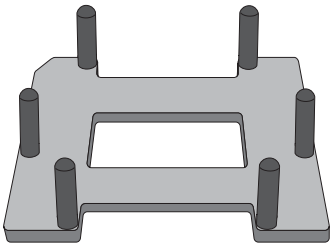
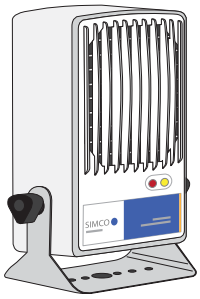
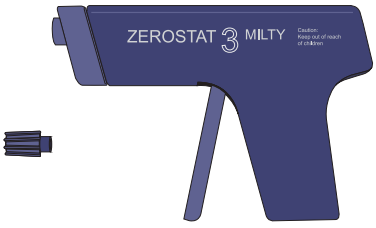
Item	Source	Image
Axiom™ Propel 96F Tracker Label[1]	952373 (Contains a sheet of 8 labels.)	
50-mL centrifuge tubes	**MLS**	
Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube	376814	
Nunc™ Conical Tube Rack	374179	
Fisherbrand™ 4-Way Tube Rack	Fisher Scientific™, 03-448-12	
BTL Safety Carrier, black **Note:** This carrier is recommended as the secondary liquid waste container for the Multidrop™ Combi.	Fisher Scientific™, 50-109-4650	

Table 1 Labware and consumable images and ordering information. *(continued)*

Item	Source	Image
Easy Peel Seal **Note:** The Easy Peel Seal is the sealing material used in the ALPS™ 3000 Automated Microplate Heat Sealer.	AB-3739	
MicroAmp™ Clear Adhesive Film	4306311	
Plate Alignment Tool	13-0401	
minION™2 Ionizing Air Blower, or equivalent	**MLS**	
GeneTitan™ ZeroStat AntiStatic Gun and Ion-Indicator Cap **Note:** The GeneTitan™ ZeroStat AntiStatic Gun can be used as an alternative if the minION™2 Ionizing Air Blower is not available.	74-0014	
Balance for gravimetric checks. • readability of 0.01 g or finer • maximum capacity of at least 300 g	**MLS**	

[1] The Axiom™ Propel 96F Tracker Label (Cat. No. 952373) contains 8 labels; 1 each is required to process using 1 Axiom™ Propel Fast Reagents kit.

Axiom™ Propel Fast Reagent Kits

IMPORTANT! The Applied Biosystems™ Axiom™ Propel 96F Fast Reagent Kits are for single use only. These large fill reagent kits are configured to include priming volumes for the Multidrop™ Combi cassettes and have been incorporated into the master mix formulations. Discard all excess reagents after use.

- Each Axiom™ Propel Fast Reagent Kit, 8x96F , Cat. No. 952372, is sufficient for 8 Axiom™ Array Plates (96-array format)
- Each Axiom™ Propel Fast Reagent Kit, 4x96F , Cat. No. 952371, is sufficient for 4 Axiom™ Array Plates (96-array format)

Component	4X kit[1]	8X kit[1]	Storage
Axiom™ Propel Reagent Kit Module 1 for 96F array plates only, 4x96F or **Axiom™ Propel Reagent Kit Module 1 for 96F array plates only, 8x96F**	**952262**	**952269**	–25°C to –15°C
Axiom™ Propel 10X Denat Solution	952176	951968	
Axiom™ Propel Neutral Solution	952173	951965	
Axiom™ Propel Water	952177	951969	
Axiom™ Propel Amp Solution	952174	951966	
Axiom™ Propel Amp Enzyme	952175	951967	
Axiom™ Propel Reagent Kit Module 2-1 for 96F or 384HT or **Axiom™ Propel Reagent Kit Module 2-1 for 96F array plates only**—Box 1 of 2	**952263**	**952337**	–25°C to –15°C
Axiom™ Propel Frag Enzyme	952181	951972	
Axiom™ Propel 10X Frag Buffer	952179	951971	
Axiom™ Propel Precip Solution 2	952178	951970	
Axiom™ Propel Hyb Buffer	952182	951973	
Axiom™ Propel Hyb Solution 1	952183	951974	

(continued)

Component	4X kit[1]	8X kit[1]	Storage
Axiom™ Propel Reagent Kit Module 2-2 for 96F or 384HT or **Axiom™ Propel Reagent Kit Module 2-2 for 96F array plates only**—Box 2 of 2	**952265**	**952338**	2°C to 8°C
Axiom™ Propel Frag Diluent	952184	951975	
Axiom™ Propel Frag Reaction Stop	952190	951976	
Axiom™ Propel Precip Solution 1	952203	951977	
Axiom™ Propel Resuspension Buffer	952206	951978	
Axiom™ Propel Hyb Solution 2	951979	951979	
Module 3	—	—	Room temperature
Axiom™ Wash Buffer A	901446	901446	
Axiom™ Wash Buffer B	901447	901447	
Axiom™ Water	901578	901578	

(continued)

Component	4X kit[1]	8X kit[1]	Storage
Axiom™ Propel Fast Wash Reagent Kit Module 4-1 for 96F or 384HT or **Axiom™ Propel Fast Wash Reagent Kit Module 4-1 for 96F array plates only**—Box 1 of 2	**952369**	**952370**	–25°C to –15°C
Axiom™ Propel Ligation Buffer	952208	951980	
Axiom™ Fast Ligation Enzyme	952367	952368	
Axiom™ Propel Ligation Solution 1	952212	951982	
Axiom™ Propel Probe Mix 1	952213	951983	
Axiom™ Propel Stain Buffer	952214	951984	
Axiom™ Propel Stabilize Solution	952215	951985	
Axiom™ Propel Reagent Kit Module 4-2 for 96F or 384HT or **Axiom™ Propel Reagent Kit Module 4-2 for 96F array plates only**—Box 2 of 2	**952268**	**952340**	2°C to 8°C
Axiom™ Propel Wash A	952218	951988	
Axiom™ Propel Probe Mix 2	952217	951987	
Axiom™ Propel Ligation Solution 2	952216	951986	
Axiom™ Propel Stain 1-A	952219	951989	
Axiom™ Propel Stain 2-A	952231	951990	
Axiom™ Propel Stabilize Diluent	952248	951991	
Axiom™ Water	952177	952177	
Axiom™ Propel Hold Buffer	952254	951992	
Axiom™ Propel Stain 1-B	952258	951993	
Axiom™ Propel Stain 2-B	952260	951994	

[1] Component Part Numbers are for identification puproses only. Kit components are not available for purchase separately.

Master mix preparation for large-fill reagent kits

IMPORTANT!

- The Applied Biosystems™ Axiom™ Propel Fast Reagent Kits (4x96F and 8x96F) are for single use only. This large fill reagent kit is configured to include priming volumes for the Multidrop™ Combi cassettes and is incorporated into the master mix formulations. Discard all excess reagents after use.
- The Axiom™ Propel Fast Reagent Kit, 8x96F is compatible with the processing of 96-array format plates only.

Additional reagents and materials required

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

Reagent	Source	Where used
2-Propanol, anhydrous, 99.5% (Isopropanol)	Sigma-Aldrich™, 278475	Stage 3: Precipitate the DNA
E-Gel™ *48* Agarose Gels, 4%	G800804	Stage 5A: In-process QC
TrackIt™ Cyan/Orange Loading Buffer	10482028	
25-bp DNA Ladder	931343	
UltraPure™ DNase/RNase-Free Distilled Water	10977023	
Reagent Alcohol, Certified, 70% (v/v) (Ethanol solution 70%, reagent grade)	Fisher Scientific™, LC222102	Multidrop™ Combi cassette cleaning

GeneTitan™ bulk consumables

GeneTitan™ trays are required for processing 96-array format plates on the GeneTitan™ MC Instrument or the GeneTitan™ MC Fast Scan Instrument. See Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation” for information on aligning and loading trays onto the GeneTitan™ MC Instrument.

Table 2 GeneTitan™ consumables kit available.

Contents[1]	Quantity	Source
GeneTitan™ 96F Barcoded Stain Trays, bulk	50	952376
GeneTitan™ Hybridization Trays, bulk	40	952357
GeneTitan™ Scan Trays, bulk	40	952358
GeneTitan™ Tray Covers, bulk (for stain trays)	50	952359

[1] See “GeneTitan™ 96-array format consumables” on page 39 for detailed descriptions of each component.

Note: All covers must have barcodes. Discard any cover without a barcode.

Table 3 Number of GeneTitan™ consumables required for processing.

Number of array plates	96-layout hybridization tray	96-layout stain tray	96-layout scan tray	96-layout scan and stain tray cover
4	4	20	4	24
8	8	40	8	48

GeneTitan™ 96-array format consumables

Note: All covers must have barcodes. Discard any cover without a barcode.

Table 4 GeneTitan™ 96-array format consumables.

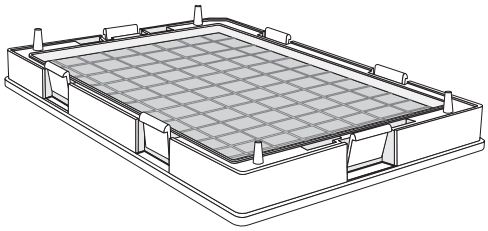
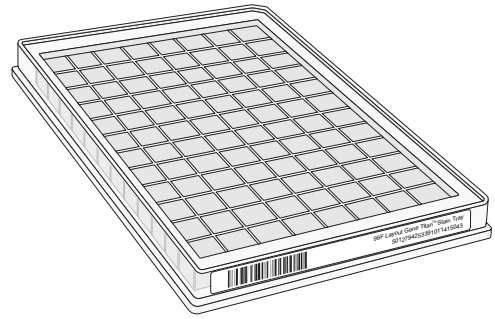
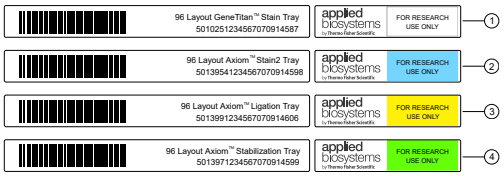
Item	Part No.	Image	Details
96-layout hybridization tray	900747		After aliquoting the denatured hybridization-ready samples into the hybridization tray, the tray should be clamped onto a 96-format array using the Plate Alignment Tool for off-line hybridization.
96-layout GeneTitan™ stain trays	952376	  ① Stain 1 tray ② Stain 2 tray ③ Ligation tray ④ Stabilization tray	The GeneTitan™ stain trays are packaged to keep them free of dust. Each GeneTitan™ stain tray is uniquely barcoded. All trays must be de-ionized to remove static electricity before dispensing GeneTitan™ reagents into the trays. See “Deionization of GeneTitan™ trays and covers” on page 222.

Table 4 GeneTitan 96-array format consumables. *(continued)*

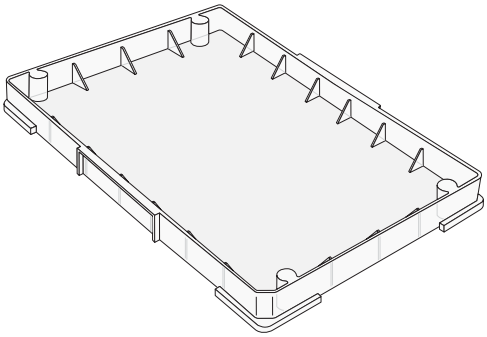
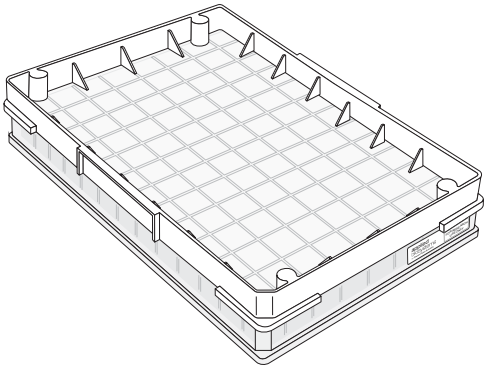
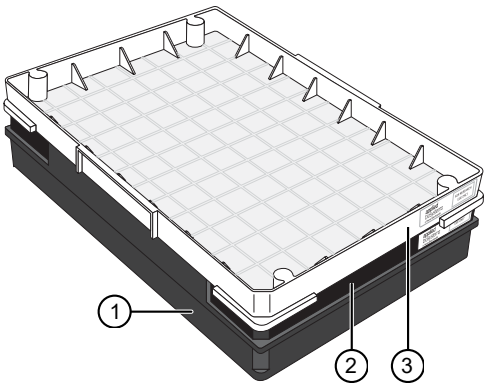
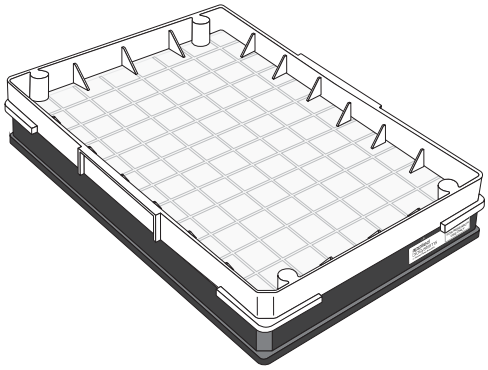
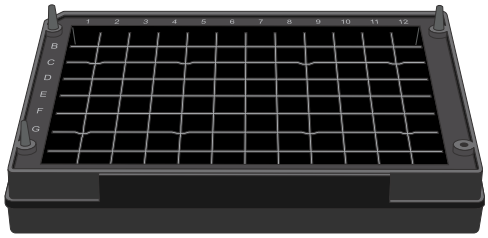
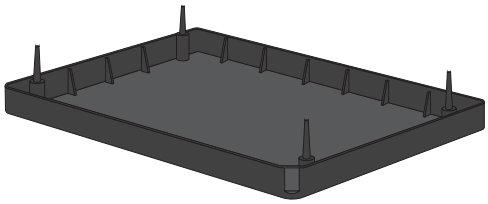
Item	Part No.	Image	Details
96-layout stain and scan tray cover	952359		The 96-layout scan and stain tray covers are provided to prevent any evaporation of the stains in stain trays and the array holding buffer in the scan tray. The GeneTitan™ scan and stain tray covers are barcoded. All stain and scan trays must be placed in the GeneTitan™ MC Instrument with the tray covers. All tray covers must be de-ionized to remove static electricity before placing the cover on the stain tray. See "Deionization of GeneTitan™ trays and covers" on page 222.
GeneTitan™ stain tray cover, shown on top of the stain tray	Tray 501025 Cover 202757		The GeneTitan™ stain trays must be placed in the GeneTitan™ MC Instrument with the GeneTitan™ stain tray covers. See "Label GeneTitan™ hybridization and reagent trays" on page 219.
Scan tray	900746 Box 501006 Pouch	 ① Scan tray protective base ② 96-layout scan tray ③ Scan tray cover	Comprised of three parts: 96-layout scan tray, a 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 use. • Remove the black scan tray protective base before loading the scan tray with the scan tray cover into the GeneTitan™ MC Instrument.

Table 4 GeneTitan 96-array format consumables. *(continued)*

Item	Part No.	Image	Details
GeneTitan™ scan tray with cover			The GeneTitan™ scan tray must be loaded with the scan tray cover into the GeneTitan™ MC Instrument. **Do not** load the scan tray with the protective base.
GeneTitan™ scan tray on black base			This combination of the GeneTitan™ scan tray on the protective black base is to be placed on the Multidrop™ Combi deck during the GeneTitan™ reagent preparation.
Black scan tray protective base			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 base is distinct from the blue array plate protective base and must not be used with the array plate. Remove the protective base from the scan tray before loading in the GeneTitan™ MC Instrument.

Other equipment, consumables, and reagents required

Microplate dispenser

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

Table 5 Multidrop™ Combi Reagent Dispenser and cassette information.

Multidrop™ Combi Reagent Dispenser	Recommended Standard Tubing Cassette (with plastic tip)
Multidrop™ Combi+ Reagent Dispenser 5840330	Standard tube plastic tip dispensing cassette 1-pack: 24072670 5-pack: 24072671 10-pack: 24072672
Multidrop™ Combi SMART+ Reagent Dispenser (includes RFID) 5840340	SMART+ standard tube dispensing cassette 1-pack: N22704

Table 6 Discontinued Multidrop™ Combi Reagent Dispenser and cassette information: Existing Multidrop™ Combi Reagent Dispenser listed in the table below continue to be compatible with the Axiom™ Propel Fast Wash Workflow, 96-Array Format using the cassettes listed.

Discontinued Instrument Models	Recommended Standard Tubing Cassette (with plastic tip)
Multidrop™ Combi Reagent Dispenser Cat. No. 5840300	Standard tube plastic tip dispensing cassette 1-pack: 24072670 5-pack: 24072671 10-pack: 24072672
Multidrop™ Combi Reagent Dispenser with SMART 2 option Cat. No. 5840320	Standard tube plastic tip dispensing cassette 1-pack: 24072670 5-pack: 24072671 10-pack: 24072672

Note: The Multidrop™ Combi SMART2 Reagent Dispenser no longer supports RFID tracking. All the cassettes, with RFID or without, can be used on either Combi+/Combi or Smart+/Smart2 instrument except that tracking capability is only available for matched RFID cassette and RFID Smart instrument.

Plate centrifuge

The plate centrifuges listed are recommended for the Axiom™ Propel Fast Wash Workflow, 96-Array Format. (See Table 7.) When centrifuging and drying pellets, the centrifuge must be able to centrifuge plates at:

- Rcf: 3,200 × *g* with an appropriate rotor-bucket combination
- Temperature: 4°C

Relative centrifugal force (rcf) is calculated using the following formula:

$rcf = (1.118 \times 10^{-5}) R S^2$

Where R is the radius of the rotor in centimeters, and S is the speed of the centrifuge in revolutions per minute.

In addition, the bottom of the rotor buckets must be soft rubber to help ensure that the 96-deepwell plates do not crack. Do not use buckets where the plates sit directly on a metal or hard plastic bottom.

Table 7 Plate centrifuge recommendations for the Axiom™ Propel Fast Wash Workflow, 96-Array Format.

Item	Source
Sorvall™ X4R Pro-MD Centrifuge, with: • TX-1000 Swinging Bucket Rotor Body • Adapter for TX-1000 Swinging Bucket Rotor • Buckets for TX-1000 Rotor	75009520 (220 V-240 V 50 Hz/230 V, 60 Hz) 75009521 (120 V, 50—60 Hz) 75009620 (220 V, 60 Hz) • 75003017 • 75007303 (pack of 4) • 75003001 (set of 4)
Eppendorf™ Centrifuge 5810 R, with: • Rotor A-4-81, with 4 MTP/Flex buckets	Fisher Scientific™, 022625501 (120 V, 50—60 Hz, 15 A) Fisher Scientific™, 022625101 (120 V, 50—60 Hz, 20 A) • Fisher Scientific™, 022638807 (rotor)

Plate sealer

The following plate sealer meets the requirements for the Axiom™ Propel Fast Wash Workflow, 96-Array Format.

Item	Source
Thermo Scientific™ ALPS™ 3000 Automated Microplate Heat Sealer[1,2]	AB3000

[1] The Easy Peel Seal is the sealing material used in the ALPS™ 3000 Automated Microplate Heat Sealer.
[2] Clean dry air (CDA) is requireor the heat sealer.

Oven requirements

We recommend using either the Thermo Scientific™ Heratherm™ Advanced Protocol Microbiological Incubator, BINDER™ ED 56 Drying and Heating Chamber, or the BINDER™ BD 56 Standard-Incubator with natural convection that are listed in the following table. If another oven is used, it must meet the following requirements.

- Be able to maintain a constant temperature of 37°C for at least 24 hours, and have a temperature accuracy of ±1°C, and
- Be able to maintain a constant temperature of 48°C for at least 24 hours, and have a temperature accuracy of ±1°C.

Item	Source
Thermo Scientific™ Heratherm™ Advanced Protocol Microbiological Incubator, capacity 66 L • 120V, 60 Hz • 230V, 50/60 Hz See note below.	 • 51028066 • 51028133
BINDER™ ED 56 Drying and Heating Chamber • ED056UL-120V Voltage: 120 V 1~60 Hz • ED056-230V Voltage: 230 V 1~50/60 Hz	 • BINDER™, 9010-0334 • BINDER™, 9010-0333
BINDER™ BD 56 Standard-Incubator with natural convection • BD056UL-120V Voltage: 120 V 1~60 Hz • BD056-230V Voltage: 230 V 1~50/60 Hz	 • BINDER™, 9010-0324 • BINDER™, 9010-0323

Note: If using the Heratherm™ Advanced Protocol Microbiological Incubator, the convection setting should be turned off.

Shakers

The following shakers are required for use in the Axiom™ Propel Fast Wash Workflow, 96-Array Format.

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

IMPORTANT! Both types of shakers (Thermo Scientific™ Digital Microplate Shaker and VWR Signature™ High-Speed Microplate Shaker) are required and are not interchangeable. Use only the shaker specified in the instructions for the assay stage.

Item	Source
Shaker, 0—1,200 rpm	
Thermo Scientific™ Digital Microplate Shaker	88882005 or 88882006
High speed shaker, 0—2,500 rpm	
VWR Signature™ High-Speed Microplate Shaker	VWR, 10027-220

Vortex mixer

A vortex mixer is required for use in the Axiom™ Propel Fast Wash Workflow, 96-Array Format.

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

Item	Source
Vortex mixer	**MLS**

Mini centrifuge

A mini centrifuge is required for use in the Axiom™ Propel Fast Wash Workflow, 96-Array Format.

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

Item	Source
Mini centrifuge	**MLS**

Liquid handler

One VIAFLO™ liquid handler is recommended for the Axiom™ Propel Fast Wash Workflow, 96-Array Format.

Note: If running both Axiom™ 96-array format plates and Axiom™ 384HT array plates and only one VIAFLO™ unit can be purchased, we recommend buying a VIAFLO™ 96 Base Unit to process both 96-array format and 384HT arrays. Refer to the Axiom™ Propel XPRES 384HT Workflow Site Preparation Guide for information on the VIAFLO™ 96 Base Unit. Contact your Field Application Scientist for more details.

Item	Source
VIAFLO™ 96 Base Unit, or VIAFLO™ 384 Base Unit with:	INTEGRA Biosciences, 6001 or 6031
☐ 96 Channel Pipetting Head (5 µL to 125 µL)	INTEGRA Biosciences, 6102
☐ Plate Holder—Three position stage (for 96 and 384 well plates)	INTEGRA Biosciences, 6230
☐ Installation and Training VIAFLO (required)	INTEGRA Biosciences, 999110

Thermal cycler recommendations

We have verified the performance of this assay using the thermal cyclers that are listed in the following table in their 96-well block configurations.

Verified thermal cyclers	Source
Applied Biosystems™ ProFlex™ 96-well PCR System[1]	4484075
Applied Biosystems™ ProFlex™ 2 × 96-well PCR System[1]	4484076

[1] The ramp rate on the thermal cycler can be programmed to 6.0C/sec (maximum).

Thermal cycler protocol

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

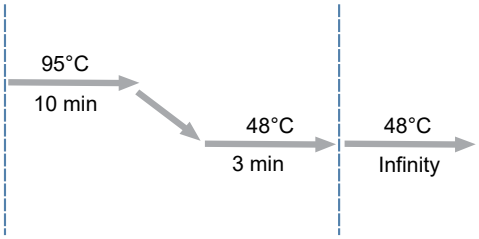


Figure 4 Axiom Denature thermal cycler protocol (Stage 6)

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

Gel Electrophoresis

See Appendix B, “Fragmentation quality control gel protocol” for gel electrophoresis equipment details.

Spectrophotometer

See Appendix C, “Sample quantification after resuspension” for spectrophotometer details.

Fume hood

Some procedures in the assay require the use of adequate local or general ventilation to keep airborne concentrations low. A fume hood is a way to achieve the desired concentration. Therefore, a fume hood is strongly recommended for several steps of this assay.

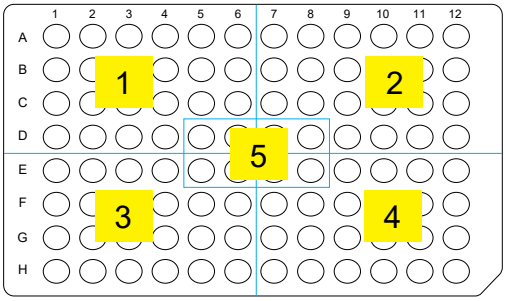
Guidelines for handling plates and reagents

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.

Vortex

- Plates: Vortex 1 second each corner, and 1 second in the center at the maximum setting.



- 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 for ~20–30 seconds. If precipitates are seen on the inside the bottle, follow the instructions in the reagent handling section for the appropriate stage.

Centrifuge

When instructed to perform a brief centrifugation step of plates or reagent vials, follow these guidelines, unless otherwise instructed.

- Plates:
  - Centrifuge plates to 675 × *g* for 30 seconds at room temperature.
  - Do not centrifuge for more than 1 minute.
- Reagent vials:
  - Briefly centrifuge for 3 seconds on the microcentrifuge.

Best practices for Axiom™ Propel Fast Wash Workflow

Preamplification/amplification staging area

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

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

General guidelines

CAUTION! To obtain optimal performance, the use of recommended instruments and procedures described in this user guide is required. Using instruments other than those recommended, such as a different microplate sealer or high-speed shaker, can affect assay performance. Additional optimization and verification is required if non-recommended instruments are used.

- Read and follow all safety precautions that are described in Appendix G, “Safety”.
- Practice standard pre- and post-lab segregation.
- Follow manufacturer's recommendations on calibration and preventive maintenance schedules for all instruments used in the workflow.
- Use a temperature monitoring system to track the performance of incubators, freezers, and refrigerators.
- To prevent liquid from overflowing from the reagent bottles, always insert the pipette slowly into the bottle.
- Ensure that the plastic consumables used in the procedures are free of defects (such as cracks) before using them.
- Mitigate static electricity by using the de-ionizing device where specified in the user guide.
- Maintain the same order of sample plate processing throughout the target preparation stages for consistency.
- Mark the plates to indicate that the reagent addition by the Multidrop™ Combi has occurred. A label, such as the one shown in the following image, is used to help tracking.

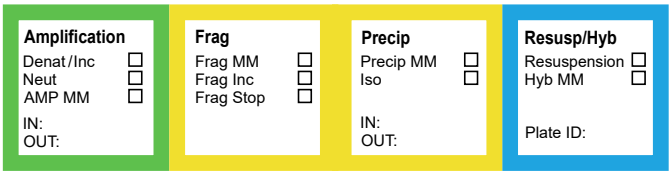


Figure 5 Axiom™ Propel 96F Tracker Label, Cat. No. 952373.

- Maintain a clear work area and designate appropriate space to help identify the plates before and after reagent dispensing.
- It is strongly recommended to use the GeneTitan™ Barcoded Stain Trays on the GeneTitan™ MC Instrument. The unique barcodes on the stain trays prevent errors when placing the trays in the GeneTitan™ MC Instrument during array processing.

Master mix preparation guidelines

- Follow the specified order of reagent addition when preparing master mixes.
- Use the recommended transfer apparatus (such as serological pipette or pipette) listed in the master mix preparation sections.
- Always use pipettes that have been calibrated.
- If multiple transfers are required to pipet the reagent, we recommend dividing the transfers into equal or simple volumes for ease of operation. Use the pipette sizes as recommended in the master mix preparation protocol for each stage.

  Example: To transfer 2.06 mL of stain 1A using a P1000 pipette, pipet 687 µL of stain 1A from the reagent bottle 3 times.
- Use conical bottles as reagent reservoirs to minimize the dead volumes in the reagent reservoir, unless otherwise instructed.

Multidrop™ Combi use guidelines

- Use dedicated cassettes for each type of master mix/reagent to prevent cross-contamination. Trace amounts of chemicals can be absorbed by the tubing.
- Before priming the Multidrop™ cassettes with reagents, ensure that the startup and gravimetric check procedures have been performed.
- Follow the recommended cleaning procedure for cassette to prevent clogging and contamination.
- Handle the dispense tips (nozzles) of the Multidrop™ cassette with care to prevent damage of the tips. Damaged tips could cause liquid stream to split and cause inaccuracy of dispense.
- Place the cassette tubing bundle at the bottom of the reagent bottle to maximize reagent usage.
- Avoid having the cassette be in contact with dust or lint—excessive exposure can cause clogging of the tubing or tips. Cover the tips with the lid when storing.
- Avoid touching any part of cassette that comes in contact with the reagent.
- When cassette is not in used for more than 1 hour, put the cassette in the "Rest" position.
- Use a sturdy tube rack or secondary container to hold the reagent tube or bottle to prevent spillage.
- Before dispensing isopropanol, always prime it through the tubing, then let it sit inside the tubing for at least 15 minutes.
- Use clean labware at the start of every run.
- Ensure that the sample plate is seated properly into the Multidrop™ stage before dispensing.
- Place a Compact in-Tool Ionizing Blower 6432E at each dispensing station and power on before dispensing reagent into the sample plates.

- If droplets are seen on the top grid of the plate, carefully blot off the droplets by laying a piece of clean laboratory tissue over the top of the plate to absorb the droplets.
- Avoid large spillage or splashing of liquid which can get into the electronic components of the instrument and cause malfunction.

IMPORTANT!

- Static electricity can cause the liquid stream to stray and result in inaccurate delivery of the reagent into the samples. The use and proper placement of the ion blower is critical to the success of the assay.
- Place consumables (96-deepwell plates, scan trays, stain trays, scan and stain tray covers) to be deionized within a distance of 12" x 36" from the ion blower for at least 10 seconds before using.

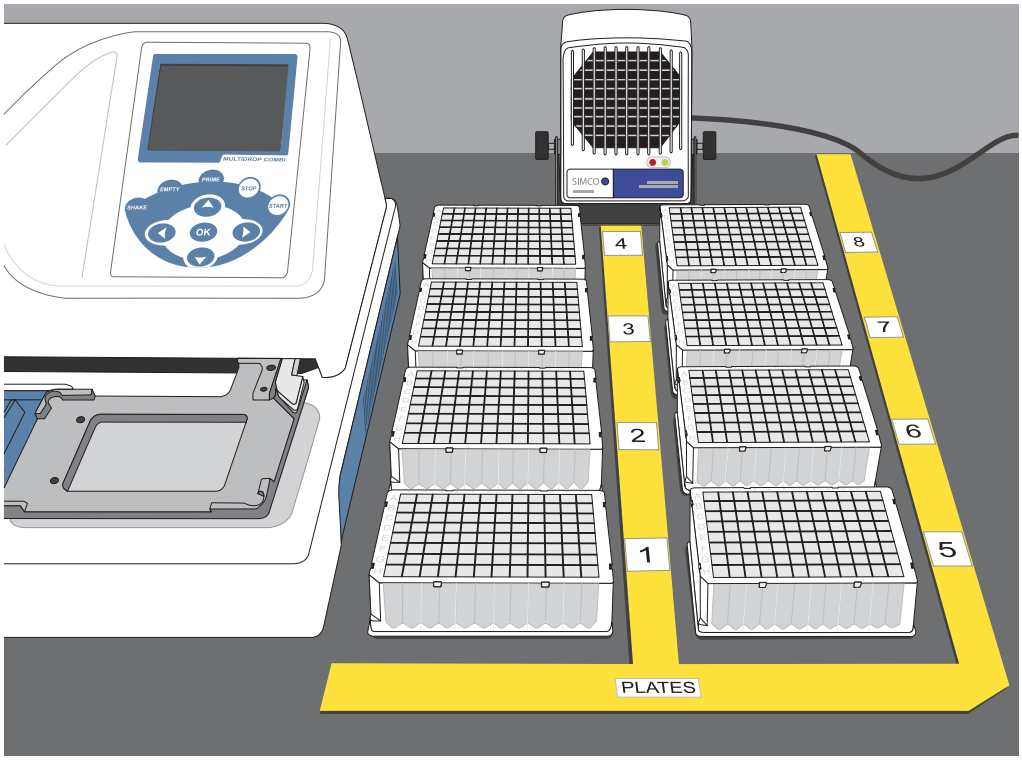


Figure 6 Ionizing air blower placement

VIAFLO guidelines

- When running a method, ensure that the stage slider is always at the center.
- Before running a method, ensure that the 3-position stage is at a locked position to the left.
- When loading tips to the 96-head, ensure that the 3-position stage is at a locked position to the right.

Plate heat sealer guidelines

- Power on the plate heat sealer, then allow the sealer to reach set temperature before starting the target preparation workflow.
- When the sealer is first powered on for the day, run a few sealing operations to ensure seal quality and proper seal alignment to the plate.
- After the sealing operation, check the seal to ensure all the grids of the wells have visible imprints on the sealing material. If misalignment is observed, follow the guidelines on correcting alignment of the seal to the plate and adjusting seal quality. (See Appendix E, “Troubleshooting”.)
- If voids (incomplete melting between the seal material and the plate) are observed, remove the seal, blot dry any visible droplets on the grids, then reseal the plate.
- Follow recommendation for cleaning and preventive maintenance that is outlined in the instrument user guide.

Shaker guidelines

- Ensure that the plates are seated properly into the shaker before running the shaker.
- Always balance the shaker when mixing.
- Ensure that you use the correct type of shaker that is specified in the protocol. Shaker types and parameters are not interchangeable.

Centrifuge guidelines

- Always balance the rotor when performing the centrifugation step.
- Ensure that the centrifuge temperature is set to the correct temperature before the start of a procedure.
- Ensure that there is sufficient time for the centrifuge to cool down to 4°C before centrifuging the Precipitation Plates.
- The bottom of the rotor buckets must be soft rubber to help ensure that the 96-deepwell plates do not crack. Do not centrifuge plates in metal or hard plastic buckets.

Incubator guidelines

- Put the plates on the racks of the incubator and do not stack plates directly on top of each other.
- Do not place the plates directly on the bottom surface of the incubator.
- After the batch of 8 plates are placed into oven for fragmentation, do not disturb the oven by opening and closing the door during the 30-minute incubation period.

Multidrop™ Combi Reagent Dispenser operations

Multidrop™ Combi protocol names and parameters

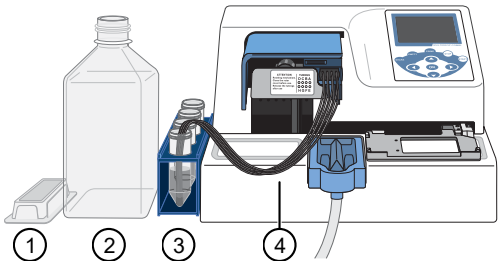
Table 8 Protocol names and parameters for the Axiom™ Propel Workflow, 96-Array Format

Protocol name	Cassette	Plate type	Set point	Speed	Plate name
96-Den-20	Standard tubing	96 DW (44mm)	20 µL	High	96-deepwell plate
96-Neu-130	Standard tubing	96 DW (44mm)	130 µL	Medium	96-deepwell plate
96-Amp-230	Standard tubing	96 DW (44mm)	230 µL	Medium	96-deepwell plate
96-Frag-60	Standard tubing	96 DW (44mm)	60 µL	Medium	96-deepwell plate
96-Stop-20	Standard tubing	96 DW (44mm)	20 µL	High	96-deepwell plate
96-Pre-220	Standard tubing	96 DW (44mm)	220 µL	Medium	96-deepwell plate
96-Iso-660	Standard tubing	96 DW (44mm)	660 µL	Medium	96-deepwell plate
96-Res-35	Standard tubing	96 DW (44mm)	35 µL	Medium	96-deepwell plate
96-Hyb-80	Standard tubing	96 DW (44mm)	80 µL	Medium	96-deepwell plate
96-Scan-150	Standard tubing	96_Scan_Tray	150 µL	High	GeneTitan™ scan tray
96-Stain-110	Standard tubing	96_Stain_Tray	110 µL	High	GeneTitan™ stain tray
96-QC-Dil-55	Standard tubing	96 standard (15mm)	55 µL	Medium	MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, Blue
96-QC-OD-90	Standard tubing	96 standard (15mm)	90 µL	Medium	OD plate
96-QC-Gel-150	Standard tubing	96 standard (15mm)	150 µL	Medium	MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, Blue

Materials required

- 50-mL conical tubes, labeled "Water 1", "Water 2", "Air", and "70% ethanol"
- Tube rack for the four 50-mL conical tubes
- 25-mL reservoir
- 70% ethanol
- DI water
- Large clean DI water bottle for cleaning the exterior of the tubing. The size of bottle must be sufficient to submerge the tubing bundle and >80% of the length of the input tubing. The 1 L Axiom™ Water container is sufficient.

IMPORTANT! Only use DI water one time to clean the exterior of *one* cassette. Discard the rinse water after use. Use fresh DI water for each individual cassette. Rinse the bottle with copious amount of DI water then fill with DI water for the cleaning step.



① 25-mL reservoir
② D. I. water bottle
③ Tube rack with four 50-mL prepared conical tubes
④ Multidrop™ Combi tubing bundle submerged at the bottom of the conical tube.

Start up the Multidrop™ Combi

The following procedure is required when the Multidrop™ cassette is used for the first time that day.

1. Install the Multidrop™ cassette and power on the Multidrop™ Combi instrument.
2. Place the tubing bundle into the "Water 1" conical tube, then press the **PRIME** button for 5 seconds (~10 mL).
3. Observe the fluid dispense to ensure that the liquid is being dispensed through all the nozzles and that there are no air bubbles in the tubing.

Perform gravimetric checks

Gravimetric checks on the Multidrop™ Combi Reagent Dispenser must be conducted daily before running the target preparation stages. Perform the check on all the dedicated reagent cassettes used for that day.

Perform gravimetric checks after the Multidrop™ Combi is primed with water, except for the isopropanol cassette. Prime the isopropanol cassette and allow the isopropanol to sit in the tubing for at least 15 minutes before dispensing. (See Table 10.)

1. On the Multidrop™ Combi, select the protocol to be used. (See “Multidrop™ Combi protocol names and parameters” on page 53.)
2. Obtain an empty 96-well plate that is specified in the protocol.
3. Place the empty 96-well plate on a scale, then tare the scale.
4. Load the tared 96-well plate onto the Multidrop™ plate carrier, then run the protocol.
5. Observe the fluid dispense to ensure that the liquid is being dispensed through all the nozzles.
6. After the dispensing is complete, remove the plate then weigh the plate containing the liquid. The weight must be within the specified range that is listed. (See “Range guidelines for gravimetric tests” on page 56.)
7. Repeat step 2—step 5 to obtain 2 more readings from the same protocol.
8. Do one of the following:
   - If the weights are within the specified range, proceed to priming the cassette with the appropriate Axiom™ reagent or reagent master mix.
   - If the weights are outside of the specified range, adjust the set point at 5-µL increment, then repeat the gravimetric check. See the *Thermo Scientific™ Multidrop™ Combi User Manual* for instructions on applying the set point. (See “Related documentation” on page 287.)

Range guidelines for gravimetric tests

Table 9 Water weight checks for "Stage 1: Amplify the genomic DNA" on page 64 (weight per plate, g) .

	Protocol name		
	96-Den-20	**96-Neu-130**	**96-Amp-230**
Cassette tubing	Standard	Standard	Standard
Plate type	96-deepwell plate	96-deepwell plate	96-deepwell plate
Liquid to use	DI water	DI water	DI water
Volume per well	**20 µL**	**130 µL**	**230 µL**
Lower limit	1.82	11.9	21.0
Target, g	1.92	12.5	22.1
Upper limit	2.02	13.1	23.2

Table 10 Weight checks for "Stage 2: Fragment the DNA" on page 71 and "Stage 3: Precipitate the DNA" on page 79 (weight per plate, g).

	Protocol name			
	96-Frag-60	**96-Stop-20**	**96-Pre-220**	**96-Iso-660**
Cassette tubing	Standard	Standard	Standard	Standard
Plate type	96-deepwell plate	96-deepwell plate	96-deepwell plate	96-deepwell plate
Liquid to use	DI water	DI water	DI water	Isopropanol[1]
Volume per well	**60 µL**	**20 µL**	**220 µL**	**660 µL**
Lower limit	5.47	1.82	19.2	44.7
Target, g	5.76	1.92	21.1	63.3
Upper limit	6.05	2.02	23.0	53.7

[1] Allow the isopropanol to sit in the tubing for at least 15 minutes before dispensing.

Table 11 Water weight checks for "Stage 5: Resuspend the pelleted DNA and prepare for hybridization" on page 85 **(weight per plate, g).**

	Protocol name	
	96-Res-35	**96-Hyb-80**
Cassette tubing	Standard	Standard
Plate type	96-deepwell plate	96-deepwell plate
Liquid to use	DI water	DI water
Volume per well	**35 µL**	**80 µL**
Lower limit	3.19	7.30
Target, g	3.36	7.68
Upper limit	3.53	8.06

Table 12 Water weight checks for "Stage 5A: In-process QC" on page 95 **(weight per plate, g).**

	Protocol name		
	96-QC-Dil-55	**96-QC-OD-90**	**96-QC-Gel-150**
Cassette tubing	Standard	Standard	Standard
Plate type	MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, Blue	Greiner Bio-One™ 96-Well UV-Star™ Plate, Flat Bottom, Chimney Style, Clear	MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, Blue
Liquid to use	DI water	DI water	DI water
Volume per well	**55 µL**	**90 µL**	**150 µL**
Lower limit	5.02	8.21	13.7
Target, g	5.28	8.64	14.4
Upper limit	5.54	9.07	15.1

Table 13 Water weight checks for "Stage 7: Preparing ligation, stain, stabilization reagent trays, and scan trays for the GeneTitan™ MC Instrument" on page 113 **(weight per plate, g).**

	Protocol name	
	96-Scan-150	**96-Stain-110**
Cassette tubing	Standard	Standard
Plate type	GeneTitan™ scan tray	GeneTitan™ stain tray
Liquid to use	DI water	DI water
Volume per well	**150 µL**	**110 µL**
Lower limit	14.0	10.1
Target, g	14.4	10.6
Upper limit	15.4	11.1

Prime the cassette

For each stage conducted at a Multidrop™ Combi Reagent Dispenser, the following steps are performed.

- Reagents and reagent master mixes are prepared before these steps.
- Before priming a reagent on the Multidrop™ Combi, always clean the cassette tubing with DI water and purge with air.

1. Lift the tubing bundle from the liquid surface, then press the **PRIME** button for ~5 seconds until DI water is purged from the tubing.

2. Prime the cassettes.
   a. Place the standard tubing bundle into the bottom of the reagent reservoir, then press the **PRIME** button until the reagent is seen flowing through all the tubing and liquid is dispensed out of all the tips. Continue holding the **PRIME** button for another 3—5 seconds to allow ~6—10 mL of reagent purging through the standard tubing cassette.

Note: Do not exceed 6 seconds of priming after the reagent starts to come out from the nozzles. Excessive priming can cause loss of reagent and lead to insufficient reagent volume for the target preparation stages.

Multidrop™ Combi cassette flush requirement

To avoid the potential for target plate batch effects, it is important to thoroughly flush reagents from the Multidrop™ Combi cassettes after each batch of plates.

For the Axiom™ Propel Fast Wash Workflow, 96-Array Format, there are 2 flush procedure depending on the reagent used in the Multidrop™ Combi.

- General reagent flush
- Isopropanol flush

Flush the Multidrop™ Combi after general reagent use

The following procedure is required to flush reagents from the Multidrop™ Combi cassette between processing plate batches.

IMPORTANT! This procedure is used for all cassettes with reagents except for the cassette used to dispense isopropanol.

---

1. Lift the tubing bundle from the liquid surface, then press the **PRIME** button for ~5 seconds until the reagent is purged out.
2. Move the tubing bundle to the “Water 1” conical tube, then press the **PRIME** button for ~5 seconds (~10 mL).
3. Lift the tubing bundle from the liquid surface then press **PRIME** for ~5 seconds until the DI water is purged out.
4. Move the tubing bundle to the “Water 2” conical tube, then press the **PRIME** button for ~5 seconds (~10 mL).
5. Lift the tubing bundle from the liquid surface, then press the **PRIME** button for ~5 seconds until the DI water is purged out. Move the tubing bundle into the “Air” conical tube.
6. Place the cassette in the “Rest” position.

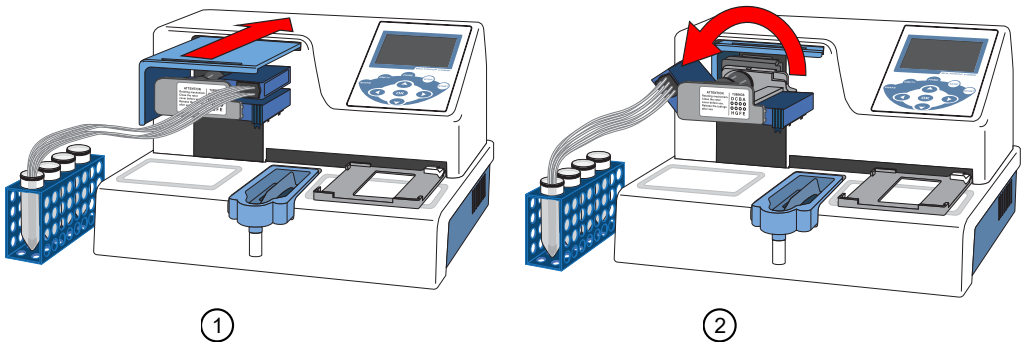


Figure 7 Multidrop™ Combi cassette “Rest” position.

① Push the cover back into the instrument.
② Move the pipetting head up and to the left into the rest position.

Flush the Multidrop™ Combi after isopropanol use

The following procedure is required to flush isopropanol from the Multidrop™ Combi cassette between processing plate batches.

1. Lift the tubing bundle from the liquid surface, then press the **PRIME** button for ~5 seconds until the reagent is purged out.
2. Place the cassette in the "Rest" position. (See Figure 7.)

Note: Before next dispensing, allow the isopropanol to sit in the tubing for at least 15 minutes.

Multidrop™ Combi Reagent Dispenser shutdown

After the last batch of plates has completed the Axiom™ Propel Fast Wash Workflow, the Multidrop™ Combi must be shut down using the correct procedure, depending on the reagent used.

- General reagent shutdown
- Isopropanol shutdown

Shut down the Multidrop™ Combi Reagent Dispenser after general reagent use

This procedure is used for all cassettes except for the cassette used to dispense isopropanol.

1. Prime the reagent out by pressing the **PRIME** button for ~5 seconds while lifting tubing bundle from solution.
2. Rinse the external tubing by submerging the tubing bundle into a large bottle that is filled with DI water, then dunk up and down 10 times. Let the tubing bundle hang inside the bottle above the liquid surface, then proceed to the next step.

   IMPORTANT! Dedicate a large bottle for each reagent cassette to prevent cross-contamination.

3. Backflush the cassette.
   a. Press the **EMPTY** button to raise the cassette.
   b. Fill a 25-mL reservoir with DI water.
   c. Submerge all tips into the filled 25-mL reservoir, then press the **EMPTY** button for ~5 seconds.
   d. Remove the reservoir from the tips. Then press the **EMPTY** button for ~5 seconds until all the water is out of the tubing.
   e. Remove the tubing bundle, then shake off excess water.
4. Place the tubing bundle into the "Water 1" conical tube, then press the **PRIME** button for ~10 seconds (20 mL).

5. Lift the tubing bundle from the liquid surface then press the **PRIME** button for ~5 seconds until the DI water is purged out.
6. Move the tubing bundle to the "Water 2" conical tube, then press the **PRIME** button for ~10 seconds (20 mL).
7. Lift the tubing bundle from the liquid surface, then press the **PRIME** button for ~5 seconds until the DI water is purged out.
8. Move the tubing bundle to the "70% EtOH" conical tube, then press the **PRIME** button for ~5 seconds (10 mL).
9. Lift the tubing bundle from the liquid surface then, press the **PRIME** button for ~5 seconds until the ethanol is purged out.
10. Remove the cassette, place the lid to cover the tips (nozzles), then store it.
11. Turn the power off.
12. Discard all remaining reagents, water, ethanol, and labware used for the target preparation and cleaning procedures.
13. Rinse the DI water bottle with copious amounts of DI water.

Shut down the Multidrop™ Combi Reagent Dispenser after isopropanol use

1. Lift the tubing bundle from the liquid surface, then press the **PRIME** button for ~5 seconds until the reagent is purged out.
2. Remove the cassette, place the lid to cover the tips (nozzles), then store it.
3. Turn the power off.
4. Discard all remaining isopropanol.

4 Target preparation with Multidrop™ Combi Reagent Dispensers for 8 plates

IMPORTANT! Eight gDNA Sample Plates must be prepared before starting DNA amplification. See Chapter 2, “Genomic DNA preparation”.

Stage 1: Amplify the genomic DNA

Equipment and labware required

Quantity	Item
Instruments	
3	Multidrop™ Combi Reagent Dispenser
3	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	ALPS™ 3000 Automated Microplate Heat Sealer
1	Compact Digital Microplate Shaker
1	Sorvall™ X4R Pro-MD Centrifuge (Set to room temperature.)
1	BINDER™ ED 56 or BINDER™ BD 56 • Must maintain a constant temperature of 37°C for at least 24 hours with a temperature accuracy of ±1°C
1	Vortex mixer
1	Electronic pipettor for serological pipettes
2	Digital timer
Labware and consumables	
As required	Easy Peel Seal (for ALPS™ 3000 Automated Microplate Heat Sealer)
1	50-mL conical tube
1	250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)
As required	Serological pipettes (5 mL and 50 mL)
As required	P1000 pipette

[1] Each reagent must use a dedicated cassette.

Input samples

Number of plates	Plate name	Content
8 (maximum)	gDNA Sample Plate	Genomic DNA samples prepared in Chapter 2, “Genomic DNA preparation” (in a 96-deepwell plate).

Note: The same eight 96-deepwell plates are processed in the workflow.

Reagent handling

Module 1, 8x96F reagents (Part No. 952269) are required from the Axiom™ Propel 8x96F Reagent Kit.

Thaw and prepare reagents according to the following table.

Table 14 Reagent handling for Stage 1 reagents.

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Reagent Kit Module 1 for 96F array plates only, 8x96F, –20°C	Axiom™ Propel 10X Denat Solution	951968	• Thaw at room temperature. This can be done as early as one day before use. • Vortex before master mix preparation.
	Axiom™ Propel Water	951969	• Thaw at room temperature. This can be done as early as one day before use.
	Axiom™ Propel Neutral Solution	951965	• Thaw at room temperature. This can be done as early as one day before use. • Vortex, then set aside until use.
	Axiom™ Propel Amp Solution	951966	• Thaw at room temperature. This can be done as early as one day before use. [1] • Vortex before master mix preparation.
	Axiom™ Propel Amp Enzyme	951967	• Keep at –20°C until ready to use. • Flick 5 times before master mix preparation.

[1] The thawed Axiom™ Propel Amp Solution solution may have white precipitate on the bottom of the bottle. Ensure that the precipitate dissolves into the solution after vortexing.

Prepare Denaturation Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 14.)

1. In a 50-mL conical tube, add reagents in the order shown.

Table 15 Denaturation Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Water	34.0 mL	50-mL serological pipette
2	Axiom™ Propel 10X Denat Solution	3.8 mL	5-mL serological pipette
	Total volume	**37.8 mL**	

2. Ensure that the cap of the conical tube is closed tightly, then vortex the Denaturation Master Mix for 5 seconds. Leave at room temperature until use.

Note: The Denaturation Master Mix can be stored at room temperature and used within 12 hours of its preparation.

Prepare the Axiom™ Propel Neutral Solution

Ensure that the Axiom™ Propel Neutral Solution is vortexed before use.

1. Use the Axiom™ Propel Neutral Solution bottle for Multidrop™ dispensing.

Table 16 Axiom™ Propel Neutral Solution for 8x96-format plates.

Reagent	Quantity	Transfer method
Axiom™ Propel Neutral Solution	Entire bottle	Use straight from bottle[1]

[1] The Axiom™ Propel Neutral Solution reagent bottle can be used for Multidrop™ Combi dispensing.

2. Leave at room temperature. Vortex before use.

Prepare the Amplification Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 14.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 17 Amplification Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Amp Solution	210.0 mL	50-mL serological pipette
2	Axiom™ Propel Amp Enzyme	4.0 mL 0.7 mL	5-mL serological pipette P1000 pipette
	Total volume	**214.7 mL**	

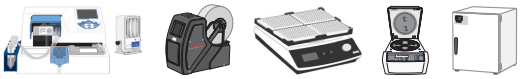
Note: The thawed Axiom™ Propel Amp Solution may have white precipitate on the bottom of the bottle. Ensure that the precipitates dissolve into the solution after vortexing.

Note: The Axiom™ Propel Amp Enzyme is a viscous solution. Pipet the solution slowly when aspirating from the reagent bottle to avoid bubble formation.

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Amplification Master Mix at least 15 times. Leave at room temperature until use.

Note: The Amplification Master Mix can be stored at room temperature and used within 2 hours of its preparation.

Stage 1 summary

Stage activities	Instruments required
• Three reagent additions with mixing—Denaturation Master Mix, Axiom™ Propel Neutral Solution, Amplification Master Mix • 10-minute denature incubation at room temperature • 22–24 hour amplification incubation at 37°C	

Perform the pre-run checklist

Before starting the workflow, the following tasks must be completed.

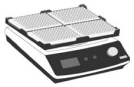
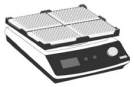
1. Multidrop™ Combi Reagent Dispenser:
   a. Ensure that the Multidrop™ Combi startup procedure has been performed. (See “Start up the Multidrop™ Combi” on page 54.)
   b. Perform gravimetric checks before running the workflow. (See “Perform gravimetric checks” on page 55.)
   c. Prime the Multidrop™ cassette with reagent. (See “Prime the cassette” on page 59.)
   d. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)
2. ALPS™ 3000 Automated Microplate Heat Sealer:
   a. Power on the ALPS™ 3000 Automated Microplate Heat Sealer, then allow it to reach 150°C.
   b. If the sealer is being used at the start of the day for this stage, do the following:
      • Allow 15 minutes for the sealer to reach 150°C.
      • Perform the sealing operation 2 to 3 times, then check to ensure that the seal quality and seal alignment are correct before starting the workflow.
3. If the gDNA Sample Plates are frozen, thaw them on the benchtop at room temperature, then centrifuge at 675 × *g* for 30 seconds to get all the droplets down.

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

Stage 1: Amplify the genomic DNA

For an 8-plate workflow, complete 2 cycles of 4 plates per cycle.

Denaturation Master Mix

Plates 1 2 3 4	Plates 5 6 7 8
①  Dispense Denaturation Master Mix • Method: 96-Den-20 • Dispense volume: 20 µL	⑥  Dispense Denaturation Master Mix • Method: 96-Den-20 • Dispense volume: 20 µL
②  Seal • Settings: 150°C, 2.5 seconds	⑦  Seal • Settings: 150°C, 2.5 seconds
③  Shake • Settings: 1,100 rpm, 30 seconds	⑧  Shake • Settings: 1,100 rpm, 30 seconds
④  Time • Start 10-minute timer **Note:** This step is time sensitive. Immediately proceed to the next step when the timer expires.	⑨  Time • Start 10-minute timer
⑤  Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds • Leave plates at room temperature after centrifugation until the timer alarms. • Continue to Step 6.	⑩  Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds • Leave plates at room temperature after centrifugation until the timer alarms.
When the 10-minute timer alarms, proceed *immediately* to the Axiom™ Propel Neutral Solution step.	• When the 10-minute timer alarms, proceed *immediately* to the "Axiom™ Propel Neutral Solution" step. • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Axiom™ Propel Neutral Solution

Plates 1 2 3 4	Plates 5 6 7 8
①  Dispense Axiom™ Propel Neutral Solution • Method: 96-Neu-130 • Dispense volume: 130 µL	⑤  Dispense Axiom™ Propel Neutral Solution • Method: 96-Neu-130 • Dispense volume: 130 µL
②  Seal • Settings: 150°C, 2.5 seconds	⑥  Seal • Settings: 150°C, 2.5 seconds
③  Shake • Settings: 1,100 rpm, 30 seconds	⑦  Shake • Settings: 1,100 rpm, 30 seconds
④  Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds • Continue to the Amplification Master Mix step	⑧  Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
	 • Continue to the Amplification Master Mix step. • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Amplification Master Mix

Plates 1 2 3 4

① 
Dispense Amplification Master Mix
- Method: 96-Amp-230
- Dispense volume: 230 µL

② 
Seal
- Settings: 150°C, 2.5 seconds

③ 
Shake
- Settings: 1,100 rpm, 30 seconds

④ 
Centrifuge
- Centrifuge at room temperature
- Settings: 675 × *g*, 30 seconds

⑤ 
Incubate
- Temperature: 37°C
- Time: 23 ±1hour
- Volume = 400 µL/well

Plates 5 6 7 8

⑥ 
Dispense Amplification Master Mix
- Method: 96-Amp-230
- Dispense volume: 230 µL

⑦ 
Seal
- Settings: 150°C, 2.5 seconds

⑧ 
Shake
- Settings: 1,100 rpm, 30 seconds

⑨ 
Centrifuge
- Centrifuge at room temperature
- Settings: 675 × *g*, 30 seconds

⑩ 
Incubate
- Temperature: 37°C
- Time: 23 ±1hour
- Volume = 400 µL/well

1 2 3 4 5 6 7 8

⑪
- Proceed directly to "Stage 2: Fragment the DNA" on page 71, or
- Store the Amplification Plates at –20°C. The frozen post-amplification plates can be stored at –20°C for up to 1 week.
- Perform the appropriate Multidrop™ procedure:
  - Flush between batches. See page 60.
  - Shutdown. See page 61.

Stage 2: Fragment the DNA

Equipment and labware required

Quantity	Item
Instruments	
2	Multidrop™ Combi Reagent Dispenser
2	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	ALPS™ 3000 Automated Microplate Heat Sealer
1	Compact Digital Microplate Shaker
1	Sorvall™ X4R Pro-MD Centrifuge (set to room temperature)
1	BINDER™ ED 56 or BINDER™ BD 56 (set to 37°C) • Must maintain a constant temperature of 37°C for at least 24 hours with a temperature accuracy of ±1°C.
1	BINDER™ ED 56 or BINDER™ BD 56 (set to 65°C)[2] • Must maintain a constant temperature of 65°C for at least 24 hours with a temperature accuracy of ±1°C.
1	Vortex mixer
1	Mini centrifuge
1	Electronic pipettor
1	P1000 pipettes
2	Digital timer
Labware and consumables	
As required	Easy Peel Seal (for ALPS™ 3000 Automated Microplate Heat Sealer)
1	250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)
As required	P1000 pipette tips
As required	Serological pipettes (10 mL and 50 mL)

[1] Each reagent must use a dedicated cassette.
[2] Only required when processing frozen Amplification Sample Plates.

Input samples

Number of plates	Plate name	Content
8 (maximum)	Amplification Plate	Amplified gDNA samples prepared in "Stage 1: Amplify the genomic DNA" on page 64 (in a 96-deepwell plate).

Reagent and plate handling

Module 2-1, 8x96F and Module 2-2, 8x96F reagents are required from the Axiom™ Propel 8x96F Reagent Kit:

- Axiom™ Propel Reagent Kit Module 2-1 for 96F array plates only, Part No. 952337
- Axiom™ Propel Reagent Kit Module 2-2 for 96F array plates only, Part No. 952338

Thaw and prepare reagents according to the following table.

Table 18 Reagent and plate handling for Stage 2 and Stage 3.

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Reagent Kit Module 2-1 for 96F array plates only –20°C	Axiom™ Propel 10X Frag Buffer	951971	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Frag Enzyme	951972	• Keep at –20°C until ready to use. • Flick 5 times, then perform brief centrifuge before master mix preparation.
	Axiom™ Propel Precip Solution 2	951970	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
Axiom™ Propel Reagent Kit Module 2-2 for 96F array plates only 4°C	Axiom™ Propel Frag Diluent	951975	• Remove from 4°C and equilibrate to room temperature on day of use. • Vortex before master mix preparation.
	Axiom™ Propel Frag Reaction Stop	951976	• Remove from 4°C and equilibrate to room temperature on day of use. • Vortex before use.
	Axiom™ Propel Precip Solution 1	951977	• Remove from 4°C and equilibrate to room temperature on day of use. • Vortex before master mix preparation.
Amplified sample plates			

Table 18 Reagent and plate handling for Stage 2 and Stage 3. *(continued)*

Module	Reagent	Component Part No.	Treatment
• If proceeding directly from the 22—24-hour amplification, continue with the fragmentation step that is described in this chapter. • If the Amplification Plates have been frozen: **a.** Place the frozen plates at room temperature to thaw. To shorten the thawing time, the frozen plates can be placed into a room temperature deionized water bath. The thawing time is ~1 hour. **b.** Wipe off excess water on the outside of the plates before continuing to the next step. **c.** Centrifuge at 675 × *g* for 30 seconds. **d.** Place the thawed Amplification Plates in the 65°C oven and incubate for 20 minutes. **e.** Transfer the Amplification Plates from the 65°C oven to the 37°C oven and incubate for 45 minutes. **Note:** Place plates on the rack in a single layer—do not stack plates directly on top of one another.			

Prepare the Fragmentation Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 18.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 19 Fragmentation Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel 10X Frag Buffer	57.0 mL	50-mL serological pipette
2	Axiom™ Propel Frag Diluent	12.8 mL	10-mL serological pipette
3	Axiom™ Propel Frag Enzyme	1.2 mL	P1000 pipette
	Total volume	**71.0 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Fragmentation Master Mix at least 15 times. Leave at room temperature until use.

Note: Use the Fragmentation Master Mix within 1 hour of its preparation.

Prepare the Axiom™ Propel Frag Reaction Stop

Ensure that the Axiom™ Propel Frag Reaction Stop is vortexed before use.

1. Use the Axiom™ Propel Frag Reaction Stop bottle for Multidrop™ dispensing.

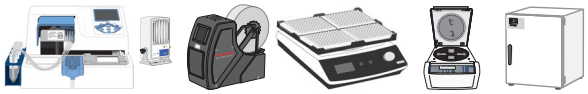
Table 20 Axiom™ Propel Frag Reaction Stop for 8x96-format plates.

Reagent	Quantity	Transfer method
Axiom™ Propel Frag Reaction Stop	Entire bottle	Use straight from bottle[1]

[1] The Axiom™ Propel Frag Reaction Stop reagent bottle can be used for Multidrop™ Combi dispensing.

2. Leave at room temperature. Vortex before use.

Stage 2 summary

Stage activities	Instruments required
• Two reagent additions with mixing—Fragmentation Master Mix, Axiom™ Propel Frag Reaction Stop • 30-minute fragmentation incubation at 37°C	

Perform the pre-run checklist

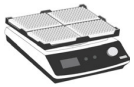
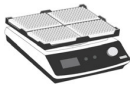
Before starting the workflow, the following tasks must be completed.

1. Multidrop™ Combi Reagent Dispenser:
   a. Ensure that the Multidrop™ Combi startup procedure has been performed. (See "Start up the Multidrop™ Combi" on page 54.)
   b. Perform gravimetric checks before running the workflow. (See "Perform gravimetric checks" on page 55.)
   c. Prime the Multidrop™ cassette with reagent. (See "Prime the cassette" on page 59.)
   d. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)
2. ALPS™ 3000 Automated Microplate Heat Sealer:
   a. Power on the ALPS™ 3000 Automated Microplate Heat Sealer, then allow it to reach 150°C.
   b. If the sealer is being used at the start of the day for this stage, do the following:
      • Allow 15 minutes for the sealer to reach 150°C.
      • Perform the sealing operation 2 to 3 times, then check to confirm that the seal quality and seal alignment are correct before starting the workflow.

Stage 2: Fragment the DNA

For an 8-plate workflow, complete 2 cycles of 4 plates per cycle.

Fragmentation Master Mix

Plates 1 2 3 4	Plates 5 6 7 8
①  Dispense Fragmentation Master Mix • Method: 96-Frag-60 • Dispense volume: 60 µL	⑧  Dispense Fragmentation Master Mix • Method: 96-Frag-60 • Dispense volume: 60 µL
②  Seal • Settings: 150°C, 2.5 seconds	⑨  Seal • Settings: 150°C, 2.5 seconds
③  Shake • Settings: 1,100 rpm, 30 seconds	⑩  Shake • Settings: 1,100 rpm, 30 seconds
④  Time • Start 30 minute timer	⑪  Time • Start 30 minute timer
⑤  Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds	⑫  Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
⑥  Incubate • Temperature: 37°C • Time: 30 minutes (Time begins at completion of shaking step.)	⑬  Incubate • Temperature: 37°C • Time: 30 minutes (Time begins at completion of shaking step.)

(continued)

Fragmentation Master Mix				
⑦		When the 30-minute timer alarms, proceed *immediately* to "Axiom™ Propel Frag Reaction Stop" step.		⑭ • When the 30-minute timer alarms, proceed *immediately* to "Axiom™ Propel Frag Reaction Stop" step. • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Axiom™ Propel Frag Reaction Stop

Plates 1 2 3 4

①  Dispense Axiom™ Propel Frag Reaction Stop
- Method: 96-Stop-20
- Dispense volume: 20 µL

②  Seal
- Settings: 150°C, 2.5 seconds

③  Shake
- Settings: 1,100 rpm, 30 seconds

④  Centrifuge
- Centrifuge at room temperature
- Settings: 675 × *g*, 30 seconds

Plates 5 6 7 8

⑤  Dispense Axiom™ Propel Frag Reaction Stop
- Method: 96-Stop-20
- Dispense volume: 20 µL

⑥  Seal
- Settings: 150°C, 2.5 seconds

⑦  Shake
- Settings: 1,100 rpm, 30 seconds

⑧  Centrifuge
- Centrifuge at room temperature
- Settings: 675 × *g*, 30 seconds

1 2 3 4 5 6 7 8

⑨
- Volume = 480 µL
- Continue to "Stage 3: Precipitate the DNA" on page 79
- Perform the appropriate Multidrop™ procedure:
  - Flush between batches. See page 60.
  - Shutdown. See page 61.

Stage 3: Precipitate the DNA

Equipment and labware required

Quantity	Item
Instruments	
2	Multidrop™ Combi Reagent Dispenser
2	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	ALPS™ 3000 Automated Microplate Heat Sealer
1	VWR Signature™ High-Speed Microplate Shaker
1	Freezer, –20°C
1	P1000 pipette
1	Fume hood
Labware and consumables	
As required	Easy Peel Seal (for ALPS™ 3000 Automated Microplate Heat Sealer)
1	Glass or polypropylene bottle, minimum 1,000 mL capacity (for isopropanol dispensing)
As required	P1000 pipette tips

[1] Each reagent must use a dedicated cassette.

Input samples

Number of plates	Plate name	Content
8 (maximum)	Fragmentation Plate	Fragmented DNA samples prepared in “Stage 2: Fragment the DNA” on page 71 (in a 96-deepwell plate).

Prepare Precipitation Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 18.)

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

1. Use the Axiom™ Propel Precip Solution 1 bottle for Multidrop™ dispensing.

Table 21 Precipitation Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Precip Solution 1	Entire bottle	Use straight from bottle[1]
2	Axiom™ Propel Precip Solution 2	Entire bottle	P1000 pipette
	Total volume	**~215 mL**	

[1] The Axiom™ Propel Precip Solution 1 reagent bottle can be used for Multidrop™ Combi dispensing.

2. Ensure that the cap of the Axiom™ Propel Precip Solution 1 bottle is closed tightly, then invert the Precipitation Master Mix at least 15 times. Leave at room temperature until use.

Note: The Precipitation Master Mix can be stored at room temperature and used within 12 hours of its preparation.

Prepare the isopropanol

Fill an appropriately sized container with the isopropanol.

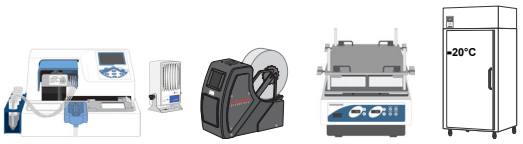
Table 22 Isopropanol for 8x96-format plates.

Reagent	Quantity
Isopropanol	850 mL[1]

[1] The quantity of isopropanol required includes usage for priming and gravimetric checks. If a flat bottom bottle is used, add 20 mL to account for the dead volume of the bottle.

IMPORTANT! The isopropanol must be primed through the cassette tubing, then allowed to equilibrate for a minimum of 15 minutes. Equilibrating the isopropanol is a highly critical step that results in the most accurate dispense volumes. Failure to follow this requirement can result in compromised assay performance.

Stage 3 summary

Stage activities	Instruments required
• Two reagent additions with high-speed mixing—Precipitation Master Mix and isopropanol. • 16–24-hour precipitation incubation at –20°C.	

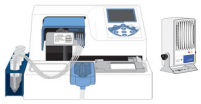
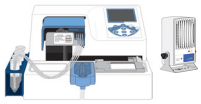
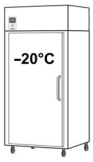
Perform the pre-run checklist

Before starting the workflow, the following tasks must be completed.

1. Multidrop™ Combi Reagent Dispenser:
   a. Ensure that the Multidrop™ Combi startup procedure has been performed. (See "Start up the Multidrop™ Combi" on page 54.)
   b. Perform gravimetric checks before running the workflow. (See "Perform gravimetric checks" on page 55.)
      Allow the isopropanol to equilibrate inside the tubing for 15 minutes before performing gravimetric checks.
   c. Prime the Multidrop™ cassette with reagent. (See "Prime the cassette" on page 59.)
   d. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)
2. ALPS™ 3000 Automated Microplate Heat Sealer:
   a. Power on the ALPS™ 3000 Automated Microplate Heat Sealer then allow it to reach 150°C.
   b. If the sealer is being used at the start of the day for this stage, do the following:
      • Allow 15 minutes for the sealer to reach 150°C.
      • Perform the sealing operation 2 to 3 times, then check to confirm that the seal quality and seal alignment are correct before starting the workflow.

Stage 3: Precipitate the DNA

For an 8-plate workflow, complete 1 cycle of 8 plates.

Precipitation		
	Plates 1 2 3 4 5 6 7 8	
1		Dispense Precipitation Master Mix • Method: 96-Pre-220 • Dispense volume: 220 µL
2		Dispense isopropanol • Method: 96-Iso-660 • Dispense volume: 660 µL
3		Seal • Settings: 150°C, 2.5 seconds
4		Shake • Settings: 2,450 rpm, 60 seconds **IMPORTANT!** Do not centrifuge the plates after shaking. Continue directly to freezing the plates at –20°C.
5		Freeze • –20°C, 16–24 hours • Volume = 1,360 µL/well • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Stage 4: Centrifuge and dry DNA pellets

Equipment required

Quantity	Item
2	Sorvall™ X4R Pro-MD Centrifuge (set at 4°C)
1	BINDER™ ED 56 or BINDER™ BD 56 (set at 37°C) • Must maintain a constant temperature of 37°C for at least 24 hours with a temperature accuracy of ±1°C.
1	Fume hood

Input samples

Number of plates	Plate name	Content
8 (maximum)	Precipitation Plate	Samples, post precipitation incubation, prepared in “Stage 3: Precipitate the DNA” (in a 96-deepwell plate).

Stage 4 summary

Stage activities	Instruments required
• Purify amplified DNA into dried pellets.	

Perform the pre-run checklist

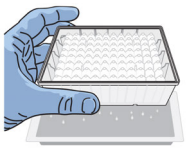
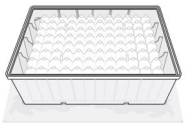
Before starting the workflow, the following must be completed.

Set each Sorvall™ X4R Pro-MD Centrifuge to 4°C, then allow the centrifuges to equilibrate before starting the centrifugation step.

Stage 4: Centrifuge and dry pellets

For an 8-plate workflow, complete 1 cycle of 8 plates. Decant under a ventilated fume hood.

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

Centrifugation		
	Plates 1 2 3 4 5 6 7 8	
①		Centrifuge at 4°C. • 3,200 × *g* for 40 minutes.
②		Gently pour off solution over a waste container and allow the liquid to drain.
③		Keep inverted for 5 minutes over laboratory tissues. Transfer the plate to a new pile of laboratory tissues twice during the 5-minute period.
④		• Dry in 37°C oven for 20 minutes. • Place the plates in the oven with wells facing up.
⑤		• Do one of the following: – Proceed directly to "Stage 5: Resuspend the pelleted DNA and prepare for hybridization" on page 85, even if some droplets of liquid remain. Leave the sample plates at room temperature. It is helpful to start preparing reagents for stage 5 during centrifuging and drying pellets. – Store the plates for resuspension later in the same day. Tightly seal the plates. – If resuspension is carried out in 4 hours, keep the plates at room temperature. – If resuspension is carried out in more than 4 hours, store the plates in a refrigerator (2–8°C). The refrigerated pellets must be equilibrated to room temperature for at least 30 minutes before proceeding to Stage 5. • To process the plates for resuspension on another day, tightly seal the plates and store at –20°C. – The frozen pellets must be equilibrated to room temperature for at least 90 minutes before proceeding to Stage 5.

Stage 5: Resuspend the pelleted DNA and prepare for hybridization

Equipment and labware required

Table 23 Equipment and labware required for Stage 5.

Quantity	Item
Instruments	
2	Multidrop™ Combi Reagent Dispenser
2	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	ALPS™ 3000 Automated Microplate Heat Sealer
1	Compact Digital Microplate Shaker
1	Sorvall™ X4R Pro-MD Centrifuge (set to room temperature)
1	Vortex mixer
1	Electronic pipettor
1	P1000 pipette
1	Fume hood
Labware and consumables	
As required	Easy Peel Seal (This is used for the ALPS™ 3000 Automated Microplate Heat Sealer.)
1	250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)
As required	Serological pipettes (5 mL and 50 mL)
As required	P1000 pipette tips

[1] Each reagent must use a dedicated cassette.

Table 24 Equipment and labware required for the Integra transfer.

Quantity	Item
Instruments	
1	VIAFLO™ 96 Base Unit or VIAFLO™ 384 Base Unit with a 96 Channel Pipetting Head (5–125 µL) and a 3 position stage installed.
Labware and consumables	
768 tips	Integra VIAFLO XYZ GripTips™
8	MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (half skirt)

Table 24 Equipment and labware required for the Integra transfer. *(continued)*

Quantity	Item
1	Corning™ Clear Polystyrene 96-Well Microplate (This is used as the plate holder for the half-skirted MicroAmp™ EnduraPlate™.)
As required	MicroAmp™ Clear Adhesive Film

Input samples

Number of plates	Plate name	Content
8 (maximum)	Pelleted Precipitation Plate	Dried DNA pellets prepared in "Stage 4: Centrifuge and dry DNA pellets" (in a 96-deepwell plate).

Reagent and plate handling

Module 2-1, 8x96F and Module 2-2, 8x96F reagents are required from the Axiom™ Propel 8x96F Reagent Kit.

- Axiom™ Propel Reagent Kit Module 2-1 for 96F array plates only, Part No. 952337
- Axiom™ Propel Reagent Kit Module 2-2 for 96F array plates only, Part No. 952338

Thaw and prepare reagents according to the following table.

Table 25 Reagent and plate handling for Stage 5.

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Reagent Kit Module 2-1 for 96F array plates only, –20°C	Axiom™ Propel Hyb Buffer	951973	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Hyb Solution 1	951974	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
Axiom™ Propel Reagent Kit Module 2-2 for 96F array plates only, 4°C	Axiom™ Propel Hyb Solution 2	951979	• Remove from 4°C and equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Resuspension Buffer	951978	• Remove from 4°C and equilibrate to room temperature on day of use. • Vortex before use.

Guidelines for pellet preparation

The equilibration of the plate of pelleted DNA, Axiom™ Propel Resuspension Buffer, and Axiom™ Propel Hyb Buffer to room temperature (18-25°C) is very critical for the success of the assay. If any of these are cooler than room temperature, pellets may not resuspend completely. This can result in compromised assay performance. Follow the guidelines below on how to work with plates with fresh, cold, or frozen pellets.

- **Fresh pellets:** A plate with fresh pellets can be kept at room temperature if proceeding with Stage 5 within 4 hours.
- **Cold pellets:** A plate with fresh pellets that are not processed within 4 hours can be transferred to a refrigerator (2-8°C) if processed during the same day. However, it is critical to equilibrate the plate to room temperature for at least 30 minutes before proceeding with Stage 5.
- **Frozen pellets:** A plate with frozen pellets must be pre-equilibrated at room temperature for at least 1.5 hours before proceeding with Stage 5.

Prepare the Axiom™ Propel Resuspension Buffer

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

1. Use the Axiom™ Propel Resuspension Buffer bottle for Multidrop™ dispensing.

Table 26 Axiom™ Propel Resuspension Buffer for 8x96-format plates.

Reagent	Quantity	Transfer method
Axiom™ Propel Resuspension Buffer	Entire bottle	Use straight from bottle[1]

[1] The Axiom™ Propel Resuspension Buffer reagent bottle can be used for Multidrop™ Combi dispensing.

2. Leave at room temperature until use.

Prepare Hybridization Master Mix

Ensure that all of the components are treated according to the reagent handling table before preparing the master mix. (See Table 25.)

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

1. In a 250-mL conical bottle, add reagents in the order shown.

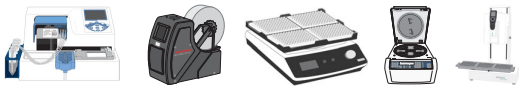
Table 27 Hybridization Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Hyb Buffer	78.0 mL	50-mL serological pipette
2	Axiom™ Propel Hyb Solution 1	0.6 mL	P1000 pipette
3	Axiom™ Propel Hyb Solution 2	9.9 mL (3.3 mL x 3)	5-mL serological pipette
	Total volume	**88.5 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, then vortex the Hybridization Master Mix for 5 seconds. Leave at room temperature until use.

Note: The Hybridization Master Mix can be stored at room temperature and used within 12 hours of its preparation.

Stage 5 summary

Stage activities	Instruments required
• Two reagent additions with mixing—Axiom™ Propel Resuspension Buffer, Hybridization Master Mix. • 10 minute shaking to resuspend the DNA pellets. • Transfer from 96-deepwell plate to half-skirted 96-well PCR plate.	

Perform the pre-run checklist

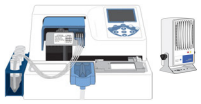
Before starting the workflow, the following tasks must be completed.

1. Multidrop™ Combi Reagent Dispenser:
   a. Ensure that the Multidrop™ Combi startup procedure has been performed. (See "Start up the Multidrop™ Combi" on page 54.)
   b. Perform gravimetric checks before running the workflow. (See "Perform gravimetric checks" on page 55.)
   c. Prime the Multidrop™ cassette with reagent. (See "Prime the cassette" on page 59.)
   d. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)
2. ALPS™ 3000 Automated Microplate Heat Sealer:
   a. Power on the ALPS™ 3000 Automated Microplate Heat Sealer then allow it to reach 150°C.
   b. If the sealer is being used at the start of the day for this stage, do the following:
      - Allow 15 minutes for the sealer to reach 150°C.
      - Perform the sealing operation 2 to 3 times, then check to ensure that the seal quality and seal alignment are correct before starting the workflow.
3. Ensure that the VIAFLO™ 96 or 384 Base Unit is clean, then power on the unit.
4. If the pellets are refrigerated or frozen, equilibrate them to room temperature before the resuspension step. (See "Guidelines for pellet preparation" on page 87.)

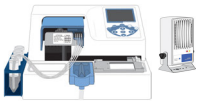
Note: The equilibration of pelleted DNA and Axiom™ Propel Resuspension Buffer to room temperature (18°C to 25°C) is critical for the resuspension process. When either is cooler than room temperature, pellets may not resuspend completely, which can result in lower performance.

Stage 5: Resuspend the pelleted DNA and prepare for hybridization

For an 8-plate workflow, complete 1 cycle of 8 plates.

Axiom™ Propel Resuspension Buffer		
	Plates 1 2 3 4 5 6 7 8	
①		Dispense Axiom™ Propel Resuspension Buffer • Method: 96-Res-35 • Dispense volume: 35 µL
②		Seal • Settings: 150°C, 2.5 seconds
③		Shake • Settings: 1,100 rpm, 10 minutes
④		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
⑤		• Continue to "Hybridization Master Mix" step. • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

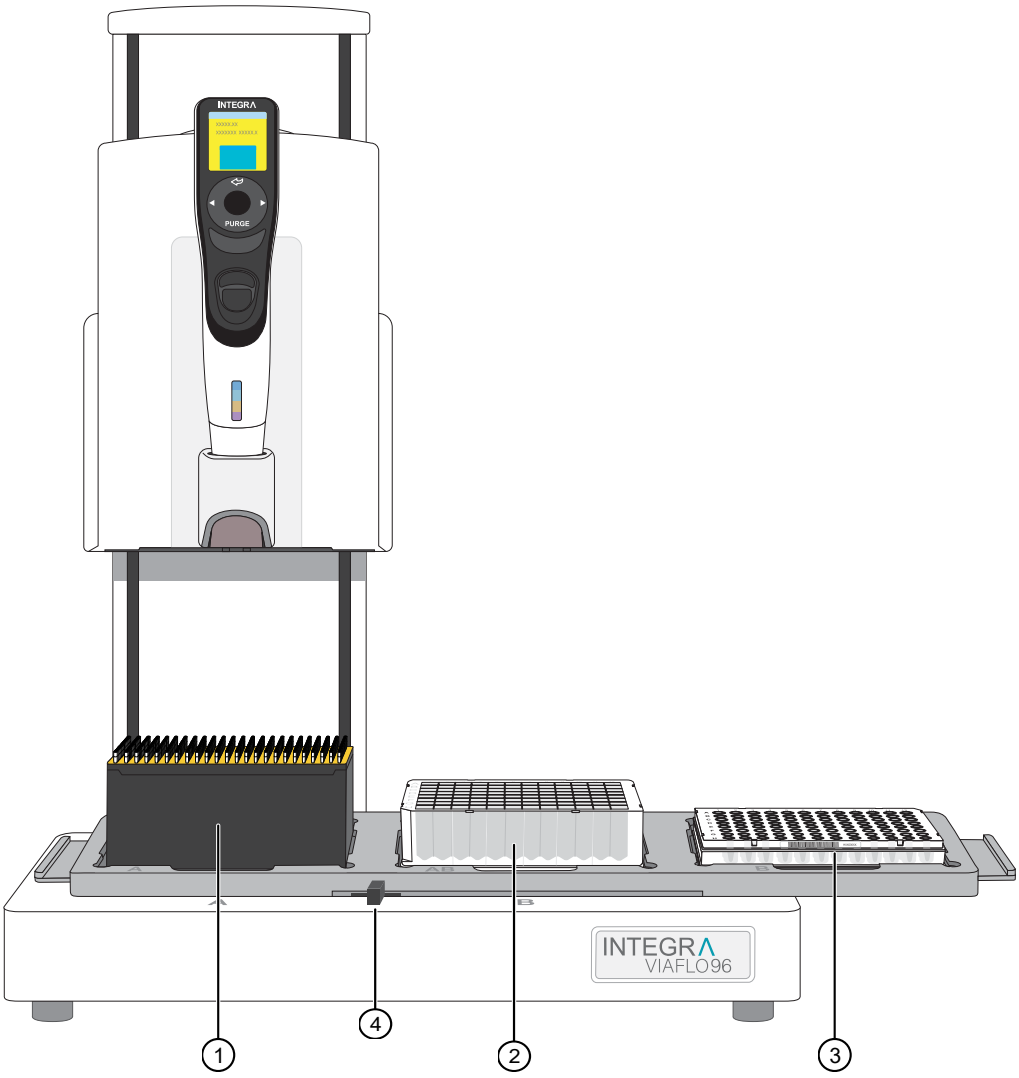
CAUTION! It is recommended that the remainder of the steps in this stage be performed under a fume hood.

Hybridization Master Mix		
	Plates 1 2 3 4 5 6 7 8	
1		Dispense Hybridization Master Mix • Method: 96-Hyb-80 • Dispense volume: 80 µL
2		Seal • Settings: 150°C, 2.5 seconds
3		Shake • Settings: 1,100 rpm, 30 seconds
4		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
5		• Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61. • Continue to: – "Transfer the hybridization-ready target to the half-skirted 96-well PCR plate" on page 92, then: – (*Recommended*) "Stage 5A: In-process QC" on page 95, and/or – "Stage 6: Denature the target and transfer to hybridization tray" on page 104

Transfer the hybridization-ready target to the half-skirted 96-well PCR plate

The *DW TO PCR* VIAFLO method is a whole plate transfer of 115 µL of the hybridization-ready target in a 96-deepwell plate to a clean, labeled half-skirted 96-well PCR plate (MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode).

1. Press the **Run** button *twice* to home the VIAFLO stage and pipetting head.
2. Place labware on the VIAFLO stage as shown in the following image.



① 384 tips
② 96-deepwell hybridization-ready sample plate
③ Half-skirted 96-well PCR plate on a plate holder
④ Stage slider

3. Slowly move the 3-position stage to the *right* for tip loading.

4. Load tips to the 96-head by adjusting the stage slider.
   a. Adjust the stage slider to the left or right, depending on the tips available for use.
      - Right—moves stage back to pick up quadrant 1 or quadrant 2 tips.
      - Left—moves stage forward to pick up quadrant 3 or quadrant 4 tips.

   b. Place one hand on the control unit handle. Maintain contact with the handle throughout the tip loading step.

   c. Gently lower the 96-head into the tip box and guide it into a set of 96 tips.

   d. After the tips are properly aligned, the indicator light of the tip load button blinks. Use the opposite hand and firmly press down on the tip load button, located at the top of the 96-head.

   e. Release the button when you hear a click. The indicator light on the button remains on.

   f. Using the control unit handle, lift the head away from the platform to provide clearance for stage loading or positioning.

5. To start method, go to the **Main Menu**, then select **Custom Programs**.

6. Under **Custom Programs**, select **DW to PCR**.

7. Move the stage slider to the center, move the 3-position stage to the *left*, then allow the 96-head to hover over the 96-deepwell sample plate.

① Tips
② 96-deepwell hybridization-ready sample plate
③ Half-skirted 96-well PCR plate on a plate holder
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

8. Press the **Run** button to run the method.
The instrument aspirates the hybridization-ready samples from the 96-deepwell sample plate, then transfers them into the half-skirted 96-well PCR plate.

9. After the run is complete, remove the 96-deepwell sample plate from position 2. Inspect the 96-deepwell sample plate to ensure that all of the samples have been transferred to the half-skirted 96-well PCR plate.

10. Eject and discard tips.

11. Proceed to "Stage 5A: In-process QC" on page 95 if running in-process QC.

Stage 5A: In-process QC

Equipment and labware required

Quantity	Item
Instruments	
1	VIAFLO™ 96 Base Unit or VIAFLO™ 384 Base Unit with a 96 Channel Pipetting Head (5–125 µL) and a 3 position stage installed.
1	Multidrop™ Combi Reagent Dispenser
2	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	Sorvall™ X4R Pro-MD Centrifuge (Set to room temperature.)
1	Vortex mixer
1	Fume hood
Labware and consumables	
768 tips	Integra VIAFLO XYZ GripTips™
16	MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, Blue
1	Corning™ Clear Polystyrene 96-Well Microplate (This is used as the plate holder for the half-skirted MicroAmp™ EnduraPlate™.)
8	Greiner Bio-One™ 96-Well UV-Star™ Plate, Flat Bottom, Chimney Style, Clear
As required	MicroAmp™ Clear Adhesive Film

[1] Each reagent must use a dedicated cassette.

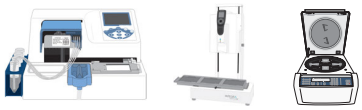
Input samples

Number	Plate name	Content
8 (maximum)	Hyb-Ready Plate	Hybridization-ready target DNA prepared in "Stage 5: Resuspend the pelleted DNA and prepare for hybridization" (in a MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode—half skirt)

Reagents required

Reagent	Volume
UltraPure™ DNase/RNase-Free Distilled Water	500 mL
Gel diluent	Prepare as instructed. (See “Prepare the gel diluent” on page 241.)

Stage 5A summary

Stage activities	Instruments required
• Three reagent dispenses—Dilution QC Plates, OD QC Plates, Gel QC Plates • Transfer hybridization-ready target from Hyb-Ready Plates to QC plates	

Perform the pre-run checklist

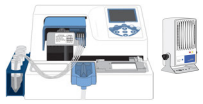
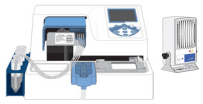
Before starting the workflow, the following tasks must be completed.

Multidrop™ Combi Reagent Dispenser:

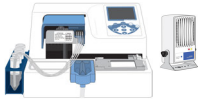
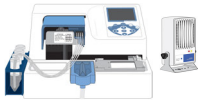
1. Ensure that the Multidrop™ Combi startup procedure has been performed. (See “Start up the Multidrop™ Combi” on page 54.)
2. Perform gravimetric checks before running the workflow. (See “Perform gravimetric checks” on page 55.)
3. Prime the Multidrop™ cassette with reagent. (See “Prime the cassette” on page 59.)
4. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)

Stage 5A: In-process QC

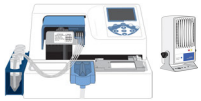
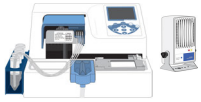
For an 8-plate workflow, complete 1 cycle of 8 plates.

Dilution QC Plates[1]		
	Plates 1 2 3 4 5 6 7 8	
1		Dispense UltraPure™ DNase/RNase-Free Distilled Water • Method: 96-QC-Dil-55 • Dispense volume: 60 µL • Volume of water required for 8 plates: 60 mL
2		After dispensing, seal the plate. Tip: After unpeeling a new adhesive seal, use the ion fan to reduce the amount of static on the sticky side of the seal. This minimizes the droplets jumping up onto the seal due to static.
3		Repeat dispense and seal all 8 plates.
4		Centrifuge all 8 plates • Setting: 675 × *g* for 30 seconds
5		Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

[1] The MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plate with Barcode, Blue is used as the Dilution QC Plate.

OD QC Plates[1]	
Plates 1 2 3 4 5 6 7 8	
①	 Dispense UltraPure™ DNase/RNase-Free Distilled Water • Method: 96-QC-OD-90 • Dispense volume: 90 µL • Volume of water required for 8 plates: 85 mL
②	 Repeat dispense, and stack all 8 plates.
③	Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

[1] The Greiner Bio-One™ 96-Well UV-Star™ Plate, Flat Bottom, Chimney Style, Clear is used as the OD QC Plate.

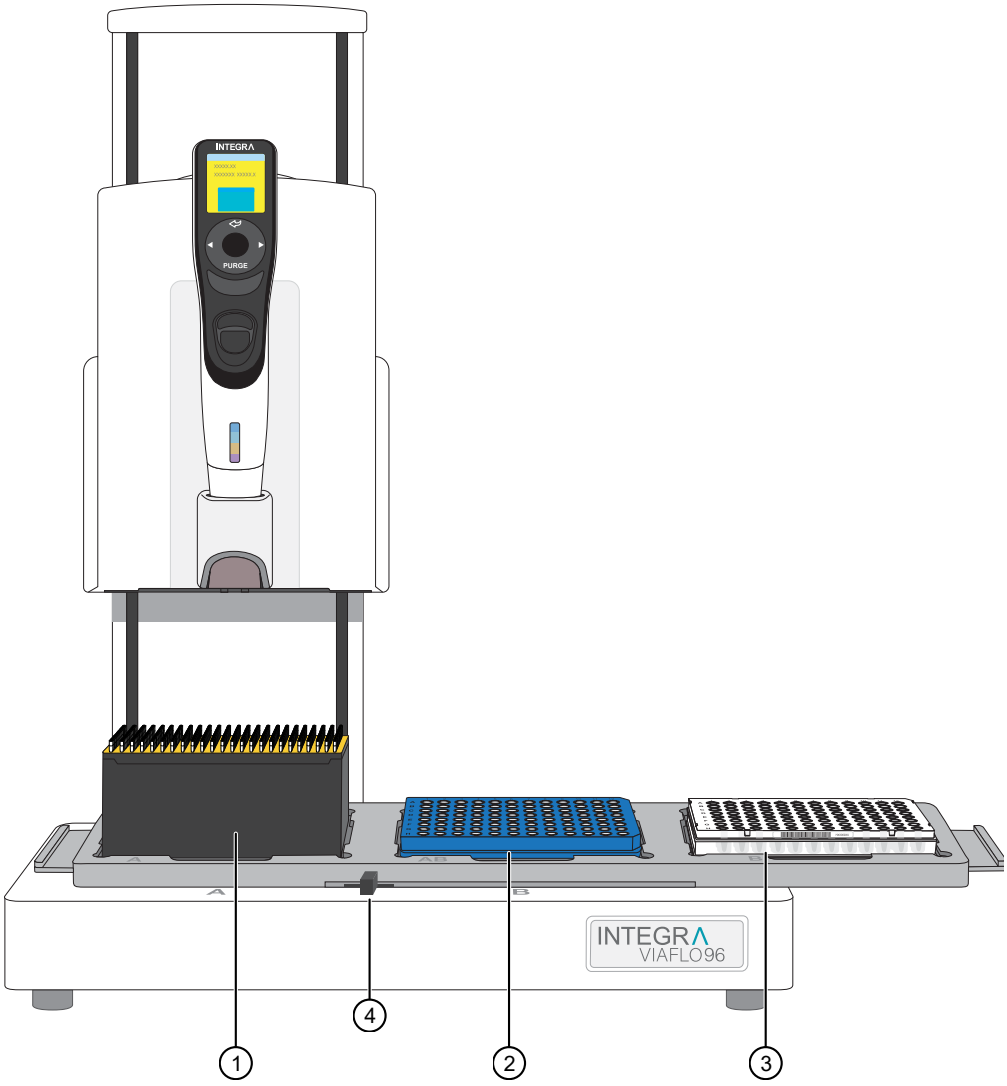
Gel QC Plates[1]	
Plates 1 2 3 4 5 6 7 8	
①	 Dispense gel diluent • 96-QC-Gel-150 • Dispense volume: 150 µL • Gel diluent required for 8 plates: 130 mL
②	 Repeat dispense, and stack all 8 plates.
③	 Continue with Integra VIAFLO
④	Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

[1] MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plate with Barcode, Blue is used as the Gel QC Plate.

Transfer hybridization-ready target in Hyb-Ready Plate to QC plate

The *PCR to QCPLATES* method transfers 5 µL of sample from Hyb-Ready Plate to the Dilution QC Plate, then 10 µL of diluted sample from the Dilution QC Plate to the OD QC Plate, and finally 5 µL of diluted sample from the Dilution QC Plate to the Gel QC Plate.

1. Place labware on the VIAFLO stage as shown in the following image.

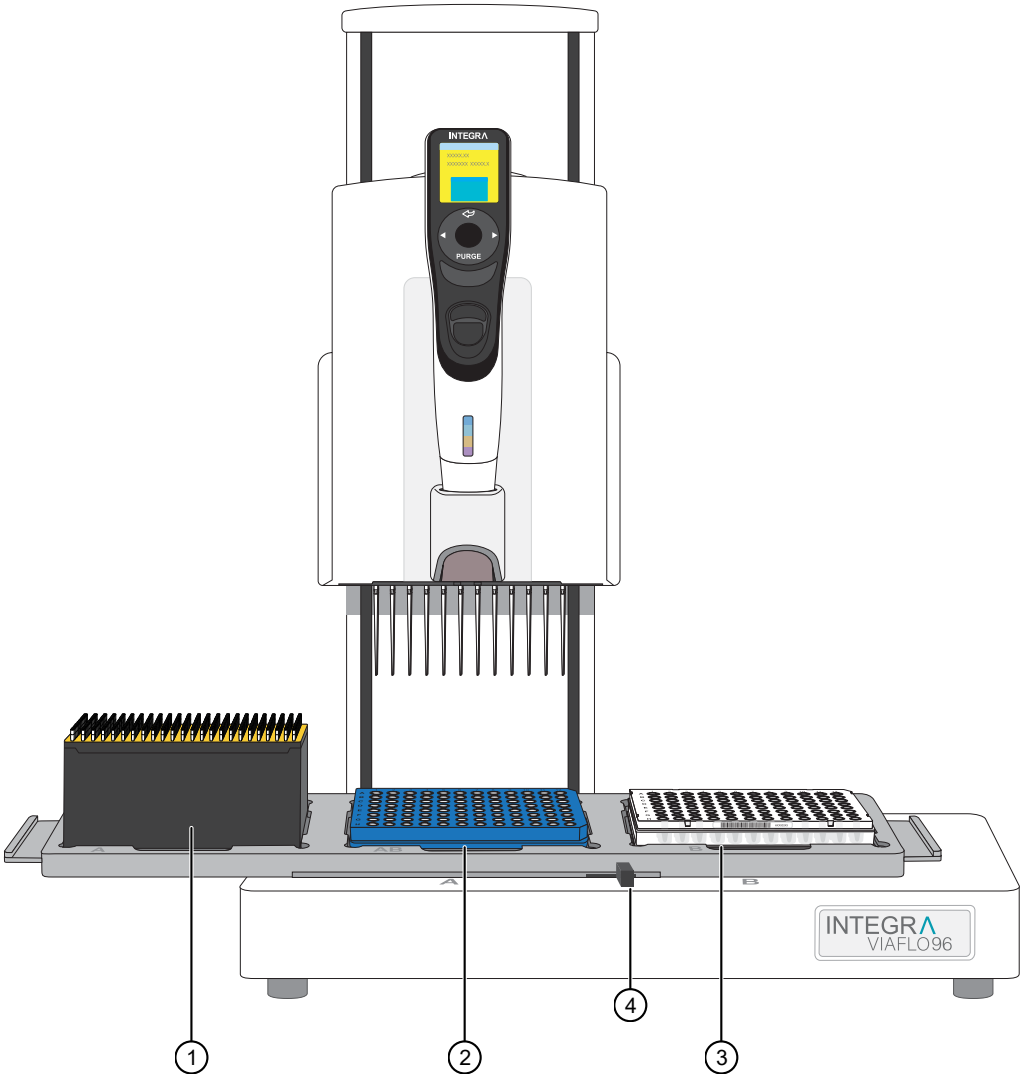


① Tips
② Dilution QC Plate
③ Hyb-Ready Plate on a plate holder
④ Stage slider

2. Slowly move the 3-plate stage to the *right* for tip loading.

3. Load tips to the 96-head by adjusting the stage slider.
    a. Adjust the stage slider to the left or right, depending on the tips available for use.
        - Right—moves stage back to pick up quadrant 1 or quadrant 2 tips.
        - Left—moves stage forward to pick up quadrant 3 or quadrant 4 tips.
    b. Place one hand on the control unit handle. Maintain contact with the handle throughout the tip loading step.
    c. Gently lower the 96-head into the tip box and guide it into a set of 96 tips.
    d. After the tips are properly aligned the indicator light of the tip load button blinks. Use the opposite hand and press down on the tip load button, located at the top of the 96-head.
    e. Release the button when you hear a click. The indicator light on the button remains on.
    f. Using the control unit handle, lift the head away from the platform to provide clearance for stage loading or positioning.
4. From the **Main Menu**, select **Custom Programs ▸ PCR to QCPLATES** to choose the method.

5. Move the stage slider to the center, move the 3-position stage to the left, then allow the 96-head to rest over the Dilution QC Plate.



① Tips
② Dilution QC Plate
③ Hyb-Ready Plate on a plate holder
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

6. Press **Run** to start the method.

7. When the run pauses, do not eject tips.

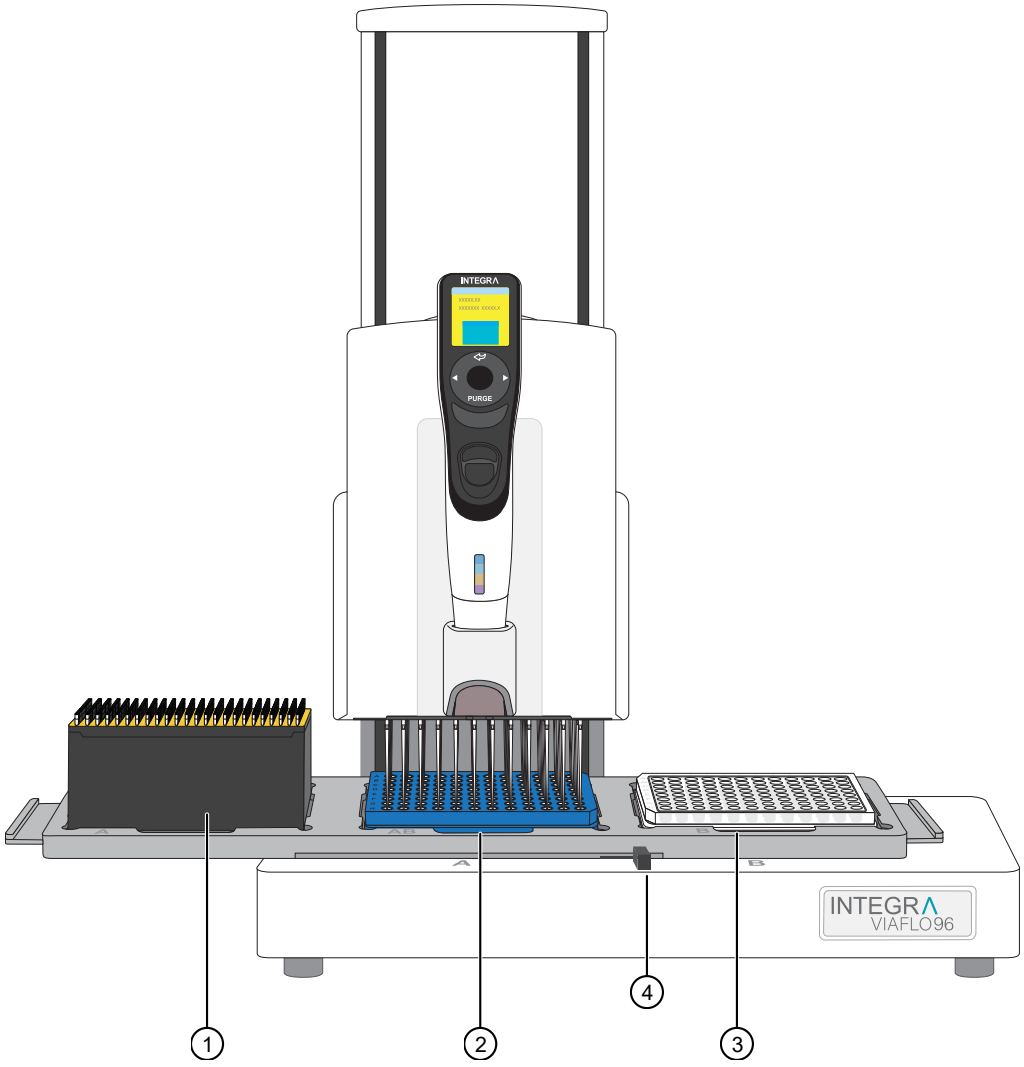
8. When prompted, remove the Hyb-Ready Plate, tightly seal, then store at –20°C.

   Tip: After unpeeling a new adhesive seal, use the ion fan to reduce the amount of static on the sticky side of the seal. This minimizes the droplets jumping up onto the seal due to static.

The Hyb-Ready Plate can be stored at –20°C for up to 2 weeks.

9. Remove the plate holder.

10. Place the OD QC Plate on position 3 where the Hyb-Ready Plate and plate holder used to be.



① Tips
② Dilution QC Plate
③ OD QC Plate
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

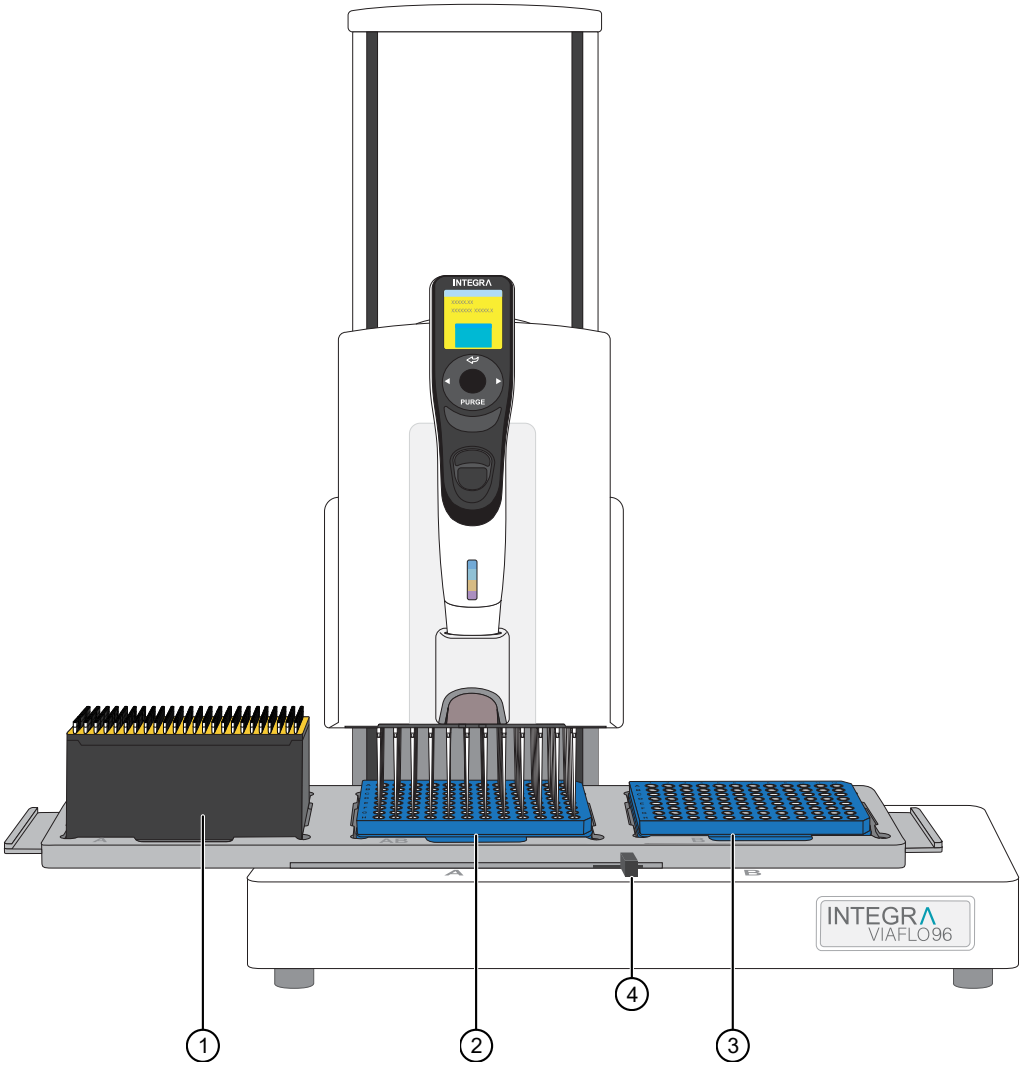
11. Resume the method by pressing the **Run** key.

12. When prompted, remove OD QC Plate, seal, then stack on benchtop.

    Tip: After unpeeling a new adhesive seal, use the ion fan to reduce the amount of static on the sticky side of the seal. This minimizes the droplets jumping up onto the seal due to static.

OD QC Plates are stacked and then later vortexed and centrifuged at 675 × *g* for 30 seconds for QC quantification.

13. Place Gel QC Plate on position 3 where the OD QC Plate used to be.



① Tips
② Dilution QC Plate
③ Gel QC Plate
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

14. Resume the method by pressing the **Run** key.

15. When complete, remove the Gel QC Plate, seal, then stack on benchtop.

    Tip: After unpeeling a new adhesive seal, use the ion fan to reduce the amount of static on the sticky side of the seal. This minimizes the droplets jumping up onto the seal due to static.

Gel QC Plates are stacked and then later vortexed and centrifuged at 675 × *g* for 30 seconds for Fragmention QC.

16. Remove Dilution QC Plate, seal, then store at 4°C.
17. Eject and discard the tips.
18. Repeat the procedure for the remaining Hyb-Ready Plates.
19. Complete the following:
    - Appendix C, “Sample quantification after resuspension”.
    - Appendix B, “Fragmentation quality control gel protocol”.

Stage 6: Denature the target and transfer to hybridization tray

Equipment and labware required

Quantity	Item
Instruments	
1	VIAFLO™ 96 Base Unit or VIAFLO™ 384 Base Unit with a 96 Channel Pipetting Head (5–125 µL) and a 3 position stage installed.
1	Fume hood
1	Thermal cycler, ProFlex™ 96-well PCR System
1	BINDER™ ED 56 or BINDER™ BD 56 (Set at 48°C) • Must maintain a constant temperature of 48°C for at least 24 hours with a temperature accuracy of ±1°C
1	Sorvall™ X4R Pro-MD Centrifuge set to room temperature (As needed)
1	Vortex mixer (As needed)
Labware and consumables	
1	Plate Alignment Tool
8	96-layout hybridization tray
768 tips	Integra VIAFLO XYZ GripTips™

Input samples

Number	Plate name	Content
8 (maximum)	Hyb-Ready Plate	Hybridization-ready target DNA prepared in "Stage 5: Resuspend the pelleted DNA and prepare for hybridization" (MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode—half skirt)

Stage 6 summary

Stage activities	Instruments required
• Denature target in thermal cycler • Transfer from Hyb-Ready Plate to hybridization tray • Off-line incubation of the array plate/hybridization tray stack at 48°C for 23.5–24 hours	

Perform the pre-run checklist

Before starting the workflow, the following tasks must be completed.

1. Ensure that the VIAFLO™ 96 or 384 Base Unit is clean and is powered on.
2. Ensure that the oven is powered on and has warmed up to 48°C.
3. Ensure that the Proflex™ thermal cycler is powered on and loaded with the **Axiom Denature** thermal cycler protocol.

Warm the array plate to room temperature

The array plate must be at room temperature before setting up hybridization.

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

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

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

Prepare hybridization-ready samples stored at –20°C

1. Warm the Hyb-Ready Plate at room temperature for 5 minutes. It is not necessary to equilibrate the plate for longer than 5 minutes.
2. Ensure 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. Tightly reseal the plate with a fresh seal.
   d. Vortex the newly sealed plate, then proceed to the next step.
3. Centrifuge for 30 seconds, then proceed to Stage 6.

Stage 6: Denature the target and transfer to hybridization tray

For an 8-plate workflow, complete 1 cycle of 8 plates.

Denature and transfer to hybridization tray		
	Plates 1 2 3 4 5 6 7 8	
①		Denature target • Protocol: **Axiom Denature**
②		Transfer from Hyb-Ready Plate to hybridization tray • Method: PCR TO HYB TRAY • Dispense volume: 105 µL
③		Incubate • Settings: 48°C, 23.5–24 hours

Transfer hybridization-ready target in Hyb-Ready Plate to hybridization tray

The *PCR TO HYB TRAY* method transfers 105 µL of denatured hybridization-ready target from the Hyb-Ready Plate to the hybridization tray.

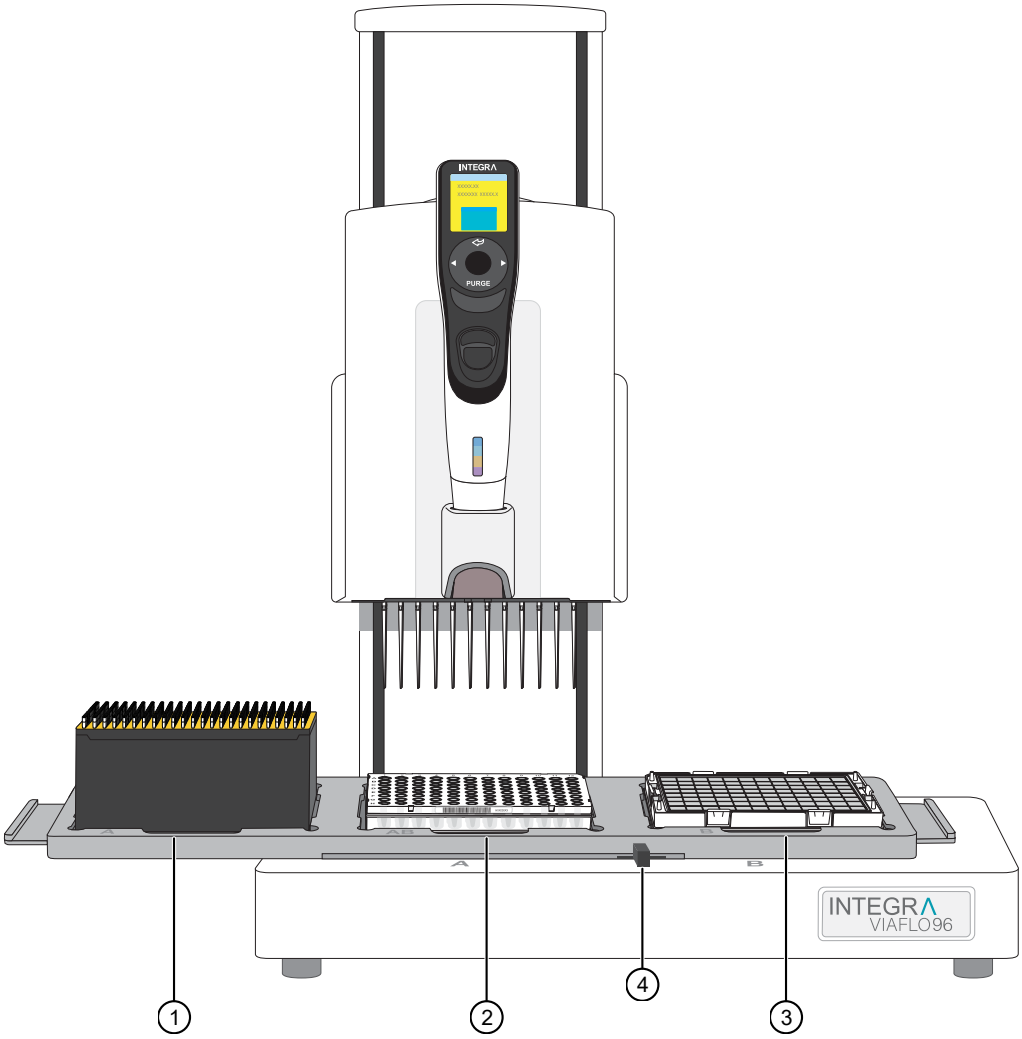
1. Run the **Axiom Denature** protocol to denature the hybridization-ready target using a thermal cycler with a heated lid:
   - 95°C for 10 minutes.
   - 48°C for 3 minutes.
   - 48°C hold.

2. Place labware on the VIAFLO stage as shown in the following image.

① Tips
② Hyb-Ready Plate on plate holder
③ Hybridization tray
④ Stage slider

3. Load tips to the 96-head by adjusting the stage slider.
    a. Adjust the stage slider to the left or right, depending on the tips available for use.
        - Right—moves stage back to pick up quadrant 1 or quadrant 2 tips.
        - Left—moves stage forward to pick up quadrant 3 or quadrant 4 tips.
    b. Place one hand on the control unit handle. Maintain contact with the handle throughout the tip loading step.
    c. Gently lower the 96-head into the tip box and guide it into a set of 96 tips.
    d. After the tips are properly aligned, the indicator light of the tip load button blinks. Use the opposite hand and press down on the tip load button, located at the top of the 96-head.
    e. Release the button when you hear a click. The indicator light on the button remains on.
    f. Using the control unit handle, lift the head away from the platform to provide clearance for stage loading or positioning.
4. From the **Main Menu**, select **Custom Programs ▸ PCR to HYB TRAY** to choose the method.

5. Move the stage slider to the center, move the 3-position stage to the left, then allow the 96-head to rest over the Hyb-Ready Plate on the plate holder.



① Tips
② Hyb-Ready Plate on plate holder
③ Hybridization tray
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

6. Press **Run** to start the method.

7. When complete, inspect the Hyb-Ready Plate to ensure that the hybridization-ready target was transferred to the hybridization tray.

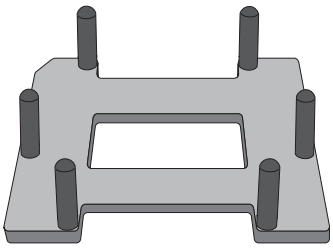
8. Ensure that there are no air bubbles present in the hybridization tray. Puncture any air bubbles that you see using a clean pipette tip. You do not need to spread the sample around the bottom of the hybridization tray wells. Sample distribution across the well occurs when the array plate is stacked together with the hybridization tray.

9. Continue to offline hybridization.

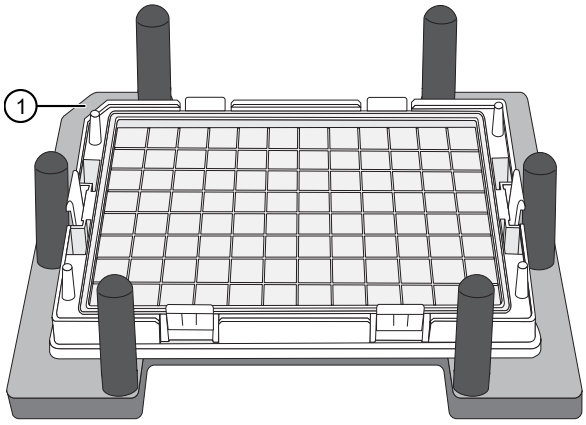
Perform array plate clamping and off-line hybridization

This procedure is performed for all plates in the workflow.

1. Place the Plate Alignment Tool flat on the table top.



2. Carefully place the hybridization tray containing the denatured hybridization target into the Plate Alignment Tool as shown in the following figure. Ensure that the hybridization tray is sitting inside the tool and that the notched corner of the hybridization tray is aligned with the notched corner of the Plate Alignment Tool.



① Notched corner of the Plate Alignment Tool and hybridization tray aligned.

3. Hold the array plate with the arrays facing down, then slowly align the array plate into the Plate Alignment Tool until the entire plate is positioned within the 6 alignment posts of the tool. Ensure that the notched corner of the arrays plate is aligned with the notched corner of the hybridization tray. (See Figure 8.)

IMPORTANT! Do not lower the array plate to the hybridization tray if the plate is not aligned within the alignment posts on the tool.

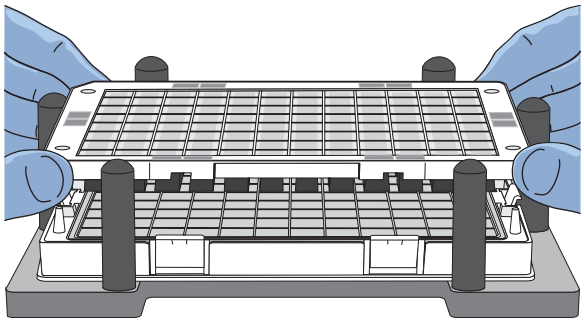


Figure 8 Place the array plate on the hybridization tray in the Plate Alignment Tool.

4. After the array plate is aligned inside the tool, slowly lower the plate towards the hybridization tray until a snapping sound is heard. The snapping sound indicates that the array plate and hybridization tray have been properly latched together.

Note: Lowering the array plate to the hybridization tray as slowly as possible prevents air bubbles from generating inside the hybridization tray.

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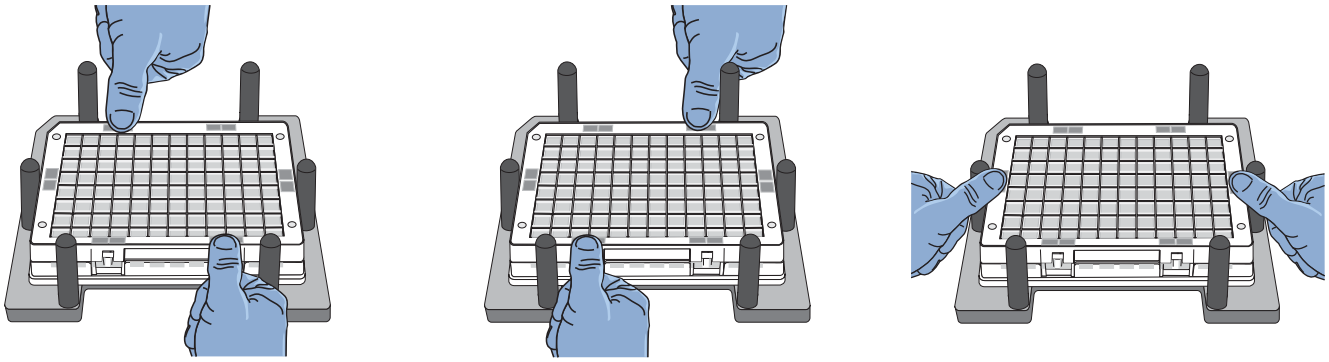
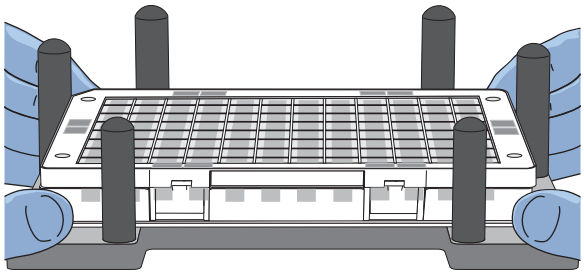
5. Position your hands as shown in the following figures, then lightly push the array plate downward to ensure that each latching mechanism is tightly closed.



6. Grip the array plate/hybridization tray stack from the hybridization tray at the bottom of the stack, then lift to remove the entire stack from the Plate Alignment Tool.



7. Inspect the stack to ensure that all clamps are fastened.

8. Keeping the array plate/hybridization tray stack level, check to ensure that there are no air bubbles trapped between the array plate and the hybridization tray.
   - If bubbles are present, tap the tray gently to dissipate the air bubbles.
9. Make a note of the array plate barcode number for later use.

   The barcode number is used to create the GeneTitan™ Array Plate Registration file and for the GeneTitan™ instrument **Wash-Scan** setup.

   Tip: Scan the barcode directly into a blank registration file or into a text program, such as Microsoft™ Notepad, Word™, or Excel™ applications.
10. Place the array plate/hybridization tray stack onto the wire racks of the hybridization oven set at 48°C.

    Note: Do not stack the array plate/hybridization tray stacks on top of one another.

    Note: Do not place the array plate/hybridization tray stack on the bottom of the hybridization oven.
11. Incubate for 23.5–24 hours. Set a timer or record the time.

Stage 7: Preparing ligation, stain, stabilization reagent trays, and scan trays for the GeneTitan™ MC Instrument

Equipment and labware required

Quantity	Item
Instruments	
5	Multidrop™ Combi Reagent Dispenser
5	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	Vortex mixer
1	Mini centrifuge
1	Electronic pipettor
1	P1000 pipettes
Labware and consumables	
4	250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)
As required	Serological pipette (5 mL, 10 mL, and 50 mL)
As required	P1000 pipette tips
40	96-format GeneTitan™ stain trays
40	96-format GeneTitan™ stain tray covers
8	96-format GeneTitan™ scan trays with covers

[1] Each reagent must use a dedicated cassette.

Reagent handling

Fast Wash Module 4-1, 8x96F and Module 4-2, 8x96F reagents are required from the Axiom™ Propel 8x96F Reagent Kit.

- Axiom™ Propel Fast Wash Reagent Kit Module 4-1 for 96F array plates only, Part No. 952370
- Axiom™ Propel Reagent Kit Module 4-2 for 96F array plates only, Part No. 952340

Thaw and prepare reagents according to the following table.

Table 28 Reagent handling for Stage 7 reagents.

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Fast Wash Reagent Kit Module 4-1 for 96F array plates only, –20°C	Axiom™ Propel Ligation Buffer	951980	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation. **Note:** White precipitate is sometimes observed when the Axiom™ Propel Ligation Buffer is thawed. The presence of some precipitate is acceptable and does not adversely impact assay performance. Use the following instructions to resuspend most precipitate before use. **1.** Vortex for 30 seconds. **2.** Examine the buffer for precipitate. **3.** If precipitate is still present, warm the bottle with your hands, then vortex again for 30 seconds.
	Axiom™ Fast Ligation Enzyme, 8X	952368	• Keep in –20°C until ready for use. • Flick 5 times before master mix preparation.
	Axiom™ Propel Ligation Solution 1	951982	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Probe Mix 1	951983	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Stain Buffer	951984	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.

Table 28 Reagent handling for Stage 7 reagents. *(continued)*

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Fast Wash Reagent Kit Module 4-1 for 96F array plates only, –20°C	Axiom™ Propel Stabilize Solution	951985	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex, then centrifuge briefly before master mix preparation.
Axiom™ Propel Reagent Kit Module 4-2 for 96F array plates only, 4°C	Axiom™ Propel Ligation Solution 2	951986	• Remove from 4°C and let equilibrate to room temperature on day of use. • Vortex before master mix preparation.
	Axiom™ Propel Probe Mix 2	951987	• Remove from 4°C and let equilibrate to room temperature, but *keep it stored away from light*. • Flick 5 times before master mix preparation.
	Axiom™ Propel Wash A	951988	• Remove from 4°C and equilibrate to room temperature. • Vortex before master mix preparation. If precipitate is present, vortex again to dissolve the precipitate.
	Axiom™ Propel Stain 1-A	951989	• Remove from 4°C and let equilibrate to room temperature on day of use. • Flick 5 times, then centrifuge briefly before master mix preparation.
	Axiom™ Propel Stain 1-B	951993	• Remove from 4°C and let equilibrate to room temperature on day of use. • Flick 5 times, then centrifuge briefly before master mix preparation.
	Axiom™ Propel Stain 2-A	951990	• Remove from 4°C and let equilibrate to room temperature on day of use. • Flick 5 times, then centrifuge briefly before master mix preparation.
	Axiom™ Propel Stain 2-B	951994	• Remove from 4°C and let equilibrate to room temperature on day of use. • Flick 5 times, then centrifuge briefly before master mix preparation.
	Axiom™ Propel Stabilize Diluent	951991	• Remove from 4°C and let equilibrate to room temperature on day of use. • Vortex before master mix preparation.

Table 28 Reagent handling for Stage 7 reagents. *(continued)*

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Reagent Kit Module 4-2 for 96F array plates only, 4°C	Axiom™ Water	952177	• Remove from 4°C and let equilibrate to room temperature on day of use.
	Axiom™ Propel Hold Buffer	951992	• Remove from 4°C and let equilibrate to room temperature on day of use. • Vortex before use.

Reagent preparation for Stage 7: Prepare GeneTitan™ reagents

You can add flexibility to the timing of array processing by making the following GeneTitan™ reagents up to 2 days (48 hours) in advance.

- Stain 1 and Stain 2 Master Mixes can be prepared up to 2 days in advance, wrapped in foil, then stored at 4°C
- Stabilization Master Mix can be prepared up to 2 days in advance and stored at 4°C
- Ligate Master Mix can be prepared with all the components listed up to 2 days in advance, except the Axiom™ Fast Ligation Enzyme, 8X. Wrap the master mix bottle in foil, then store at 4°C. Axiom™ Fast Ligation Enzyme, 8X must be kept at –20°C and added only when ready for use.

The following sections provide detailed procedures for master mix preparation.

Prepare Ligate Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 28.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 29 Ligate Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Ligation Buffer	71.0 mL	50-mL serological pipette
2	Axiom™ Propel Ligation Solution 1	14.1 mL	10-mL serological pipette
3	Axiom™ Propel Probe Mix 1	11.3 mL	10-mL serological pipette
4	Axiom™ Propel Probe Mix 2	11.3 mL	10-mL serological pipette
5	Axiom™ Propel Ligation Solution 2	3.4 mL	5-mL serological pipette
6	Axiom™ Fast Ligation Enzyme, 8X	4.0 mL 0.25 mL	5-mL serological pipette P1000 pipette
	Total volume	**114.95 mL**	

2. Ensure that the cap of the reservoir is closed tightly, then invert the Ligate Master Mix at least 15 times to mix.
3. Protect the Ligate Master Mix from direct light by covering with aluminum foil.
4. Leave the master mix at room temperature and use within 1 hour of its preparation.

Ligate Master Mix—advance preparation procedure

To provide flexibility for the workflow, the master mix can be made up to 2 days (48 hours) in advance, but without the Axiom™ Fast Ligation Enzyme, 8X.

1. Add the reagents 1–5 from Table 29.
2. Invert the conical tube at least 15 times to mix.
3. Protect the Ligate Master Mix from direct light by covering with aluminum foil, then store at 4°C.

IMPORTANT! Label the 250-mL conical tube to indicate that the content contains "*No Enzyme*".

4. On the day of use, remove from the 4°C storage, then allow the Ligate Master Mix to equilibrate to room temperature. Equilibration can take up to 30 minutes.
5. Add the Axiom™ Fast Ligation Enzyme, 8X to the conical tube.
6. Invert the tube at least 15 times to mix.
7. Leave the master mix at room temperature to allow it equilibrate to room temperature. The prepared Ligate Master Mix can be left at room temperature within 1 hour of its preparation.

Prepare Stain 1 Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 28.)

Note: Two stain trays of Stain 1 Master Mix are required per 96 samples, therefore, a higher volume of master mix is required.

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 30 Stain 1 Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Wash A	199.0 mL	50-mL serological pipette
2	Axiom™ Propel Stain Buffer	4.1 mL	5-mL serological pipette
3	Axiom™ Propel Stain 1-A	2.06 mL	P1000 pipette
4	Axiom™ Propel Stain 1-B	2.06 mL	P1000 pipette
	Total volume	**207.22 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Stain 1 Master Mix at least 15 times to mix.
3. Protect the Stain 1 Master Mix from direct light by covering with aluminum foil.
4. Store the prepared master mix in one of the following ways:
   - Store at room temperature and use within 2 hours of its preparation.
   - **Optional:** To provide flexibility for the workflow, the master mix can be made up to 2 days (48 hours) in advance, then stored in the dark at 4°C. On the day of use, remove from the 4°C storage, then allow the master mix to equilibrate to room temperature. Equilibration can take up to 30 minutes.

Prepare Stain 2 Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 28.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 31 Stain 2 Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Wash A	109.0 mL	50-mL serological pipette
2	Axiom™ Propel Stain Buffer	2.3 mL	5-mL serological pipette
3	Axiom™ Propel Stain 2-A	1.1 mL	P1000 pipette
4	Axiom™ Propel Stain 2-B	1.1 mL	P1000 pipette
	Total volume	**113.5 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Stain 2 Master Mix at least 15 times to mix.

3. Protect the Stain 2 Master Mix from direct light by covering with aluminum foil.

4. Store the prepared master mix in one of the following ways:
   - Store at room temperature and use within 2 hours of its preparation.
   - **Optional:** To provide flexibility for the workflow, the master mix can be made up to 2 days (48 hours) in advance, then stored in the dark at 4°C. On the day of use, remove from the 4°C storage, then allow the master mix to equilibrate to room temperature. Equilibration can take up to 30 minutes.

Prepare Stabilization Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 28.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 32 Stabilization Master Mix for 8x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Water	101.0 mL	50-mL serological pipette
2	Axiom™ Propel Stabilize Diluent	11.4 mL	10-mL serological pipette
3	Axiom™ Propel Stabilize Solution	1.4 mL	P1000 pipette
	Total volume	**113.8 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, vortex for 5 seconds, then leave at room temperature until use.

3. Store the prepared master mix in one of the following ways:
   - Store at room temperature and use within 2 hours of its preparation.
   - **Optional:** To provide flexibility for the workflow, the master mix can be made up to 2 days (48 hours) in advance, then stored at 4°C. On the day of use, remove from the 4°C storage, then allow the master mix to equilibrate to room temperature. Equilibration can take up to 30 minutes.

Prepare the Axiom™ Propel Hold Buffer

Ensure that the Axiom™ Propel Hold Buffer is vortexed before use.

1. Use the Axiom™ Propel Hold Buffer bottle for Multidrop™ dispensing.

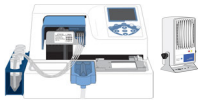
Table 33 Axiom™ Propel Hold Buffer for 8x96-format plates.

Reagent	Quantity	Transfer method
Axiom™ Propel Hold Buffer	Entire bottle	Use straight from bottle[1]

[1] The Axiom™ Propel Hold Buffer reagent bottle can be used for Multidrop™ Combi dispensing.

2. Leave at room temperature until use.

Stage 7 summary

Stage activities	Instruments required
Five reagent dispenses—Ligation, Stain 1, Stain 2, Stabilization, Axiom™ Propel Hold Buffer	

Prepare the GeneTitan™ trays and covers

Familiarity with handling GeneTitan™ is required. If needed, review the proper techniques provided in this document. See Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation”.

1. Collect and label the stain trays as described in the following table.

Quantity per array plate	Tray type	Label[1]
1	Ligation Tray 	*Lig*
2	Stain 1 Tray 	*Stain 1* or *S1*
1	Stain 2 Tray 	*Stain 2* or *S2*
1	Stabilization Tray 	*Stbl*

[1] It is critical that you write on the proper location of the stain/reagent trays. See “Label the GeneTitan™ reagent trays” on page 220.

2. Collect enough covers for the trays (5 x the number of array plates).

3. Place the labeled stain trays and covers next to the Multidrop™ Combi. Ensure that each labeled stain tray is placed next to the correct Multidrop™ Combi dispensing the appropriate/corresponding reagent.

   For example, stain trays labeled “Stain 2” are placed next to the Multidrop™ Combi dispensing the Stain 2 Master Mix.

4. Deionize the stain trays and covers by placing them directly in front of the ion blower and allowing the air to stream across them for at least 10 seconds.

   Alternatively, trays and covers can be deionized manually using the GeneTitan™ ZeroStat AntiStatic Gun. See “Manual deionization of GeneTitan™ trays and covers” on page 222.

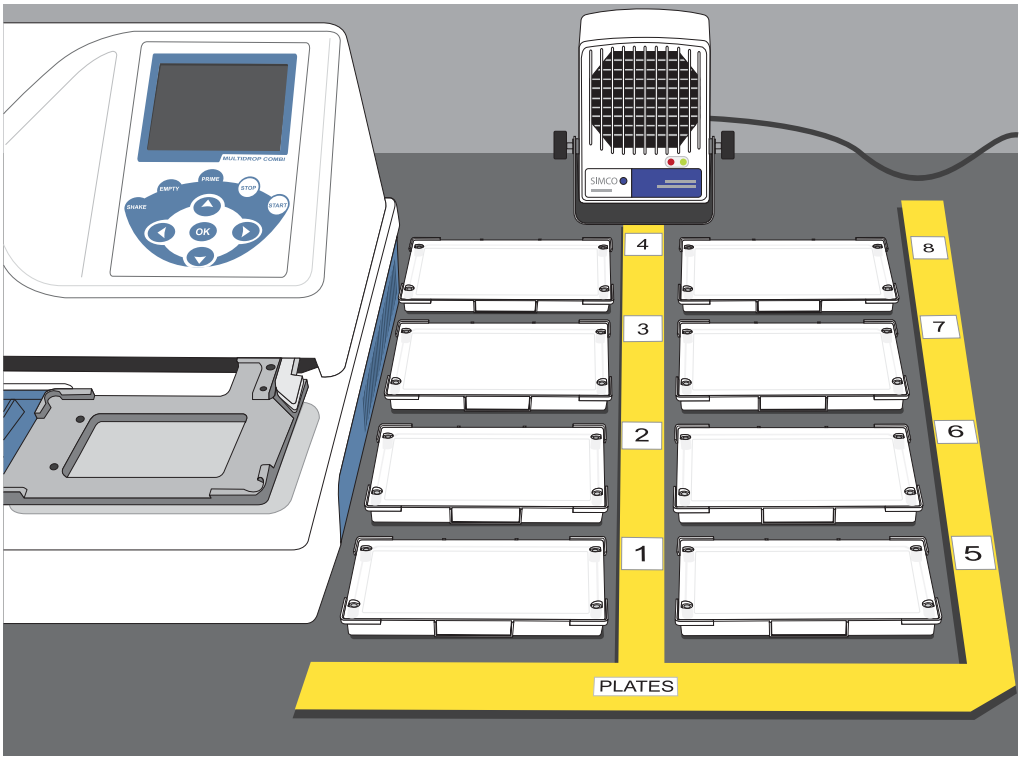


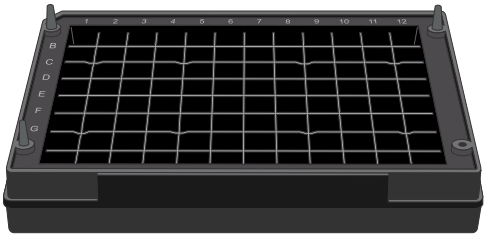
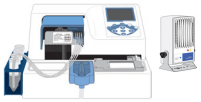
Figure 9 Placement and positioning of stain tray covers for deionization with the ionizing air blower.

Perform the pre-run checklist

Before starting the workflow, the following tasks must be completed.

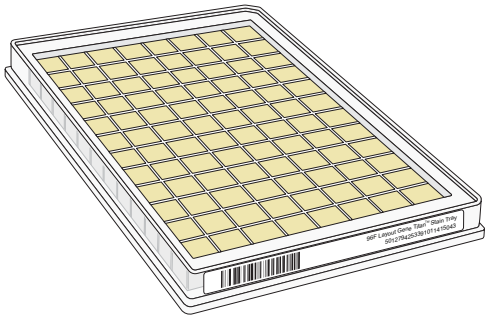
1. Ensure that the Multidrop™ Combi startup procedure has been performed. (See “Start up the Multidrop™ Combi” on page 54.)
2. Perform gravimetric checks before running the workflow. (See “Perform gravimetric checks” on page 55.)
3. Prime the Multidrop™ cassette with reagent. (See “Prime the cassette” on page 59.)
4. Ensure that each labeled stain tray is placed next to the correct Multidrop™ Combi dispensing the corresponding reagent.
5. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)

Stage 7: Prepare GeneTitan™ reagents

Scan tray dispense	
Plates 1 2 3 4 5 6 7 8	
	
⚠	**CAUTION!** Do not remove the Scan Tray from its protective black base until loading onto the GeneTitan™ MC Instrument.
①	 Dispense Axiom™ Propel Hold Buffer • Method: 96-Scan-150 • Dispense volume: 150 µL
②	✓ Examine the Scan Tray. • If bubbles are present, puncture them with a pipette tip. • If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.
③	ℹ Place the Scan Tray cover onto the Scan Tray.
④	ℹ • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61. • Continue to "Stain tray dispense—Ligation Master Mix"

Stain tray dispense—Ligation Master Mix

Plates: 1 2 3 4 5 6 7 8

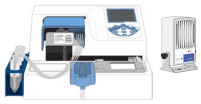


- Place the ion blowers at the dispensing station. If ion blowers are not available, the GeneTitan™ ZeroStat AntiStatic Gun can be used to individually deionize the stain trays and covers. (See "Deionization of GeneTitan™ trays and covers" on page 222.)

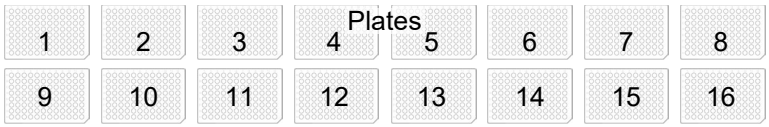
IMPORTANT! Deionze the trays and covers by ion blowers or GeneTitan™ ZeroStat AntiStatic Gun before dispensing the reagents.

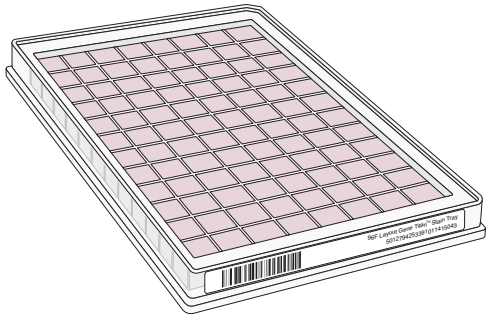
IMPORTANT! Ensure that the stain trays labeled "Lig" are used for the Multidrop™ Combi dispensing the Ligation Master Mix.

IMPORTANT! If the Ligation Master Mix was made a day in advance, ensure that the Axiom™ Fast Ligation Enzyme, 8X is added to the mixture before dispensing.

①		Dispense Ligation Master Mix • Method: 96-Stain-110 • Dispense volume: 110 µL
②	✓	Examine the Ligation Tray. • If bubbles are present, puncture them with a pipette tip. • If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.
③	ⓘ	• Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61. • Place a deionized stain tray cover on top of each Ligation stain tray. Continue to "Stain tray dispense—Stain 1 Master Mix" • Protect the trays from light if not immediately loading onto the GeneTitan™ MC Instrument.

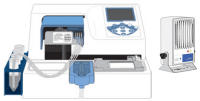
Stain tray dispense—Stain 1 Master Mix





IMPORTANT! Deionze the trays and covers by ion blowers or GeneTitan™ ZeroStat AntiStatic Gun before dispensing the reagents.

IMPORTANT! Ensure that the stain trays labeled “Stain 1” or “S1” are used for the Multidrop™ Combi dispensing the Stain 1 Master Mix.

① 

Dispense Stain 1 Master Mix

- Method: 96-Stain-110
- Dispense volume: 110 µL

② ✓

Examine the Stain 1 Tray.

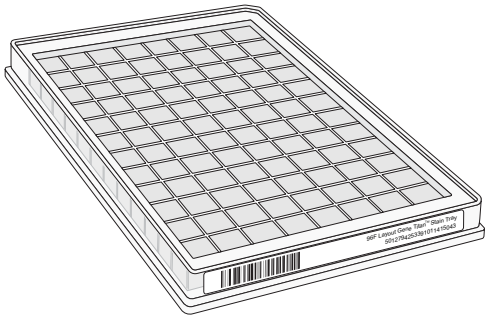
- If bubbles are present, puncture them with a pipette tip.
- If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.

③ ⓘ

- Perform the appropriate Multidrop™ procedure:
  - Flush between batches. See page 60.
  - Shutdown. See page 61.
- Place a deionized stain tray cover on top of each Stain 1 stain tray. Continue to “Stain tray dispense—Stain 2 Master Mix”
- Protect the trays from light if not immediately loading onto the GeneTitan™ MC Instrument.

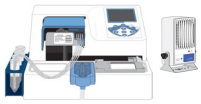
Stain tray dispense—Stain 2 Master Mix

Plates: 1 2 3 4 5 6 7 8



IMPORTANT! Deionze the trays and covers by ion blowers or GeneTitan™ ZeroStat AntiStatic Gun before dispensing the reagents.

IMPORTANT! Ensure that the stain trays labeled “Stain 2” or “S2” are used for the Multidrop™ Combi dispensing the Stain 2 Master Mix.

1. 

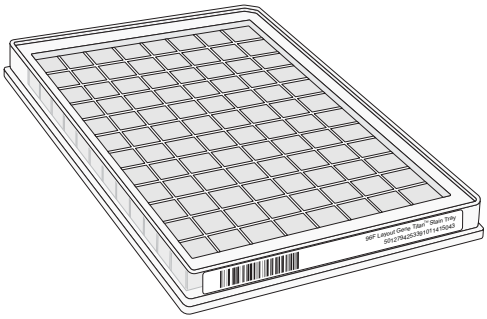
   Dispense Stain 2 Master Mix
   - Method: 96-Stain-110
   - Dispense volume: 110 µL

2. Examine the Stain 2 Tray.
   - If bubbles are present, puncture them with a pipette tip.
   - If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.

3. - Perform the appropriate Multidrop™ procedure:
     - Flush between batches. See page 60.
     - Shutdown. See page 61.
   - Place a deionized stain tray cover on top of each Stain 2 stain tray. Continue to “Stain tray dispense—Stabilization Master Mix”.
   - Protect the trays from light if not immediately loading onto the GeneTitan™ MC Instrument.

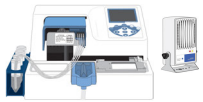
Stain tray dispense—Stabilization Master Mix

Plates: 1 2 3 4 5 6 7 8



IMPORTANT! Deionze the trays and covers by ion blowers or GeneTitan™ ZeroStat AntiStatic Gun before dispensing the reagents.

IMPORTANT! Ensure that the stain trays labeled “Stbl” are used for the Multidrop™ Combi dispensing the Stabilization Master Mix.

1		Dispense Stabilization Master Mix • Method: 96-Stain-110 • Dispense volume: 110 µL
2	✓	Examine the Stabilization Tray. • If bubbles are present, puncture them with a pipette tip. • If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.
3	ⓘ	Place a deionized stain tray cover on top of each Stabilization stain tray.
4	ⓘ	Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

5 Target preparation with Multidrop™ Combi Reagent Dispensers for 4 plates

The instructions that are provided in this chapter are for processing 4 plates or less. When working with fewer than 4 plates, stop the dispensing after the sample containing plates are processed.

Ensure that the centrifuge and shaker are balanced.

IMPORTANT! Four gDNA Sample Plates or fewer must be prepared before starting DNA amplification. See Chapter 2, “Genomic DNA preparation”.

Stage 1: Amplify the genomic DNA

Equipment and labware required

Quantity	Item
Instruments	
3	Multidrop™ Combi Reagent Dispenser
3	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	ALPS™ 3000 Automated Microplate Heat Sealer
1	Compact Digital Microplate Shaker
1	Sorvall™ X4R Pro-MD Centrifuge (Set to room temperature.)
1	BINDER™ ED 56 or BINDER™ BD 56 • Must maintain a constant temperature of 37°C for at least 24 hours with a temperature accuracy of ±1°C
1	Vortex mixer
1	Electronic pipettor for serological pipettes
1	Digital timer
Labware and consumables	
As required	Easy Peel Seal (for ALPS™ 3000 Automated Microplate Heat Sealer)
1	50-mL conical tube
1	250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)
As required	Serological pipettes (25 mL and 50 mL)
As required	P1000 pipette

[1] Each reagent must use a dedicated cassette.

Input samples

Number of plates	Plate name	Content
4 (maximum)	gDNA Sample Plate	Genomic DNA samples prepared in Chapter 2, “Genomic DNA preparation” (in a 96-deepwell plate).

Note: The same four 96-deepwell plates are processed in the workflow.

Reagent handling

Module 1, 4x96F reagents (Part No. 952262) are required from the Axiom™ Propel 4x96F Reagent Kit.

Thaw and prepare reagents according to the following table.

Table 34 Reagent handling for Stage 1 reagents.

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Reagent Kit Module 1 for 96F array plates only, 4x96F, –20°C	Axiom™ Propel 10X Denat Solution	952176	• Thaw at room temperature. This can be done as early as one day before use. • Vortex before master mix preparation.
	Axiom™ Propel Water	952177	• Thaw at room temperature. This can be done as early as one day before use.
	Axiom™ Propel Neutral Solution	952173	• Thaw at room temperature. This can be done as early as one day before use. • Vortex before master mix preparation.
	Axiom™ Propel Amp Solution	952174	• Thaw at room temperature. This can be done as early as one day before use. [1] • Vortex before master mix preparation.
	Axiom™ Propel Amp Enzyme	952175	• Keep at –20°C until ready to use. • Flick 5 times before master mix preparation.

[1] The thawed Axiom™ Propel Amp Solution solution may have white precipitate on the bottom of the bottle. Ensure that the precipitate dissolves into the solution after vortexing.

Prepare Denaturation Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 34.)

1. In a 50-mL conical tube, add reagents in the order shown.

Table 35 Denaturation Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Water	25.0 mL	25-mL serological pipette
2	Axiom™ Propel 10X Denat Solution	2.8 mL	P1000
	Total volume	**27.8 mL**	

2. Ensure that the cap of the conical tube is closed tightly, then vortex the Denaturation Master Mix for 5 seconds. Leave at room temperature until use.

Note: The Denaturation Master Mix can be stored at room temperature and used within 12 hours of its preparation.

Prepare the Axiom™ Propel Neutral Solution

Ensure that the Axiom™ Propel Neutral Solution is vortexed before use.

1. Use the Axiom™ Propel Neutral Solution bottle for Multidrop™ dispensing.

Table 36 Axiom™ Propel Neutral Solution for 4x96-format plates.

Reagent	Quantity	Transfer method
Axiom™ Propel Neutral Solution	Entire bottle	Use straight from bottle[1]

[1] The Axiom™ Propel Neutral Solution reagent bottle can be used for Multidrop™ Combi dispensing.

2. Leave at room temperature. Vortex before use.

Prepare the Amplification Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 34.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 37 Amplification Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Amp Solution	114.0 mL	50-mL serological pipette
2	Axiom™ Propel Amp Enzyme	2.53 mL	P1000 pipette
	Total volume	**116.53 mL**	

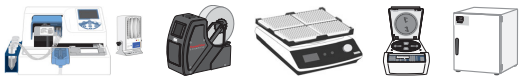
Note: The thawed Axiom™ Propel Amp Solution solution may have white precipitate on the bottom of the bottle. Ensure that the precipitates dissolve into the solution after vortexing.

Note: The Axiom™ Propel Amp Enzyme is a viscous solution. Pipette the solution slowing when aspirating from the reagent bottle to avoid bubble formation.

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Amplification Master Mix at least 15 times. Leave at room temperature.

Note: The Amplification Master Mix can be stored at room temperature and used within 2 hours of its preparation.

Stage 1 summary

Stage activities	Instruments required
• Three reagent additions with mixing—Denaturation Master Mix, Axiom™ Propel Neutral Solution, Amplification Master Mix • 10-minute denature incubation at room temperature • 22–24 hour amplification incubation at 37°C	

Perform the pre-run checklist

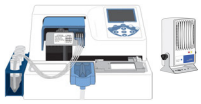
Before starting the workflow, the following tasks must be completed.

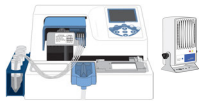
1. Multidrop™ Combi Reagent Dispenser:
   a. Ensure that the Multidrop™ Combi startup procedure has been performed. (See "Start up the Multidrop™ Combi" on page 54.)
   b. Perform gravimetric checks before running the workflow. (See "Perform gravimetric checks" on page 55.)
   c. Prime the Multidrop™ cassette with reagent. (See "Prime the cassette" on page 59.)
   d. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)
2. ALPS™ 3000 Automated Microplate Heat Sealer:
   a. Power on the ALPS™ 3000 Automated Microplate Heat Sealer, then allow it to reach 150°C.
   b. If the sealer is being used at the start of the day for this stage, do the following:
      - Allow 15 minutes for the sealer to reach 150°C.
      - Perform the sealing operation 2 to 3 times, then check to ensure that the seal quality and seal alignment are correct before starting the workflow.
3. If the gDNA Sample Plates are frozen, thaw them on the benchtop at room temperature, then centrifuge at 675 × *g* for 30 seconds to get all the droplets down.

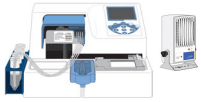
IMPORTANT! The gDNA samples must be brought to room temperature before proceeding with denaturation.

Stage 1: Amplify the genomic DNA

For a 4-plate workflow, complete 1 cycle of 4 plates.

Denaturation Master Mix		
	Plates 1 2 3 4	
1		Dispense Denaturation Master Mix • Method: 96-Den-20 • Dispense volume: 20 µL
2		Seal • Settings: 150°C, 2.5 seconds
3		Shake • Settings: 1,100 rpm, 30 seconds
4		Time • Start 10-minute timer **Note:** This step is time sensitive. Immediately proceed to the next step when the timer expires.
5		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds • Leave plates at room temperature after centrifugation until the timer alarms.
6		• When the 10-minute timer alarms, proceed *immediately* to the "Axiom™ Propel Neutral Solution" step. • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Axiom™ Propel Neutral Solution		
		Plates 1 2 3 4
1		Dispense Axiom™ Propel Neutral Solution • Method: 96-Neu-130 • Dispense volume: 130 µL
2		Seal • Settings: 150°C, 2.5 seconds
3		Shake • Settings: 1,100 rpm, 30 seconds
4		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
5		• Continue to the Amplification Master Mix step. • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Amplification Master Mix		
	Plates 1 2 3 4	
1		Dispense Amplification Master Mix • Method: 96-Amp-230 • Dispense volume: 230 µL
2		Seal • Settings: 150°C, 2.5 seconds
3		Shake • Settings: 1,100 rpm, 30 seconds
4		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
5		Incubate • Temperature: 37ºC • Time: 23 ±1hour • Volume = 400 µL/well
6		After the 22—24-hour incubation, do one of the following. • Proceed directly to "Stage 2: Fragment the DNA" on page 137, or • Store the Amplification Plates at –20°C. The frozen post-amplification plates can be stored at –20°C for up to 1 week. Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

Stage 2: Fragment the DNA

Equipment and labware required

Quantity	Item
Instruments	
2	Multidrop™ Combi Reagent Dispenser
2	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	ALPS™ 3000 Automated Microplate Heat Sealer
1	Compact Digital Microplate Shaker
1	Sorvall™ X4R Pro-MD Centrifuge (set to room temperature)
1	BINDER™ ED 56 or BINDER™ BD 56 (set to 37°C) • Must maintain a constant temperature of 37°C for at least 24 hours with a temperature accuracy of ±1°C.
1	BINDER™ ED 56 or BINDER™ BD 56 (set to 65°C)[2] • Must maintain a constant temperature of 65°C for at least 24 hours with a temperature accuracy of ±1°C.
1	Vortex mixer
1	Mini centrifuge
1	Electronic pipettor
1	P1000 pipettes
1	Digital timer
Labware and consumables	
As required	Easy Peel Seal (for ALPS™ 3000 Automated Microplate Heat Sealer)
1	250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)
As required	P1000 pipette tips
As required	Serological pipettes (10 mL and 50 mL)

[1] Each reagent must use a dedicated cassette.
[2] Only required when processing frozen Amplification Plates.

Input samples

Number of plates	Plate name	Content
4 (maximum)	Amplification Plate	Amplified gDNA samples prepared in "Stage 1: Amplify the genomic DNA" on page 129 (in a 96-deepwell plate).

Reagent handling

Module 2-1, 4x96F and Module 2-2, 4x96F reagents are required from the Axiom™ Propel 4x96F Reagent Kit.

- Axiom™ Propel Reagent Kit Module 2-1 for 96F or 384HT, Part No. 952263
- Axiom™ Propel Reagent Kit Module 2-2 for 96F or 384HT, Part No. 952265

Thaw and prepare reagents according to the following table.

Table 38 Reagent handling for Stage 2 and Stage 3 reagents.

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Reagent Kit Module 2-1 for 96F or 384HT –20°C	Axiom™ Propel 10X Frag Buffer	952179	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Frag Enzyme	952181	• Keep at –20°C until ready to use. • Flick 5 times, then perform brief centrifuge before master mix preparation.
	Axiom™ Propel Precip Solution 2	952178	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
Axiom™ Propel Reagent Kit Module 2-2 for 96F or 384HT 4°C	Axiom™ Propel Frag Diluent	952184	• Remove from 4°C and equilibrate to room temperature on day of use. • Vortex before master mix preparation.
	Axiom™ Propel Frag Reaction Stop	952190	• Remove from 4°C and equilibrate to room temperature on day of use. • Vortex before use.
	Axiom™ Propel Precip Solution 1	952203	• Remove from 4°C and equilibrate to room temperature on day of use. • Vortex before master mix preparation.
Amplified sample plates			

Table 38 Reagent handling for Stage 2 and Stage 3 reagents. *(continued)*

Module	Reagent	Component Part No.	Treatment
• If proceeding directly from the 22—24-hour amplification, continue with the fragmentation step that is described in this chapter. • If the Amplification Plates have been frozen: **a.** Place the frozen plates at room temperature to thaw. To shorten the thawing time, the frozen plates can be placed into a room temperature deionized water bath. The thawing time is ~1 hour. **b.** Wipe off excess water on the outside of the plates before continuing to the next step. **c.** Centrifuge at 675 × *g* for 30 seconds. **d.** Place the thawed Amplification Plates in the 65°C oven and incubate for 20 minutes. **e.** Transfer the Amplification Plates from the 65°C oven to the 37°C oven and incubate for 45 minutes. **Note:** Place plates on the rack in a single layer—do not stack plates directly on top of one another.			

Prepare the Fragmentation Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 38.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 39 Fragmentation Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel 10X Frag Buffer	36.0 mL	50-mL serological pipette
2	Axiom™ Propel Frag Diluent	8.1 mL	10-mL serological pipette
3	Axiom™ Propel Frag Enzyme	0.79 mL	P1000 pipette
	Total volume	**44.89 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Fragmentation Master Mix at least 15 times to mix. Leave at room temperature until use.

Note: Use the Fragmentation Master Mix within 1 hour of its preparation.

Prepare the Axiom™ Propel Frag Reaction Stop

Ensure that the Axiom™ Propel Frag Reaction Stop is vortexed before use.

1. Use the Axiom™ Propel Frag Reaction Stop bottle for Multidrop™ dispensing.

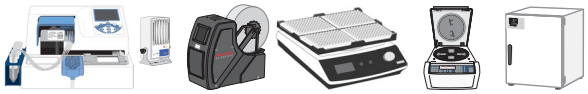
Table 40 Axiom™ Propel Frag Reaction Stop for 4x96-format plates.

Reagent	Quantity	Transfer method
Axiom™ Propel Frag Reaction Stop	Entire bottle	Use straight from bottle[1]

[1] The Axiom™ Propel Frag Reaction Stop reagent bottle can be used for Multidrop™ Combi dispensing.

2. Leave at room temperature. Vortex before use.

Stage 2 summary

Stage activities	Instruments required
• Two reagent additions with mixing—Fragmentation Master Mix, Axiom™ Propel Frag Reaction Stop • 30-minute fragmentation incubation at 37°C	

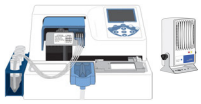
Perform the pre-run checklist

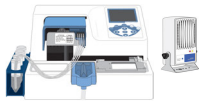
Before starting the workflow, the following tasks must be completed.

1. Multidrop™ Combi Reagent Dispenser:
   a. Ensure that the Multidrop™ Combi startup procedure has been performed. (See "Start up the Multidrop™ Combi" on page 54.)
   b. Perform gravimetric checks before running the workflow. (See "Perform gravimetric checks" on page 55.)
   c. Prime the Multidrop™ cassette with reagent. (See "Prime the cassette" on page 59.)
   d. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)
2. ALPS™ 3000 Automated Microplate Heat Sealer:
   a. Power on the ALPS™ 3000 Automated Microplate Heat Sealer, then allow it to reach 150°C.
   b. If the sealer is being used at the start of the day for this stage, do the following:
      • Allow 15 minutes for the sealer to reach 150°C.
      • Perform the sealing operation 2 to 3 times, then check to confirm that the seal quality and seal alignment are correct before starting the workflow.

Stage 2: Fragment the DNA

For a 4-plate workflow, complete 1 cycle of 4 plates.

Fragmentation Master Mix		
	Plates 1 2 3 4	
①		Dispense Fragmentation Master Mix • Method: 96-Frag-60 • Dispense volume: 60 µL
②		Seal • Settings: 150°C, 2.5 seconds
③		Shake • Settings: 1,100 rpm, 30 seconds
④		Time • Start 30-minute timer
⑤		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
⑥		Incubate • Temperature: 37°C • Time: 30 minutes (Time begins at completion of shaking step.)
⑦		• When the 30-minute timer alarms, proceed *immediately* to "Axiom™ Propel Frag Reaction Stop" step. • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Axiom™ Propel Frag Reaction Stop		
	Plates 1 2 3 4	
1		Dispense Axiom™ Propel Frag Reaction Stop • Method: 96-Stop-20 • Dispense volume: 20 µL
2		Seal • Settings: 150°C, 2.5 seconds
3		Shake • Settings: 1,100 rpm, 30 seconds
4		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
5		• Volume = 480 µL • Continue to "Stage 3: Precipitate the DNA" on page 144 • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Stage 3: Precipitate the DNA

Equipment and labware required

Quantity	Item
Instruments	
2	Multidrop™ Combi Reagent Dispenser
2	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	ALPS™ 3000 Automated Microplate Heat Sealer
1	VWR Signature™ High-Speed Microplate Shaker
1	Freezer, –20°C
1	P1000 pipette
1	Fume hood
Labware and consumables	
As required	Easy Peel Seal (for ALPS™ 3000 Automated Microplate Heat Sealer)
1	Glass or polypropylene bottle, minimum 1,000 mL capacity (for isopropanol dispensing)
As required	P1000 pipette tips

[1] Each reagent must use a dedicated cassette.

Input samples

Number of plates	Plate name	Content
4 (maximum)	Fragmentation Plate	Fragmented DNA samples prepared in "Stage 2: Fragment the DNA" on page 137 (in a 96-deepwell plate).

Prepare Precipitation Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 38.)

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

1. Use the Axiom™ Propel Precip Solution 1 bottle for Multidrop™ dispensing.

Table 41 Precipitation Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Precip Solution 1	Entire bottle	Use straight from bottle[1]
2	Axiom™ Propel Precip Solution 2	Entire bottle	P1000 pipette
	Total volume	**~116 mL**	

[1] The Axiom™ Propel Precip Solution 1 reagent bottle can be used for Multidrop™ Combi dispensing.

2. Ensure that the cap of the Axiom™ Propel Precip Solution 1 bottle is closed tightly, then invert the Precipitation Master Mix at least 15 times to mix. Leave at room temperature until use.

Note: The Precipitation Master Mix can be stored at room temperature and used within 12 hours of its preparation.

Prepare the Isopropanol

Fill an appropriately sized container with the isopropanol.

Table 42 Isopropanol for 4x96-format plates.

Reagent	Quantity
Isopropanol	550 mL[1]

[1] The quantity of isopropanol required includes usage for priming and gravimetric checks. If a flat bottom bottle is used, add 20 mL to account for the dead volume of the bottle.

IMPORTANT! The isopropanol must be primed through the cassette tubing, then allowed to equilibrate for a minimum of 15 minutes. Equilibrating the isopropanol is a highly critical step that results in the most accurate dispense volumes. Failure to follow this requirement can result in compromised assay performance.

Stage 3 summary

Stage activities	Instruments required
• Two reagent additions with high-speed mixing—Precipitation Master Mix and isopropanol. • 16–24-hour precipitation incubation at –20°C.	

Perform the pre-run checklist

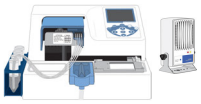
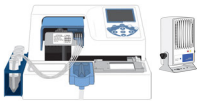
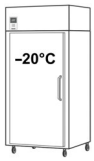
Before starting the workflow, the following tasks must be completed.

1. Multidrop™ Combi Reagent Dispenser:
   a. Ensure that the Multidrop™ Combi startup procedure has been performed. (See "Start up the Multidrop™ Combi" on page 54.)
   b. Perform gravimetric checks before running the workflow. (See "Perform gravimetric checks" on page 55.)

      Allow the isopropanol to equilibrate inside the tubing for 15 minutes before performing gravimetric checks.
   c. Prime the Multidrop™ cassette with reagent. (See "Prime the cassette" on page 59.)
   d. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)
2. ALPS™ 3000 Automated Microplate Heat Sealer:
   a. Power on the ALPS™ 3000 Automated Microplate Heat Sealer then allow it to reach 150°C.
   b. If the sealer is being used at the start of the day for this stage, do the following:
      - Allow 15 minutes for the sealer to reach 150°C.
      - Perform the sealing operation 2 to 3 times, then check to confirm that the seal quality and seal alignment are correct before starting the workflow.

Stage 3: Precipitate the DNA

For a 4-plate workflow, complete 1 cycle of 4 plates.

Precipitation		
	Plates 1 2 3 4	
1		Dispense Precipitation Master Mix • Method: 96-Pre-220 • Dispense volume: 220 µL
2		Dispense isopropanol • Method: 96-Iso-660 • Dispense volume: 660 µL
3		Seal • Settings: 150°C, 2.5 seconds
4		Shake • Settings: 2,450 rpm, 60 seconds **IMPORTANT!** Do not centrifuge the plates after shaking. Continue directly to freezing the plates at –20°C.
5		Freeze • –20°C, 16–24 hours • Volume = 1,360 µL/well • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

Stage 4: Centrifuge and dry DNA pellets

Equipment required

Quantity	Item
2	Sorvall™ X4R Pro-MD Centrifuge (set at 4°C)
1	BINDER™ ED 56 or BINDER™ BD 56 (set at 37°C) • Must maintain a constant temperature of 37°C for at least 24 hours with a temperature accuracy of ±1°C.
1	Fume hood

Input samples

Number of plates	Plate name	Content
4 (maximum)	Precipitation Plate	Samples, post precipitation incubation, prepared in “Stage 3: Precipitate the DNA” (in a 96-deepwell plate).

Stage 4 summary

Stage activities	Instruments required
• Purify amplified DNA into dried pellets.	

Perform the pre-run checklist

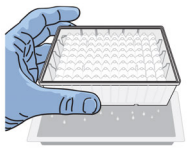
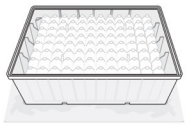
Before starting the workflow, the following must be completed.

Set each Sorvall™ X4R Pro-MD Centrifuge to 4°C, then allow the centrifuges to equilibrate before starting the centrifugation step.

Stage 4: Centrifuge and dry pellets

For a 4-plate workflow, complete 1 cycle of 4 plates. Decant under a ventilated fume hood.

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

Centrifugation		
	Plates 1 2 3 4	
①		Centrifuge at 4°C. • 3,200 × *g* for 40 minutes.
②		Gently pour off solution over a waste container and allow the liquid to drain.
③		Keep inverted for 5 minutes over laboratory tissues. Transfer the plate to a new pile of laboratory tissues twice during the 5-minute period.
④		• Dry in 37°C oven for 20 minutes. • Place the plates in the oven with wells facing up.
⑤		• Do one of the following: – Proceed directly to "Stage 5: Resuspend the pelleted DNA and prepare for hybridization" on page 85, even if some droplets of liquid remain. Leave the sample plates at room temperature. It is helpful to start preparing reagents for stage 5 during centrifuging and drying pellets. – Store the plates for resuspension later in the same day. Tightly seal the plates. – If resuspension is carried out in 4 hours, keep the plates at room temperature. – If resuspension is carried out in more than 4 hours, store the plates in a refrigerator (2–8°C). The refrigerated pellets must be equilibrated to room temperature for at least 30 minutes before proceeding to Stage 5. • To process the plates for resuspension on another day, tightly seal the plates and store at –20°C. The pellets can be stored at –20°C for one day. – The frozen pellets must be equilibrated to room temperature for at least 90 minutes before proceeding to Stage 5.

Stage 5: Resuspend the pelleted DNA and prepare for hybridization

Equipment and labware required

Table 43 Equipment and labware required for Stage 5.

Quantity	Item
Instruments	
2	Multidrop™ Combi Reagent Dispenser
2	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	ALPS™ 3000 Automated Microplate Heat Sealer
1	Compact Digital Microplate Shaker
1	Sorvall™ X4R Pro-MD Centrifuge (set to room temperature)
1	Vortex mixer
1	Electronic pipettor
1	P1000 pipette
1	Fume hood
Labware and consumables	
As required	Easy Peel Seal (This is used for the ALPS™ 3000 Automated Microplate Heat Sealer.)
1	250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)
As required	Serological pipettes (5 mL and 50 mL)
As required	P1000 pipette tips

[1] Each reagent must use a dedicated cassette.

Table 44 Equipment and labware required for the Integra transfer.

Quantity	Item
Instruments	
1	VIAFLO™ 96 Base Unit or VIAFLO™ 384 Base Unit with a 96 Channel Pipetting Head (5–125 µL) and a 3 position stage installed.
Labware and consumables	
384 tips	Integra VIAFLO XYZ GripTips™
4	MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (half skirt)

Table 44 Equipment and labware required for the Integra transfer. *(continued)*

Quantity	Item
1	Corning™ Clear Polystyrene 96-Well Microplate (This is used as the plate holder for the half-skirted MicroAmp™ EnduraPlate™.)
As required	MicroAmp™ Clear Adhesive Film

Input samples

Number of plates	Plate name	Content
4 (maximum)	Pelleted Precipitation Plate	Dried DNA pellets prepared in “Stage 4: Centrifuge and dry DNA pellets” (in a 96-deepwell plate).

Reagent handling

Module 2-1, 4x96F and Module 2-2, 4x96F reagents are required from the Axiom™ Propel 4x96F Reagent Kit.

- Axiom™ Propel Reagent Kit Module 2-1 for 96F or 384HT, Part No. 952263
- Axiom™ Propel Reagent Kit Module 2-2 for 96F or 384HT, Part No. 952265

Thaw and prepare reagents according to the following table.

Table 45 Reagent handling for Stage 5 reagents.

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Reagent Kit Module 2-1 for 96F or 384HT –20°C	Axiom™ Propel Hyb Buffer	952182	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Hyb Solution 1	952183	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
Axiom™ Propel Reagent Kit Module 2-2 for 96F or 384HT 4°C	Axiom™ Propel Hyb Solution 2	951979	• Remove from 4°C and equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Resuspension Buffer	952206	• Remove from 4°C and equilibrate to room temperature on day of use. • Vortex before use.

Guidelines for pellet preparation

The equilibration of the plate of pelleted DNA, Axiom™ Propel Resuspension Buffer, and Axiom™ Propel Hyb Buffer to room temperature (18-25°C) is very critical for the success of the assay. If any of these are cooler than room temperature, pellets may not resuspend completely. This can result in compromised assay performance. Follow the guidelines below on how to work with plates with fresh, cold, or frozen pellets.

- **Fresh pellets:** A plate with fresh pellets can be kept at room temperature if proceeding with Stage 5 within 4 hours.
- **Cold pellets:** A plate with fresh pellets that are not processed within 4 hours can be transferred to a refrigerator (2-8°C) if processed during the same day. However, it is critical to equilibrate the plate to room temperature for at least 30 minutes before proceeding with Stage 5.
- **Frozen pellets:** A plate with frozen pellets must be pre-equilibrated at room temperature for at least 1.5 hours before proceeding with Stage 5.

Prepare the Axiom™ Propel Resuspension Buffer

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

1. Use the Axiom™ Propel Resuspension Buffer bottle for Multidrop™ dispensing.

Table 46 Axiom™ Propel Resuspension Buffer for 4x96-format plates.

Reagent	Quantity	Transfer method
Axiom™ Propel Resuspension Buffer	Entire bottle	Use straight from bottle[1]

[1] The Axiom™ Propel Resuspension Buffer reagent bottle can be used for Multidrop™ Combi dispensing.

2. Leave at room temperature until use.

Prepare Hybridization Master Mix

Ensure that all of the components are treated according to the reagent handling table before preparing the master mix. (See Table 45.)

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

1. In a 250-mL conical bottle, add reagents in the order shown.

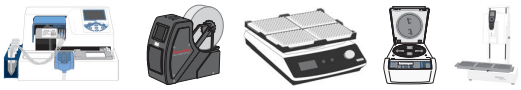
Table 47 Hybridization Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Hyb Buffer	47.0 mL	50-mL serological pipette
2	Axiom™ Propel Hyb Solution 1	0.333 mL	P1000 pipette
3	Axiom™ Propel Hyb Solution 2	6.0 mL (3 mL x 2)	5-mL serological pipette
	Total volume	**53.33 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, then vortex the Hybridization Master Mix for 5 seconds. Leave at room temperature until use.

Note: The Hybridization Master Mix can be stored at room temperature and used within 12 hours of its preparation.

Stage 5 summary

Stage activities	Instruments required
• Two reagent additions with mixing—Axiom™ Propel Resuspension Buffer, Hybridization Master Mix. • 10 minute shaking to resuspend the DNA pellets. • Transfer from 96-deepwell plate to half-skirted 96-well PCR plate.	

Perform the pre-run checklist

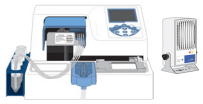
Before starting the workflow, the following tasks must be completed.

1. Multidrop™ Combi Reagent Dispenser:
   a. Ensure that the Multidrop™ Combi startup procedure has been performed. (See "Start up the Multidrop™ Combi" on page 54.)
   b. Perform gravimetric checks before running the workflow. (See "Perform gravimetric checks" on page 55.)
   c. Prime the Multidrop™ cassette with reagent. (See "Prime the cassette" on page 59.)
   d. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)
2. ALPS™ 3000 Automated Microplate Heat Sealer:
   a. Power on the ALPS™ 3000 Automated Microplate Heat Sealer then allow it to reach 150°C.
   b. If the sealer is being used at the start of the day for this stage, do the following:
      - Allow 15 minutes for the sealer to reach 150°C.
      - Perform the sealing operation 2 to 3 times, then check to ensure that the seal quality and seal alignment are correct before starting the workflow.
3. Ensure that the VIAFLO™ 96 or 384 Base Unit is clean, then power on the unit.
4. If the pellets are refrigerated or frozen, equilibrate them to room temperature before the resuspension step. (See "Guidelines for pellet preparation" on page 87.)

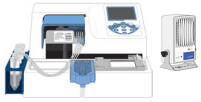
Note: The equilibration of pelleted DNA and Axiom™ Propel Resuspension Buffer to room temperature (18°C to 25°C) is critical for the resuspension process. When either is cooler than room temperature, pellets may not resuspend completely, which can result in lower performance.

Stage 5: Resuspend the pelleted DNA and prepare for hybridization

For a 4-plate workflow, complete 1 cycle of 4 plates.

Axiom™ Propel Resuspension Buffer		
		Plates 1 2 3 4
1		Dispense Axiom™ Propel Resuspension Buffer • Method: 96-Res-35 • Dispense volume: 35 µL
2		Seal • Settings: 150°C, 2.5 seconds
3		Shake • Settings: 1,100 rpm, 10 minutes
4		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
5		• Continue to "Hybridization Master Mix" step. • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61.

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

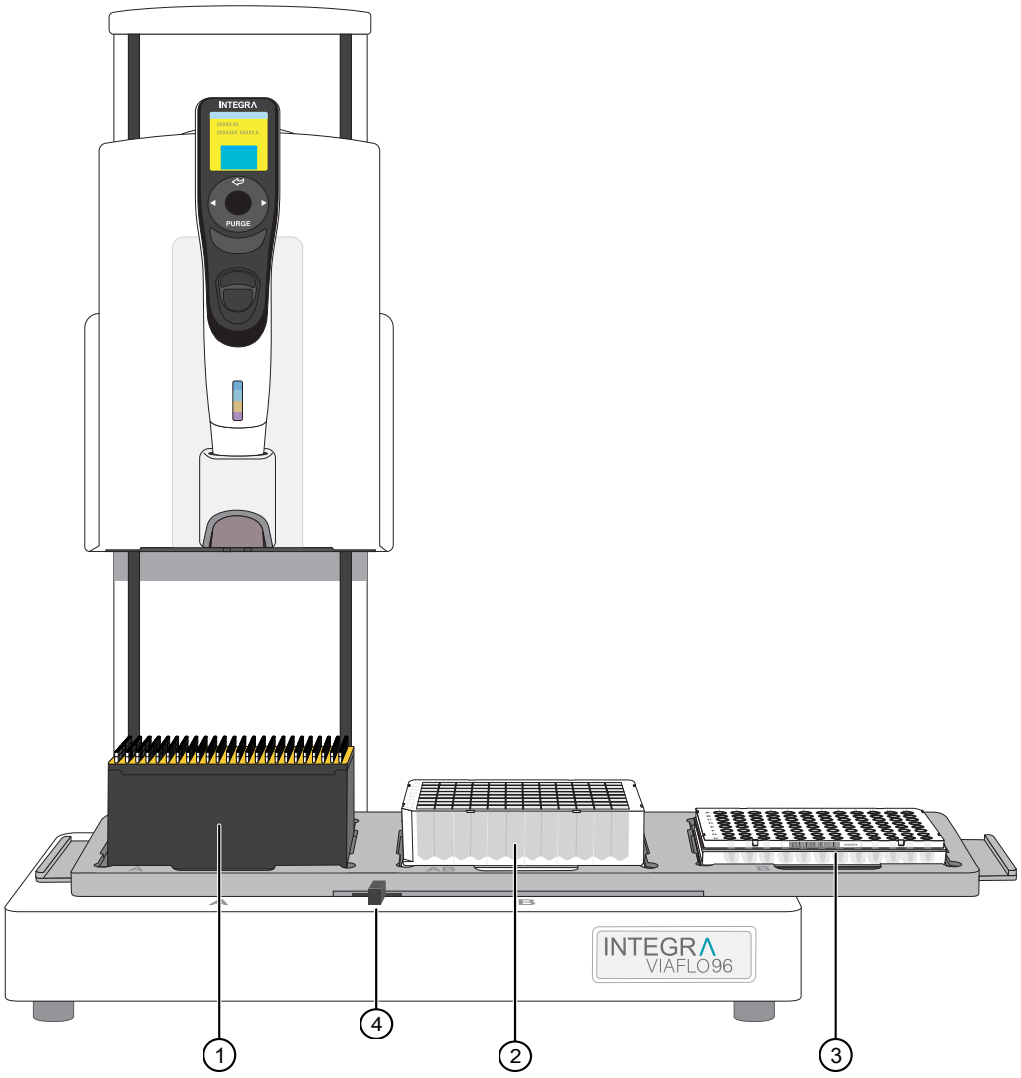
Hybridization Master Mix		
	Plates 1 2 3 4	
1		Dispense Hybridization Master Mix • Method: 96-Hyb-80 • Dispense volume: 80 µL
2		Seal • Settings: 150°C, 2.5 seconds
3		Shake • Settings: 1,100 rpm, 30 seconds
4		Centrifuge • Centrifuge at room temperature • Settings: 675 × *g*, 30 seconds
5		• Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61. • Continue to: – "Transfer the hybridization-ready target to the half-skirted 96-well PCR plate" on page 157, then: – (*Recommended*) "Stage 5A: In-process QC" on page 161, and/or – "Stage 6: Denature the target and transfer to hybridization tray" on page 171

Transfer the hybridization-ready target to the half-skirted 96-well PCR plate

The *DW TO PCR* VIAFLO method is a whole plate transfer of 115 µL of the hybridization-ready target in a 96-deepwell plate to a clean, labeled half-skirted 96-well PCR plate (MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode).

1. Press the **Run** button *twice* to home the VIAFLO stage and pipetting head.

2. Place labware on the VIAFLO stage as shown in the following image.

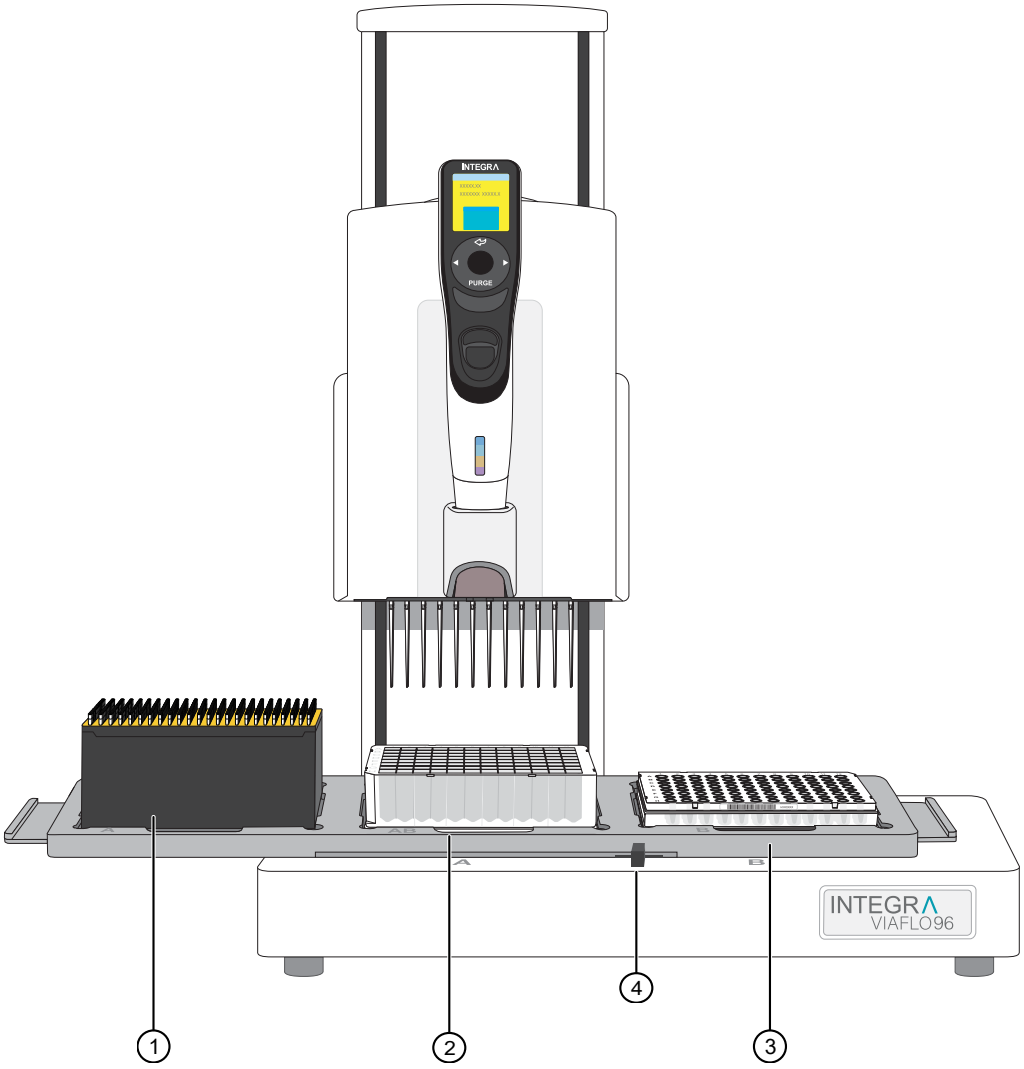


① 384 tips
② 96-deepwell hybridization-ready sample plate
③ Half-skirted 96-well PCR plate on a plate holder
④ Stage slider

3. Slowly move the 3-position stage to the *right* for tip loading.

4. Load tips to the 96-head by adjusting the stage slider.
    a. Adjust the stage slider to the left or right, depending on the tips available for use.
        - Right—moves stage back to pick up quadrant 1 or quadrant 2 tips.
        - Left—moves stage forward to pick up quadrant 3 or quadrant 4 tips.
    b. Place one hand on the control unit handle. Maintain contact with the handle throughout the tip loading step.
    c. Gently lower the 96-head into the tip box and guide it into a set of 96 tips.
    d. After the tips are properly aligned, the indicator light of the tip load button blinks. Use the opposite hand and firmly press down on the tip load button, located at the top of the 96-head.
    e. Release the button when you hear a click. The indicator light on the button remains on.
    f. Using the control unit handle, lift the head away from the platform to provide clearance for stage loading or positioning.
5. To start method, go to the **Main Menu**, then select **Custom Programs**.
6. Under **Custom Programs**, select **DW to PCR**.

7. Move the stage slider to the center, move the 3-position stage to the *left*, then allow the 96-head to hover over the 96-deepwell sample plate.



① Tips
② 96-deepwell hybridization-ready sample plate
③ Half-skirted 96-well PCR plate on a plate holder
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

8. Press the **Run** button to run the method.
The instrument aspirates the hybridization-ready samples from the 96-deepwell sample plate, then transfers them into the half-skirted 96-well PCR plate.

9. After the run is complete, remove the 96-deepwell sample plate from position 2. Inspect the 96-deepwell sample plate to ensure that all of the samples have been transferred to the half-skirted 96-well PCR plate.

10. Eject and discard tips.

11. Proceed to “Stage 5A: In-process QC” on page 95 if running in-process QC.

Stage 5A: In-process QC

Equipment and labware required

Quantity	Item
Instruments	
1	VIAFLO™ 96 Base Unit or VIAFLO™ 384 Base Unit with a 96 Channel Pipetting Head (5–125 µL) and a 3 position stage installed.
1	Multidrop™ Combi Reagent Dispenser
2	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	Sorvall™ X4R Pro-MD Centrifuge (Set to room temperature.)
1	Vortex mixer
1	Fume hood
Labware and consumables	
384 tips	Integra VIAFLO XYZ GripTips™
8	MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plates with Barcode, Blue
1	Corning™ Clear Polystyrene 96-Well Microplate (This is used as the plate holder for the half-skirted MicroAmp™ EnduraPlate™.)
4	Greiner Bio-One™ 96-Well UV-Star™ Plate, Flat Bottom, Chimney Style, Clear
As required	MicroAmp™ Clear Adhesive Film

[1] Each reagent must use a dedicated cassette.

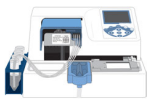
Input samples

Number	Plate name	Content
4 (maximum)	Hyb-Ready Plate	Hybridization-ready target DNA prepared in "Stage 5: Resuspend the pelleted DNA and prepare for hybridization" on page 150 (in a MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode—half skirt)

Reagents required

Reagent	Volume
UltraPure™ DNase/RNase-Free Distilled Water	500 mL
Gel diluent	Prepare as instructed. (See "Prepare the gel diluent" on page 241.)

Stage 5A summary

Stage activities	Instruments required
• Three reagent dispenses—Dilution QC Plates, OD QC Plates, Gel QC Plates • Transfer hybridization-ready target from Hyb-Ready Plates to QC plates	  

Perform the pre-run checklist

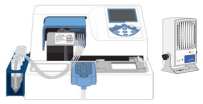
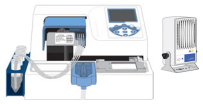
Before starting the workflow, the following tasks must be completed.

Multidrop™ Combi Reagent Dispenser:

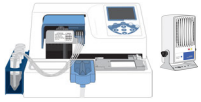
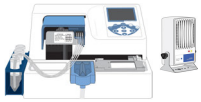
1. Ensure that the Multidrop™ Combi startup procedure has been performed. (See “Start up the Multidrop™ Combi” on page 54.)
2. Perform gravimetric checks before running the workflow. (See “Perform gravimetric checks” on page 55.)
3. Prime the Multidrop™ cassette with reagent. (See “Prime the cassette” on page 59.)
4. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)

Stage 5A: In-process QC

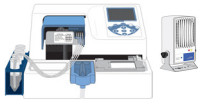
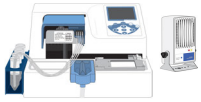
For a 4-plate workflow, complete 1 cycle of 4 plates.

Dilution QC Plates[1]		
	Plates 1 2 3 4	
1		Dispense UltraPure™ DNase/RNase-Free Distilled Water • Method: 96-QC-Dil-55 • Dispense volume: 55 µL • Volume of water required for 4 plates: 35 mL
2		After dispensing, seal the plate. Tip: After unpeeling a new adhesive seal, use the ion fan to reduce the amount of static on the sticky side of the seal. This minimizes the droplets jumping up onto the seal due to static.
3		Repeat dispense and seal all 4 plates.
4		Centrifuge all 4 plates • Setting: 675 × *g* for 30 seconds
5		Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

[1] The MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plate with Barcode, Blue is used as the Dilution QC Plate.

OD QC Plates[1]		
	Plates 1 2 3 4	
1		Dispense UltraPure™ DNase/RNase-Free Distilled Water • Method: 96-QC-OD-90 • Dispense volume: 90 µL • Volume of water required for 4 plates: 50 mL
2		Repeat dispense, and stack all 4 plates.
3		Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

[1] The Greiner Bio-One™ 96-Well UV-Star™ Plate, Flat Bottom, Chimney Style, Clear is used as the OD QC Plate.

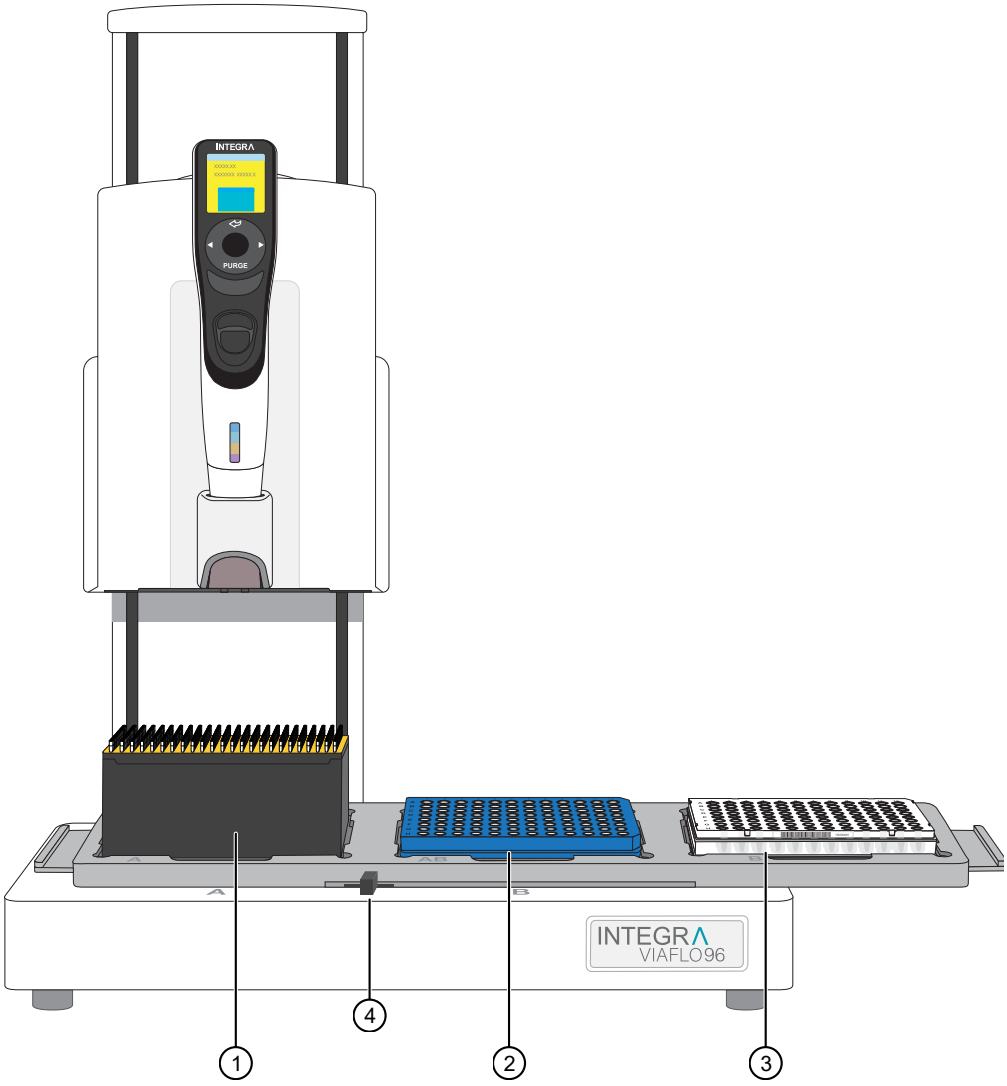
Gel QC Plates[1]		
	Plates 1 2 3 4	
1		Dispense gel diluent • 96-QC-Gel-150 • Dispense volume: 150 µL • Volume of gel diluent required for 4 plates: 70 mL
2		Repeat dispense, and stack all 4 plates.
3		Continue with Integra VIAFLO
4		Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

[1] MicroAmp™ EnduraPlate™ Optical 96-Well Full-Skirted Plate with Barcode, Blue is used as the Gel QC Plate.

Transfer hybridization-ready target in Hyb-Ready Plate to QC plate

The *PCR to QCPLATES* method transfers 5 µL of sample from Hyb-Ready Plate to the Dilution QC Plate, then 10 µL of diluted sample from the Dilution QC Plate to the OD QC Plate, and finally 5 µL of diluted sample from the Dilution QC Plate to the Gel QC Plate.

1. Place labware on the VIAFLO stage as shown in the following image.

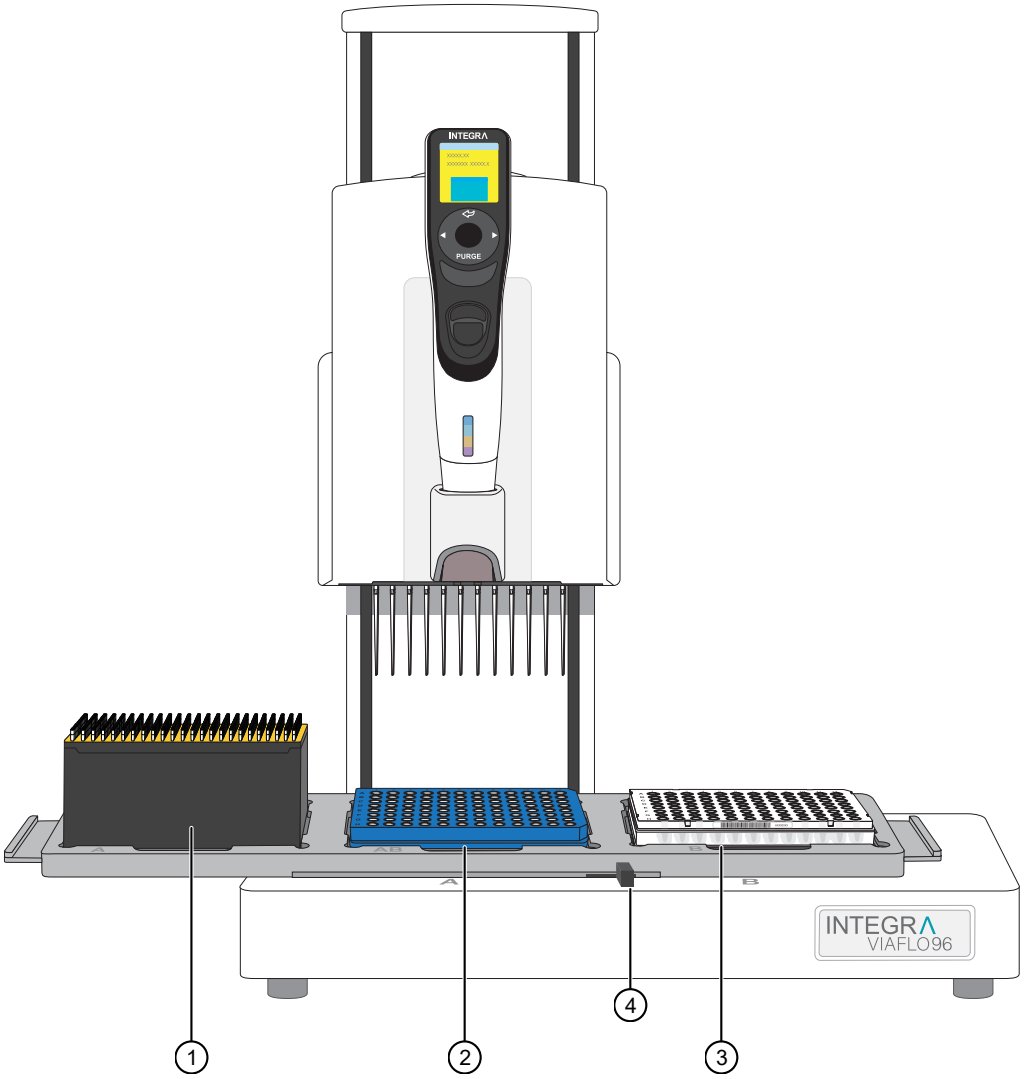


① Tips
② Dilution QC Plate
③ Hyb-Ready Plate on a plate holder
④ Stage slider

2. Slowly move the 3-plate stage to the *right* for tip loading.

3. Load tips to the 96-head by adjusting the stage slider.
   a. Adjust the stage slider to the left or right, depending on the tips available for use.
      - Right—moves stage back to pick up quadrant 1 or quadrant 2 tips.
      - Left—moves stage forward to pick up quadrant 3 or quadrant 4 tips.
   b. Place one hand on the control unit handle. Maintain contact with the handle throughout the tip loading step.
   c. Gently lower the 96-head into the tip box and guide it into a set of 96 tips.
   d. After the tips are properly aligned the indicator light of the tip load button blinks. Use the opposite hand and press down on the tip load button, located at the top of the 96-head.
   e. Release the button when you hear a click. The indicator light on the button remains on.
   f. Using the control unit handle, lift the head away from the platform to provide clearance for stage loading or positioning.
4. From the **Main Menu**, select **Custom Programs ▸ PCR to QCPLATES** to choose the method.

5. Move the stage slider to the center, move the 3-position stage to the left, then allow the 96-head to rest over the Dilution QC Plate.



① Tips
② Dilution QC Plate
③ Hyb-Ready Plate on a plate holder
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

6. Press **Run** to start the method.

7. When the run pauses, do not eject tips.

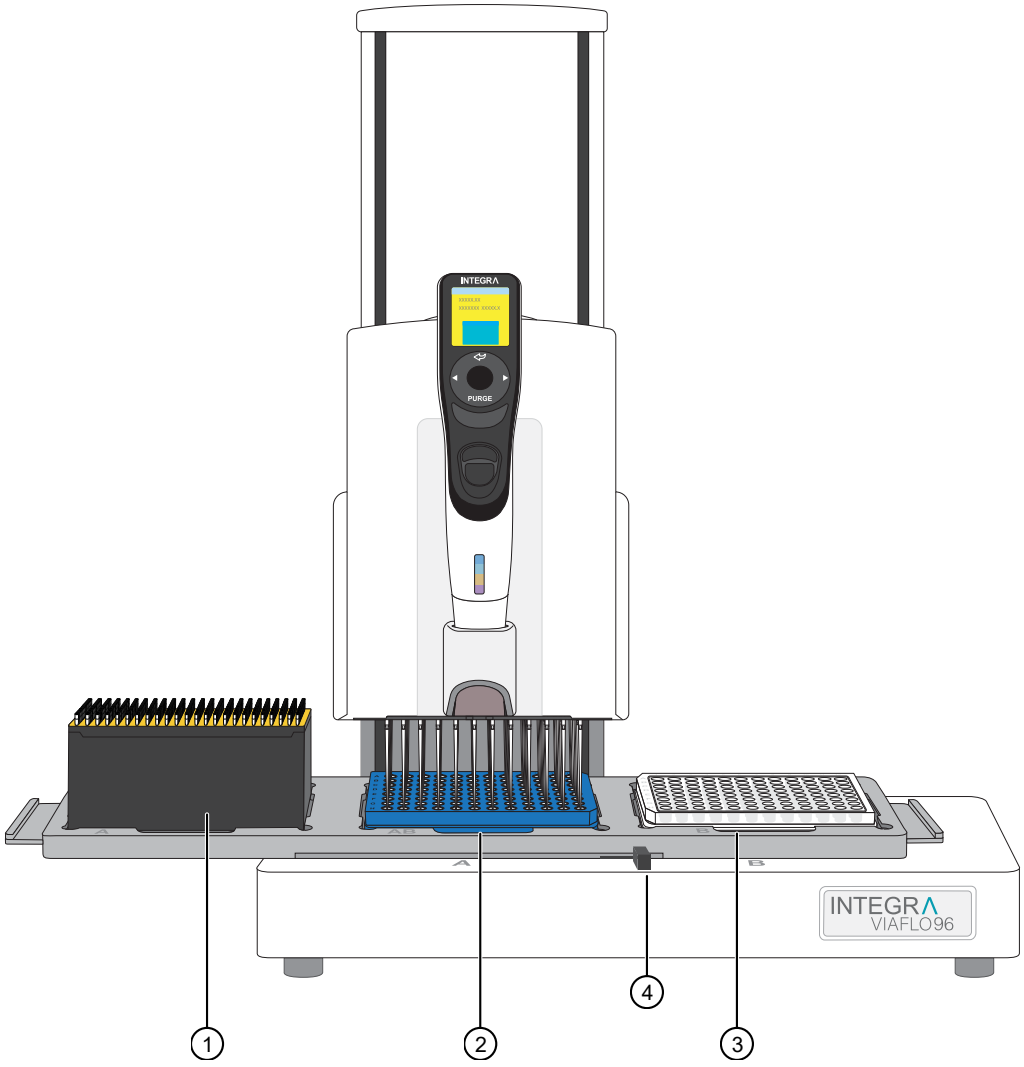
8. When prompted, remove the Hyb-Ready Plate, tightly seal, then store at –20°C.

   Tip: After unpeeling a new adhesive seal, use the ion fan to reduce the amount of static on the sticky side of the seal. This minimizes the droplets jumping up onto the seal due to static.

The Hyb-Ready Plate can be stored at –20°C for up to 2 weeks.

9. Remove the plate holder.

10. Place the OD QC Plate on position 3 where the Hyb-Ready Plate and plate holder used to be.



① Tips
② Dilution QC Plate
③ OD QC Plate
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

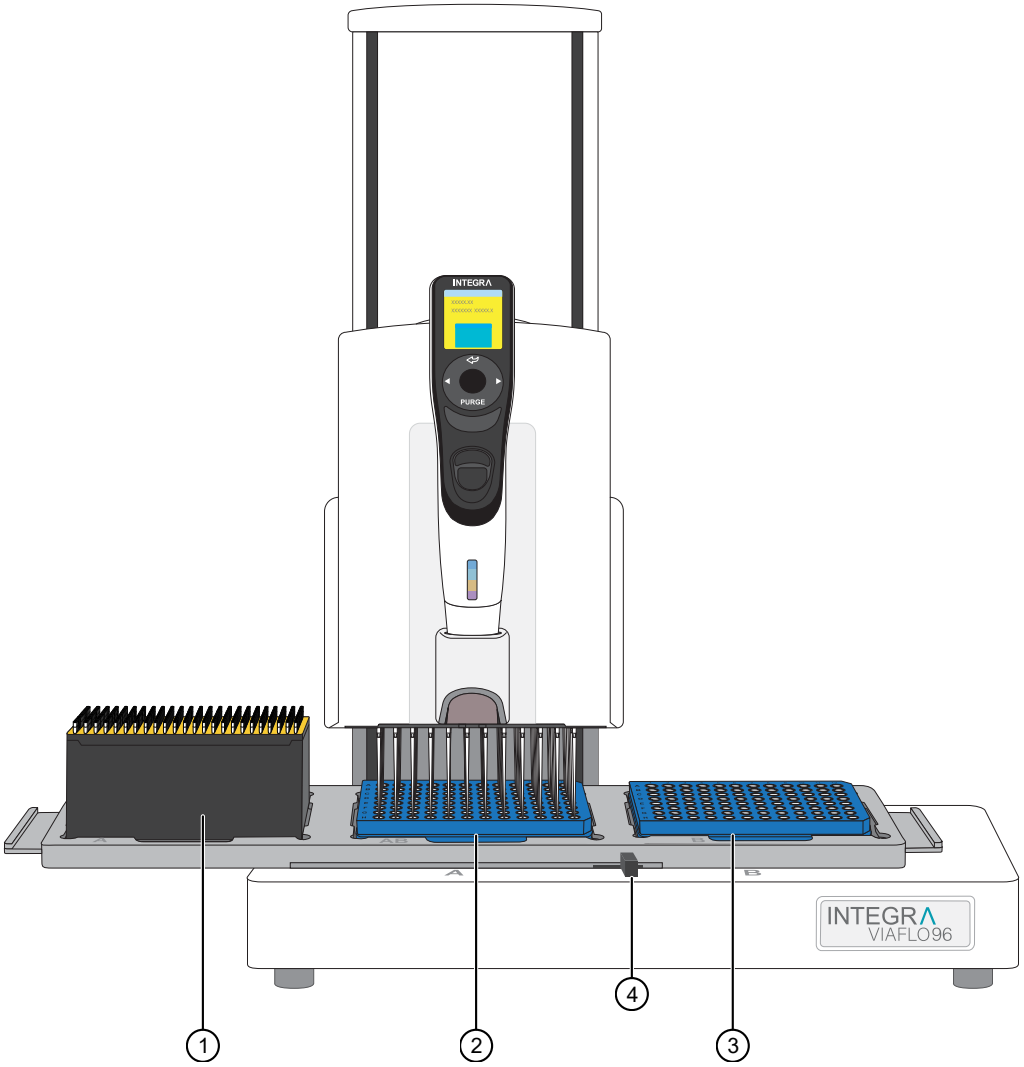
11. Resume the method by pressing the **Run** key.

12. When prompted, remove OD QC Plate, seal, then stack on benchtop.

    Tip: After unpeeling a new adhesive seal, use the ion fan to reduce the amount of static on the sticky side of the seal. This minimizes the droplets jumping up onto the seal due to static.

OD QC Plates are stacked and then later vortexed and centrifuged at 675 × *g* for 30 seconds for QC quantification.

13. Place Gel QC Plate on position 3 where the OD QC Plate used to be.



① Tips
② Dilution QC Plate
③ Gel QC Plate
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

14. Resume the method by pressing the **Run** key.

15. When complete, remove the Gel QC Plate, seal, then stack on benchtop.

    Tip: After unpeeling a new adhesive seal, use the ion fan to reduce the amount of static on the sticky side of the seal. This minimizes the droplets jumping up onto the seal due to static.

Gel QC Plates are stacked and then later vortexed and centrifuged at 675 × *g* for 30 seconds for Fragmention QC.

16. Remove Dilution QC Plate, seal, then store at 4°C.

17. Eject and discard the tips.

18. Repeat the procedure for the remaining Hyb-Ready Plates.

19. Complete the following:

    - Appendix C, “Sample quantification after resuspension”.
    - Appendix B, “Fragmentation quality control gel protocol”.

Stage 6: Denature the target and transfer to hybridization tray

Equipment and labware required

Quantity	Item
Instruments	
1	VIAFLO™ 96 Base Unit or VIAFLO™ 384 Base Unit with a 96 Channel Pipetting Head (5–125 µL) and a 3 position stage installed.
1	Fume hood
1	Thermal cycler, ProFlex™ 96-well PCR System
1	BINDER™ ED 56 or BINDER™ BD 56 (Set at 48°C) • Must maintain a constant temperature of 48°C for at least 24 hours with a temperature accuracy of ±1°C
1	Sorvall™ X4R Pro-MD Centrifuge set to room temperature (As needed)
1	Vortex mixer (As needed)
Labware and consumables	
1	Plate Alignment Tool
4	96-layout hybridization tray
384 tips	Integra VIAFLO XYZ GripTips™

Input samples

Number	Plate name	Content
4 (maximum)	Hyb-Ready Plate	Hybridization-ready target DNA prepared in "Stage 5: Resuspend the pelleted DNA and prepare for hybridization" (MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode—half skirt

Stage 6 summary

Stage activities	Instruments required
• Denature target in thermal cycler • Transfer from Hyb-Ready Plate to hybridization tray • Off-line incubation of the array plate/hybridization tray stack at 48°C for 23.5–24 hours	

Perform the pre-run checklist

Before starting the workflow, the following tasks must be completed.

1. Ensure that the VIAFLO™ 96 or 384 Base Unit is clean and is powered on.
2. Ensure that the oven is powered on and has warmed up to 48°C.
3. Ensure that the Proflex™ thermal cycler is powered on and loaded with the **Axiom Denature** thermal cycler protocol.

Warm the array plate to room temperature

The array plate must be at room temperature before setting up hybridization.

1. Remove the array plate packaging from the 4°C refrigerated storage.
2. Open the array plate box, then remove the pouch containing the array plate and protective base. Do not open the pouch.
3. Equilibrate the unopened pouch on the bench for at least 25 minutes.
4. During, or at the end of the array warm up time, open the pouch and scan the array plate barcode into the GeneTitan™ Array Plate Registration file.
   See Appendix D, “Register samples in GeneChip™ Command Console™”.

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

Prepare hybridization-ready samples stored at –20°C

1. Warm the Hyb-Ready Plate at room temperature for 5 minutes. It is not necessary to equilibrate the plate for longer than 5 minutes.
2. Ensure 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. Tightly reseal the plate with a fresh seal.
   d. Vortex the newly sealed plate, then proceed to the next step.
3. Centrifuge for 30 seconds, then proceed to Stage 6.

Stage 6: Denature the target and transfer to hybridization tray

For a 4-plate workflow, complete 1 cycle of 4 plates.

Denature and hybridization transfer		
	Plates 1 2 3 4	
1		Denature target • Protocol: **Axiom Denature**
2		Transfer from Hyb-Ready Plate to hybridization tray • Method: PCR TO HYB TRAY • Dispense volume: 105 µL
3		Incubate • Settings: 48°C, 23.5–24 hours

Transfer hybridization-ready target in Hyb-Ready Plate to hybridization tray

The *PCR TO HYB TRAY* method transfers 105 µL of denatured hybridization-ready target from the Hyb-Ready Plate to the hybridization tray.

1. Run the **Axiom Denature** protocol to denature the hybridization-ready target using a thermal cycler with a heated lid:
   - 95°C for 10 minutes.
   - 48°C for 3 minutes.
   - 48°C hold.

2. Place labware on the VIAFLO stage as shown in the following image.

① Tips
② Hyb-Ready Plate on plate holder
③ Hybridization tray
④ Stage slider

3. Load tips to the 96-head by adjusting the stage slider.
   a. Adjust the stage slider to the left or right, depending on the tips available for use.
      - Right—moves stage back to pick up quadrant 1 or quadrant 2 tips.
      - Left—moves stage forward to pick up quadrant 3 or quadrant 4 tips.
   b. Place one hand on the control unit handle. Maintain contact with the handle throughout the tip loading step.
   c. Gently lower the 96-head into the tip box and guide it into a set of 96 tips.

d. After the tips are properly aligned, the indicator light of the tip load button blinks. Use the opposite hand and press down on the tip load button, located at the top of the 96-head.

e. Release the button when you hear a click. The indicator light on the button remains on.

f. Using the control unit handle, lift the head away from the platform to provide clearance for stage loading or positioning.

4. From the **Main Menu**, select **Custom Programs ▸ PCR to HYB TRAY** to choose the method.

5. Move the stage slider to the center, move the 3-position stage to the left, then allow the 96-head to rest over the Hyb-Ready Plate on the plate holder.

① Tips
② Hyb-Ready Plate on plate holder
③ Hybridization tray
④ Stage slider

IMPORTANT! The stage slider lever must be centered and the 3-position stage must be in the correct position before starting the run.

6. Press **Run** to start the method.

7. When complete, inspect the Hyb-Ready Plate to ensure that the hybridization-ready target was transferred to the hybridization tray.

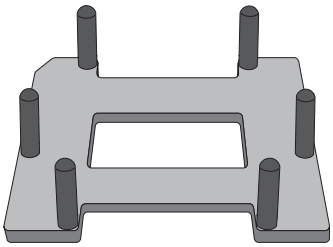
8. Ensure that there are no air bubbles present in the hybridization tray. Puncture any air bubbles that you see using a clean pipette tip. You do not need to spread the sample around the bottom of the hybridization tray wells. Sample distribution across the well occurs when the array plate is stacked together with the hybridization tray.

9. Continue to offline hybridization.

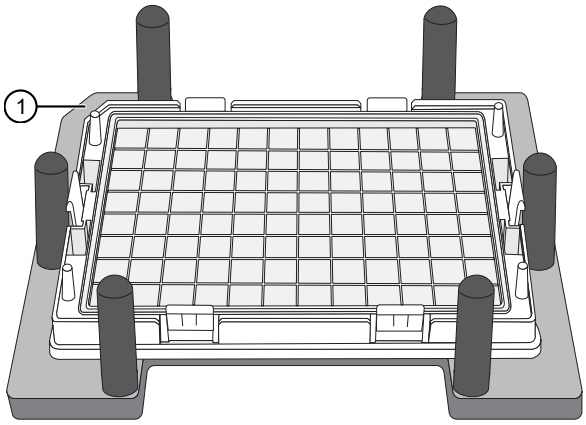
Perform array plate clamping and off-line hybridization

This procedure is performed for all plates in the workflow.

1. Place the Plate Alignment Tool flat on the table top.



2. Carefully place the hybridization tray containing the denatured hybridization target into the Plate Alignment Tool as shown in the following figure. Ensure that the hybridization tray is sitting inside the tool and that the notched corner of the hybridization tray is aligned with the notched corner of the Plate Alignment Tool.



(1) Notched corner of the Plate Alignment Tool and hybridization tray aligned.

3. Hold the array plate with the arrays facing down, then slowly align the array plate into the Plate Alignment Tool until the entire plate is positioned within the 6 alignment posts of the tool. Ensure that the notched corner of the arrays plate is aligned with the notched corner of the hybridization tray. (See Figure 10.)

IMPORTANT! Do not lower the array plate to the hybridization tray if the plate is not aligned within the alignment posts on the tool.

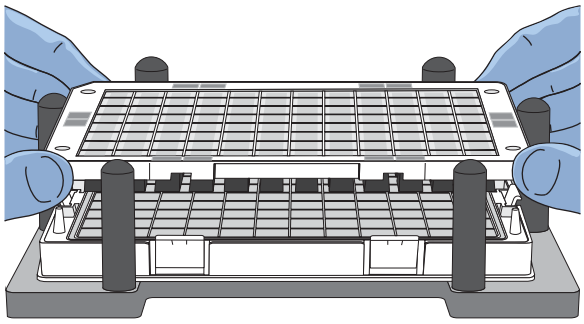
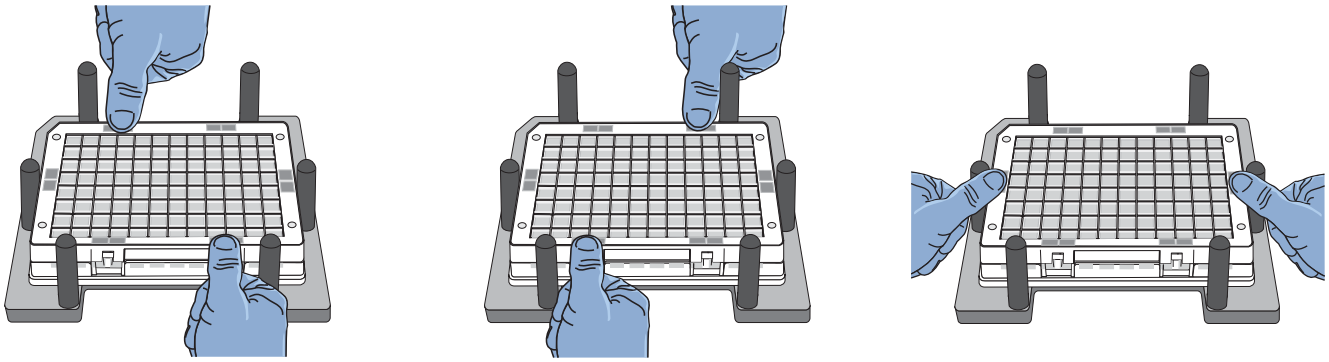


Figure 10 Place the array plate on the hybridization tray in the Plate Alignment Tool.

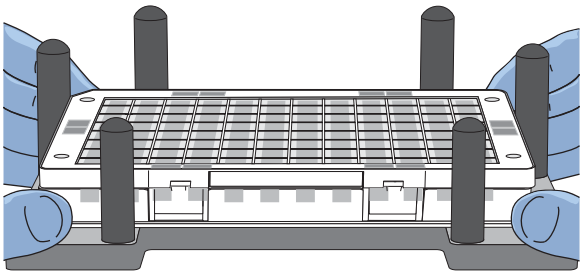
4. After the array plate is aligned inside the tool, slowly lower the plate towards the hybridization tray until a snapping sound is heard. The snapping sound indicates that the array plate and hybridization tray have been properly latched together.

Note: Lowering the array plate to the hybridization tray as slowly as possible prevents air bubbles from generating inside the hybridization tray.

5. Position your hands as shown in the following figures, then lightly push the array plate downward to ensure that each latching mechanism is tightly closed.



6. Grip the array plate/hybridization tray stack from the hybridization tray at the bottom of the stack, then lift to remove the entire stack from the Plate Alignment Tool.



7. Inspect the stack to ensure that all clamps are fastened.

8. Keeping the array plate/hybridization tray stack level, check to ensure that there are no air bubbles trapped between the array plate and the hybridization tray.

   - If bubbles are present, tap the tray gently to dissipate the air bubbles.

9. Make a note of the array plate barcode number for later use.

   The barcode number is used to create the GeneTitan™ Array Plate Registration file and for the GeneTitan™ instrument **Wash-Scan** setup.

   Tip: Scan the barcode directly into a blank registration file or into a text program, such as Microsoft™ Notepad, Word™, or Excel™ applications.

10. Place the array plate/hybridization tray stack onto the wire racks of the hybridization oven set at 48°C.

    Note: Do not stack the array plate/hybridization tray stacks on top of one another.

    Note: Do not place the array plate/hybridization tray stack on the bottom of the hybridization oven.

11. Incubate for 23.5–24 hours. Set a timer or record the time.

Stage 7: Preparing ligation, stain, stabilization reagent trays, and scan trays for the GeneTitan™ MC Instrument

Equipment and labware required

Quantity	Item
Instruments	
5	Multidrop™ Combi Reagent Dispenser
5	Multidrop™ Combi SMART 2 Standard tube dispensing cassette[1]
1	Vortex mixer
1	Mini centrifuge
1	Electronic pipettor
1	P1000 pipettes
Labware and consumables	
4	250-mL conical bottle (Nunc™ 250 mL Wide Mouth Conical Centrifuge Tube)
As required	Serological pipette (5 mL, 10 mL, and 50 mL)
As required	P1000 pipette tips
20	96-format GeneTitan™ stain trays
20	96-format GeneTitan™ stain tray covers
4	96-format GeneTitan™ scan trays with covers

[1] Each reagent must use a dedicated cassette.

Reagent handling

Fast Wash Module 4-1, 4x96F and Module 4-2, 4x96F reagents are required from the Axiom™ Propel 4x96F Reagent Kit:

- Axiom™ Propel Fast Wash Reagent Kit Module 4-1 for 96F or 384HT, Part No. 952369
- Axiom™ Propel Reagent Kit Module 4-2 for 96F or 384HT, Part No. 952268

Thaw and prepare reagents according to the following table.

Table 48 Reagent handling for Stage 7 reagents.

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Fast Wash Reagent Kit Module 4-1 for 96F or 384HT –20°C	Axiom™ Propel Ligation Buffer	952208	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation. **Note:** White precipitate is sometimes observed when the Axiom™ Propel Ligation Buffer is thawed. The presence of some precipitate is acceptable and does not adversely impact assay performance. Use the following instructions to resuspend most precipitate before use. **1.** Vortex for 30 seconds. **2.** Examine the buffer for precipitate. **3.** If precipitate is still present, warm the bottle with your hands, then vortex again for 30 seconds.
	Axiom™ Fast Ligation Enzyme	952367	• Keep in –20°C until ready for use. • Flick 5 times before master mix preparation.
	Axiom™ Propel Ligation Solution 1	952212	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Probe Mix 1	952213	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Stain Buffer	952214	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Stabilize Solution	952215	• Thaw and equilibrate to room temperature on the day of use, or • Thaw at 4°C the day before then equilibrate to room temperature on the day of use. • Vortex, then centrifuge briefly before master mix preparation.

Table 48 Reagent handling for Stage 7 reagents. *(continued)*

Module	Reagent	Component Part No.	Treatment
Axiom™ Propel Reagent Kit Module 4-2 for 96F or 384HT 4°C	Axiom™ Propel Ligation Solution 2	952216	• Remove from 4°C and let equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Propel Probe Mix 2	952217	• Remove from 4°C and let equilibrate to room temperature, but *keep it stored away from light*. • Flick 5 times before master mix preparation.
	Axiom™ Propel Wash A	952218	• Remove from 4°C and equilibrate to room temperature. • Vortex before master mix preparation. If precipitate is present, vortex again to dissolve the precipitate.
	Axiom™ Propel Stain 1-A	952219	• Remove from 4°C and let equilibrate to room temperature on the day of use. • Flick 5 times, then centrifuge briefly before master mix preparation.
	Axiom™ Propel Stain 1-B	952258	• Remove from 4°C and let equilibrate to room temperature on the day of use. • Flick 5 times, then centrifuge briefly before master mix preparation.
	Axiom™ Propel Stain 2-A	952231	• Remove from 4°C and let equilibrate to room temperature on the day of use. • Flick 5 times, then centrifuge briefly before master mix preparation.
	Axiom™ Propel Stain 2-B	952260	• Remove from 4°C and let equilibrate to room temperature on the day of use. • Flick 5 times, then centrifuge briefly before master mix preparation.
	Axiom™ Propel Stabilize Diluent	952248	• Remove from 4°C and let equilibrate to room temperature on the day of use. • Vortex before master mix preparation.
	Axiom™ Water	952177	• Remove from 4°C and let equilibrate to room temperature on the day of use.
	Axiom™ Propel Hold Buffer	952254	• Remove from 4°C and let equilibrate to room temperature on the day of use. • Vortex before use.

Reagent preparation for Stage 7: Prepare GeneTitan™ reagents

You can add flexibility to the timing of array processing by making the following GeneTitan™ reagents up to 2 days (48 hours) in advance.

- Stain 1 and Stain 2 Master Mixes can be prepared up to 2 days in advance, wrapped in foil, then stored at 4°C.
- Stabilization Master Mix can be prepared up to 2 days in advance and stored at 4°C.
- Ligate Master Mix can be prepared with all the components listed up to 2 days in advance, except the Axiom™ Fast Ligation Enzyme. Wrap the master mix bottle in foil, then store at 4°C. Axiom™ Fast Ligation Enzyme must be kept at –20°C and added only when ready for use.

IMPORTANT! Add the Axiom™ Fast Ligation Enzyme (#6) only if 2 GeneTitan™ MC Instruments instruments (GTMC) are available to process *and* stain trays for both array plates can be loaded into GTMC within 1 hour. If there is only 1 GeneTitan™ MC Instrument available to process (only 1 array plate can be processed at a time), see "Guidance to process 1 array plate at a time on the GeneTitan™ MC instrument" on page 185. Consult with your Field Application Scientist for further guidance.

The following sections provide detailed procedures for master mix preparation.

Prepare Ligate Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 48.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 49 Ligate Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Ligation Buffer	41.0 mL	50-mL serological pipette
2	Axiom™ Propel Ligation Solution 1	8.1 mL	10-mL serological pipette
3	Axiom™ Propel Probe Mix 1	6.5 mL	10-mL serological pipette
4	Axiom™ Propel Probe Mix 2	6.5 mL	10-mL serological pipette
5	Axiom™ Propel Ligation Solution 2	1.95 mL	P1000 pipette
6	Axiom™ Fast Ligation Enzyme[1]	2.45 mL	P1000 pipette
	Total volume	**66.5 mL**	

[1] See the IMPORTANT note in the section, "Reagent preparation for Stage 7: Prepare GeneTitan™ reagents" on page 182.

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Ligate Master Mix at least 15 times to mix.
3. Protect the Ligate Master Mix from direct light by covering with aluminum foil.
4. Leave the master mix at room temperature, dispense into the ligation tray, then load into the GeneTitan™ MC Instrument within 1 hour of its preparation.

Ligate Master Mix—advance preparation procedure

To provide flexibility for the workflow and to accommodate the number of GeneTitan™ MC Instruments that are available to process array plates, the Ligation Master Mix can be made up to 2 days (48 hours) in advance, but without the Axiom™ Fast Ligation Enzyme.

Prepare Stain 1 Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 28.)

Note: Two stain trays of Stain 1 Master Mix are required per 96 samples, therefore, a higher volume of master mix is required.

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 50 Stain 1 Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Wash A	108.0 mL	50-mL serological pipette
2	Axiom™ Propel Stain Buffer	2.3 mL	5-mL serological pipette
3	Axiom™ Propel Stain 1-A	1.13 mL	P1000 pipette
4	Axiom™ Propel Stain 1-B	1.13 mL	P1000 pipette
	Total volume	**112.56 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Stain 1 Master Mix at least 15 times to mix.
3. Protect the Stain 1 Master Mix from direct light by covering with aluminum foil.
4. Store the prepared master mix in one of the following ways:
   - Store at room temperature and use within 2 hours of its preparation.
   - **Optional:** To provide flexibility for the workflow, the master mix can be made up to 2 days (48 hours) in advance, then stored in the dark at 4°C. On the day of use, remove from the 4°C storage, then allow the master mix to equilibrate to room temperature. Equilibration can take up to 30 minutes.

Prepare Stain 2 Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 48.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 51 Stain 2 Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Wash A	63.0 mL	50-mL serological pipette
2	Axiom™ Propel Stain Buffer	1.31 mL	P1000 pipette
3	Axiom™ Propel Stain 2-A	0.66 mL	P1000 pipette
4	Axiom™ Propel Stain 2-B	0.66 mL	P1000 pipette
	Total volume	**65.63 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, then invert the Stain 2 Master Mix at least 15 times to mix.

3. Protect the Stain 2 Master Mix from direct light by covering with aluminum foil.

4. Store the prepared master mix in one of the following ways:
   - Store at room temperature and use within 2 hours of its preparation.
   - **Optional:** To provide flexibility for the workflow, the master mix can be made up to 2 days (48 hours) in advance, then stored in the dark at 4°C. On the day of use, remove from the 4°C storage, then allow the master mix to equilibrate to room temperature. Equilibration can take up to 30 minutes.

Prepare Stabilization Master Mix

Ensure that all components are treated according to the reagent handling table before preparing the master mix. (See Table 48.)

1. In a 250-mL conical bottle, add reagents in the order shown.

Table 52 Stabilization Master Mix for 4x96-format plates.

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Water	58.0 mL	50-mL serological pipette
2	Axiom™ Propel Stabilize Diluent	6.5 mL	10-mL serological pipette
3	Axiom™ Propel Stabilize Solution	0.815 mL	P1000 pipette
	Total volume	**65.3 mL**	

2. Ensure that the cap of the conical bottle is closed tightly, vortex for 5 seconds, then leave at room temperature until use.

3. Store the prepared master mix in one of the following ways:
   - Store at room temperature and use within 2 hours of its preparation.
   - **Optional:** To provide flexibility for the workflow, the master mix can be made up to 2 days (48 hours) in advance, then stored at 4°C. On the day of use, remove from the 4°C storage, then allow the master mix to equilibrate to room temperature. Equilibration can take up to 30 minutes.

Prepare the Axiom™ Propel Hold Buffer

Ensure that the Axiom™ Propel Hold Buffer is vortexed before use.

1. Use the Axiom™ Propel Hold Buffer bottle for Multidrop™ dispensing.

Table 53 Axiom™ Propel Hold Buffer for 4x96-format plates.

Reagent	Quantity	Transfer method
Axiom™ Propel Hold Buffer	Entire bottle	Use straight from bottle[1]

[1] The Axiom™ Propel Hold Buffer reagent bottle can be used for Multidrop™ Combi dispensing.

2. Leave at room temperature until use.

Guidance to process 1 array plate at a time on the GeneTitan™ MC instrument

The GeneTitan™ MC master mixes and reagents can be split into smaller aliquots to provide flexibility in the Axiom™ Propel Fast Wash Workflow when only 1 GeneTitan™ MC instrument is available. There will not be enough volume in GT to use the Multidrop™ instrument to dispense them. These aliquots will instead be dispensed manually using a multichannel pipette.

Prepare the Intermediate Ligate Master Mix (without enzyme)

1. Prepare the Intermediate Ligate Master Mix with all components except the Axiom™ Fast Ligation Enzyme.

Table 54 Intermediate Ligate Master Mix (no enzyme).

Order of addition	Reagent	Quantity	Transfer method
1	Axiom™ Propel Ligation Buffer	41.0 mL	50-mL serological pipette
2	Axiom™ Propel Ligation Solution 1	8.1 mL	10-mL serological pipette
3	Axiom™ Propel Probe Mix 1	6.5 mL	10-mL serological pipette
4	Axiom™ Propel Probe Mix 2	6.5 mL	10-mL serological pipette
5	Axiom™ Propel Ligation Solution 2	1.95 mL	P1000 pipette
	Total volume	**64.05 mL**	

2. Prepare three 16-mL aliquots of the Intermediate Ligate Master Mix from its original 250-mL container into 50-mL conical tubes. Keep the remaining 16 mL of Intermediate Ligate Master Mix in its original 250-mL container. This will serve as the 4th aliquot of Intermediate Ligate Master Mix.

3. Protect the Intermediate Ligate Master Mix aliquots from direct light by covering the tubes with aluminum foil, then store at 4°C.

IMPORTANT!

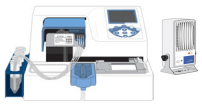
- Label the Intermediate Ligate Master Mix aliquot tubes to indicate that the content contains "No Enzyme".
- The Intermediate Ligate Master Mix aliquots must be used within 2 days (48 hours) of preparation.

4. 3. To prepare Ligate Master Mix for 1 array plate:
   a. Remove one 16-mL aliquot of Intermediate Ligate Master Mix from 4°C storage, then allow it to equilibrate to room temperature
   b. Add 613 µL of Axiom™ Fast Ligation Enzyme to the aliquot to complete the Ligate Master Mix.
   c. Invert the Ligate Master Mix aliquot container at least 15 times to mix.
   d. Leave the Ligate Master Mix at room temperature. Use a multichannel pipette to manually dispense 110 µL/well of the Ligate Master Mix into a stain tray. Load the stain tray into the GeneTitan™ MC instrument within 1 hour.

Prepare GeneTitan™ reagents for master mixes and aliquot for 1 array plate

Reagents	Tray type	Volume per well	Number of trays required	Volume to aliquot
Stain 1 Master MIx	Stain tray, 96F	110 µL	2	28 mL
Stain 2 Master MIx	Stain tray, 96F	110 µL	1	16 mL
Stabilization Master Mix	Stain tray, 96F	110 µL	1	16 mL
Axiom Hold Buffer	Scan tray, 96F	150 µL	1	20 mL

Stage 7 summary

Stage activities	Instruments required
Five reagent dispenses—Ligation, Stain 1, Stain 2, Stabilization, Axiom™ Propel Hold Buffer	

Prepare the GeneTitan™ trays and covers

Familiarity with handling GeneTitan™ is required. If needed, review the proper techniques provided in this document. See Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation”.

1. Collect and label the stain trays as described in the following table.

Quantity per array plate	Tray type	Label[1]
1	Ligation Tray 	*Lig*
2	Stain 1 Tray 	*Stain 1* or *S1*
1	Stain 2 Tray 	*Stain 2* or *S2*
1	Stabilization Tray 	*Stbl*

[1] It is critical that you write on the proper location of the stain/reagent trays. See “Label the GeneTitan™ reagent trays” on page 220.

2. Collect enough covers for the trays (5 x the number of array plates).

3. Place the labeled stain trays and covers next to the Multidrop™ Combi. Ensure that each labeled stain tray is placed next to the correct Multidrop™ Combi dispensing the appropriate/corresponding reagent.

   For example, stain trays labeled “Stain 2” are placed next to the Multidrop™ Combi dispensing the Stain 2 Master Mix.

4. Deionize the stain trays and covers by placing them directly in front of the ion blower and allowing the air to stream across them for at least 10 seconds.

   Alternatively, trays and covers can be deionized manually using the GeneTitan™ ZeroStat AntiStatic Gun. See “Manual deionization of GeneTitan™ trays and covers” on page 222.

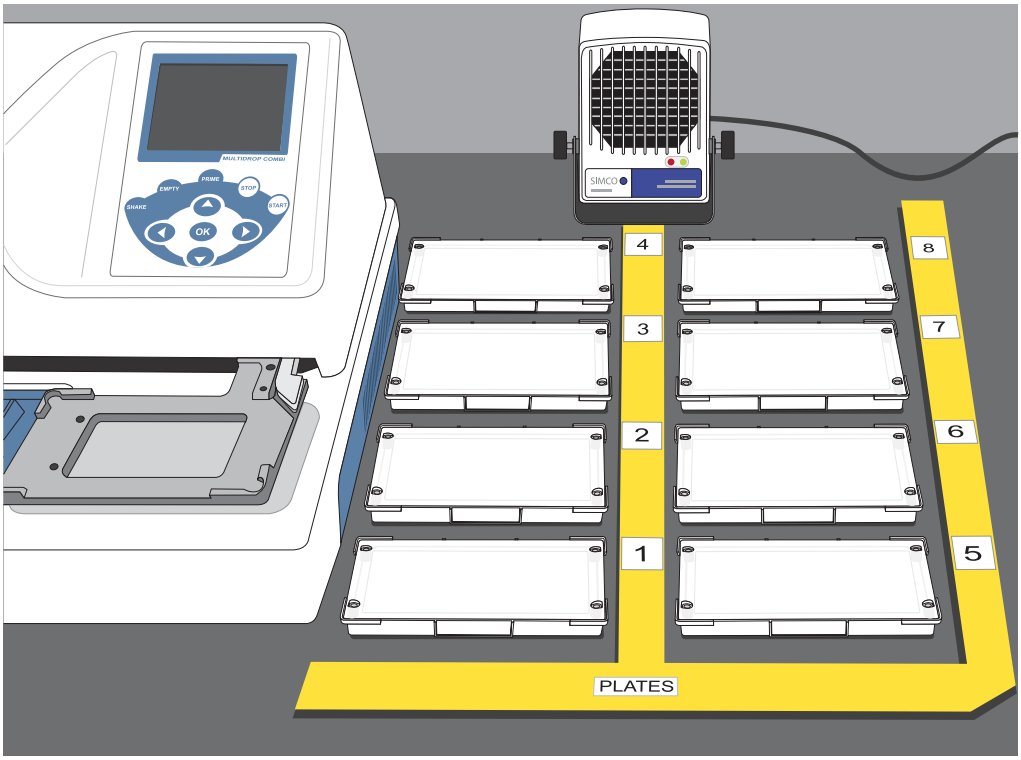


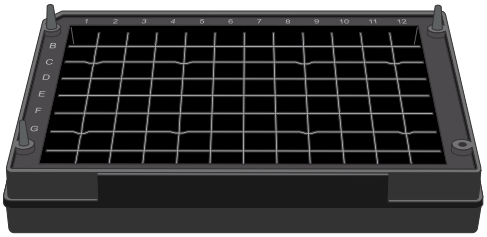
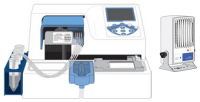
Figure 11 Placement and positioning of stain tray covers for deionization with the ionizing air blower.

Perform the pre-run checklist

Before starting the workflow, the following tasks must be completed.

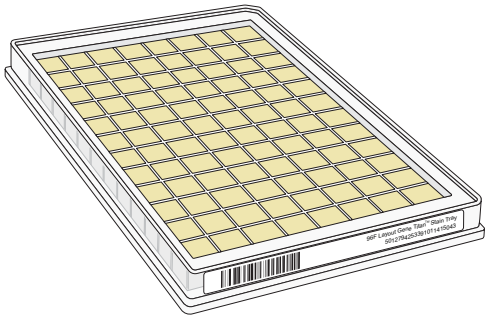
1. Ensure that the Multidrop™ Combi startup procedure has been performed. (See “Start up the Multidrop™ Combi” on page 54.)
2. Perform gravimetric checks before running the workflow. (See “Perform gravimetric checks” on page 55.)
3. Prime the Multidrop™ cassette with reagent. (See “Prime the cassette” on page 59.)
4. Ensure that each labeled stain tray is placed next to the correct Multidrop™ Combi dispensing the corresponding reagent.
5. Move the ion blowers to each dispensing station, then power them on. (Figure 6 on page 51.)

Stage 7: Prepare GeneTitan™ reagents

Scan Tray dispense	
	
	
⚠	**CAUTION!** Do not remove the Scan Tray from its protective black base until loading onto the GeneTitan™ MC Instrument.
①	 Dispense Axiom™ Propel Hold Buffer • Method: 96-Scan-150 • Dispense volume: 150 µL
②	✓ Examine the Scan Tray. • If bubbles are present, puncture them with a pipette tip. • If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.
③	ℹ Place the Scan Tray cover onto the Scan Tray.
④	ℹ • Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61. • Continue to "Stain tray dispense—Ligation Master Mix"

Stain tray dispense—Ligation Master Mix

Plates 1 2 3 4

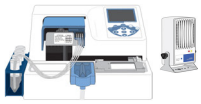


- Place the ion blowers at the dispensing station. If ion blowers are not available, the GeneTitan™ ZeroStat AntiStatic Gun can be used to individually deionize the stain trays and covers. (See "Deionization of GeneTitan™ trays and covers" on page 222.)

IMPORTANT! Deionze the trays and covers by ion blowers or GeneTitan™ ZeroStat AntiStatic Gun before dispensing the reagents.

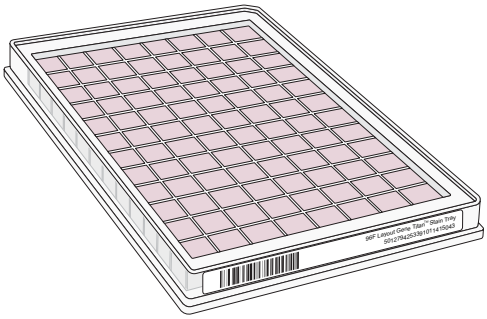
IMPORTANT! Ensure that the stain trays labeled "Lig" are used for the Multidrop™ Combi dispensing the Ligation Master Mix.

IMPORTANT! If the Ligation Master Mix was made a day in advance, ensure that the Axiom™ Fast Ligation Enzyme is added to the mixture before dispensing.

①		Dispense Ligation Master Mix • Method: 96-Stain-110 • Dispense volume: 110 µL
②	✓	Examine the Ligation Tray. • If bubbles are present, puncture them with a pipette tip. • If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.
③	ⓘ	• Perform the appropriate Multidrop™ procedure: – Flush between batches. See page 60. – Shutdown. See page 61. • Place a deionized stain tray cover on top of each Ligation stain tray. Continue to "Stain tray dispense—Stain 1 Master Mix". • Protect the trays from light if not immediately loading onto the GeneTitan™ MC Instrument.

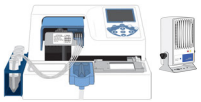
Stain tray dispense—Stain 1 Master Mix

Plates: 1 2 3 4 5 6 7 8



IMPORTANT! Deionze the trays and covers by ion blowers or GeneTitan™ ZeroStat AntiStatic Gun before dispensing the reagents.

IMPORTANT! Ensure that the stain trays labeled “Stain 1” or “S1” are used for the Multidrop™ Combi dispensing the Stain 1 Master Mix.

1 

Dispense Dispense Stain 1 Master Mix

- Method: 96-Stain-110
- Dispense volume: 110 µL

2 ✓

Examine the Stain 1 Tray.

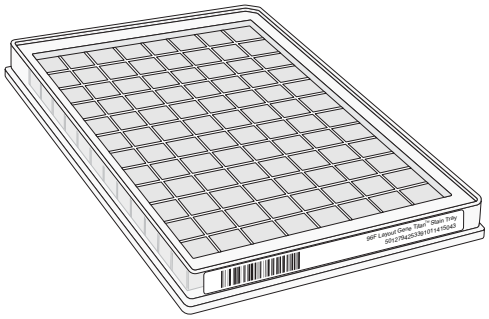
- If bubbles are present, puncture them with a pipette tip.
- If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.

3 ⓘ

- Perform the appropriate Multidrop™ procedure:
  - Flush between batches. See page 60.
  - Shutdown. See page 61.
- Place a deionized stain tray cover on top of each Stain 1 stain tray. Continue to “Stain tray dispense—Stain 2 Master Mix”.
- Protect the trays from light if not immediately loading onto the GeneTitan™ MC Instrument.

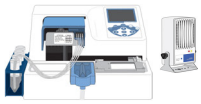
Stain tray dispense—Stain 2 Master Mix

Plates 1 2 3 4



IMPORTANT! Deionze the trays and covers by ion blowers or GeneTitan™ ZeroStat AntiStatic Gun before dispensing the reagents.

IMPORTANT! Ensure that the stain trays labeled “Stain 2” or “S2” are used for the Multidrop™ Combi dispensing the Stain 2 Master Mix.

1 

Dispense Stain 2 Master Mix

- Method: 96-Stain-110
- Dispense volume: 110 µL

2 ✓

Examine the Stain 2 Tray.

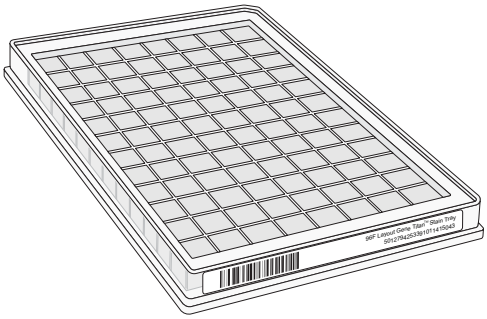
- If bubbles are present, puncture them with a pipette tip.
- If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.

3 ⓘ

- Perform the appropriate Multidrop™ procedure:
  - Flush between batches. See page 60.
  - Shutdown. See page 61.
- Place a deionized stain tray cover on top of each Stain 2 stain tray. Continue to “Stain tray dispense—Stabilization Master Mix”.
- Protect the trays from light if not immediately loading onto the GeneTitan™ MC Instrument.

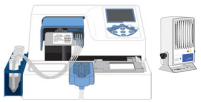
Stain tray dispense—Stabilization Master Mix

Plates 1 2 3 4



IMPORTANT! Deionze the trays and covers by ion blowers or GeneTitan™ ZeroStat AntiStatic Gun before dispensing the reagents.

IMPORTANT! Ensure that the stain trays labeled “Stbl” are used for the Multidrop™ Combi dispensing the Stabilization Master Mix.

1		Dispense Stabilization Master Mix • Method: 96-Stain-110 • Dispense volume: 110 µL
2	✓	Examine the Stabilization Tray. • If bubbles are present, puncture them with a pipette tip. • If droplets of liquid splashed onto the well dividers, use a laboratory tissue and gently dab the droplet on top of the tray divider to remove the droplet without disturbing the liquid inside the tray.
3	ⓘ	Place a deionized stain tray cover on top of each Stabilization stain tray.
4	ⓘ	Perform the appropriate Multidrop™ procedure: • Flush between batches. See page 60. • Shutdown. See page 61.

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

IMPORTANT! Contact a Thermo Fisher Scientific Field Application Scientist for the appropriate GeneChip™ Command Console™ library files to enable the GeneTitan™ MC Instrument to run the accelerated wash protocol.

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

Note: Review Appendix A, "Recommended techniques for GeneTitan™ MC Instrument operation" for details on array processing setup options and consumable handling.

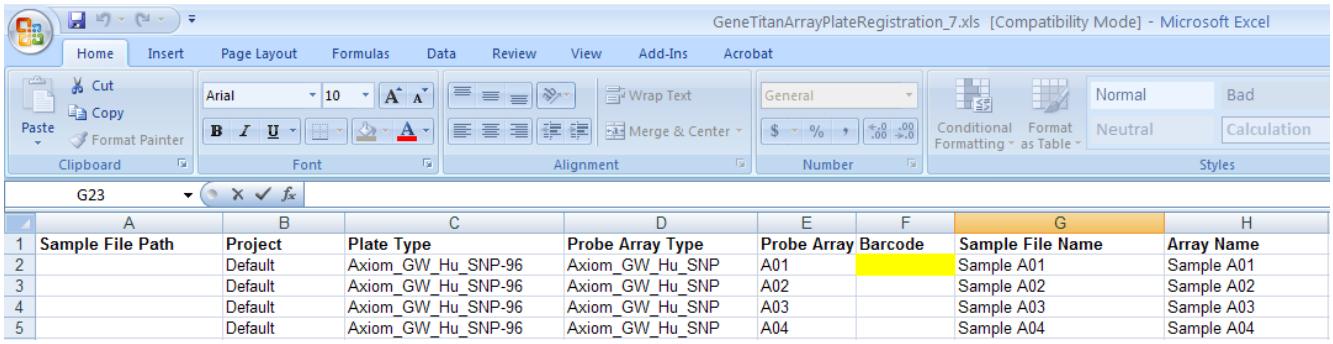
Create and upload a GeneTitan™ Array Plate Registration file

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

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

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

1. If you have already created a batch registration file but have not yet uploaded the file to GCC, open the file, then go to step 6.
2. From the **Launcher** window, open **GCC Portal** ▸ **Samples** ▸ **GeneTitan™ Array Plate Registration**.
3. In the GeneTitan™ Array Plate Registration window, select the **GeneTitan Array Plate Type** to be processed from the dropdown list.
4. Click **Download**.
5. In the **Samples** tab of the GeneTitan™ Array Plate Registration file, enter a unique name for each sample (Sample File Name) and any additional information. Additional information on the GeneTitan™ Array Plate Registration file is in the *GeneChip™ Command Console™ User Guide* (Pub. No. MAN0027771)
6. Scan the array plate barcode into the yellow **Barcode** field, column **F**. See Figure 12.
7. Save the file.
   By default, the file is saved in the **Applied Biosystems Download** folder.
8. Return to the GCC Portal GeneTitan™ Array Plate Registration page.
   a. Click **Browse**, navigate to the array plate registration file, then click **Open**.
   b. Under **Step 3**, click **Upload**, wait for the information to load, then click **Save** found at the bottom of the next window that is displayed.



	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 12 Example of a GeneTitan™ Array Plate Registration file.

Run Wash-Scan

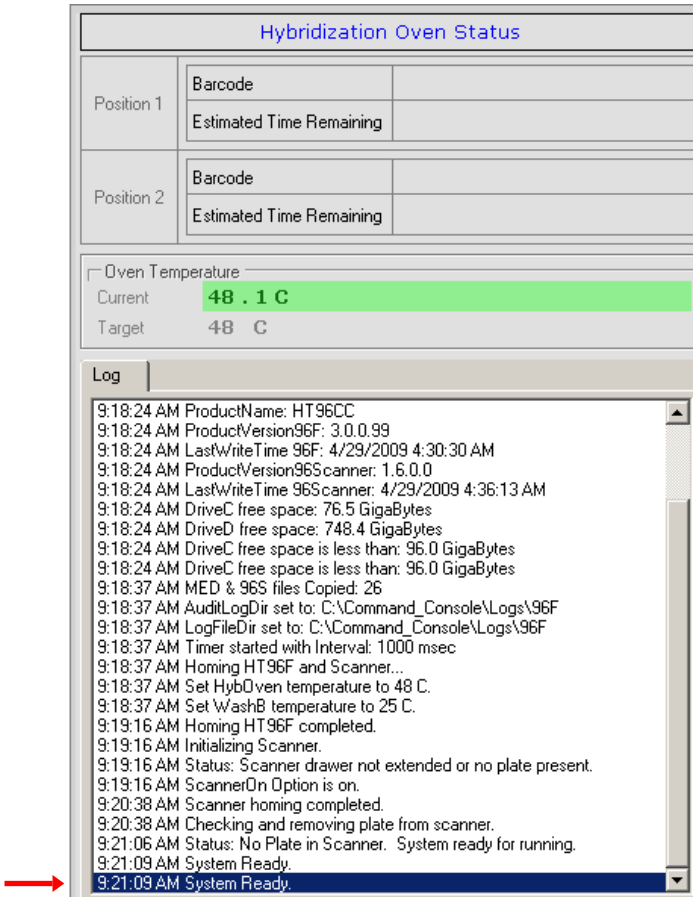
The Wash-Scan option is often used when the array is hybridized in an off-line oven in a high throughput lab where multiple loadings are done within a working day. The Hyb-Wash-Scan option can be used if running 1 array at a time. See “Hyb-Wash-Scan” on page 226.

The following reagents from the Axiom™ Propel Fast Reagent Kit are required for this procedure.

- Axiom™ Wash Buffer A, Part No. 901446
- Axiom™ Wash Buffer B, Part No. 901447
- Axiom™ Water, Part No. 901578

1. Verify that the correct hybridization time for the “Array Plate/Hyb Tray Stack” has been achieved. Keep the “Array Plate/Hyb Tray Stack” in the off-line oven until you are ready to place it into the GeneTitan™ MC Instrument.

2. From the **GCC Launcher** and select **GCC GeneTitan Control**.
   The "System Ready" is indicated at the bottom of the status pane.



3. When the system has initialized and “System Ready” status appears, click to select the **System Setup** tab.

4. Select **Wash-Scan** from the **Setup Option** dropdown list.

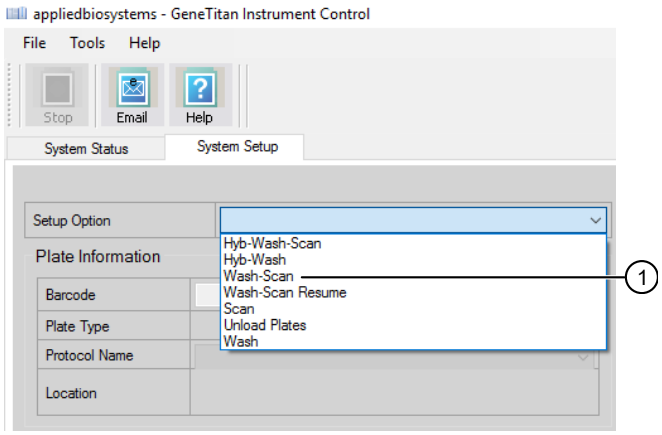


Figure 13 Wash–Scan setup option.

5. Enter the barcode of the array plate to be processed.

   During this step, the array plate must remain in the hybridization oven. The barcode can be retrieved from the registration file that was created before the start of the off-line hybridization.

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

   IMPORTANT! For executing the accelerated wash protocol, ensure that the selected **Protocol Name** has the format: "XXXXXX_fastwash.protocol", where "XXXXXX" is the array part number. Failure to select the correct protocol can result in compromised assay performance, and a delay with the timing of the array processing workflow.

7. Refill the GeneTitan™ bottles with the following reagents.

   Note: Invert the Axiom™ Wash Buffer A and Axiom™ Wash Buffer B bottles 2–3 times to mix before filling the GeneTitan™ bottles.

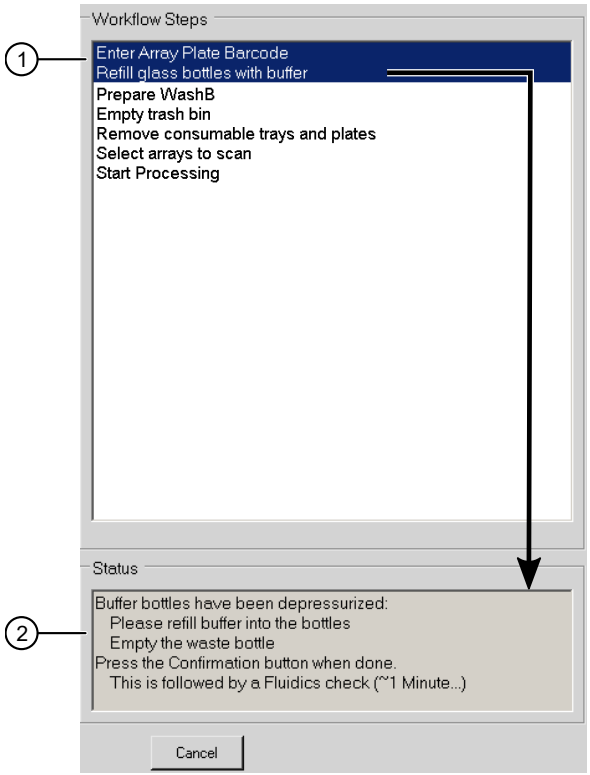
   - Wash A: fill with Axiom™ Wash Buffer A—keep at 2 L full.
   - Wash B: fill with Axiom™ Wash Buffer B—use all 600 mL of Axiom™ Wash Buffer B.
   - Rinse: fill with Axiom™ Water—keep at 1 L full.

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

   We strongly recommend refilling these bottles every time you are prompted to do so.
   If the volume in any of the GeneTitan™ bottles become too low during a run, an error message is displayed. However, if you wait to fill the bottle at the time of the error message, the instrument cannot complete the step that was in progress.

8. Empty the GeneTitan™ bottle labeled Waste.

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

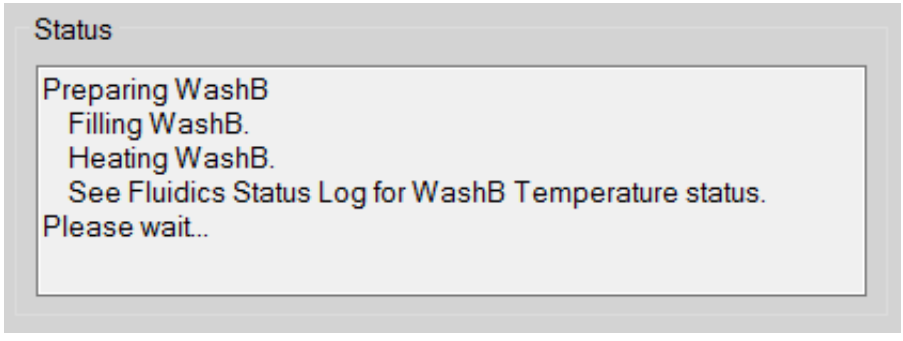


① **Workflow Step**

② Specific instructions for the current workflow step appear in the **Status** pane.

10. Prepare the Axiom™ Wash Buffer B for the **Wash-Scan** workflow.

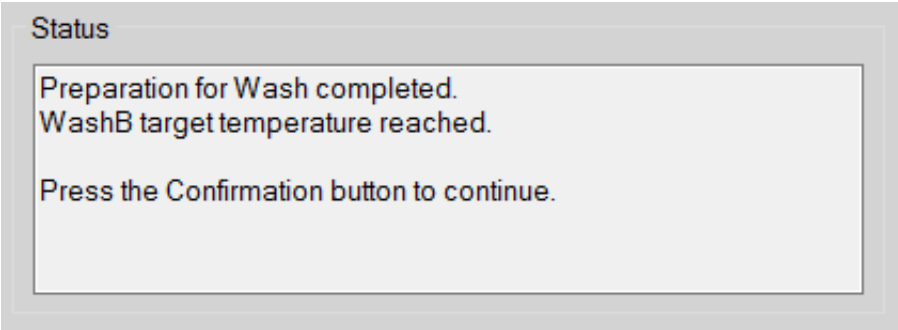
    Warming up the Axiom™ Wash Buffer B takes 25–30 minutes. Therefore, this step must be completed at least 30 minutes before to the completion of the 24-hour hybridization time.



Note: During this waiting time, we recommend that you prepare the reagents for "Stage 7: Preparing ligation, stain, and stabilization reagent trays for the GeneTitan™ MC Instrument".

WARNING! *Do not* load reagents onto the GeneTitan™ MC Instrument until you are prompted by GeneTitan™ Instrument Control Software.

11. After the temperature has been reached, you are prompted to press the Confirmation button on GeneTitan™ MC Instrument to continue (blinking light blue button found at the top center of the GeneTitan™ Instrument).



12. Empty the trash bin.
    a. Open the trash bin and empty.
    b. If already empty, the trash bin remains locked and the **Status** pane reads "Trash bin is empty".
    c. Press the blue confirmation button to continue.
13. Remove consumable trays and plates.
    a. Remove used trays and plates when drawers open.
    b. If there are no consumables to remove, the **Status** window reads "Drawers are empty".
    c. Press the blue confirmation button on the GeneTitan™ Instrument to continue.

14. Load consumable trays and plates. Follow the prompts in the **Status** window to load the reagent trays (scan, stain, ligation, and stabilization).
    a. When drawer 2 opens:
        - Left side: Scan tray with cover. Remove the protective black base from the scan tray immediately before loading. Do not load the protective black base.
        - When complete, press the blue confirmation button on the GeneTitan™ Instrument to continue.

IMPORTANT! Before installing the consumables into the instrument, ensure that the fingers are retracted. Do not lay the consumables on top of the drawer fingers.

You must 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. When the drawer is open and the fingers are not retracted, the instrument is not functioning correctly. Notify a Field Service Engineer if the fingers do not retract automatically.

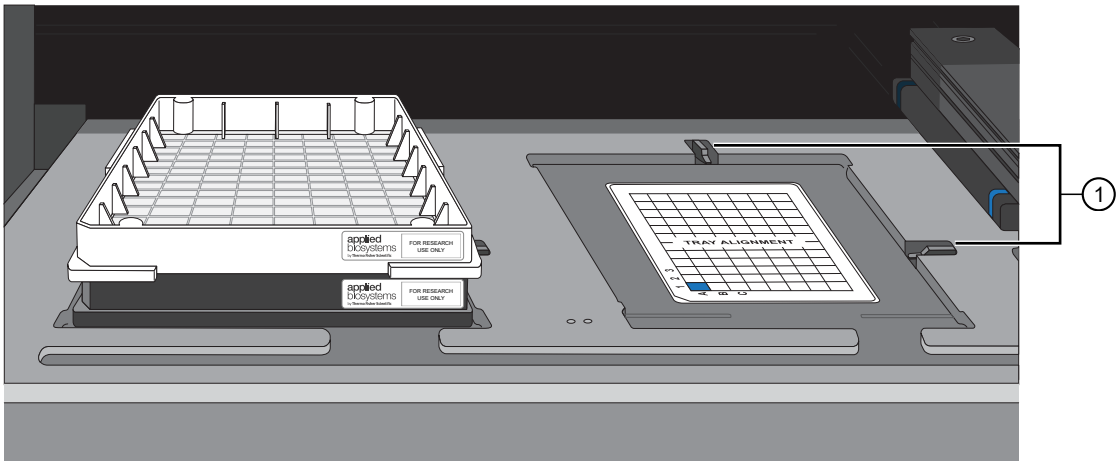


Figure 14 Drawer 2, left side: Scan tray with cover.

(1) Locations of drawer tabs, or "fingers".

b. When drawer 3 opens:

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

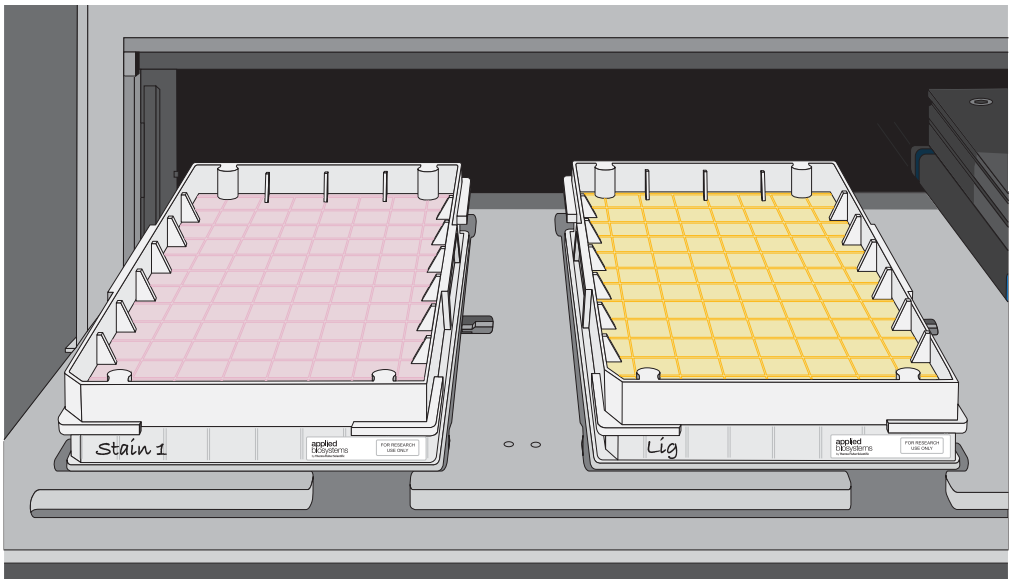


Figure 15 Drawer 3, left side: Stain 1 tray with cover. Drawer 3, right side: Ligation tray with cover.

c. When drawer 4 opens:

- Left side: Stain 2 tray with cover.
- Right side: Stabilization tray with cover.
- Press the blue confirmation button on the GeneTitan™ Instrument to continue.

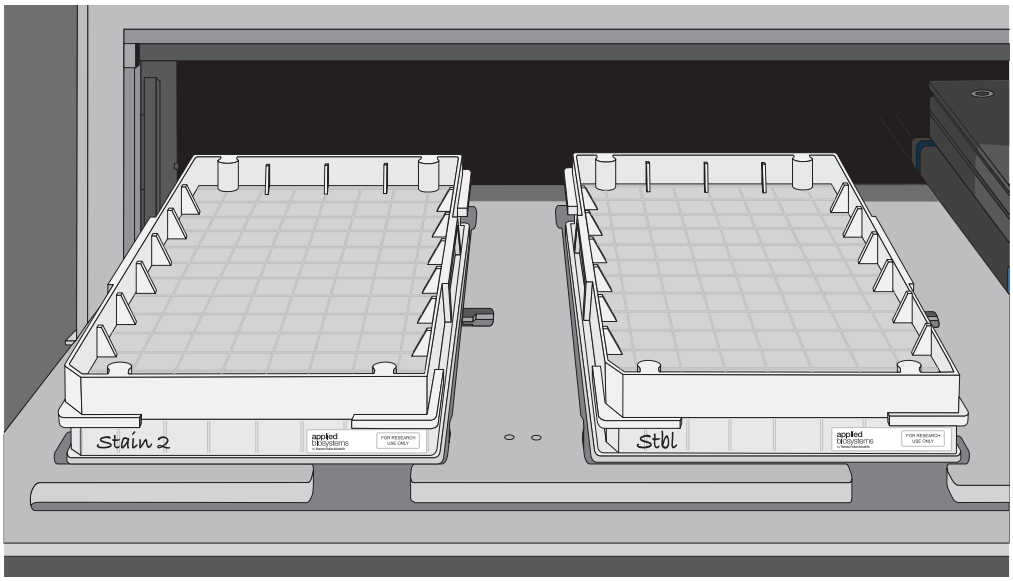


Figure 16 Drawer 4, left side: Stain 2 tray with cover. Drawer 4 right side: Stabilization tray with cover.

d. When drawer 5 opens:

- Left side: Stain 1 tray with cover.
- Press the blue confirmation button on the GeneTitan™ Instrument to continue.

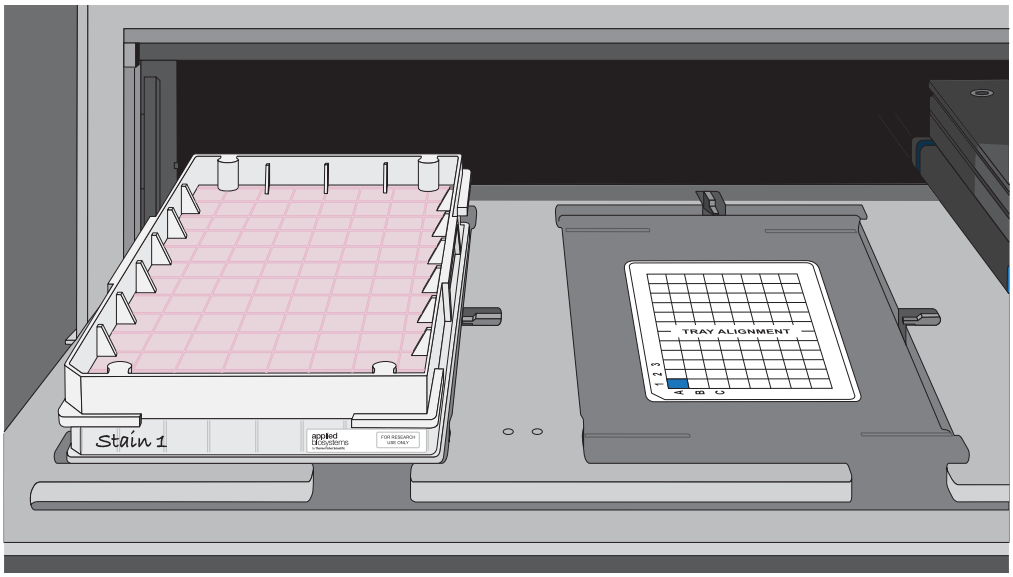


Figure 17 Drawer 5, left side: Stain 1 tray with cover.

e. The Array Plate/Hyb Tray Stack is loaded in drawer 6.

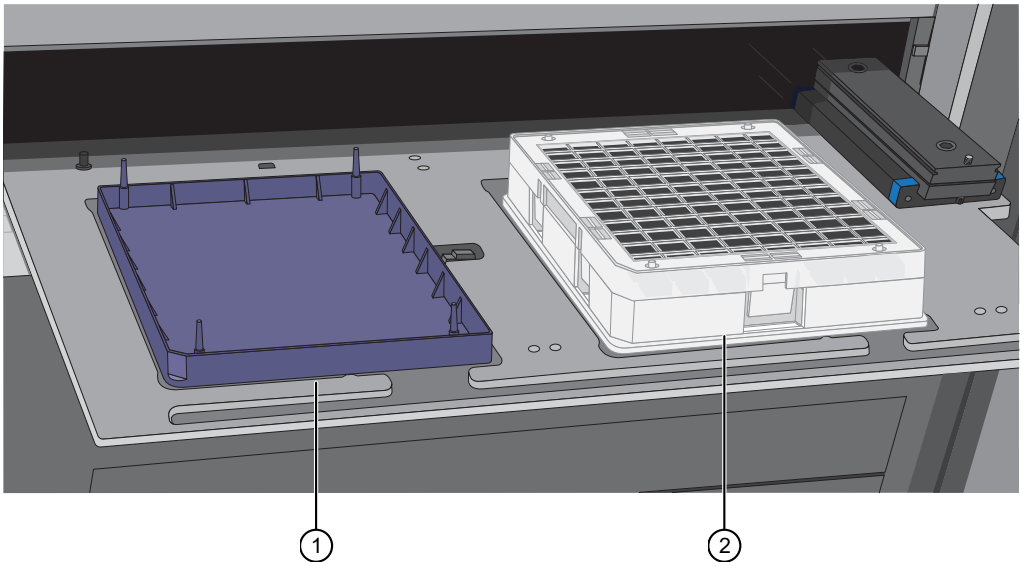


Figure 18 Blue protective base and the array plate/hybridization tray stack.

① Blue protective base.
② Array plate/hybridization tray stack

IMPORTANT! A blue base must be present on the left side of drawer 6 at all times when an array plate is being processed in the fluidics.

15. Select the arrays that you want to scan.

16. Start processing.

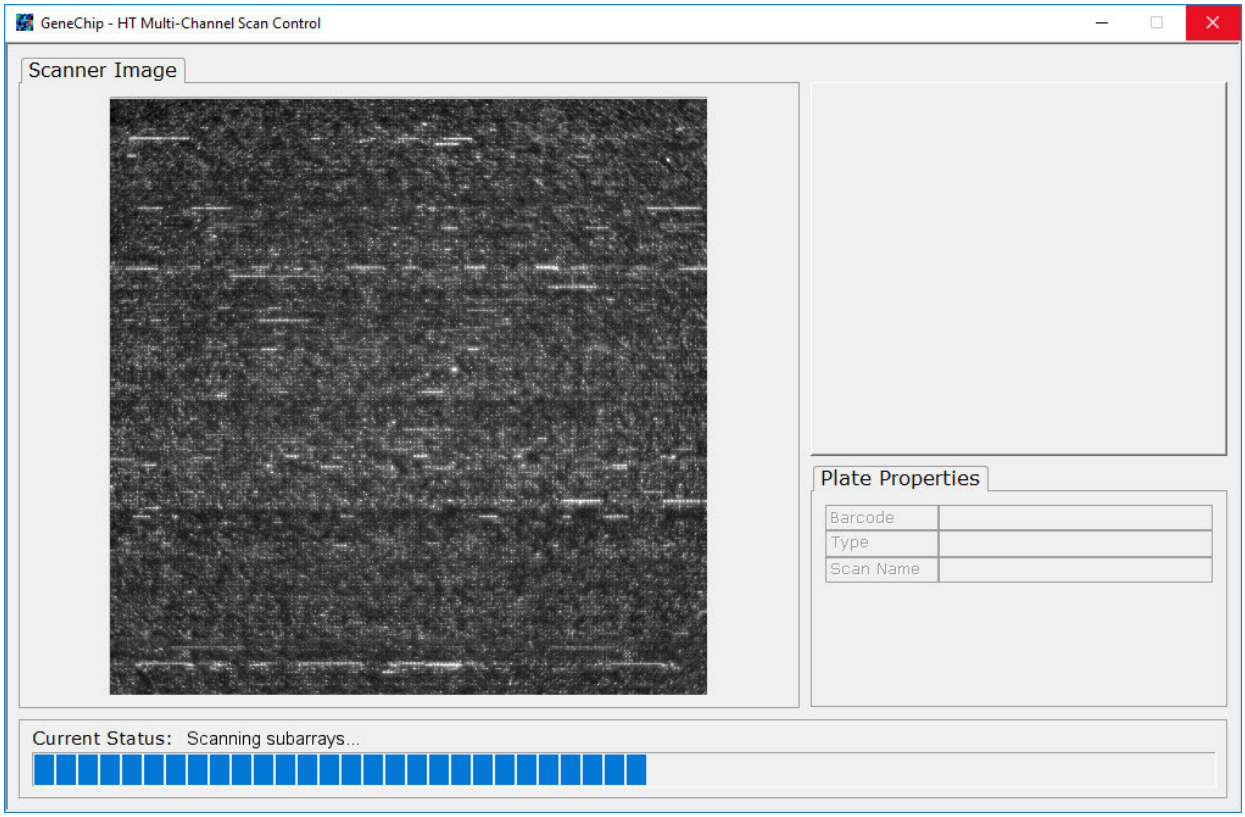
Scan workflow

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

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

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

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



Queue another plate for Wash-Scan

This technique can be used in high throughput workflows to maximize the capacity of the GeneTitan™ MC Instruments.

- GeneTitan™ MC Fast Scan Instrument
  - Load the next plate to be washed and scanned immediately after the previous plate has finished washing (~5 hours).
- GeneTitan™ MC Instrument
  - Load the second plate to be washed and scanned immediately after the first plate has finished washing (~5 hours).
  - Load the third and subsequent plates for **Wash-Scan** at a ~5.5-hour interval, or after the plate being processed in the scanner has completed.

Shut down the GeneTitan™ MC Instrument

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

1. From the **System Setup** window, open the **Setup Options** dropdown list, then select **Unload Plates**.
2. Unload all the consumables as prompted.
3. Power off the GeneTitan™ MC Instrument by opening **Tools** ▸ **Shutdown**.
4. Exit the GCC software if it does not close automatically.

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

7 High throughput with the Axiom™ Propel Fast Wash Workflow

Overview

The Axiom™ Propel Fast Wash Workflow is uniquely designed to accommodate the growing demand for higher throughput of Axiom™ array plates. The workflow features the Thermo Scientific™ Multidrop™ Combi Reagent Dispenser and the Applied Biosystems™ GeneTitan™ MC Instrument, along with other common laboratory equipment, to achieve the tasks required for multi-array plate processing.

With the recommended equipment, 6 operators can process ~100 plates of 96 samples in a 5-day work week. This chapter provides example equipment and labor schedules for 2 different production levels of Axiom™ 96-array format plates.

IMPORTANT! The successful execution of this workflow requires experienced operators and careful timing.

The requirements and schedules presented in this chapter are intended as general guidelines for developing an individualized plan to fit your needs. Contact your Thermo Fisher Scientific FAS representative for help with planning the scale-up of the Axiom™ Propel Fast Wash Workflow.

Considerations for customization of the workflows

GeneTitan™ MC Instrument throughput

The wash-stain and scan steps of the Axiom™ Propel Fast Wash Workflow are completed with the GeneTitan™ Multi-Channel (MC) Instrument, with the following assumptions:

- Wash-stain time = ~4 hours per plate
- Scan time on GeneTitan™ MC Fast Scan Instrument = ~3.5 hours per plate
- Scan time on GeneTitan™ MC Instrument = ~5.5 hours per plate

The following figure illustrates the GeneTitan activities for a typical 8-hour workday running 96-format array plates in the GeneTitan™ MC Fast Scan Instrument vs. GeneTitan™ MC Instrument.

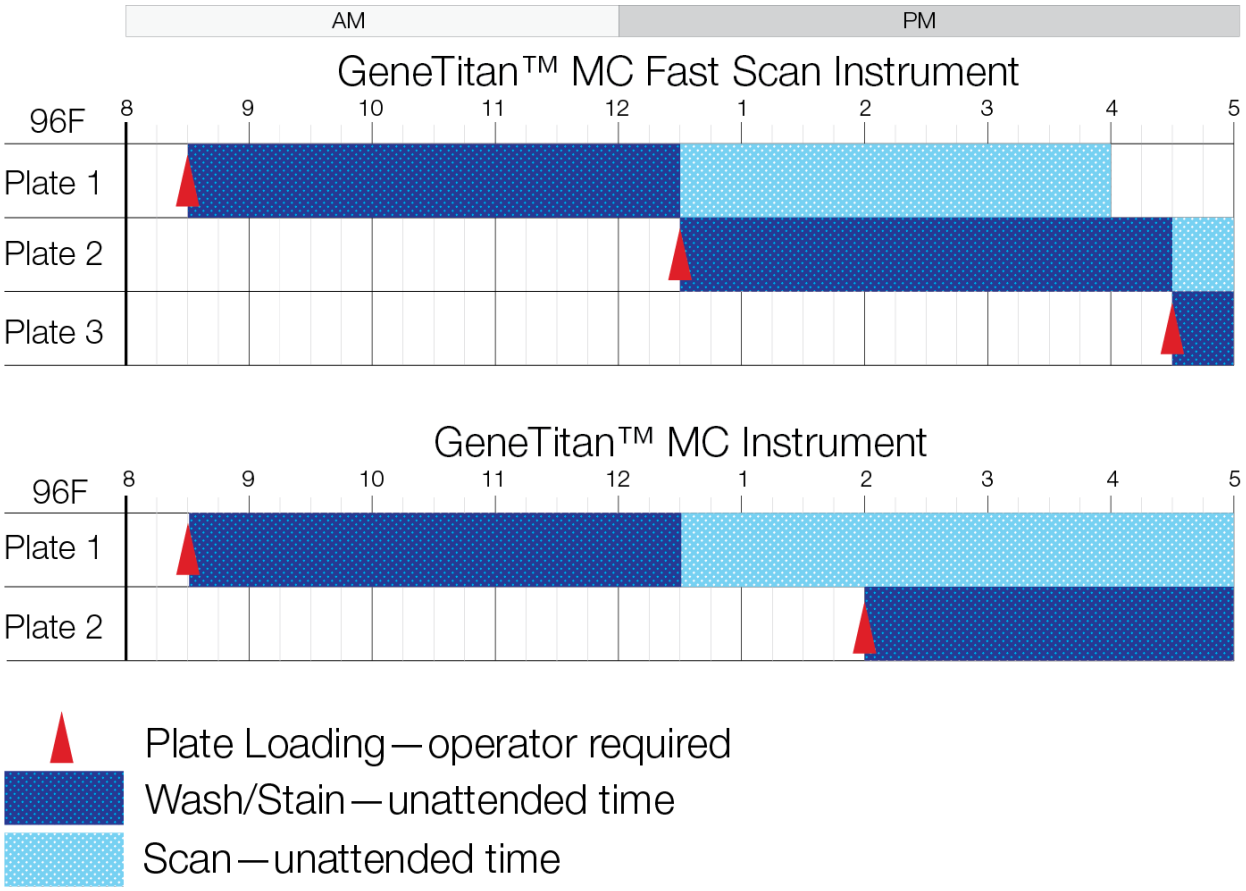


Figure 19 GeneTitan™ MC Fast Scan Instrument vs. GeneTitan™ MC Instrument times for 96-format array plates.

Target preparation with the Axiom™ Propel Fast Wash Workflow

Table 55 Task duration for target preparation

Task	Plates	Operators	Allotted time[1]	Unattended time
Amplification	8	2	1 hour	22–24 hours
Fragmentation and precipitation	8	2	1 hour, 15 minutes	16–24 hours
Centrifugation and drying	8	1	1 hour, 30 minutes	1 hour
Resuspension of hybridization-ready targets	8	1	45 minutes	N/A
In-process quality control	8	1	1 hour	N/A
Hybridization transfer and hybridization	4 6	1	30 minutes 40 minutes	23.5–24 hours
GTMC preparation	4 6	2 2	1 hour 1 hour, 15 minutes	N/A
Washing on GTMC	1	N/A	N/A	~4 hours
Scanning on GeneTitan™ MC Fast Scan	1	N/A	N/A	~3.5 hours
Scanning on GTMC	1	N/A	N/A	~5.5 hours

[1] The allotted time is the the amount of time provided to complete each task in the assay workflow schedule. However, the actual time needed to run each task is less than the allotted time.

Practices to promote effiency

When used, the following practices increase the efficiency of the workflow when running in an ultrahigh throughput environment.

- Keep all required instruments in close proximity to their use in the laboratory, and position the instruments to enable a smooth workflow.
- Thaw the necessary reagents the day before use. See the appropriate stage "Reagent handling" table in the target preparation chapter. See:
  - Chapter 4, “Target preparation with Multidrop™ Combi Reagent Dispensers for 8 plates”
  - Chapter 5, “Target preparation with Multidrop™ Combi Reagent Dispensers for 4 plates”
- Prepare the master mixes ahead of time. For example, advanced preparation of some GeneTitan™ reagents reduces the morning start-up time for Stage 7. See:
  - “Stage 7: Preparing ligation, stain, stabilization reagent trays, and scan trays for the GeneTitan™ MC Instrument” on page 113
  - “Stage 7: Preparing ligation, stain, stabilization reagent trays, and scan trays for the GeneTitan™ MC Instrument” on page 179
- Utilize the Wash B warm-up time (25–30 minutes) on the GeneTitan™ MC Instrument to perform tasks such as setting up the Multidrop™ Combi instruments to dispense the stain trays and scan trays.
- Maximize the GeneTitan™ MC Instrument throughput by loading subsequent plates as soon as the instrument is available. For further information, review the following procedure. (See “Queue another plate for Wash-Scan” on page 205.)

32x96-array format plates per week

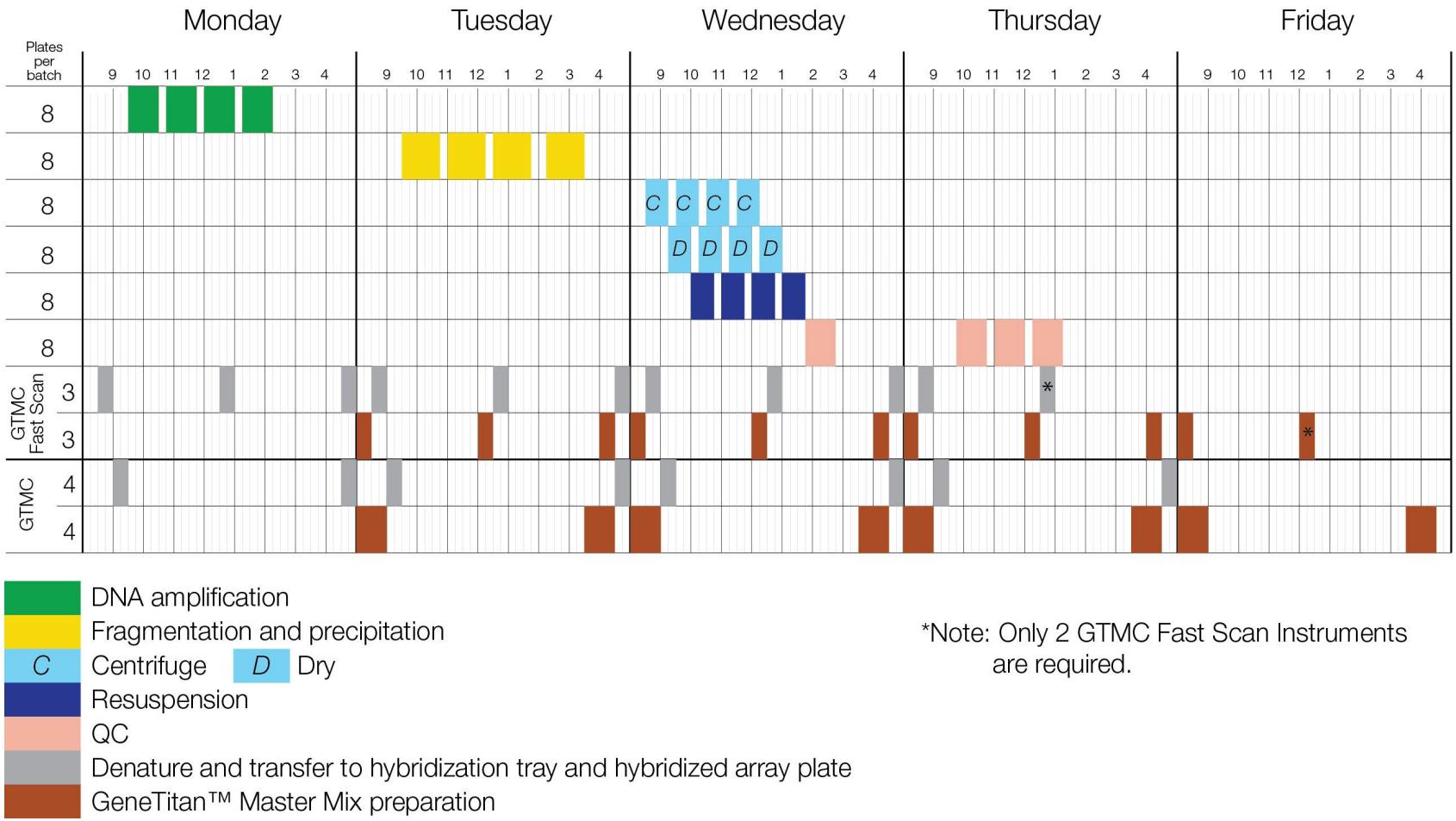
Requirements and output

Table 56 Requirements to process 32 plates of 96 samples in 1 week

Requirement	Quantity
Instruments	
GeneTitan™ MC Fast Scan Instrument	3
GeneTitan™ MC Instrument	4
Multidrop™ Combi Reagent Dispenser	5 (Five reagent dispensers are required to prepare all of the GeneTitan™ reagents simultaneously to achieve the Wash-Stain of 4 array plates at a time.)
ALPS™ 3000 Automated Microplate Heat Sealer	1
Compact Digital Microplate Shaker	1
VWR Signature™ High-Speed Microplate Shaker	1
Sorvall™ X4R Pro-MD Centrifuge	3 • One for room temperature centrifugation • Two with refrigeration for 4°C centrifugation
BINDER™ oven	2 • One at 37°C. This incubator can be used for the amplification, fragmentation, and pellet drying stages. • One at 48°C for off-line hybridization.
ProFlex™ 96-well PCR System thermal cycler	1 (with 2x96 adapter)
INTEGRA Biosciences VIAFLO™ liquid handler, or similar	1

Day-by-day activities

Note: The following schedule is intended as a general guideline. Contact your Thermo Fisher Scientific FAS representative for help with planning an individualized scale-up of the Axiom™ Propel Fast Wash Workflow.

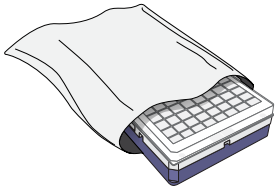
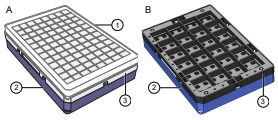


Note: The GeneTitan™ MC Fast Scan Instrument allows 1 additional plate loading in 1 shift as illustrated in the above schedule to reduce the number of GTMC instruments required

A Recommended techniques for GeneTitan™ MC Instrument operation

This chapter describes the recommended techniques and procedures to follow when using the GeneTitan™ MC Instrument or the GeneTitan™ MC Fast Scan Instrument for the fluidics processing and array scanning steps of the Axiom™ Propel Fast Wash Workflow, 96-Array Format. Being familiar with these techniques helps to ensure the success of the assay. Detailed safety information and instruction on using the GeneTitan™ MC Instrument is in the *GeneTitan™ Multi-Channel Instrument User Guide* and the *GeneChip™ Command Console™ User Guide*.

Array plate packaging

Item	Image	Details
Axiom™ Array Plate	  ① Shipping cover (to be discarded) ② Array plate protective base ③ Array plate	The array plate package includes the following: • 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. • 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. • Desiccant pack: The desiccant pack can be discarded after the array plate is removed from the pouch.

Proper tray alignment and placement

Proper alignment and loading of plates, covers, and trays is critical when using the GeneTitan™ MC Instrument. Each plate, cover, and tray has 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 Instrument drawer.

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

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

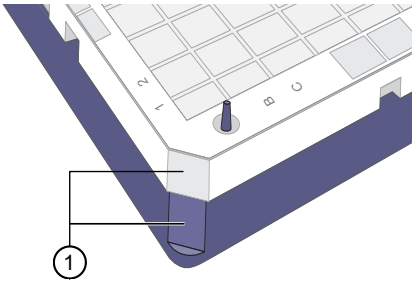


Figure 20 Notched corners aligned.

① Notched corner of array plate that is aligned with notched corner of blue base.

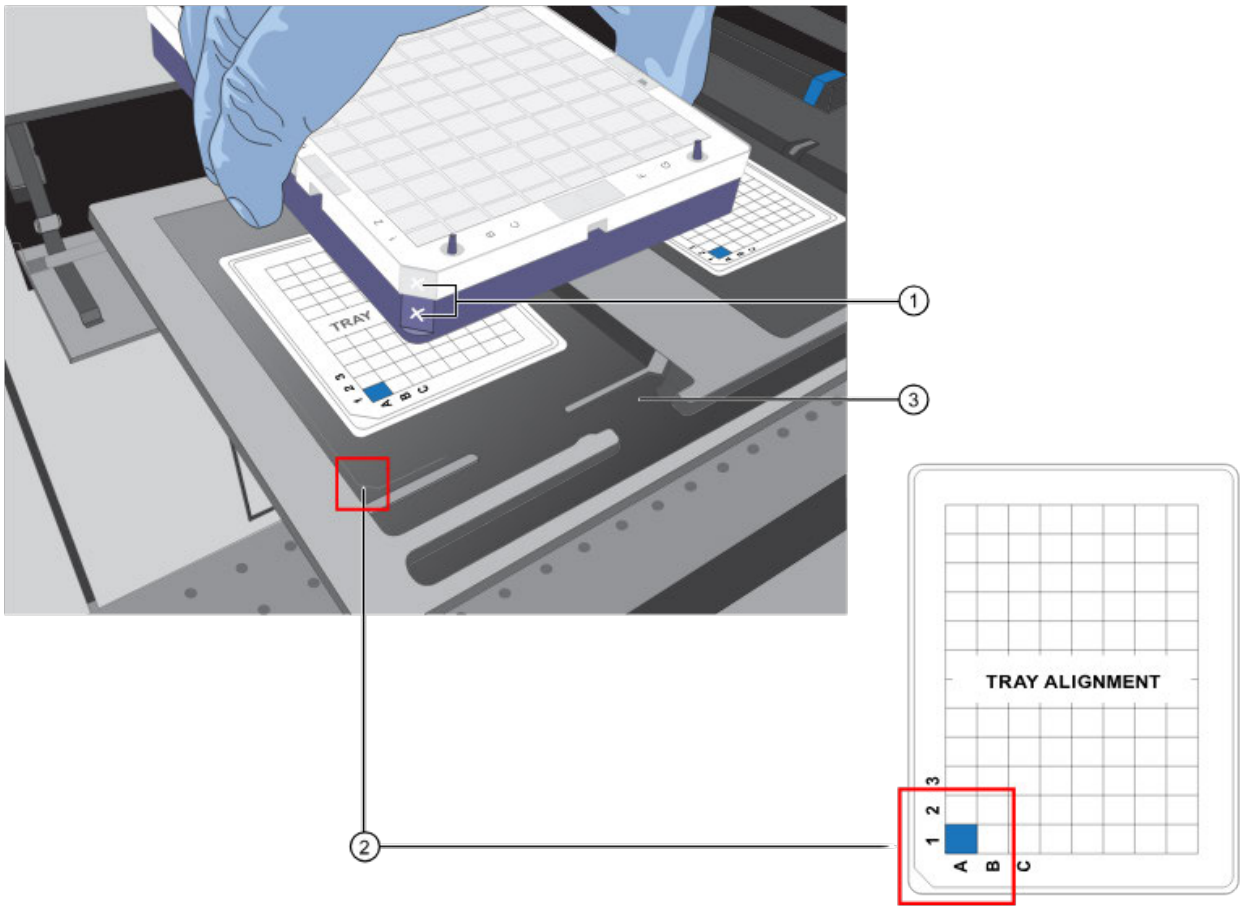


Figure 21 Notched corners marked and aligned with tray alignment guide.

① Notched corners of the array plate and blue base marked with a permanent marker to help ensure proper alignment and loading.

② The notched corner of all plates, bases, and covers and must be seated in the front left corner of the drawer, as indicated the Tray Alignment guide.

③ Plates and trays must be seated in this groove.

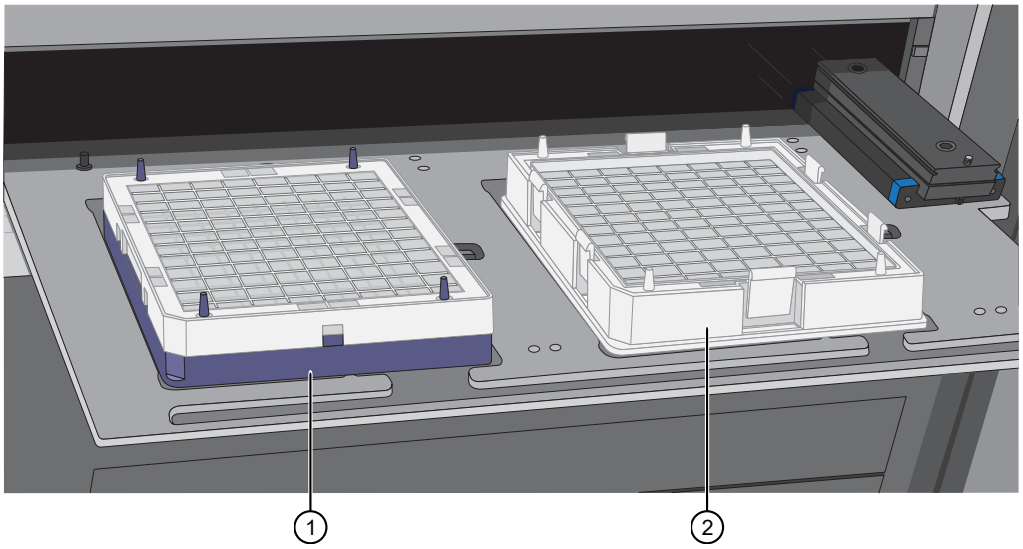


Figure 22 Array plate with protective blue base and the hybridization tray aligned properly loaded into drawer 6.

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

Proper orientation of consumables

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

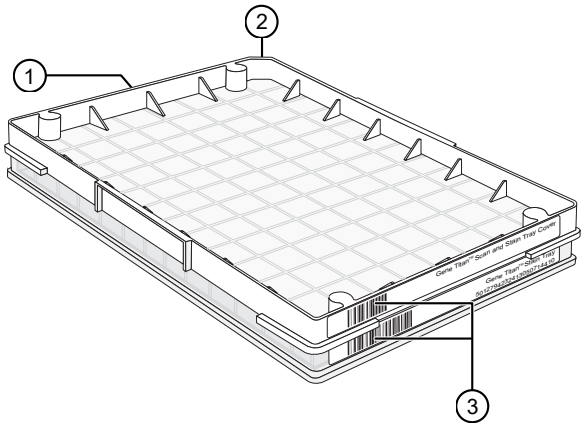


Figure 23 Example shows consumables that must be rotated and loaded on the drawer so that the barcodes face into the instrument.

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

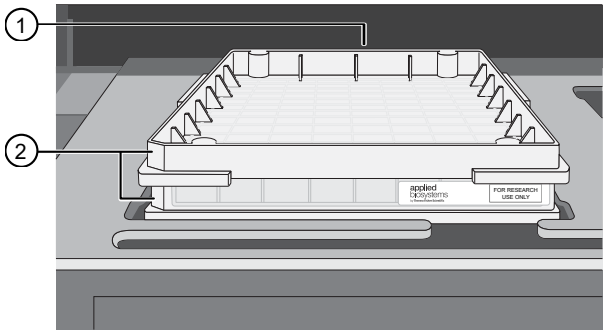


Figure 24 Example of properly loaded GeneTitan™ tray consumables. A GeneTitan™ stain tray and the stain tray cover are shown in this example.

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

Drawer tabs in the GeneTitan™ MC Instrument

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

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

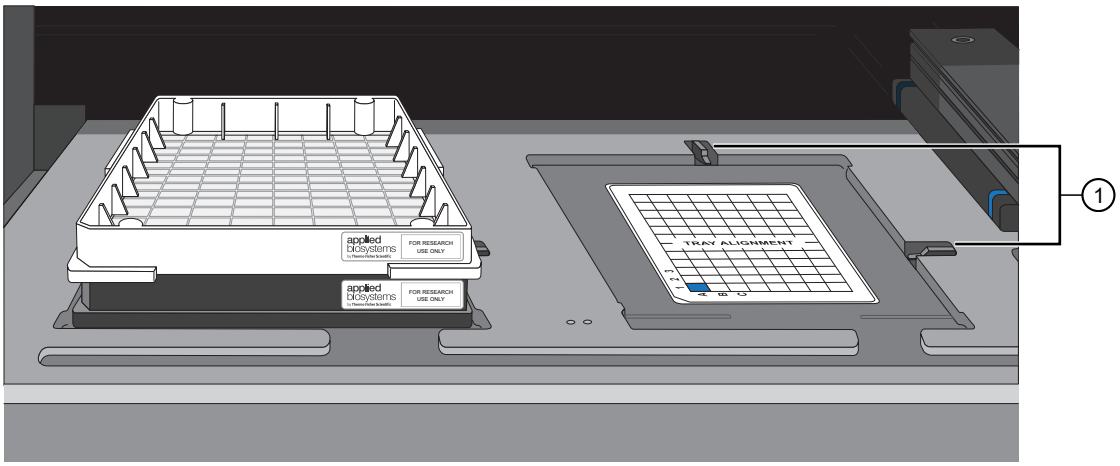


Figure 25 Location of drawer tabs, or fingers.

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

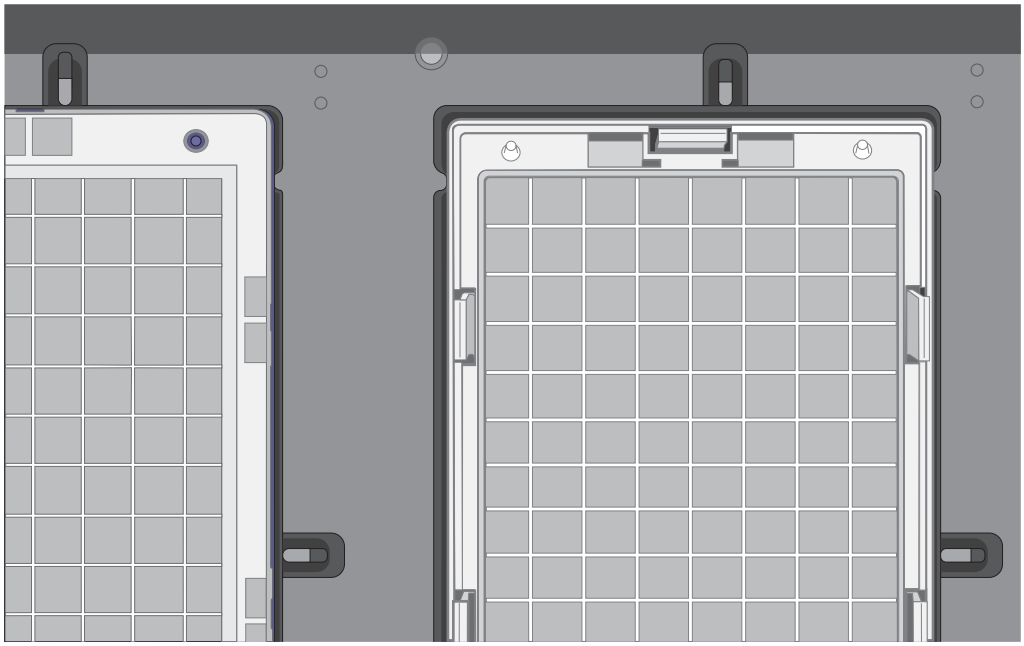


Figure 26 Trays are placed on the drawer when the tabs, or fingers, are retracted.

Stain trays and covers

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

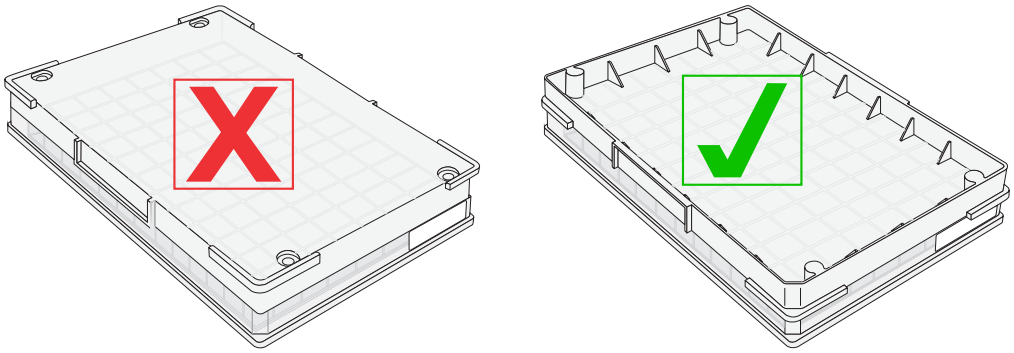


Figure 27 Placement of covers on trays.

Label GeneTitan™ hybridization and reagent trays

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan™ Multi-Channel Instrument, it is helpful to label each tray in a way that identifies its contents.

IMPORTANT! It is important that you write only on the correct locations of the correct sides of hybridization and stain trays. **Do not** write in any other location, because writing can obstruct sensors inside the GeneTitan™ Multi-Channel Instrument and result in experiment failure. To support correct placement of lids onto stain trays, and trays onto the GeneTitan™ Multi-Channel Instrument, you can also label the notched corner of the trays and lids.

Label the GeneTitan™ 96-layout hybridization tray

Label the GeneTitan™ 96-layout hybridization tray on the front part of the short side of the tray, next to the notch at the left, as shown in the following image. The proper section for labeling is nearest to the notched corner, corresponding to the A1 and B1 wells.

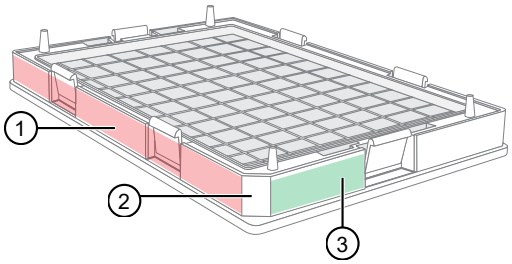


Figure 28 Correct area to label the GeneTitan™ 96-layout hybridization tray.

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

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

Label the GeneTitan™ reagent trays

You can label the GeneTitan™ reagent trays on the left side of the front of the tray as shown in the following image. The correct side is nearest to the notched corner, corresponding to the A1 through C1 wells.

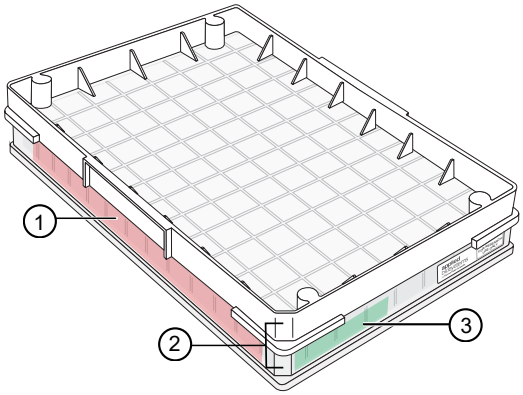
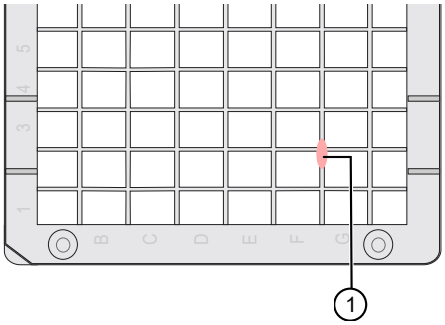


Figure 29 Correct area to label the GeneTitan™ reagent trays (96-layout stain tray with cover shown).

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

Guidelines for aliquoting reagents to GeneTitan™ trays

IMPORTANT! Droplets near or on the top of the well dividers can cause the cover to stick to the tray during GeneTitan™ MC Instrument processing. Remember to deionize the stain trays and the covers before aliquoting master mixes.



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

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

Deionization of GeneTitan™ trays and covers

When dispensing the GeneTitan™ reagents using the Multidrop™ Combi Reagent Dispenser, we recommend using the Compact in-Tool Ionizing Blower 6432E at the dispense station. The stain and scan tray covers can also be deionized by using the ion blowers. The GeneTitan™ ZeroStat AntiStatic Gun (Cat. No. 74-0014) can also be used to deionize GeneTitan™ stain trays and covers manually.

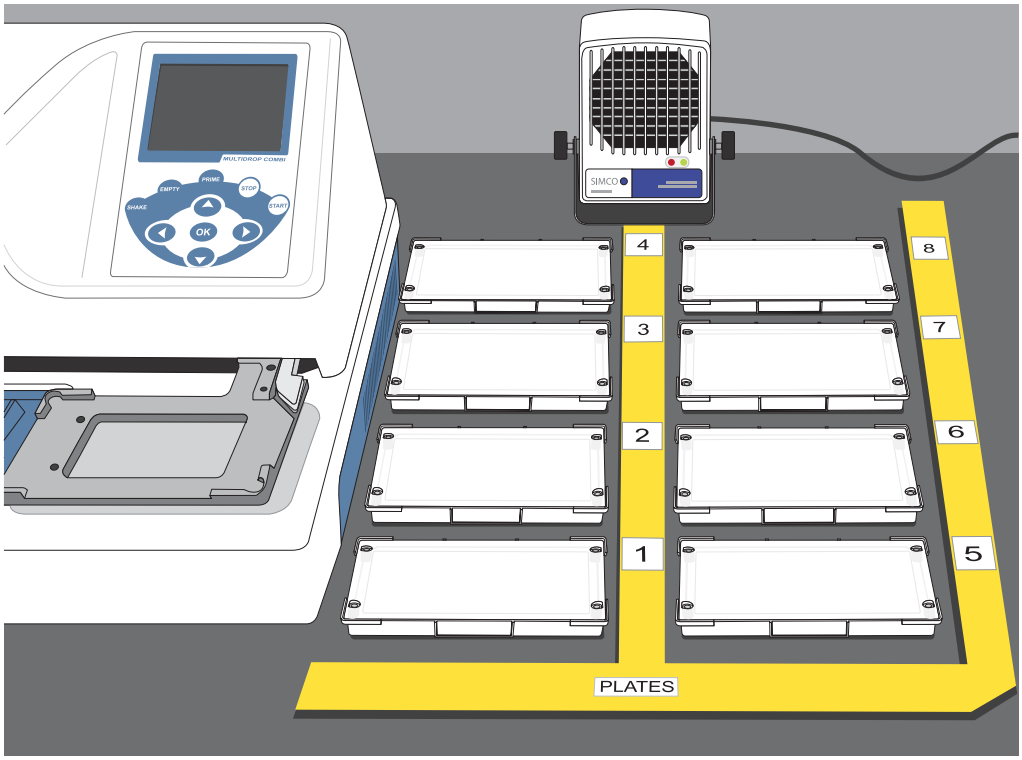


Figure 30 Placement and positioning of stain tray covers for deionization with the ionizing air blower.

Manual deionization of GeneTitan™ trays and covers

IMPORTANT! Except for the array plate, scan tray, and the hybridization tray, you must deionize all GeneTitan™ stain trays, stain tray covers, and the scan tray cover using an antistatic gun. Always deionize before you fill the trays with reagents and before you place the covers on the trays. Deionization removes the static electricity. 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.

Deionize the inner surface of each tray and cover:

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

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

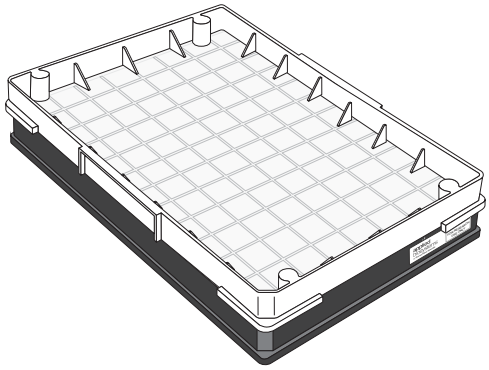


Figure 31 96-format scan tray and cover. Deionize only the cover

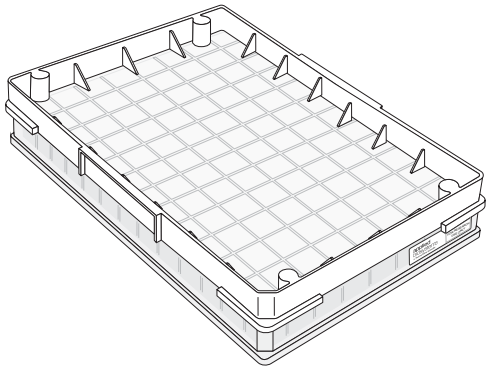


Figure 32 96-format stain tray and cover. Deionize the cover and the tray

Deionize GeneTitan™ trays and covers

WARNING! The deionization steps damage the arrays on the plate. Before using the antistatic gun, ensure that the array plates remain in their protective pouch and placed away from the deionization area.

Place the scan tray and hybridization tray away from the area where you are performing deionization.

During this procedure, treat the plate or cover as if it were divided into 6 sections. See Figure 33.

1. Place a laboratory tissue on the benchtop.
2. Place the stain tray on a table top. Use the antistatic gun, then squeeze, then release the trigger slowly 3 times over the center of each section, squeezing for approximately 2 seconds, then releasing for approximately 2 seconds.

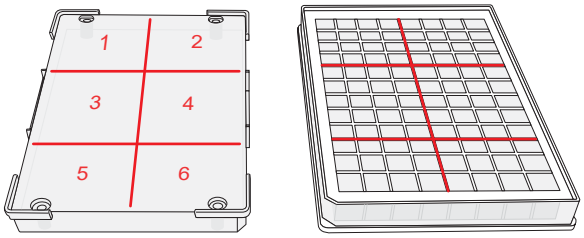


Figure 33 Deionization zones for GeneTitan™ 96-format trays and covers.

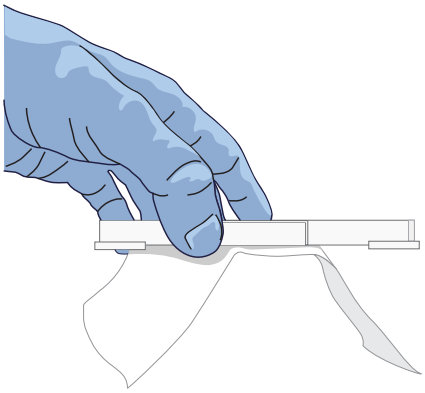
Ensure that a stream of ionized particles settles on all wells of the stain tray to dissipate the static electricity.

3. Place the stain tray cover on the tissue with the flat surface facing upward.

4. From ~13 mm away, aim the antistatic gun at the flat surface of the stain tray cover, then squeeze the trigger. As you squeeze the trigger, move the gun across the cover so that the stream of ionized particles settles on all areas of the cover to dissipate the static electricity.

   Squeeze and release the trigger slowly 3 times over each section, squeezing for approximately 2 seconds and releasing for approximately 2 seconds.

5. Place the treated cover or tray on the laboratory tissue, then lift it up.



6. Do one of the following:

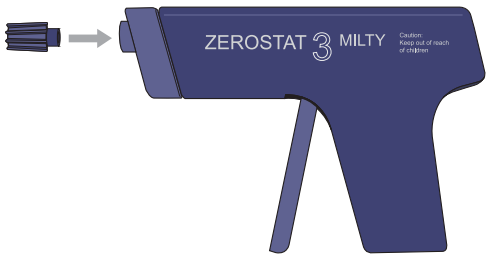
   - If the tissue does not cling to the plastic, proceed with the protocol.
   - If the tissue clings to the plastic, then repeat step 2 through step 5. If the tissue continues to cling to the plastic, test the device using the ion-indicator cap to determine if the unit is still releasing ions. Otherwise, consider replacing the unit.

Ion-indicator cap

The GeneTitan™ ZeroStat AntiStatic Gun includes an ion-indicator cap. The cap is a device that is used to test the release of ions when the antistatic gun is in use.

Test the antistatic gun for ion release

1. Insert the ion-indicator cap into the nose of the GeneTitan™ ZeroStat AntiStatic Gun.



IMPORTANT! Do not leave the ion-indicator cap on the antistatic gun when deionizing trays and covers.

2. Slowly squeeze the release trigger, then observe the discharge through the viewing window on the ion-indicator cap.

   A visible light is observed in the viewing window on the cap when charged ions are discharged.

3. If you cannot see a light through the viewing window, replace the antistatic gun as it is unusable.

   Each GeneTitan™ ZeroStat AntiStatic Gun produces approximately 50,000 trigger operations, which are sufficient for approximately 200-250 runs on the GeneTitan™ MC Instrument.

4. If you can see a light through the viewing window, then have determined that the gun is functional, remove the cap from the gun before deionizing a tray or cover.

Best practice guidelines for GeneTitan™ reagent bottles

Follow these best practices for re-filling GeneTitan™ reagent bottles if processing 2 or more array plates on the same day.

1. When prompted to refill or replenish the glass bottles, follow these guidelines:
   - Axiom™ Wash Buffer A: Refill the GeneTitan™ Wash A bottle to a minimum of 2 L up to approximately 2/3rds of the glass bottle volume.
   - Axiom™ Wash Buffer B: Refill the GeneTitan™ Wash B bottle up to the 1-L mark.
   - Axiom™ Water: Refill the GeneTitan™ Rinse bottle up to the 1-L mark.
2. Do not overfill the bottles.

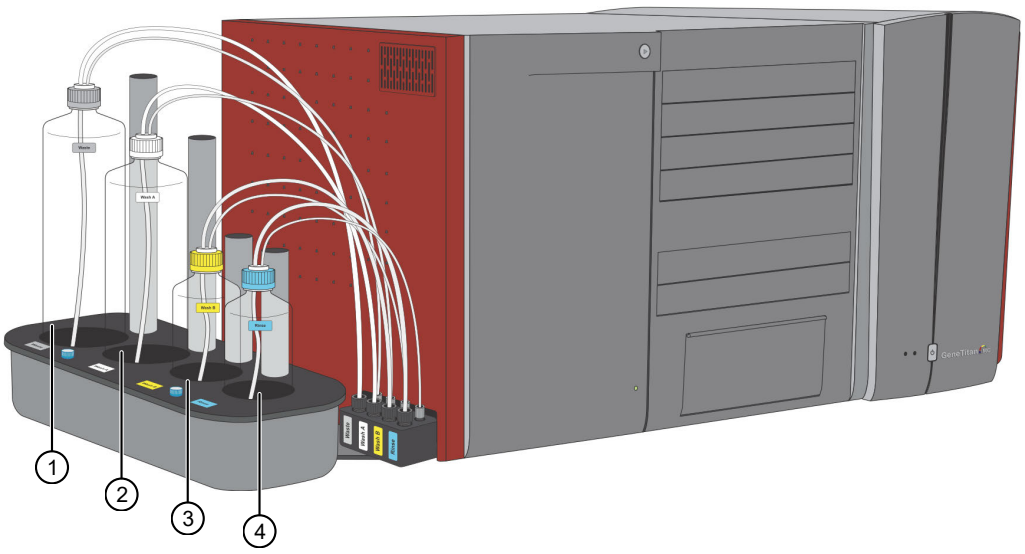


Figure 34 GeneTitan™ Multi-Channel (MC) Instrument reagent bottles.

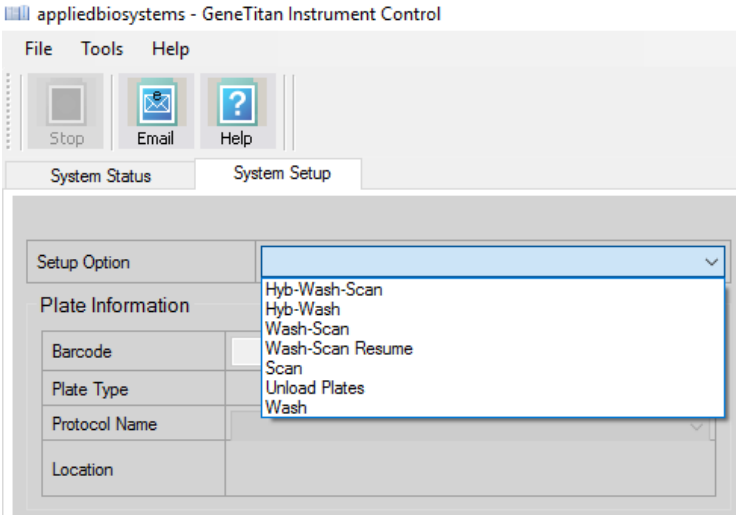
① Waste bottle
② Wash A bottle
③ Wash B bottle
④ Rinse bottle

Setup options for array plate processing

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

- Hybridization
- Wash and Stain
- Imaging (Scan)

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



Hyb-Wash-Scan

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

- **Hyb**: The array plate is moved to the hybridization oven inside the instrument. Each denatured sample in the hybridization tray is hybridized to an array on the array plate.
  - Time that is required for 96 samples = 23.5–24 hours
- **Wash**: Samples on arrays are ligated, washed, stained, and stabilized.
  - Time that is required for 96 samples = ~4 hours
- **Scan**: The array plate is moved to the imaging device in the GeneTitan™ MC Instrument and each array is scanned.
  - Time that is required for 96 samples = ~3.5 to ~5.5 hours. Scan time varies with instrument type and GCC version.

Hyb-Wash

When the **Hyb-Wash** setup option is selected, processing stops after the array has gone through fluidics processing. Use this option if an array plate cannot be scanned on the same GeneTitan™ MC Instrument as the one used for hybridization and fluidics processing.

1. If the array plate cannot be scanned immediately after the **Hyb-Wash** process is complete, store the array plate following these steps:
   a. Wrap the array plate (in the scan tray with black protective base) in aluminum foil to protect from light. No lid is required.

      Do not tilt or invert the plate stack. If tilted or inverted, the Hold Buffer spills out of the tray. To prevent liquid spillage, keep the plate stack level when handling it. Do not touch the bottom optical surface of the scan tray.

   b. Store at 4°C.

   c. Scan the array plate within 1 week.

2. When ready to scan, prepare the array plate following these steps:
   a. Protect the plate from light.

   b. Bring the plate to room temperature for approximately 50 minutes.

   c. Remove the aluminum foil, then load the plate onto the GeneTitan™ MC Instrument.

Wash-Scan

Note: The **Wash-Scan** option is available in GCC version 6.1 or later.

Use the **Wash-Scan** option if:

- The array plate was hybridized in an oven separate from the GeneTitan™ MC Instrument.
- To bypass the hybridization step and perform only the wash/stain and scan steps.

  Note: If the **Wash-Scan** option is selected, it usually takes 25–30 minutes to warm up the Wash B.

Note: Ensure that the Continuous Wash-Scan process is enabled. Contact your local FAS to perform this procedure.

Queue another plate for Wash-Scan

This technique can be used in high throughput workflows to maximize the capacity of the GeneTitan™ MC Instruments.

- GeneTitan™ MC Fast Scan Instrument
  - Load the next plate to be washed and scanned immediately after the previous plate has finished washing (~5 hours).
- GeneTitan™ MC Instrument
  - Load the second plate to be washed and scanned immediately after the first plate has finished washing (~5 hours).
  - Load the third and subsequent plates for **Wash-Scan** at a ~5.5-hour interval, or after the plate being processed in the scanner has completed.

Wash

The **Wash** workflow enables you to bypass the scan step, performing only wash and stain.

Note: When the **Wash** option is selected, allow 25–30 minutes to warm up the Wash B.

IMPORTANT! After the **Wash** workflow is complete, scan the array plate as soon as possible. Array plate data can be affected when the plate is not scanned immediately after washing.

Wash-Scan Resume

Use the **Wash-Scan Resume** option if fluidics processing has been interrupted (for example, a power failure at your facility). This allows you to resume an interrupted workflow at any point in the **Wash** stage.

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

The step at which the run was aborted is identified by:

- Viewing the **System Status** window if you are aborting the last plate through the fluidics system.
- Starting the **Resume** process.

Select **Wash/Scan Resume** from the **System Setup** tab, then follow the prompts to unload and reload all drawers.

The trays are loaded. It is up to you to determine whether to load fresh reagents or reuse the trays already in the GeneTitan™ Multi-Channel (MC) Instrument. Base your decision on the step where the problem occurred.

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

- Load new stain trays with fresh reagents.
- Load a new scan tray.

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

Scan

Use the scan option:

- To rescan an entire array plate or specific arrays on a plate that failed to scan for reasons such as bubbles or gridding failure.
- If you have hybridized and performed the fluidics processes on a different GeneTitan™ MC Instrument than the one currently being used for the scan, or at a different time.
- To queue a second plate for scanning. Using the scan option allows you to start a second scan workflow although another scan workflow is already running.

Queue a second plate for scanning

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

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

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

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

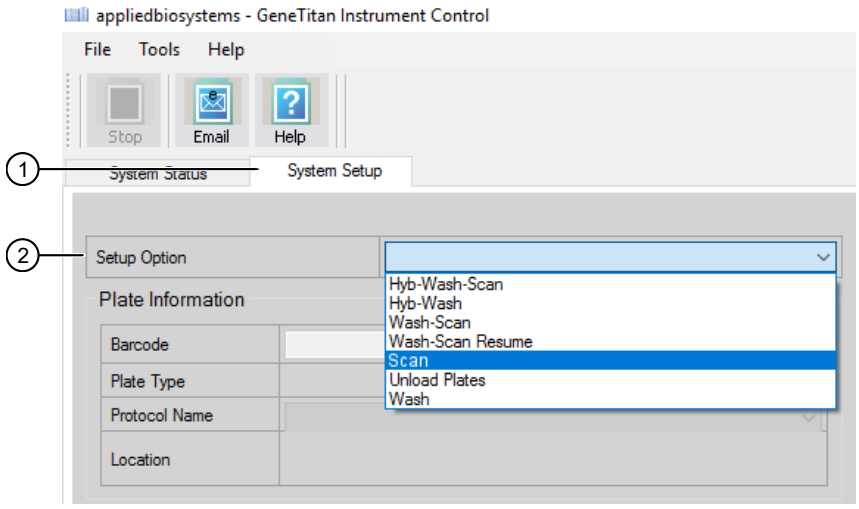


Figure 35 Scan setup option to process a second array plate.

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

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

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

5. Follow the instructions in the **Status** pane and empty the trash bin if needed, then press the blue confirmation button on the instrument to continue.

6. Place the array plate on top of a scan tray in the correct orientation such that notched corner of the array plate and scan tray are aligned.

7. Load the array plate/scan tray combination in drawer 2 of the GeneTitan™ MC Instrument, on the left or right side, as instructed in the **Status** pane. Ensure that the array plate/scan tray combination is loaded in the correct orientation in the drawer. If needed, see Figure 20 for further information on the proper alignment and loading of plates, covers, and trays in the GeneTitan™ MC Instrument.

8. When ready, press the blue confirmation button on the instrument.

9. Select the arrays to scan in the **Array Selection** section in the upper right corner of the window, then click **Next**.

10. In the **Start Processing** confirmation message, click **OK** to continue.
    The second queued plate runs after the first scan finishes and the scanner is available.

Unload Plates

Use the **Unload Plates** option to unload plates and trays from the instrument when processing is complete or has been aborted.

Load an array plate and hybridization tray into the GeneTitan™ MC Instrument (for Hyb-Wash-Scan or Hyb-Wash)

1. When drawer 6 opens, load the array plate and hybridization tray in the following manner:
   a. Examine the wells of the hybridization tray for bubbles, then puncture any bubbles with a clean pipette tip.

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

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

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

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

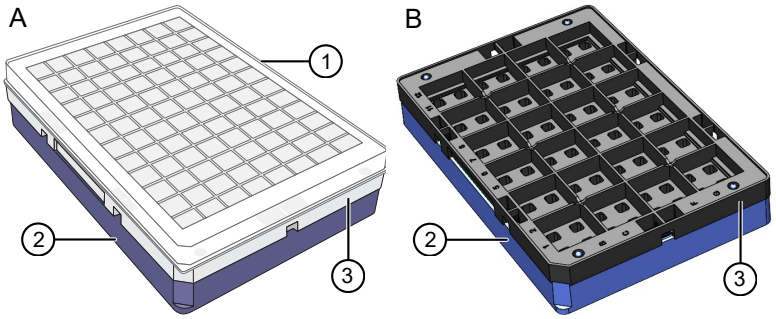


Figure 36 Array plate components, as shipped (2 configurations).

① Clear 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.

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

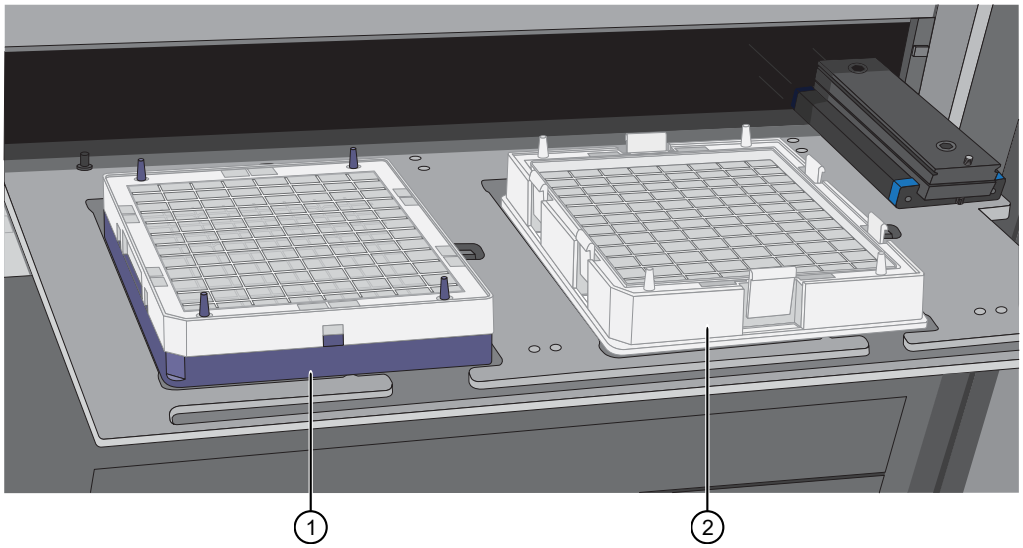


Figure 37 Array plate on protective blue base and the hybridization tray properly loaded into drawer 6.

① Array plate on protective base.
② Hybridization tray.

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

CAUTION! The notched corner of each plate, cover, and tray must be aligned. When loading onto the GeneTitan™ MC Instrument, the notched edge plates, covers, and trays must be aligned as indicated by the Tray Alignment guide in the drawer.

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

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

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

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

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

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

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

5. Verify the plate clamping step to ensure that the array plate is securely fastened to the hybridization tray. Remove the plate stack from the drawer and place on a bench top. Using your thumbs, press the array plate downward following the positions that are specified in Figure 38. *No clicking sound indicates proper clamping.*

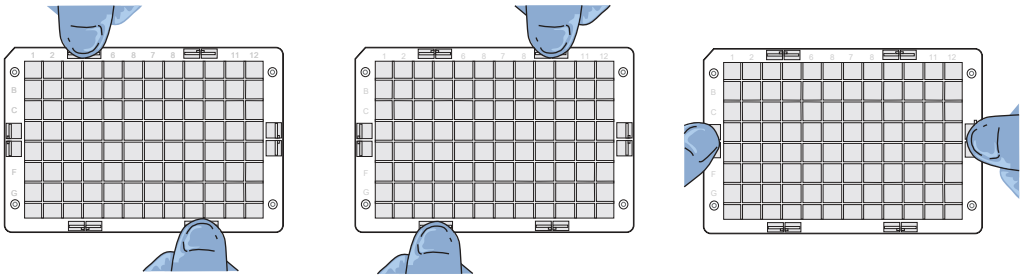


Figure 38 Clamping verification steps.

6. Inspect the array plate for bubbles.
   a. **Keeping the plate stack level**, inspect the bottom for bubbles under the arrays—*do not* tilt or invert the plates.

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

7. Return the plate stack to the drawer with the notched corner facing you, then press the confirmation button on the GeneTitan™ Instrument to proceed.

8. A message may be displayed if plate orientation is not correct or if the hybridization tray barcode cannot be read. If this message appears, complete one or both of the following actions.
   - Check the loading of the array plate, then click **OK**.
   - If the instrument continues to have problems reading the barcode and after ensuring that the correct trays have been placed in the proper orientation, click **Skip**.

9. Continue to “Load a second array plate and hybridization tray onto the GeneTitan™ MC Instrument” on page 234.

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

When a second array plate and hybridization tray can be loaded

After processing starts on the first plate stack, you have a specific length of time during which you can load another array plate and hybridization tray. This length of time is displayed above the **Hybridization Oven Status** pane (Figure 39). You cannot load another hybridization tray before or after this time.

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

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

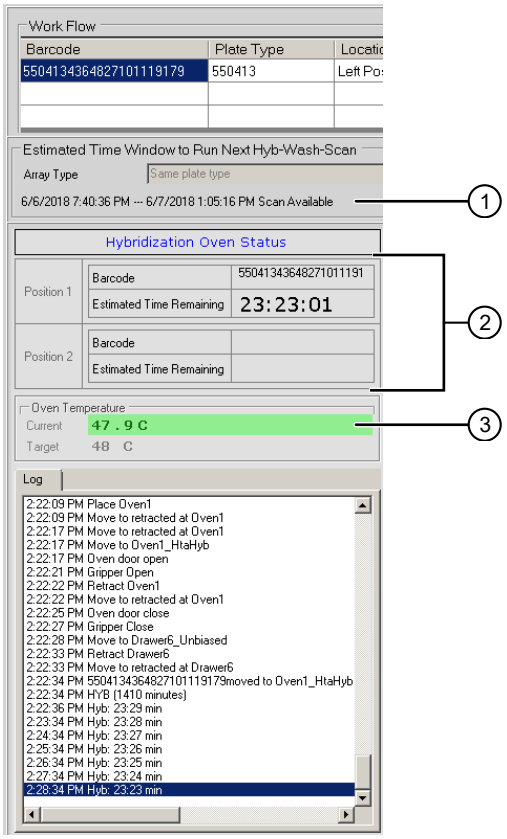


Figure 39 When to load a second array plate and hybridization tray based on oven status information.

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

② Position of plate stack in the hybridization oven. Only 1 plate is being processed in this figure. As such, position 2 is blank.

Position 1—left side of the oven

Position 2—right side of the oven

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

Load a second array plate and hybridization tray

1. Select the **System Setup** tab.
2. Load an array plate and hybridization tray in the same manner as the previous plate and tray were loaded.
   a. Scan or manually enter the array plate barcode, then click **Next**.
   b. Load the array plate with the blue protective base and the hybridization tray without the cover, then press the confirmation button.
   c. Select the arrays to scan, then click **Next**.

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

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

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

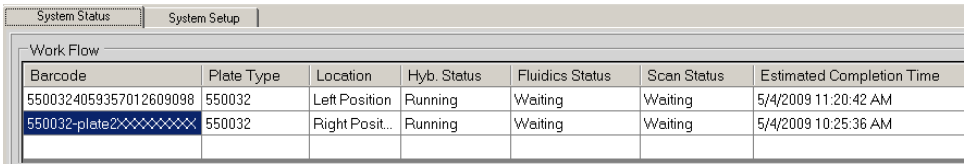


Figure 40 Example of the Work Flow pane when 2 plates are loaded and are in the hybridization oven.

When to abort a process

If needed, the processing of array plates can be aborted.

If a plate is in the fluidics station, the abort process can take up to 3 minutes. The status window displays “AbortRequested” and then changes to “Aborted”.

A clamped array plate/hybridization tray stack that is aborted while it is in the oven or in drawer 6 is moved to drawer 1.

To retrieve the array plate and related consumables after the instrument aborts a process, take the following actions as needed.

- Use the **Unload Plates** option.
- Start another run. That forces the system to unload the aborted plates.

An instrument-initiated abort can occur for the following reasons.

- The plates are improperly placed.
- The uninterruptible power supply (UPS) detects a long power interruption, draining the UPS to 75% power.
- The equipment malfunctions.

When the system aborts the processing, follow the instructions that are displayed in the user interface.

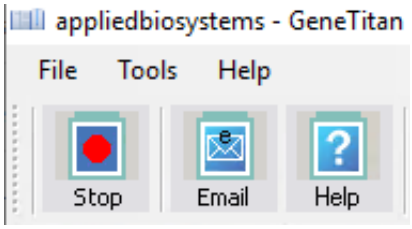
The operator can initiate an abort on 1 plate and the gripper can continue to process other plates in the instrument.

Abort a process

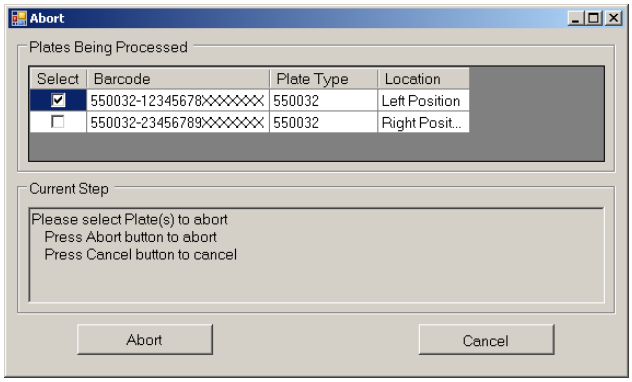
Use the following procedure to abort a process underway in the GeneTitan™ MC Instrument.

Note: If reagents are loading, do not use this method. Instead, click **Cancel** in the reagent load step.

1. Click **Stop** in the upper left corner of the **Instrument Control** window.



2. In the **Abort** dialog box, select the array plate to abort, then click **Abort**.



3. In the confirmation box, click **Yes**.

4. Wait until the status of the array plate in the **Work Flow** pane in the **Instrument Control** display changes from “AbortRequest” to “Aborted”.

Note: If reagents are loading, abort the plate by clicking **Cancel** in the reagent load step.

Note: If the gripper is required to complete the abort process, the plate remains in the “AbortRequest” state until the gripper becomes available.

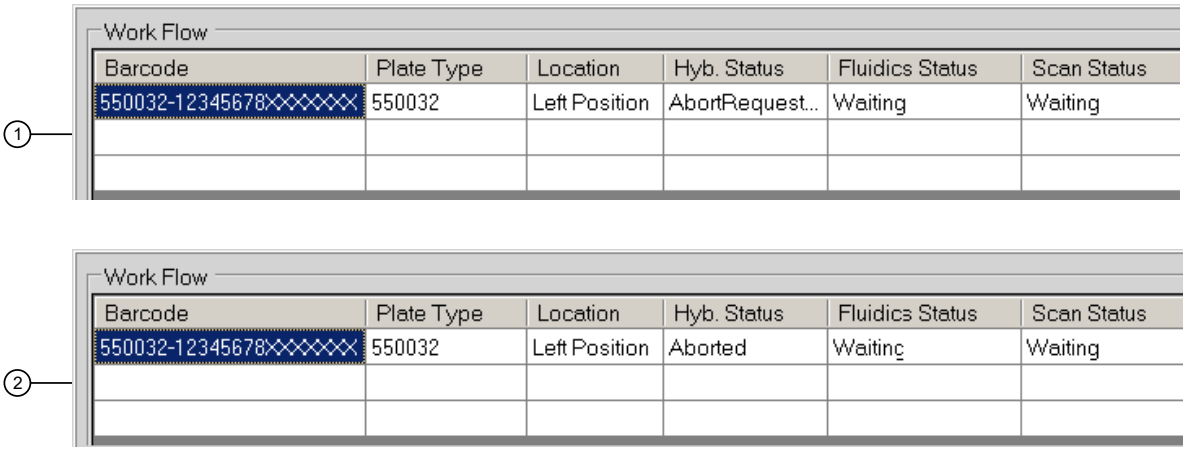


Figure 41 The Work Flow pane.

1. Shows that the abort has been requested.
2. Shows that the abort has been completed.

5. After the abort process is completed, do one of the following to retrieve the array plate and related consumables.
   - In the **Setup Option** list, select **Unload Plates**.
   - Start to load a new array plate.

Email notifications from the GeneTitan™ MC Instrument

You can configure the GeneChip™ Command Console™ software to send email notifications about the GeneTitan™ MC Instrument status. It is critical that you know when the instrument requires attention for sample handling or troubleshooting. Rapid notification can lessen the risk of sample loss.

The system can notify you when a process starts, completes, aborts, or encounters an error.

For instructions on setting up notifications, see the *GeneChip™ Command Console™ User Guide*.

GeneTitan™ MC Instrument lamp

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

The GeneTitan™ Instrument Control software provides a timer that indicates the remaining useful life of the bulb and notifies you when it requires replacement. It is important to adhere to the warnings specified in the *GeneTitan™ Multi-Channel Instrument User Guide*.

See the user guide for the Lambda LS and Smart controller system. Never manually switch the lamp and the controller on or off. The GeneTitan™ Instrument Control software manages the lamp activity and switches the lamp on and off as required. It takes 10 minutes to warm up the lamp. In idle mode, the lamp remains on for 2 hours before it is automatically switched off if there are no more plates being transferred from the fluidics to the imaging station. This automatic switching is by design and is intended behavior. Do not try to save the lamp life by powering off the switch on the lamp.

Note: The power switch on the shutter box must always be ON. The OPEN/CLOSE switch on the shutter box must always be at the AUTO position.

B Fragmentation quality control gel protocol

Equipment required

"MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Gel Imager	**MLS**
Pipette, multichannel or single-channel P20	**MLS**
Plate centrifuge	**MLS**
Vortexer	**MLS**

E-Gel™ and reagents required

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

Item	Source
E-Gel™ Power Snap Plus Electrophoresis Device	G9110
iBright™ CL750 Imaging System	A44116
Invitrogen™ E-Gel™ *48* Agarose Gels, 4%	G800804
Applied Biosystems™ 25-bp DNA Ladder, or a similar product prepared as instructed by the manufacturer	931343
Invitrogen™ TrackIt™ Cyan/Orange Loading Buffer	10482028
Invitrogen™ UltraPure™ DNase/RNase-Free Distilled Water	10977023

Consumables required

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

Item	Source
Adhesive film—use one of the following: • Applied Biosystems™ MicroAmp™ Clear Adhesive Film • Microseal™ 'B' PCR Plate Sealing Film	 • 4306311 • Bio-Rad™, MSB1001
Pipette tips	Same brand as pipettor

Prepare the gel diluent

A 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer can be used in Stage 5: Resuspend the pelleted DNA and prepare for hybridization. Adjust the formulation proportionally to prepare the required quantity of gel diluent for the test.

1. Add 1,000 µL of TrackIt™ Cyan/Orange Loading Buffer to 99-mL nuclease-free water. Total volume 100 mL.
2. Mix well.
3. Store at room temperature.

Dilute the TrackIt™ Cyan/Orange Loading Buffer

A 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer can be used in QC checks stage.

1. Add 500 µL of TrackIt™ Cyan/Orange Loading Buffer to 49.5-mL nuclease-free water. Total volume equals 50 mL.
2. Mix well.
3. Store at room temperature.

Dilute the 25-bp DNA Ladder

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

1. Add 25 µL of the 25-bp DNA Ladder to 125 µL of UltraPure™ DNase/RNase-Free Distilled Water.
2. Mix well.
3. Store at 4°C until use.

Run the fragmentation QC gel

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

1. Tightly seal the Gel QC Plate that is produced during Stage 5A: In-process QC.
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 30 seconds.
3. Power on the electrophoresis device.
4. Place the E-Gel™ *48* Agarose Gel onto the electrophoresis unit.
5. Remove 2 combs from the gel.
6. Load 15 µL from user-selected wells of the Gel QC Plate onto the gel.
7. Load 15 µL of 25 bp DNA ladder into the marker wells (M).
8. Load 15-µL nuclease-free water into any unused wells.
9. Run the gel for 19 minutes.
10. Capture a gel image.

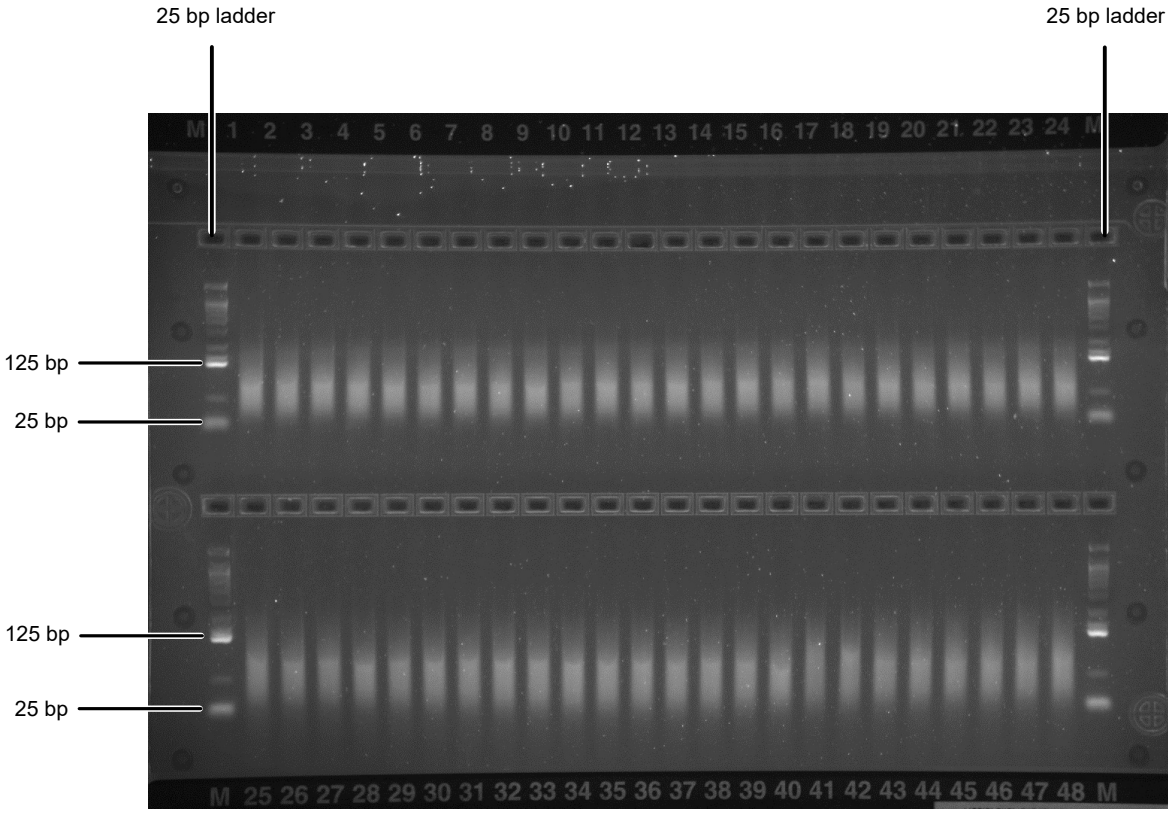


Figure 42 Fragments fall between 125 bp and 25 bp on a successful gel image.

C

Sample quantification after resuspension

Equipment required

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

Quantity	Item	Source
1	Multiskan™ SkyHigh Microplate Spectrophotometer	A51119500C

Spectrophotometer

Specifications: Must be able to read DNA samples using UV/VIS absorbance setting at 260 nm, 280 nm and 320 nm wavelengths.

We recommend using the following spectrophotometer, or equivalent.

Item	Source
Multiskan™ SkyHigh Microplate Spectrophotometer	A51119500C

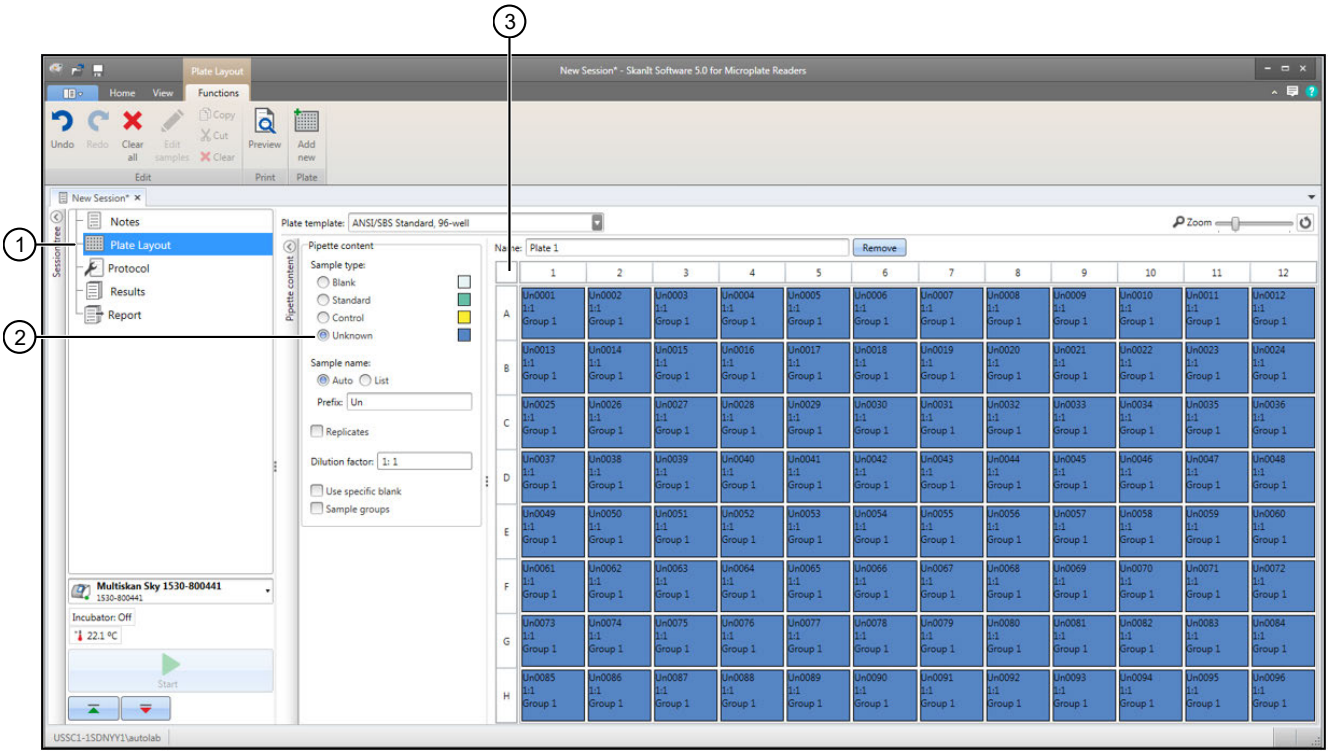
Quantify the diluted samples

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 252.

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. Then assign 260 nm, 280 nm, and 320-nm wavelengths to be measured.

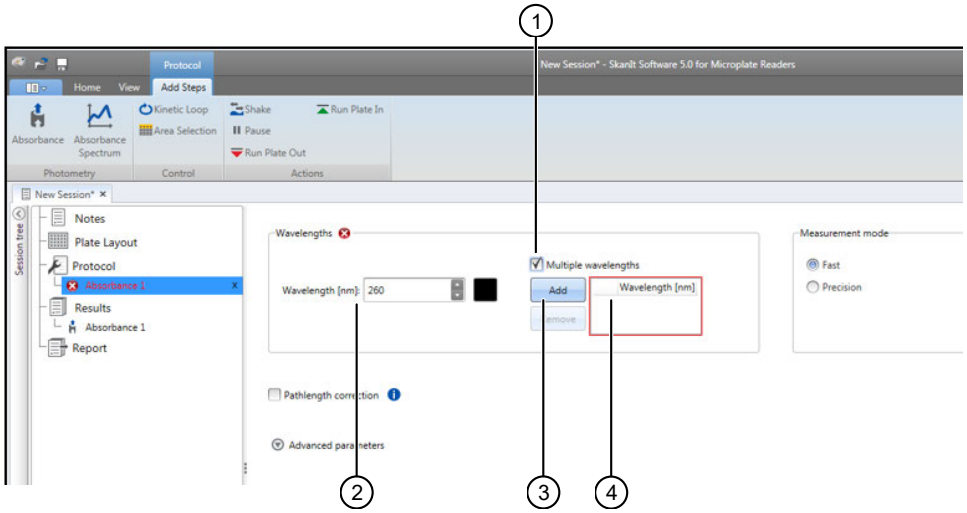


Figure 43 Assign wavelengths

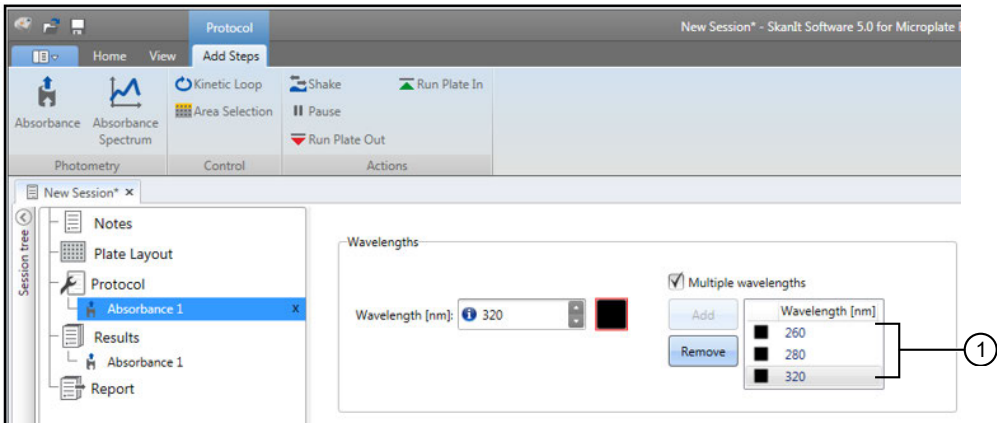
① **Multiple wavelengths** checkbox
② **Wavelength [nm]** field
③ **Add**
④ **Wavelengths [nm]** box

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.

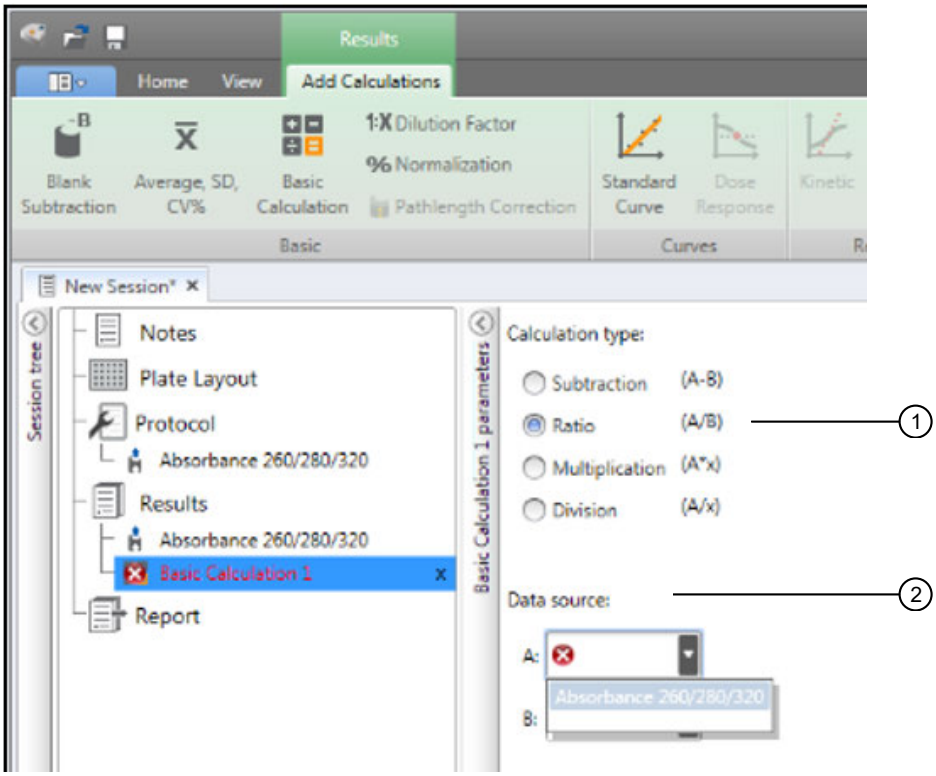
6. Repeat step 5 to add 280 nm and 320-nm wavelengths. When complete, 260, 280, and 320 appears in the **Wavelengths [nm]** box.



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**.

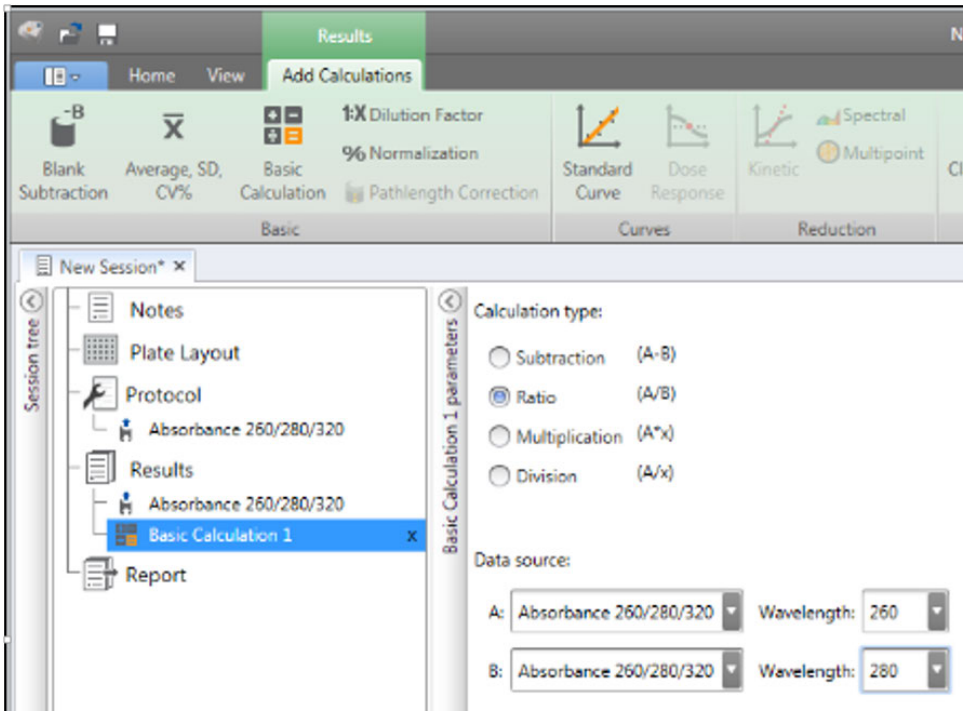


a. Under **Results** in the session tree pane, click **Absorance 260/280/320**.

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.

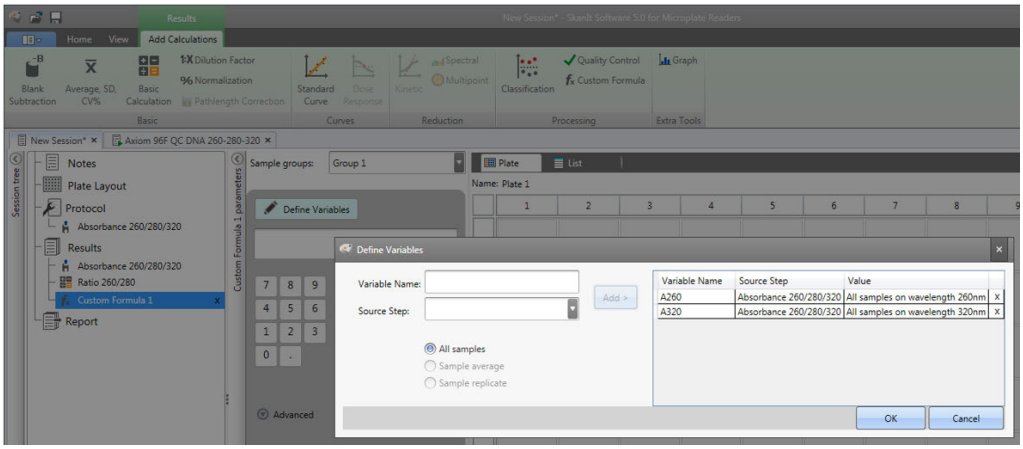
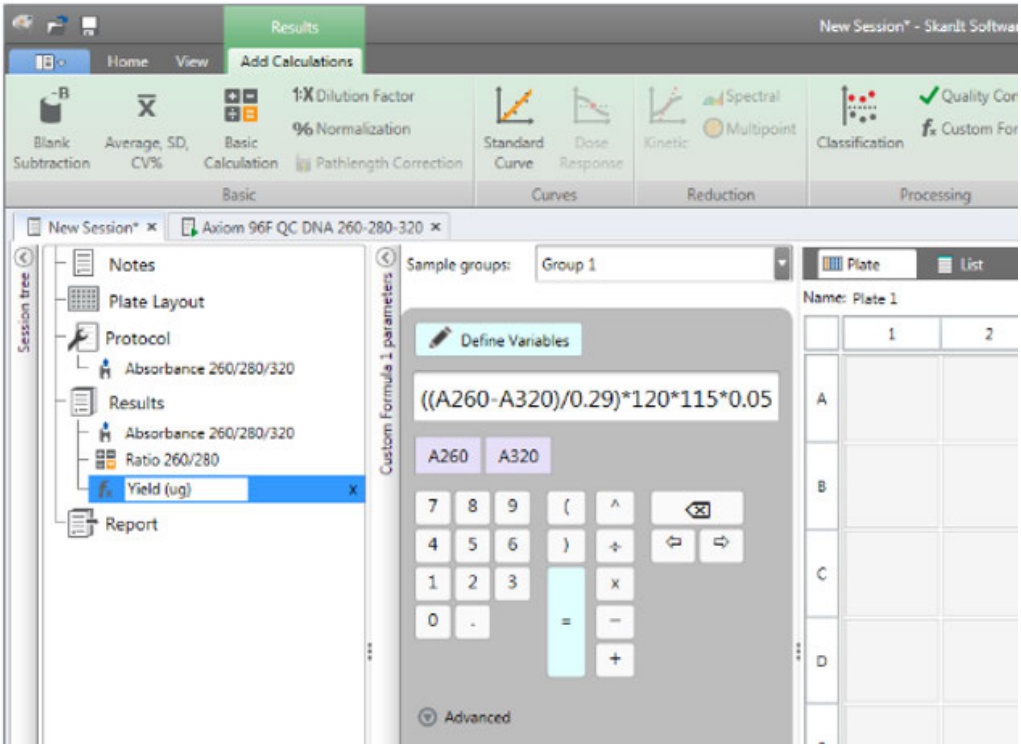


Figure 44 Define Variables window with 2 new variables added

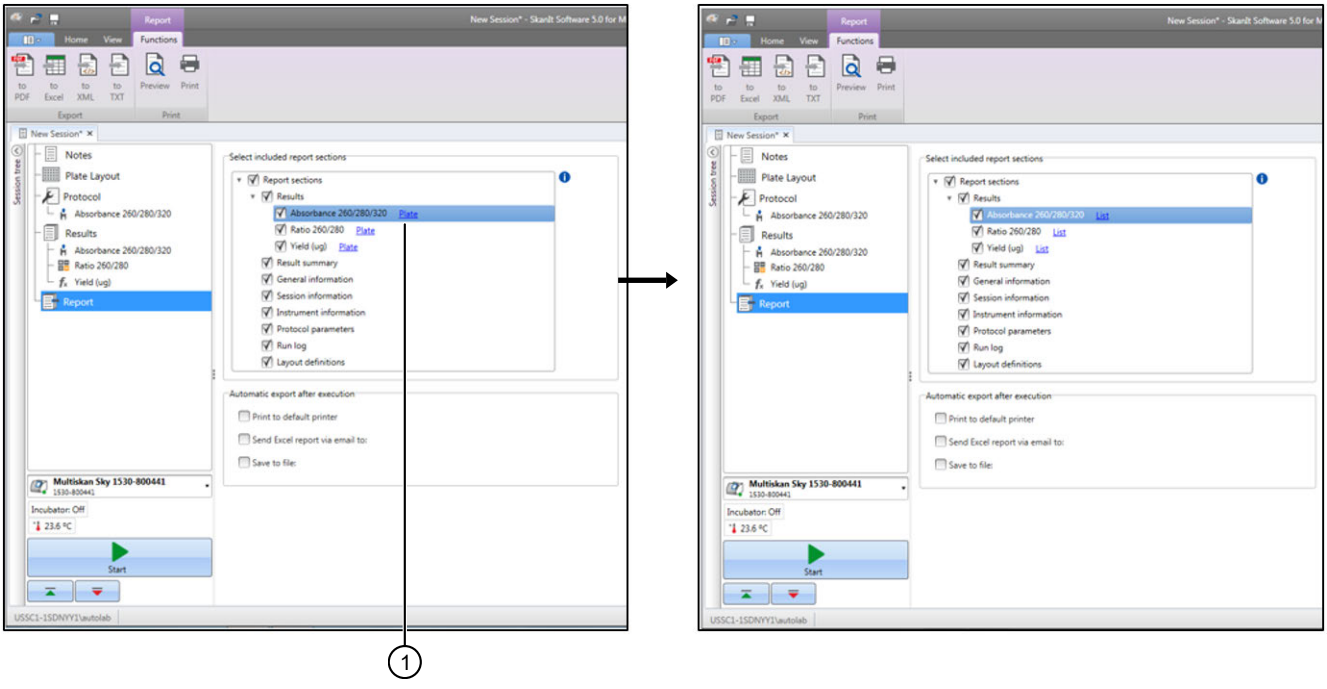
d. In the Custom Formula screen, enter the following DNA yield equation. Use the purple **A260** and **A320** buttons to enter them into the equation.

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

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



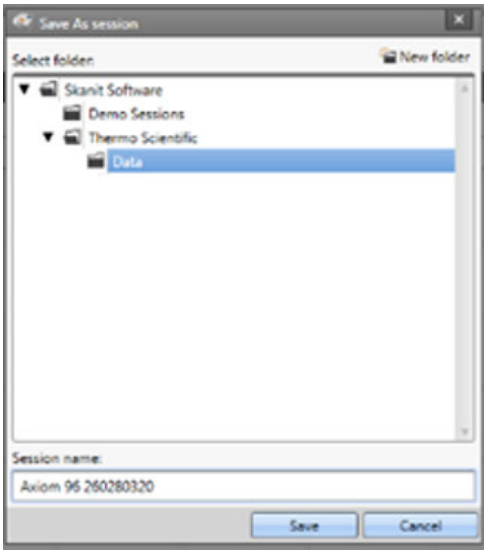
f. Click **Report** in the left pane. Results of the calculation are provided in either Plate or List format. Click **Plate** to change the results to **List** format.



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**.



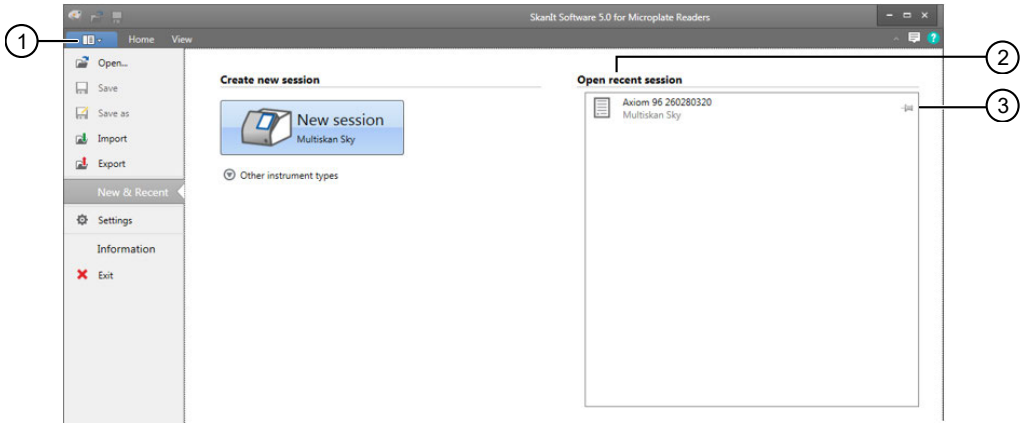
13. Click **Save**.
The session is available to be used to read the Axiom™ 96-well OD QC Plates.

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 on 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™ Propel Fast Wash Workflow, 96-Array Format. If the median yield for the plate is <1,200 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 0.59 and an A_{260}-A_{320} value of approximately 0.53.

Plate reader guidelines for sample quantification

When performing sample quantification, the plate reader must be calibrated to facilitate 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”.

D

Register samples in GeneChip™ Command Console™

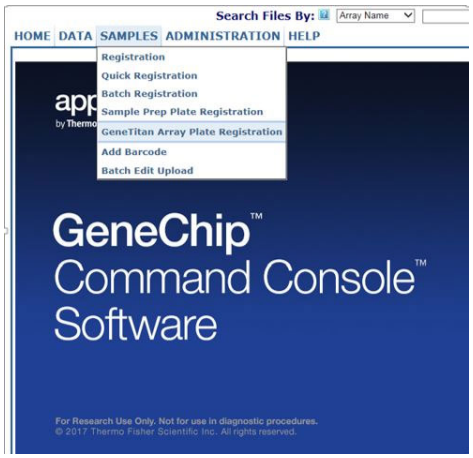
GeneTitan™ Array Plate Registration file

A GeneTitan™ Array Plate Registration file is a Microsoft™ Excel™ spreadsheet that includes information on the samples that you are processing on a single array plate. This information includes the array plate format, the array plate barcode, and the sample file names for tracking the samples that are loaded onto a particular array plate.

Note: The GeneTitan™ Array Plate Registration file uses the *.xls Microsoft™ Excel™ file extension. Do not use the *.xlsx file extension.

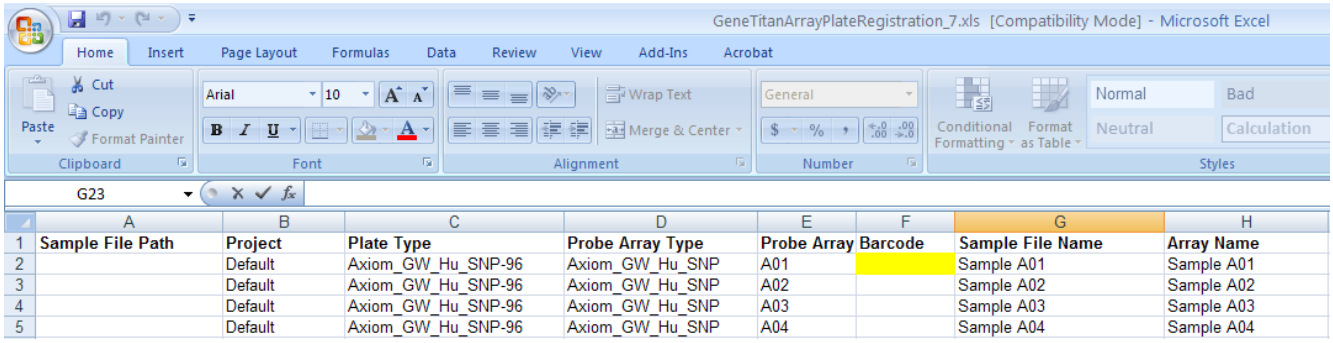
Create a GeneTitan™ Array Plate Registration file

1. In GCC Portal, click **Samples** ▸ **GeneTitan Array Plate Registration**.



2. Create a new template in GCC that includes fields required for sample traceability.
3. Select the array plate to be processed on the GeneTitan™ MC Instrument.
4. Select the newly created template that contains the fields that are required for traceability.
5. Select the **GeneTitan Array Plate Type** from the dropdown list.
6. Select the project where the sample registration data and all associated data files are saved.
7. Click **Download**.

8. Click the Microsoft™ Excel™ icon to open the spreadsheet.



	A	B	C	D	E	F	G	H
1	**Sample File Path**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array**	**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

9. In the **Sample File Name** column, enter a unique name for each sample and any additional information, such as array plate barcode.

Note: The array plate's barcode can be scanned into the **Barcode** field. The barcode is stored in the sample file for each array.

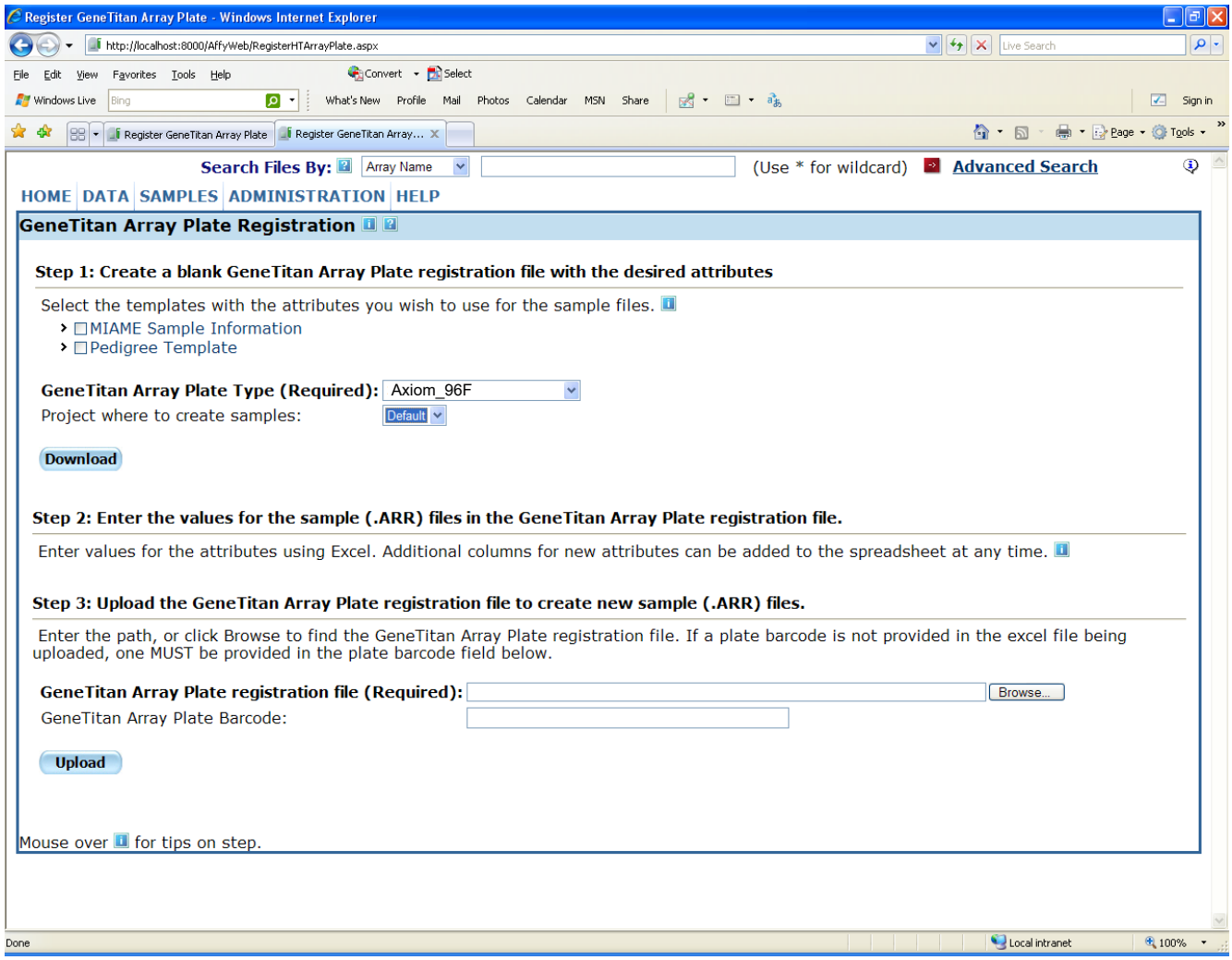
---

10. Complete one of the following:
    - If you are ready to load the array plate onto the GeneTitan™ MC Instrument, scan the array plate barcode into column F, then proceed to step 11.
    - If you are not ready to load the array plate onto the GeneTitan™ MC Instrument, proceed to step 11.

11. Follow these steps to save the file:
    a. Click **File** ▸ **Save As**.
    b. Enter a name for the array plate registration file.
    c. Click **Save**.

12. Follow these steps when you are ready to load the array plate onto the GeneTitan™ MC Instrument.
    a. Click **Browse**, navigate to the GeneTitan™ Array Plate Registration file, then click **Open**.
    b. Scan the array plate barcode, if it has not already been scanned, and save the registration file.

c. Click **Upload**, wait for the information to load, then click **Save** found at the bottom of the next window that is displayed.



E

Troubleshooting

Multidrop™ Combi Reagent Dispenser

Observation	Possible cause	Recommended action
Gravimetric weight check out of range	• Cassette is not primed properly. • Wear of cassette tubing over time.	• Ensure that the cassette is primed properly before performing the gravimetric check. • Verify that each tip of the cassette is dispensing properly. • Adjust the Multidrop™ Combi dispense volume up or down, accordingly. See “Adjust Multidrop™ Combi dispense volume” on page 257.
	Wrong solution used for gravimetric checks. If water is used for the isopropanol cassette check, the values will be different due to density differences.	Use the correct type of solution described in “Range guidelines for gravimetric tests” on page 56.
Dispensed liquid strays and does not go into the wells	Static electricity.	Ensure ion blowers are in place and powered on at the dispense station.
	Tips are clogged.	Perform the priming and backflush to remove the foreign materials inside the tips. (“Prime the cassette” on page 59. “Flush the Multidrop™ Combi after general reagent use” on page 60.)
	Tips are damaged.	Replace the Multidrop™ Combi dispensing cassette.

Adjust Multidrop™ Combi dispense volume

1. Select the **Main** tab on the Multidrop™ Combi screen.
2. Select the protocol name then press **OK**.
3. Using the up/down arrows, select the dispense volume setting, then press **OK**.
4. Use the up/down arrows on the instrument to decrease or increase the dispense volume, then press **OK**.
   - For cassettes with standard tubing, the resolution of the volume adjustment is 5 µL.
5. After adjusting the dispense volume on the Multidrop™ Combi, save the changes to the method.
   a. Press **OK** on the protocol name.
   b. Press the right arrow on the Multidrop to edit the protocol.
   c. Do not change the protocol name. Use the arrows on the Multidrop to select **Save** , then press **OK** to save.
   After pressing **OK**, the display goes back to the main screen.
6. Repeat the gravimetric testing to confirm that the dispense weight is within the specified limits. (See “Range guidelines for gravimetric tests” on page 56.)

Thermo Scientific™ ALPS™ 3000 Automated Microplate Heat Sealer

Observation	Possible cause	Recommended action
Seal is misaligned on the plate	The nut to position the roll of Easy Peel Seal is loose.	See “Align the ALPS™ 3000 seal roll” on page 258.
Bubble is seen on the film after sealing	Liquid spray is on the grids of the 96-deepwell plate, or the 96-deepwell plate is not flat.	Peel the seal off off of the plate, then inspect for liquid on the grid. If liquid is present, lay a clean laboratory tissue over the grid, then dab dry. Reseal the plate. If after several attempts bubbles persist, manually transfer the contents of the wells to a new 96-deepwell plate with a multichannel pipette, then continue the process. Use clean pipette tips for each transfer.
Seal is too light (light imprint or uneven imprint) or too heavy (seal material sticks to the webs of the plate during peel-off)	Temperature fluctuation of the heating element.	Increase or decrease the sealing temperature by 1°C, then check the qualitative seal strength. If proper sealing cannot be achieved within 145°C to 155°C for 2.5 seconds, contact customer support.

ALPS™ 3000 Automated Microplate Heat Sealer

Thermo Fisher Scientific recommends the ALPS™ 3000 Automated Microplate Heat Sealer for use with ultrahigh-throughput workflows, such as the Axiom™ Propel Fast Wash Workflow, 96-Array Format.

Align the ALPS™ 3000 seal roll

The roll of plate sealing material can drift out of alignment, resulting in a seal that doesn't cover the top or bottom of the plate.

A nut inside the spindle axle (2) holds the adjusting hub (1) in place, as shown in the left figure below. When the nut is loosened, the adjusting hub can be moved, as shown in the following figure on the right side.

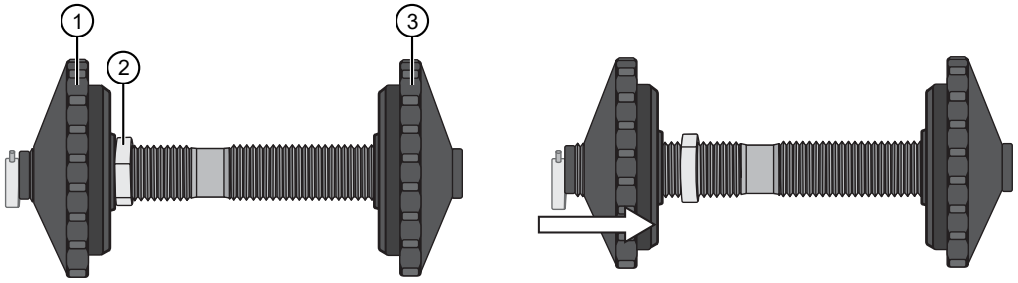


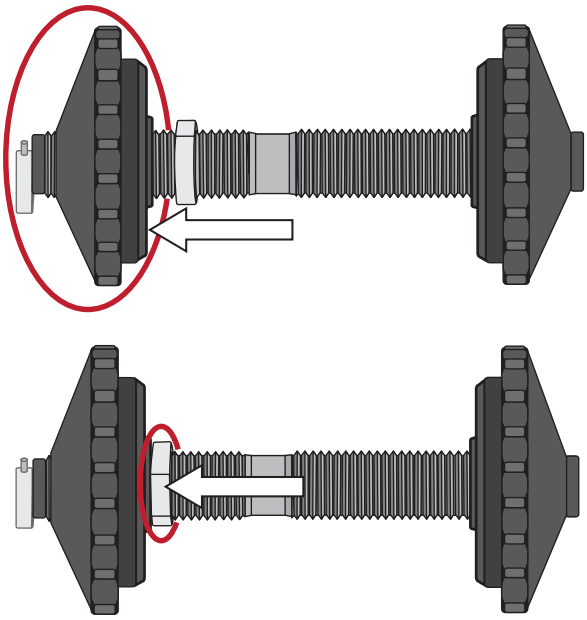
Figure 45 The spindle assembly, located in the back of the plate sealer.

① Adjusting hub
② Nut
③ Fixed hub

Align the seal roll—Seal does not cover bottom of the plate

If the seal does not cover the bottom of the plate (with the plate oriented so that A1 is at the back right corner), follow the steps below.

1. Remove the spindle from the back of the plate sealer.
2. Rotate the adjusting hub so that it moves to the left on the spindle axle. The nut might need to be loosened before the adjusting hub will rotate.
3. Using a wrench, lock the adjusting hub in place by tightening the nut against it.



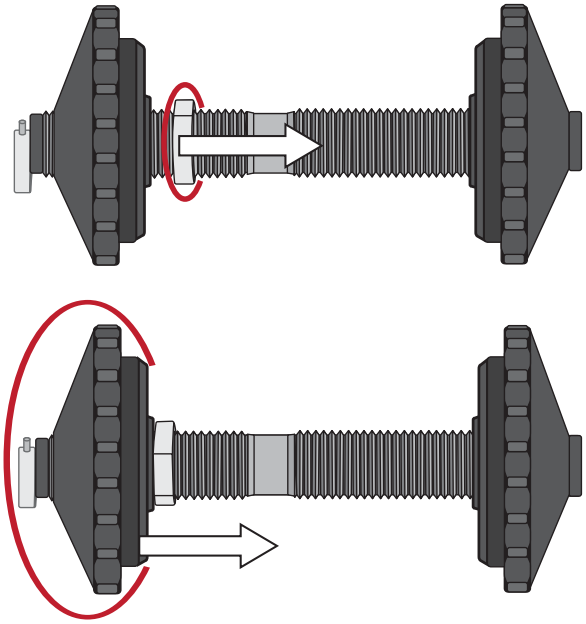
IMPORTANT! Move the adjusting hub in small increments. Test the seal after each adjustment.

Align the seal roll—Seal does not cover top of the plate

If the seal does not cover the top of the plate (with the plate oriented so that A1 is at the back right corner), follow the steps below.

1. Remove the spindle from the back of the plate sealer.
2. Rotate the adjusting hub so that it moves to the right on the spindle axle.
3. Move the locking nut to the right if necessary, as shown in left figure, before moving the adjusting hub.

4. Using a wrench, lock the adjusting hub in place by tightening the nut against it.



IMPORTANT! Move the adjusting hub in small increments. Test the seal after each adjustment.

Clean the ALPS™ 3000 Automated Microplate Heat Sealer

Periodically, clean the 2 linear slides and the 2 slide rests with 70% ethanol.

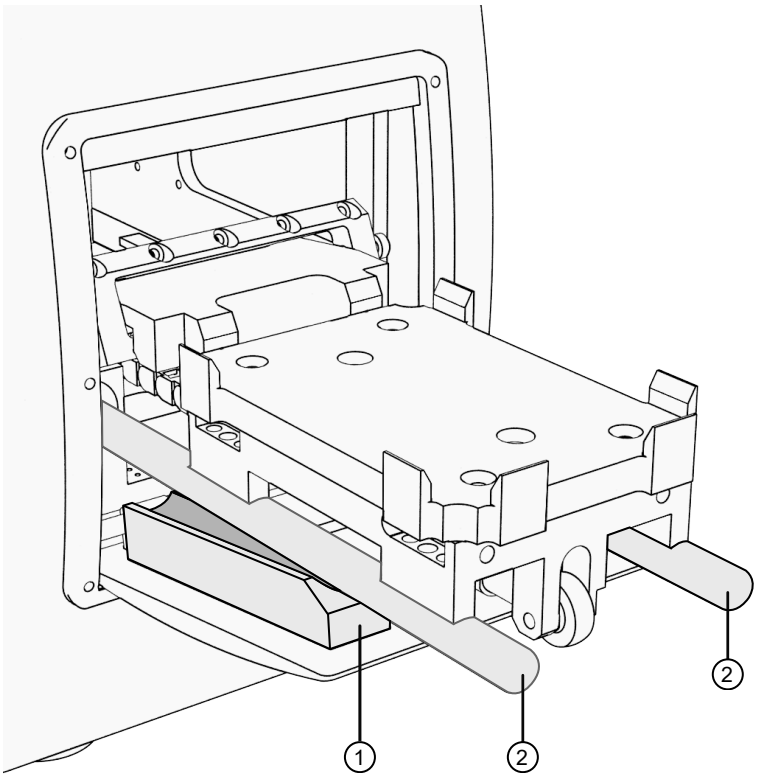


Figure 46 The ALPS™ 3000 slides and slide rests.

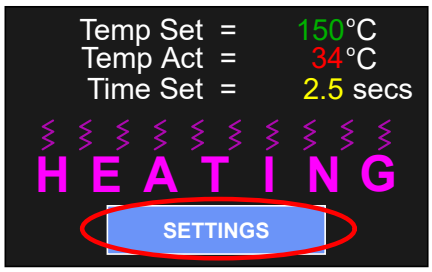
① Slide rest (left, right not shown)
② Linear slide (left and right)

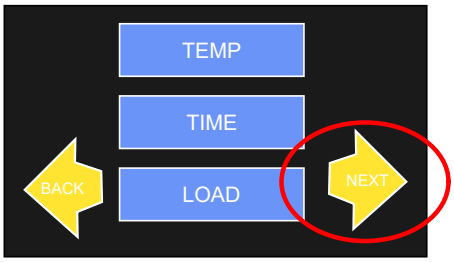
Park the ALPS™ 3000 Automated Microplate Heat Sealer

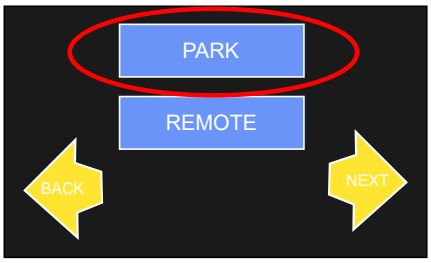
If the sealer will not be used for an extended period of time, put the sealer in the "Park" position to prevent the exposed parts from collecting dust and particles.

Before parking the instrument, ensure that there is not a plate in the plate shuttle.

1. Press **Settings** ▸ **Next** ▸ **Park**.







2. Remove the roll of sealing material, then press **NEXT**.

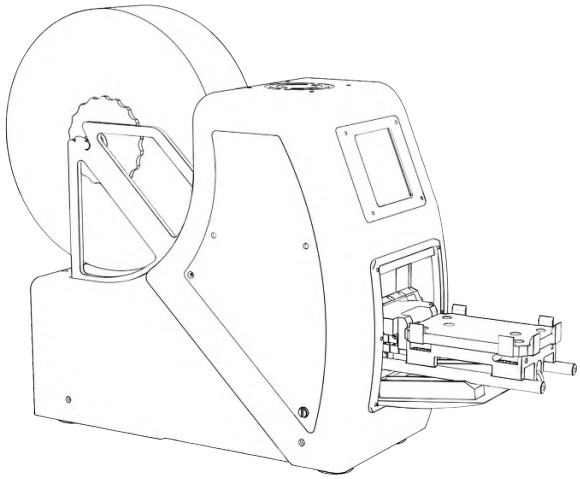
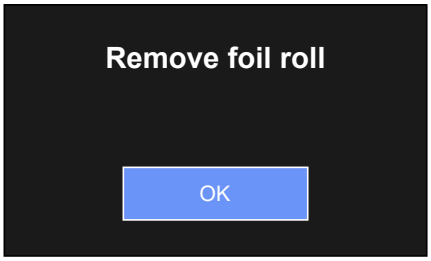


Figure 47 A roll of sealing material on the instrument.

3. After removing the roll, click **OK**.



After pressing **OK**, the plate seal nest retracts into the ALPS 3000, then the door closes.

GeneTitan™ MC Instrument support files for troubleshooting

Log files

The different GeneChip™ Command Console™ (GCC) components generate log files. The logs provide a record of the tasks performed by the different components, such as the migration tools and installer. These log files provide useful information for troubleshooting problems. Sometimes these files are required by your field application scientist (FAS), field service engineer (FSE), or the Thermo Fisher Scientific call center to help with troubleshooting.

GeneChip™ Command Console™ log files

The following files are generated by the GeneTitan™ MC Instrument. All the GCC log files are from the following path: `C:\Command_Console\Logs`.

Log file type	Description
`Systemlog.xml`	XML file with system information.
`DEC.log`	Text file with information on the use of the Data Exchange Console (DEC).
`DECError.log`	Text file with information on errors created while using DEC.

Other GeneChip™ Command Console™ files

The following GCC files and requests are sometimes used by FAS or FSE for troubleshooting.

- Library files (*.PARAMS, *.MASTER, *.WORKFLOW, *.SMD, *.MEDIA) in `C:\Command_Console\Library`, excluding the large analysis library files (CDF, PSI, GRC).
- Provide a list of all sub folders and their contents under the library files folder that is in `C:\Command_Console\Library`. Ensure that there are no duplicate library files, as these files can cause problems
- GCC system configuration file that is found at `C:\Command_Console\Configuration\Calvin.System.config`.
- Pending job order files that are in `C:\Command_Console\Jobs`
- Other GCC related information, such as
  - The number of files under `C:\Command_Console\Data`, including sub directory.
  - If the system is a networked system or a stand-alone system.
  - Other applications that are installed on the system, such as antivirus application, Microsoft™ Office™ , and Internet Explorer® versions.

GCC log files for GeneTitan™ MC Instrument systems

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

GeneTitan™ MC Instrument fluidics

- `C:\Command_Console\Logs\96F\`
  - Subdirectories are named by date (for example, Log7-29-2016)

    Collect all dated directories and contents from the time the GeneTitan™ application was started, not just from the date of the event. Some logging goes into files from the date the application started so these files can be critical for troubleshooting.

    All the log directories from the date the run was started to the date of the event are essential.
- `C:\Command_Console\Logs\96F\FluidicErrorLog` - all files in this directory.

GeneTitan™ MC Instrument imaging device

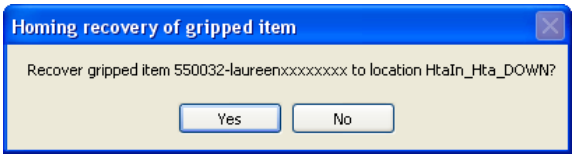
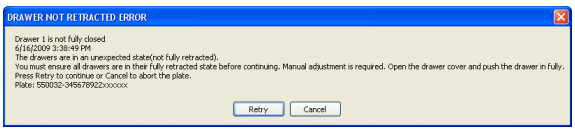
- `C:\Affymetrix\GeneChipHTScanControlMC\Log` - collect all dated directories and contents from the time the GeneTitan™ application was started.
- `C:\Affymetrix\GeneChipHTScanControlMC\RunLog` - collect all dated directories and contents from the time the GeneTitan™ application was started.

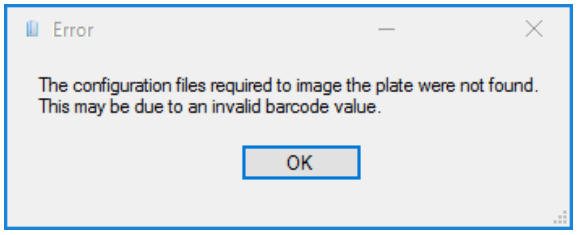
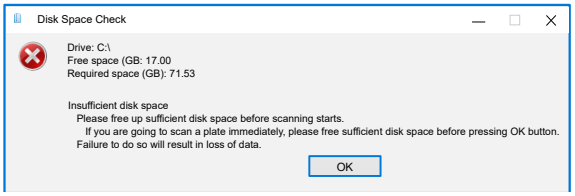
Troubleshooting the GeneTitan™ MC Instrument

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

For software errors that do not involve hardware crashes, the most common solution is to close or exit the application, then restart it. If the same error occurs, close the application and power off the computer, then restart. If the error still occurs, power off the GeneTitan™ MC Instrument, then restart.

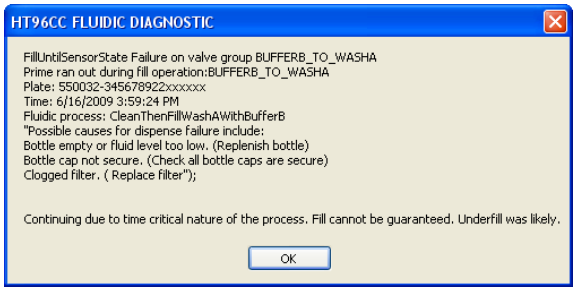
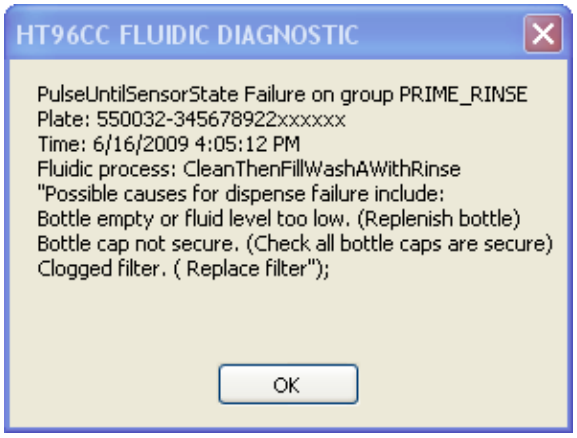
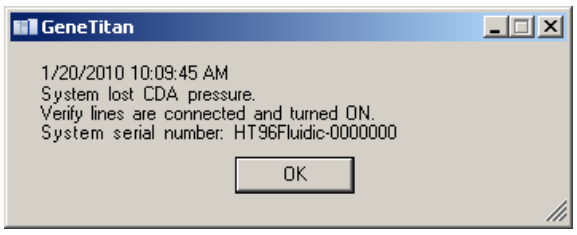
Observation	Possible cause	Recommended action
Plate trapped in the GeneTitan™ MC Instrument	• Plate (or plate with lid) not properly loaded in drawer. • Notched edge of lid and plate not aligned. • Gripper failed to retrieve plate. • System requires adjustment.	1. Restart the GeneTitan™ MC Instrument by unplugging and reconnecting power cord. 2. Run the **Unload Plates** setup option. 3. If the plate remains trapped in the instrument, call Thermo Fisher Scientific support.
Computer frozen	• Too many processes running. • Attempting to transfer data while an array plate is being scanned (imaged).	Restart the computer and unload all of the plates. • Plates in the hybridization station: finish hybridization off line. • Plate in the scanner: rescan using **Scan Only** function. • Plate in fluidics: use **Wash/Scan Resume** to resume the fluidics process. **IMPORTANT!** Do not manually, or through the GCC transfer utility, move any data associated with the current plate that is being processed/scanned.
Fluidics aborted	• System-initiated abort: power loss. • User-initiated abort: incorrect protocol selected.	Follow the recommendations and instructions under "Wash-Scan Resume" on page 228.

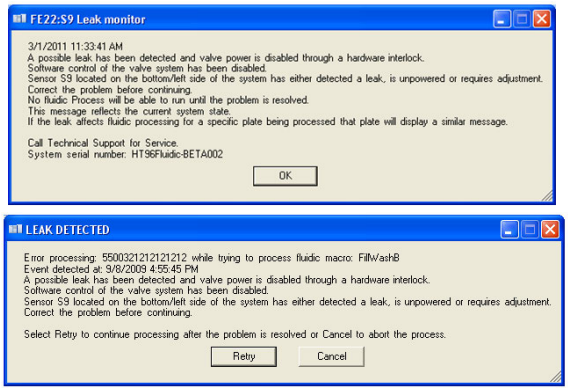
Observation	Possible cause	Recommended action
Homing recovery of gripped item 	Indicates that an item is in the gripper, and normal startup of the GeneTitan™ MC Instrument is not possible. The item must be removed from the instrument before you can start processing array plates.	Recommendation: click **Yes**. If you click **No**, nothing occurs. Homing will not complete and you will not be able to use the system. The item that is held by the gripper is moved to either: • Drawer 2—plates and trays • Trash Bin—covers The drawer names reflect the location (left or right) and the drawer number (1 through 6). Examples: Drawer2L_Hta_DOWN = Scan tray on left side of drawer 2 HtaHyb = Clamped 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 that the item held. An example is drawer1R_HtaHyb_DOWN indicating it is an array plate with a hybridization tray or Drawer2L_ScanHta_Pk_DOWN indicating it is an array plate with a scan tray
Drawer not retracted error 	The drawer that is listed in the message is not fully closed.	Manually push the drawer back into the instrument until it is fully closed. There are 2 stop positions with audible clicks. Push until you hear the second click and the drawer is fully seated.

Observation	Possible cause	Recommended action
Array registration error message 	The protocol file for the array plate barcode could not be found.	Check that the array plate barcode has been entered correctly. Ensure that the library files required for the type of array plate you are using have been installed, and are installed in the correct directory. Restart the GeneTitan™ Instrument control software after library files have been installed.
Insufficient disk space notice  This message appears when you first initialize the software and instrument, or when you select arrays for imaging.	There is not sufficient memory on the computer hard drive to save the data from an array plate.	Free up sufficient disk space before starting imaging with the GeneTitan™ MC Instrument.

GeneTitan™ MC Instrument fluidic diagnostic messages

Observation	Possible cause	Recommended action
Failed prime  If this message is displayed during a water wash step, array processing has been compromised. If this message is displayed during cleanup, array processing is okay, but cleanup will not be complete.	The fluid level is either too low or the bottle is empty.	Always ensure that the GeneTitan™ bottles containing Axiom™ Wash Buffer A and Axiom™ Water are above the 50% mark when setting up the system to process an array plate. We recommend that all 600 mL of the Axiom™ Wash Buffer B from the Axiom™ Propel Fast Reagent Kit be emptied into the GeneTitan™ Wash B bottle when setting up the system to process a plate. Using all 600 mL of the Axiom™ Wash Buffer B helps ensure that the bottle is filled to more than the requisite 35% of Wash B bottle volume.

Observation	Possible cause	Recommended action
Fluidics diagnostic dispense error  BUFFER*x* = Buffer bottle A, B, or Rinse. WASH*x* = Wash A or B reservoir in the fluidics station.  	Reagent bottle is empty or too low.	Replenish fluid level in the Rinse or Wash Bottle B to the 1-L mark. Do not overfill. **IMPORTANT!** Only replenish bottles when prompted by the UI. Replenishing during fluidic processing can cause system malfunction including overflowing inside the system and more problems. The only thing to do when a plate is running is to ensure that bottle caps are secure.
		Replenish fluid level in Wash Bottle A to 2 L.
	GeneTitan™ reagent bottle cap is loose.	Fasten the bottle cap.
	The GeneTitan™ reagent bottle filter is clogged.	Replace the filter. See “Bottle filter replacement” on page 273.
Loss in CDA pressure  	The instrument experienced a loss in Clean Dry Air (CDA) pressure.	Ensure that all lines are connected and turned on.
		Ensure that the facility CDA or the portable CDA compressor is in working condition. See the *GeneTitan™ Multi-Channel Instrument Site Preparation Guide* for the portable compressor model that has been verified with the GeneTitan™ MC Instrument.
		Contact your local field application specialist and notify the engineer about the error message.

Observation	Possible cause	Recommended action
Leak detected Leak checks are performed at application startup and any time a fluidic process, such as priming, filling, draining, is performed. Leak detection is a hard-wired sensor that shuts off fluid flow without software control. Leaks are normally confined to the drip pan found inside the system. 	• System malfunction. • The GCC application being manually closed using Windows™ Task Manager during a fill operation resulting in an application exit without stopping flow.	Contact Thermo Fisher Scientific support. The system cannot be used for fluidic processing until the problem is resolved.
Filter change required error message The software displays warning messages for the filter in each reagent bottle when it detects a problem or shows a trend of increased fill times during fluid fill operations. When an error is detected, a message box is displayed along with the information on the specific operation (dispense-related check or fill-related check).	One or more reagent bottle filters are clogged or worn out.	Change all 3 reagent bottle filters, even if only 1 is reported as problematic. See “Bottle filter replacement” on page 273.

F

GeneTitan™ Multi-Channel Instrument care

Overview

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

- The GeneTitan™ Multi-Channel (MC) Instrument must be positioned on a sturdy level bench away from extremes in temperature and away from moving air.
- Always run a Shutdown protocol when the instrument is off or unused overnight or longer to prevent salt crystals from forming in the fluidics system.
- Always use deionized (DI) water to prevent contamination of the lines. Swap out old buffers with freshly prepared buffer at each system startup.

IMPORTANT! Before performing maintenance power off the instrument to avoid injury if an electrical malfunction occurs.

Maintenance

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

The following tasks must be performed regularly to help ensure that the imaging device remains in working order.

Monthly

Wipe down the outer surface of the imaging device with a dry cloth.

Every 6 months

1. Replace the cooling fan air filters at the rear of the instrument.
2. Replace the Micropore™ filters in the Wash A, Wash B, and Rinse bottles. If you run 4-8 plates/week, then replace the Micropore™ filters more frequently.

Outer enclosure fan filters

Cleaning schedule

The GeneTitan™ fan filter cartridge must be cleaned at least every 90 days of service. Note that in some service locations, the presence of excessive dust or particulate matter can require cleaning the cartridge more often than 90 days.

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

Figure 48 GeneTitan™ fan filter cartridge.

Clean the GeneTitan™ MC Instrument fan filter

Contact your field service engineer for GeneTitan™ fan filter ordering information when new filters are required.

Number of filters that are required per GeneTitan™ MC Instrument: 3

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

Bottle filter replacement

The bottles that are used in GeneTitan™ MC Instrument contain a filter to remove particulates that can exist in the buffers and DI water. The filters in the 3 GeneTitan™ fluidics bottles (Wash A, Wash B, and Rinse) must be replaced when the filters are clogged.

When the instrument detects an increase in the amount of time that is required to perform the fill operations, a **Filter Change Required** message window opens. The message window provides information on fluid dispense errors that were detected for any of the bottles during a dispense operation. All 3 filters must be changed when a warning is displayed for any of the 3 filters.

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

After changing the filters in all 3 bottles using the procedure that is described in this section, press the **Yes** button to continue. If you select to ignore the error message, press the **No** button. This warning message is displayed each time GeneChip™ Command Console™ instrument control software is launched. You can also experience data quality problems when particulate matter is not trapped by the filters because they are clogged.

We recommend having 3 spare filters on hand in the event the filters must be replaced.

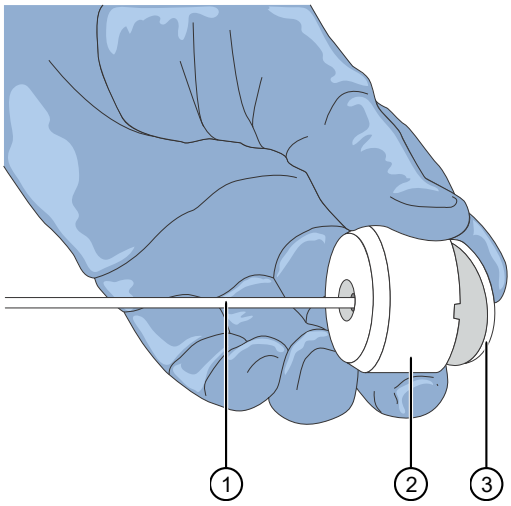


Figure 49 Components of the reagent buffer supply line and filter.

① Buffer supply line
② Filter holder
③ Filter

Remove and inspect the reagent bottle filters

1. Loosen, then remove the cap on the bottle.
2. Carefully remove the filter from the end of the filter body (see Figure 49).

3. Visually inspect the filter. If one of the filters appears to have a concentration of dirt or contaminate in it, discard it. Replace the filter in all 3 reagent bottles with a new one.
4. Replace the cap on the reagent bottle when finished.

Replace fluidics bottle filter

GeneTitan™ Fluidics Bottle Filter part details:

Thermo Fisher Scientific Cat. No. 01-0671

1. Loosen the reagent bottle cap, then remove the draw tube.
2. Carefully remove the filter from the end of the filter body.
3. Insert a new filter into the end of the filter holder.
4. Replace the cap on the reagent bottle, then tighten it.
5. Repeat these steps for each bottle.

IMPORTANT! Replace 1 filter at a time to ensure the correct connection of the buffer supply tube to its respective bottle. The color of the buffer supply tubing matches the bottle color code.

Xenon lamp replacement in the GeneTitan™ MC Instrument

This section applies to the GeneTitan™ MC Instrument.

After the normal life expectancy of the lamp has expired, the software application alerts you to the requirement to replace the lamp. The lamp replacement procedure is simple but good health and safety precautions must be followed.

CAUTION! Do not try to replace the lamp when a plate is being processed either in the fluidics or scanner system.

Lamp life/imaging device status notices

The **Imaging Device Status** pane displays lamp life and imaging device status notices for the GeneTitan™ MC Instrument.

In normal operation, the pane displays the hours of life that is left in the lamp.

Imaging Device Status	
Barcode	
Estimated Time Remaining	
Lamp Life Remaining	163 hours

A red or yellow notice is displayed when the lamp life is getting short.

Imaging Device Status	
Barcode	
Estimated Time Remaining	
Lamp Life Remaining	-1 hours -- Replace lamp as soon as possible

A red notice is also displayed when the imaging device is offline.

Imaging Device Status	
Barcode	
Estimated Time Remaining	
Scanner Status	Offline: scanning is not available.

Note: The 300-watt xenon lamp in the GeneTitan™ MC Instrument is warranted for 500 hours. The instructions to remove and replace the lamp are found in “Remove the xenon lamp” on page 276, and “Replace the xenon lamp” on page 277. After changing the lamp, you must manually reset the lamp life clock.

Remove the xenon lamp

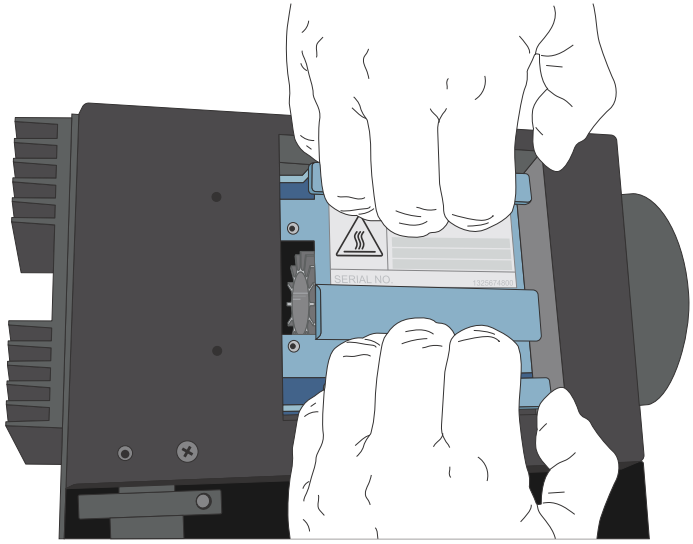
WARNING! Power off the lamp using the switch in the rear of the unit and then disconnect the power cord. Allow to cool before attempting to replace the lamp.

1. Unscrew the 4 retaining bolts with your fingers.

① Remove these 4 bolts.

2. Remove, then set aside the warning cover to reveal the xenon lamp that is contained inside.

3. Place a hand on each side of the blue plastic flange, then lift out the lamp in a vertical motion. Both hands must be used to remove the lamp. Apply equal pressure on each side of the lamp and gently lift.

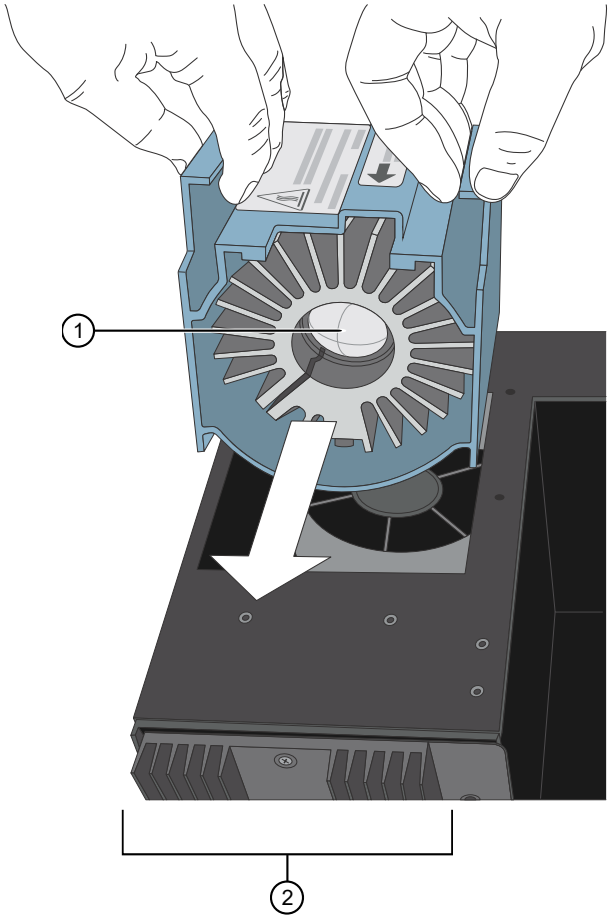


Replace the xenon lamp

A new Cermax™ Xenon Arc Lamp (Cat. No. 01-0740) is required for this procedure.

IMPORTANT! Ensure that you install the lamp in the correct orientation.

1. Hold the lamp by the blue plastic flanges. Ensure that the lamp bulb faces inward toward the rear heat sink on the unit, and then vertically lower the lamp to install.



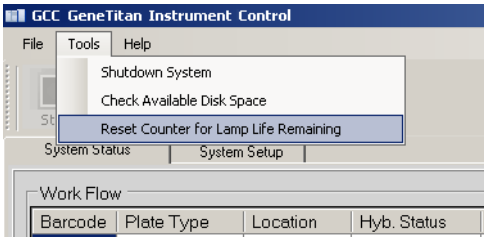
① Xenon bulb faces away from the fan and towards the heat sink.
② Heat sink on the Lambda LS unit.

2. Replace the warning cover, then hand tighten the bolts.

Reset the lamp life counter

Using the **GCC GeneTitan Instrument Control** module accessed from the **Launcher** window, you must alert the software that the lamp has been replaced so that the hours of the lamp counter are reset. This menu option is only available when the system is not processing any plates.

1. Select **Tools** ▸ **Reset Counter for Lamp Life Remaining**.



2. Click **Yes** in the message window to reset the counter.

G

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit thermofisher.com/support.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Risk of electrical shock.

(continued)

Symbol and description
CAUTION! Hot surface.
CAUTION! Potential biohazard.
CAUTION! Ultraviolet light.

Symbole et description
MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
MISE EN GARDE ! Risque de choc électrique.
MISE EN GARDE ! Surface chaude.
MISE EN GARDE ! Danger biologique potentiel.
MISE EN GARDE ! Rayonnement ultraviolet.

Additional safety symbols

Symbol and description
CAUTION! Moving parts.
CAUTION! Moving parts.
CAUTION! Moving parts.
CAUTION! Moving parts.

(continued)

Symbol and description
CAUTION! Moving parts.
CAUTION! Piercing hazard.
CAUTION! Sharp edges.
CAUTION! Potential slipping hazard.
CAUTION! Potential overhead hazard.
CAUTION!

Symbole et description
MISE EN GARDE ! Parties mobiles.
MISE EN GARDE ! Parties mobiles.
MISE EN GARDE ! Parties mobiles.
MISE EN GARDE ! Parties mobiles.
MISE EN GARDE ! Parties mobiles.
MISE EN GARDE ! Danger de perforation.
MISE EN GARDE ! Bords coupants.
MISE EN GARDE ! Danger de glisser potentiel.

(suite)

Symbole et description
MISE EN GARDE ! Danger en surplomb potentiel.
MISE EN GARDE !
 electrostatic

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Earth (ground) terminal
	Protective conductor terminal (main ground)
	Direct current
	Alternating current
	Both direct and alternating current

Conformity symbols

Conformity mark	Description
     	INDICATES CONFORMITY WITH SAFETY REQUIREMENTS FOR CANANDA AND U.S.A.
  	INDICATES CONFORMITY WITH CHINA RoHS REQUIREMENTS.
	INDICATES CONFORMITY WITH AUSTRALIAN STANDARDS FOR ELECTRICAL SAFETY AND ELECTROMAGNETIC COMPATIBILITY.
 	INDICATES CONFORMITY WITH SAFETY REQUIREMENTS FOR JAPAN.
	INDICATES CONFORMITY WITH THE WEEE DIRECTIVE 2012/19/EU. **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Chemical safety

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

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

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.

- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! **HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! **4L Reagent and Waste Bottle Safety.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication number	Description
Applied Biosystems™ *Axiom™ Propel Fast Wash Workflow, 96-Array Format User Guide*	MAN0019450	Provides guidance on reagents, instruments, and supplies needed to run the Axiom™ Propel Workflow, 96-Array Format with the Thermo Scientific™ Multidrop™ Combi Reagent Dispenser.
Applied Biosystems™ *Axiom™ 2.0 gDNA Sample Preparation Quick Reference*	MAN0017720	An abbreviated reference on preparing the genomic DNA sample.
Applied Biosystems™ *GeneTitan™ MC Protocol for Axiom™ Array Plate Processing Quick Reference*	MAN0017718	An abbreviated reference for processing Axiom™ Array Plates with the GeneTitan™ Multi-Channel Instrument.
Thermo Scientific™ *Multidrop™ Combi User Manual*	N05616	This document detailing the safety information, setup, use, maintenance, and troubleshooting for the Multidrop™ Combi Reagent Dispenser.
Applied Biosystems™ *GeneTitan™ Multi-Channel Instrument User Guide*	MAN0027694	The GeneTitan™ Multi-Channel (MC) Instrument automates array processing from target hybridization to data generation by combining a hybridization oven, fluidics processing, and state-of-the-art imaging device into a single benchtop instrument. This document detailing the use, care, and maintenance for the GeneTitan™ MC.
Applied Biosystems™ *GeneTitan™ Multi-Channel Instrument Site Preparation Guide*	MAN0025571	Provides guidance on creating and maintaining the proper environment needed for the GeneTitan™ MC Instrument.
Thermo Scientific™ *ALPS™ 3000 Automated Laboratory Plate Sealer User Manual*	EXT0002597	Instructions about the setup and use of the ALPS™ 3000 Automated Microplate Heat Sealer.

(continued)

Document	Publication number	Description
Recommended Alternative Microarray Consumables Quick Reference	MAN0019853	A quick reference document identifying recommended alternative replacement consumables for use in microarray assays.
Software and analysis		
Applied Biosystems™ *GeneChip™ Command Console™ User Guide*	MAN0027771	This user guide provides instructions about using Applied Biosystems™ GeneChip™ Command Console™ software (GCC) used to control GeneChip™ instrument systems. GeneChip™ Command Console™ software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip™ arrays.
Applied Biosystems™ *Axiom™ Analysis Suite User Guide*	MAN0027928	Axiom™ Analysis Suite advances genotyping data analysis with a single-source software package to enable complete genotyping analysis of all Axiom™ arrays. This document provides instructions about using the software to automate the Best Practices Workflow to facilitate accurate results in a single step for export in PLINK, VCF, or TXT formats.
Applied Biosystems™ *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™ Analysis Power Tools and SNPolisher R package to perform quality control analysis (QC) for samples and plates, SNP filtering before downstream analysis, and advanced genotyping methods.

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