Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow

USER GUIDE

for use with:
Axiom™ Precision Medicine Diversity Research Array Plate
Axiom™ 2.0 Plus Reagent Kit 96F
Applied Biosystems™ NIMBUS® Target Preparation Instrument

Catalog Numbers 951958 and 951960
Publication Number MAN0018331
Revision F00

ThermoFisher SCIENTIFIC

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Products manufactured at this site:

- Axiom™ Precision Medicine Diversity Research Array Plate

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Products manufactured at this site:

- Axiom™ 2.0 Plus Reagent Kit 96F

Revision history: MAN0018331 F00 (English)

Revision	Date	Description
F00	24 April 2024	• Added information regarding the two 96-array plate configurations. • Replaced the Mother E-Base™ Device and Daughter E-Base™ Device with the E-Gel™ Power Snap Plus Electrophoresis Device and iBright™ CL750 Imaging System.
E.0	28 September 2023	• Updated product information. • Updated *Related Documentation*.
D.0	10 April 2023	Added information regarding the optional use of the Fisher Scientific™, Nunc™ 96-Well Polypropylene DeepWell™ Storage Plate and adapter, as well as the associated new kits: • Axiom™ NIMBUS® Starter Pack-NC, 952493. • Axiom™ Starter Kit for Applied Biosystems™ NIMBUS® Instrument–NC Combo Kit, 952496. • NIMBUS® Upgrade Kit for NC, 952495.
C.0	28 April 2021	• Added options for using alternative 96-well round deepwell plates and 96-well PCR plates. • Added Axiom™ NIMBUS® 2.0 Consumables Kit v2. • Changed method name to Axiom™ 96 Sample Automated Target Preparation Solution. • Added optional Thermo Scientific™ Digital Microplate Shaker, set at 900 rpm for 10 minutes.

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

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1 Overview

Introduction

The Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow is a workflow for high throughput microarray genotyping. The workflow includes:

- Automated DNA target preparation on the Applied Biosystems™ NIMBUS® Target Preparation Instrument: DNA amplification, fragmentation, precipitation, and resuspension of the target in hybridization cocktail.
- Transfer of hybridization-ready target DNA to the Applied Biosystems™ GeneTitan™ Multi-Channel (MC) Instrument followed by automated, hands-free hybridization, staining, washing, and scanning.
- Processing of CEL files that are generated by the GeneTitan™ MC Instrument, using the Axiom™ Genotyping Algorithm version 1 (Axiom GT1), available through Applied Biosystems™ Analysis Power Tools 2.10.2 or later, or Axiom™ Analysis Suite 4.0.1 or later.

The Axiom™ 2.0 Plus Reagent Kit 96F provides all necessary reagents for target preparation in volumes that are optimized for processing on the NIMBUS® Target Preparation Instrument.

About the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow for PMD Array

The Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 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 for high-throughput genotyping and is used in large-scale national precision medicine studies worldwide. The Axiom™ Precision Medicine Diversity Research Array (PMD Array) is a genotyping resource that is designed to drive deeper scientific insights and offers high coverage and accuracy across all ancestral populations. It offers ~900,000 markers including ~4,600 pharmacogenomics research variants in ~1,200 genes that include core functional pharmacogenomic content including markers from Clinical Pharmacogenomics Implementation Consortium (CPIC) guidelines, Pharmacogenomics Knowledge Base (PharmGKB), markers in Very Important Pharmacogenes (VIP), PharmGKB markers, and PharmaADME core markers. The ADME category includes alleles with known relevance to drug metabolism.

The Axiom™ 2.0 Plus Assay is a multiplex genotyping assay which combines the proven Axiom™ chemistry with the incorporation of a multiplex PCR step to overcome some complexities that are associated with genotyping in regions of high sequence homology for highly predictive markers in genes including: CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2C8, SULT1A1 and CYP2D6. The Axiom™ 2.0 Plus Assay is not for use with PharmacoScan™ Array Plates. It uses Axiom™ 2.0 catalog and custom designs that are identified for use with Axiom™ 2.0 Plus Assay.

The automated workflow that is described in this user guide uses the Axiom™ 96 Sample Automated Target Preparation Solution method on the Applied Biosystems™ NIMBUS® Target Preparation Instrument and for GeneTitan™ reagent preparation. The multiplex PCR (mPCR) and mPCR spike-in steps are not part of the automated method and must be executed off-deck, manually.

Assay highlights

This user guide introduces an option for a 3-hour DNA target precipitation step to enable a faster assay turnaround time, going from sample to CEL file generation within 72 hours. The 3-hour precipitation step shortens “Stage 2: Fragment and precipitate the DNA” to enable the operator to advance to “Stage 3: Centrifuge and dry pellets”, followed by “Stage 4A and 4B: Resuspension and hybridization preparation”, “Stage 4C: Perform sample QC”, and the start of “Stage 5: Prepare the hybridization tray” on page 105 to start hybridization on the GeneTitan™ MC Instrument on day 2 of the assay workflow. Note that this workflow option requires approximately 9–10 hours to complete the combined day 2 activities (fragmentation to initiation of hybridization on the GeneTitan™ MC Instrument).

In addition, a recommended workflow is presented to support the processing of 3 plates per week using the shortened DNA precipitation step. See Chapter 8, “3-plate workflow for Axiom™ PMD Array Plates using a 3-hour DNA precipitation step” for details regarding equipment and operator assumptions to support this workflow.

The standard Axiom™ 2.0 Plus Assay workflow, in which the DNA is precipitated overnight, provides a convenient stopping point to support single operator assay execution of 1 plate within an 8-hour workday. Recommended workflows are presented to support the processing of 3 plates per week using 3-hour DNA precipitation, and 8 plates per week using the standard overnight DNA precipitation. See the appropriate chapter for details regarding equipment and operator assumptions to support

these workflows. (Chapter 8, “3-plate workflow for Axiom™ PMD Array Plates using a 3-hour DNA precipitation step”, and Chapter 7, “8-plate workflow for Axiom™ PMD Array Plates”.)

Axiom™ 2.0 Plus Reagent Kit 96F

Each Axiom™ 2.0 Plus Reagent Kit 96F (Cat. No. 951960) is sufficient for 1 Axiom™ Precision Medicine Diversity Research Array Plate.

Note: If running the Axiom™ 2.0 Plus Assay without the mPCR step, use the Axiom™ 2.0 Reagent Kit (Cat. No. 901758).

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

Multiplate workflows

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

- Incubation for DNA amplification is 22–24 hours.
- Hybridization in the GeneTitan™ MC Instrument is 23.5–24 hours.
- Reagent trays for wash/stain/imaging must be prepared as hybridization finishes.
- Limits to when a second hybridization tray and array plate can be loaded into the GeneTitan™ MC Instrument.

Using the automated target preparation protocol, workflows are available for processing 8 array plates per work week, or 3 array plates per week.

- Chapter 7, “8-plate workflow for Axiom™ PMD Array Plates”
- Chapter 8, “3-plate workflow for Axiom™ PMD Array Plates using a 3-hour DNA precipitation step”

Overview of the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow (with mPCR step)

	gDNA plate setup 5 ng/µL of gDNA		
Day 1	▼	▼	Manual procedure
	10 µL/well	20 µL/well	10 µL/well from the gDNA plate is used for Stage 1A, and 20 µL/well from the gDNA plate is used for Stage 1B
Day 1	**Stage 1A: mPCR (manual)** ▼	**Stage 1B: Amplify the genomic DNA (automated)** ▼	**23 ±1 hour amplification incubation at 37°C. Optional stopping point.**
Day 2	mPCR Plate	► Amplification Plate ▼	10 µL mPCR spike-in to Amplification Plate
Day 2	“Stage 2: Fragment and precipitate the DNA” on page 63 ▼		Approximate time: 16-24 hours (or optional 3-hour precipitation step) –20°C incubation of Precipitation Plate
Day 3	“Stage 3: Centrifuge and dry pellets” on page 78 ▼		Optional stopping point.
Day 3	“Stage 4A: Prepare the resuspension buffer” on page 83 “Stage 4B: Prepare Hybridization Master Mix” on page 90 “Stage 4C: Perform sample QC” on page 97 ▼		
Day 3	“Stage 5: Prepare the hybridization tray” on page 105 ▼		23.5 to 24-hour array hybridization in the GeneTitan™ MC Instrument
Day 4	“Stage 6: Prepare GeneTitan™ reagent trays” on page 113		
	Array processing		
Day 5	Chapter 6, “Process array plates with the GeneTitan™ instrument” *Array processing is completed with the GeneTitan™ MC Instrument and GeneChip™ Command Console™ software v4.3 or later.*		Fluidics: 5 hours Scan: ~7.5 hours

Overview of the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow (without mPCR step)

	gDNA plate setup 5 ng/µL of gDNA	
Day 1	▼ 20 µL/well	Manual procedure 20 µL/well from the gDNA plate is used for Stage 1B
Day 1	**"Stage 1B: Amplify the genomic DNA"** on page 52 **(automated)** ▼	**23 ±1 hour amplification incubation at 37°C.** **Optional stopping point.**
Day 2	"Stage 2: Fragment and precipitate the DNA" on page 63 ▼	Approximate time: 16-24 hours (or optional 3-hour precipitation step) – 20°C incubation of Precipitation Plate
Day 3	"Stage 3: Centrifuge and dry pellets" on page 78 ▼	Optional stopping point.
Day 3	"Stage 4A: Prepare the resuspension buffer" on page 83 "Stage 4B: Prepare Hybridization Master Mix" on page 90 "Stage 4C: Perform sample QC" on page 97 ▼	
Day 3	"Stage 5: Prepare the hybridization tray" on page 105 ▼	23.5 to 24-hour array hybridization in the GeneTitan™ MC Instrument.
Day 4	"Stage 6: Prepare GeneTitan™ reagent trays" on page 113	
	Array processing	
Day 5	Chapter 6, "Process array plates with the GeneTitan™ instrument" *Array processing is completed with the GeneTitan™ MC Instrument and GeneChip™ Command Console™ software v4.3 or later.*	Fluidics: 5 hours Scan: ~7.5 hours

2 Required materials

Plate centrifuge

One plate centrifuge is required for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow. See the *Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow Site Preparation Guide—Applied Biosystems™ NIMBUS® Target Preparation Instrument*, for an appropriate plate centrifuge. When centrifuging and drying pellets, the centrifuge must meet the following requirements:

- Rcf: 3,200 × *g* (4,000 rpm for the Eppendorf™ 5810R with the rotor configuration that is described in the *Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow Site Preparation Guide—Applied Biosystems™ NIMBUS® Target Preparation Instrument*).
- Temperature: 4°C and room temperature.

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

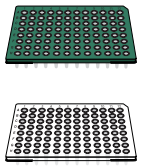
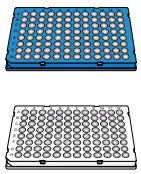
Table 1 Plate centrifuge recommendations for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow.

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)

Labware and consumables for multiplex PCR

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.

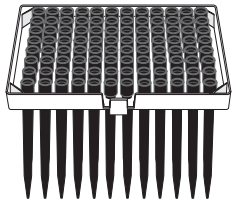
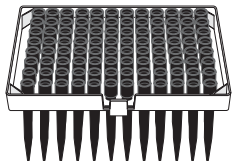
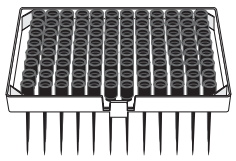
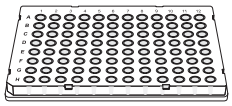
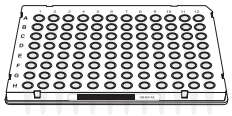
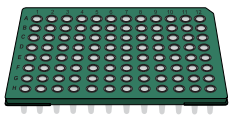
Note: Labware required for the mPCR step is not included in the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437) and must be ordered separately.

Labware	Source	Image
Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted	One of the following: • Bio-Rad™ PCR plates (Cat. Nos. HSS9641 (green) or HSS9601 (clear)) • MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354)	
Hard-Shell™ 96-Well PCR Plate, low profile, full skirted See “PCR plate type by thermal cycler for the mPCR step” on page 41.	Bio-Rad™, Cat. Nos. HSP9631 (blue) or HSP9601 (white)	
15-mL conical-bottom centrifuge tube, polypropylene	**MLS**	
Matrix™ Reagent Reservoir, 25 mL	Cat. No. 14-387-069	
96-well Block Cooling Chamber for 0.2 mL tubes, 96 holes (4 for 1.5 mL and 6 for 0.5 mL tubes), Dim.: 6 1/8"L x 3 1/8"W x 1" H	Diversified Biotech™, Cat. No. CHAM-1000	
Adhesive film	Use one of the following: • Applied Biosystems™ MicroAmp™ Clear Adhesive Film (Cat. No. 4306311) • Microseal™ 'B' PCR Plate Sealing Film (Bio-Rad™, Cat. No.MSB1001)	

Labware used on the deck

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! There are 2 types of compatible “Round deepwell plates” that can be used on the NIMBUS® Instrument. Each type of “Round deepwell plate” has a specific plate adapter. The correct plate adapter must be paired with the correct “Round deepwell plate” to prevent part interference, which can lead assay failures.

Labware	Source	Image
CO-RE® II Filter Tips, 1,000 µL Conductive 1,000 µL filter pipette tips in frames.	Hamilton™ Robotics (Cat. No. 235905)	
CO-RE® II Filter Tips, 300 µL Conductive 300 µL filter pipette tips in frames.	Hamilton™ Robotics (Cat. No. 235903)	
CO-RE® II Filter Tips, 50 µL Conductive 50 µL filter pipette tips in frames.	Hamilton™ Robotics (Cat. No. 235948)	
96-well full-skirt plate	One of the following: • Available as a component of the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437). • Purchased separately, Fisher Scientific™ (Cat. No. 14-222-326).	
96 half-skirt plate Also called Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted.	One of the following: • Available as a component of the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437). • Purchased separately, half- skirt plate (Cat. Nos. 4483354 or 4483352). • Purchased separately, half-skirt plate (Bio-Rad™, Cat. Nos. HSS9641 (green) or HSS9601 (clear)). • Purchased separately, MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354).	 

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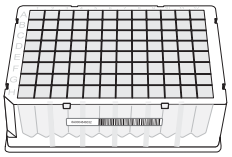
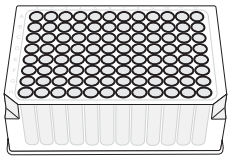
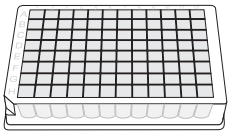
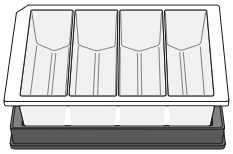
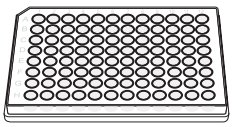
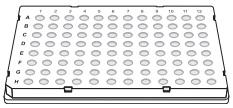
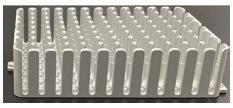
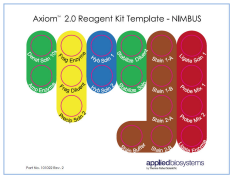
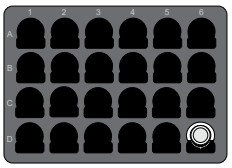
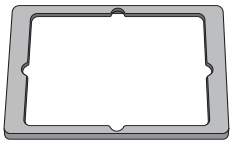
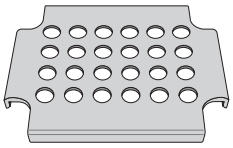
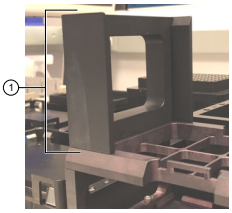
Labware	Source	Image
Square deepwell plate	One of the following: • Available as a component of the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437). • Purchased separately, deepwell plate (Cat. No. AB0932).	
Round deepwell plate	One of the following: • Available as a component of the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437). • Purchased separately, Nunc™ 96-Well Polypropylene DeepWell™ Storage Plate (sterile) (Cat. No. 12-565-605). • Purchased separately, Axygen™ 96-well Round Deepwell Plate (sterile, PDW20CS), (Cat. No. 14-222-354). • Purchased separately, Axygen™ 96-well Round Deepwell Plate (nonsterile, PDW20C), (Cat. No. 14-222-353). **Note:** • Use the correct plate adapter for each 96-deepwell plate type. See Table 2. • The Nunc™ plates are components of the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437).	
Square 1.2-mL plate	One of the following: • Available as a component of the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437). • Purchased separately, square plate (Thomas Scientific™, Cat. No.OX1263).	
4-column reservoir with reservoir frame	One of the following reservoirs: • Available as a component of the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437). • Purchased separately, reservoir (Thomas Scientific™, Cat. No. EK-2119). Reservoir frame: Available as a component of the Axiom™ NIMBUS® Starter Pack-NC (Cat. No. 952493).	 **Note:** The 4-column reservoir must always be placed on a reservoir frame when on the NIMBUS® Instrument deck.

(continued)

Labware	Source	Image
96-well UV plate	One of the following: • Available as a component of the Axiom™ Consumables Kit for QC (Cat. No. 902909). • Purchased separately, UV plate (Cat. No. 07-200-623).	
Alpillo™ Plate Cushion and Alpillo™ Extension	One of the following: • Available as a component of the Axiom™ NIMBUS® Starter Pack-NC (Cat. No. 952493). • Purchased separately: plate cushion (Alpaqua™, Cat. No. A000007) and extension (Alpaqua™, Cat. No. K000018).	 The image shown displays the plate cushion and extension assembled.
Pedestal, 35.48 mm Designed specifically for the Axiom™ 96 Sample Automated Target Preparation Solution method to fasten the Alpillo™ Plate Cushion and extension on deck position 5.	Available as a component of the Axiom™ NIMBUS® Starter Pack-NC (Cat. No. 952493).	
96 Half-Skirt Plate Holder Used as holder for 96 half-skirt plate.	One of the following: • Available as a component of the Axiom™ NIMBUS® Starter Pack-NC (Cat. No. 952493). • Purchased separately, skirt plate holder (Bio-Rad™, Cat. No.TRC 9601).	
Thermoshake Deepwell Plate Adapter Adapter plate for the Nunc™ round deepwell plate.	One of the following: • Available as a component of the Axiom™ NIMBUS® Starter Pack-NC (Cat. No. 952493) and the NIMBUS® Upgrade Kit for NC (Cat. No. 952495). • Purchased separately, plate adapter (INHECO™, Cat. No. 3200648). **Note:** Use the correct plate adapter for each 96-deepwell plate type. See Table 2.	
Axiom™ 2.0 Reagent Kit cooling block template Designed specifically for use with the NIMBUS® Instrument and the Axiom™ 2.0 Reagent Kit.	Available as a component of the Axiom™ NIMBUS® Starter Pack-NC (Cat. No. 952493).	

(continued)

Labware	Source	Image
Beckman Coulter™ 24-Position Tube Rack With one 11 mm tube insert in position D6.	Tube rack (Beckman Coulter™, Cat. No. 373661) and insert (Beckman Coulter™, Cat. No.373696).	
Plate collar	Included as part of the NIMBUS® Instrument configuration.	
Tube collar	Included as part of the NIMBUS® Instrument configuration.	
Tip loading tool	Included as part of the NIMBUS® Instrument configuration.	 ① Tip loading tool attached to deck.
GeneTitan™ ZeroStat AntiStatic Gun and Ion-Indicator Cap	Cat. No. 74-0014	
96-well Block Cooling Chamber for 0.2 mL tubes, 96 holes (four for 1.5 mL and six for 0.5 mL tubes), Dim.: 6 1/8”L x 3 1/8”W x 1” H	Diversified Biotech™, Cat. No. CHAM-1000	
Adhesive film	Use one of the following: • Applied Biosystems™ MicroAmp™ Clear Adhesive Film, 4306311 • Microseal™ 'B' PCR Plate Sealing Film, Bio-Rad™, MSB1001	

Correct adapter for the 96-round deepwell plate type used

Table 2 Adapter for the 96-round deepwell plate type.

96-round deepwell plate	Adapter to use
Fisher Scientific™, Nunc™ 96-Well Polypropylene DeepWell™ Storage Plate (sterile), Cat. No. 12-565-605.	INHECO™, 3200648. Also available in the Axiom™ NIMBUS® Starter Pack-NC (Cat. No.952493).
Axygen™ 96-well Round Deepwell Plate (sterile, PDW20CS), Cat. No. 14-222-354.	INHECO™ (Cat. No. 3200390).
Axygen™ 96-well Round Deepwell Plate (nonsterile, PDW20C), Cat. No. 14-222-353.	

Axiom™ consumables kits for the NIMBUS® Instrument

Table 3 Axiom™ Starter Kit for Applied Biosystems™ NIMBUS® Instrument–NC Combo Kit (Cat. No. 952496)

Component	Cat. No.
Axiom™ NIMBUS® 2.0 Consumables Kit v2	952437
Axiom™ NIMBUS® Starter Pack-NC	952493
Axiom™ Consumables Kit for QC	902909

Table 4 Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437).

Component[1]	Part No. [2]	Number	
		Per run	Per kit[3]
96-well full-skirt plate	203023	7	30
96 half-skirt plate	203244	1	4
Square deepwell plate	203016	3	12
Round deepwell plate	203367	2	8
Square 1.2-mL plate	203031	1	4
4-column reservoir	203056	9	36

[1] This consumables kit does not include a 96-well UV plate. This plate is in the Axiom™ Consumables Kit for QC (Cat. No. 902909).
[2] Plate part numbers are provided for identification purposes only.
[3] The kit provides sufficient quantities of consumables for 4 runs of 96-format array plates.

Table 5 Axiom™ NIMBUS® Starter Pack-NC (Cat. No. 952493)

Component	Quantity per run
96 Half-Skirt Plate Holder	1
Thermoshake Deepwell Plate Adapter	1
Alpillo™ Plate Cushion	1
Alpillo™ Extension	1
Axiom™ 2.0 Reagent Kit Template	1
Pedestal, 35.48 mm	1
Reservoir holder	2
Axiom™ 96 Consumables Kit for Applied Biosystems™ NIMBUS® 2.0	1
Axiom™ Consumables Kit for QC	1
2-mL tubes	25

Table 6 Axiom™ Consumables Kit for QC (Cat. No. 902909).

Component	Part No.	Number	
		Per run	Per kit
96-well UV plate	202919	1	25

Axiom™ GeneTitan™ Consumables Kit

Each Axiom™ GeneTitan™ Consumables Kit is sufficient to process one 96-array format plate. These trays are required for processing 96-array format plates on the GeneTitan™ MC Instrument. See Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation” for information on aligning and loading trays onto the GeneTitan™ MC Instrument.

Table 7 Axiom™ GeneTitan™ Consumables Kit (Cat. No. 901606).

Contents[1]	Amount	Storage
96-layout stain tray	5	Room temperature
96-layout hybridization tray	1	
96-layout scan tray	1	
96-layout scan and stain tray cover	6	

[1] See Table 8 for detailed descriptions of each component.

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

Table 8 Axiom™ GeneTitan™ tray consumables (from the Axiom™ GeneTitan™ Consumables Kit (Cat. No. 901606).

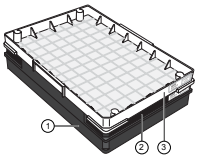
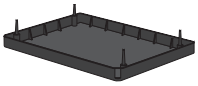
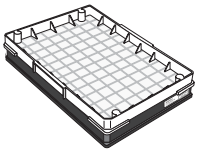
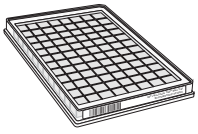
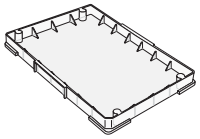
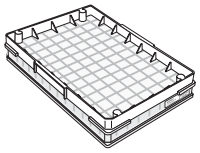
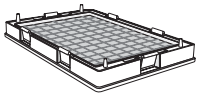
Item	Part No.	Image	Details
Scan tray	900746 Box 501006 Pouch	 ① Scan tray protective base ② 96-layout scan tray ③ Scan tray cover	The scan tray package includes the scan tray, a cover, and a black protective base. • Cover the scan tray with the scan tray cover before placing the tray in the GeneTitan™ MC Instrument. • The scan tray must be protected at all times from damage or exposure to dust. The scan tray must be in the black plate base at all times except when loaded into the GeneTitan™ MC Instrument. • The black scan tray protective base in the package is used to protect the bottom of the scan tray glass from damage. Remove the protective base from the scan tray before loading the scan tray with the scan tray cover in the GeneTitan™ MC Instrument.
GeneTitan™ scan tray on black base			This combination of the GeneTitan™ scan tray on the protective black base is to be placed on the NIMBUS® Instrument deck during the GeneTitan™ reagent preparation method.
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.
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.
96-layout GeneTitan™ stain trays	9016110 Kit (5 stain trays) 501025 Tray		The GeneTitan™ stain trays are packaged in zip-top bags to keep them free of dust. Each GeneTitan™ stain tray is uniquely barcoded. All trays must be de-ionized to remove static electricity before placing the trays on the NIMBUS® Instrument deck for the prepare GeneTitan™ reagent trays step. See “Deionization of GeneTitan™ trays and covers” on page 209.

Table 8 Axiom GeneTitan tray consumables (from the Axiom GeneTitan Consumables Kit (Cat. No. 901606). *(continued)*

Item	Part No.	Image	Details
96-layout stain and scan tray cover	202757		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 cover. All tray covers must be de-ionized to remove static electricity before placing the cover on the tray. See “Deionization of GeneTitan™ trays and covers” on page 209.
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 cover. See “Label GeneTitan™ hybridization and reagent trays” on page 208.
96-layout hybridization tray	900747		After aliquoting the denatured hybridization-ready samples into the hybridization tray, the tray should be immediately loaded into the GeneTitan™ MC Instrument with the barcode facing away from the operator, that is, the barcode should be on the back side.

3 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.

The Axiom™ 2.0 Plus Reagent Kit 96F is used for this protocol. Genomic DNA Standard (Ref 103) (Cat. No. 951957), available separately, meets the DNA requirements that are outlined in this chapter and can be used as a control. The size and purity of sample gDNA can be compared with the size and purity of the control DNA to evaluate sample quality. Routinely use the control DNA as an experimental positive control and for troubleshooting purposes.

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

Sources of genomic DNA

The following sources of human gDNA have been successfully tested in the laboratories at Thermo Fisher Scientific for DNA that meets the requirements for the Axiom™ 2.0 Plus Assay.

- Whole blood
- Buccal cell
- Saliva
- Cell lines

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

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

General requirements

- Starting DNA must be double-stranded for accurate concentration determination.
- DNA must be of high purity. DNA must be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (that is, EDTA). The gDNA extraction 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 31.
- 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 advised to evaluate gDNA by gel electrophoresis. Evaluating the gDNA by gel electrophoresis is of particular importance for DNA extracted from saliva and buccal cells, sample types prone to DNA degradation.

Special requirements

Preamplification area

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

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

Ideally, this 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™ 2.0 Plus Assay) and degraded gDNA samples. For gDNA that is degraded perform a test experiment to investigate the performance of the samples in the Axiom™ 2.0 Plus Assay before starting any large-scale genotyping projects.

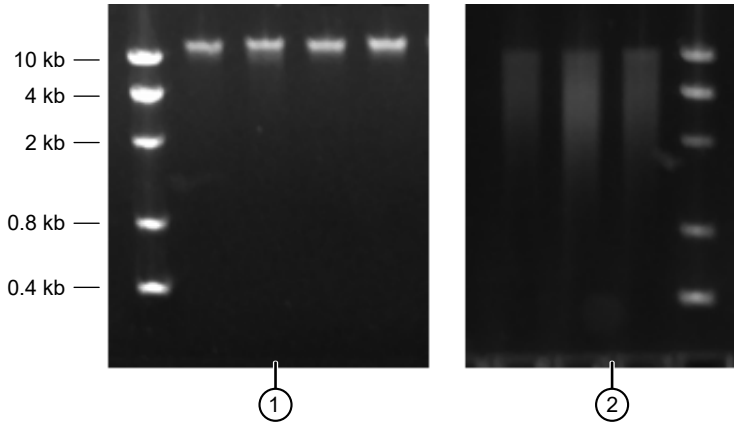


Figure 1 Gel images with intact gDNA and degraded gDNA.

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 mPCR and DNA amplification stages.

Note: If you are not performing the mPCR step, prepare only the genomic DNA sample plate for "Stage 1B: Amplify the genomic DNA".

The genomic DNA (gDNA) you process using the Axiom™ 2.0 Plus Assay must meet the general requirements that are listed earlier in this chapter. The amount of gDNA is 50 ng for the mPCR step and 100 ng for the Axiom™ 2.0 Plus whole-genome amplification step.

Genomic DNA input requirements

Table 9 Genomic DNA input requirements.

Sample type	Volume per well	Input mass per well	gDNA concentration
Stage 1A: mPCR	10 µL	50 ng	5 ng/µL
Stage 1B: DNA amplification	20 µL	100 ng	5 ng/µL

Time required

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

Equipment, consumables, and reagents required

Unless otherwise indicated, all materials are available through **thermofisher.com**. "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	Ice bucket, filled with ice
1 each	Pipettes: single-channel P10 or P20 Optional: multichannel P10 or P20
As required	Pipette tips

(continued)

Quantity	Item
1	One of the following: • Fisher Scientific™, Nunc™ 96-Well Polypropylene DeepWell™ Storage Plate, 12-565-605. • Axygen™ 96-well Round Deepwell Plate (sterile, PDW20CS), Fisher Scientific™, 14-222-354. • Axygen™ 96-well Round Deepwell Plate (nonsterile, PDW20C), Fisher Scientific™, 14-222-353. **IMPORTANT!** Depending on the type of 96-well round deepwell plate used, the plate must be used with the corresponding thermal shake adapter. See Table 2.
1	For the Applied Biosystems™ GeneAmp™ PCR System 9700, Applied Biosystems™ Veriti™ Thermal Cycler, and Applied Biosystems™ ProFlex™ PCR System, use one of the following plates. • Bio-Rad™ 96-well PCR plates (Cat. Nos. HSS9641 (green) or HSS9601 (clear)). • MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354).
	For the Eppendorf™ Mastercycler™ pro S, use Bio-Rad™ 96-well PCR plate (Cat. Nos. HSP9631 (blue) or HSP9601 (white)).
1	Plate centrifuge
1	Plate spectrophotometer (required only if no OD measurements available for samples)
1	Vortexer

Reagents required

Reagent	Source
User-supplied	
Genomic DNA Standard (Ref 103), 10 ng/µL	951957
Low EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	75793
Quant-iT™ PicoGreen™ dsDNA Assay Kit	P7589

Thaw samples and control

Thaw the following components to room temperature:

- gDNA samples
- Genomic DNA Standard (Ref 103)

To thaw, either:

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

Quantify and dilute test sample gDNA

1. Gently vortex (50% maximum), then centrifuge the gDNA samples and Genomic DNA Standard (Ref 103).

2. *Recommendation*: Quantify each sample (for example, using the Quant-iT™ PicoGreen™ dsDNA Assay Kit).

3. Using Low EDTA TE Buffer, dilute each sample to a concentration of 5 ng/µL.

   Dilute gDNA to a final concentration of 5 ng/µL in a 35 µL volume. The assay requires 20 µL (5 ng/µL) for DNA AMP and 10 µL (5 ng/µL) for the mPCR step.

4. Seal, vortex, then centrifuge.

Note: Do not dilute the Genomic DNA Standard (Ref 103). It is already at a working concentration.

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

Aliquot the diluted samples and control

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

Note: Ensure that the gDNA is well mixed before plating.

Aliquot diluted samples and control to the round deepwell plate for DNA amplification

1. Aliquot 20 µL of each diluted gDNA sample to the round deepwell plate.
2. Pipet 20 µL of Genomic DNA Standard (Ref 103) control. We recommend including at least 1 positive control on each plate.
3. Seal, then centrifuge.

Aliquot diluted samples and control to the mPCR Sample Plate

1. Aliquot 10 µL of each diluted gDNA sample to the 96-well PCR plate.

   Ensure that you are using the appropriate 96-well PCR plate for your thermal cycler. See Table 10.
2. Pipet 10 µL of Genomic DNA Standard (Ref 103). We recommend including at least 1 positive control on each plate.
3. Seal, then centrifuge.

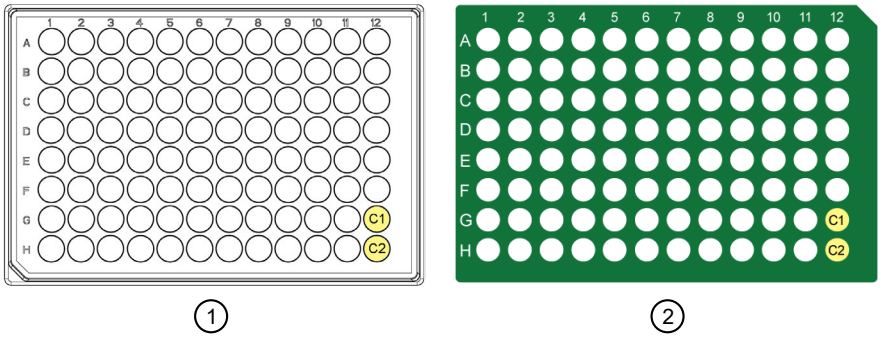


Figure 2 Aliquot genomic DNA to the Amplification Sample Plate and mPCR Sample Plate.

① Amplification Sample Plate: Round deepwell plate, 20 µL/well.

② mPCR Sample Plate: 96-well PCR plate, 10 µL/well.

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

Freeze or proceed

Do one of the following:

- Store the sample plates at –20°C.
- Proceed to DNA amplification for automated target preparation. See Chapter 5, "Multiplex PCR and target preparation with the Applied Biosystems™ NIMBUS® Target Preparation Instrument".

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

GeneTitan™ Array Plate Registration file

Each array plate has a barcode for tracking and each row and column number identifies an individual array. The GeneTitan™ Array Plate Registration file is where you enter the sample information for each individual array of the array plate to be run. It is important to create and upload a GeneTitan™ Array Plate Registration file *before* loading the array plate and hybridization tray onto the GeneTitan™ Multi-Channel (MC) Instrument 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).

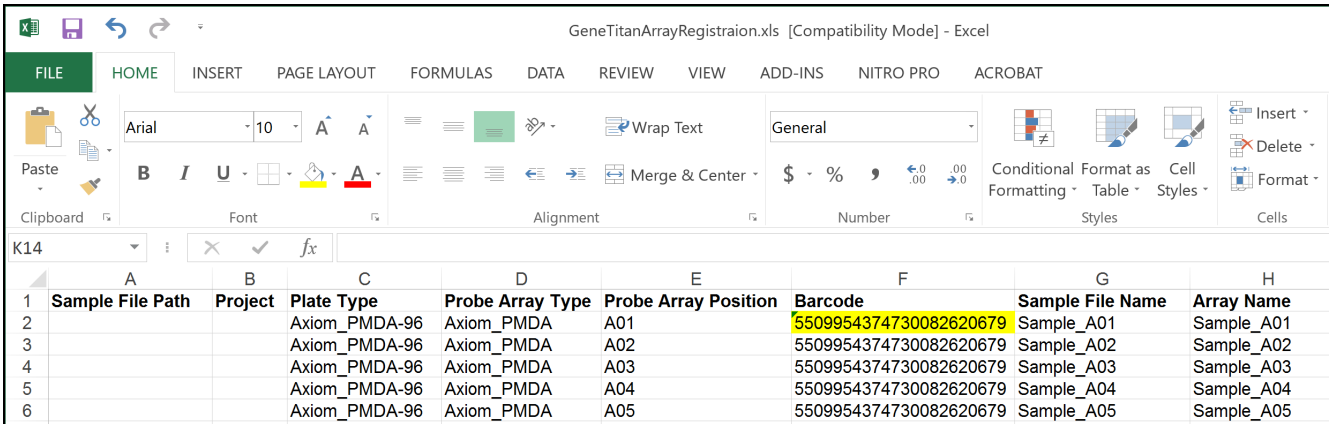
Create and save a GeneTitan™ Array Plate Registration file

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™ instrument”.



	A	B	C	D	E	F	G	H
1	**Sample File Path**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array Position**	**Barcode**	**Sample File Name**	**Array Name**
2			Axiom_PMDA-96	Axiom_PMDA	A01	550995437473008262067 9	Sample_A01	Sample_A01
3			Axiom_PMDA-96	Axiom_PMDA	A02	550995437473008262067 9	Sample_A02	Sample_A02
4			Axiom_PMDA-96	Axiom_PMDA	A03	550995437473008262067 9	Sample_A03	Sample_A03
5			Axiom_PMDA-96	Axiom_PMDA	A04	550995437473008262067 9	Sample_A04	Sample_A04
6			Axiom_PMDA-96	Axiom_PMDA	A05	550995437473008262067 9	Sample_A05	Sample_A05

Figure 3 Example of a GeneTitan™ Array Plate Registration file for Axiom™ Precision Medicine Diversity Research Array Plate.

4 Set up the Applied Biosystems™ NIMBUS® Target Preparation Instrument

This chapter contains information describing the procedures, equipment, and materials required for running the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow. To ensure operator safety and assay performance, operators must be familiar with this content before starting target preparation.

Guidelines for use

Pipette tips

The following table provides the pipette tip usage for 1 full run of the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow on the NIMBUS® Target Preparation Instrument.

Step	CO-RE® II Filter Tips, 1,000 µL	CO-RE® II Filter Tips, 300 µL	CO-RE® II Filter Tips, 50 µL
DNA amplification	24 tips	120 tips	—
Fragmentation	33 tips	114 tips	—
Resuspension	—	104 tips	—
Hybridization preparation	14 tips	105 tips	—
Sample QC	—	16 tips	192 tips
Hybridization transfer	—	96 tips	—
GeneTitan™ preparation 1	47 tips	7 tips	—
GeneTitan™ preparation 2	28 tips	1 tip	—
Totals	**146 tips**	**563 tips**	**192 tips**

Guidelines for handling plates and tubes

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

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

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

  Blot-dry: Before sealing plates, check the top of the plate to ensure that there are no droplets. If droplets are present, blot-dry the top of the plate before sealing to ensure a tight seal.
  - To remove droplets before sealing, overlay a sheet of laboratory tissue across the top of the plate and gently pat down to dry.
  - Lift the sheet off the plate and discard. Ensure that the top of the plate is dry and seal the plate as usual.
- **Vortex reagent vials**:

  Vortex 3 times for 1 second each time at the maximum setting.
- **Vortex plates**:
  - Vortex deepwell plates for 5 seconds in each of the 5 sectors. See the sectors in Figure 4.
  - Vortex PCR plates for 2 seconds in each of the 5 sectors.

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

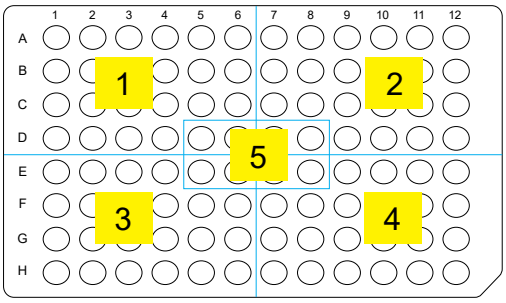


Figure 4 The 5 plate sectors.

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

Guidelines for a run on the NIMBUS® Instrument

Observe the following guidelines when running the assay on the NIMBUS® Instrument.

- Position all plates and the chilled reagent block with A1 in the upper left corner of the frame.
- Vortex and centrifuge briefly all reagent tubes (except enzymes) before placing in cooling block to avoid loss of solution volume to the cap and sides of the tube.
- Remove caps from the reagent tubes and press tubes into the cooling block to ensure that they are fully seated.
- All labware and reagents must be in the proper locations as illustrated in the deck setup prompt.

Thermal cycler recommendations and protocols

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.

	Thermal cycler protocol	
Verified thermal cyclers	**Axiom 2.0 Plus mPCR**	**Axiom 2.0 Plus Denature**
Applied Biosystems™ ProFlex™ System	✓	✓
Applied Biosystems™ Veriti™ Thermal Cycler	✓	✓
Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block)	✓	✓
Applied Biosystems™ 2720 Thermal Cycler	No	✓
Bio-Rad™ DNA Engine Tetrad™ 2 PTC-0240G	No	✓
Eppendorf™ Mastercycler™ pro S	✓	✓

IMPORTANT! Always use the heated lid option when programming protocols. The **Axiom 2.0 Plus mPCR** protocol was verified using the "9600 mode" on the Applied Biosystems™ GeneAmp™ PCR System 9700, Applied Biosystems™ Veriti™ Thermal Cycler, and Applied Biosystems™ ProFlex™ System thermal cyclers. The "Safe" mode was used for the Eppendorf™ Mastercycler™ pro S. See the appropriate thermal cycler user guide for programming information.

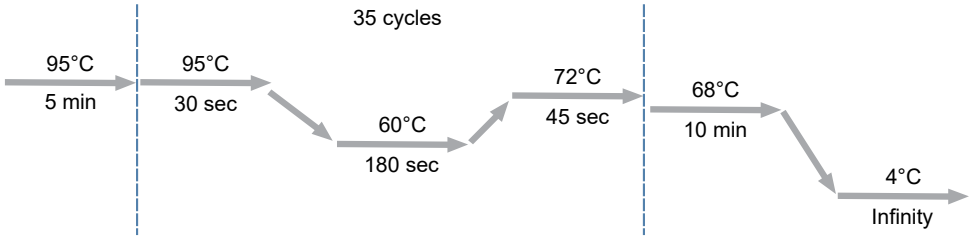


Figure 5 Axiom 2.0 Plus mPCR thermal cycler protocol (Stage 1A).

The mPCR step of the Axiom™ 2.0 Plus Assay has been verified with the Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block) Applied Biosystems™ Veriti™ Thermal Cycler, Applied Biosystems™ ProFlex™ System, and Eppendorf™ Mastercycler™ pro S. Use of other

thermal cyclers for this stage can result in assay failure and violate the array and reagent replacement policy.

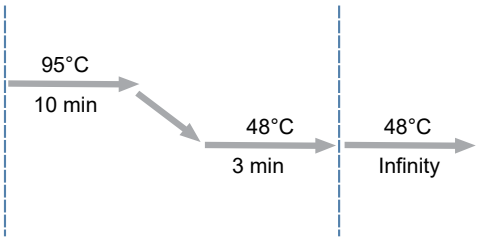


Figure 6 Axiom 2.0 Plus Denature thermal cycler protocol (Stage 5).

WARNING! Evaporation during denaturation can negatively affect assay performance. Use the recommended thermal cycler consumables and sealing film to eliminate condensation and evaporation. For thermal cyclers with variable lid tension (such as the Bio-Rad™ PTC-200 or Tetrad™ 0240), follow the instructions in the appropriate thermal cycler user guide for adjusting lid tension.

Note: Two thermal cyclers are required if running the 3 plates per week or the 8 plates per week automated target preparation workflow.

PCR plate type by thermal cycler for the mPCR step

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

Table 10 PCR plate type and seal for thermal cyclers

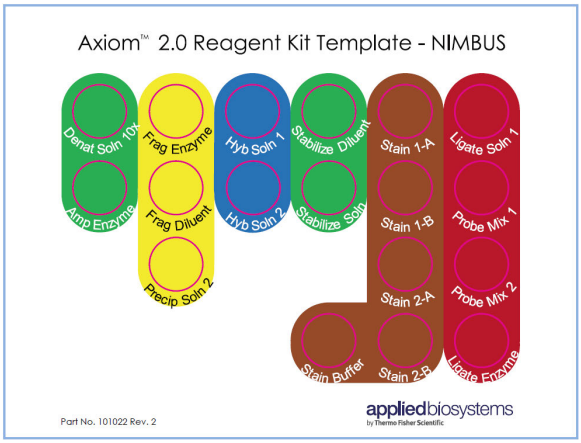
Thermal cycler model	PCR plate type	Seal[1]
Applied Biosystems™ ProFlex™ PCR System Applied Biosystems™ Veriti™ Thermal Cycler Applied Biosystems™ GeneAmp™ PCR System 9700	One of the following plates: MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (Cat. No. 4483354) Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641 or HSS9601)	MicroAmp™ Clear Adhesive Film (Cat. No. 4306311)
Eppendorf™ Mastercycler™ pro S	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (Cat. No. HSP9631 or HSP9601)	

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

Deck setup for reagent tubes

Cooling block template

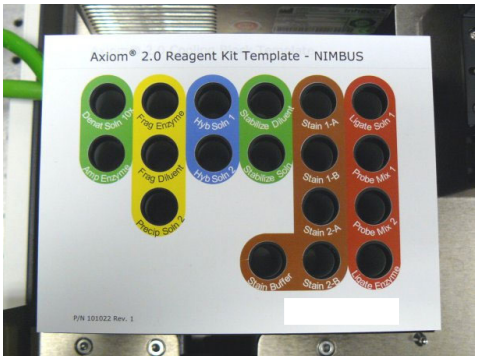
The Axiom™ 2.0 Reagent Kit Template fits precisely on top of the INHECO™ CPAC device. Using this template helps ensure the proper placement of reagent tubes onto the block for each method.



Assemble the cooling block template with reagent tubes and tube collar

1. Place the Axiom™ 2.0 Reagent Kit Template directly on top of the CPAC device with the 24-tube rack adapter.

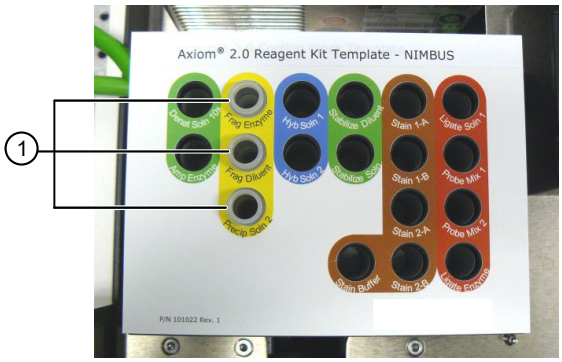
①



②

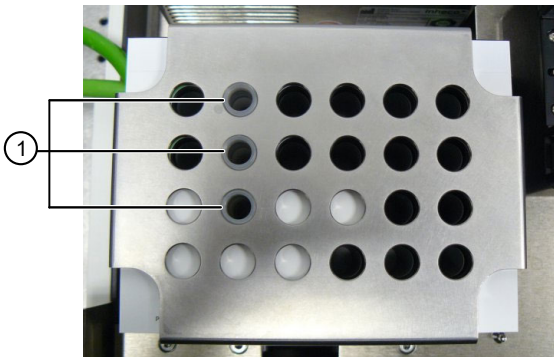
① CPAC device with 24-tube rack adapter.
② Axiom™ 2.0 Reagent Kit Template placed on top.

2. Insert the appropriate reagent tubes for the method, as indicated in the user interface and in this user guide. Press the reagent tubes into the block to ensure that they are fully seated.



① Three fragmentation reagent tubes that are inserted into tube block.

3. Place the tube collar over the reagent template and reagent tubes.



① Three reagent tubes.

5 Multiplex PCR and target preparation with the Applied Biosystems™ NIMBUS® Target Preparation Instrument

The Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow uses the Axiom™ 96 Sample Automated Target Preparation Solution method on the NIMBUS® Instrument for target preparation and GeneTitan™ reagent preparation to process 96 samples at a time. Note that the multiplex PCR (mPCR) and mPCR spike-in steps are not part of the automated method and are executed off deck, manually.

Note: If performing the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow without the mPCR step, skip all the mPCR activities and proceed directly to "Stage 1B: Amplify the genomic DNA" on page 52 to start target preparation.

IMPORTANT! The NIMBUS® software method for running the assay is available for Microsoft™ Windows™ 7 and Windows™ 10 operating systems. The Windows™ 10 and Windows™ 7 methods have different version numbers, but the workflow and user interface information are identical.

Stage 1A: Perform multiplex PCR (mPCR)

IMPORTANT! Before proceeding to mPCR or DNA amplification, perform the gDNA preparation. See Chapter 3, “Genomic DNA preparation”.

IMPORTANT! Preamplification preparation must take place in a dedicated area such as a biosafety hood with dedicated pipettes, tips, and vortex. See “Preamplification area” on page 29.

Time required

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

[1] For 96 samples.

Input required

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

See Chapter 3, “Genomic DNA preparation”.

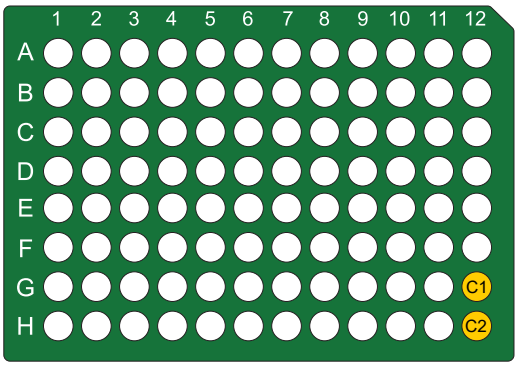


Figure 7 mPCR Sample Plate.

Materials, labware, and reagents required

Equipment and labware required for Stage 1A

Quantity	Item
Labware and consumables	
As required	Adhesive seals for 96-well plate (MicroAmp™ Clear Adhesive Film)
1	Marker, fine point, permanent
1	96-well plate holder
1	15-mL conical tube (RNase/DNase-free)
Equipment	
1	Vortexer
1	96-well metal chamber, cooled to 4°C
1	Plate centrifuge
1	Ice bucket with ice
1	Thermal cycler programmed with the **Axiom 2.0 Plus mPCR** protocol. See “Thermal cycler recommendations and protocols” on page 40.
1 each	Pipettes: • Single-channel P200 • Single-channel P1000 • Multichannel P200
As required	Pipette tips

Reagents required for Stage 1A

Quantity	Reagent and cap color	Module
From the Axiom™ 2.0 Plus Reagent Kit 96F		
1 tube	◯10X Primer Mix	Module A—mPCR Primers, –20°C (Part No. 912896)
User supplied from the QIAGEN™ Multiplex PCR *Plus* Kit (100)[1] (Cat. No. 206152)		
3 tubes	2X Multiplex PCR Master Mix	
1 tube	5X Q-Solution	
1 tube	RNase-free Water	

[1] CoralLoad Dye in kit is not needed for Axiom™ 2.0 Plus Assay.

Prepare for mPCR

1. Power on the thermal cycler that is programmed with **Axiom 2.0 Plus mPCR** protocol. See Figure 5.

   Ensure that the heated lid option has been selected.

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

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

IMPORTANT! gDNA samples must be 10-µL volume at a concentration of 5 ng/µL in a 96-well PCR plate. See “Aliquot diluted samples and control to the mPCR Sample Plate” on page 35.

---

3. Thaw, then prepare the reagents from Module A—mPCR Primers (Part No. 912896), of the Axiom™ 2.0 Plus Reagent Kit 96F.
   a. Thaw the 10X Primer Mix on the benchtop at room temperature, then place on ice.

   b. Vortex, then centrifuge briefly before use.

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

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

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

IMPORTANT!

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

Prepare the mPCR Master Mix

1. Label a 15-mL conical tube “mPCR”.
2. To the mPCR tube, add the reagents that are listed in the following table and in the following order:

Table 11 mPCR Master Mix.

Reagent	One reaction	120 reactions
RNase-free Water	2 µL	240 µL
Q-Solution	4 µL	480 µL
10X mPCR Primer Mix	4 µL	480 µL
2X QIAGEN™ Multiplex PCR Master Mix	20 µL	2,400 µL
Total	**30 µL**	**3,600 µL**

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

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

Set up the mPCR Reaction Plate

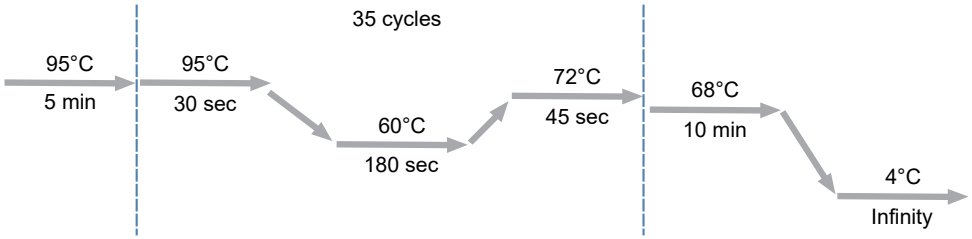
1. Confirm that the 96-well PCR plate is labeled “mPCR”, then include the date and any other desired experimental details.
2. Place the plate in a 96-well metal chamber that had been stored at 4°C.
3. Carefully pour the prepared mPCR Master Mix into a 25-mL reservoir.
4. Use a P200 multichannel pipette to carefully transfer 30 µL of mPCR Master Mix into the mPCR Sample Plate.
   Final volume of each well is 40 µL.
5. Seal the plate with adhesive seal, ensuring that the seal is firmly pressed down to prevent sample contamination during mixing and evaporation during PCR cycling.
6. Vortex the plate for 2 seconds in each quadrant, twice. See “Guidelines for handling plates and tubes” on page 39.

7. Centrifuge at 2,000 rpm for 30 seconds.
8. Return the plate to the cold 96-well metal chamber until the plate can be loaded onto the thermal cycler.
9. Load the plate onto the thermal cycler within 5 minutes.
10. Discard all leftover reagents, including any remaining unused QIAGEN™ reagents.

Run the Axiom 2.0 Plus mPCR thermal cycler protocol

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

1. Place mPCR Reaction Plate on thermal cycler, then run the **Axiom 2.0 Plus mPCR** protocol.
   See Figure 5.



2. After the mPCR Reaction Plate has been placed on the thermal cycler, proceed to “Stage 1B: Amplify the genomic DNA”.

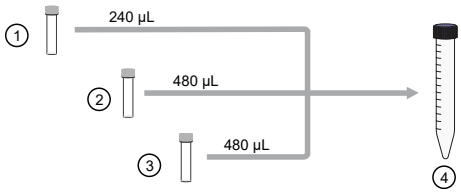
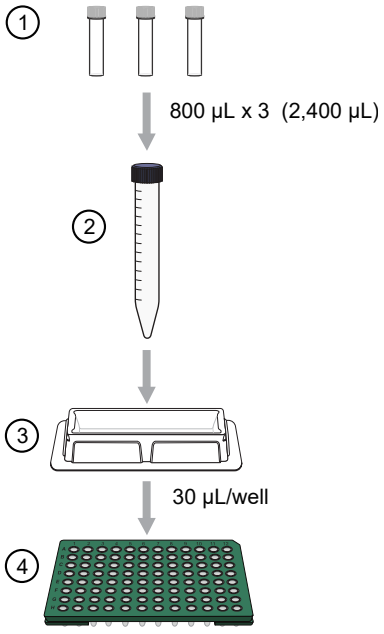
Store the mPCR Product Plate

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

1. After the **Axiom 2.0 Plus mPCR** thermal cycler protocol is complete, remove the plate from the thermal cycler, then centrifuge the plate briefly. Ensure that the plate is well sealed to prevent evaporation during storage.
2. Do one of the following:
   - For mPCR spike-in and fragmentation on another day, store the mPCR Product Plate at –20°C.
   - For mPCR spike-in and fragmentation within 4 hours, store the mPCR Product Plate in a refrigerator (2–8°C).

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

Workflow for Stage 1A: Multiplex PCR

Prepare the mPCR Master Mix–part 1	
 ① RNase-free water ② Q-solution ③ mPCR Primer Mix ④ 15-mL tube	Add all components to a 15-mL tube, vortex briefly, then centrifuge briefly.
Prepare the mPCR Master Mix–part 2	
 ① Three vials of 2X QIAGEN Multiplex PCR Master Mix ② 15-mL tube from part 1 ③ Matrix™ Reagent Reservoir ④ mPCR Sample Plate	**1.** Transfer 800 µL from 3 vials of 2X QIAGEN Multiplex PCR Master Mix into the 15-mL tube from part 1. **2.** Invert tube 10 times to mix, then centrifuge briefly. **3.** Pour contents of the 15-mL tube into the reservoir. **4.** Add 30 µL mPCR Master Mix to each well using a P200 12-channel pipette. Seal the mPCR Reaction Plate tight, vortex, then centrifuge briefly. Final volume: 40 µL/well.

Run the Multiplex PCR thermal cycler protocol	
	Time estimate: ~3.5 hours **Note:** When the thermal cycler program is finished, the mPCR Reaction Plate is now known as the mPCR Product Plate.

Stage 1B: Amplify the genomic DNA

Before proceeding to DNA amplification, complete genomic DNA preparation. (See Chapter 3, "Genomic DNA preparation".)

Time required

Note: A 22–24 hour incubation is required at the end of this stage.

Activity	Time
Hands-on time	~30 minutes
NIMBUS® Instrument—DNA Amplification	~30 minutes
Incubation	23 ±1 hour
Total	**~24 hours**

Input required

The Amplification Sample Plate of genomic DNA samples in a round deepwell plate.

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
Equipment and consumables	
As required	Adhesive seals for 96-well plates
1	Marker, fine point, permanent
As required	Laboratory tissues
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Oven • >3 array plates per week—we recommend using the Thermo Scientific™ Heratherm™ Advanced Protocol Microbiological Incubator, or the BINDER™ ED 56 • ≤3 array plates per week—okay to use the GeneChip™ Hybridization Oven 645 or the BINDER™ ED 56
1	Plate centrifuge
1	Vortexer
Labware	
2	96-well full-skirt plate[1]

(continued)

Quantity	Item
2	4-column reservoir[1]
1	Round deepwell plate[1]
1	Square deepwell plate[1]
1 frame	CO-RE® II Filter Tips, 1,000 µL
2 frames	CO-RE® II Filter Tips, 300 µL
1	Alpillo™ Plate Cushion and extension
2	Reservoir frame
2	Plate collar
1	Tube collar

[1] From the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437).

Reagents required for Stage 1B

Reagent and cap color	Module
Reagents and cap color from the Axiom™ 2.0 Plus Reagent Kit 96F (Cat. No. 951960)	
Axiom™ 2.0 Denat Soln 10X	Module 1, –20°C Part No. 901711
Axiom™ 2.0 Neutral Soln	
Axiom™ 2.0 Amp Soln	
Axiom™ 2.0 Amp Enzyme	
Axiom™ Water	

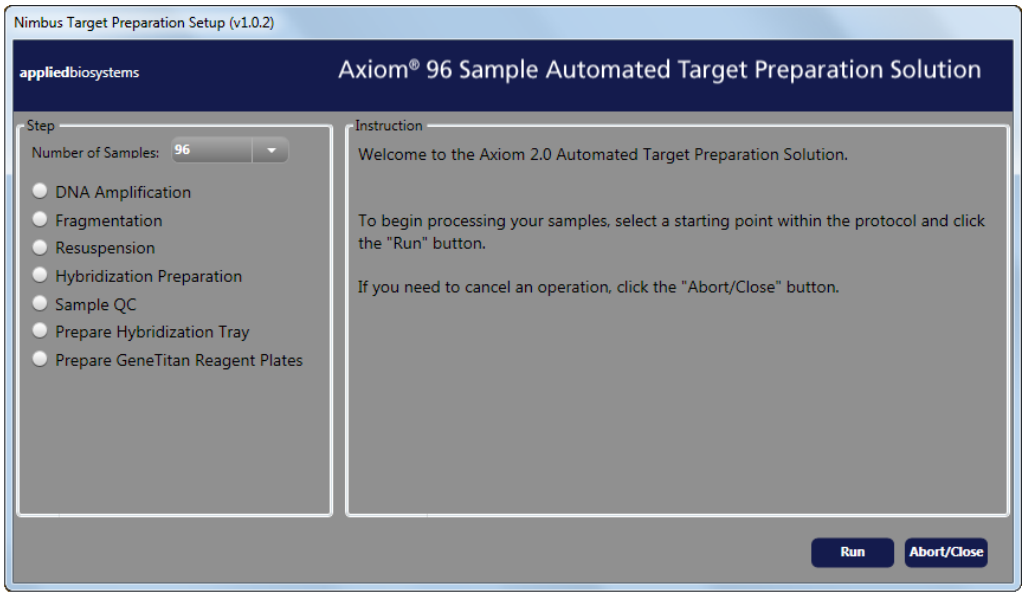
Perform the prerun checklist

The following actions are required before the first operation of the Applied Biosystems™ NIMBUS® Target Preparation Instrument at the start of the day or the start of a run. Some or all these steps can be required depending on the current state of the workstation.

1. Power on the NIMBUS® Instrument workstation.
2. Ensure that all the peripherals are powered on.
   - Multi TEC Controller (controls the CPAC cooling unit and the Thermoshake device).
   - Computer workstation

Note: The CPAC device takes approximately 20 minutes to cool to 4°C. Allow the CPAC device to cool to temperature before placing reagents in the cooling block.

3. Double-click the Axiom 2.0 Assay icon on your desktop to open the NIMBUS Target Preparation Setup window.



4. Continue with the instructions in the sections that follow.

IMPORTANT! Do not run a method at this point.

Prepare for DNA amplification

1. Thaw the Sample Plate (containing 100 ng or 5 ng/µL of gDNA for human).

Note: Do not place a frozen Sample Plate directly on the workstation deck.

2. Thaw the following reagents on the benchtop at room temperature:

- Axiom™ 2.0 Denat Soln 10X
- Axiom™ 2.0 Neutral Soln
- Axiom™ 2.0 Amp Soln
- Axiom™ Water

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

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

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

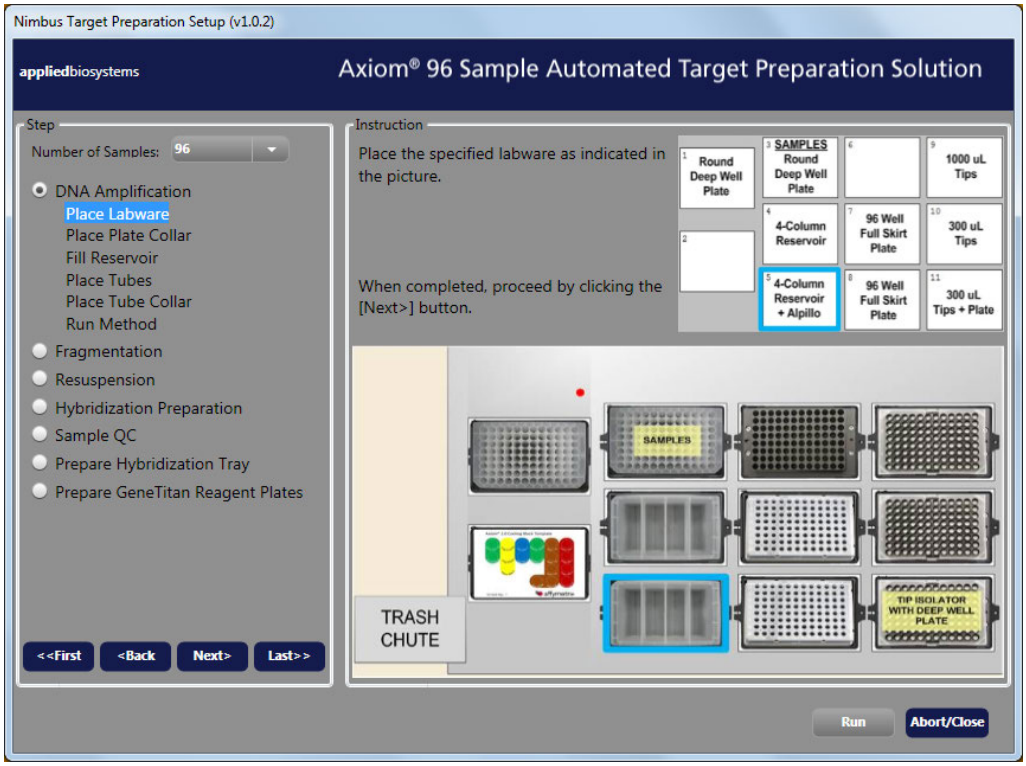
5. Preheat the oven to 37°C.

   We recommend using one of these ovens:
   - BINDER™ ED 56
   - GeneChip™ Hybridization Oven 645 (Do not rotate plates; switch off the rotisserie in the oven.)

Run the DNA amplification step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **DNA Amplification**, then click **Run**.

2. Click **Yes** in the confirmation window to start the DNA amplification step.



3. Place the labware on the deck as instructed.

Note: The 4-column reservoir must always be on a reservoir frame when placed on the NIMBUS® Instrument deck.

4. Place a square deepwell plate underneath the tip isolator and a rack of 300-µL tips on top in deck position 11. See Figure 8.

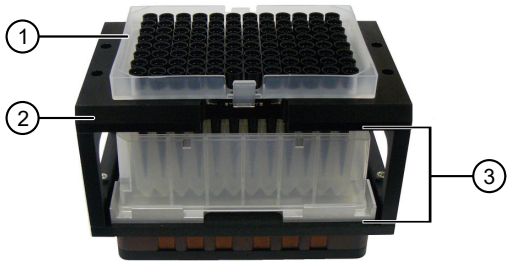
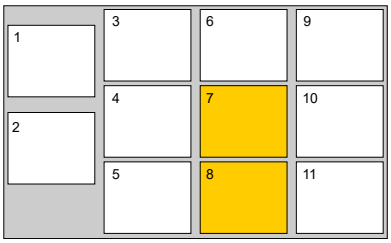


Figure 8 Tip isolator setup.

(1) CO-RE® II Filter Tips, 300 µL
(2) Tip isolator frame
(3) Square deepwell plate as a tip isolator

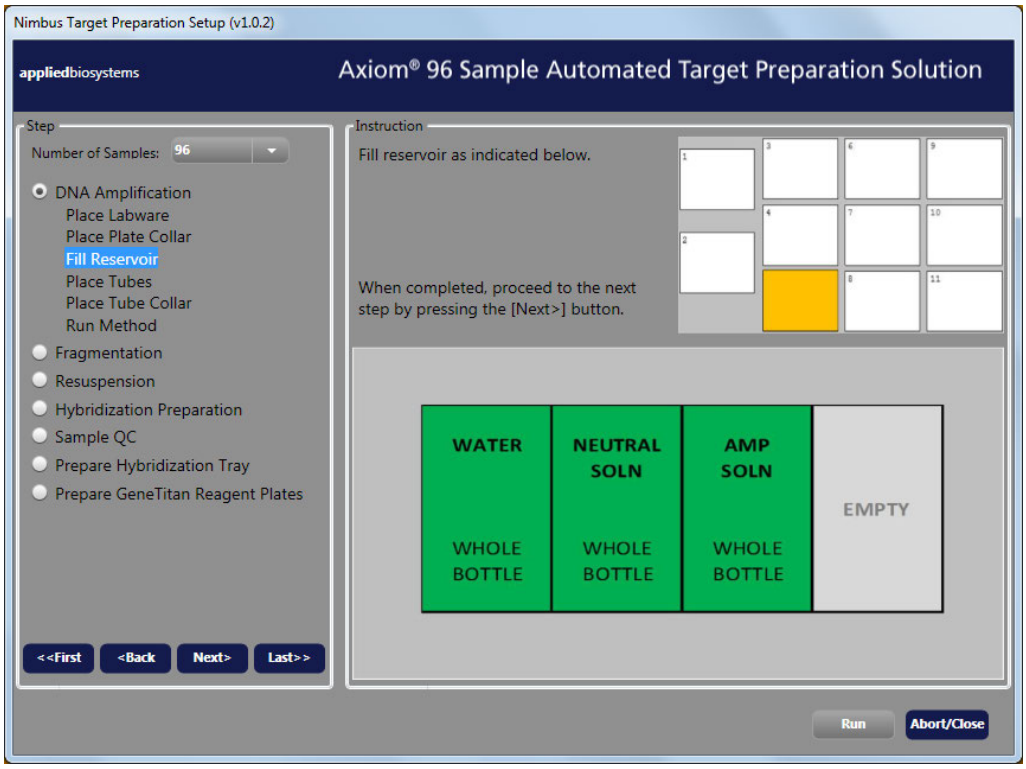
5. When labware setup is complete, click **Next** to continue.

6. Place a plate collar on top of the plates in deck position 7 and deck position 8, as instructed in the **DNA Amplification—Place Plate Collar** window.



Place plate collars on the plates in the deck positions that are highlighted in orange.

7. After placing the plate collars, click **Next**.



8. Fill the reservoir in deck position 5 with the reagents as directed in the **DNA Amplification—Fill Reservoir** window, then click **Next**.

9. Place the Axiom™ 2.0 Denat Soln 10X and Axiom™ 2.0 Amp Enzyme reagent tubes in the cooling block as directed in the **DNA Amplification—Place Tubes** window and the following figure.

IMPORTANT! Immediately before placing the Axiom™ 2.0 Amp Enzyme on the reagent cooling block, gently flick the tube with your finger 2 to 3 times to mix, then centrifuge briefly. Do not vortex.

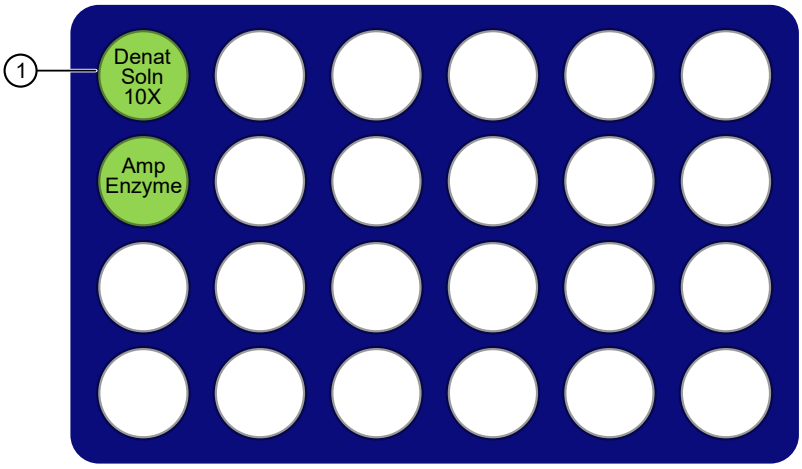


Figure 9 Placement of reagents in cooling block for DNA amplification. Diagram of the cooling block without the reagent template.

(1) A1 position in the upper left corner

IMPORTANT!

- Position all reagents in the reagent cooling block with A1 in the upper left corner of the frame.
- Centrifuge all reagent tubes before placing in block to avoid loss of solution volume to the cap and sides of the tube.
- Press reagent tubes into the block to ensure that they are fully seated.

10. After the reagents have been placed in the cooling block, click **Next**.

11. Place the tube collar on the cooling block in deck position 2, then click **Next**.

12. Check the deck layout to ensure that all labware and reagents are in the proper locations.

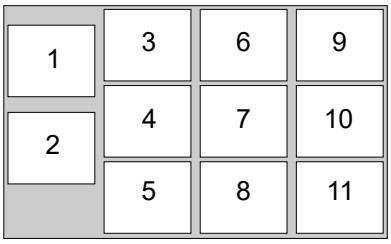
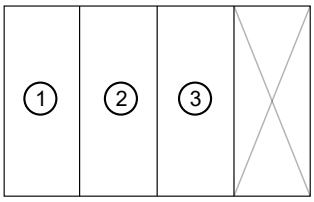


Figure 10 NIMBUS® Instrument deck positions.

Table 12 Labware and reagent locations on the deck for the DNA amplification step.

Deck position	Labware	Reagent or sample
1	Round deepwell plate	—
2	Cooling block + tube collar	See Figure 9
3	Round deepwell plate	gDNA samples
4	4-column reservoir + reservoir frame	4-column reservoir is empty

Table 12 Labware and reagent locations on the deck for the DNA amplification step. *(continued)*

Deck position	Labware	Reagent or sample
5	4-column reservoir + reservoir frame + Alpillo™	 ① Axiom™ Water ② Axiom™ 2.0 Neutral Soln ③ Axiom™ 2.0 Amp Soln
7	96-well full-skirt plate + plate collar	—
8	96-well full-skirt plate + plate collar	—
9	CO-RE® II Filter Tips, 1,000 µL	—
10	CO-RE® II Filter Tips, 300 µL	—
11	CO-RE® II Filter Tips, 300 µL + Square deepwell plate	—

Note: If the physical deck does not match the deck layout that is described in Table 12, either modify the physical deck to match or click **Abort/Close** in the **DNA Amplification—Run Method** window.

13. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start.
    The DNA Amplification method runs until the Amplification Master Mix has been added to the sample plate. After complete, instructions that are shown in the **DNA Amplification—Cleanup** window.

14. Click **Finish** when deck cleanup is complete, then click **Yes** in the **Finish Confirmation** window.

15. Save or discard the labware as instructed.

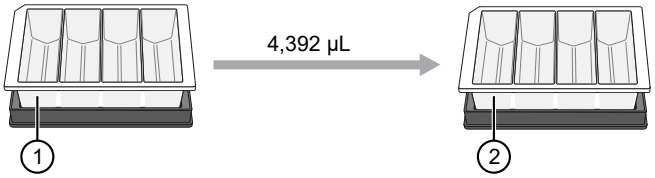
16. Remove the sample plate from deck position 3.
    a. Blot the top of the plate with a laboratory tissue to remove any droplets present.
    b. Tightly seal the plate.
    c. Vortex, then centrifuge the plate.
    d. Place in a preheated oven, then incubate at 37°C for 23 ±1 hour.

    Note: If using the GeneChip™ Hybridization Oven 645, place the plate on the bottom of the oven. Do not rotate plates; switch off the rotisserie in the oven.

e. After 22–24 hours of incubation, do one of the following:
   - Proceed directly to "Stage 2: Fragment and precipitate the DNA" on page 63.
   - Tightly seal and store the amplified DNA samples at –20°C.

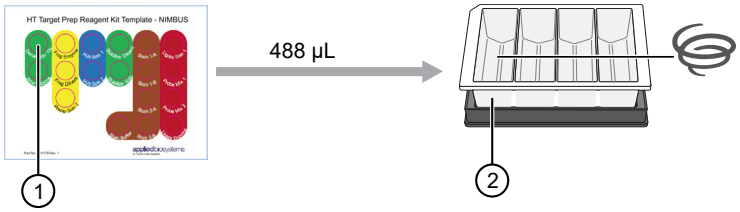
Workflow for Stage 1B: DNA amplification

Transfer Axiom™ Water to D1 MM Reservoir



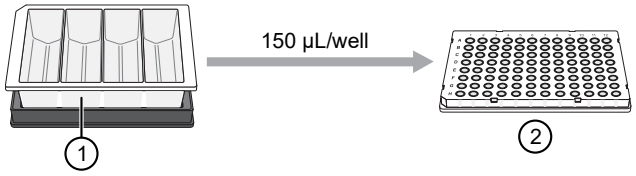
① Axiom™ Water, column 1 (deck position 5)
② D1 MM Reservoir, column 1 (deck position 4)

Transfer Axiom™ 2.0 Denat Soln 10X to D1 MM Reservoir



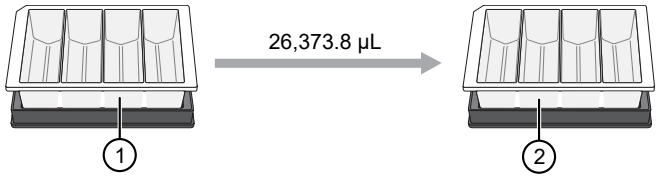
① Axiom™ 2.0 Denat Soln 10X (deck position 2)
② D1 MM Reservoir, column 1 (deck position 4)
Mix

Prepare N1 Distribution Plate



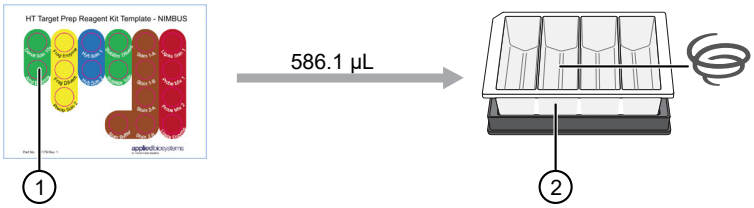
① Axiom™ 2.0 Neutral Soln, column 2 (deck position 5)
② N1 Distribution Plate (deck position 8)

Transfer Axiom™ 2.0 Amp Soln to Amp MM



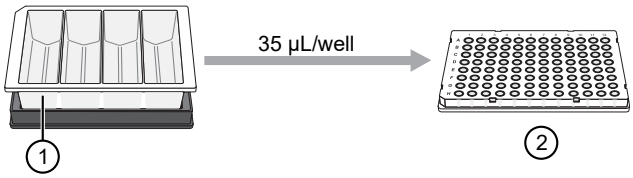
① Axiom™ 2.0 Amp Soln, column 3 (deck position 5)
② Amp MM Reservoir, column 2 (deck position 4)

Transfer Axiom™ 2.0 Amp Enzyme to Amp MM



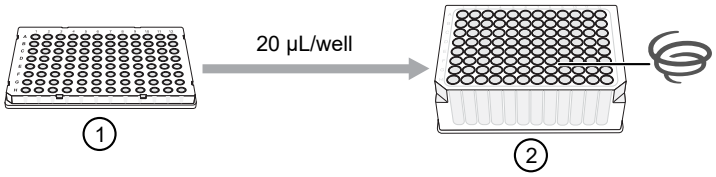
① Axiom™ 2.0 Amp Enzyme (deck position 2)
② Amp MM Reservoir, column 2 (deck position 4)
Mix

Prepare D1 Distribution Plate



① D1 MM Reservoir, column 1 (deck position 4)
② D1 Distribution Plate (deck position 7)

Transfer D1 to Sample Plate

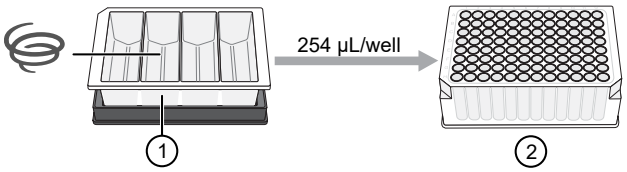


① D1 Distribution Plate (deck position 7)
② Sample Plate (deck position 3)
Mix

Incubate Sample Plate on deck for 10 minutes at room temperature.

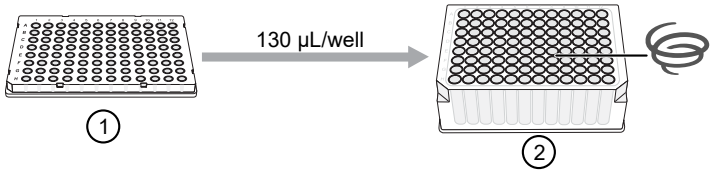
This step occurs during the 10 minute on-deck incubation.

Prepare Amp Distribution Plate



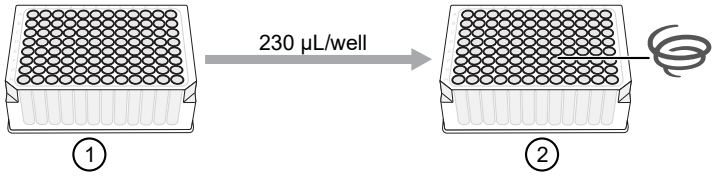
① Amp MM Reservoir in column 2 (deck position 4)
② Amp MM Dist Plate (deck position 1)
Mix

Transfer N1 to Sample Plate



① N1 Dist Plate (deck position 8)
② Sample Plate (deck position 3)
Mix

Transfer Amp MM to Sample Plate



① Amp MM Dist Plate (deck position 1)
② Sample Plate (deck position 3)
Mix

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

Stage 2: Fragment and precipitate the DNA

Time required

Activity	Time
Hands-on time	~25 minutes ~50 minutes if frozen amplified DNA from Stage 1
NIMBUS® Instrument—Fragmentation • Deactivation incubation—20 minutes to deactivate the amplification reaction and 20 minutes to equilibrate to the fragmentation temperature • Fragmentation incubation—30 minutes	~1.5 hours
Off-line precipitation incubation at –20°C	3 hours (optional), or overnight (16–18 hours)
Total (does not include precipitation time)	**~2–2.5 hours**

Input required

The Sample Plate of amplified DNA from Stage 1 in a round deepwell plate.

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
Equipment and consumables	
As required	Adhesive seals for 96-well plates
1	Benchtop cooler, chilled to –20°C
1	Freezer, –20°C
1	Ice bucket, filled with ice
As required	Laboratory tissues
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Oven preheated to 37°C
1	Plate centrifuge
1	Vortexer (for plates and microtubes)

(continued)

Quantity	Item
Labware	
2	4-column reservoir[1]
1	96-well full-skirt plate[1]
1	Square 1.2-mL plate[1]
2	Square deepwell plate[1]
1 frame	CO-RE® II Filter Tips, 1,000 µL
2 frames	CO-RE® II Filter Tips, 300 µL
1	Alpillo™ Plate Cushion and extension
2	Reservoir frame
1	Plate collar
1	Tube collar

[1] From the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No. 952437).

Reagents required

Reagent and cap color	Module
From the Axiom™ 2.0 Plus Reagent Kit 96F	
Axiom™ Frag Enzyme (leave at –20°C until ready to use)	Module 2-1, –20°C Part No. 901528
Axiom™ 10X Frag Buffer	
Axiom™ Precip Soln 2	
Axiom™ Frag Diluent	Module 2-2, 2—8°C Part No. 901529
Axiom™ Frag Rxn Stop	
Axiom™ Precip Soln 1	
User-supplied	
Isopropanol, 99.5%, 70 mL	—

Perform the prerun checklist

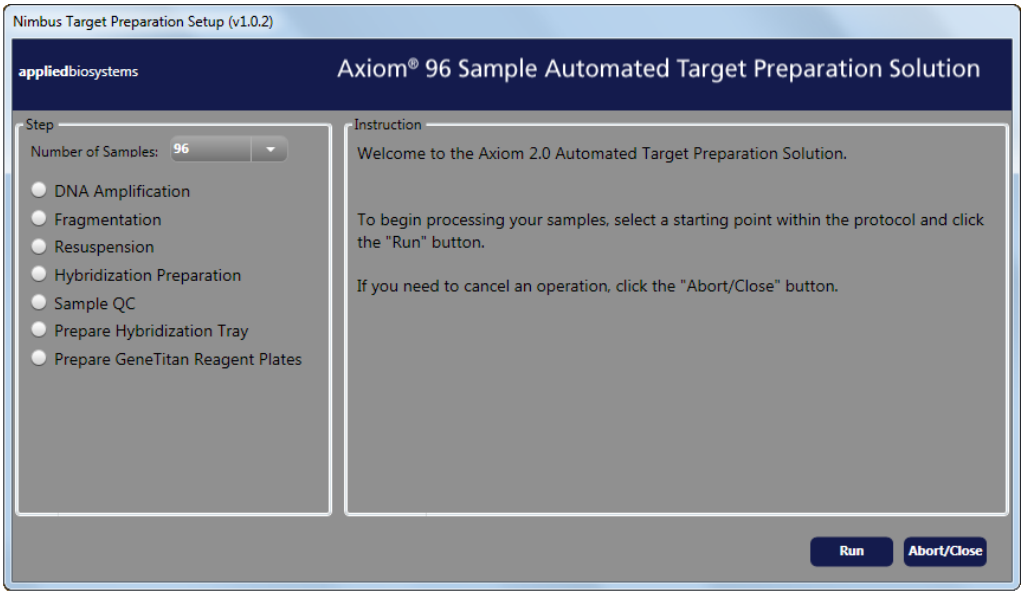
The following actions are required before the first operation of the Applied Biosystems™ NIMBUS® Target Preparation Instrument at the start of the day or the start of a run. Some or all these steps can be required depending on the current state of the workstation.

1. Power on the NIMBUS® Instrument workstation.

2. Ensure that all the peripherals are powered on.
   - Multi TEC Controller (controls the CPAC cooling unit and the Thermoshake device).
   - Computer workstation

   Note: The CPAC device takes approximately 20 minutes to cool to 4°C. Allow the CPAC device to cool to temperature before placing reagents in the cooling block.

3. Double-click the Axiom 2.0 Assay icon on your desktop to open the NIMBUS Target Preparation Setup window.



4. Continue with the instructions in the sections that follow.

IMPORTANT! Do not run a method at this point.

Prepare for fragmentation

Prepare amplified DNA Sample Plate

The following steps are for preparing a plate of amplified samples that has been frozen.

1. Place the deepwell plate in a small bath of room temperature ultra-pure water.
2. Leave the plate in the water bath for ~50 minutes until all wells have thawed.
3. Centrifuge the plate at 1,000 rpm for 30 seconds.
4. To avoid cross-contamination of wells during vortexing:
   a. Remove the seal, then blot the top of the plate with a laboratory tissue.
   b. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 seconds to mix.
6. Centrifuge the plate at 1,000 rpm for 30 seconds.

Thaw and prepare the mPCR Reaction Plate

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

1. Thaw the plate at room temperature for approximately 20 minutes or until all samples are thawed.
2. Centrifuge the plate at 1,000 rpm for 30 seconds.
3. To avoid cross-contamination of wells during vortexing, remove the seal, then tightly reseal the plate using a fresh seal.
4. Vortex the plate for 10 seconds to mix.
5. Centrifuge at 1,000 rpm for 30 seconds.

Thaw and prepare the reagents

1. Thaw the following reagents on the benchtop at room temperature:
   - Axiom™ 10X Frag Buffer
   - Axiom™ Precip Soln 2
2. Vortex all reagents (except Axiom™ Frag Enzyme), then place on ice.
   - Vortex, then briefly centrifuge the Axiom™ Precip Soln 2 before placing onto deck.
   - For the Axiom™ Frag Enzyme, leave at –20°C until ready to use. Just before placing the enzyme on the deck, gently flick the tube 3 times to mix, then centrifuge briefly.

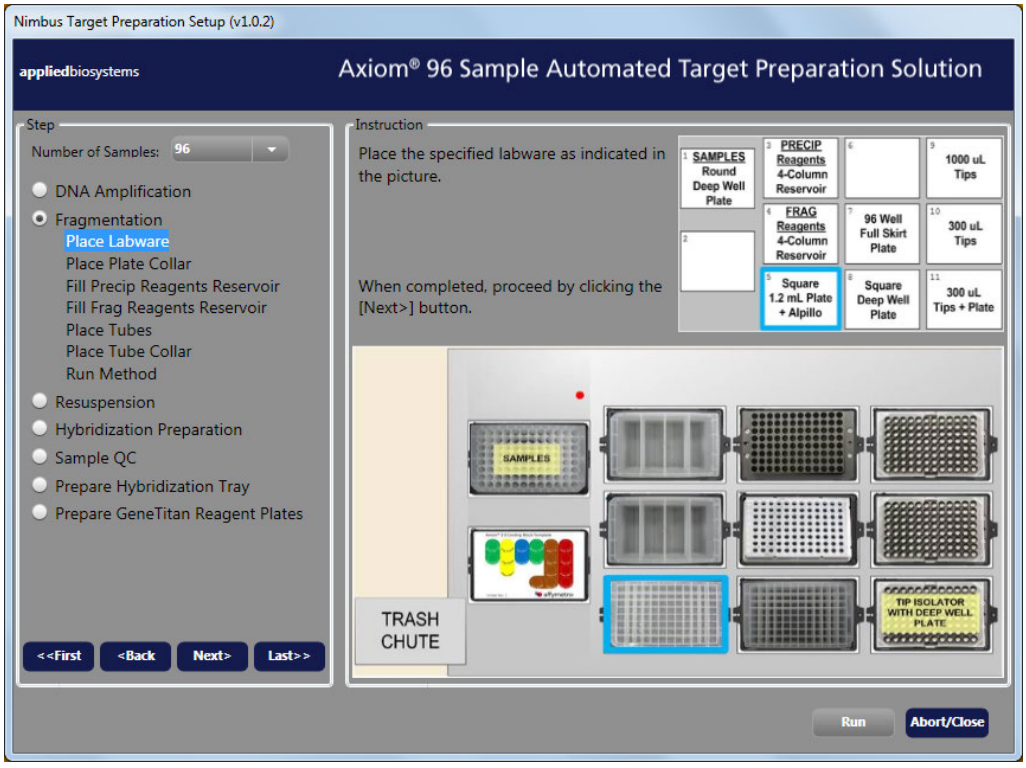
Perform mPCR spike-in to Amplification Plate

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

1. Transfer 10 µL of the mPCR reactions into the corresponding well of the amplified DNA Sample Plate. Pipette up and down a few times to ensure complete liquid transfer from pipette tip.
2. Seal the plate. Ensure that the seal is securely attached to the plate to minimize evaporation during next steps.
3. Thoroughly mix by vortexing plate for 30 seconds, then centrifuge briefly.
4. Immediately proceed to the next step. ("Run the fragmentation step, then precipitate samples" on page 67.)

Run the fragmentation step, then precipitate samples

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Fragmentation**, then click **Run**.
2. Click **Yes** in the confirmation window to start the fragmentation step.



3. Place the labware on the deck as instructed.

IMPORTANT! Remove the seal from the sample plate before placing on the deck.

Note: The 4-column reservoir must always be on a reservoir frame when placed on the NIMBUS® Instrument deck.

4. Place a square deepwell plate underneath the tip isolator frame and a rack of 300-µL tips on top in deck position 11 (Figure 11).

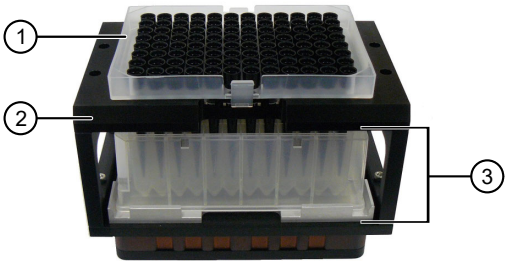
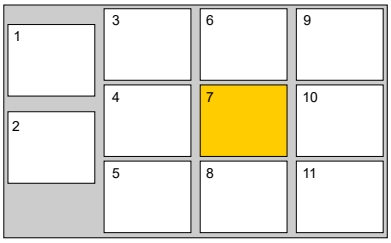


Figure 11 Tip isolator setup.

① CO-RE® II Filter Tips, 300 µL
② Tip isolator frame
③ Square deepwell plate as a tip isolator

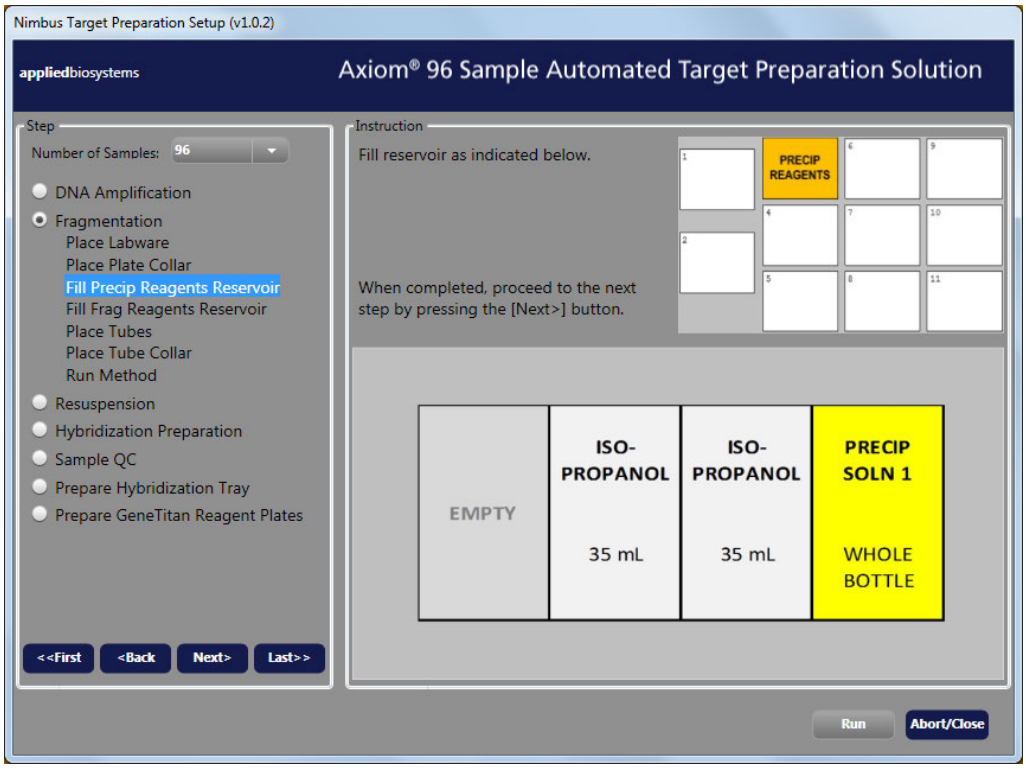
5. When labware setup is complete, click **Next** to continue.

6. Place a plate collar on top of the plate in deck position 7 as instructed in the **Fragmentation—Place Plate Collar** window.

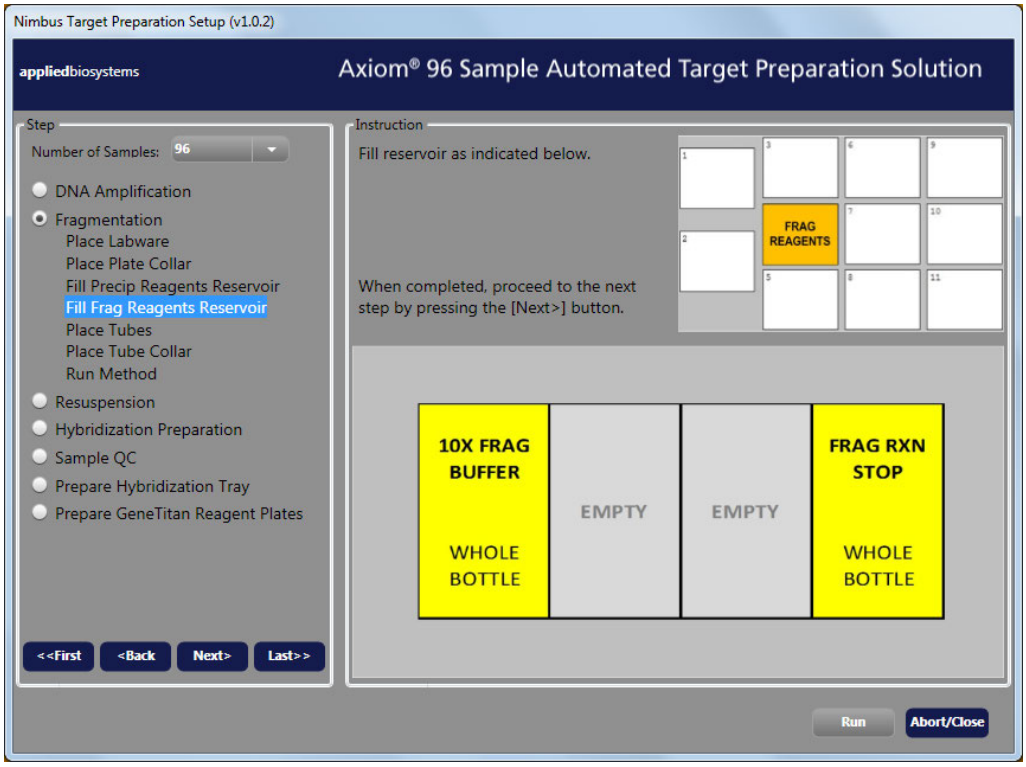


Place a plate collar on the plate in the deck position that is highlighted in orange.

7. After placing the plate collar, click **Next**.



8. Fill the reservoir in deck position 3 with the reagents as directed in the **Fragmentation—Fill Precip Reagents Reservoir** window, then click **Next**.



9. Fill the reservoir in deck position 4 with the reagents as directed in the **Fragmentation—Fill Frag Reagents Reservoir** window, then click **Next**.

10. Place the Axiom™ Frag Enzyme, Axiom™ Frag Diluent, and Axiom™ Precip Soln 2 reagent tubes in the cooling block as directed in the **Fragmentation—Place Tubes** window and the following figure.

IMPORTANT! Immediately before placing the Axiom™ Frag Enzyme on the reagent cooling block, gently flick the tube with your finger 2 to 3 times to mix, then centrifuge. Do not vortex.

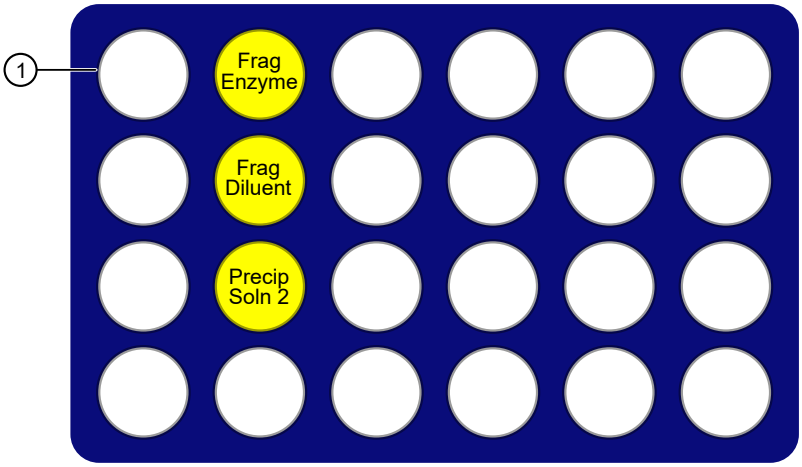


Figure 12 Placement of reagents in cooling block for fragmentation. Diagram of the cooling block without the reagent template.

① A1 position in the upper left corner

IMPORTANT!

- Axiom™ Frag Enzyme—Flick to mix 3 times, then centrifuge immediately before placing on the cooling block.
- Position all reagents in the reagent cooling block with A1 in the upper left corner of the frame.
- Centrifuge all reagent tubes before placing in block to avoid loss of solution volume to the cap and sides of the tube.
- Press reagent tubes into the block to ensure that they are fully seated.

11. After the reagents have been placed in the cooling block, click **Next**.

12. Place the tube collar on the cooling block in deck position 2, then click **Next**.

13. Check the deck layout to ensure that all labware and reagents are in the proper locations.

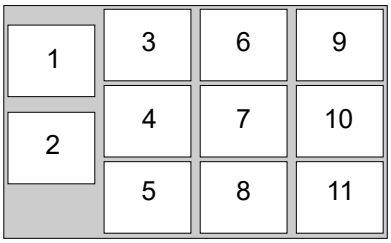
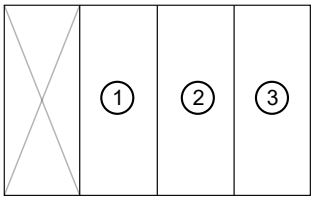
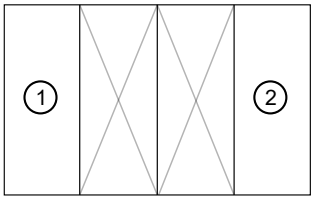


Figure 13 NIMBUS® Instrument deck positions.

Table 13 Labware and reagent locations on the deck for the fragmentation step.

Deck position	Labware	Reagent or sample
1	Round deepwell plate	Amplified gDNA
2	Cooling block + tube collar	See Figure 12
3	4-column reservoir + reservoir frame (Precipitation reagents)	 ① 35 mL isopropanol ② 35 mL isopropanol ③ Axiom™ Precip Soln 1
4	4-column reservoir + reservoir frame (Fragmentation reagents)	 ① Axiom™ 10X Frag Buffer ② Axiom™ Frag Rxn Stop
5	Square 1.2-mL plate + Alpillo™	—
7	96-well full-skirt plate + plate collar	—
8	Square deepwell plate	—
9	CO-RE® II Filter Tips, 1,000 µL	—
10	CO-RE® II Filter Tips, 300 µL	—
11	CO-RE® II Filter Tips, 300 µL + Square deepwell plate	—

Note: If the physical deck does not match the deck layout that is described in Table 13, either modify the physical deck to match or click **Abort/Close** in the **Fragmentation—Run Method** window.

14. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start.

    The fragmentation method starts. The sample plate is incubated at 65°C to inactivate amplification. When complete the **Fragmentation—Cleanup** window appears.

15. Remove the Sample Plate from deck position 8.

    The Sample Plate is now known as the Precipitation Plate.

    a. Blot the top of the plate with a laboratory tissue, then seal tightly.

    b. Place the plate in a –20°C freezer overnight to precipitate the DNA.

A new option for DNA target precipitation is to incubate the plate in the –20°C freezer for 3 hours, instead of overnight. This shortened precipitation allows you to proceed to Stage 3: Centrifuge and dry pellets, followed by stages 4A–C: Resuspension, hybridization preparation, sample QC, and Stage 5: Prepare the hybridization tray Stage 5: Prepare the hybridization tray to start hybridization on the GTMC on day 2 of the assay workflow.

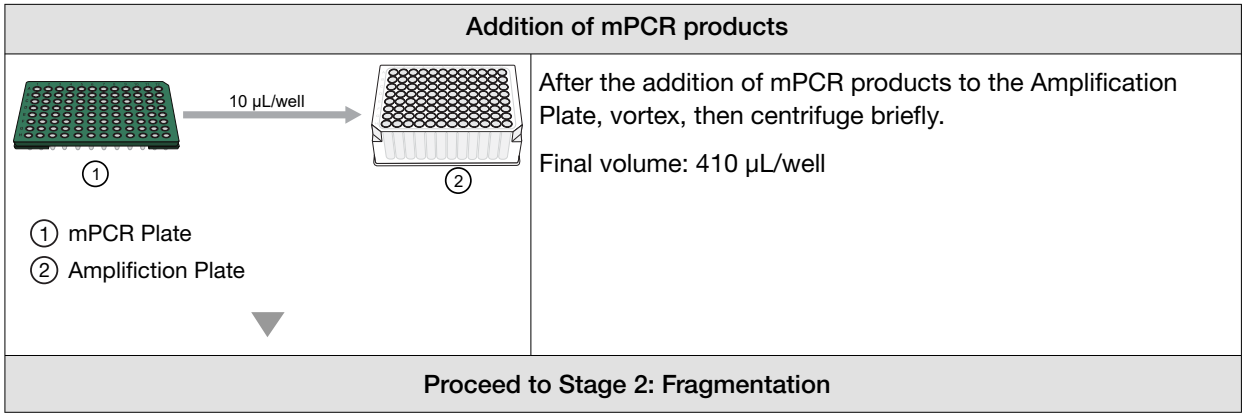
Note: If possible, designate a shelf in a –20°C freezer where the plates can be left undisturbed. In addition, the freezer must not be subjected to frequent temperature excursions.

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

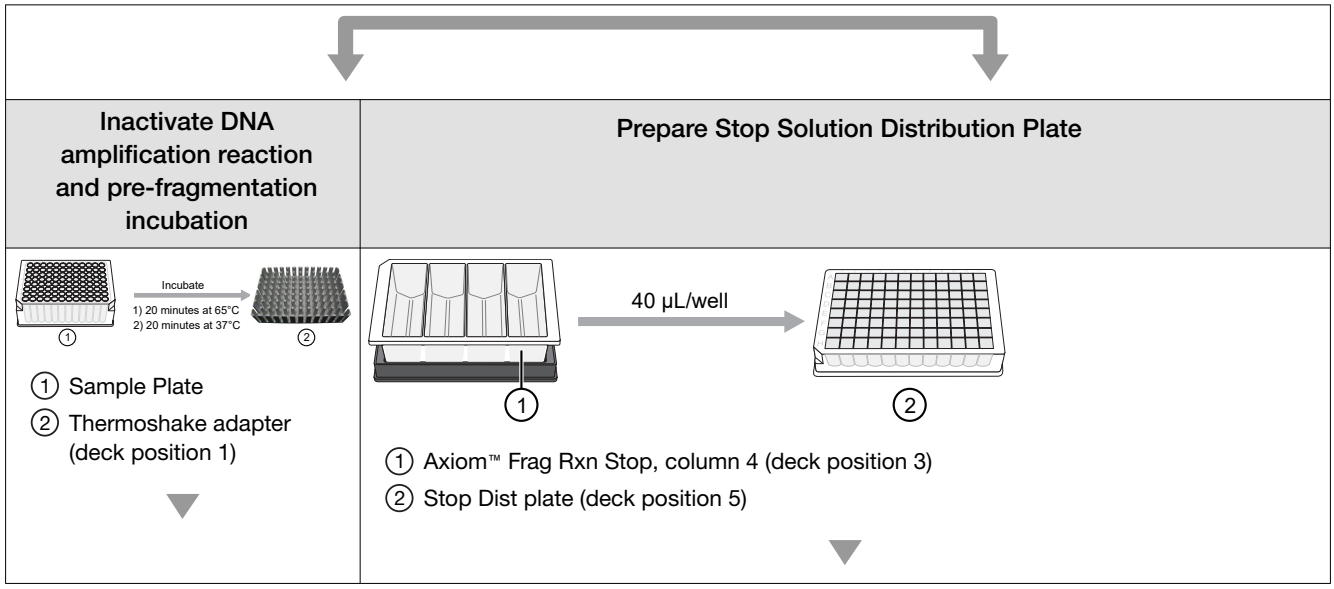
16. Save or discard the labware as instructed.

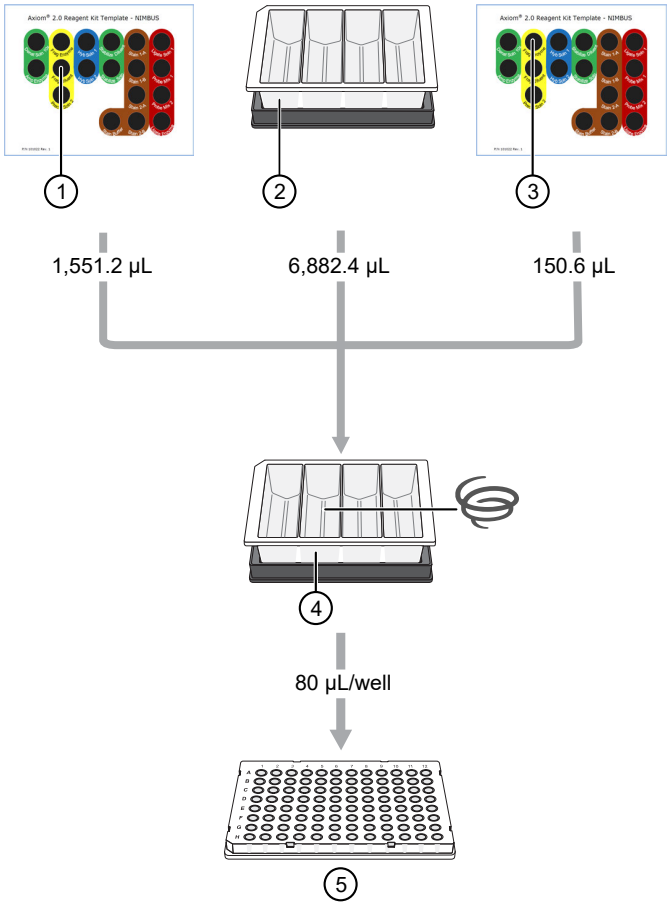
17. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.

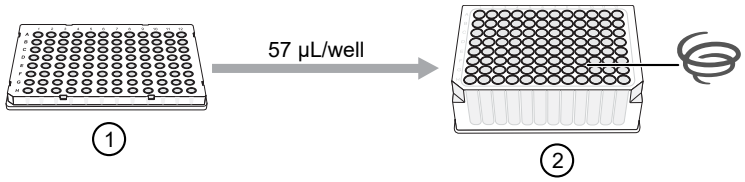
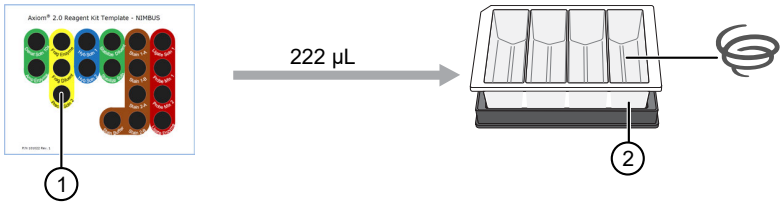
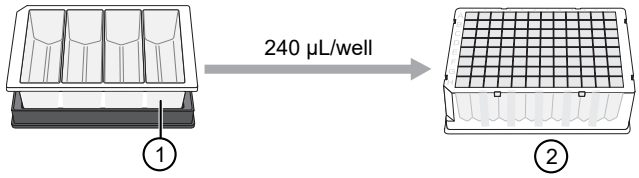
Workflow for mPCR spike-in to Amplification Plate

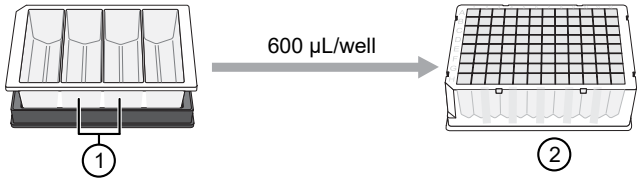


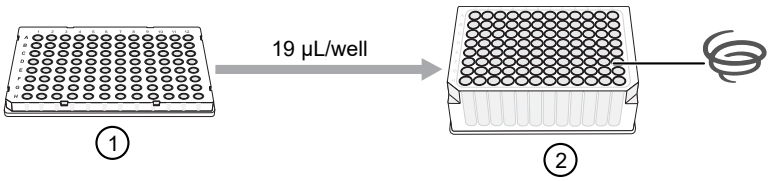
Workflow for Stage 2: Fragmentation

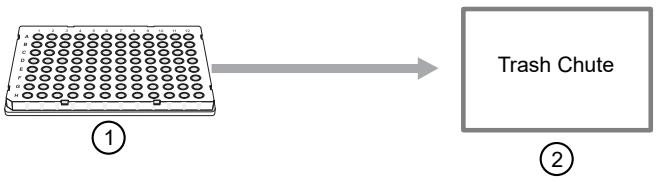


	Prepare Fragmentation Master Mix
	 ① Axiom™ Frag Diluent (deck position 2) ② Axiom™ 10X Frag Buffer, column 1 (deck position 4) ③ Axiom™ Frag Enzyme (deck position 2) ④ Fragmentation Master Mix reservoir, column 2 (deck position 4) ⑤ Frag MM Dist Plate (deck position 7) Mix

	Transfer Fragmentation Master Mix to Sample Plate
Incubate on Thermoshake adapter (deck position 1) 30 minutes at 37°C	 ① Frag MM Dist Plate (deck position 7) ② Sample Plate (deck position 1) Mix
	Add Axiom™ Precip Soln 2 to Axiom™ Precip Soln 1
	 ① Axiom™ Precip Soln 2 (deck position 2) ② Axiom™ Precip Soln 1, column 4 (deck position 3) Mix
	Add Precipitation Solution to Distribution Plate
	 ① Precipitation Solution, column 4 (deck position 3) ② Precip Plate (deck position 8)

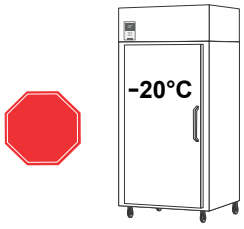
<table>
<tr><td></td><td>Add Isopropanol to Distribution Plate</td></tr>
<tr><td> 
End of fragmentation incubation.</td><td> 

① Isopropanol, columns 2 and 3 (deck position 3)
② Precip Dist Plate (deck position 8)</td></tr>
<tr><td></td><td>Transfer Stop Solution to Sample Plate</td></tr>
<tr><td></td><td> 

① Stop Dist Plate (deck position 5)
② Sample Plate (deck position 1)
Mix</td></tr>
<tr><td></td><td>Discard Stop Distribution Plate</td></tr>
<tr><td></td><td> 

① Stop Dist Plate (deck position 5)
② Trash chute</td></tr>
<tr><td></td><td>Move Sample Plate from Thermoshake to deck position 5</td></tr>
<tr><td></td><td> 

① Thermoshake adapter (deck position 1)
② Sample Plate to deck position 5</td></tr>
</table>

	Transfer Sample Mix to Precipitation Plate
	 ① Sample Plate (deck position 5) ② Precip Plate (deck position 8) Mix
	Offline precipitation
	Incubate precipitation plate containing samples at –20°C freezer overnight, or 3 hours (optional). 

Stage 3: Centrifuge and dry pellets

Time required

Activity	Time
Hands-on time	~10 minutes
Centrifugation	40 minutes
Drying	25 minutes
Total	**~75 minutes**

Input required

One plate of precipitated samples from Stage 2 in a square deepwell plate.

Equipment and consumables required

Quantity	Item
As required	Adhesive seals for 96-well plates
As required	Laboratory tissues
1	Plate centrifuge precooled to 4°C
1	Oven preheated to 37°C

Centrifuge and dry the pellets

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

Note: Keep the centrifuge ready at 4°C.

1. Power on the oven and preheat to 37°C.
2. Transfer the Precipitation Plate from the –20°C freezer to a pre-chilled centrifuge.

3. Centrifuge the plate for 40 minutes at 4°C at 3,200 x *g*.

   4,000 rpm for the Eppendorf 5810R centrifuge with the rotor configuration that is described in the *Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow Site Preparation Guide—Applied Biosystems™ NIMBUS® Target Preparation Instrument*.

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

4. Immediately after the 40-minute centrifugation time, empty the liquid from the plate using the following steps:
   a. Remove the seal.
   b. Invert the plate over a waste container, then allow the liquid to drain.

      Note: It is normal for the intensity of the blue color between pellets to vary and the color variation does not indicate any significant differences in the yield of precipitated DNA.

   c. While still inverted, gently press the plates on a pile of laboratory tissues on a bench, then allow them to drain for 5 minutes. Transfer the plates to a new pile of tissues twice during the 5-minute time frame.

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

   Note: If using a GeneChip™ Hybridization Oven 645, place the plate on the bottom of the oven. Do not rotate plates; switch off the rotisserie in the oven.

6. Do one of the following:
   - Proceed directly to "Stage 4A and 4B: Resuspension and hybridization preparation" on page 80, even if some droplets of liquid remain. Leave the sample plate at room temperature. It is helpful to start preparing reagents for Stage 4A and 4B while centrifuging and drying pellets.
   - Store the plates for resuspension later in the same day. Tightly seal the plates.
     - If resuspension is carried out within 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).
   - Store the plates for resuspension on another day. Tightly seal the plate and store at –20°C.

Stage 4A and 4B: Resuspension and hybridization preparation

In Stage 4A: Resuspension, the Axiom™ Resusp Buffer is added to the plate of pelleted DNA by the Applied Biosystems™ NIMBUS® Target Preparation Instrument. The samples are then resuspended by shaking off-deck. During the off-deck resuspension, you must use this time to prepare the deck for Stage 4B: Hybridization preparation, to maintain the timing that is required to run the multiplate workflow.

We recommend thawing the Module 2-1 and Module 2-2 reagents before starting Stage 4A to minimize any time lapses between stages. The resuspension step must be immediately followed by hybridization preparation (see "Stage 4B: Prepare Hybridization Master Mix" on page 90).

Time required

Activity	Time
Off-deck shaking	10 minutes
Hands-on time	~15 minutes
Frozen pellet equilibration to room temperature	1.5 hours
NIMBUS® Instrument—Resuspension	4 minutes
NIMBUS® Instrument—Hybridization preparation	15 minutes
Total	**~2.25 hours**

Input required

- **Stage 4A: Resuspension**—Pelleted DNA from Stage 3 in a square deepwell plate.
- **Stage 4B: Hybridization preparation**—Resuspended DNA from Stage 4A in a square deepwell plate.

Materials, labware, and reagents required

Equipment and consumables required

Quantity	Item
Equipment and consumables	
As required	Adhesive seals for 96-well plates
1	Shaker, either: • Thermo Scientific™ Digital Microplate Shaker • Thermo Scientific™ Compact Digital Microplate Shaker • Boekel Scientific™ Jitterbug™

(continued)

Quantity	Item
1	Benchtop cooler, chilled to –20°C
1	Ice bucket, filled with ice
1	Marker, fine point, permanent
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Plate centrifuge at room temperature
1	Vortexer

Labware required

Quantity Stage 4A	Quantity Stage 4B	Item
1	1	96-well full-skirt plate[1]
—	1	96 half-skirt plate[1]
—	1	96 plate holder[1]
1	1	4-column reservoir[1]
—	1 frame	CO-RE® II Filter Tips, 1,000 µL
2 frames	2 frames	CO-RE® II Filter Tips, 300 µL
1	1	Alpillo™ Plate Cushion and extension
1	1	Reservoir frame
2	2	Plate collar
—	1	Tube collar

[1] From the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No.952437).

Reagents required

Reagent and cap color	Module	Stage used
From the Axiom™ 2.0 Plus Reagent Kit 96F		
Axiom™ Hyb Buffer	Module 2-1, –20°C (Part No. 901528)	Stage 4B
Axiom™ Hyb Soln 1		Stage 4B
Axiom™ Resusp Buffer	Module 2-2, 2–8°C (Part No. 901529)	Stage 4A
Axiom™ Hyb Soln 2		Stage 4B

Thaw reagents

Note: Prepare the reagents when indicated as you move through the procedure for each stage.

1. Thaw the reagents for Stage 4A: Resuspension.
   - Warm the Axiom™ Resusp Buffer on the benchtop at room temperature for at least 1 hour.
2. Thaw the reagents for Stage 4B: Hybridization preparation.
   - Thaw the Axiom™ Hyb Soln 1 on the benchtop at room temperature.
   - Place the Axiom™ Hyb Buffer and the Axiom™ Hyb Soln 2 on ice.

Stage 4A: Prepare the resuspension buffer

The resuspension stage must be immediately followed by "Stage 4B: Prepare Hybridization Master Mix" on page 90.

Frozen pellets and Axiom™ Resusp Buffer

Guidelines for preparation of frozen pellets and Axiom™ Resusp Buffer

The equilibration of resuspension buffer and pellets to room temperature (18°C to 25°C) is critical for the success of the Axiom™ 2.0 Plus Assay. When either the pellets or buffer is cooler than room temperature, the pellets might not resuspend completely. Pellets that are not resuspended can result in compromised assay performance. Observe following guidelines on how to work with plates with fresh, cold, or frozen pellets.

- If proceeding with the resuspension and hybridization preparation protocol within 4 hours, plates with fresh pellets can be kept at room temperature.
- Plates with fresh pellets that are not processed within 4 hours can be transferred to a refrigerator (2–8°C) for a few hours only if processed within a day. However, it is critical to equilibrate the plate to room temperature for at least 30 minutes before proceeding with the resuspension and hybridization protocol.
- Plates with frozen pellets must be pre-equilibrated at room temperature for at least 1.5 hours before proceeding with the resuspension and hybridization protocol. For example, plates with frozen pellets are used on day 5 of the 8-plate workflow.
- The Axiom™ Resusp Buffer needs at least 1 hour to equilibrate to room temperature.

Prepare frozen pellets and Axiom™ Resusp Buffer

IMPORTANT! The pellets and the resuspension buffer must be at room temperature before proceeding with this step.

Equilibrate the pellets and Axiom™ Resusp Buffer to room temperature (18°C to 25°C) following the information in "Guidelines for preparation of frozen pellets and Axiom™ Resusp Buffer" on page 83.

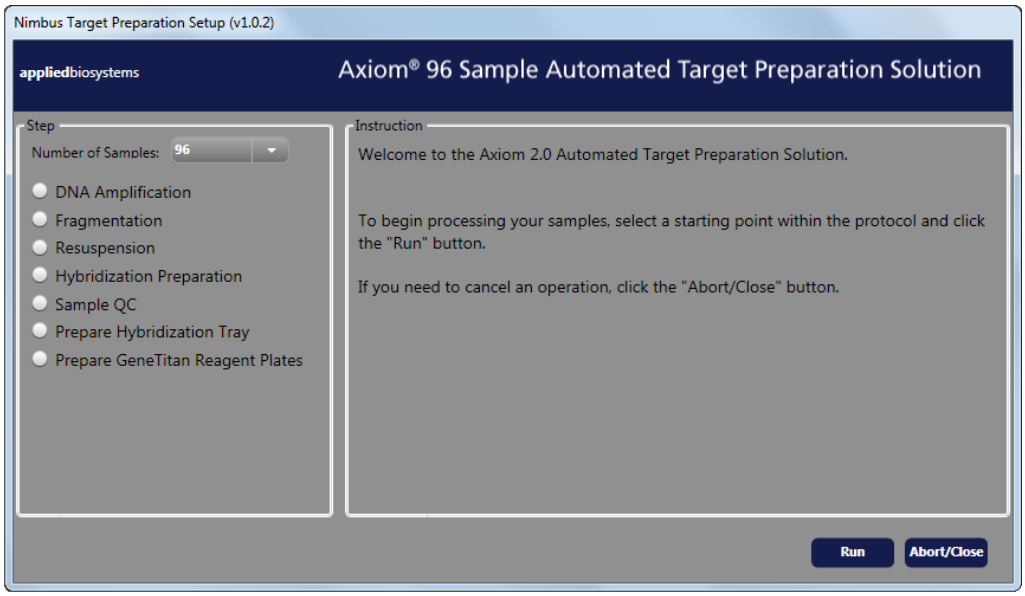
Perform the prerun checklist

The following actions are required before the first operation of the Applied Biosystems™ NIMBUS® Target Preparation Instrument at the start of the day or the start of a run. Some or all these steps can be required depending on the current state of the workstation.

1. Power on the NIMBUS® Instrument workstation.
2. Ensure that all the peripherals are powered on.
   - Multi TEC Controller (controls the CPAC cooling unit and the Thermoshake device).
   - Computer workstation

   Note: The CPAC device takes approximately 20 minutes to cool to 4°C. Allow the CPAC device to cool to temperature before placing reagents in the cooling block.

3. Double-click the Axiom 2.0 Assay icon on your desktop to open the NIMBUS Target Preparation Setup window.



4. Continue with the instructions in the sections that follow.

IMPORTANT! Do not run a method at this point.

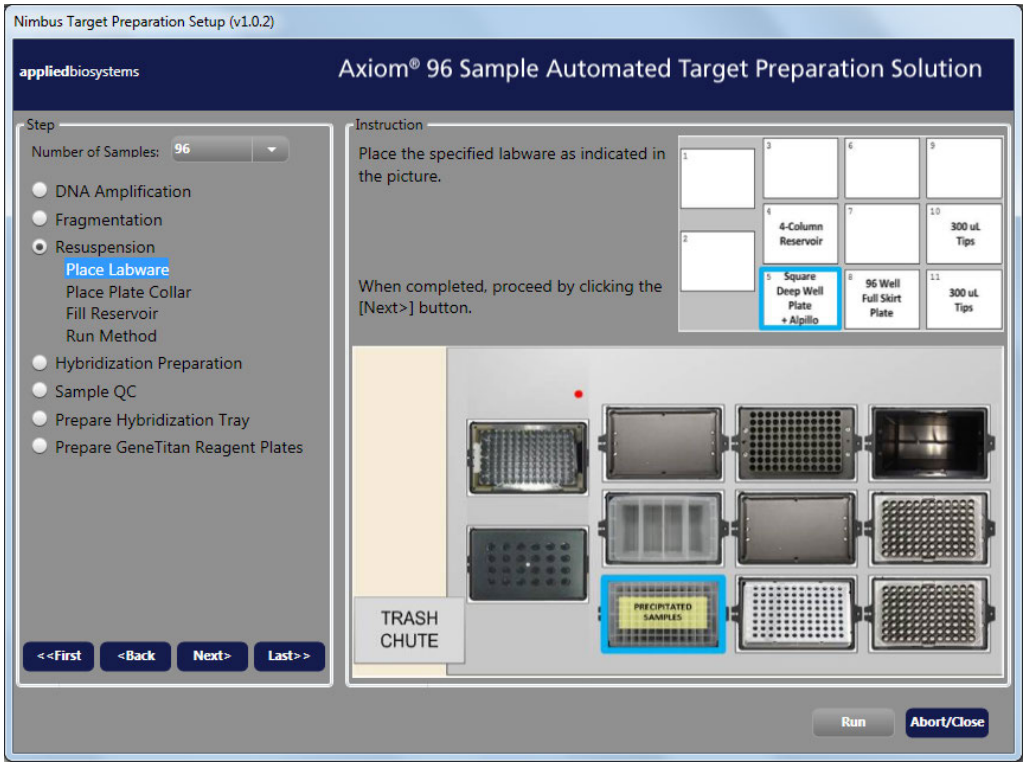
Prepare resuspension reagents

Note: The Axiom™ Resusp Buffer must be warmed on the benchtop at room temperature for at least 1 hour as instructed in the section “Thaw reagents” on page 82.

Vortex the Axiom™ Resusp Buffer that has been warmed to room temperature.

Run the resuspension step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Resuspension**, then click **Run**.
2. Click **Yes** in the confirmation window to start the resuspension step.



3. Place the labware on the deck as instructed.

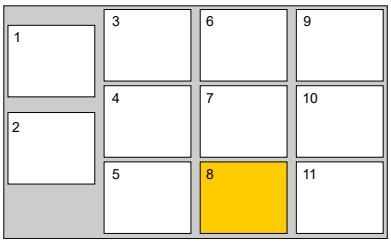
IMPORTANT! If needed, remove the seal from the sample plate before placing on the deck.

Note: The 4-column reservoir must always be on a reservoir frame when placed on the NIMBUS® Instrument deck.

Note: A square deepwell plate is not required under the tip isolator in deck position 11 for this stage.

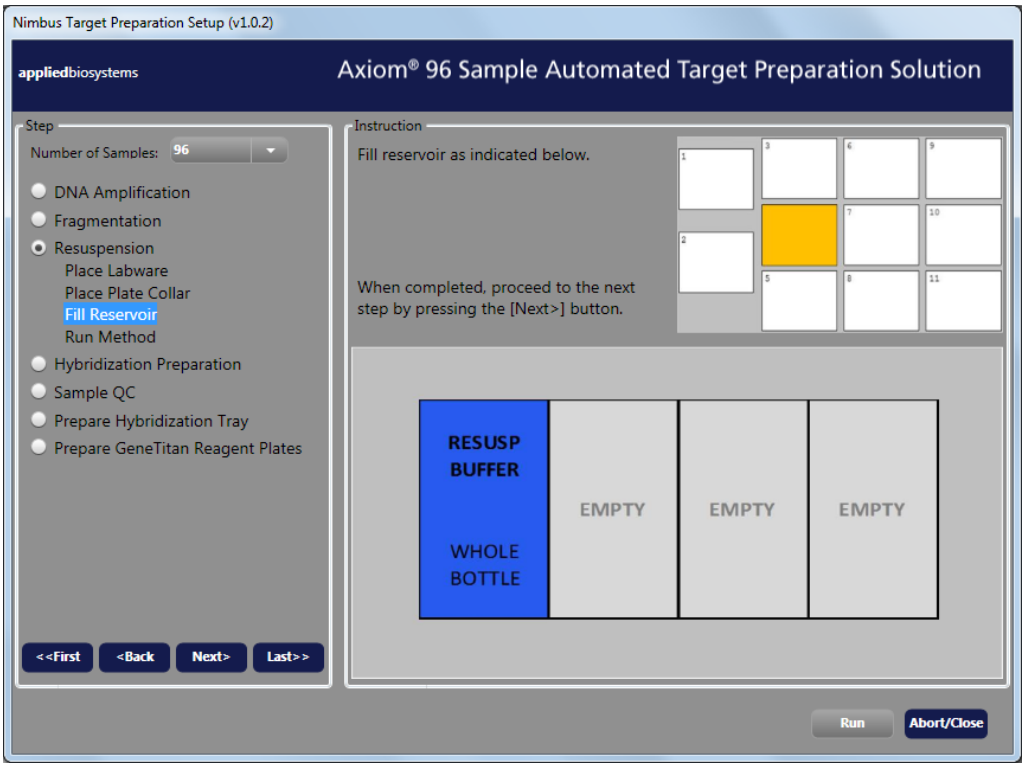
4. When labware setup is complete, click **Next** to continue.

5. Place a plate collar on top of the plate in deck position 8 as instructed in the **Resuspension—Place Plate Collar** window.



Place a plate collar on the plate in the deck position that is highlighted in orange.

6. After placing the plate collar, click **Next**.



7. Fill the reservoir in deck position 4 with Axiom™ Resusp Buffer, as directed in the **Resuspension—Fill Reservoir** window, then click **Next**.

8. Check the deck layout to ensure that all labware and reagents are in the proper locations.

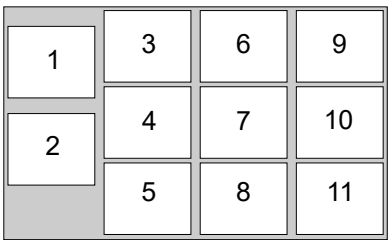
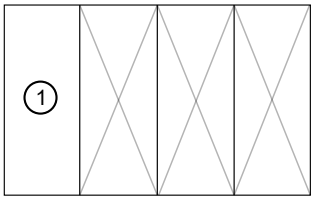


Figure 14 NIMBUS® Instrument deck positions.

Table 14 Labware and reagent locations on the deck for the resuspension step.

Deck position	Labware	Reagent or sample
4	4-column reservoir + reservoir frame	 ① Axiom™ Resusp Buffer
5	Square deepwell plate + Alpillo™	Precipitated and pelleted samples
8	96-well full-skirt plate + plate collar	—
10	CO-RE® II Filter Tips, 300 µL	—
11	CO-RE® II Filter Tips, 300 µL	—

Note: If the physical deck does not match the deck layout that is described in Table 14, either modify the physical deck to match or click **Abort/Close** in the **Resuspension—Run Method** window.

9. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start the resuspension step.
   The resuspension method starts. When complete the **Resuspension—Cleanup** window appears.

10. Remove the Sample Plate from deck position 5.
    This plate contains the DNA pellets in Axiom™ Resusp Buffer.
    a. Blot the top of the plate with a laboratory tissue to remove droplets present.
    b. Seal the plate tightly.

11. Save or discard the labware as instructed.

12. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.

13. Immediately proceed to “Resuspend the samples by off-deck shaking” on page 88.

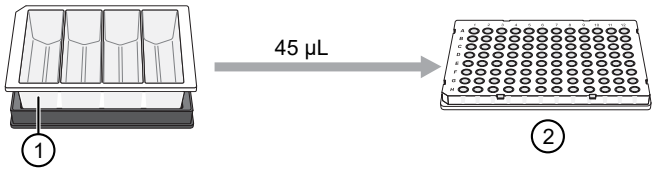
Resuspend the samples by off-deck shaking

After completion of the on-deck method to aliquot the Axiom™ Resusp Buffer to the square deepwell plate containing the DNA pellets, resuspension is carried out by shaking off-deck.

1. Seal the plate tightly.
   Blue pellets should be visible at the bottom of the wells.
2. Place the sample plate onto one of the following shakers for 10 minutes:
   - Thermo Scientific™ Digital Microplate Shaker set at 900 rpm.
   - Thermo Scientific™ Compact Digital Microplate Shaker, set at 900 rpm.
   - Boekel Scientific™ Jitterbug™, set at speed of 7.
3. Inspect the plate from the bottom. If the pellets are not dissolved, repeat the shaking step.
4. Centrifuge the plate in a room temperature centrifuge at 1,000 rpm for 30 seconds.
5. Proceed to “Stage 4B: Prepare Hybridization Master Mix” on page 90.

Workflow for Stage 4A: Resuspension

Transfer Axiom™ Resusp Buffer to Distribution Plate

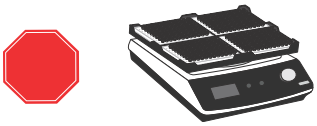


① Axiom™ Resusp Buffer, column 1 (deck position 4)
② Resusp Buffer Dist Plate (deck position 8)

Transfer Axiom™ Resusp Buffer to Precipitation Plate for off-deck pellet resuspension

① Resusp Buffer Dist Plate (deck position 8)
② Precipitation Plate (deck position 5)

Off-deck pellet resuspension on plate shaker (10 minutes)



Stage 4B: Prepare Hybridization Master Mix

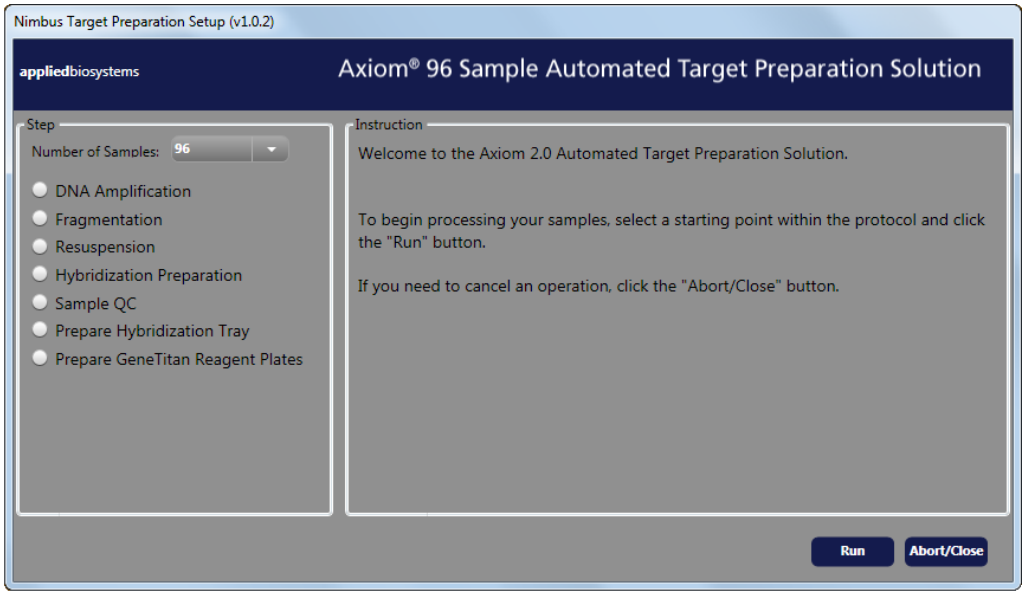
Perform the off-deck shaking to resuspend the samples before starting this stage.

Perform the prerun checklist

The following actions are required before the first operation of the Applied Biosystems™ NIMBUS® Target Preparation Instrument at the start of the day or the start of a run. Some or all these steps can be required depending on the current state of the workstation.

1. Power on the NIMBUS® Instrument workstation.
2. Ensure that all the peripherals are powered on.
   - Multi TEC Controller (controls the CPAC cooling unit and the Thermoshake device).
   - Computer workstation

   Note: The CPAC device takes approximately 20 minutes to cool to 4°C. Allow the CPAC device to cool to temperature before placing reagents in the cooling block.
3. Double-click the Axiom 2.0 Assay icon on your desktop to open the NIMBUS Target Preparation Setup window.



4. Continue with the instructions in the sections that follow.

IMPORTANT! Do not run a method at this point.

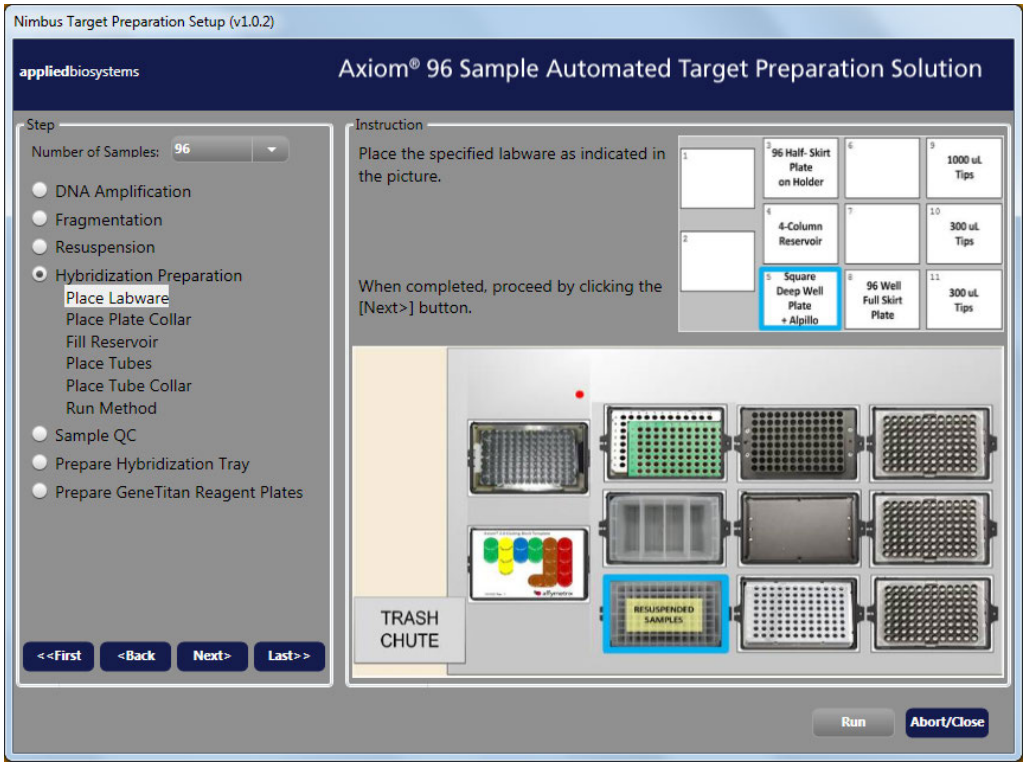
Prepare hybridization preparation reagents

Note: The Axiom™ Hyb Buffer, Axiom™ Hyb Soln 1, and Axiom™ Hyb Soln 2 must be thawed and stored on ice, as instructed in the section "Thaw reagents" on page 82.

1. Vortex the Axiom™ Hyb Buffer.
2. Vortex, then centrifuge the Axiom™ Hyb Soln 1 and Axiom™ Hyb Soln 2.

Run the hybridization preparation step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Hybridization Preparation**, then click **Run**.
2. Click **Yes** in the confirmation window to start the hybridization preparation step.



3. Place the labware on the deck as instructed.

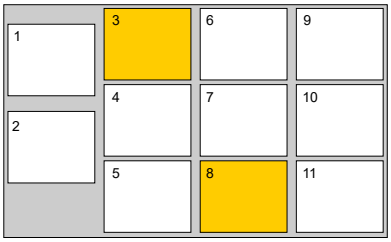
IMPORTANT! Remove the seal from the sample plate before placing on the deck.

Note: The 4-column reservoir must always be on a reservoir frame when placed on the NIMBUS® Instrument deck.

Note: A square deepwell plate is not required under the tip isolator in deck position 11 for this stage.

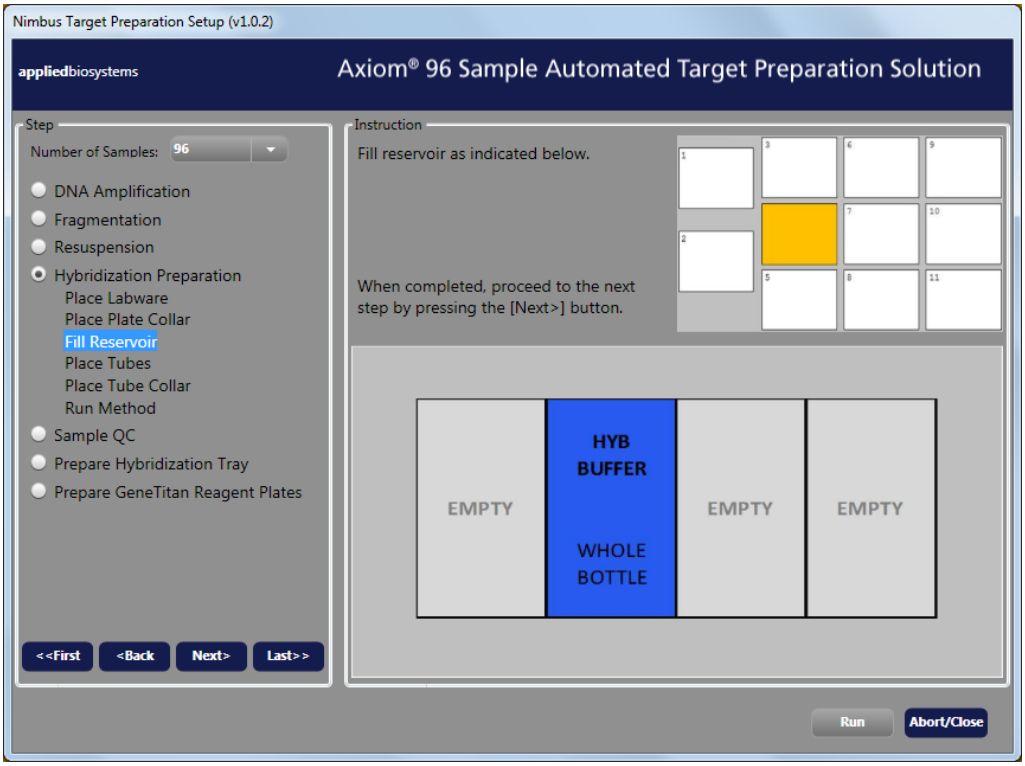
4. When labware setup is complete, click **Next** to continue.

5. Place a plate collar on top of the plates in deck position 3 and position 8 as instructed in the **Hybridization Preparation—Place Plate Collar** window.



Place plate collars on the plates in deck the positions that are highlighted in orange.

6. After placing the plate collars, click **Next**.



7. Fill the reservoir in deck position 4 with Axiom™ Hyb Buffer , as directed in the **Hybridization Preparation—Fill Reservoir** window, then click **Next**.

8. Place the Axiom™ Hyb Soln 1 and Axiom™ Hyb Soln 2 reagent tubes in the cooling block as directed in the **Hybridization Preparation—Place Tubes** window and the following figure.

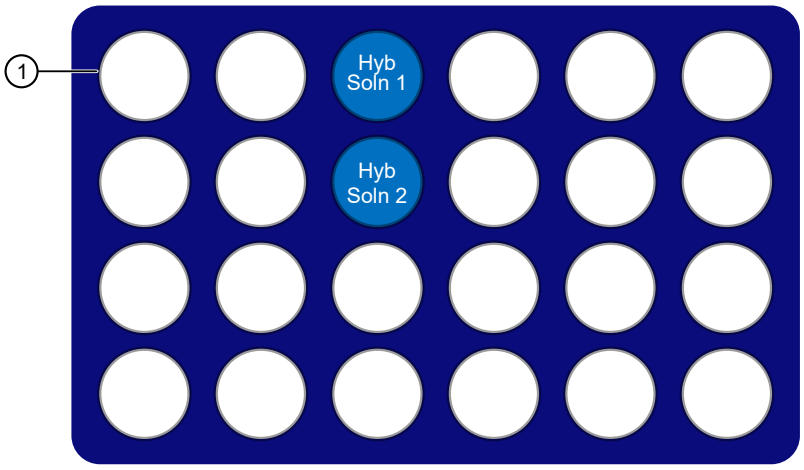


Figure 15 Placement of reagents in cooling block for hybridization preparation. Diagram of the cooling block without the reagent template.

① A1 position in the upper left corner

IMPORTANT!

- Position all reagents in the reagent cooling block with A1 in the upper left corner of the frame.
- Briefly centrifuge all reagent tubes before placing in block to avoid loss of solution volume to the cap and sides of the tube.
- Press reagent tubes into the block to ensure that they are fully seated.

9. After the reagents have been placed in the cooling block, click **Next**.

10. Place the tube collar on the cooling block in deck position 2, then click **Next**.

11. Check the deck layout to ensure that all labware and reagents are in the proper locations.

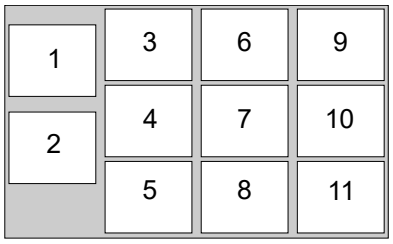
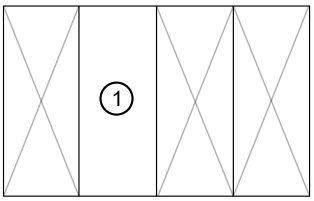


Figure 16 NIMBUS® Instrument deck positions.

Table 15 Labware and reagent locations on the deck for the hybridization preparation step.

Deck position	Labware	Reagent or sample
2	Cooling block + tube collar	See Figure 15
3	96 half-skirt plate on holder + plate collar	—

Table 15 Labware and reagent locations on the deck for the hybridization preparation step. *(continued)*

Deck position	Labware	Reagent or sample
4	4-column reservoir + reservoir frame	 ① Axiom™ Hyb Buffer
5	Square deepwell plate + Alpillo™	Resuspended DNA pellets
8	96-well full-skirt plate + plate collar	—
9	CO-RE® II Filter Tips, 1,000 µL	—
10	CO-RE® II Filter Tips, 300 µL	—
11	CO-RE® II Filter Tips, 300 µL + Square deepwell plate	—

Note: If the physical deck does not match the deck layout that is described in Table 15, either modify the physical deck to match or click **Abort/Close** in the **Hybridization Preparation—Run Method** window.

12. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start the fragmentation step.
    The hybridization method starts. When complete the **Hybridization Preparation—Cleanup** window appears.

13. Remove the Sample Plate from deck position 3.
    a. Blot the top of the plate with a laboratory tissue to remove any droplets.
    b. Seal the plate tightly.

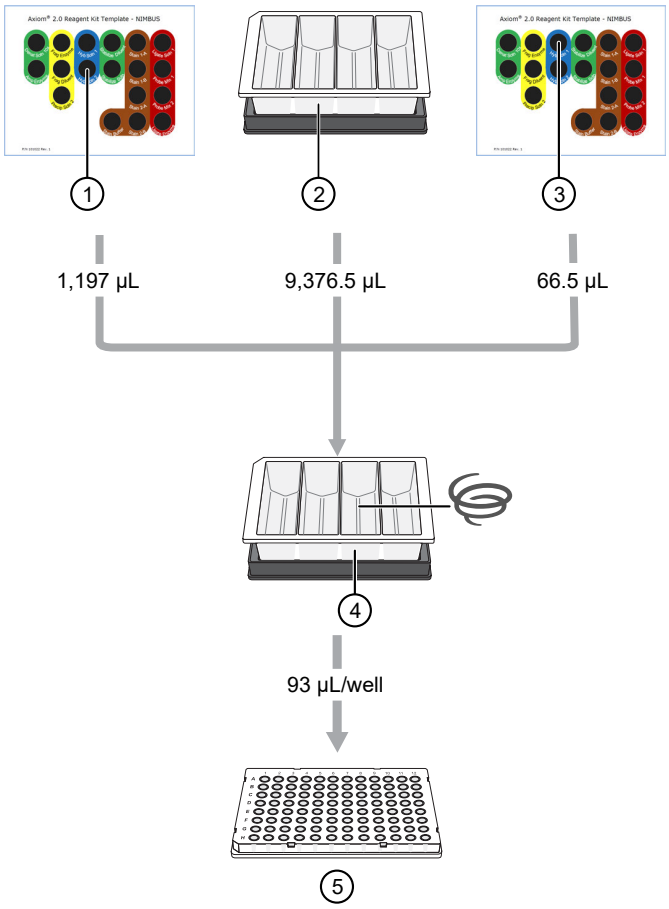
14. Save or discard the labware as instructed.

15. When deck cleanup is complete, click **Finish**, then click **Yes** in the confirmation window.

16. Do one of the following:
    - Proceed directly to "Stage 4C: Perform sample QC" on page 97.
    - Tightly seal the Sample Plate and store at –20°C.

Workflow for Stage 4B: Hybridization preparation

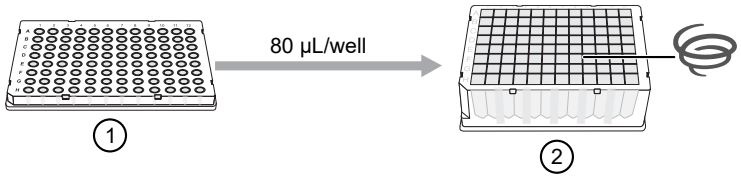
Prepare Hybridization Master Mix



① Axiom™ Hyb Soln 1 (deck position 2)
② Axiom™ Hyb Buffer, column 2 (deck position 4)
③ Axiom™ Hyb Soln 2 (deck position 2)
④ Hybridization Master Mix reservoir, column 3 (deck position 4)
⑤ Hyb MM Dist Plate (deck position 8)

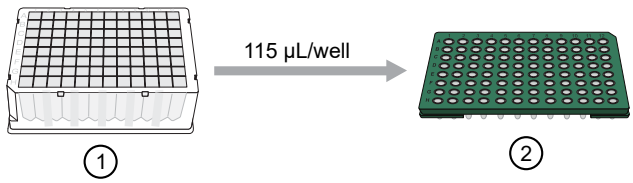
Mix

Transfer Hybridization Master Mix to Precipitation Plate



① Hyb MM Dist Plate (deck position 8)
② Precipitation Plate (deck position 5)
Mix

Transfer Hybridization Samples to Hyb-Ready Plate



① Precipitation Plate (deck position 5)
② Hybridization ready samples (deck position 3)

Process QC Plates

Stage 4C: Perform sample QC

Note: We strongly recommend that you run 2 quality process controls during this step:

- A gel to verify successful fragmentation
- An OD quantification of each resuspended sample

Time required

Activity	Time
Hands-on time	~5 minutes ~15 minutes if frozen from Stage 4B
NIMBUS® Instrument—Sample QC	~12 minutes
Total	**~25 minutes**

Input required

One plate of hybridization ready samples from Stage 4B.

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
Equipment and consumables	
As required	Adhesive seals for 96-well plates
1	Marker, fine point, permanent
1	Plate centrifuge, at room temperature
1	Vortexer
Labware	
2	96-well full-skirt plate[1]
1	4-column reservoir[1]
1	Reservoir frame[2]
1	96-well UV plate[3]
1 frame	CO-RE® II Filter Tips, 300 µL
2 frames	CO-RE® II Filter Tips, 50 µL

(continued)

Quantity	Item
1	Alpillo™ Plate Cushion and extension
3	Plate Collar

[1] From the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No.952437).
[2] From the Axiom™ NIMBUS® Starter Pack-NC (Cat. No. 952493).
[3] From the Axiom™ Consumables Kit for QC (Cat. No. 902909).

Reagents required

Quantity	Reagent	Source
User-supplied		
20 mL	Gel diluent: TrackIt™ Cyan/Orange Loading Buffer, diluted 100-fold	10482028
20 mL	Water, Nuclease-free, Molecular Biology Grade, Ultrapure, for OD and gel plate preparation	71786

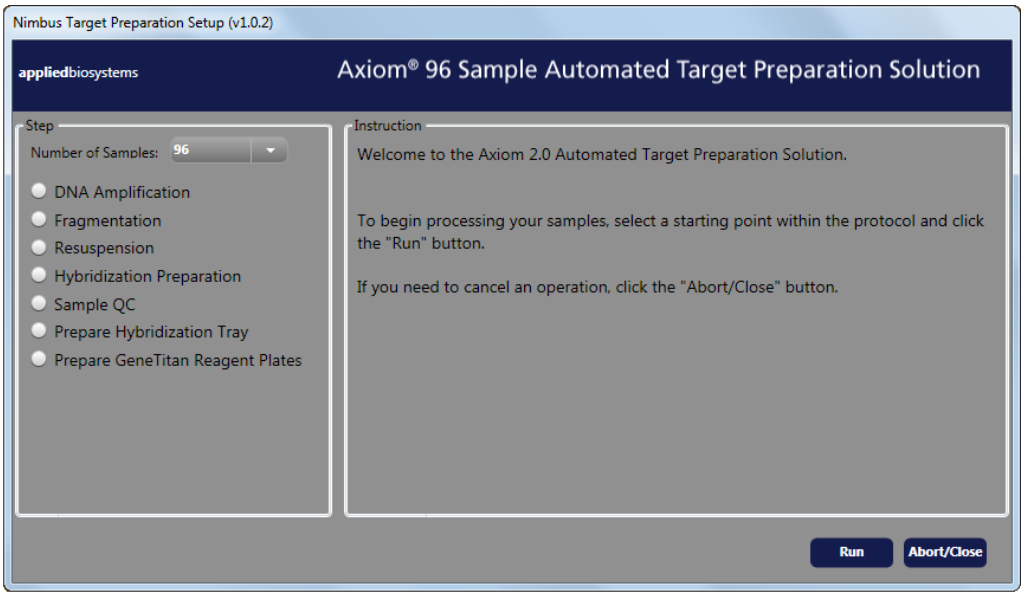
Perform the prerun checklist

The following actions are required before the first operation of the Applied Biosystems™ NIMBUS® Target Preparation Instrument at the start of the day or the start of a run. Some or all these steps can be required depending on the current state of the workstation.

1. Power on the NIMBUS® Instrument workstation.
2. Ensure that all the peripherals are powered on.
   - Multi TEC Controller (controls the CPAC cooling unit and the Thermoshake device).
   - Computer workstation

Note: The CPAC device takes approximately 20 minutes to cool to 4°C. Allow the CPAC device to cool to temperature before placing reagents in the cooling block.

3. Double-click the Axiom 2.0 Assay icon on your desktop to open the NIMBUS Target Preparation Setup window.



4. Continue with the instructions in the sections that follow.

IMPORTANT! Do not run a method at this point.

Prepare the Hyb-Ready Plate

Prepare a frozen Hyb-Ready Plate

1. Remove Hyb-Ready Plate from –20°C storage, then allow plate to warm to room temperature for 5 minutes. It is not necessary to equilibrate the plate for a longer length of time.
2. Ensure that the Hyb-Ready Plate is sealed well. If the plate is not sealed well:
   a. Briefly centrifuge the plate and carefully remove the old seal.
   b. If there is condensation on the top of the plate, blot dry gently with a laboratory tissue.
   c. Use a fresh seal and tightly reseal the plate.
3. Vortex the plate for 30 seconds to mix.
4. Centrifuge at 1,000 rpm for 30 seconds.

Prepare the Hyb-Ready Plate when proceeding directly from Stage 4A or 4B

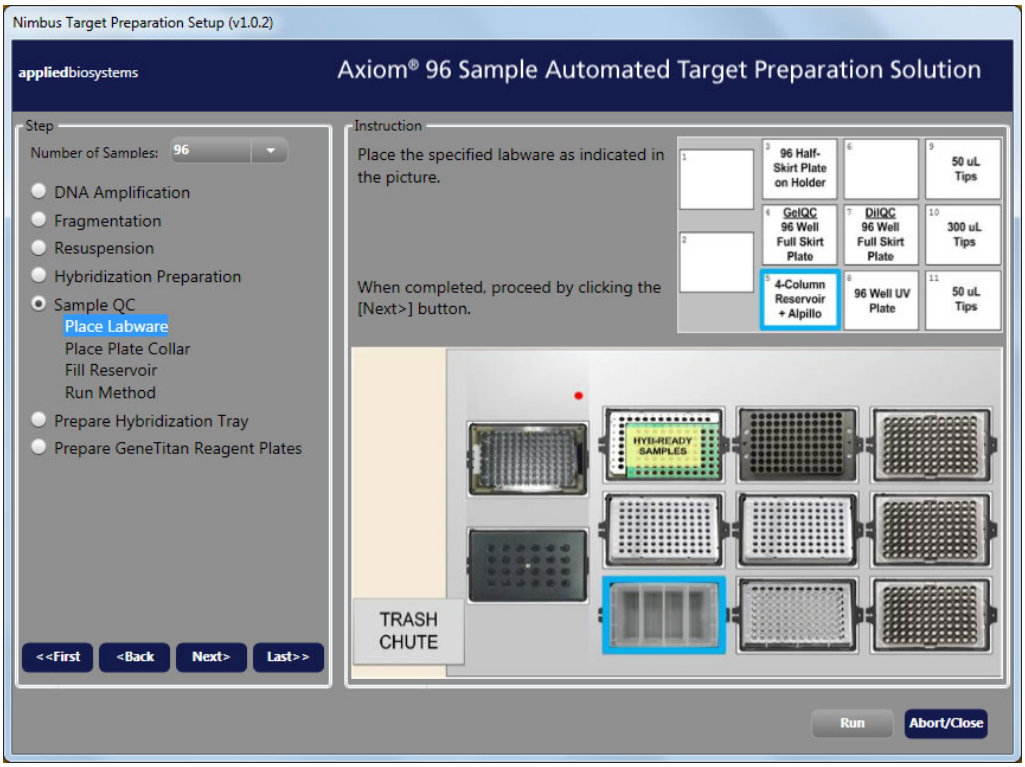
1. Tightly seal the sample plate.
2. Vortex the plate for 1 second each corner, and 1 second in the center at the maximum setting.
3. Centrifuge at 1,000 rpm for 30 seconds.

Prepare gel diluent

The TrackIt™ Cyan/Orange Loading Buffer, diluted 100-fold, is called "Gel Diluent" in this method. See Appendix C, “Fragmentation quality control gel protocol” for dilution instructions.

Run the sample QC step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Sample QC**, then click **Run**.
2. Click **Yes** in the confirmation window to start the sample QC step.



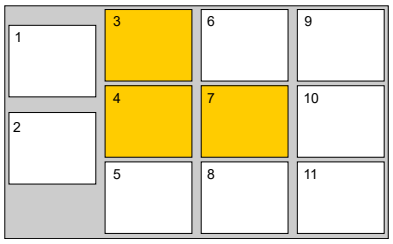
3. Place the labware on the deck as instructed.

Note: The 4-column reservoir must always be on a reservoir frame when placed on the NIMBUS® Instrument deck.

Note: A square deepwell plate is not required under the tip isolator in deck position 11 for this stage.

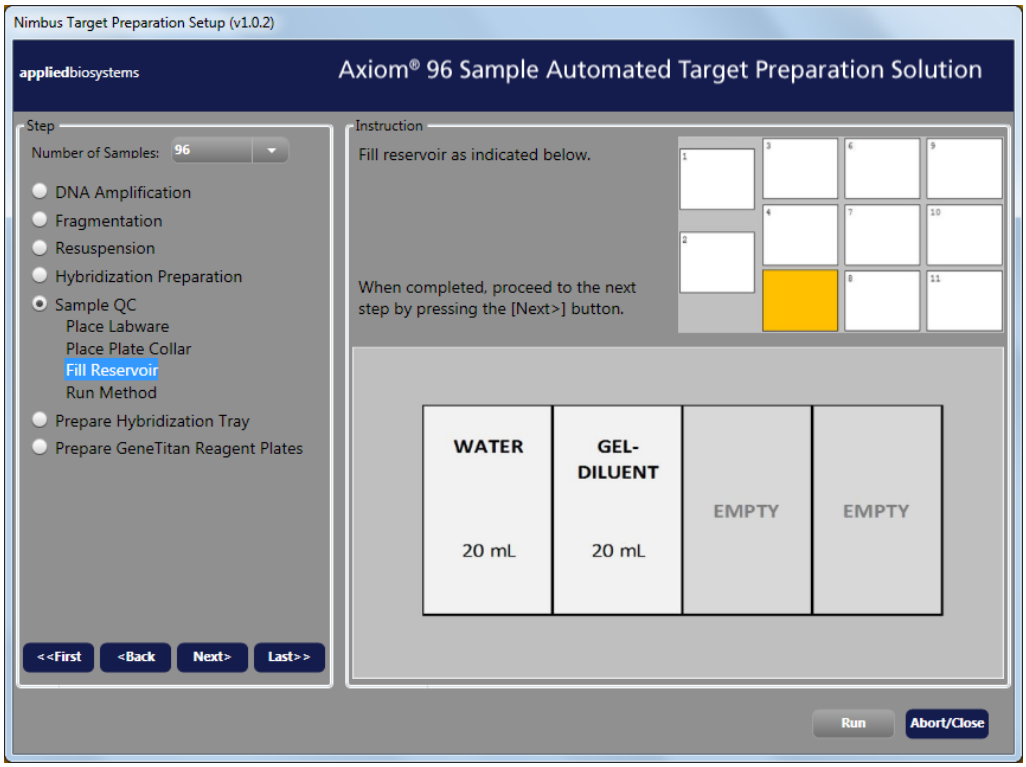
4. When labware setup is complete, click **Next** to continue.

5. Place a plate collar on top of the plates deck positions 3, 4, and 7, as instructed in the **Sample QC—Place Plate Collar** window.



Place plate collars on the plates in deck the positions that are highlighted in orange.

6. After placing the plate collars, click **Next**.



7. Fill the reservoir in deck position 5 with the reagents as directed in the **Sample QC—Fill Reservoir** window, then click **Next**.

8. Check the deck layout to ensure that all labware and reagents are in the proper locations.

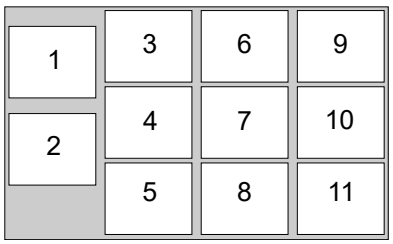
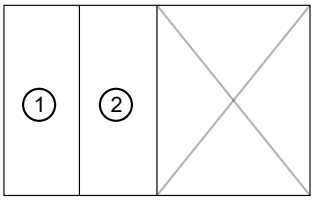


Figure 17 NIMBUS® Instrument deck positions.

Table 16 Labware and reagent locations on the deck for the sample QC step.

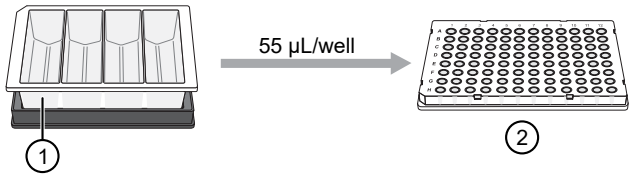
Deck position	Labware	Reagent or sample
3	96 half-skirt plate on holder + plate collar	Hybridization ready samples
4	96-well full-skirt plate + plate collar	—
5	4-column reservoir + reservoir frame + Alpillo™	 ① 20 mL water ② 20 mL gel diluent
7	96-well full-skirt plate + plate collar	—
8	96-well UV plate	—
9	CO-RE® II Filter Tips, 50 µL	—
10	CO-RE® II Filter Tips, 300 µL	—
11	CO-RE® II Filter Tips, 50 µL	—

Note: If the physical deck does not match the deck layout that is described in Table 16, either modify the physical deck to match or click **Abort/Close** in the **Sample QC—Run Method** window.

9. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start the sample QC step.
   The sample QC method starts. When complete the **Sample QC—Cleanup** window appears.
10. Remove the Sample Plate from position 3. Tightly seal the Hyb-Ready Plate.
11. Save or discard the labware as instructed.
12. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.
13. Run the fragmentation gel and OD quantification process controls.
    For instructions and guidelines on evaluating gel and OD results, see:
    - Appendix C, “Fragmentation quality control gel protocol”.
    - Appendix D, “Sample quantification after resuspension”.
14. Do one of the following:
    - If the GeneTitan™ MC Instrument is free, and if the gel and OD quantification results are good, proceed directly to “Stage 5: Prepare the hybridization tray” on page 105.
    - If the GeneTitan™ MC Instrument is not free, then store the sealed Hyb-Ready Plate at –20°C.

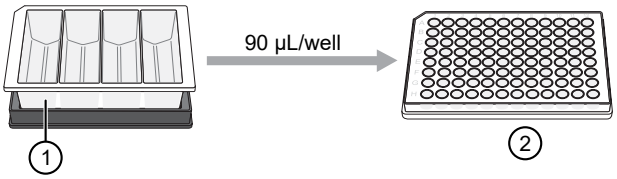
Workflow for Stage 4C: Sample QC

Fill Dilution QC Plate with water



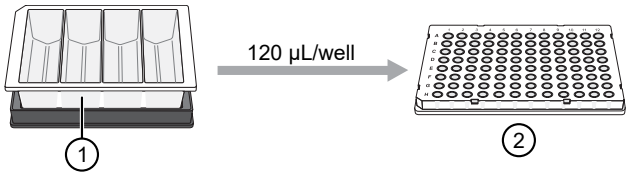
① Water reservoir, column 1 (deck position 5)
② Dilution QC Plate (deck position 7)

Fill OD QC Plate with water



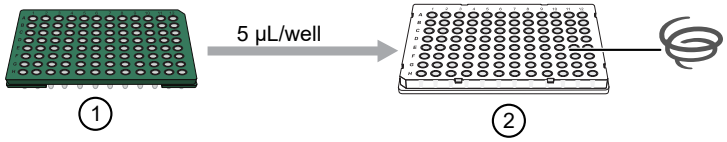
① Water reservoir, column 1 (deck position 5)
② OD QC Plate (deck position 8)

Fill Gel QC Plate with gel diluent



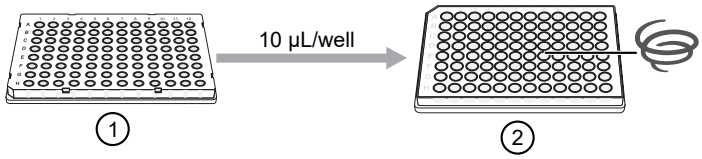
① Gel diluent, column 2 (deck position 5)
② Gel QC Plate (deck position 4)

Transfer hybridization ready samples to Dilution QC Plate



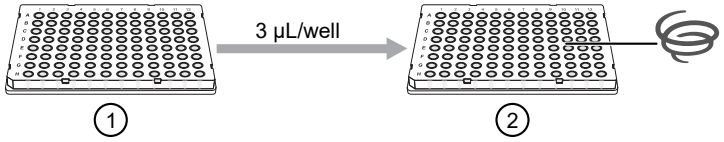
① Hybridization ready samples (deck position 3)
② Dilution QC Plate (deck position 7)
Mix

Transfer diluted sample to OD QC Plate



① Dilution QC Plate (deck position 7)
② OD QC Plate (deck position 8))
Mix

Transfer diluted sample to Gel QC Plate



① Dilution QC Plate (deck position 7)
② Gel QC Plate (deck position 4)
Mix

Perform offline analysis

Stage 5: Prepare the hybridization tray

About Stage 5: Prepare the hybridization tray

Proceed to Stage 5 in 1 of 2 ways:

- Directly from Stage 4 without interruption.
- With samples that have been stored at –20°C after Stage 4.

To perform Stage 5:

- If the Hyb-Ready Plate was stored at –20°C, go to “Sample plate and array plate preparation” on page 107.
- If proceeding directly from the end of Stage 4C: Sample QC, go to “Denature the samples” on page 109.

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

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

Time required

Table 17 Sample denaturation and hybridization tray transfer.

Activity	Time
Hands-on time	~10 minutes
Off-deck step: Denaturation in a thermal cycler	15 minutes
NIMBUS® Instrument—Prepare Hybridization Tray	1 minute
Total	**~30 minutes**

Table 18 Hybridization.

Activity	Time
Hands-on time	~45 minutes, including denaturation time
Hybridization in the GeneTitan™ MC Instrument	23.5 hours to 24 hours

Input required

One plate of hybridization-ready samples from Stage 4B.

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
Equipment	
1	GeneTitan™ MC Instrument
1	Thermal cycler programmed with the **Axiom 2.0 Plus Denature** protocol[1]
1	96-well Block warmed in a 48°C oven[2]
Labware	
1	Axiom™ Precision Medicine Diversity Research Array Plate (96-format)
1	Hybridization tray[3]
1 frame	CO-RE® II Filter Tips, 300 µL
1	Alpillo™ Plate Cushion and extension
1	Plate collar

[1] See *Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow Site Preparation Guide—Applied Biosystems™ NIMBUS® Target Preparation Instrument* for a list of recommended thermal cyclers.

[2] The block coming out the 48°C oven is warm to the touch. Gloves and mitts can be used if it feels too hot.

[3] From the Axiom™ GeneTitan™ Consumables Kit.

Reagents required

Quantity	Reagent	Module
From the Axiom™ 2.0 Plus Reagent Kit 96F		
2 bottles/1 L	Axiom™ Wash Buffer A	Module 3, room temperature
1 bottle	Axiom™ Wash Buffer B	
1 bottle	Axiom™ Water	

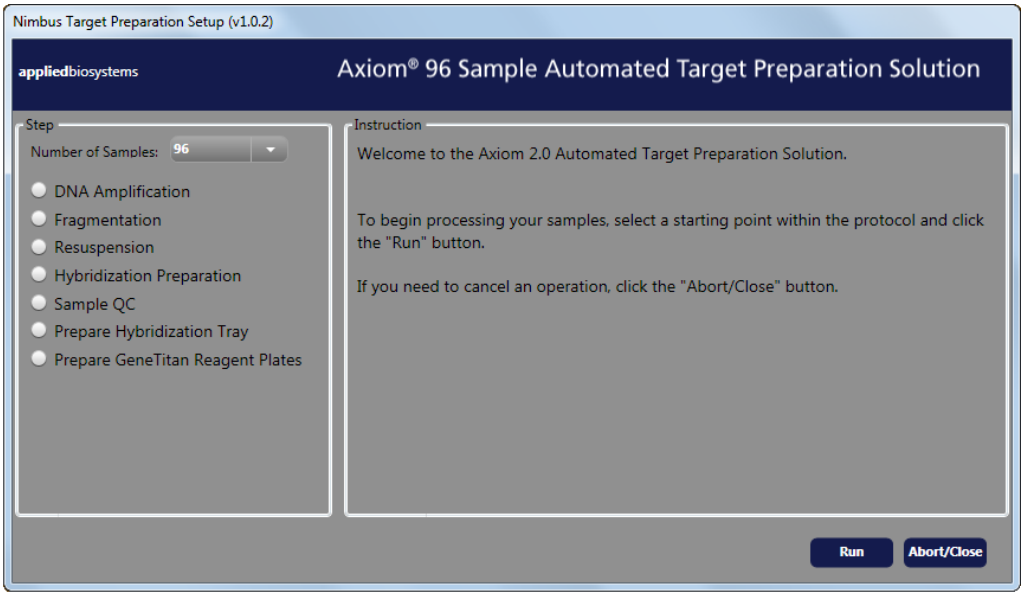
Perform the prerun checklist

The following actions are required before the first operation of the Applied Biosystems™ NIMBUS® Target Preparation Instrument at the start of the day or the start of a run. Some or all these steps can be required depending on the current state of the workstation.

1. Power on the NIMBUS® Instrument workstation.
2. Ensure that all the peripherals are powered on.
   - Multi TEC Controller (controls the CPAC cooling unit and the Thermoshake device).
   - Computer workstation

Note: The CPAC device takes approximately 20 minutes to cool to 4°C. Allow the CPAC device to cool to temperature before placing reagents in the cooling block.

3. Double-click the Axiom 2.0 Assay icon on your desktop to open the NIMBUS Target Preparation Setup window.



4. Continue with the instructions in the sections that follow.

IMPORTANT! Do not run a method at this point.

Sample plate and array plate preparation

Prepare samples that have been stored at –20°C

1. Warm up the Hyb-Ready Plate at room temperature for 5 minutes.
   It is not necessary to equilibrate the plate for a longer length of time.
2. Check to ensure that the Hyb-Ready Plate is well sealed. If the plate is not well sealed:
   a. Briefly centrifuge the plate and carefully remove the old seal.
   b. If there is condensation on the top of the plate, blot dry with a laboratory tissue.
   c. Use a fresh seal and tightly reseal the plate.
3. Vortex the Hyb-Ready Plate briefly, then centrifuge at 1,000 rpm for 30 seconds.
4. Place the Hyb-Ready Plate at room temperature.

Prepare the array plate

1. Warm the array plate on the benchtop before setting up hybridization on the GeneTitan™ MC Instrument.
2. Leave the unopened array plate in the pouch at room temperature for at least 25 minutes.
3. At the end of the array warm-up time, open the pouch, then scan the array plate barcode into the GeneTitan™ Array Plate Registration file.

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

Prepare the GeneTitan™ MC Instrument

Before you denature the Denaturation Plate samples, ensure that the GeneTitan™ MC Instrument is ready for use. Follow the instructions in "Stage 2—Hybridize plates in the GeneTitan™ MC Instrument" on page 136 and Appendix B, "Register samples in GeneChip™ Command Console™". The following is a brief summary of the steps to perform.

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

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

   Note: When creating the GeneTitan™ Array Plate Registration file, you can add the barcode of the hybridization tray as a sample file attribute. Adding the barcode as an attribute enables traceability in the system. See the *GeneChip™ Command Console™ User Guide*, for details on adding attributes to sample files.
4. From **GCC GeneTitan Instrument Control**, select the **System Setup** tab.
5. For **Setup Option**, select **Hyb-Wash-Scan**.
6. Click **Next**.
7. Complete the following in the **Plate information** section:
   a. **Barcode**: Scan or manually enter the array plate barcode, then click **Next**.
   b. **Protocol Name**: Select the protocol name, then click **Next**.
8. Fill the Wash A, Wash B, and Rinse bottles with Axiom™ Wash Buffer A, Axiom™ Wash Buffer B, and Axiom™ Water, respectively.
9. Empty the Waste bottle.

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

11. Open the trash bin and empty, then press the confirmation button to continue.
    If already empty, the trash bin remains locked and the **Status** pane reads "Trash bin is empty".

12. Remove used trays and plates when drawers open, then press the confirmation button to continue.
    If there are no consumables to remove, the **Status** pane reads "Drawers are empty".

Denature the samples

1. Double-check that the 96-well heat block is being pre-heated in a 48°C oven. If not, preheat it now.

2. Ensure that the thermal cycler is powered on and the **Axiom 2.0 Plus Denature** protocol with the heated lid option is selected.

3. Open the lid of the thermal cycler, then place the sealed Hyb-Ready Plate on the thermal cycler. Check the integrity of the seal as evaporation during denaturation can negatively affect assay performance.

4. Close the lid.
   For thermal cyclers with variable lid tension (such as the Bio-Rad™ DNA Engine Tetrad™ 2), follow manufacturer's instructions for adjusting lid tension.

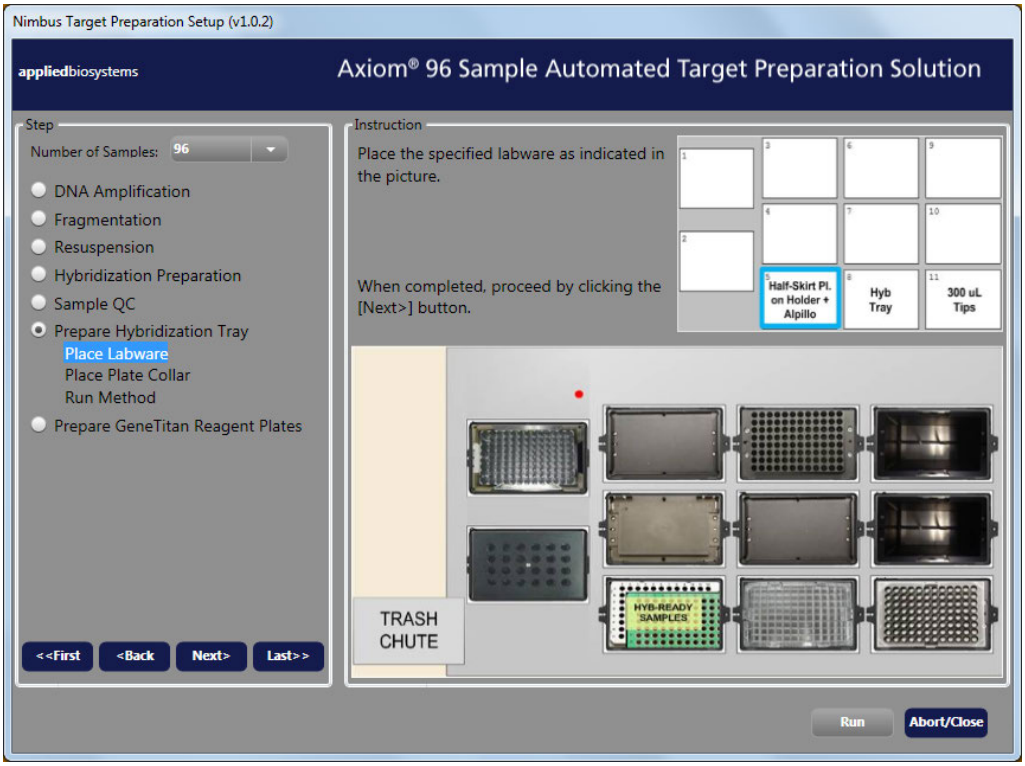
5. Start the **Axiom 2.0 Plus Denature** protocol.
   a. 95°C for 10 minutes
   b. 48°C for 3 minutes
   c. 48°C hold
      Use the heated lid option when setting up or running the protocol.

IMPORTANT! Avoid leaving denatured samples at room temperature for any length of time. When you are ready to transfer the plate from the thermal cycler to the NIMBUS® Instrument deck at the end of the Axiom 2.0 Plus Denature protocol, place it on a heat block pre-heated at 48°C. Placing the plate on a heat block minimizes sample cooling as you return to the instrument deck.

Run the prepare hybridization tray step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Prepare Hybridization Tray**, then click **Run**.

2. Click **Yes** in the confirmation window to start the prepare hybridization tray step.



3. Place the labware on the deck as instructed.

4. After setting pipette tips and hybridization tray on deck, return to the thermal cycler and to retrieve the denatured Hyb-Ready Plate after the Axiom 2.0 Plus Denature protocol is completed.

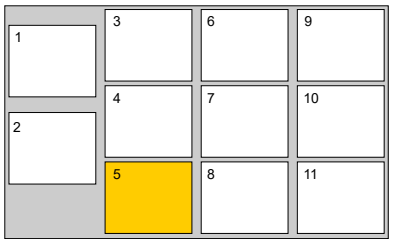
5. Carry the denatured Hyb-Ready Plate in a 48°C pre-warmed metal chamber to the NIMBUS® Instrument deck, then remove the seal, then place it on the deck at position 5.

IMPORTANT! Avoid letting the samples sit at room temperature for an extended length of time after denaturation. Do not remove the denatured Hyb-Ready Plate from the thermal cycler unless both the GeneTitan™ MC Instrument and NIMBUS® workstation are ready.

Note: A square deepwell plate is not required under the tip isolator in deck position 11 for this stage.

6. Click **Next** immediately after the denatured Hyb-Ready Plate is placed on the deck.

7. Place a plate collar on top of the plate in deck position 5, as instructed in the **Prepare Hybridization Tray—Place Plate Collar** window.



Place a plate collar on the plates in deck the position that is highlighted in orange.

8. After placing the plate collar, click **Next**.

9. Check the deck layout to ensure that all labware and reagents are in the proper locations.

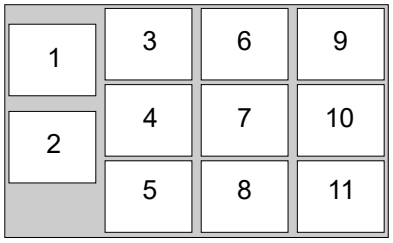


Figure 18 NIMBUS® Instrument deck positions.

Table 19 Labware and reagent locations on the deck for the prepare hybridization tray step.

Deck position	Labware	Reagent or sample
5	96 half-skirt plate on holder + plate collar + Alpillo™	Denatured hybridization ready samples
8	Hybridization tray	—
11	CO-RE® II Filter Tips, 300 µL	—

Note: If the physical deck does not match the deck layout that is described in Table 19, either modify the physical deck to match or click **Abort/Close** in the **Prepare Hybridization Tray—Run Method** window.

CAUTION! It is critical that you write only on the proper location of the hybridization tray (on the edge in front of wells A1 and B1). Do not write on any other side, because the writing can interfere with sensors inside the GeneTitan™ MC Instrument and result in experiment failure. See Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation”.

10. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start the Prepare Hybridization Tray step.

    The prepare hybridization tray step starts. When complete, the **Prepare Hybridization Tray—Cleanup** window appears.

11. Immediately remove the hybridization tray from deck position 8 and load into the GeneTitan™ MC Instrument, then follow the instructions for discarding used labware and tips on the deck.

12. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.

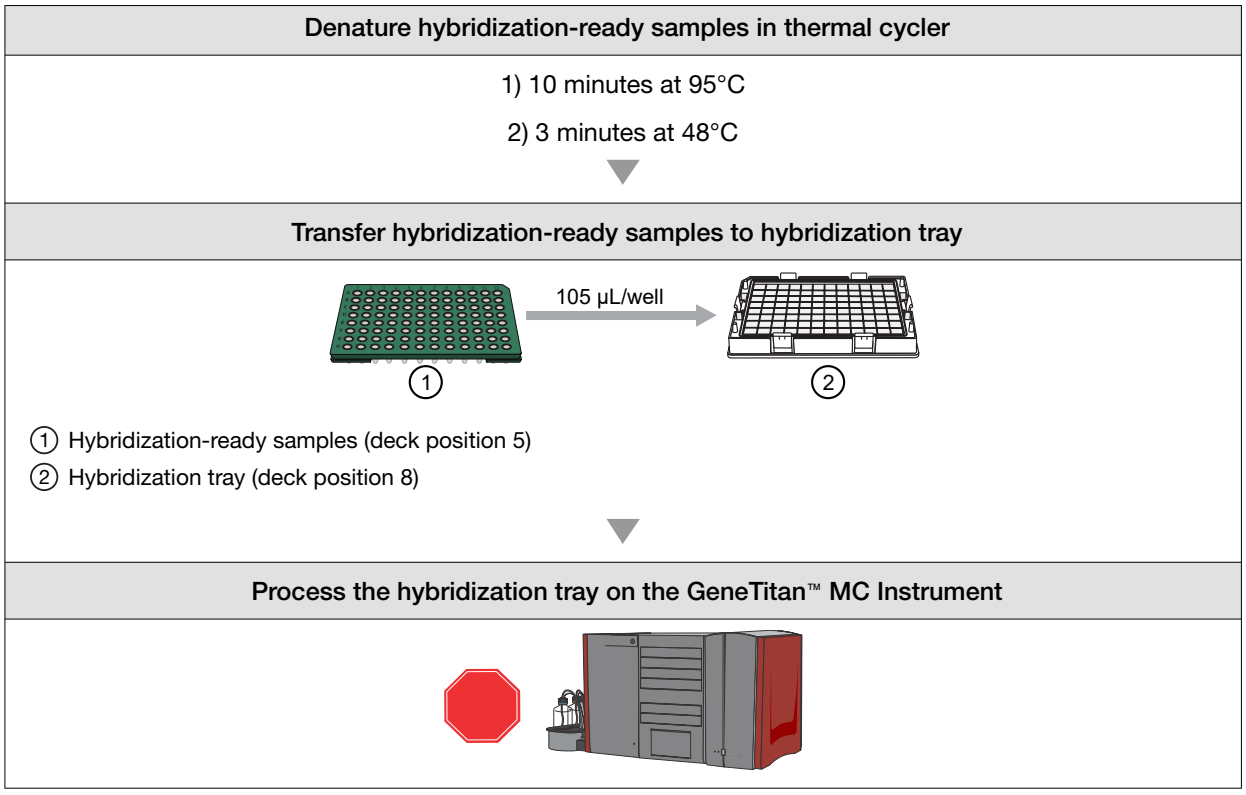
Load the hybridization tray and array plate into the GeneTitan™ MC Instrument

1. Examine the hybridization tray to ensure that there are no air bubbles present. Puncture any air bubbles that you see using a clean pipette tip for each sample.

2. Load the hybridization tray and array plate into the GeneTitan™ MC Instrument following the instructions in "Stage 2—Hybridize plates in the GeneTitan™ MC Instrument" on page 136.

3. Near the end of the 23.5 to 24-hour hybridization, proceed to "Stage 6: Prepare GeneTitan™ reagent trays" on page 113.

Workflow for Stage 5: Prepare the hybridization tray



Stage 6: Prepare GeneTitan™ reagent trays

The reagent trays that are prepared in this stage are for the continued processing of an array plate that is:

- Already on the GeneTitan™ MC Instrument.
- Has completed the hybridization stage.
- Is ready for transfer to the fluidics area.

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

The method for Stage 6 consists of 2 parts:

- Part 1: Preparation of the scan tray, stain 2 tray, and the stabilize tray.
- Part 2: Preparation of the stain 1-1 and stain 1-2 trays and the ligation tray.

After part 1 of the method is completed, a dialog box for labware change appears prompting you to remove labware from specific deck positions, then replace them with new labware. After the labware change, the run proceeds with part 2 of the method.

Time required

Activity	Time
Prepare reagents (thaw and organize reagents)	~30 minutes
Hands-on time	~15 minutes
NIMBUS® Instrument—Prepare GeneTitan™ reagent trays (with labware change) • Runtime for part 1 • Runtime for part 2	~45 minutes • 21 minutes • 16 minutes
Total	**~90 minutes**

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
Equipment and consumables	
As required	Laboratory tissues
1	Marker, fine point, permanent
1	Ice bucket filled with ice

(continued)

Quantity	Item
1	CPAC reagent template
1	Cooler for enzyme
1	GeneTitan™ MC Instrument
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Vortexer
1	GeneTitan™ ZeroStat AntiStatic Gun
Labware	
1	Scan tray with cover and protective base[1]
5	Stain tray[1]
5	Cover for stain tray[1]
2	4-column reservoir[2]
2	Reservoir frame
1	24-Position Tube Rack (with one tube insert at D06)
1	Tube collar
1	Alpillo™ Plate Cushion and extension
1 frame	CO-RE® II Filter Tips, 1,000 µL
2 frames	CO-RE® II Filter Tips, 300 µL

[1] From the Axiom™ GeneTitan™ Consumables Kit (Cat. No. 901606).

[2] From the Axiom™ NIMBUS® 2.0 Consumables Kit v2 (Cat. No.952437).

Reagents required and reagent handling

Prepare reagents according to the following table.

Module	Reagent and cap color	Thaw, then place on ice	Place on ice	Place at room temp	Deck loading instructions
Module 4-1 –20°C	Axiom™ Ligate Buffer			✓	Vortex for 30 seconds. [1] Pour in reservoir.
	Axiom™ Ligate Enzyme	⊗ Do not thaw. Keep at –20°C until ready to use.			**Immediately before use:** Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Ligate Soln 1	✓			Vortex, then centrifuge briefly. Place in the cooling block.
	Axiom™ Probe Mix 1	✓			Vortex, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stain Buffer	✓			Vortex, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stabilize Soln	✓			Vortex, then centrifuge briefly. Place in the cooling block.
Module 4-2 2°C to 8°C	Axiom™ Ligate Soln 2			✓	Vortex, then centrifuge briefly. Place in 24-Position Tube Rack.
	Axiom™ Probe Mix 2[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Wash A			✓	Vortex for 30 seconds.[1] Pour in reservoir.
	Axiom™ Stain 1-A[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stain 1-B[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stain 2-A[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stain 2-B[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stabilize Diluent		✓		Vortex, then centrifuge briefly.[1] Place in the cooling block.

(continued)

Module	Reagent and cap color	Thaw, then place on ice	Place on ice	Place at room temp	Deck loading instructions
Module 4-2 2°C to 8°C	Axiom™ Water			✓	Pour in reservoir.
	Axiom™ Hold Buffer[2]			✓	Vortex for 30 seconds. Pour in reservoir.
Estimated reagent thawing time is ~1 hour.					

[1] Check for precipitate. If precipitate is present, repeat the vortex and centrifuge step.
[2] These solutions are light sensitive. Keep tubes out of direct light for a prolonged length of time.

Notes on handling reagents with precipitates

Prepare Axiom™ Wash A

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

Prepare Axiom™ Stabilize Diluent

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

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

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

Prepare Axiom™ Ligate Buffer

White precipitate is sometimes observed when the Axiom™ Ligate Buffer is thawed. The presence of some precipitate is okay and does not adversely impact assay performance. Use the following instructions to resuspend most precipitate before use.

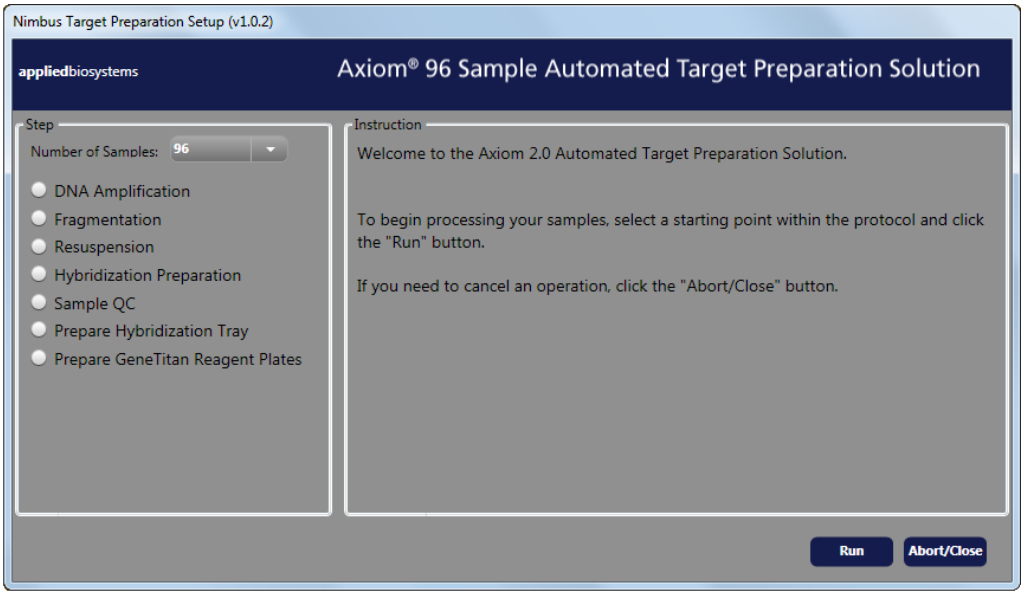
1. Vortex for 30 seconds.
2. Examine the buffer for precipitate.
3. If precipitate is still present, warm the bottle with your hands, then vortex again for 30 seconds.
4. Pour the Axiom™ Ligate Buffer into the appropriate reagent reservoir.

Perform the prerun checklist

The following actions are required before the first operation of the Applied Biosystems™ NIMBUS® Target Preparation Instrument at the start of the day or the start of a run. Some or all these steps can be required depending on the current state of the workstation.

1. Power on the NIMBUS® Instrument workstation.
2. Ensure that all the peripherals are powered on.
   - Multi TEC Controller (controls the CPAC cooling unit and the Thermoshake device).
   - Computer workstation

   Note: The CPAC device takes approximately 20 minutes to cool to 4°C. Allow the CPAC device to cool to temperature before placing reagents in the cooling block.
3. Double-click the Axiom 2.0 Assay icon on your desktop to open the NIMBUS Target Preparation Setup window.



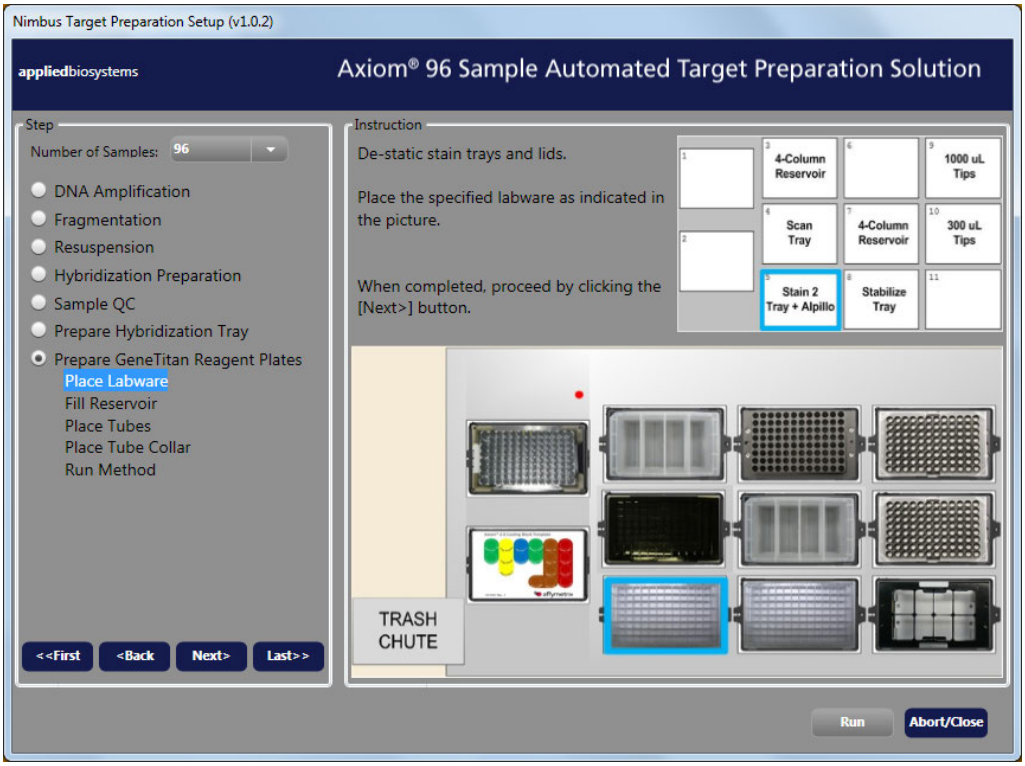
4. Continue with the instructions in the sections that follow.

IMPORTANT! Do not run a method at this point.

Run the prepare GeneTitan™ reagent trays step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Prepare GeneTitan Reagent Plates**, then click **Run**.

2. Click **Yes** in the confirmation window to start the prepare GeneTitan™ reagent trays step.



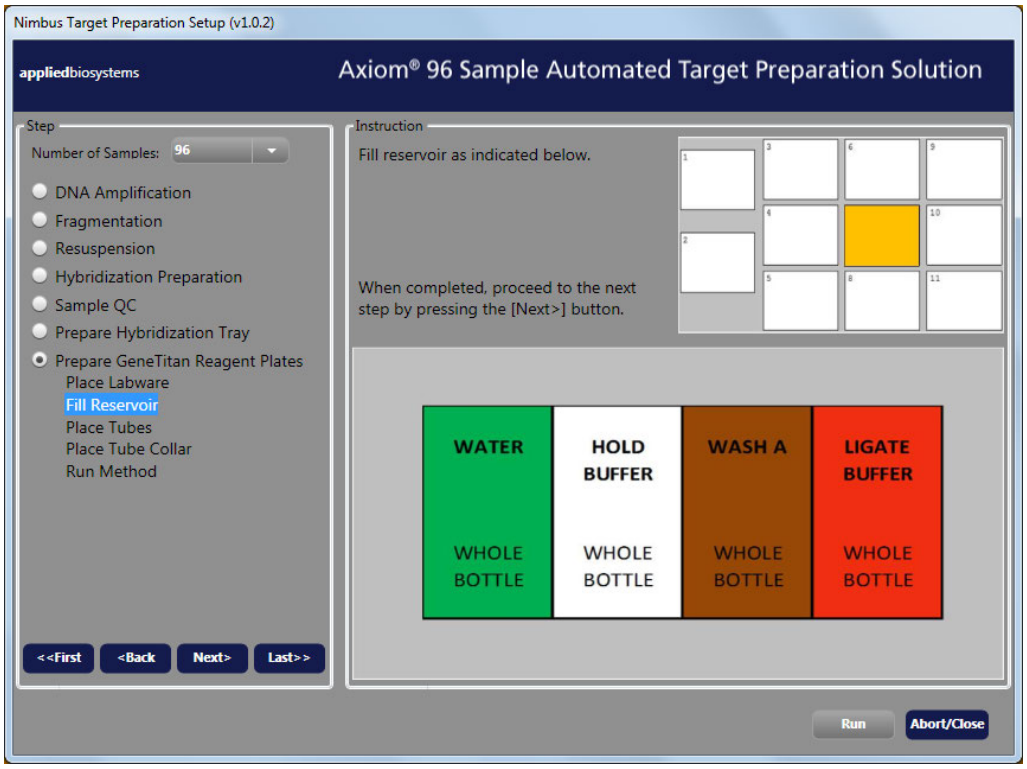
3. Label, then deionize the GeneTitan™ stain trays and covers.
   - See “Label the GeneTitan™ reagent trays” on page 209.
   - See “Deionization of GeneTitan™ trays and covers” on page 209.

IMPORTANT! It is important to deionize the GeneTitan™ reagent trays and covers to remove any static electricity on the trays and covers. Static attraction by the trays can prevent the tray cover from being lifted by the instrument gripper.

4. Place the labware on the NIMBUS® Instrument deck as instructed.

Note: The 4-column reservoir must always be on a reservoir frame when placed on the deck.

5. When labware setup is complete, click **Next** to continue.



6. Fill the reservoir in deck position 7 with the following reagents, as directed in the **Prepare GeneTitan Reagent Plates—Fill Reservoir** window, then click **Next**.

   - Axiom™ Water
   - Axiom™ Hold Buffer
   - Axiom™ Wash A
   - Axiom™ Ligate Buffer

7. Place the reagent tubes in the cooling block as directed in the **Prepare GeneTitan Reagent Plates—Place Tubes** window and the following figure.

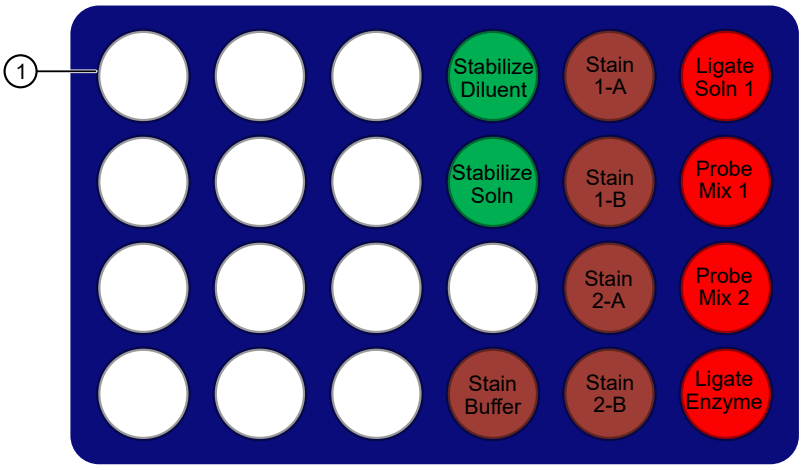


Figure 19 Placement of reagents in cooling block for GeneTitan reagent trays preparation—part 1. Diagram of the cooling block without the reagent template.

(1) A1 position in the upper left corner.

IMPORTANT!

- Position all reagents in the reagent cooling block with A1 in the upper left corner of the frame.
- Centrifuge all reagent tubes before placing in block to avoid loss of solution volume to the cap and sides of the tube.
- Press reagent tubes into the block to ensure that they are fully seated.

8. After the reagents have been placed in the cooling block, click **Next**.

9. Place the tube collar on the cooling block in deck position 2, then click **Next**.

10. Check the deck layout to ensure that all labware and reagents are in the proper locations.

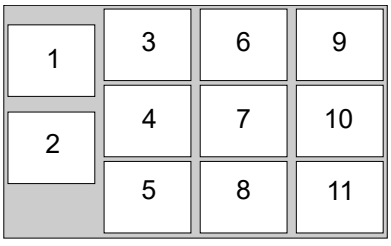
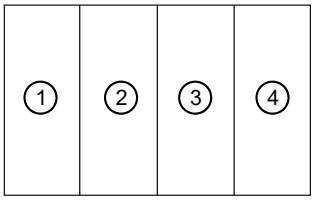


Figure 20 NIMBUS® Instrument deck positions.

Table 20 Labware and reagent locations on the deck for GeneTitan™ reagent trays preparation—part 1.

Deck position	Labware	Reagent or sample
2	Cooling block + tube collar	See Figure 19
3	4-column reservoir + reservoir frame	4-column reservoir is empty
4	Scan tray on protective base[1]	—
5	Stain tray, Stain 2[1,2] + Alpillo™	—
7	4-column reservoir + reservoir frame	 ① Axiom™ Water ② Axiom™ Hold Buffer ③ Axiom™ Wash A ④ Axiom™ Ligate Buffer
8	Stain tray, "Stbl"[1,2]	—
9	CO-RE® II Filter Tips, 1,000 µL	—
10	CO-RE® II Filter Tips, 300 µL	—

[1] Included in Axiom™ GeneTitan™ Consumables Kit.

[2] Label each of these stain trays as described in "Label GeneTitan™ hybridization and reagent trays" on page 208. For example, label the stain tray to be placed in deck position 5 with the word "Lig". This tray will contain the Ligation Master Mix.

Note: If the physical deck does not match the deck layout that is described in Table 20, either modify the physical deck to match or click **Abort/Close** in the **Prepare GeneTitan Reagent Plates—Run Method** window.

11. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start.

    The method starts.

    While the method is running, deionize the GeneTitan™ covers and the 3 remaining GeneTitan™ stain trays.

    When part 1 is complete, the **Labware Change** window appears.

12. Remove the following labware:

IMPORTANT! Ensure that the GeneTitan™ reagent tray covers have been deionized before placing them on the reagent trays.

a. Remove the Scan Tray from deck position 4. Cover, then set aside for placement into the GeneTitan™ MC Instrument.

b. Remove the Stain 2 Tray from deck position 5. Cover, then set aside for placement into the GeneTitan™ MC Instrument.

c. Remove the Stabilize Tray from deck position 8. Cover, then set aside for placement into the GeneTitan™ MC Instrument.

d. Remove the 4-column reservoir and reservoir frame from deck position 7. Discard the 4-column reservoir. Save the reservoir frame for reuse.

13. Add the following labware:

IMPORTANT! Ensure that the GeneTitan™ stain trays have been deionized before placing them on the deck.

a. Place the Ligate Tray in deck position 4.

b. Place the Stain 1-1 Tray in deck position 5.

c. Place the 24-Position Tube Rack in deck position 7 with tube insert in position D6.

d. Place the Stain 1-2 Tray in deck position 8.

14. Place the Axiom™ Ligate Soln 2 in the tube rack in position D6.

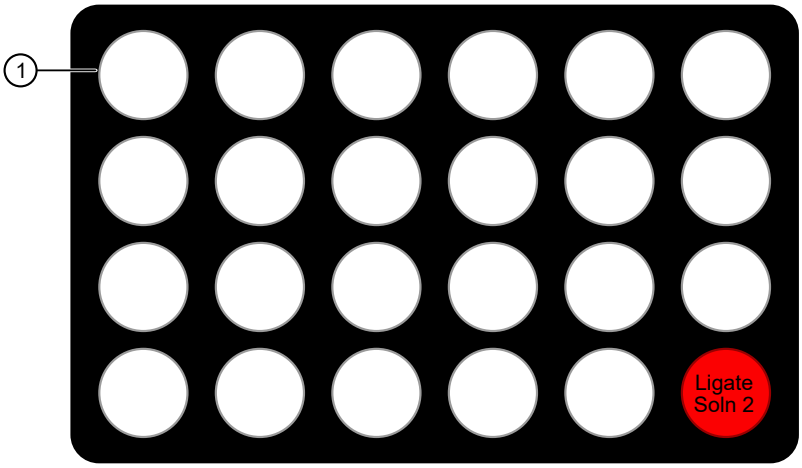


Figure 21 Placement of reagents in 24-Position Tube Rack for GeneTitan reagent trays preparation —part 2.

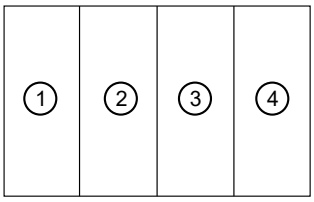
① A1 position in the upper left corner

15. Check the deck layout to ensure that all labware and reagents are in the proper deck locations for GeneTitan reagent trays preparation step—part 2.

Table 21 Labware and reagent locations on the deck for GeneTitan™ reagent trays preparation —part 2.

Deck position	Labware	Reagent or sample
2	Cooling block + tube collar	See Figure 19

Table 21 Labware and reagent locations on the deck for GeneTitan reagent trays preparation—part 2. *(continued)*

Deck position	Labware	Reagent or sample
3	4-column reservoir + reservoir frame	 ① Stain1 MM ② Stain2 MM ③ Stab MM ④ Ligation MM
4	Stain tray, lig[1,2]	—
5	Stain tray, Stain 1[1,2] (first of 2 with Stain 1)	—
7	24-Position Tube Rack	Axiom™ Ligate Soln 2 in rack position D6.
8	Stain tray, Stain 1[1,2] (second of 2 with Stain 1)	—

[1] Included in Axiom™ GeneTitan™ Consumables Kit.

[2] Label each of these stain trays as described in "Label GeneTitan™ hybridization and reagent trays" on page 208. For example, label the stain tray to be placed in deck position 5 with the word "Lig". This tray will contain the Ligation Master Mix.

16. When ready, click **Continue**.

    The method continues. The Stain 1 Master Mix is prepared and aliquoted into 2 stain trays. The NIMBUS® Instrument prepares the Ligation Master Mix and aliquots it into the Ligation Tray. When complete, the **Prepare GeneTitan Reagent Plates step—Deck Cleanup** window appears.

17. Remove the following trays from the deck:

    - Ligation Tray from deck position 4, then cover.
    - Stain 1-1 Tray from deck position 5, then cover.
    - Stain 1-2 Tray from deck position 8, then cover.

18. Gather the reagent trays that were previously set aside during this method:

    - Scan Tray and cover.
    - Stain 2 Tray and cover.
    - Stabilize Tray and cover.

19. Examine each tray to:

    - Ensure all the wells have been filled. If any wells do not contain reagents, then manually add reagents to these wells.
    - Ensure that there are no air bubbles present. Puncture any air bubbles using a pipette tip.

IMPORTANT!

- Avoid touching the sides or the top of the wells with the pipette tips. Droplets near, or on the top of the well dividers can cause the lid to stick to the tray during GeneTitan™ processing.
- 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.

20. Transfer the reagent trays, scan tray to the GeneTitan™ MC Instrument and load.

    See "Stage 3—Ligate, wash, stain, and scan" on page 148 continue the process on the GeneTitan™ MC Instrument.

21. Return to the NIMBUS® Instrument, then clear the deck. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.

IMPORTANT! Immediately load the reagent trays onto the GeneTitan™ MC Instrument. Do not leave denatured samples or reagent trays at room temperature for any length of time.

Stage 5 and stage 6 for the 8-plate workflow

When processing arrays in an 8-plate workflow on the GeneTitan™ MC Instrument, it is necessary to prepare reagents for GeneTitan™ fluidics processing of 1 plate which has already finished hybridization while preparing a second plate to start hybridization. Proper timing is required for performing these 2 stages of the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow concurrently.

- "Stage 5: Prepare the hybridization tray" on page 105 is performed for the plate about to start hybridization in the GeneTitan™ MC Instrument.
- "Stage 6: Prepare GeneTitan™ reagent trays" on page 113 is performed for the plate that has already finished hybridization and about to start fluidics processing in the GeneTitan™ MC Instrument.

Coupling these processes occurs on day 2, day 3, and day 4 of the 8 plate workflow. Day 1 of the workflow uses stage 5 only, whereas day 5 of the workflow uses stage 6 only.

Couple stage 5 and stage 6 in a multiplate workflow

The following steps outline the necessary procedure for coupling Stage 5 and Stage 6 when running a multiplate workflow.

1. Start "Stage 6: Prepare GeneTitan™ reagent trays" on page 113 for the plate that is already in the GeneTitan™ MC Instrument hybridization oven. Carry out the steps of:
   a. "Perform the prerun checklist" on page 53.
   b. Prepare reagents as instructed in "Reagents required and reagent handling" on page 115.
   c. "Run the prepare GeneTitan™ reagent trays step" on page 118.
2. While the NIMBUS® Instrument is running the GeneTitan™ reagent preparation step, start denaturation of the hybridization ready samples for the plate that is to be hybridized next. Follow the instructions in "Stage 5: Prepare the hybridization tray" on page 105 for the steps of:
   a. "Prepare samples that have been stored at –20°C" on page 107.

b. "Prepare the array plate" on page 108.

c. "Denature the samples" on page 109.

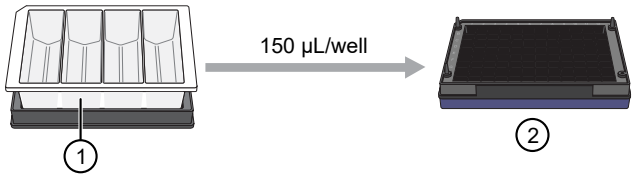
Note: Avoid letting the hybridization ready samples sit at room temperature or in the thermal cycler for an extended amount of time after denaturation. Do not start denaturation at the same time as the GeneTitan™ reagent preparation.

3. While the NIMBUS® Instrument and hybridization ready sample denaturation are running, ensure that the GeneTitan™ MC Instrument is ready for use by following the instructions given in Appendix B, "Register samples in GeneChip™ Command Console™" and "Stage 3—Ligate, wash, stain, and scan" on page 148. A brief summary of steps to be performed is:
   a. Upload your GeneTitan™ array plate registration file now. If you do not upload your samples before scanning the array plate barcode, the software assigns names to your samples.
   b. At the GCC GeneTitan™ Control software prompts:
      - Fill the Wash A, Wash B and Rinse bottles.
      - Empty the Waste bottle.
      - Remove consumable trays and plates as instructed, except for the blue base. Leave the blue array plate base in drawer 6 although the base is empty.
4. After completion of the GeneTitan™ reagent preparation run on the NIMBUS® Instrument, follow the steps that are described in "Stage 6: Prepare GeneTitan™ reagent trays" on page 113 in the target preparation chapter, and in "Stage 3—Ligate, wash, stain, and scan" on page 148 of the array processing chapter to load reagent trays for the plate which has already finished hybridization:
   a. Remove reagent trays from the NIMBUS® Instrument deck, then inspect for under filled wells and bubbles.
   b. Cover the reagent trays.
   c. Load reagent trays into the GeneTitan™ MC Instrument. After loading the reagent trays, click **YES** at the "Do you want to load another plate" prompt.

   IMPORTANT! Ensure to click **YES** if hybridization of a subsequent plate is planned.
5. Clear the NIMBUS® Instrument deck, then follow instructions in "Run the prepare hybridization tray step" on page 110 to set up the deck transfer the denatured hybridization ready samples from the thermal cycler to the deck, then run the method.
6. Immediately after completion of the transfer samples to hybridization tray step, load the hybridization tray and array plate into the GeneTitan™ MC Instrument (see "Load a second array plate and hybridization tray" on page 145).
7. Return to the NIMBUS® Instrument, then clear the deck as described in the on-screen prompt.

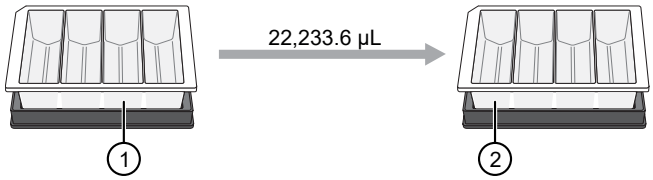
Workflow for Stage 6: Prepare GeneTitan™ reagent trays

Transfer Axiom™ Hold Buffer to scan tray



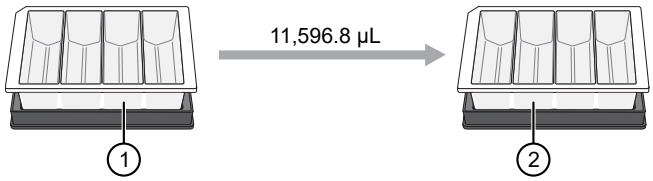
① Axiom™ Hold Buffer reservoir, column 2 (deck position 7)
② Scan tray on base (deck position 4)

Transfer Axiom™ Wash A to Stain 1 Reservoir



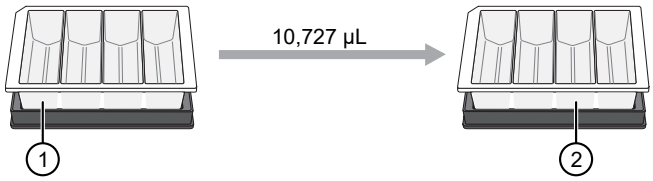
① Axiom™ Wash A, column 3 (deck position 7)
② Stain 1 Master Mix Reservoir, column 1 (deck position 3)

Transfer Axiom™ Wash A to Stain 2 Reservoir



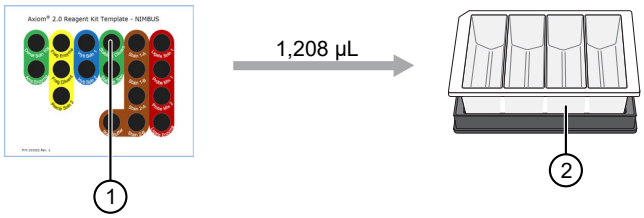
① Axiom™ Wash A, column 3 (deck position 7)
② Stain 2 Master Mix Reservoir, column 2 (deck position 3)

Transfer Axiom™ Water to Stabilize Master Mix Reservoir



① Axiom™ Water, column (deck position 7)
② Stabilize Master Mix Reservoir, column 3 (deck position 3)

TransferAxiom™ Stabilize Diluent to Stabilize Master Mix Reservoir



① Axiom™ Stabilize Diluent, (deck position 2)
② Stabilize Master Mix Reservoir, column 3 (deck position 3)

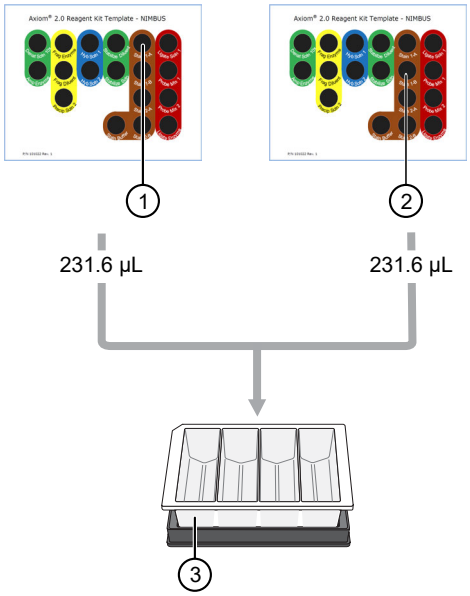
Transfer Axiom™ Stain Buffer to Stain 1 Reservoir

① Axiom™ Stain Buffer, (deck position 2)
② Stain 1 Master Mix Reservoir, column 1 (deck position 3)

Transfer Axiom™ Stain Buffer to Stain 2 Reservoir

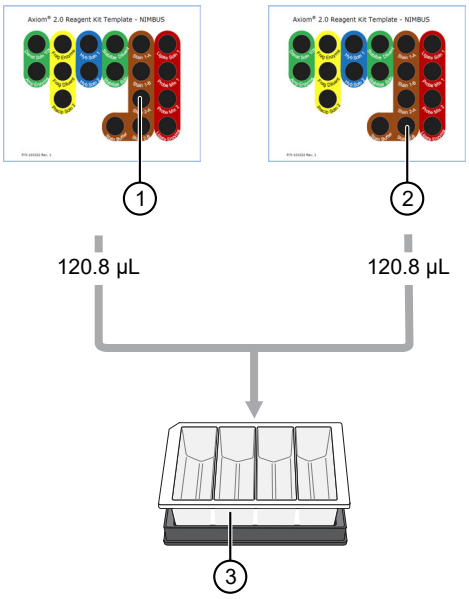
① Axiom™ Stain Buffer, (deck position 2)
② Stain 2 Master Mix Reservoir, column 3 (deck position 3)

Transfer Axiom™ Stain 1-A and Axiom™ Stain 1-B to Stain 1 Master Mix Reservoir



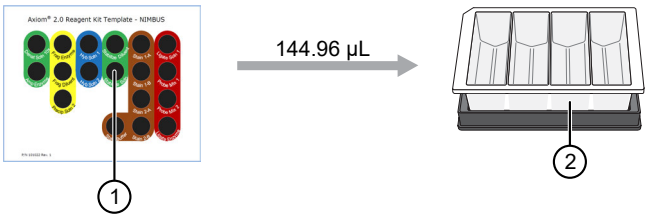
① Axiom™ Stain 1-A (deck position 2)
② Axiom™ Stain 1-B (deck position 2)
③ Stain 1 Master Mix Reservoir, column 1 (deck position 3)

Transfer Axiom™ Stain 2-A and Axiom™ Stain 2-B to Stain 2 Master Mix Reservoir



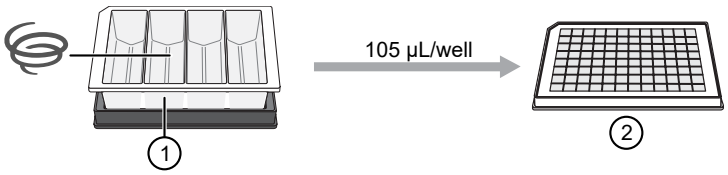
① Axiom™ Stain 2-A (deck position 2)
② Axiom™ Stain 2-B (deck position 2)
③ Stain 2 Master Mix Reservoir, column 2 (deck position 3)

Transfer Axiom™ Stabilize Soln to Stabilize Master Mix Reservoir



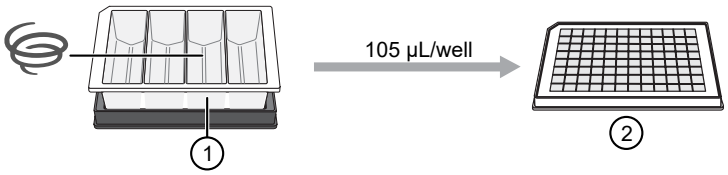
① Axiom™ Stabilize Soln (deck position 2)
② Stabilize Master Mix Reservoir, column 3 (deck position 3)

Prepare Stain 2 Tray



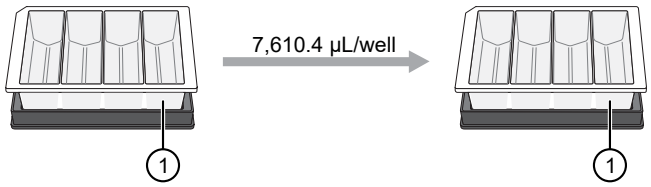
① Stain 2 Master Mix Reservoir, column 2 (deck position 3)
② Stain 2 Tray (deck position 5)
Mix

Prepare Stabilize Solution Tray



① Stabilize Master Mix Reservoir, column 3 (deck position 3)
② Stabilize Tray (deck position 8)
Mix

Transfer Axiom™ Ligate Buffer to Ligation Master Mix Reservoir

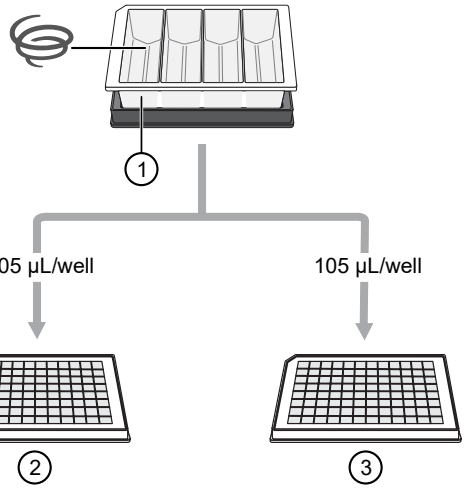


① Axiom™ Ligate Buffer, column 4 (deck position 7)
② Ligation Master Mix Reservoir, column 4 (deck position 3)

User intervention required

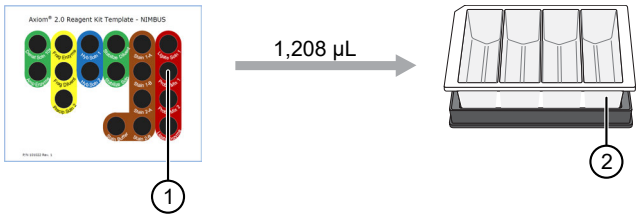
1. Remove the following items from the deck:
   - Scan Tray (deck position 4)
   - Stain 2 Tray (deck position 5)
   - Stabilize Solution Tray (deck position 8)
   - 4-column Reservoir + Reservoir Frame (deck position 7)
2. Load onto the deck the following items:
   - Stain 1-1 Tray (deck position 5)
   - Stain 1-2 Tray (deck position 8)
   - Ligation Tray (deck position 4)
   - 24-Position Tube Rack (deck position 7)

Prepare Stain 1 Trays



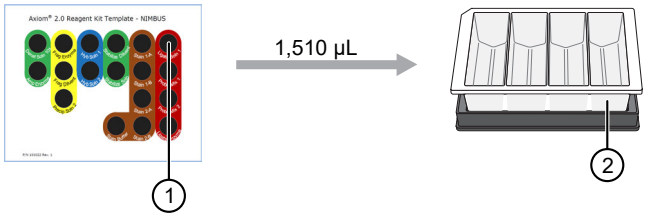
① Stain 1 Master Mix Reservoir, column 1 (deck position 3)
② Stain 1-1 Tray (deck position 5)
③ Stain 1-2 Tray (deck position 8)
Mix

Transfer Axiom™ Probe Mix 1 to Ligation Master Mix Reservoir



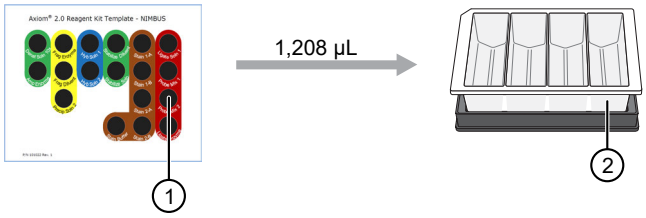
① Axiom™ Probe Mix 1 (deck position 2)
② Ligation Master Mix Reservoir, column 4 (deck position 3)

Transfer Axiom™ Ligate Soln 1 to Ligation Master Mix Reservoir



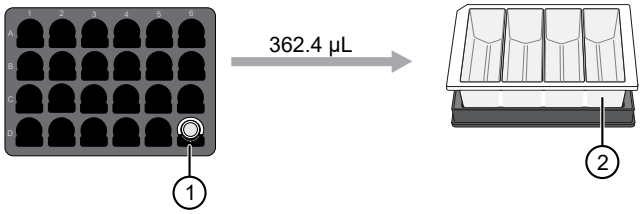
① Axiom™ Ligate Soln 1 (deck position 2)
② Ligation Master Mix Reservoir, column 4 (deck position 3)

Transfer Axiom™ Probe Mix 2 to Ligation Master Mix Reservoir



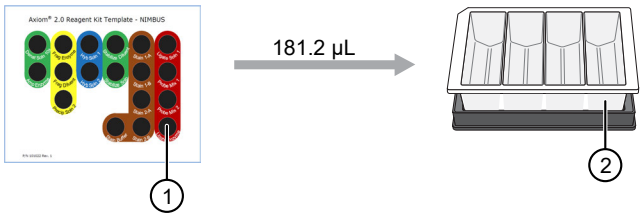
① Axiom™ Probe Mix 2 (deck position 2)
② Ligation Master Mix Reservoir, column 4 (deck position 3)

Transfer Axiom™ Ligate Soln 2 to Ligation Master Mix Reservoir



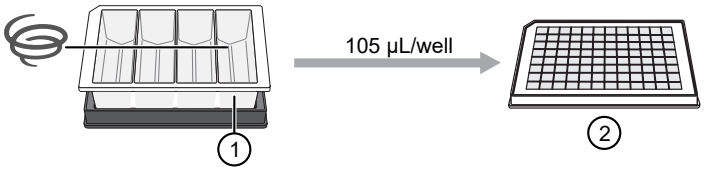
① Axiom™ Ligate Soln 2 (deck position 7)
② Ligation Master Mix Reservoir, column 4 (deck position 3)

Transfer Axiom™ Ligate Enzyme to Ligation Master Mix Reservoir



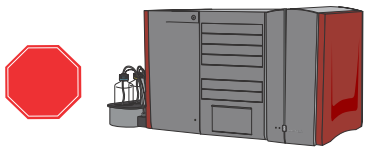
① Axiom™ Ligate Enzyme (deck position 2)
② Ligation Master Mix Reservoir, column 4 (deck position 3)

Prepare Ligation Tray



① Ligation Master Mix Reservoir, column 4 (deck position 3)
② Ligation Tray (deck position 4)
Mix

Process plates on the GeneTitan™ MC Instrument



Load stain and scan trays onto the GeneTitan™ MC Instrument.

6 Process array plates with the GeneTitan™ 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.

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

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

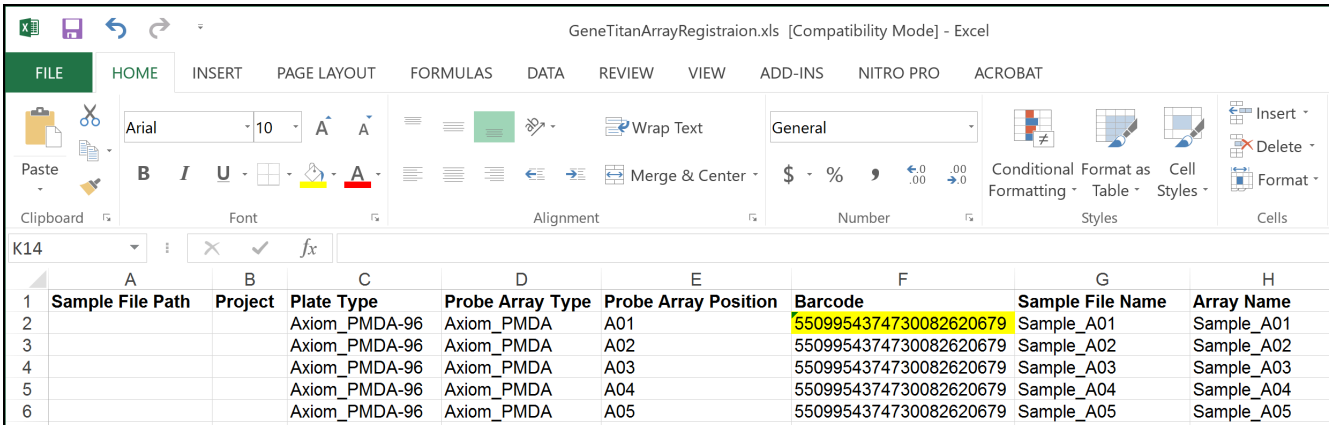
Stage 1—Create and upload a GeneTitan™ Array Plate Registration file

A batch registration file must be created and uploaded with GeneChip™ Command Console™ (GCC) software before you start "Stage 2—Hybridize plates in the GeneTitan™ MC Instrument" on page 136. This file contains information critical for data file generation during scanning, and for tracking the experimental results for each sample loaded onto an array plate. This file can be created at any time before loading the array plate and hybridization tray onto the GeneTitan™ MC Instrument.

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

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

1. If you have already created, then saved a batch registration file but have not yet uploaded the file to GCC, open the file, then go to step 6.
2. From the **Launcher** window, open **GCC Portal ▸ Samples ▸ GeneTitan™ Array Plate Registration**.
3. In the GeneTitan™ Array Plate Registration window, from the **GeneTitan Array Plate Type** list, select the plate type to be processed.
4. Click **Download**.
5. In the **Samples** tab of the GeneTitan™ Array Plate Registration file, enter a unique name for each sample (**Sample File Name**) and any additional information.
   Additional information on the GeneTitan™ Array Plate Registration file is in the *GeneChip™ Command Console™ User Guide*.
6. Scan the array plate barcode into the yellow **Barcode** field, column **F**.
   See Figure 22.
7. Scan the barcode of the hybridization tray if the array plate registration file template includes a column for the hybridization tray barcode.
8. Save the file.
   By default, the file is saved in the `Applied Biosystems Download` folder.
9. Return to the GCC Portal GeneTitan™ Array Plate Registration page.
   a. Click **Browse**, navigate to the array plate registration file, then click **Open**.
   b. Under **Step 3**, click **Upload**, wait for the information to load, then click **Save** found at the bottom of the next window that is displayed.



	A	B	C	D	E	F	G	H
1	**Sample File Path**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array Position**	**Barcode**	**Sample File Name**	**Array Name**
2			Axiom_PMDA-96	Axiom_PMDA	A01	550995437473008262067 9	Sample_A01	Sample_A01
3			Axiom_PMDA-96	Axiom_PMDA	A02	550995437473008262067 9	Sample_A02	Sample_A02
4			Axiom_PMDA-96	Axiom_PMDA	A03	550995437473008262067 9	Sample_A03	Sample_A03
5			Axiom_PMDA-96	Axiom_PMDA	A04	550995437473008262067 9	Sample_A04	Sample_A04
6			Axiom_PMDA-96	Axiom_PMDA	A05	550995437473008262067 9	Sample_A05	Sample_A05

Figure 22 Example of a GeneTitan™ Array Plate Registration file.

Stage 2—Hybridize plates in the GeneTitan™ MC Instrument

Materials, labware, and reagents required

Reagents required

The following reagents from the Axiom™ 2.0 Plus Reagent Kit 96F are required for the hybridization step.

Reagent	Module
Axiom™ Wash Buffer A (both bottles, 1 L)	Module 3, room temperature
Axiom™ Wash Buffer B	
Axiom™ Water	

Materials required

- Hybridization tray containing denatured samples.

  Note: The denatured samples must be transferred to the hybridization tray only after the GeneTitan™ MC Instrument is ready for loading.

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

Warm array plate to room temperature

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

1. Remove the array plate packaging from the 4°C refrigerated storage.
2. Open the array plate box, then remove the pouch containing the array plate and protective base. Do not open the pouch.
3. Equilibrate the unopened pouch on the bench for at least 25 minutes.
4. At the end of the array warm-up time, open the pouch, then scan the array plate barcode into the batch registration file.
   See “Stage 1—Create and upload a GeneTitan™ Array Plate Registration file” on page 134.

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

Set up the instrument

1. Select **GCC GeneTitan Control** from the GCC **Launcher**.
   The system initializes. After initialization, the **System Status** tab is selected, and the status of the hybridization oven is displayed at the bottom of the Log panel. The status reads <*Time of day*> *System Ready.*

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

IMPORTANT! Do not close the scanner application by right-clicking and selecting the **Close** option. This method causes the scanner application to exit abnormally and delay in processing the next plate. The correct way to close the application is described in “Shut down the GeneTitan™ MC Instrument” on page 155.

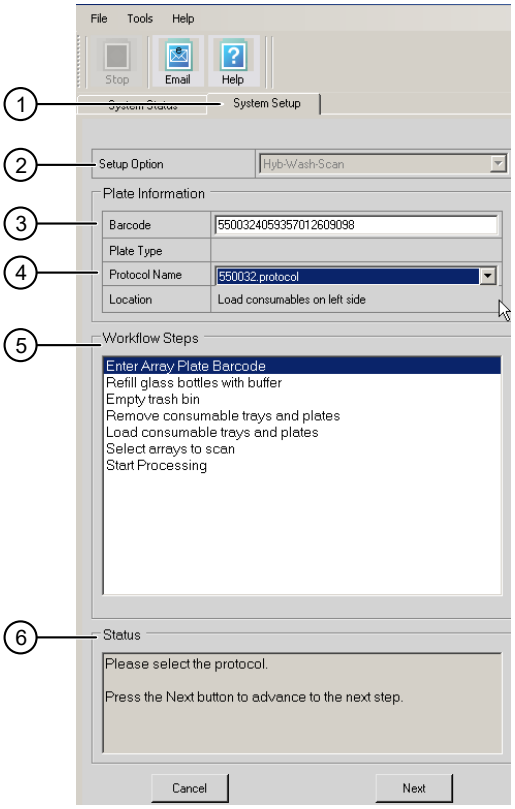


Figure 23 System Setup tab.

① **System Setup** tab.

② **Setup Option**: Access a list of the various options available for processing array plates.

③ **Barcode**: The array plate barcode. The barcode can be scanned or entered manually.

④ **Protocol Name**: The list of protocols that are displayed is based on the first 6 digits of the array plate barcode. Only the protocols that are valid for the array plate type that is loaded are displayed.

⑤ **Workflow Steps**: This field displays an overview of the user actions that are required to process an array plate that is based on the **Setup Option** selected.

⑥ **Status**: This field displays the actions that must be performed to prepare or unload the GeneTitan™ MC Instrument for the selected **Setup Option**.

After each action, click **Next** or press the blinking confirmation button on the GeneTitan™ MC Instrument to continue.

2. Select **Hyb-Wash-Scan** from the **Setup Option** list.

   Other options available are described under “Setup options for array plate processing” on page 212.

3. Click **Next**.

Note: A message is displayed when insufficient disk space is available. Delete or move DAT files to another location to free up sufficient disk space for the data that are generated by 8 Axiom™ Precision Medicine Diversity Research Array Plates. One 96-array plate requires ~80 GB of disk space.

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

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

   Note: If an error message is displayed after entering the array plate barcode, do the following:
   - Ensure that the library files for the type of array plate you are using are correctly installed.
   - Library files must be installed before launching the GeneTitan™ MC Instrument. If a library file must be installed, exit the GeneTitan™ MC Instrument, install libraries, and relaunch the GeneTitan™ MC Instrument.
   - Try manually entering the array plate barcode.

   ---

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

6. Refill the bottles with the following reagents.

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

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

   All 600 mL of the Axiom™ Wash Buffer B from the Axiom™ 2.0 Plus Reagent Kit 96F must be emptied into the GeneTitan™ Wash B bottle when setting up the system.

   - Using all of the Axiom™ Wash Buffer B contents from the reagent kit ensures that the GeneTitan™ Wash B bottle is filled to more than the minimum requisite 35% of bottle volume.
   - If you intend to load 2 array plates on the same day, fill the Wash B bottle to the 1-L mark (use 2 bottles from the Axiom™ 2.0 Plus Reagent Kit 96F).

   Do not overfill the bottles.

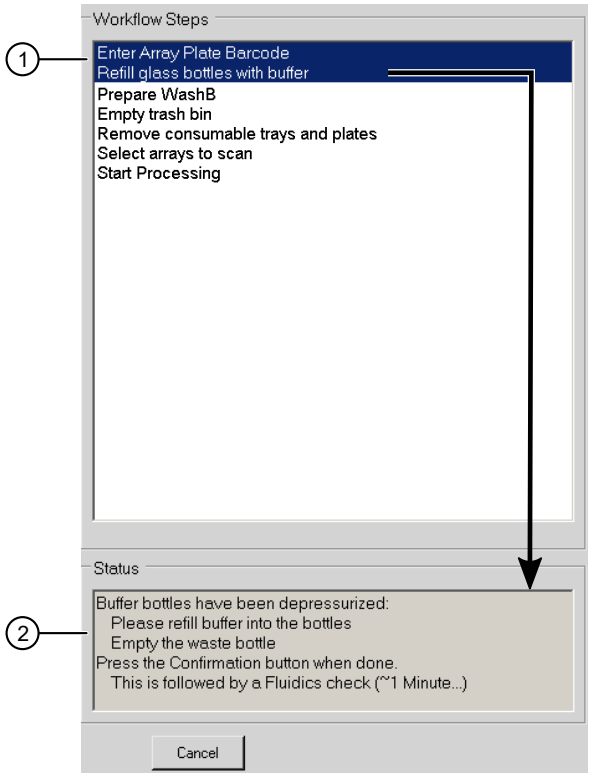
   - The maximum volume for the Wash B and Rinse bottles is 1 L. Fill to the 1-L mark only.
   - The maximum volume for the Wash A bottle is 2 L.

   We strongly recommend refilling these bottles every time you are prompted to do so. If the volume in any of these bottles becomes too low during a run, a message is displayed. However, even if you fill the bottle then, the instrument cannot complete the step that was in progress.

7. Empty the waste bottle.

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

   A fluidics check is run (~1 minute).



① **Workflow Step**

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

9. 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 confirmation button to continue.
10. Remove consumable trays and plates.
    a. Remove used trays and plates when drawers open.
    b. If no consumables are in the drawers to remove, the **Status** window reads "Drawers are empty".
    c. Press the confirmation button to continue.
11. Continue to “Load an array plate and hybridization tray into the GeneTitan™ MC Instrument (for Hyb-Wash-Scan or Hyb-Wash)” on page 141 when prompted by the GCC software.

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.

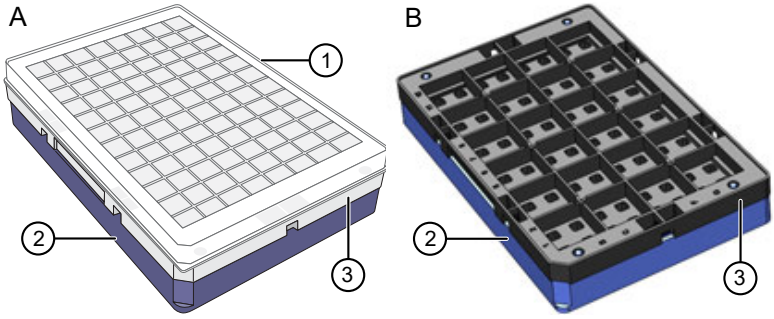


Figure 24 Array plate components, as shipped (2 configurations).

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.

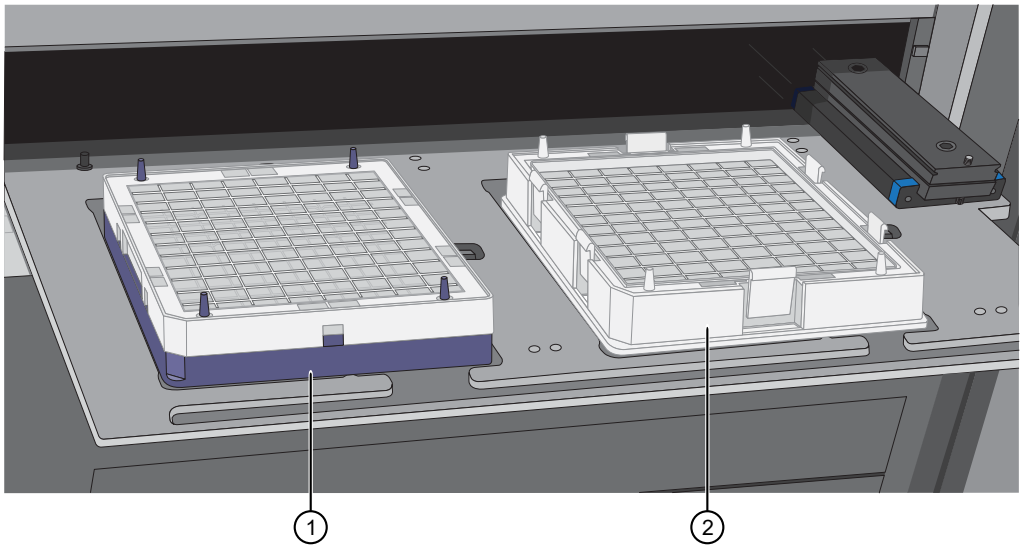


Figure 25 Array plate on protective blue base and the hybridization tray properly loaded into drawer 6.

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 26. *No clicking sound indicates proper clamping.*

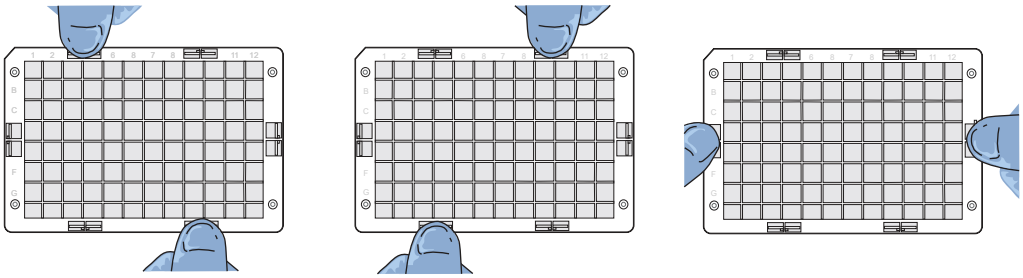


Figure 26 Clamping verification steps.

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 144.

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 27). 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 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.

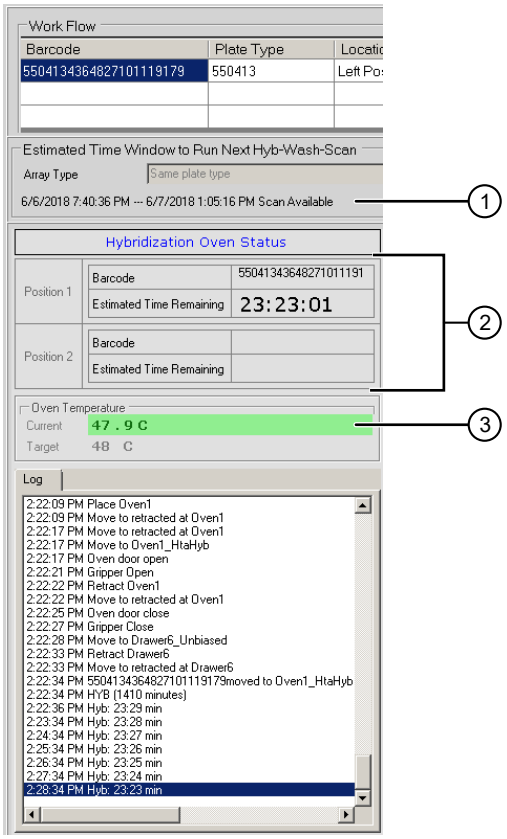


Figure 27 When to load a second array plate and hybridization tray based on oven status information.

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.

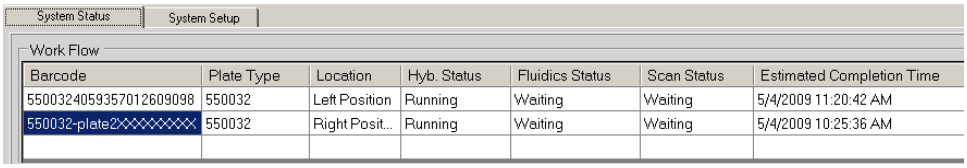


Figure 28 Example of the Work Flow pane when 2 plates are loaded and are in the hybridization oven.

Queue a second plate for scanning

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

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

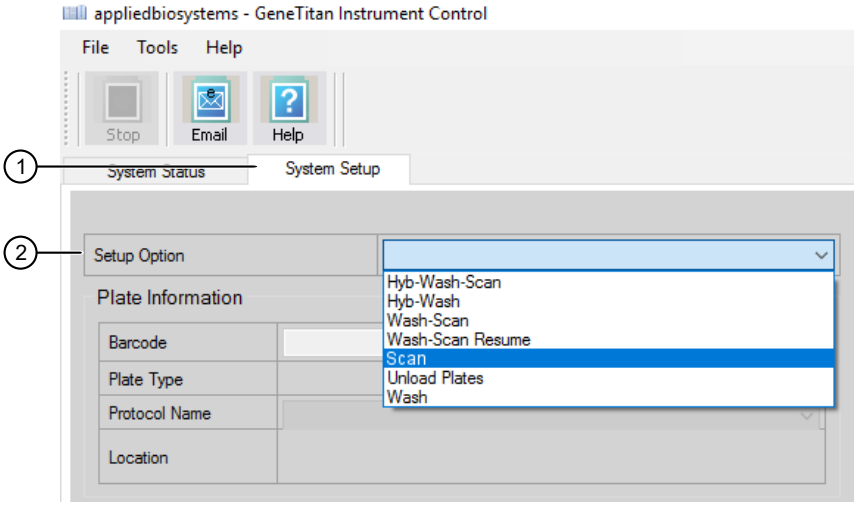


Figure 29 Scan setup option to process a second array plate.

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

3. Click **Next** in the lower left 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 confirmation button on the instrument to continue.

6. Place the array plate on top of a scan tray, which has already been filled with holding buffer, in the correct orientation, with the notched corner of the array plate and scan tray aligned.

7. Load the array plate/scan tray stack 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 stack is loaded in the correct orientation in the drawer.

   For further information on the proper alignment and loading of plates, covers, and trays in the GeneTitan™ MC Instrument, see “Proper tray alignment and placement” on page 203.

8. When ready, press the 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 becomes available.

Stage 3—Ligate, wash, stain, and scan

The GeneTitan™ tray loading process

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

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

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

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

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

Load trays in GeneTitan™ MC Instrument

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

   b. Empty the trash bin.

   c. Remove consumable trays and plates as instructed, except for the blue base. Leave the blue array plate base in drawer 6 although the base is empty.

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

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

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

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

a. When drawer 2 opens:
   - Left side: Scan tray with cover. Remove the protective black base from the scan tray immediately before loading. Do not load the protective black base.
   - When complete, press the confirmation button on the GeneTitan™ MC 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 your Field Service Engineer if the fingers do not retract automatically.

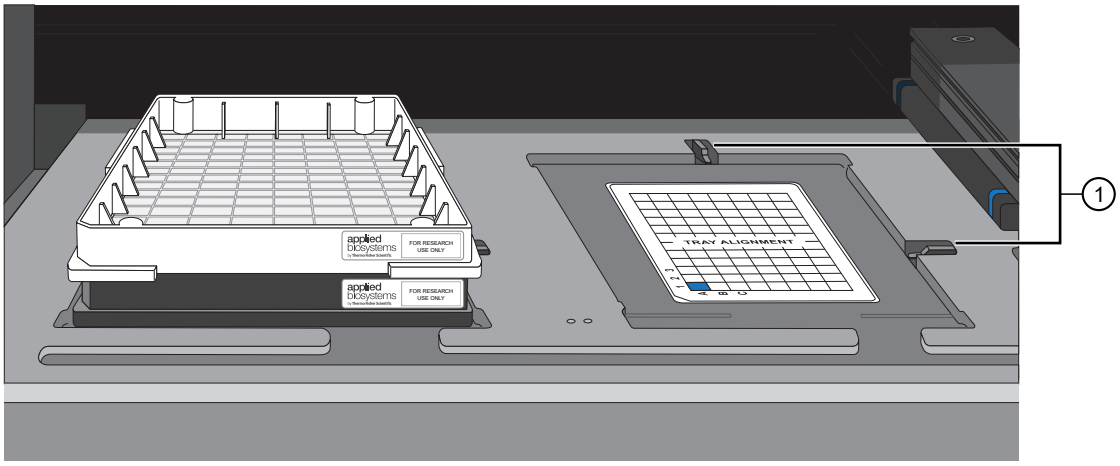


Figure 30 Drawer 2, left side: Scan tray with cover.

(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 confirmation button on the GeneTitan™ MC Instrument to continue.

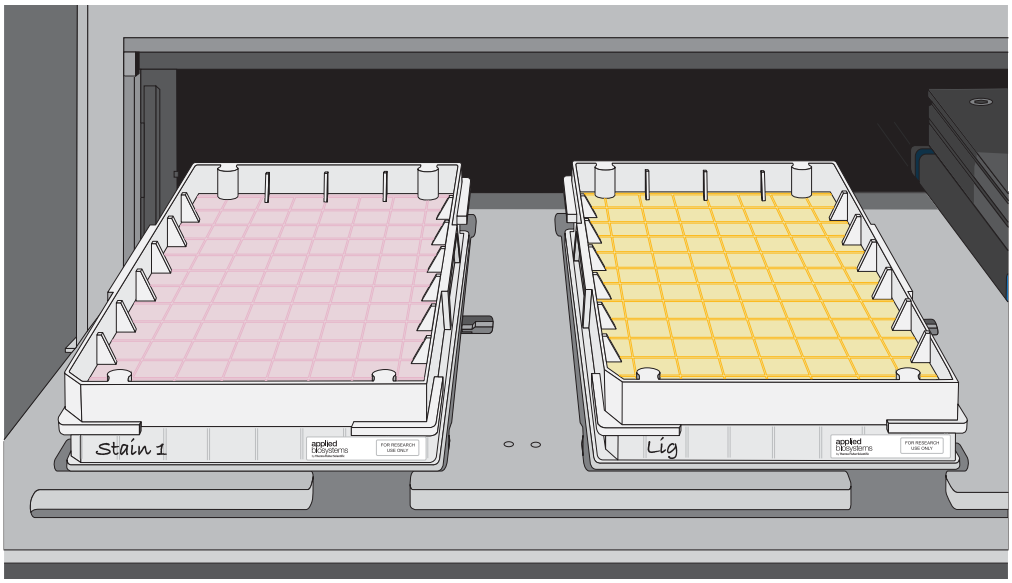


Figure 31 Drawer 3, left side: Stain 1 tray with cover. Drawer 3, right side: Ligation tray with cover.

c. When drawer 4 opens:

- Left side: Stain 2 tray with cover.
- Right side: Stabilization tray with cover.
- Press the confirmation button on the GeneTitan™ MC Instrument to continue.

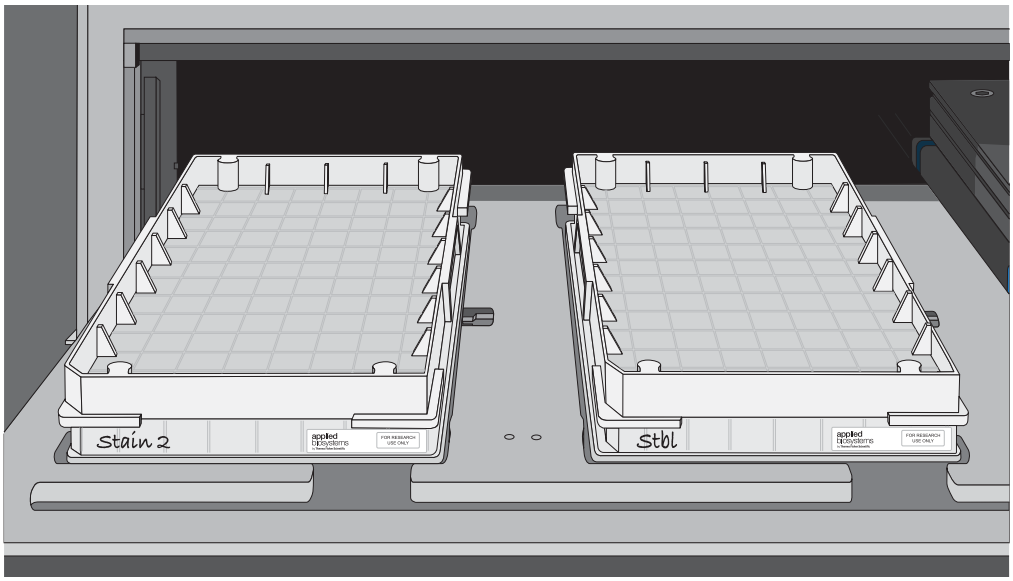


Figure 32 Drawer 4, left side: Stain 2 Tray with cover. Drawer 4 right side: Stabilization Tray.

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

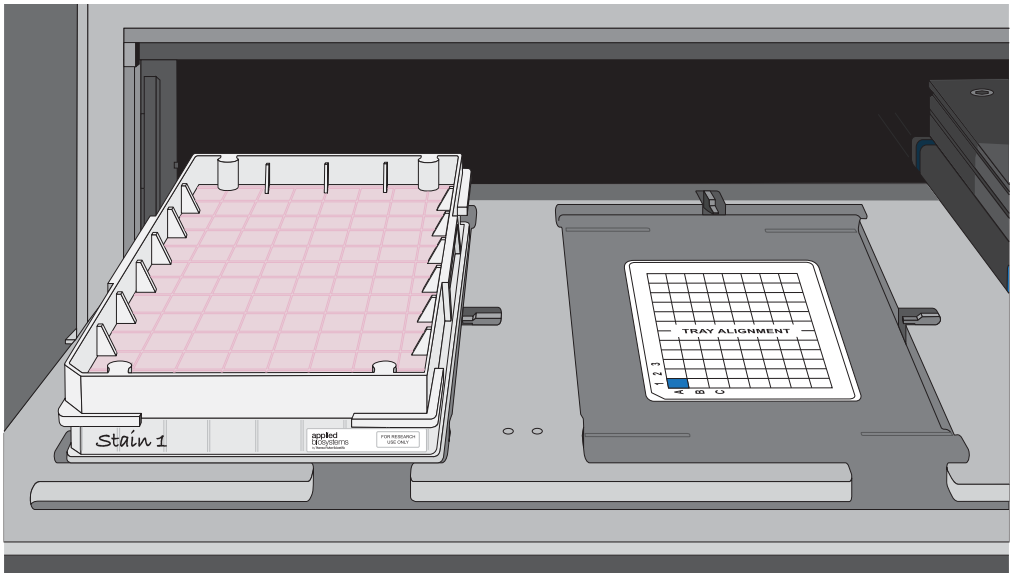


Figure 33 Drawer 5, left side: Stain 1 tray with cover.

3. At the **WorkFlow Option** window prompt, click **Yes** if you want to load another array plate and hybridization tray.
4. In the **Setup Option**, select **Setup Another Run**, then click **Next**.
5. Scan or manually enter the array plate barcode, then click **Next**.
6. Select a protocol, then click **Next**.
7. When drawer 6 opens:
   a. Remove the blue cover from the previous array plate.
   b. Load a new array plate and new blue base on the left side of the drawer, then load a new hybridization tray on the right side of the drawer.
   c. Press the confirmation button.
   d. Click **OK** to continue.
   e. When drawer 6 opens, ensure that the plate stack is securely clamped.
      If needed, review the clamping procedure. (See “Load an array plate and hybridization tray into the GeneTitan™ MC Instrument (for Hyb-Wash-Scan or Hyb-Wash)” on page 141.)
   f. Press the confirmation button.

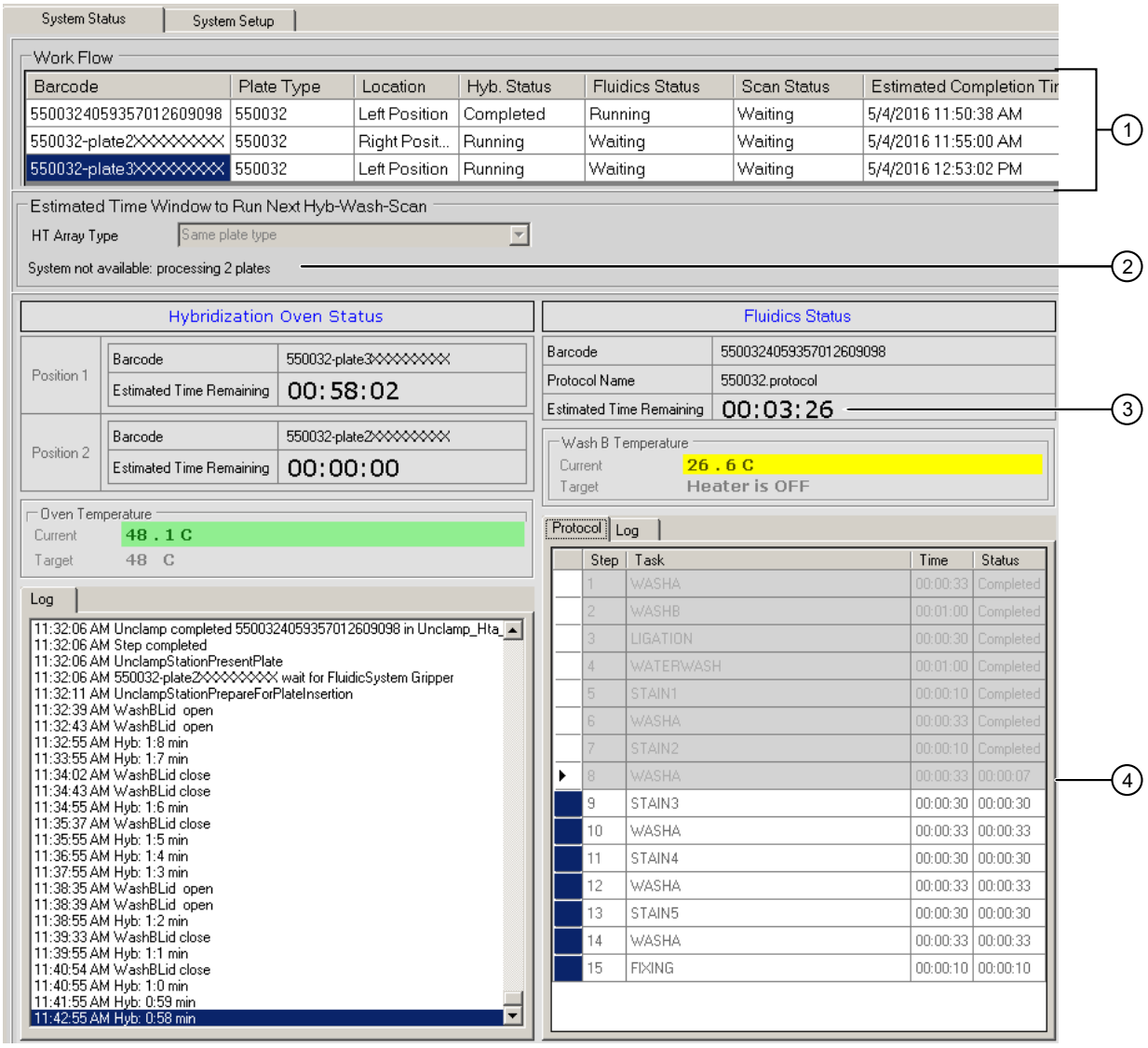
GeneTitan™ MC Instrument internal array plate activity

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

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

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

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



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

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

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

④ The step that is currently executing in fluidics.

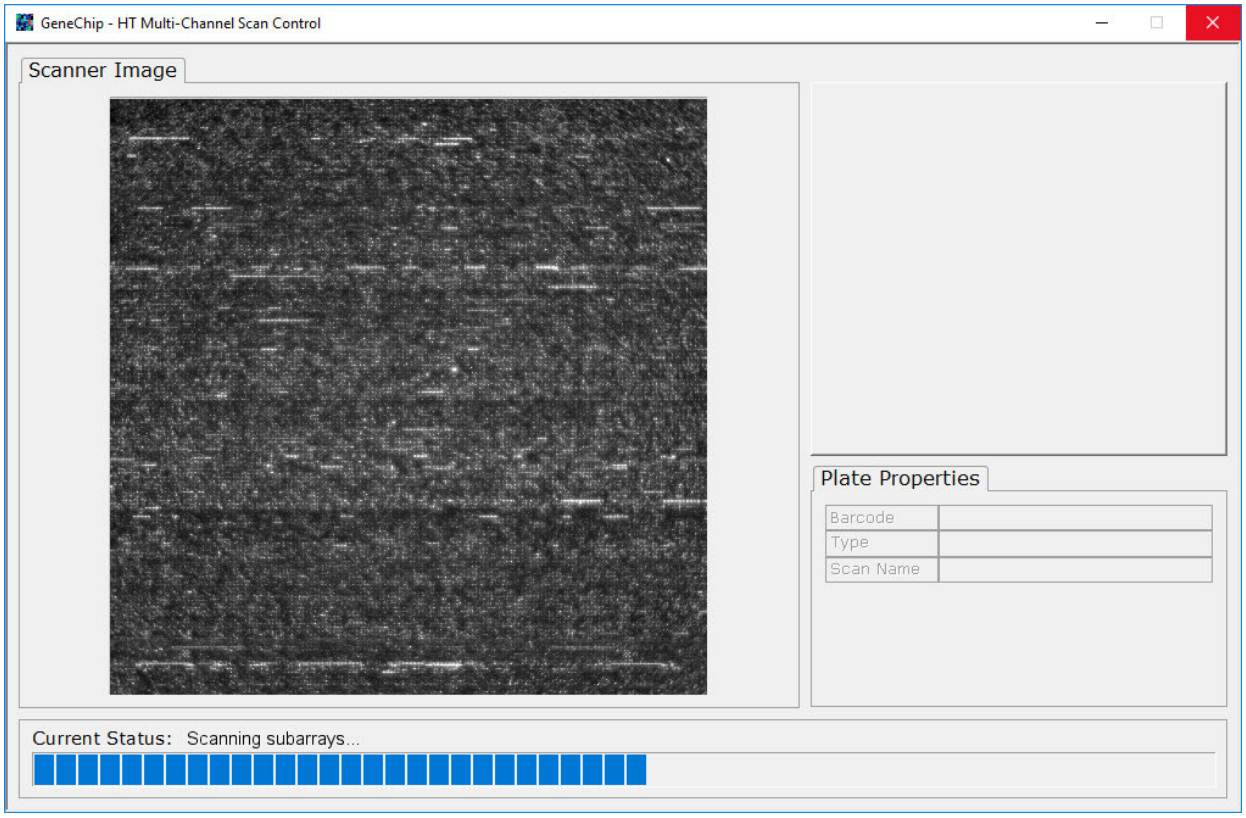
Scan workflow

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.



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 8-plate workflow for Axiom™ PMD Array Plates

The Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 8-plate workflow allows array processing in the GeneTitan™ MC Instrument on 8 sample plates per 5-day work week at the same time that target preparation is performed on 8 more sample plates. Samples that are prepared one week can be hybridized the following week to continue the workflow. This chapter includes tables that present the timing of the steps that are required to perform this workflow in a 5-day work week, 10 hours per day.

The workflow that is presented is under the following assumptions:

- Three FTEs: 1 dedicated mPCR operator and 2 operators for the NIMBUS® Target Preparation Instrument protocols.
- One verified NIMBUS® Instrument, 1 verified GeneTitan™ MC Instrument, and 2 verified thermal cyclers available in laboratory.

IMPORTANT! Previous experience with the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow and careful timing is critical for the successful execution of this workflow.

This chapter assumes user familiarity with all procedures for target preparation and array processing using the NIMBUS® Target Preparation Instrument and the GeneTitan™ MC Instrument.

Note: If performing the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow without the mPCR step, skip all the mPCR activities and proceed directly to “Stage 1B: Amplify the genomic DNA” to start target preparation.

Overview of the 8-plate workflow

For continual processing of plates, a summary of the activities by week is:

- Week 1
  - Target preparation—perform target preparation on eight sample plates to create 8 hybridization ready plates.
- Week 2 (and beyond)
  - Array processing—hybridize the 8 plates that were prepared in week 1.
  - Target preparation—perform target preparation on 8 more sample plates. Array processing for these hybridization ready plates can be carried out the following week.

A scheme illustrating the timing that is required for the second week of this workflow is given in Figure 34 and Figure 35, and steps that are performed on the NIMBUS® Instrument and GeneTitan™ MC Instrument are listed in Table 22 and Table 23. Throughout this chapter, plates undergoing the target preparation on the NIMBUS® Instrument are given letters A through H whereas plates undergoing array processing on the GeneTitan™ MC Instrument is given numbers 1 through 8. Hybridization ready plates that are prepared on the NIMBUS® Instrument can be processed on arrays in any order on the GeneTitan™ MC Instrument. Detailed day-by-day instructions are given in additional figures and tables in this chapter.

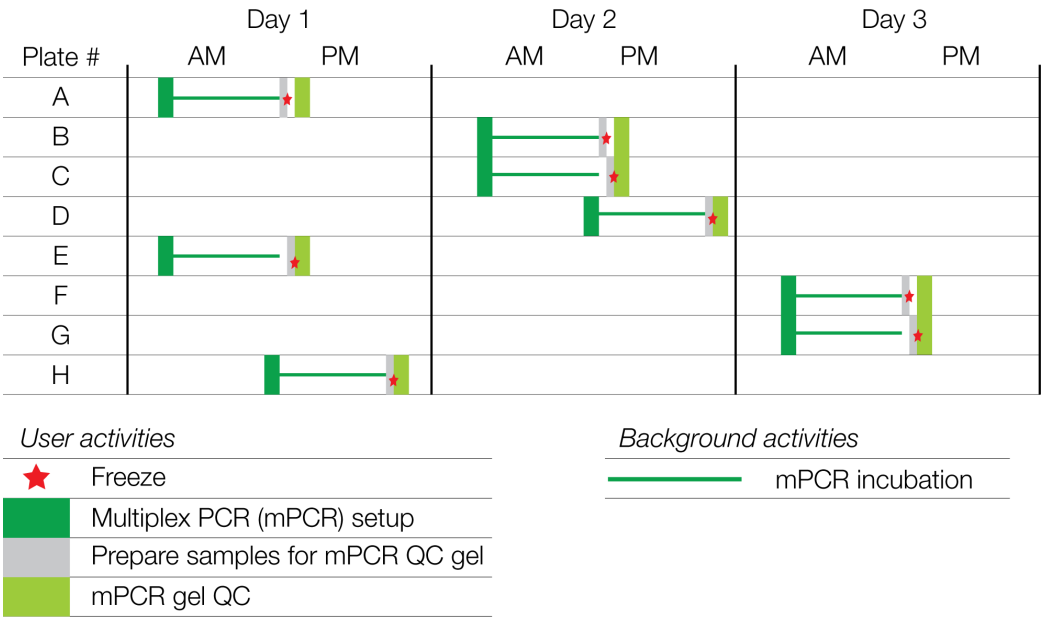


Figure 34 Week 2: mPCR activities for Axiom™ 2.0 Plus Assay simultaneous 8-plate workflow.

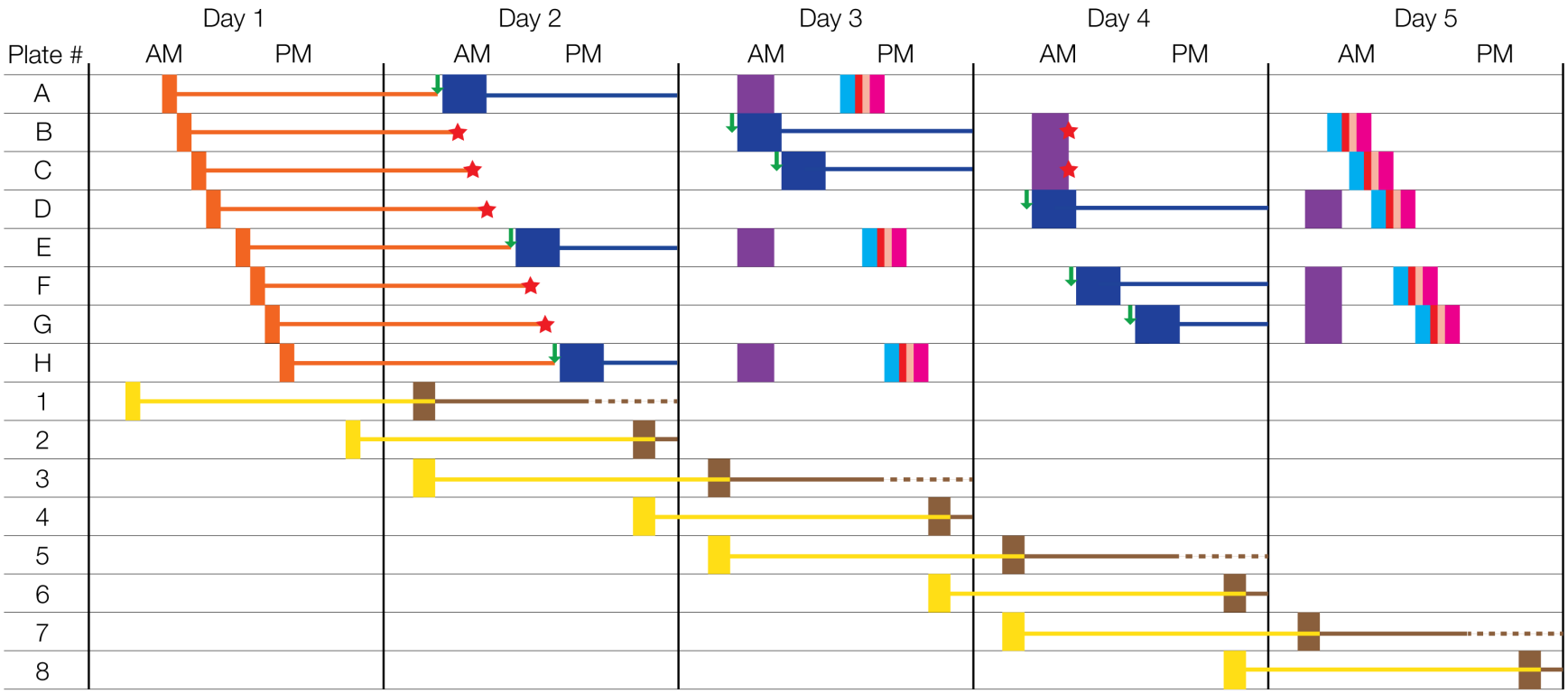


Figure 35 Week 2: Activities for NIMBUS® Instrument and GeneTitan™ MC Instrument simultaneous 8-plate workflow.

Overview of the manual mPCR and automated target preparation steps

Table 22 Overview of the manual mPCR and automated target preparation steps for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow on the NIMBUS® Instrument.

Day	Activity	Plates
1	• mPCR 3 plates of genomic DNA. • Amplify 8 plates of genomic DNA.	• A, E, H • A–H
2	• mPCR 3 plates of genomic DNA. • Fragment and precipitate 3 plates amplified DNA on day 1. • Freeze 5 plates of amplified DNA for fragmentation later in the week.	• B, C, D • A, E, H • B, C, D, F, G
3	• mPCR 2 plates of genomic DNA. • Fragment and precipitate 2 plates of frozen amplified DNA on day 2. • Centrifuge/dry, resuspend, prepare hybridization-ready sample plate, sample QC, and off-deck QC (OD, gel QC run) 3 plates precipitated on day 2.	• F, G • B, C • A, E, H
4	• Fragment and precipitate the 3 remaining plates of frozen amplified DNA on day 2. • Centrifuge/dry and freeze pellets for 2 plates precipitated on day 3.	• D, F, G • B, C
5	• Resuspend, prepare hybridization-ready sample plate, sample QC, and off-deck QC (OD, gel QC run) 2 plates of frozen pellets from day 4. • Centrifuge/dry, resuspend, prepare hybridization-ready sample plate, sample QC, and off-deck QC (OD, gel QC run) 3 plates precipitated on day 4.	• B, C • D, F, G

IMPORTANT! Maintaining consistent timing during the setup of the GeneTitan™ MC Instrument is critical to containing the user interventions of the 8-plate workflow in the work day. After one process starts late, there is little opportunity to catch up until the end of the workflow.

Table 23 Overview of the array processing steps for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow on the GeneTitan™ MC Instrument.

Day	Activity	Plates
1	• Hybridize 2 plates of denatured hybridization ready samples.	• 1 and 2
2	• Load reagent trays in GTMC for fluidics and imaging of plates that are loaded on day 1. • Hybridize 2 plates of denatured hybridization ready samples.	• 1 and 2 • 3 and 4
3	• Load reagent trays in GTMC for fluidics and imaging of plates that are loaded on day 2. • Hybridize 2 plates of denatured hybridization ready samples.	• 3 and 4 • 5 and 6

Table 23 Overview of the array processing steps for the Axiom 2.0 Plus Assay 96-Array Format Automated Workflow on the GeneTitan MC Instrument. *(continued)*

Day	Activity	Plates
4	• Load reagent trays in GTMC for fluidics and imaging of plates that are loaded on day 3. • Hybridize 2 plates of denatured hybridization ready samples.	• 5 and 6 • 7 and 8
5	• Load reagent trays in GTMC for fluidics and imaging of plates that are loaded on day 4.	• 7 and 8

Time required for assay steps

The time that is required of the manually performed multiplex PCR activities is listed in Table 24.

The time that is required for the steps that are involved in target preparation and array processing in the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow is given in Table 25. This table also indicates where detailed instructions for each step can be found.

These steps include:

- Deck setup, run time, and deck cleanup on the NIMBUS® Instrument.
- Preparation and loading of the GeneTitan™ MC Instrument.
- Off-deck steps of centrifugation and drying pellets, DNA pellet resuspension on a microplate shaker, running the QC gel, and reading the OD plate.

Times for thawing reagents are given separately in the detailed day by day instructions in the next section.

Table 24 Time required for Axiom™ multiplex PCR associated steps.

Operation	Time required
Multiplex PCR setup	
Thaw mPCR reagents and prepare mPCR reactions (see "Prepare the mPCR Master Mix" on page 48).	60 minutes
mPCR incubation	
Run mPCR protocol on thermal cycler (see Figure 5).	~3.5 hours[1]
mPCR gel QC	
Prepare samples for mPCR QC gel, and then freeze reaction plate (see "Store the mPCR Product Plate" on page 49).	15 minutes
mPCR gel QC (see Appendix E, "mPCR quality control gel protocol").	30 minutes

Table 24 Time required for Axiom multiplex PCR associated steps. *(continued)*

Operation	Time required
mPCR spike-in	
Thaw mPCR reaction plate (see "Thaw and prepare the mPCR Reaction Plate" on page 66).	20 minutes
Spike mPCR reactions into corresponding WGA plate (see "Perform mPCR spike-in to Amplification Plate" on page 67).	10 minutes

[1] Thermal cycler run time can vary slightly between models.

Table 25 Time required for individual operations involved in the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow for the NIMBUS® Instrument.

Operation	Time required
Target preparation steps	
DNA amplification (see "Stage 1B: Amplify the genomic DNA" on page 52) followed by a 23 ±1-hour incubation in a 37°C oven.	30 minutes
Fragmentation (see "Stage 2: Fragment and precipitate the DNA" on page 63) followed by overnight precipitation in a –20°C freezer.	90 minutes
Off-deck centrifugation and drying pellets (see "Stage 3: Centrifuge and dry pellets" on page 78).	75 minutes
Resuspension and hybridization preparation (see "Stage 4A: Prepare the resuspension buffer" on page 83 and "Stage 4B: Prepare Hybridization Master Mix" on page 90).	30 minutes
Sample QC (see "Stage 4C: Perform sample QC" on page 97).	15 minutes
Off-deck QC: Fragmentation Gel and OD quantification (see Appendix C, "Fragmentation quality control gel protocol" and Appendix D, "Sample quantification after resuspension").	45 minutes
Array processing steps	
Prepare hybridization tray • Sample denaturation off-deck and transfer to hybridization tray on the NIMBUS® Instrument (see "Stage 5: Prepare the hybridization tray" on page 105). • Load Hybridization Tray into the GeneTitan™ MC Instrument, start hybridization.	• 30 minutes • 23.5–24 hours
GeneTitan™ reagent tray preparation & loading. • GeneTitan™ reagent preparation on the NIMBUS® Instrument (see "Stage 6: Prepare GeneTitan™ reagent trays" on page 113). • Load reagent trays into GeneTitan™ MC Instrument (see "Stage 3—Ligate, wash, stain, and scan" on page 148).	45 minutes

Table 25 Time required for individual operations involved in the Axiom 2.0 Plus Assay 96-Array Format Automated Workflow for the NIMBUS Instrument. *(continued)*

Operation	Time required
Concurrent hybridization setup and reagent tray preparation & loading (see "Couple stage 5 and stage 6 in a multiplate workflow" on page 124). • GeneTitan™ reagent preparation on the NIMBUS® Instrument. • Load reagent trays into GeneTitan™ MC Instrument. • Sample denaturation and transfer to hybridization tray on the NIMBUS® Instrument. • Load hybridization tray into GeneTitan™ MC Instrument.	90 minutes

Table 26 GeneTitan™ MC Instrument processing times.

Step on the GeneTitan™ MC Instrument	Time required
Hybridization in the GTMC oven at 48°C.	23.5 to 24 hours
Fluidics processing.	5 hours
Imaging 96-array format plates.	7.5 hours

The hybridization time for a 96-array format plate in the GeneTitan™ MC Instrument is 23.5 hours to 24 hours (Table 26). This time frame provides a 30-minute window during which the instrument control software prompts you to load the reagents that are required for washing and staining. Loading the reagent trays into the GeneTitan™ MC Instrument at the mid-point of this 30-minute window is recommended to allow the wash procedures to start 24 hours after the start of hybridization. If catch-up time is required during the 8-plate workflow, start the loading of reagents at the onset of this 30-minute window (that is, immediately after prompted by the software).

Thaw frozen plates of amplified DNA

Five of the plates (B, C, D, F, and G) in the workflow are frozen at the end of the 23-hour DNA amplification stage on day 2. These plates must be thawed before performing the fragmentation step on day 3 or 4 using the following instructions.

1. Place the deepwell plate in a small water bath.

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

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

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

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

   b. Tightly reseal the plate with a fresh seal.

c. Vortex the plate for 30 seconds to mix.

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

Thaw plates with frozen pellets

Two of the plates (B and C) in the workflow are frozen at the end of centrifugation and drying pellets on day 4. These plates must be pre-equilibrated at room temperature for at least 1.5 hours before proceeding with the resuspension and hybridization protocol on day 5.

1. Leave the plate on the bench for 1.5 hours until all wells have equilibrated to room temperature.

2. If needed, centrifuge the plate at 1,000 rpm for 30 seconds to bring down any droplets or condensation that is generated from thawing the plate.

Target preparation and array processing for the 8-plate workflow

Day 1 activities

Multiplex PCR activities

- Manually prepare 3 plates of mPCR (plates A, E, and H) from genomic DNA.
- Run mPCR QC gel (plates A, E, and H).
- Freeze mPCR reaction plates after mPCR reaction run is complete (plates A, E, and H).

NIMBUS® Instrument

- Sample denaturation and prepare hybridization tray on 2 plates of target prepared the previous week (plates 1 and 2).
- Amplify 8 new plates of genomic DNA (plates A through H).

GeneTitan™ MC Instrument

- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization (plates 1 and 2).

Reagent and plate handling

- Start thawing the amplification reagents, particularly the Axiom™ 2.0 Amp Soln, and Axiom™ Water, 60 minutes before the start of each reaction.
- Before each hybridization setup step, warm the array plate to room temperature unopened in its pouch for at least 25 minutes before loading the array plate and hybridization tray into the GeneTitan™ MC Instrument.
- Start warming the Hyb-Ready Plate at room temperature for at least 5 minutes before the hybridization setup step.

Note: All amplifications must be set up on day 1 to allow for a 23 ±1-hour amplification incubation for each plate.

Table 27 Day 1 activities for multiplex PCR.

Activity	Plate	Instrument	Time estimates	
			Start time	End time
Thaw mPCR reagents and mPCR reaction setup.	A, E	—	8:30 a.m.	9:30 a.m.
Run mPCR reactions.	A, E	Thermal cycler[1]	9:30 a.m.	1:00 p.m.
Thaw mPCR reagents and mPCR reaction setup.	H	—	12:00 p.m.	1:00 p.m.
Run mPCR reactions.	H	Thermal cycler[1]	1:00 p.m.	4:30 p.m.
Prepare samples for mPCR QC gel and freeze mPCR reaction plate.	A	—	1:00 p.m.	1:15 p.m.
Prepare samples for mPCR QC gel and freeze mPCR reaction plate.	E	—	1:15 p.m.	1:30 p.m.
mPCR gel QC.	A, E[2]	E-gel system	1:30 p.m.	2:00 p.m.
Prepare samples for mPCR QC gel and freeze mPCR reaction plate.	H	—	4:30 p.m.	4:45 p.m.
mPCR gel QC.	H	E-gel system	4:45 p.m.	5:15 p.m.

[1] The thermal cycler must be an approved thermal cycler verified for the Axiom™ 2.0 Plus Assay. Thermal cycler run time can vary slightly between models.

[2] Assumes mPCR QC gels are run concurrently.

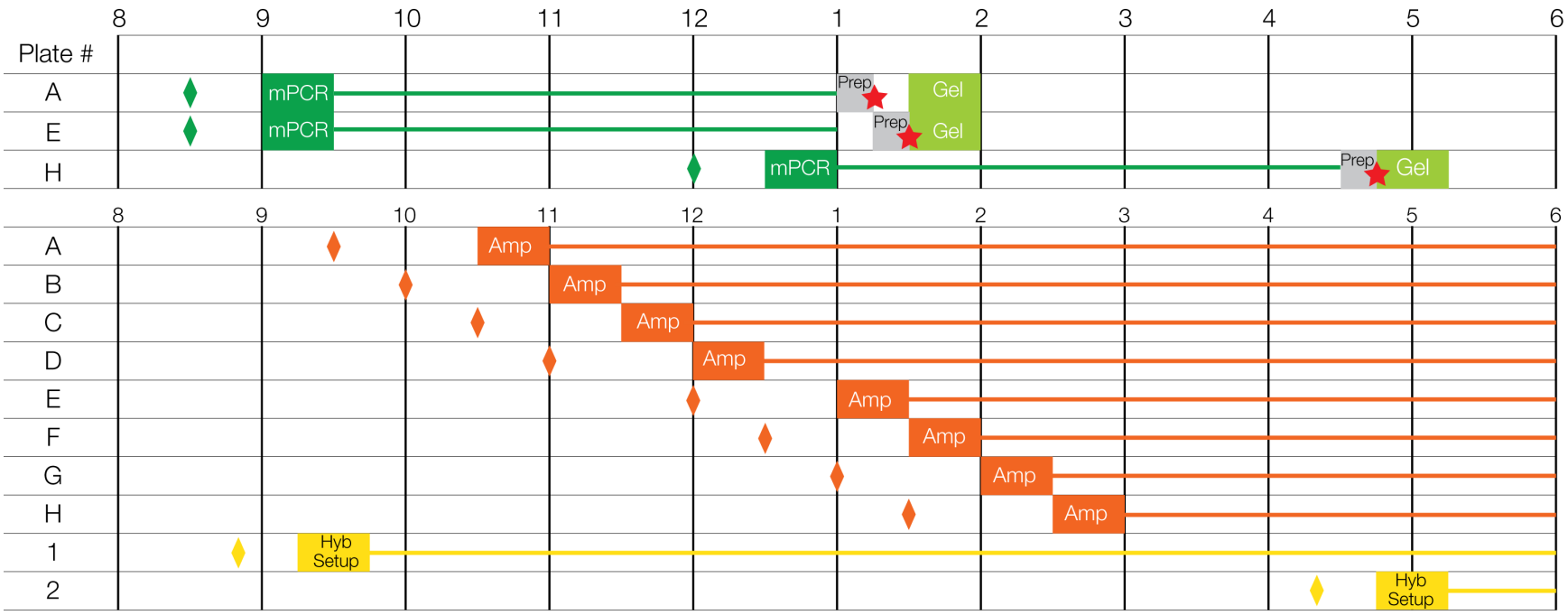
Table 28 Day 1 NIMBUS® activities.

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Hybridization setup • Denature • Transfer to hybridization tray	1	• Off-line • NIMBUS	9:15 a.m.	9:45 a.m.
Hybridization	1	GTMC	9:45 a.m.	9:45 a.m.—day 2
DNA amplification	A	NIMBUS	10:30 a.m.	11:00 a.m.
DNA amplification	B	NIMBUS	11:00 a.m.	11:30 a.m.
DNA amplification	C	NIMBUS	11:30 a.m.	12:00 p.m.
DNA amplification	D	NIMBUS	12:00 p.m.	12:30 p.m.
DNA amplification	E	NIMBUS	1:00 p.m.	1:30 p.m.
DNA amplification	F	NIMBUS	1:30 p.m.	2:00 p.m.
DNA amplification	G	NIMBUS	2:00 p.m.	2:30 p.m.
DNA amplification	H	NIMBUS	2:30 p.m.	3:00 p.m.

Table 28 Day 1 NIMBUS activities. *(continued)*

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Hybridization setup • Denature • Transfer to hybridization tray	2	• Off-line • NIMBUS	4:45 p.m.	5:15 p.m.
Hybridization	2	GTMC	5:15 p.m.	5:15 p.m.—day 2

[1] See Table 24, Table 25, and Table 26 for sections of the user guide containing detailed descriptions for each activity.
[2] GTMC = GeneTitan™ MC Instrument. NIMBUS = NIMBUS® Target Preparation Instrument.



User activities

- Prepare reagents required for the operation
- Warm array plate to room temperature
- Freeze
- Multiplex PCR (mPCR) setup
- Prepare samples for mPCR QC gel
- mPCR gel QC
- DNA amplification setup
- Hybridization setup (denature and transfer to hybridization tray)

Background activities

- mPCR incubation
- Amplification incubation
- Hybridization in the GeneTitan™ MC Instrument

Figure 36 Day 1 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 8-plate workflow.

Day 2 activities

Multiplex PCR activities

- Thaw 3 mPCR reaction plates from day 1 for mPCR spike-in into corresponding DNA amplification plate (plates A, E, and H).
- Manually prepare 3 plates of mPCR (plates B, C, and D) from genomic DNA.
- Run mPCR QC gel (plates B, C, and D).
- Freeze mPCR reaction plates after mPCR reaction run is complete (plates B, C, and D).

NIMBUS® Instrument

- Sample denaturation and prepare hybridization tray on 2 plates of target prepared the previous week (plates 3 and 4).
- Prepare reagent trays for the GeneTitan™ MC Instrument (for plates 1 and 2 already on the GeneTitan™ MC Instrument).
- Fragment and precipitate plates A, E, and H.
- Freeze amplified plates B, C, D, F, and G after each plate has incubated at 37°C for 23 hours.

GeneTitan™ MC Instrument

- Load reagent trays for plates 1 and 2. These plates are moved from the hybridization oven to the fluidics area. After fluidics, the plates move to the imaging area of the instrument.
- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization (plates 3 and 4).

Reagent and plate handling

- Start thawing the fragmentation reagents 30 minutes before the start of each fragmentation step.
- Start preparing the reagents 45 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.
- Before each hybridization setup step, warm the array plate to room temperature unopened in its pouch for at least 25 minutes before loading the array plate and hybridization tray into the GeneTitan™ MC Instrument.
- Start thawing the mPCR reaction plate at room temperature for 20 minutes before mPCR spike-in step.
- Start warming the Hyb-Ready Plate at room temperature for at least 5 minutes before the hybridization setup step.

Note:

- Plates A, E, and H are fragmented and precipitated on day 2 without freezing to preserve the 23 ±1-hour amplification incubation.
- Store plates B, C, D, F, and G at –20°C following 23 ±1 hour of amplification reaction incubation.
- Precipitation is carried out at –20°C overnight. If space allows, keep plates in a single layer during overnight precipitation.

Table 29 Day 2 activities for multiplex PCR.

Activity	Plate	Instrument	Time estimates	
			Start time	End time
Thaw mPCR reagents and mPCR reaction setup.	B, C	—	9:00 a.m.	10:00 a.m.
Run mPCR reactions.	B, C	Thermal cycler[1]	10:00 a.m.	1:30 p.m.
Thaw mPCR reagents and mPCR reaction setup.	D	—	12:30 p.m.	1:30 p.m.
Run mPCR reactions.	D	Thermal cycler[1]	1:30 p.m.	5:00 p.m.
Prepare samples for mPCR QC gel and freeze mPCR Reaction Plate.	B	—	1:30 p.m.	1:45 p.m.
Prepare samples for mPCR QC gel and freeze mPCR Reaction Plate.	C	—	1:45 p.m.	2:00 p.m.
mPCR gel QC.	B, C[2]	E-gel system	2:00 p.m.	2:30 p.m.
Prepare samples for mPCR QC gel and freeze mPCR Reaction Plate.	D	—	5:00 p.m.	5:15 p.m.
mPCR gel QC.	D	E-gel system	5:15 p.m.	5:45 p.m.
Operator 2				
Thaw mPCR Reaction Plate.	A	—	9:30 a.m.	9:50 a.m.
mPCR spike-in into Amplification Plate.	A	—	9:50 a.m.	10:00 a.m.
Thaw mPCR Reaction Plate.	E	—	12:00 p.m.	12:20 p.m.
mPCR spike-in into Amplification Plate.	E	—	12:20 p.m.	12:30 p.m.
Thaw mPCR Reaction Plate.	H	—	1:30 p.m.	1:50 p.m.
mPCR spike-in into Amplification Plate.	H	—	1:50 p.m.	2:00 p.m.

[1] The thermal cycler must be an approved thermal cycler verified for the Axiom™ 2.0 Plus Assay. Thermal cycler run time can vary slightly between models.

[2] Assumes mPCR QC gels are run concurrently.

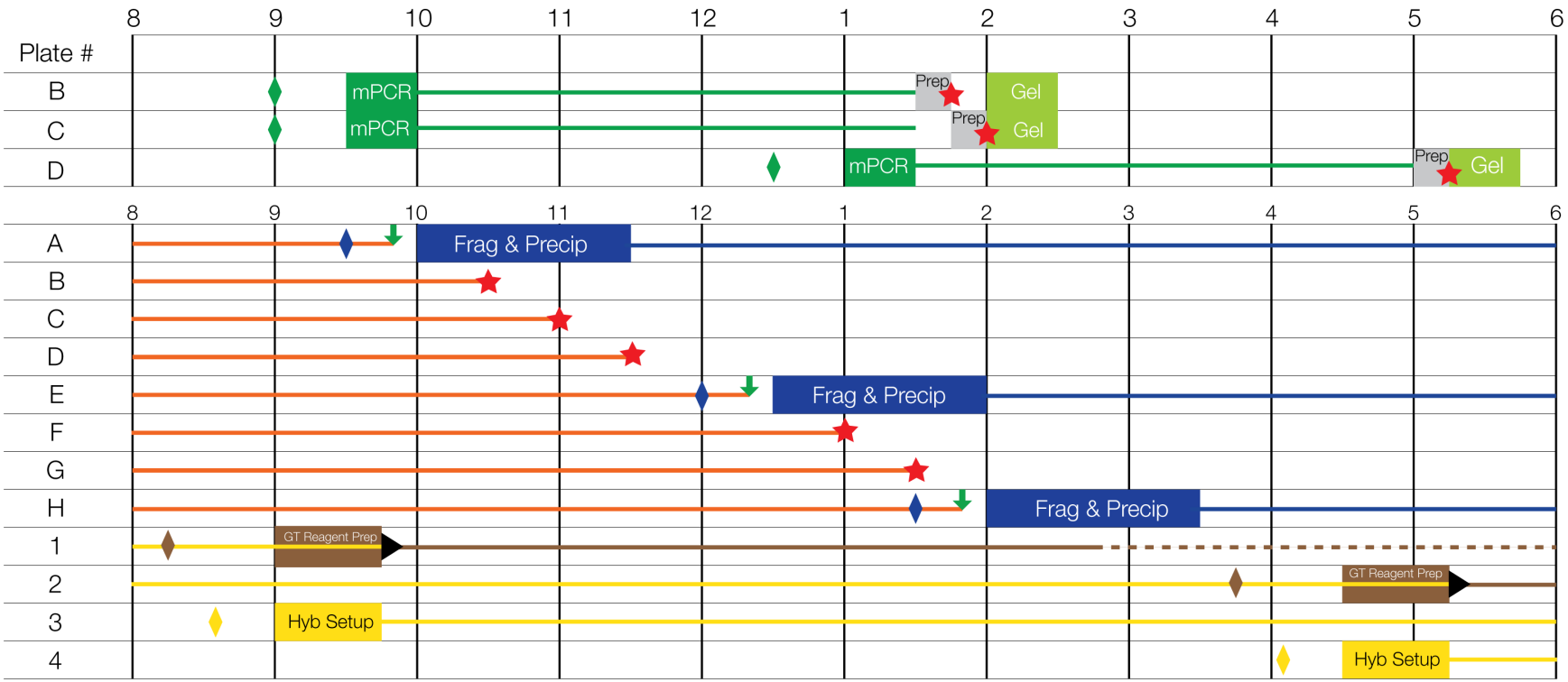
Table 30 Day 2 activities for NIMBUS® Instrument and GeneTitan™ MC Instrument.

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Thaw reagents for GeneTitan™ reagent tray preparation	1	—	8:15 a.m.	9:00 a.m.
Concurrent steps: • GeneTitan™ reagent tray preparation and loading • Denature & hybridization setup	 • 1 • 3	 • NIMBUS/GTMC • Off-line/NIMBUS	9:00 a.m.	9:45 a.m.

Table 30 Day 2 activities for NIMBUS Instrument and GeneTitan MC Instrument. *(continued)*

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Hybridization	3	GTMC	9:45 a.m.	9:45 a.m.—day 3
Fragmentation & precipitation	A	NIMBUS	10:00 a.m.	11:30 a.m.
Freeze (–20°C) at end of 23-hour DNA amplification	B	Freezer	10:30 a.m.	
Freeze (–20°C) at end of 23-hour DNA amplification	C	Freezer	11:00 a.m.	
Freeze (–20°C) at end of 23-hour DNA amplification	D	Freezer	11:30 a.m.	
Fragmentation & precipitation	E	NIMBUS	12:30 p.m.	2:00 p.m.
Freeze (–20°C) at end of 23-hour DNA amplification	F	Freezer	1:00 p.m.	
Freeze (–20°C) at end of 23-hour DNA amplification	G	Freezer	1:30 p.m.	
Fragmentation & precipitation	H	NIMBUS	2:00 p.m.	3:30 p.m.
Thaw reagents for GeneTitan™ reagent tray preparation	2	—	3:45 p.m.	4:30 p.m.
Concurrent steps: • GeneTitan™ reagent tray preparation and loading • Denature & hybridization setup	 • 2 • 4	 • NIMBUS/GTMC • Off-line/NIMBUS	4:30 p.m.	5:15 p.m.
Hybridization	4	GTMC	5:15 p.m.	5:15 p.m.—day 3

[1] See Table 24, Table 25, and Table 26 for sections of the user guide containing detailed descriptions for each activity.
[2] GTMC = GeneTitan™ MC Instrument. NIMBUS = NIMBUS® Target Preparation Instrument.



User activities

- mPCR spike-in
- Prepare reagents required for the operation (including mPCR Reaction Plate)
- GeneTitan™ Instrument loading
- Multiplex PCR (mPCR) setup
- Prepare samples for mPCR QC gel
- mPCR gel QC
- Fragmentation and precipitation
- Hybridization setup (denature and transfer to hybridization tray)
- GeneTitan™ reagent tray preparation
- Warm array plate to room temperature
- Freeze

Background activities

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

Figure 37 Day 2 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 8-plate workflow.

Day 3 activities

Multiplex PCR activities

- Thaw 2 mPCR reaction plates from day 2 for mPCR spike-in into its corresponding DNA amplification plate (plates B and C).
- Manually prepare 2 plates of mPCR (plates F and G) from genomic DNA.
- Run mPCR QC gel (plates F and G).
- Freeze mPCR reaction plates after mPCR reaction run is complete (plates F and G).

NIMBUS® Instrument

- Sample denaturation and prepare hybridization tray on 2 plates of target prepared the previous week (plates 5 and 6).
- Prepare reagent trays for the GeneTitan™ MC Instrument (for plates 3 and 4 already on the GeneTitan™ MC Instrument).
- Fragment and precipitate plates B and C.
- Resuspend, prepare hybridization-ready sample plates, and sample QC plates A, E, and H.

Off-deck activities

- Centrifuge and dry plates A, E, and H.
- Resuspension—off-deck pellet resuspension on a microplate shaker for plates A, E, and H.
- Off-deck QC—fragmentation gel and OD quantification for plates A, E, and H.

GeneTitan™ MC Instrument

- Load reagent trays for plates 3 and 4. These plates are moved from the hybridization oven to the fluidics area. After fluidics, the plates move to the imaging area of the instrument.
- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization (plates 5 and 6).

Reagent and plate handling

- Start thawing the fragmentation reagents 30 minutes before the start of each fragmentation step. Thaw the corresponding mPCR reaction plate for mPCR spike-in now.
- Amplified plates that were frozen on day 2 (plates B and C) must be thawed before use in the fragmentation step. See “Thaw frozen plates of amplified DNA” on page 162.
- Start preparing the resuspension reagents at least 60 minutes before the start of the resuspension step. Resuspension reagents for both plates A, D, and G can be prepared simultaneously to save time.
- Start preparing the reagents 45 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.
- Before each hybridization setup step, warm the array plate to room temperature unopened in its pouch for at least 25 minutes before loading the array plate and hybridization tray into the GeneTitan™ MC Instrument.
- Start thawing the mPCR reaction plate at room temperature for 20 minutes before mPCR spike-in step.
- Start warming the Hyb-Ready Plate at room temperature for at least 5 minutes before the hybridization setup step.

Note:

- Following the centrifuging and drying step, plates A, E, and H can be kept at room temperature before the resuspension and hybridization preparation on the NIMBUS® Instrument (seal the plates, then unseal before placing on the NIMBUS® Instrument deck). If needed, review guidelines on storing pellets and warming plates of pellets if there is a delay between pellet drying and resuspension. See "Guidelines for preparation of frozen pellets and Axiom™ Resusp Buffer" on page 83.
- Precipitation is carried out at –20°C overnight. If space allows, keep plates in a single layer during overnight precipitation.

Table 31 Day 3 activities for multiplex PCR.

Activity	Plate	Instrument	Time estimates	
			Start time	End time
Thaw mPCR reagents and mPCR reaction setup.	F, G	—	9:00 a.m.	10:00 a.m.
Run mPCR reactions.	F, G	Thermal cycler[1]	10:00 a.m.	1:30 p.m.
Prepare samples for mPCR QC gel and freeze mPCR reaction plate.	F	—	1:30 p.m.	1:45 p.m.
Prepare samples for mPCR QC gel and freeze mPCR reaction plate.	G	—	1:45 p.m.	2:00 p.m.
mPCR gel QC.	F, G[2]	E-gel system	2:00 p.m.	2:30 p.m.
Operator 2				
Thaw mPCR Reaction Plate.	B	—	9:30 a.m.	9:50 a.m.
mPCR spike-in into Amplification Plate.	B	—	9:50 a.m.	10:00 a.m.
Thaw mPCR Reaction Plate.	C	—	11:00 a.m.	11:20 a.m.
mPCR spike-in into Amplification Plate.	C	—	11:20 a.m.	11:30 a.m.

[1] The thermal cycler must be an approved model that is verified for the Axiom™ 2.0 Plus Assay. Thermal cycler run time can vary slightly between models.

[2] Assumes mPCR QC gels are run concurrently.

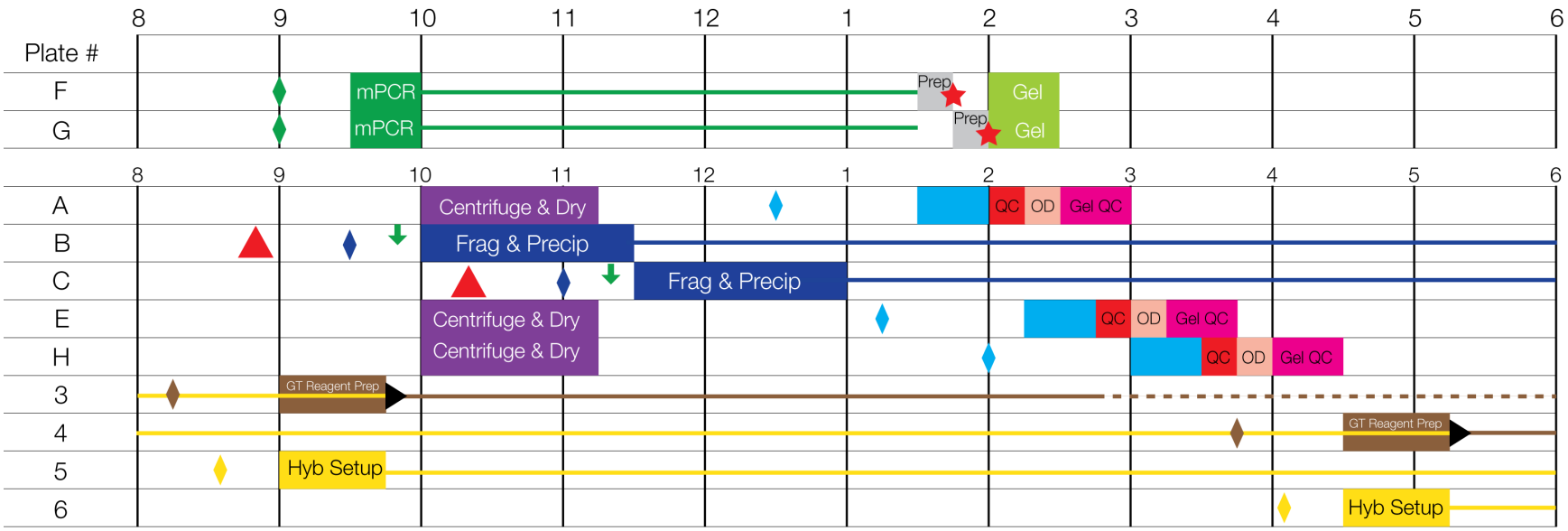
Table 32 Day 3 activities for NIMBUS® Instrument and GeneTitan™ MC Instrument.

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Thaw reagents for GeneTitan™ reagent tray preparation	3	—	8:15 a.m.	9:00 a.m.
Concurrent steps: • GeneTitan™ reagent tray preparation and loading • Denature & hybridization setup	• 3 • 5	• NIMBUS/GTMC • Off-line/NIMBUS	9:00 a.m.	9:45 a.m.

Table 32 Day 3 activities for NIMBUS Instrument and GeneTitan MC Instrument. *(continued)*

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Hybridization	5	GTMC	9:45 a.m.	9:45 a.m.—day 4
Thaw DNA Amplification Plate	B	—	8:50 a.m.	9:50 a.m.
Fragmentation & precipitation	B	NIMBUS	10:00 a.m.	11:30 a.m.
Off-deck centrifugation and drying pellets	A, E, H	Centrifuge/Oven	10:00 a.m.	11:15 a.m.
Thaw DNA Amplification Plate	C	—	10:20 a.m.	11:20 a.m.
Fragmentation & precipitation	C	NIMBUS	11:30 a.m.	1:00 p.m.
Resuspension and hybridization preparation	A	Off-line/NIMBUS	1:30 p.m.	2:00 p.m.
Sample QC	A	NIMBUS	2:00 p.m.	2:15 p.m.
Off-deck OD	A	Plate reader/ spectrophotometer	2:15 p.m.	2:30 p.m.
Resuspension and hybridization preparation	E	Off-line/NIMBUS	2:15 p.m.	2:45 p.m.
Sample QC	E	NIMBUS	2:45 p.m.	3:00 p.m.
Off-deck OD	E	Plate reader/ spectrophotometer	3:00 p.m.	3:15 p.m.
Resuspension and hybridization preparation	H	Off-line/NIMBUS	3:00 p.m.	3:30 p.m.
Sample QC	H	NIMBUS	3:30 p.m.	3:45 p.m.
Off-deck OD	H	Plate reader/ spectrophotometer	3:45 p.m.	4:00 p.m.
Gel QC run	A	E-gel system	2:30 p.m.	3:00 p.m.
Gel QC run	E	E-gel system	3:15 p.m.	3:45 p.m.
Gel QC run	H	E-gel system	4:00 p.m.	4:30 p.m.
Thaw reagents for GeneTitan™ reagent tray preparation	4	—	3:45 p.m.	4:30 p.m.
Concurrent steps: • GeneTitan™ reagent tray preparation and loading • Denature & hybridization setup	• 4 • 6	• NIMBUS/GTMC • Off-line/NIMBUS	4:30 p.m.	5:15 p.m.
Hybridization	6	GTMC	5:15 p.m.	5:15 p.m.—day 4

[1] See Table 24, Table 25, and Table 26 for sections of the user guide containing detailed descriptions for each activity.
[2] GTMC = GeneTitan™ MC Instrument. NIMBUS = NIMBUS® Target Preparation Instrument.



User activities

- mPCR spike-in
- Multiplex PCR (mPCR) setup
- Prepare samples for mPCR QC gel
- mPCR gel QC
- Fragmentation and precipitation
- Off-deck centrifugation and drying pellets
- Resuspension, hybridization preparation
- Sample QC on the NIMBUS Instrument Deck
- Off-deck OD
- Gel QC Run
- Hybridization setup (denature & transfer to hybridization tray)
- GeneTitan™ reagent tray preparation

User activities

- Warm array plate to room temperature
- Prepare reagents required for the operation (including mPCR Reaction Plate)
- Freeze
- Thaw DNA Amplification Plate
- GeneTitan™ Instrument loading

Background activities

- mPCR incubation
- Hybridization in the GeneTitan™ MC Instrument
- Precipitation incubation
- Fluidics processing in the GeneTitan™ MC Instrument
- Imaging in the GeneTitan™ MC Instrument

Figure 38 Day 3 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 8-plate workflow.

Day 4 activities

Multiplex PCR activities

- Thaw 3 mPCR reaction plates from day 2 and day 3 for mPCR spike-in into its corresponding DNA amplification plate (plates D, F, and G).

NIMBUS® Instrument

- Sample denaturation and prepare hybridization tray on 2 plates of target prepared the previous week (plates 7 and 8).
- Prepare reagent trays for the GeneTitan™ MC Instrument (for plates 5 and 6 already on the GeneTitan™ MC Instrument).
- Fragment and precipitate plates D, F, and G.

Off-deck activities

- Centrifuge and dry plates B and C.
- Freeze precipitation plates B and C after centrifugation and drying pellets.

GeneTitan™ MC Instrument

- Load reagent trays for plates 5 and 6. These plates are moved from the hybridization oven to the fluidics area. After fluidics, the plates move to the imaging area of the instrument.
- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization (plates 7 and 8).

Reagent and plate handling

- Start thawing the fragmentation and precipitation reagents 30 minutes before the start of each fragmentation step. The precipitation reagents can be prepared at the same time or during fragmentation.
- Plates of amplified DNA that were frozen on day 2 (plates D, F, and G) must be thawed before use in fragmentation. Follow the instructions in “Thaw frozen plates of amplified DNA” on page 162.
- Start preparing the reagents 45 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.
- Before each hybridization setup step, warm the array plate to room temperature unopened in its pouch for at least 25 minutes before loading the array plate and hybridization tray into the GeneTitan™ MC Instrument.
- Start thawing the mPCR reaction plate at room temperature for 20 minutes before mPCR spike-in step.
- Start warming the Hyb-Ready Plate at room temperature for at least 5 minutes before the hybridization setup step.

Note:

- Precipitation is carried out at –20°C overnight. If space allows, keep plates in a single layer during overnight precipitation.
- After being centrifuged and dried, plates B and C are sealed and stored at –20°C.

Table 33 Day 4 activities for multiplex PCR.

Activity	Plate	Instrument	Time estimates	
			Start time	End time
Thaw mPCR Reaction Plate.	D	—	9:30 a.m.	9:50 a.m.
mPCR spike-in into Amplification Plate.	D	—	9:50 a.m.	10:00 a.m.
Thaw mPCR Reaction Plate.	F	—	11:00 a.m.	11:20 a.m.
mPCR spike-in into Amplification Plate.	F	—	11:20 a.m.	11:30 a.m.
Thaw mPCR Reaction Plate.	G	—	1:00 p.m.	1:20 p.m.
mPCR spike-in into Amplification Plate.	G	—	1:20 p.m.	1:30 p.m.

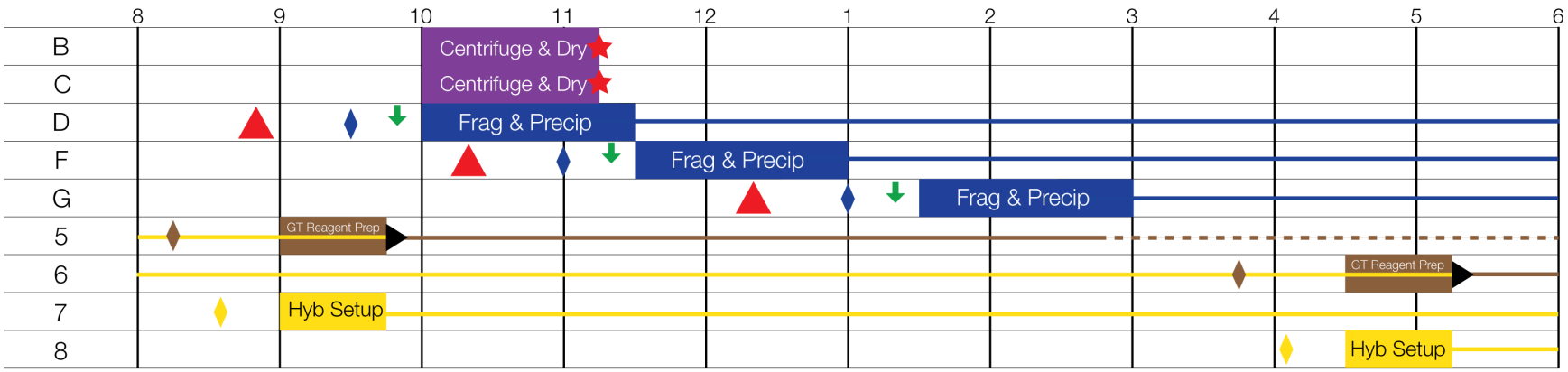
Table 34 Day 4 activities for NIMBUS® Instrument and GeneTitan™ MC Instrument.

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Thaw reagents for GeneTitan™ reagent tray preparation	5	—	8:15 a.m.	9:00 a.m.
Concurrent steps: • GeneTitan™ reagent tray preparation and loading • Denature & hybridization setup	 • 5 • 7	 • NIMBUS/GTMC • Off-line/NIMBUS	9:00 a.m.	9:45 a.m.
Hybridization	7	GTMC	9:45 a.m.	9:45 a.m.—day 5
Thaw DNA Amplification Plate	D	—	8:50 a.m.	9:50 a.m.
Fragmentation & precipitation	D	NIMBUS	10:00 a.m.	11:30 a.m.
Off-deck centrifugation, drying pellets, and freeze	B	Centrifuge/Oven Freezer	10:00 a.m. 11:15 a.m.	11:15 a.m. 8:30 a.m.—day 5
Off-deck centrifugation, drying pellets, and freeze	C	Centrifuge/Oven Freezer	10:00 a.m. 11:15 a.m.	11:15 a.m. 9:15 a.m.—day 5
Thaw DNA Amplification Plate	F	—	10:20 a.m.	11:20 a.m.
Fragmentation & precipitation	F	NIMBUS	11:30 a.m.	1:00 p.m.
Thaw DNA Amplification Plate	G	—	12:20 p.m.	1:20 p.m.
Fragmentation & precipitation	G	NIMBUS	1:30 p.m.	3:00 p.m.
Thaw reagents for GeneTitan™ reagent tray preparation	6	—	3:45 p.m.	4:30 p.m.

Table 34 Day 4 activities for NIMBUS Instrument and GeneTitan MC Instrument. *(continued)*

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Concurrent steps: • GeneTitan™ reagent tray preparation and loading • Denature & hybridization setup	• 6 • 8	• NIMBUS/GTMC • Off-line/NIMBUS	4:30 p.m.	5:15 p.m.
Hybridization	6	GTMC	5:15 p.m.	5:15 p.m.—day 5

[1] See Table 24, Table 25, and Table 26 for sections of the user guide containing detailed descriptions for each activity.
[2] GTMC = GeneTitan™ MC Instrument. NIMBUS = NIMBUS® Target Preparation Instrument.



User activities

	Prepare reagents required for the operation (including mPCR Reaction Plate)
	Warm array plate to room temperature
	mPCR spike-in
	Freeze
	Thaw DNA Amplification Plate
	GeneTitan™ Instrument loading
	Fragmentation and precipitation
	Off-deck centrifugation and drying pellets
	Hybridization setup (denature & transfer to hybridization tray)
	GeneTitan™ reagent tray preparation

Background activities

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

Figure 39 Day 4 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 8-plate workflow.

Day 5 activities

NIMBUS® Instrument

- Prepare reagent trays for the GeneTitan™ MC Instrument (for plates 7 and 8 already on the GeneTitan™ MC Instrument).
- Resuspend, prepare hybridization-ready sample plates, and sample QC plates B, C, D, F, and G.

Off-deck activities

- Centrifuge and dry plates D, F, and G.
- Resuspension—off-deck pellet resuspension on a microplate shaker for plates B, C, D, F and G.
- Off-deck QC—fragmentation gel and OD quantification for plates B, C, D, F, and G.

GeneTitan™ MC Instrument

- Load reagent trays for plates 7 and 8. These plates are moved from the hybridization oven to the fluidics area. After fluidics, the plates move to the imaging area of the instrument.

Reagent and plate handling

- Start preparing the resuspension reagents at least 60 minutes before the start of the resuspension step. To save time, resuspension reagents for plates B, C, D, F, and G can be prepared at the same time.
- The precipitation plates with pellets (plates B and C) that are stored at –20°C must be equilibrated at room temperature for 90 minutes before starting the resuspension and hybridization preparation step.
- Start preparing the reagents 45 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.

Note: Following centrifuging and drying, plates D, F, and G can be kept at room temperature before resuspension on the NIMBUS® Instrument (seal the plates, then unseal before placing on the NIMBUS® Instrument deck). See “Guidelines for preparation of frozen pellets and Axiom™ Resusp Buffer” on page 83 for further guidelines on storing pellets and warming plates of pellets if there is a delay between pellet drying and resuspension.

Table 35 Day 5 activities for NIMBUS® Instrument and GeneTitan™ MC Instrument.

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Thaw reagents for GeneTitan™ reagent tray preparation	7	—	8:15 a.m.	9:00 a.m.
GeneTitan™ reagent tray preparation and loading	7	GTMC	9:00 a.m.	9:45 a.m.
Warm Precipitation Plate to room temperature	B	—	8:30 a.m.	10:00 a.m.
Off-deck centrifugation and drying pellets	D, F, G	Centrifuge/Oven	9:15 a.m.	10:30 a.m.
Resuspension and hybridization preparation	B	Off-line/NIMBUS	10:00 a.m.	10:30 a.m.
Sample QC	B	NIMBUS	10:30 a.m.	10:45 a.m.

Table 35 Day 5 activities for NIMBUS Instrument and GeneTitan MC Instrument. *(continued)*

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Off-deck OD	B	Plate reader/ spectrophotometer	10:45 a.m.	11:00 a.m.
Warm Precipitation Plate to room temperature	C	—	9:15 a.m.	10:45 a.m.
Resuspension and hybridization preparation	C	Off-line/NIMBUS	10:45 a.m.	11:15 a.m.
Sample QC	C	NIMBUS	11:15 a.m.	11:30 a.m.
Off-deck OD	C	Plate reader/ spectrophotometer	11:30 a.m.	11:45 a.m.
Resuspension and hybridization preparation	D	Off-line/NIMBUS	11:30 a.m.	12:00 p.m.
Sample QC	D	NIMBUS	12:00 p.m.	12:15 p.m.
Off-deck OD	D	Plate reader/ spectrophotometer	12:15 p.m.	12:30 p.m.
Resuspension and hybridization preparation	F	Off-line/NIMBUS	12:15 p.m.	12:45 p.m.
Sample QC	F	NIMBUS	12:45 p.m.	1:00 p.m.
Off-deck OD	F	Plate reader/ spectrophotometer	1:00 p.m.	1:15 p.m.
Resuspension and hybridization preparation	G	Off-line/NIMBUS	1:00 p.m.	1:30 p.m.
Sample QC	G	NIMBUS	1:30 p.m.	1:45 p.m.
Off-deck OD	G	Plate reader/ spectrophotometer	1:45 p.m.	2:00 p.m.
Gel QC run	B	E-gel system	11:00 a.m.	11:30 a.m.
Gel QC run	C	E-gel system	11:45 a.m.	12:15 p.m.
Gel QC run	D	E-gel system	12:30 p.m.	1:00 p.m.
Gel QC run	F	E-gel system	1:15 p.m.	1:45 p.m.
Gel QC run	G	E-gel system	2:00 p.m.	2:30 p.m.
Thaw reagents for GeneTitan™ reagent tray preparation	8	—	3:45 p.m.	4:30 p.m.
GeneTitan™ reagent tray preparation and loading	8	GTMC	4:30 p.m.	5:15 p.m.

[1] See Table 24, Table 25, and Table 26 for sections of the user guide containing detailed descriptions for each activity.

[2] GTMC = GeneTitan™ MC Instrument. NIMBUS = NIMBUS® Target Preparation Instrument.

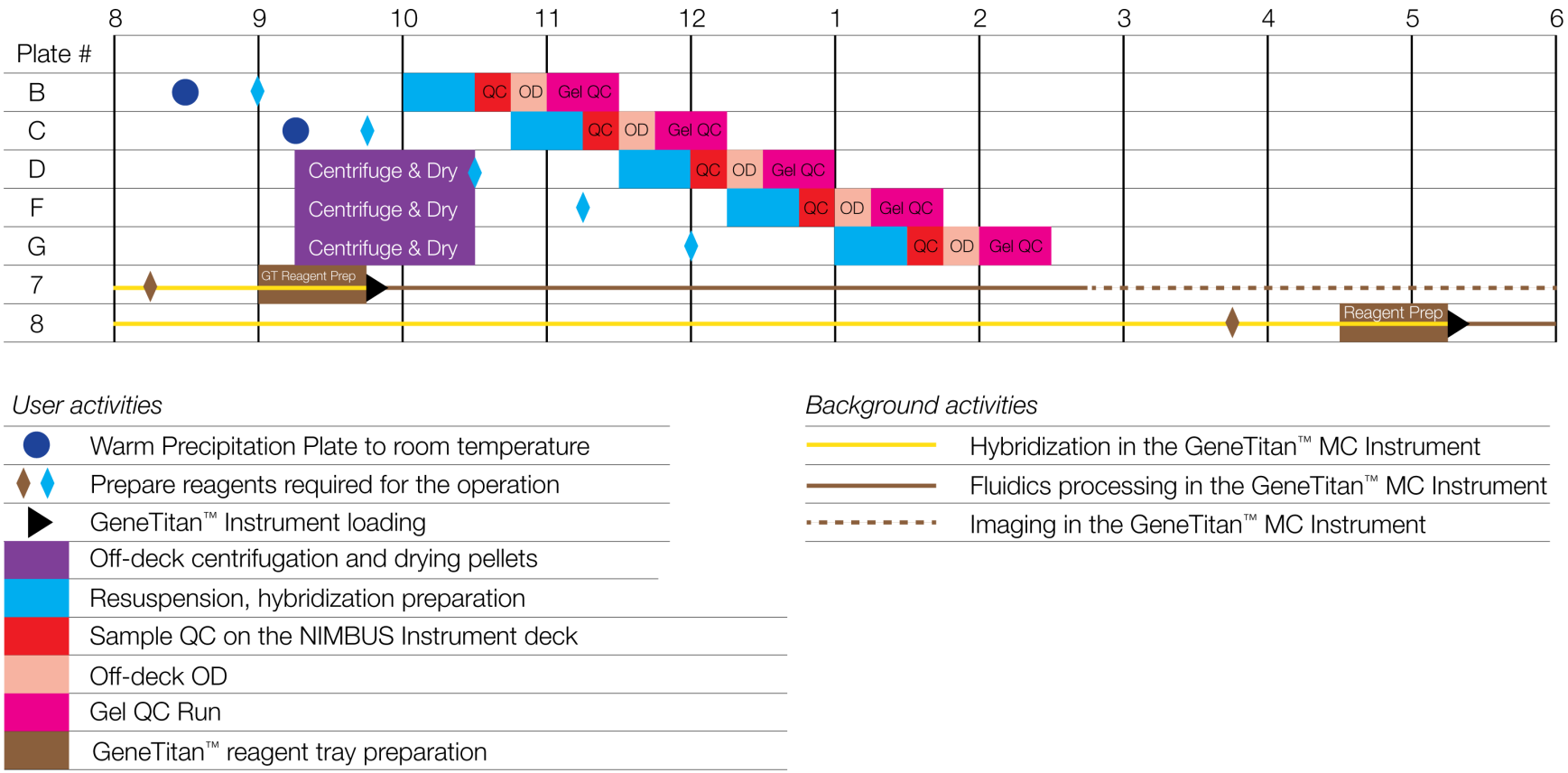


Figure 40 Day 5 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 8-plate workflow.

8 3-plate workflow for Axiom™ PMD Array Plates using a 3-hour DNA precipitation step

The Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 3-plate workflow for the Applied Biosystems™ NIMBUS® Instrument using the 3-hour DNA precipitation step allows faster assay turnaround time. Using this workflow, CEL files for plate 1 are available in 72 hours, and CEL files for all 3 plates are available by day 5. One to 2 people can process 3 Axiom™ PMD Array Plates in 1 work week. This chapter describes the timing of the steps for each sample and array plate that are required to perform this workflow. Note that this workflow requires approximately 9–10-hour workdays. The workflow was verified under the following assumptions:

- One dedicated mPCR operator on day 1, 1 to 2 operators for NIMBUS® Instrument protocols on day 1–day 4
- One verified NIMBUS® Instrument, 1 verified GeneTitan™ Multi-Channel Instrument, and 2 verified thermal cyclers available in laboratory.

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

This chapter assumes user familiarity with all procedures for target preparation and array processing using the GeneTitan™ MC Instrument and the NIMBUS® Instrument, as described in this user guide.

Note: If performing the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow without the mPCR step, skip all the mPCR activities and proceed directly to “Stage 1B: Amplify the genomic DNA” to start target preparation.

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

A scheme illustrating the timing that is required for the 3-plate workflow is given in Figure 41 and Figure 42. The 3 plates are referred to as plates A, B, and C for the target preparation and the GeneTitan™ array processing steps. The daily activities, which include manual mPCR, automated target preparation on the NIMBUS® Instrument, and array processing on the GeneTitan™ MC Instrument are listed in Table 36. Detailed day-by-day instructions are given in this chapter.

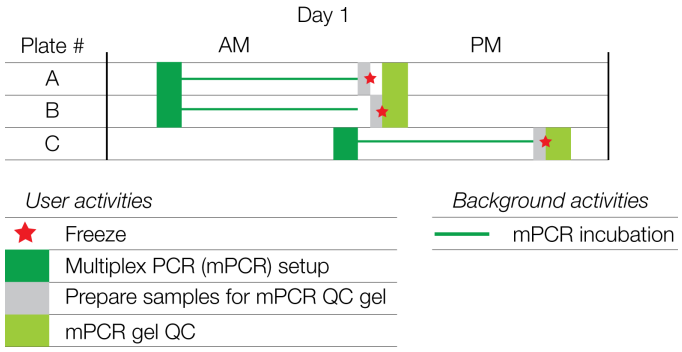


Figure 41 Multiplex PCR activities for Axiom™ 2.0 Plus Assay 3-plate workflow .

Figure 42 Activities for the NIMBUS® Instrument and GeneTitan™ MC Instrument 3-plate workflow with 3-hour precipitation.

The 3 plates are referred to as plates A, B, and C in the target preparation and in the GeneTitan™ array processing steps. The steps must be performed in the order and with the timing that is described in this chapter. On day 1, it is recommended that 1 person is responsible for setting up the whole-genome amplification for all 3 plates and that another person is responsible for setting up and running mPCR for all 3 plates. It is also helpful to have 2 people working together on day 2 and day 3.

Table 36 Daily steps for Axiom™ 2.0 Plus Assay 3-plate workflow with 3-hour precipitation.

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

IMPORTANT! Maintaining consistent timing during GeneTitan™ MC Instrument setup is critical to containing the user interventions of the 3-plate workflow within the work day. When 1 process starts late, there is little opportunity to catch up until the end of the workflow.

Time required for assay steps

The length of time of the manually performed multiplex PCR activities is listed in Table 37.

The time that is required for the steps that are involved in target preparation and array processing in the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow is given in Table 38. This table also indicates where detailed instructions for each step can be found.

These steps include:

- Deck setup, run time, and deck cleanup on the NIMBUS® Instrument.
- Preparation and loading of the GeneTitan™ MC Instrument.
- Off-deck steps of centrifugation and drying pellets, DNA pellet resuspension on a microplate shaker, running the QC gel, and reading the OD plate.

Times for thawing reagents are given separately in the detailed day by day instructions in the next section.

Table 37 Time required for Axiom™ multiplex PCR-associated steps.

Operation	Time required
Multiplex PCR set up	
Thaw mPCR reagents and prepare mPCR reactions (see "Prepare the mPCR Master Mix" on page 48).	60 minutes
mPCR incubation	
Run mPCR protocol on thermal cycler (see Figure 5).	~3.5 hours[1]
mPCR gel QC	
Prepare samples for mPCR QC gel, and then Freeze reaction plate (see "Store the mPCR Product Plate" on page 49).	15 minutes
mPCR gel QC (see Appendix E, "mPCR quality control gel protocol").	30 minutes
mPCR spike-in	
Thaw mPCR reaction plate (see "Thaw and prepare the mPCR Reaction Plate" on page 66)	20 minutes
Spike mPCR reactions into corresponding WGA plate (see "Perform mPCR spike-in to Amplification Plate" on page 67).	10 minutes

[1] Thermal cycler run time can vary slightly between models.

Table 38 Time required for individual operations involved in the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow for the NIMBUS® Instrument.

Operation	Time required
Target preparation steps	
DNA amplification (see "Stage 1B: Amplify the genomic DNA" on page 52) followed by a 23 ±1 hour incubation in a 37°C oven.	30 minutes
Fragmentation (see "Stage 2: Fragment and precipitate the DNA" on page 63) followed by 3-hour precipitation in a –20°C freezer.	90 minutes 3 hours
Off-deck centrifugation and drying pellets (see "Stage 3: Centrifuge and dry pellets" on page 78).	75 minutes
Resuspension and hybridization preparation (see "Stage 4A: Prepare the resuspension buffer" on page 83 and "Stage 4B: Prepare Hybridization Master Mix" on page 90).	30 minutes
Sample QC (see "Stage 4C: Perform sample QC" on page 97)	15 minutes
Off-deck QC: Fragmentation Gel and OD quantification (see Appendix C, "Fragmentation quality control gel protocol" and Appendix D, "Sample quantification after resuspension").	45 minutes

Table 38 Time required for individual operations involved in the Axiom 2.0 Plus Assay 96-Array Format Automated Workflow for the NIMBUS Instrument. *(continued)*

Operation	Time required
Array processing steps	
Prepare hybridization tray. • Sample denaturation off-deck and transfer to hybridization tray on the NIMBUS® Instrument (see "Stage 5: Prepare the hybridization tray" on page 105). • Load hybridization tray into the GeneTitan™ MC Instrument, start hybridization.	• 30 minutes • 23.5–24 hours
GeneTitan™ reagent tray preparation and loading. • GeneTitan™ reagent preparation on the NIMBUS® Instrument (see "Stage 6: Prepare GeneTitan™ reagent trays" on page 113). • Load reagent trays into GeneTitan™ MC Instrument (see "Stage 3—Ligate, wash, stain, and scan" on page 148).	45 minutes
Concurrent hybridization setup and reagent tray preparation & loading (see "Couple stage 5 and stage 6 in a multiplate workflow" on page 124). • GeneTitan™ reagent preparation on the NIMBUS® Instrument. • Load reagent trays into GeneTitan™ MC Instrument. • Sample denaturation and transfer to hybridization tray on the NIMBUS® Instrument. • Load hybridization tray into GeneTitan™ MC Instrument.	90 minutes

Table 39 GeneTitan™ MC Instrument processing times.

Step on the GeneTitan™ MC Instrument	Time required
Hybridization in the GTMC oven at 48°C.	23.5 to 24 hours
Fluidics processing.	5 hours
Imaging 96-array format plates.	7.5 hours

The hybridization time for a 96-array format plate in the GeneTitan™ MC Instrument is 23.5 hours to 24 hours (Table 39). This time frame provides a 30-minute window during which the instrument control software prompts you to load the reagents that are required for washing and staining. Loading the reagent trays into the GeneTitan™ MC Instrument at the mid-point of this 30-minute window is recommended to allow the wash procedures to start 24 hours after the start of hybridization. If catch-up time is required during the 3-plate workflow, start the loading of reagents at the onset of this 30-minute window (that is, immediately after prompted by the software).

Thaw the frozen plate of amplified DNA

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

1. Place the deepwell plate in a small water bath.

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

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

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

4. Avoid cross-contamination of wells during vortexing.
   a. Remove the seal, then blot the top of the plate with a laboratory tissue.
   b. Tightly reseal the plate with a fresh seal.
   c. Vortex the plate for 30 seconds to mix.

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

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

Note: The day 1 schedule is written for 2 people working together. One person sets up the whole-genome amplification for all 3 plates on the NIMBUS® Instrument, and the other person sets up and runs the mPCR reactions for all 3 plates. When the mPCR run is complete, the mPCR QC gel can be run (optional), and then the plates are frozen until needed. It is also recommended to have 2 people working together on day 2 and day 3. Note that the 3-hour precipitation workflow requires approximately 9 to 10 hours per day, over a 4-day time frame.
Genomic DNA sample for amplification must be properly prepared. (See Chapter 3, "Genomic DNA preparation".)

Day 1 activities

Multiplex PCR activities

- Manually prepare 3 plates of mPCR (plates A, B, and C) from genomic DNA. Two thermal cyclers are required.
- Run mPCR QC gel (plates A, B, and C).
- Freeze mPCR reaction plates after mPCR reaction run is complete (plates A, B, and C).

NIMBUS® Instrument

- Amplify 3 plates of genomic DNA (plates A, B, and C).

Reagent and plate handling

- Start thawing the amplification reagents, particularly the Axiom™ 2.0 Amp Soln, and Axiom™ Water, 60 minutes before the start of each reaction.
- Thaw 3 plates of genomic DNA (plates A, B, C) at room temperature and then briefly centrifuge before the start of each reaction.

Note: All amplifications must be set up on day 1 to allow for a 23 ±1-hour amplification incubation in a 37°C oven for each plate.

Table 40 Day 1 activities for Multiplex PCR.

Activity	Plate	Instrument	Time estimates	
			Start time	End time
Thaw mPCR reagents and mPCR reaction setup.	A, B	—	8:30 a.m.	9:30 a.m.
Run mPCR reactions.	A, B	Thermal cycler[1]	9:30 a.m.	1:00 p.m.
Thaw mPCR reagents and mPCR reaction setup.	C	—	12:00 p.m.	1:00 p.m.
Run mPCR reactions.	C	Thermal cycler[1]	1:00 p.m.	4:30 p.m.
Prepare samples for mPCR QC gel and freeze mPCR reaction plate.	A	—	1:00 p.m.	1:15 p.m.

Table 40 Day 1 activities for Multiplex PCR. *(continued)*

Activity	Plate	Instrument	Time estimates	
			Start time	End time
Prepare samples for mPCR QC gel and freeze mPCR reaction plate.	B	—	1:15 p.m.	1:30 p.m.
mPCR gel QC.	A, B[2]	E-gel system	1:30 p.m.	2:00 p.m.
Prepare samples for mPCR QC gel and freeze mPCR reaction plate.	C	—	4:30 p.m.	4:45 p.m.
mPCR gel QC.	C	E-gel system	4:45 p.m.	5:15 p.m.

[1] The thermal cycler must be an approved model that is verified for the Axiom™ 2.0 Plus Assay. Thermal cycler run time can vary slightly between models.

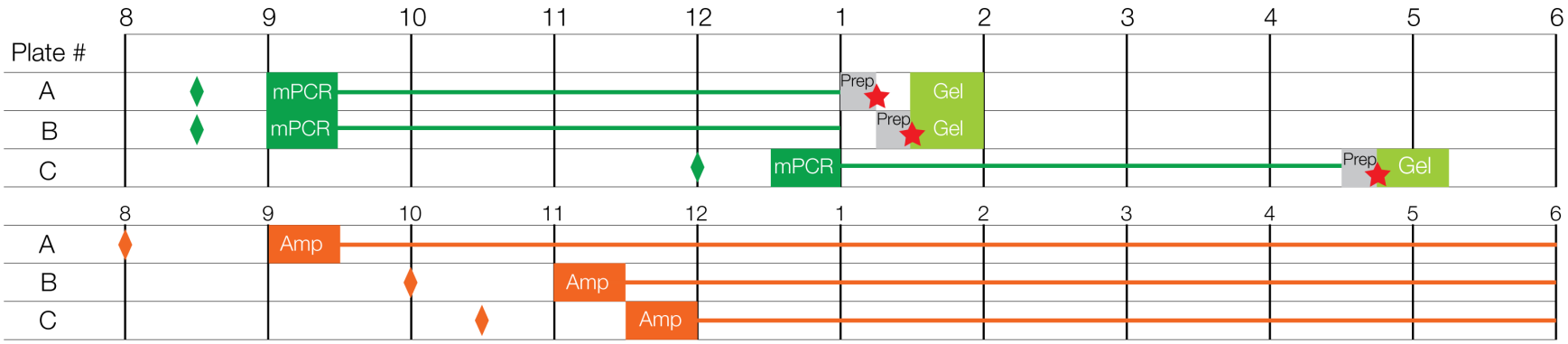
[2] Assumes mPCR QC gels are run concurrently.

Table 41 Day 1 NIMBUS® activities.

Activity	Plate	Instrument[1]	Time estimates	
			Start time	End time
DNA amplification.	A	NIMBUS	9:00 a.m.	9:30 a.m.
DNA amplification.	B	NIMBUS	11:00 a.m.	11:30 a.m.
DNA amplification.	C	NIMBUS	11:30 a.m.	12:00 p.m.

[1] NIMBUS = NIMBUS® Target Preparation Instrument.

Day 1 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 3-plate workflow with 3-hour precipitation



User activities

	Thaw mPCR reagents
	Prepare reagents for DNA amplification
	Freeze
	Multiplex PCR (mPCR) setup
	Prepare samples for mPCR QC gel
	mPCR gel QC
	DNA amplification setup

Background activities

	mPCR incubation
	Amplification incubation

Day 2 activities

Multiplex PCR activities

- Thaw 2 mPCR reaction plates from day 1 for mPCR spike-in into corresponding DNA amplification plates (plates A and B).

NIMBUS® Instrument

- Sample denaturation and prepare hybridization tray for plate A.
- Fragment and precipitate (plates A and B).
- Resuspend, prepare hybridization-ready sample plate, and sample QC (plates A and B).
- Perform denaturation and hybridization setup on plate A.

Off-deck activities

- Precipitation is carried out at –20°C for 3 hours (plates A and B).
- Off-deck centrifuge, dry pellets (plates A and B).
- Freeze amplified plate (C) after the plate has incubated at 37°C for 23 hours.
- Store Hyb-Ready Plate at –20°C until hybridization on day 3 (plate B).
- Centrifuge and dry plates A and B.
- Resuspension—off-deck pellet resuspension on a microplate shaker for plates A and B.
- Off-deck QC—fragmentation gel and OD quantification for plates A and B.

GeneTitan™ MC Instrument

- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization (plate A).

Reagent and plate handling

- Start thawing the fragmentation reagents 30 minutes before the start of each fragmentation step. Thaw the corresponding mPCR reaction plate for mPCR spike-in now.
- Start preparing the resuspension reagents at least 60 minutes before the start of the resuspension step. Resuspension reagents for plates A and B can be prepared simultaneously to save time.
- Before the hybridization setup step, warm the array plate to room temperature unopened in its pouch for at least 25 minutes before loading the array plate and hybridization tray into the GeneTitan™ MC Instrument.

Note:

- Plates A and B are fragmented and precipitated on day 2 without freezing to preserve the 23-hour amplification incubation.
- Store plate C at –20°C following 23 hours of amplification reaction incubation.
- Precipitation is carried out at –20°C for 3 hours. If space allows, keep plates in a single layer during precipitation. In addition, the freezer should not be subjected to frequent temperature excursions.

Table 42 Day 2 activities for Multiplex PCR.

Activity	Plate	Instrument	Time estimates	
			Start time	End time
Thaw mPCR reaction plate.	A	—	8:00 a.m.	8:20 a.m.
mPCR spike-in into DNA Amplification Plate.	A	—	8:20 a.m.	8:30 a.m.
Thaw mPCR reaction plate.	B	—	10:00 a.m.	10:20 a.m.
mPCR spike-in into DNA Amplification Plate.	B	—	10:20 a.m.	10:30 a.m.

Table 43 Day 2 NIMBUS® activities.

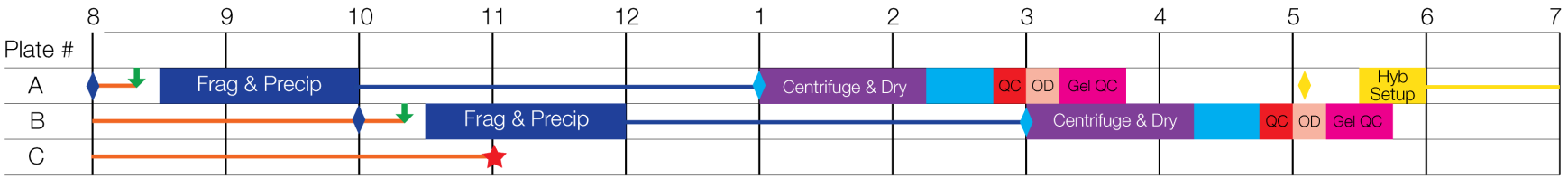
Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Fragmentation & precipitation.	A	NIMBUS	8:30 a.m.	10:00 a.m.
Incubate Precipitation Plate at –20°C.	A	Freezer	10:00 a.m.	1:00 p.m.
Fragmentation & precipitation.	B	NIMBUS	10:30 a.m.	12:00 p.m.
Freeze (–20°C) at end of 23-hour DNA amplification.	C	Freezer	11:00 a.m.	8:20 a.m.—day 3
Incubate Precipitation Plate at –20°C.	B	Freezer	12:00 p.m.	3:00 p.m.
Off-deck centrifugation and drying pellets.	A	Centrifuge/Oven	1:00 p.m.	2:15 p.m.
Resuspension and hybridization preparation.	A	Off-line/NIMBUS	2:15 p.m.	2:45 p.m.
Sample QC.	A	NIMBUS	2:45 p.m.	3:00 p.m.
Off-deck centrifugation and drying pellets.	B	Centrifuge/Oven	3:00 p.m.	4:15 p.m.
Off-deck OD.	A	Plate Reader/ Spectrophotometer	3:00 p.m.	3:15 p.m.
Gel QC run.	A	E-gel system	3:15 p.m.	3:45 p.m.
Resuspension and hybridization preparation.	B	Off-line/NIMBUS	4:15 p.m.	4:45 p.m.
Sample QC.	B	NIMBUS	4:45 p.m.	5:00 p.m.
Off-deck OD.	B	Plate Reader/ Spectrophotometer	5:00 p.m.	5:15 p.m.
Gel QC run.	B	E-gel system	5:15 p.m.	5:45 p.m.
Freeze the Hyb-Ready Plate at –20°C.	B	Freezer	5:45 p.m.	8:55 a.m.—day 3

Table 43 Day 2 NIMBUS activities. *(continued)*

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Hybridization setup. • Denature. • Transfer to hybridization tray.	A	• Off-line • NIMBUS	5:30 p.m.	6:00 p.m.
Hybridization.	A	GTMC	6:00 p.m.	6:00 p.m.—day 3

[1] See Table 37, Table 38, and Table 39 for sections of the user guide containing detailed descriptions for each activity.
[2] GTMC = GeneTitan™ MC Instrument. NIMBUS = NIMBUS® Target Preparation Instrument.

Day 2 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 3-plate workflow with 3-hour precipitation



User activities

♦ ♦	Prepare reagents required for the operation (including mPCR Reaction Plate)
▼	mPCR spike-in
★	Freeze
♦	Warm array plate to room temperature
	Fragmentation and precipitation
	Off-deck centrifugation and drying pellets
	Resuspension and hybridization preparation
	Sample QC on the NIMBUS Instrument Deck
	Off-deck OD
	Gel QC Run
	Hybridization setup (denature & transfer to hybridization tray)

Background activities

	Amplification incubation
	Precipitation incubation
	Hybridization in the GeneTitan™ MC Instrument

Day 3 activities

Multiplex PCR activities

- Thaw mPCR reaction plates from day 1 for mPCR spike-in into its corresponding DNA Amplification Plate (plate C).

NIMBUS® Instrument

- Sample denaturation and prepare hybridization tray for plate B and C.
- Fragment and precipitate plate C.
- Resuspend, prepare hybridization-ready sample plate, and sample QC plate C.
- Prepare GeneTitan™ reagent trays for plate A.

Off-deck activities

- Off-deck centrifuge, dry pellets (plates C).
- Resuspension—off-deck pellet resuspension on a microplate shaker (plate C).
- Off-deck QC—fragmentation gel and OD quantification for plate C.
- Precipitation is carried out at –20°C for 3 hours (plate C).

GeneTitan™ MC Instrument

- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization (plates B and C).
- Load GeneTitan™ reagent trays for plate A into the GeneTitan™ MC Instrument. Plate A is moved from the hybridization oven to the fluidics area. After fluidics, plate A moves to the imaging area of the instrument.

Reagent and plate handling

- Start thawing the fragmentation reagents 30 minutes before the start of each fragmentation step. Thaw the corresponding mPCR reaction plate for mPCR spike-in now.
- Amplified plate that was frozen on day 2 (plate C) must be thawed before use in fragmentation. Follow the procedure that is described in “Thaw the frozen plate of amplified DNA” on page 188.
- Start preparing the resuspension reagents at least 60 minutes before the start of the resuspension step.
- Start preparing the reagents 45 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.
- Before each hybridization setup step, warm the array plate to room temperature unopened in its pouch for at least 25 minutes before loading the array plate and hybridization tray into the GeneTitan™ MC Instrument.
- Start thawing the mPCR reaction plate at room temperature for 20 minutes before mPCR spike-in step.
- Start warming the Hyb-Ready Plate at room temperature for at least 5 minutes before the hybridization setup step.

Note:

- Precipitation is carried out at –20°C for 3 hours. If space allows, keep plates in a single layer during precipitation. In addition, the freezer must not be subjected to frequent temperature excursions.

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

Table 44 Day 3 activities for Multiplex PCR.

Activity	Plate	Instrument	Time estimates	
			Start time	End time
Thaw mPCR reaction plate.	C	—	9:00 a.m.	9:20 a.m.
mPCR spike-in into DNA Amplification Plate.	C	—	9:20 a.m.	9:30 a.m.

Table 45 Day 3 NIMBUS® activities.

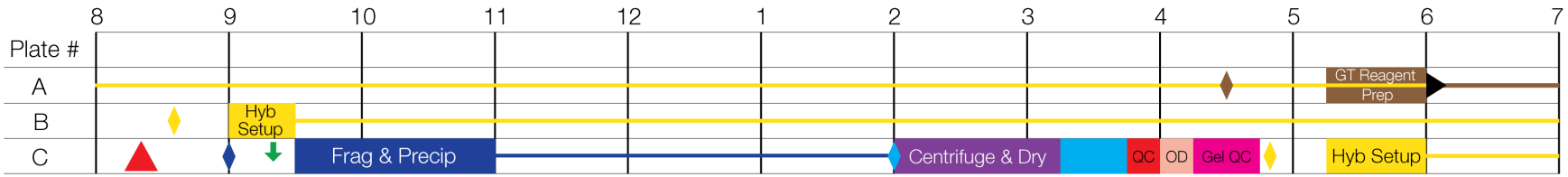
Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Thaw DNA Amplification Plate.	C	—	8:20 a.m.	9:20 a.m.
Hybridization setup. • Denature. • Transfer to hybridization tray.	B	• Off-line • NIMBUS	9:00 a.m.	9:30 a.m.
Hybridization.	B	GTMC	9:30 a.m.	9:30 a.m.—day 4
Fragmentation & precipitation.	C	NIMBUS	9:30 a.m.	11:00 a.m.
Incubate Precipitation Plate at –20°C.	C	Freezer	11:00 a.m.	2:00 p.m.
Off-deck centrifugation and drying pellets.	C	Centrifuge/Oven	2:00 p.m.	3:15 p.m.
Resuspension and hybridization preparation.	C	Off-line/NIMBUS	3:15 p.m.	3:45 p.m.
Sample QC.	C	NIMBUS	3:45 p.m.	4:00 p.m.
Off-deck OD.	C	Plate Reader/ Spectrophotometer	4:00 p.m.	4:15 p.m.
Gel QC run	C	E-gel system	4:15 p.m.	4:45 p.m.
Thaw reagents for GeneTitan™ reagent tray preparation.	A	—	4:30 p.m.	5:15 p.m.

Table 45 Day 3 NIMBUS activities. *(continued)*

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Concurrent steps: • GeneTitan™ reagent tray preparation and loading. • Denature & hybridization setup.	• A • C	• NIMBUS/GTMC • Off-line/NIMBUS	5:15 p.m.	6:00 p.m.
Hybridization.	C	GTMC	6:00 p.m.	6:00 p.m.—day 4

[1] See Table 37, Table 38, and Table 39 for sections of the user guide containing detailed descriptions for each activity.
[2] GTMC = GeneTitan™ MC Instrument. NIMBUS = NIMBUS® Target Preparation Instrument.

Day 3 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 3-plate workflow with 3-hour precipitation



User activities

▲	Thaw DNA Amplification Plate
♦	Warm array plate to room temperature
♦♦	Prepare reagents required for the operation (including mPCR Reaction Plate)
▼	mPCR spike-in
▶	GeneTitan™ Instrument loading
	Fragmentation and precipitation
	Off-deck centrifugation and drying pellets
	Resuspension, hybridization preparation
	Sample QC on the NIMBUS Instrument Deck
	Off-deck OD
	Gel QC Run
	Hybridization setup (denature & transfer to hybridization tray)
	GeneTitan™ reagent tray preparation

Background activities

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

Day 4 activities

NIMBUS® Instrument

- Prepare GeneTitan™ reagent trays for plates B and C.

GeneTitan™ MC Instrument

- Load GeneTitan™ reagent trays for plates B and C into the GeneTitan™ MC Instrument. These plates are moved from the hybridization oven to the fluidics area. After fluidics, the plates move to the imaging area of the instrument.

Reagent and plate handling

- Start preparing the reagents 45 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.

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

Table 46 Day 4 NIMBUS® activities.

Activity[1]	Plate	Instrument[2]	Time estimates	
			Start time	End time
Thaw reagents for GeneTitan™ reagent tray preparation.	B	—	8:00 a.m.	8:45 a.m.
GeneTitan™ reagent tray preparation and loading.	B	NIMBUS/GTMC	8:45 a.m.	9:30 a.m.
Thaw reagents for GeneTitan™ reagent tray preparation.	C	—	4:30 p.m.	5:15 p.m.
GeneTitan™ reagent tray preparation and loading.	C	NIMBUS/GTMC	5:15 p.m.	6:00 p.m.

[1] See Table 37, Table 38, and Table 39 for sections of the user guide containing detailed descriptions for each activity.
[2] GTMC = GeneTitan™ MC Instrument. NIMBUS = NIMBUS® Target Preparation Instrument.

Day 4 activities for the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow 3-plate workflow with 3-hour precipitation

User activities	
♦	Thaw reagents for GeneTitan reagent tray preparation
▶	GeneTitan™ Instrument loading
■	GeneTitan™ reagent tray preparation

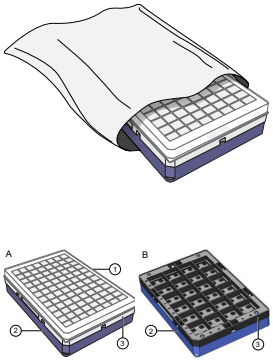
Background activities	
———	Hybridization in the GeneTitan™ MC Instrument
———	Fluidics processing in the GeneTitan™ MC Instrument
·····	Imaging in the GeneTitan™ MC Instrument

A

Recommended techniques for GeneTitan™ MC Instrument operation

This chapter describes the recommended techniques and procedures to follow when using the GeneTitan™ MC Instrument or the GeneTitan™ MC Fast Scan Instrument for the fluidics processing and array scanning steps of the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow. Being familiar with these techniques helps to ensure the success of the assay. Detailed safety information and instruction on using the GeneTitan™ MC Instrument is in the *GeneTitan™ Multi-Channel Instrument User Guide* and the *GeneChip™ Command Console™ User Guide*.

Array plate packaging

Item	Image	Details
Axiom™ Precision Medicine Diversity Research 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.

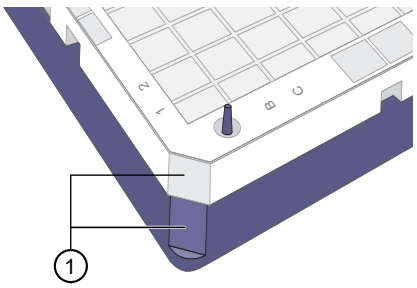


Figure 43 Notched corners aligned.

① Notched corner of array plate that is aligned with notched corner of blue base.

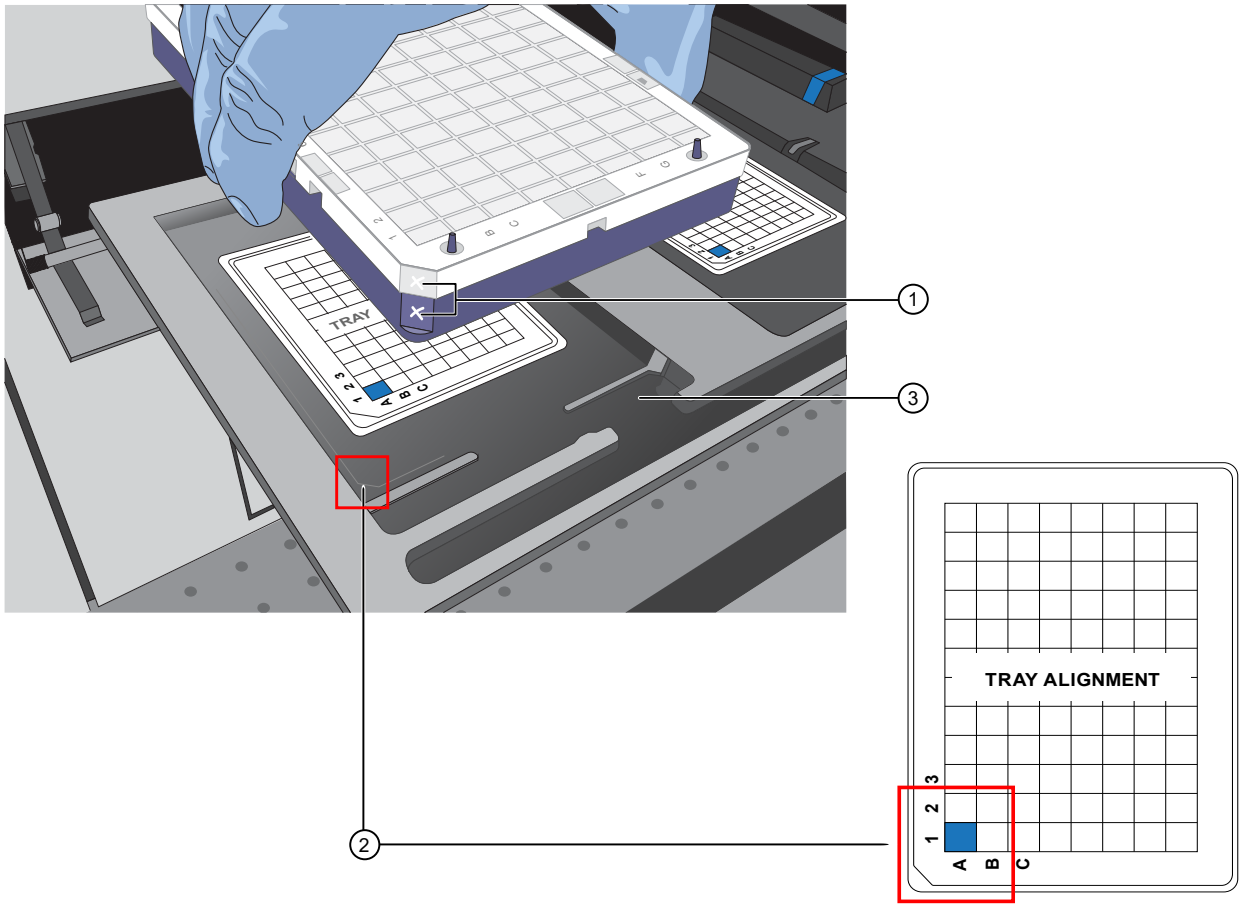


Figure 44 Notched corners marked and aligned with tray alignment guide.

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.

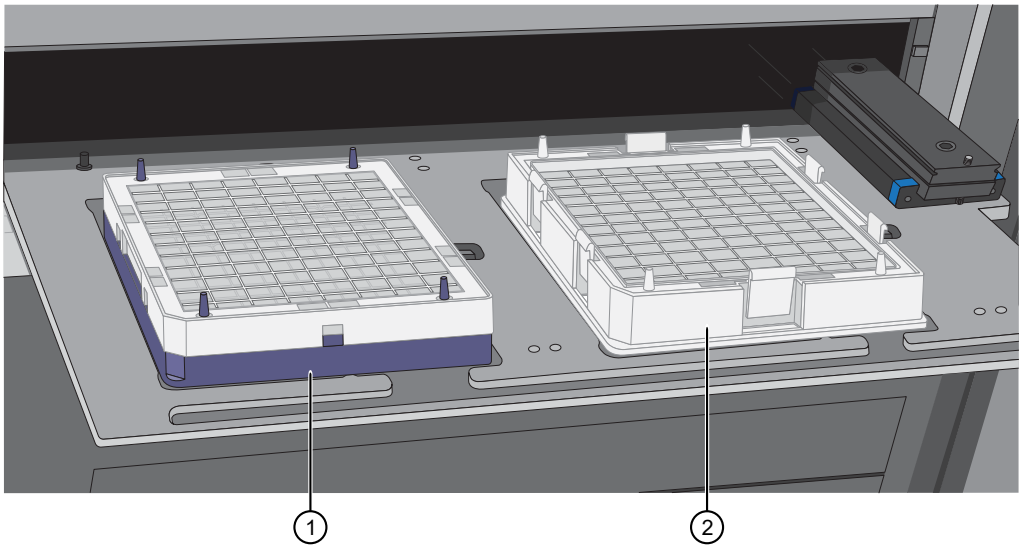


Figure 45 Array plate with protective blue base and the hybridization tray aligned properly loaded into drawer 6.

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

Scan tray

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

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

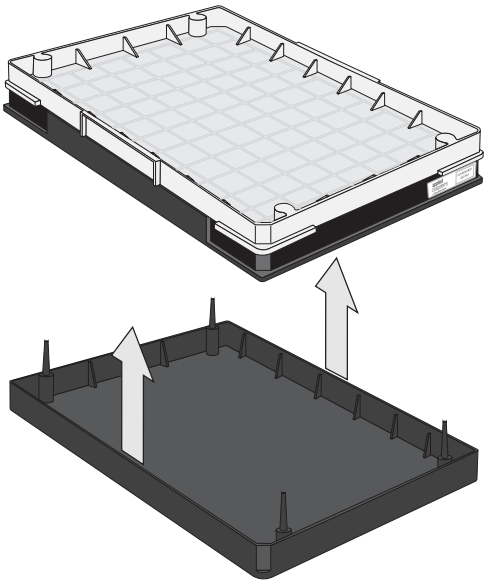


Figure 46 Remove the scan tray and cover from the base before loading in the GeneTitan™ Instrument.

Proper orientation of consumables

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

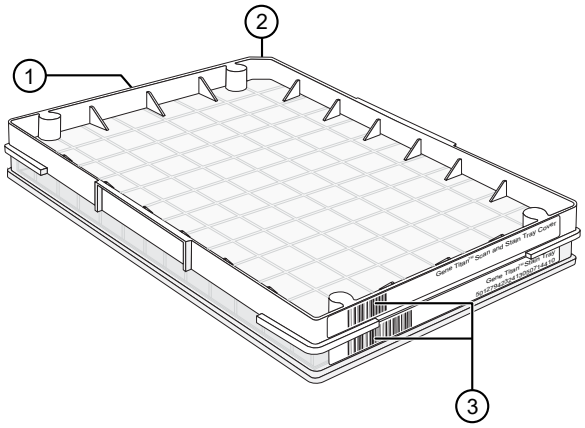


Figure 47 Example shows consumables that must be rotated and loaded on the drawer so that the barcodes face into the instrument.

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.

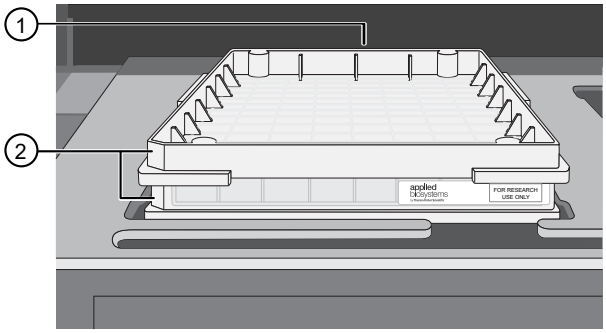


Figure 48 Example of properly loaded GeneTitan™ tray consumables. A GeneTitan™ stain tray and the stain tray cover are shown in this example.

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.

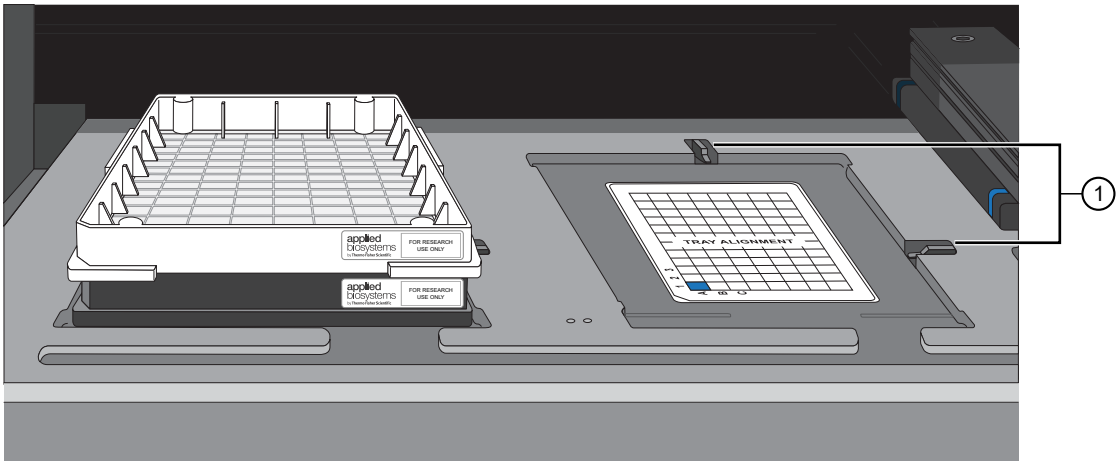


Figure 49 Location of drawer tabs, or fingers.

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

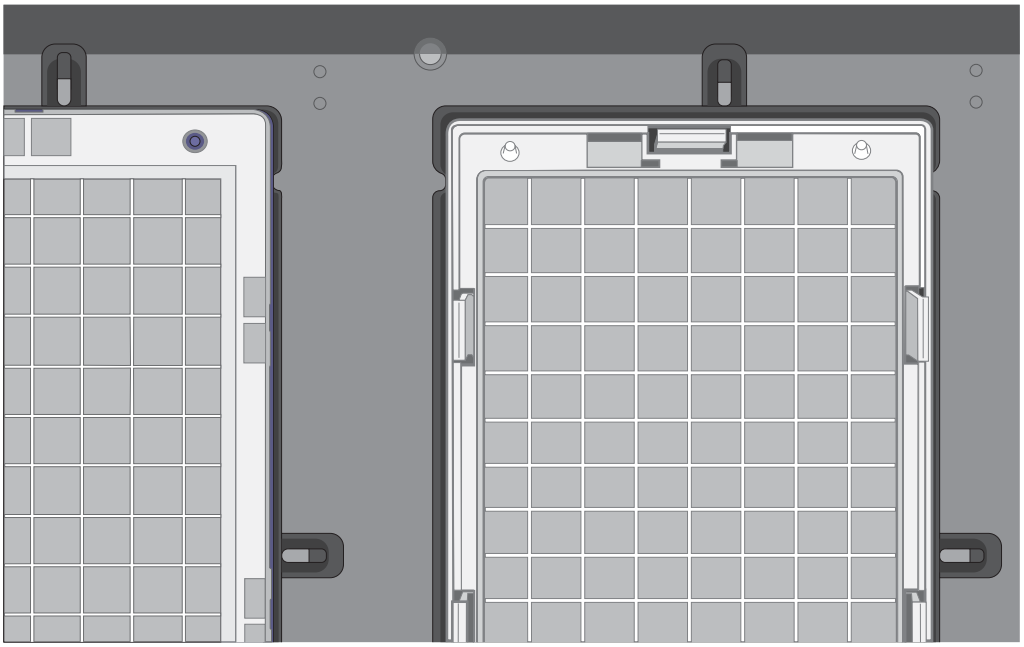


Figure 50 Trays are placed on the drawer when the tabs, or fingers, are retracted.

Stain trays and covers

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

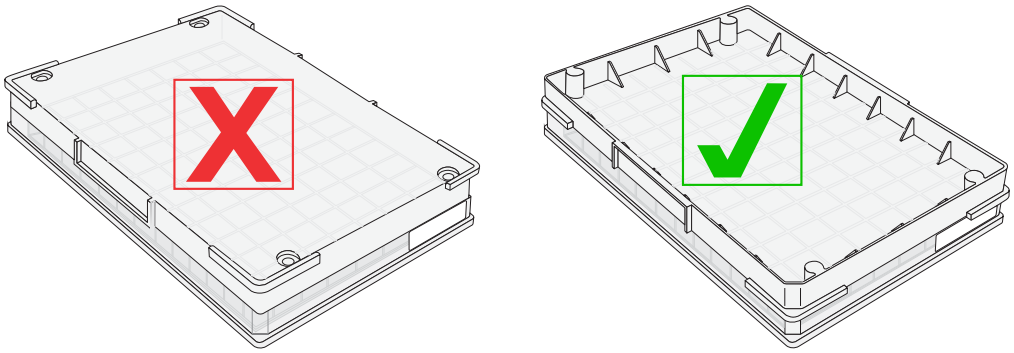


Figure 51 Placement of covers on trays.

Label GeneTitan™ hybridization and reagent trays

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

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

Label 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.

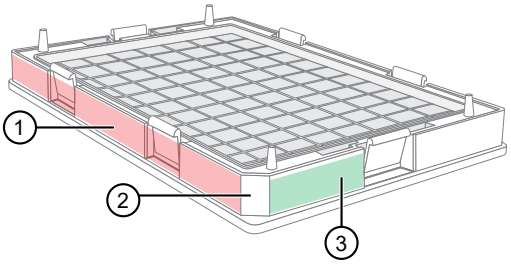


Figure 52 Correct area to label the GeneTitan™ 96-layout hybridization tray.

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.

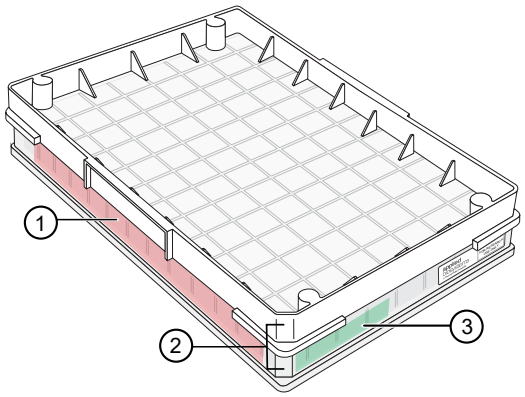


Figure 53 Correct area to label the GeneTitan™ reagent trays (96-layout stain tray with cover shown).

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.

Deionization of GeneTitan™ trays and covers

We recommend the use of the GeneTitan™ ZeroStat AntiStatic Gun (Cat. No. 74-0014) to deionize GeneTitan™ MC Instrument stain 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.

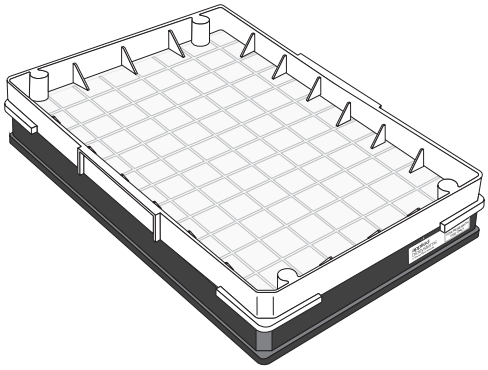


Figure 54 96-format scan tray and cover. Deionize only the cover.

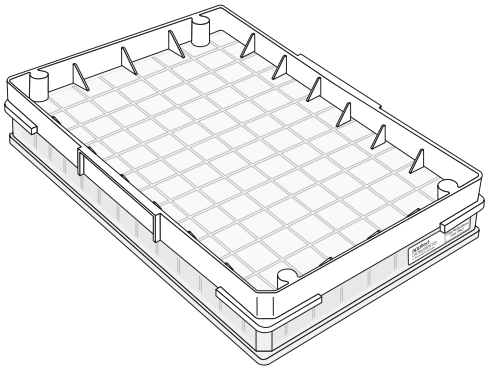


Figure 55 96-format stain tray and cover. Deionize the cover and the tray.

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 56.

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.

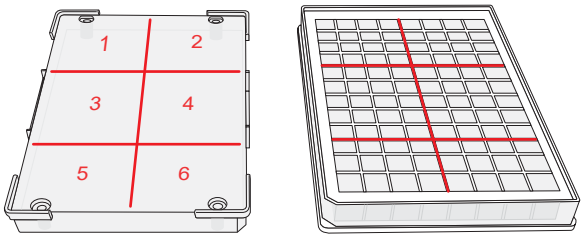
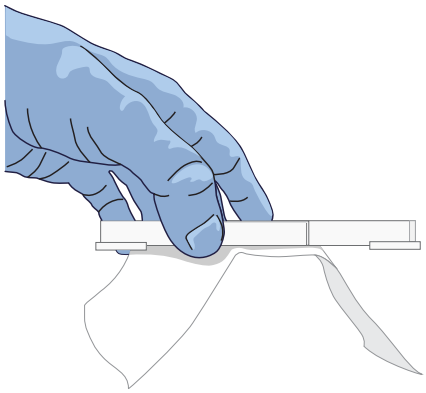


Figure 56 Deionization zones for GeneTitan™ 96-format trays and covers.

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.



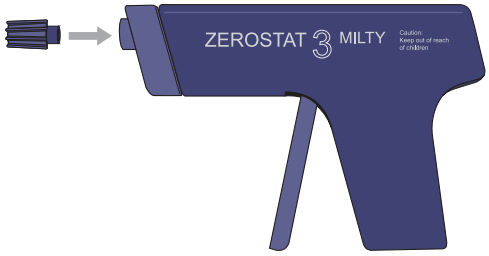
6. Do one of the following:
   - If the tissue does not cling to the plastic, proceed with the protocol.
   - If the tissue clings to the plastic, then repeat step 2 through step 5. If the tissue continues to cling to the plastic, test the device using the ion-indicator cap to determine if the unit is 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.

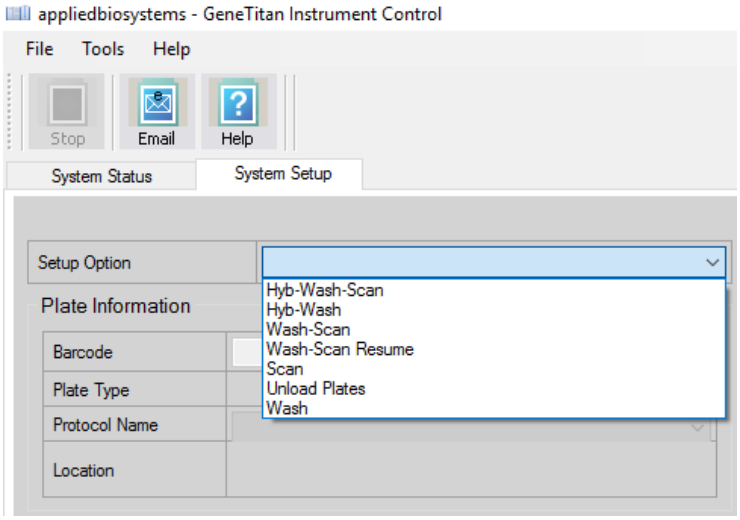
4. If you can see a light through the viewing window, then have determined that the gun is functional, remove the cap from the gun before deionizing a tray or cover.

Setup options for array plate processing

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

- Hybridization
- Wash and Stain
- Imaging (Scan)

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



Hyb-Wash-Scan

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

- **Hyb**: The array plate is moved to the hybridization oven inside the instrument. Each denatured sample in the hybridization tray is hybridized to an array on the array plate.
  - Time that is required for 96 samples = 23.5 hours
- **Wash**: Samples on arrays are ligated, washed, stained, and stabilized.
  - Time that is required for 96 samples = ~5 hours
- **Scan**: The array plate is moved to the imaging device in the GeneTitan™ MC Instrument and each array is scanned.
  - Time that is required for 96 samples = ~3.5 to ~5.5 hours. Scan time varies with instrument type and GCC software 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 level when handling it. Do not touch the bottom optical surface of the scan tray.

   b. Store at 4°C.

   c. Scan the array plate in 3 days or less.

2. When ready to scan, prepare the array plate following these steps:
   a. Protect the plate from light.
   b. Bring the plate to room temperature for approximately 50 minutes.
   c. Remove the aluminum foil, then load the plate onto the GeneTitan™ MC Instrument.

Wash-Scan

Note: The **Wash-Scan** option is available in GCC version 6.1 or later.

Use the **Wash-Scan** option if:

- The array plate was hybridized in an oven separate from the GeneTitan™ MC Instrument.
- To bypass the hybridization step and perform only the wash/stain and scan steps.

  Note: If the **Wash-Scan** option is selected, it usually takes 25–30 minutes to warm up the Wash B.

Note: Ensure that the Continuous Wash-Scan process is enabled. Contact your local FAS to perform this procedure.

Wash-Scan Resume

Use the **Wash-Scan Resume** option if fluidics processing has been interrupted (for example, a power failure at your facility). This allows you to resume an interrupted workflow at any point in the **Wash** stage.

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

The step at which the run was aborted is identified by:

- Viewing the **System Status** window if you are aborting the last plate through the fluidics system.
- Starting the **Resume** process.

Select **Wash/Scan Resume** from the **System Setup** tab, then follow the prompts to unload and reload all drawers.

The trays are loaded. It is up to you to determine whether to load fresh reagents or reuse the trays already in the GeneTitan™ Multi-Channel (MC) Instrument. Base your decision on the step where the problem occurred.

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

- Load new stain trays with fresh reagents.
- Load a new scan tray.

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

Resume step

For **Resume**, select the step at which to resume plate processing. You can select any step that has not yet been started.

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

Scan

Use the scan option:

- 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.

Unload Plates

Use the **Unload Plates** option to unload plates and trays from the instrument when processing is complete or has been aborted.

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.

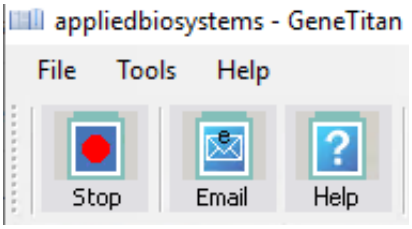
The operator can initiate an abort on 1 plate and the gripper can continue to process other plates in the instrument.

Abort a process

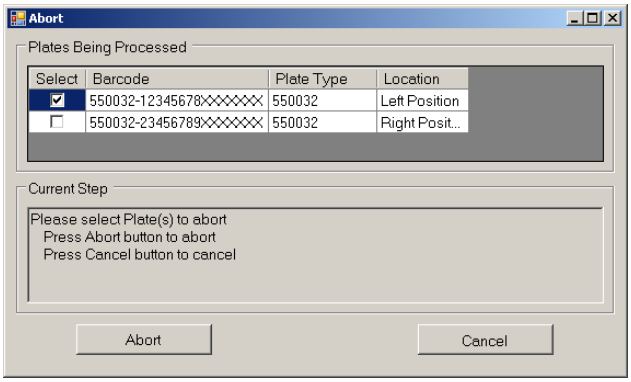
Use the following procedure to abort a process underway in the GeneTitan™ MC Instrument.

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.

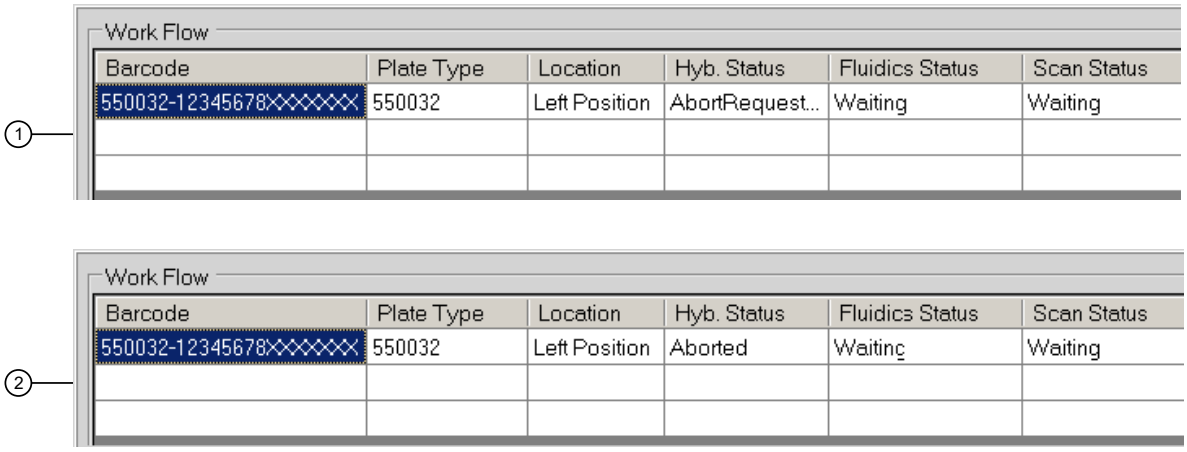


Figure 57 The Work Flow pane.

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

Register samples in GeneChip™ Command Console™

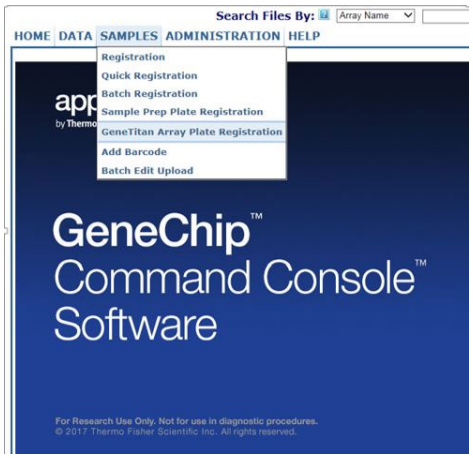
GeneTitan™ Array Plate Registration file

A GeneTitan™ Array Plate Registration file is a Microsoft™ Excel™ spreadsheet that includes information on the samples that you are processing on a single array plate. This information includes the array plate format, the array plate barcode, and the sample file names for tracking the samples that are loaded onto a particular array plate.

Note: The GeneTitan™ Array Plate Registration file uses the *.xls Microsoft™ Excel™ file extension. Do not use the *.xlsx file extension.

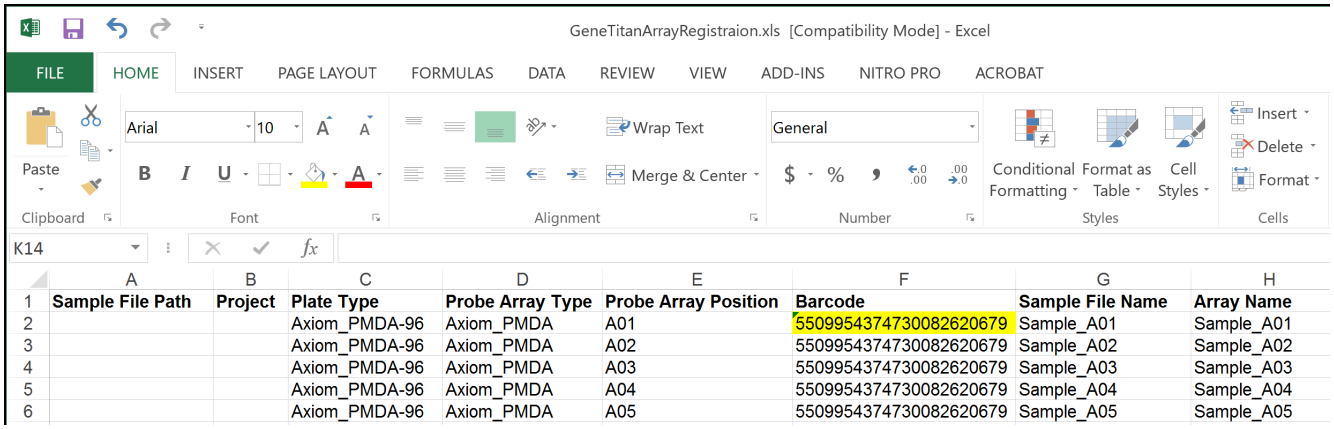
Create a GeneTitan™ Array Plate Registration file

1. In GCC Portal, click **Samples** ▸ **GeneTitan Array Plate Registration**.



2. Create a new template in GCC that includes fields required for sample traceability.
3. Select the array plate to be processed on the GeneTitan™ MC Instrument.
4. Select the newly created template that contains the fields that are required for traceability.

5. Select the **GeneTitan Array Plate Type** from the dropdown list.
6. Select the project where the sample registration data and all associated data files are saved.
7. Click **Download**.
8. Click the Microsoft™ Excel™ icon to open the spreadsheet.



	A	B	C	D	E	F	G	H
1	**Sample File Path**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array Position**	**Barcode**	**Sample File Name**	**Array Name**
2			Axiom_PMDA-96	Axiom_PMDA	A01	550995437473008262067 9	Sample_A01	Sample_A01
3			Axiom_PMDA-96	Axiom_PMDA	A02	550995437473008262067 9	Sample_A02	Sample_A02
4			Axiom_PMDA-96	Axiom_PMDA	A03	550995437473008262067 9	Sample_A03	Sample_A03
5			Axiom_PMDA-96	Axiom_PMDA	A04	550995437473008262067 9	Sample_A04	Sample_A04
6			Axiom_PMDA-96	Axiom_PMDA	A05	550995437473008262067 9	Sample_A05	Sample_A05

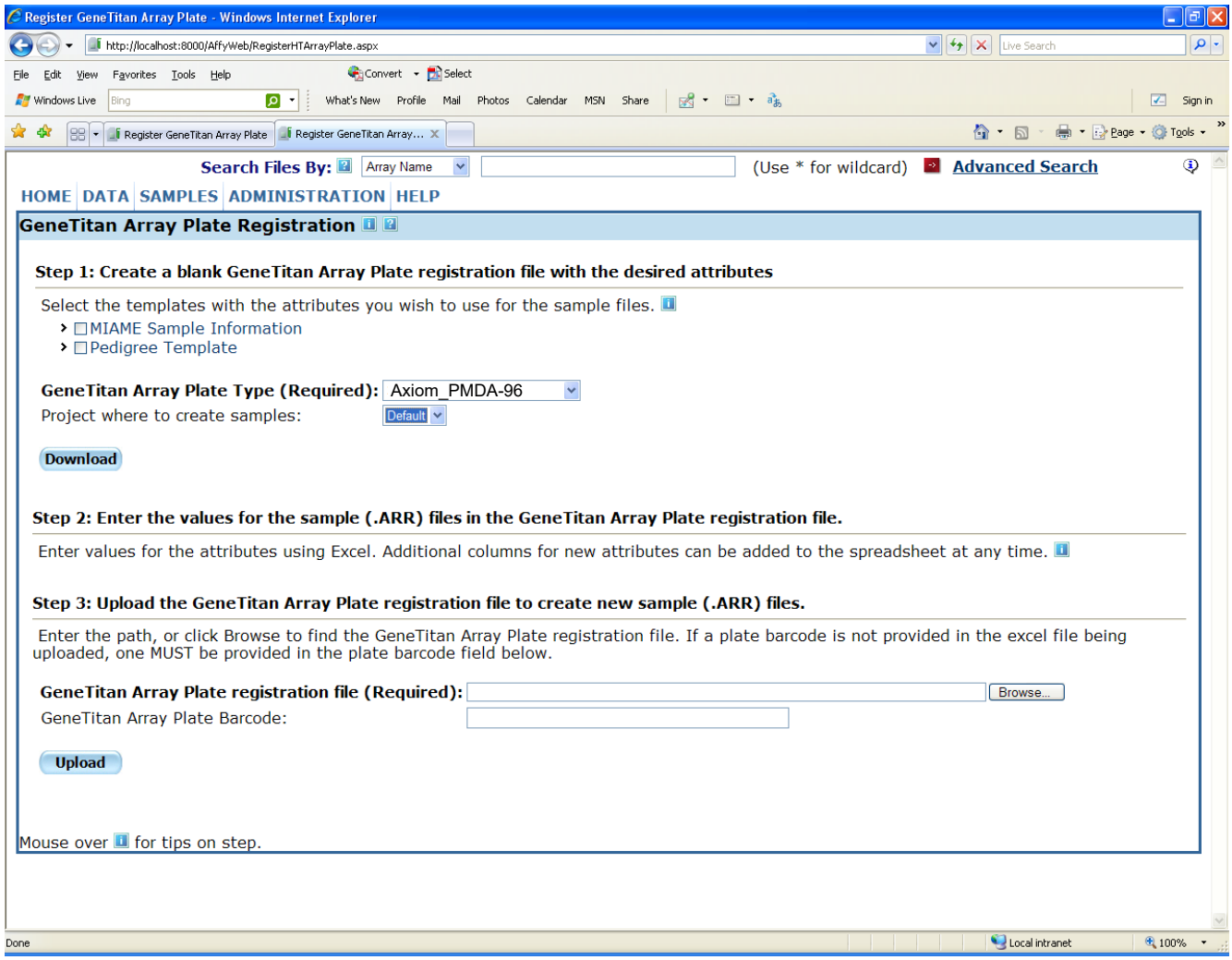
9. In the **Sample File Name** column, enter a unique name for each sample and any additional information, such as array plate barcode.

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.



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 Laboratories™, MSB1001
Pipette tips	Same brand as pipettor

Prepare the gel diluent

Dilute the TrackIt™ Cyan/Orange Loading Buffer

A 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer can be used in 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

Follow the product instructions for the dilution method.

Run the fragmentation QC gel

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

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

1. Tightly seal the Gel QC Plate that is prepared during automated target preparation.
2. Vortex the center of the plate for 3 seconds. Centrifuge at 1,000 rpm for 30 seconds to get the droplets down.
3. Connect an E-Base™ device to an electrical outlet.
4. Push the **Power/Prg** button on each to ensure the gel base is in **EG** mode, not EP mode.
5. Take the gel out of the pouch, then remove the combs.
6. Place the E-Gel™ *48* Agarose Gel into the base unit.

7. Load 20 µL from each well of the Gel QC Plate onto the gel.
8. Load 25 bp DNA Ladder into the marker wells (M).
9. Load 20-µL nuclease-free water into any unused wells.
10. Run the gels for 22 minutes.
11. Capture a gel image.

Fragmentation QC gel images should look similar to the gel shown in Figure 58.

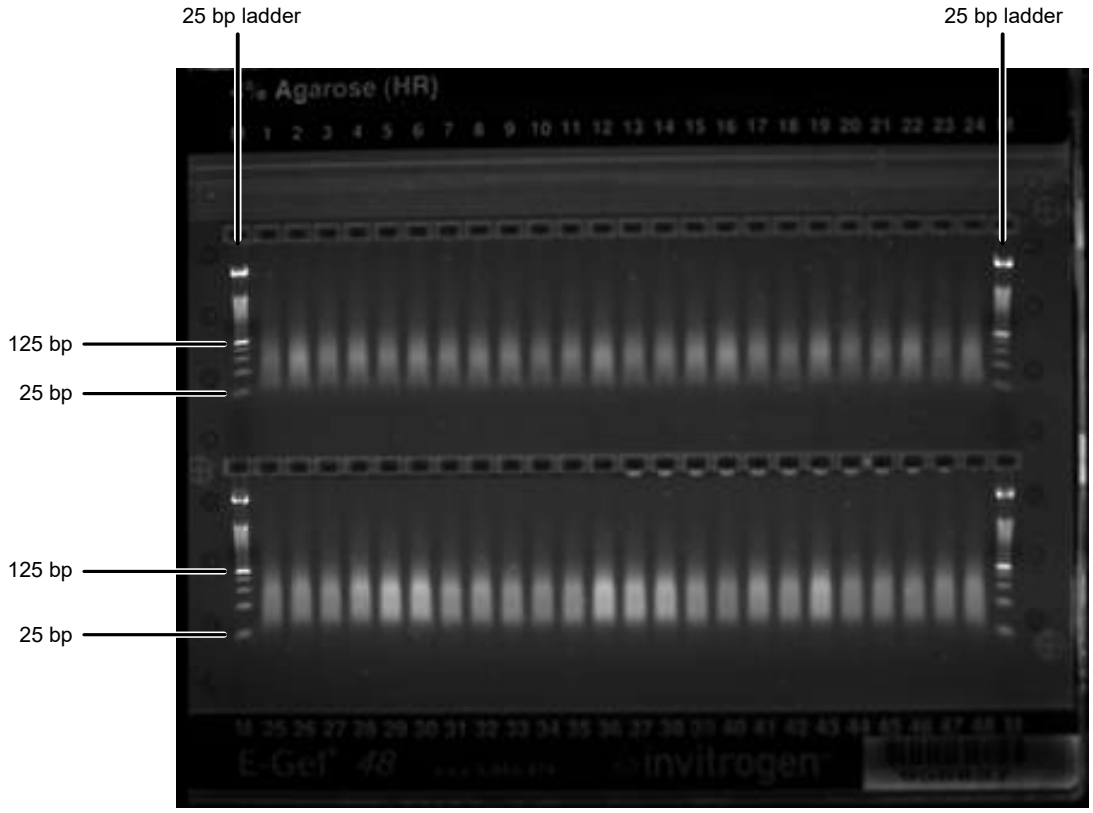


Figure 58 Fragments fall between 125 bp and 25 bp on a successful gel image.

D

Sample quantification after resuspension

Equipment required

Spectrophotometer

We recommend that you use one of the following spectrophotometers, or the equivalent.

Item	Source
Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer	51119600
SpectraMax® Plus 384 Microplate Reader	Molecular Devices™, PLUS 384
DTX 880 Multimode Detector with genomic filter slide	Beckman Coulter™, Detector, 987921 Filter slide, A30184

Quantify the diluted samples

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

For OD quantification, readings must be taken at wavelengths of 260 nm, 280 nm, and 320 nm. See “Plate reader guidelines for sample quantification” on page 226.

OD yield evaluation guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow. 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 0.50.

Plate reader guidelines for sample quantification

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

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

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

Where:

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

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

The optical path length depends on the type of plate and can depend on the spectrophotometer used. Check the recommendations for the path length for your instrument and plate type or for recommendations on how to measure this quantity. The SpectraMax® Plus 384 Microplate Reader can use an automated path length detection system. Consult the SpectraMax® Plus 384 Microplate Reader user guide for more information.

E mPCR quality control gel protocol

The mPCR quality control gel protocol is an optional procedure. It is meant only as a *qualitative* examination of the mPCR reaction to confirm that amplification has occurred. Gene copy number differences result in differences in DNA band patterns and amplicon intensities, and therefore, sample to sample variation can be observed.

Materials required

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

Item	Source
Equipment	
Gel imager	**MLS**
Pipette, multichannel and single-channel	**MLS**
Plate centrifuge	**MLS**
Vortexer	**MLS**
E-Gel and reagents	
E-Gel™ Power Snap Plus Electrophoresis Device	G9110
iBright™ CL750 Imaging System	A44116
E-Gel™ *48* Agarose Gels, 2%	G800802
TrackIt™ Cyan/Orange Loading Buffer	10482028
Thermo Scientific™ TE Buffer, 1X Solution pH 8.0, Low EDTA, Molecular Biology Grade	Fisher Scientific™, AAJ75793AE
50 bp DNA Ladder	New England BioLabs™, N3236S
Diluted TrackIt™ Cyan/Orange Loading Buffer [1]	

(continued)

Item	Source
Consumables	
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 pipette)	**MLS**
96-well PCR plate	**MLS**
1.7-mL microcentrifuge tube	**MLS**

[1] See "Dilute the TrackIt™ Cyan/Orange Loading Buffer" on page 223.

Prepare the 50 bp DNA Ladder

The following steps prepare a 250-fold dilution of the New England BioLabs™ 50 bp DNA Ladder (4 ng/µL final concentration).

1. In a 1.7-mL microcentrifuge tube, add 1 µL of 50 bp DNA Ladder to 249 µL of 100-fold diluted TrackIt™ Cyan/Orange Loading Buffer.
2. Vortex the tube to mix well. Briefly centrifuge to get the droplets down.

Prepare mPCR samples for gel analysis

1. If frozen, thaw the mPCR Product Plate on benchtop at room temperature.
2. Ensure that the plate seal is affixed tightly, vortex the plate, then centrifuge briefly.
3. Prepare the mPCR samples for gel analysis by diluting samples of the mPCR Product Plate 120-fold.
   a. Prepare the mPCR Dilution QC Plate (12-fold dilution from mPCR Product Plate).
      1. Obtain a 96-well PCR plate, then aliquot 22 µL of low EDTA TE buffer per well.
      2. Transfer 2 µL of the mPCR product to the appropriate wells of the 96-well PCR plate with TE buffer.
      3. Seal the plate, vortex, then centrifuge briefly.

b. Prepare the mPCR Gel QC Plate (10-fold dilution from the mPCR Dilution QC Plate).

1. Obtain a 96-well PCR plate, then aliquot 18 µL of gel diluent (TrackIt™ Cyan/Orange Loading Buffer diluted 100-fold).
2. Transfer 2 µL of the mPCR Dilution QC Plate to the appropriate wells of the 96-well plate with gel diluent.
3. Seal, vortex, then centrifuge briefly.
4. Proceed immediately to the next step.
   See "Run the mPCR QC gel" on page 229.

4. Store the mPCR Product Plate.
   See "Store the mPCR Product Plate" on page 49.

Run the mPCR QC gel

This protocol is based on running QC gels for 96 samples. Two E-Gel™ *48* Agarose Gels, 2% are needed.

1. Tightly seal the mPCR Gel QC Plate.
2. Vortex the plate for 3 seconds. Briefly centrifuge to get the droplets down.
3. Power on one or more electrophoresis devices.
4. Remove 2 combs from the gel.
5. Place the E-Gel™ *48* Agarose Gel into the electrophoresis unit.
6. Load 15 µL from each well of the mPCR Gel QC Plate into its designated wells.
7. Load 15 µL of diluted 50-bp ladder into the marker wells (M).
8. Load 15-µL nuclease-free water into any unused wells.

9. Run the gels for 25 minutes.

10. Image the gel.

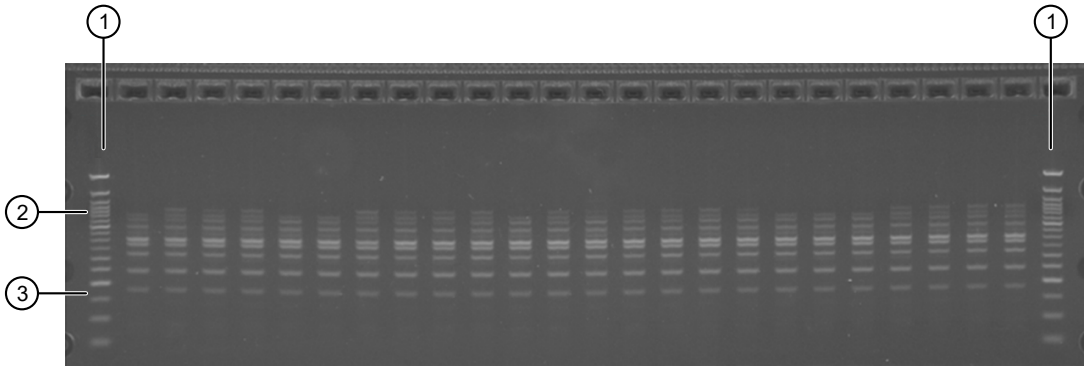


Figure 59 Example of a typical mPCR QC E-gel. All samples were amplified and show multiple DNA bands that fall between 150 bp and 700 bp.

① 50-bp ladder
② 700 bp
③ 150 bp

Note: Variation in DNA band patterns and intensities can be observed from sample to sample due to gene copy number differences. The mPCR QC Gel is meant to be a qualitative examination of the mPCR reaction to confirm that amplification has occurred for each sample.

F

Troubleshooting

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.

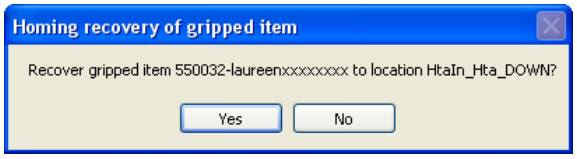
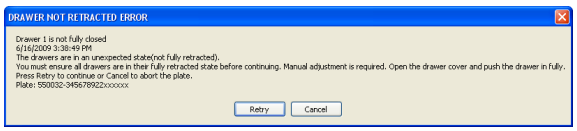
Troubleshooting the GeneTitan™ MC Instrument

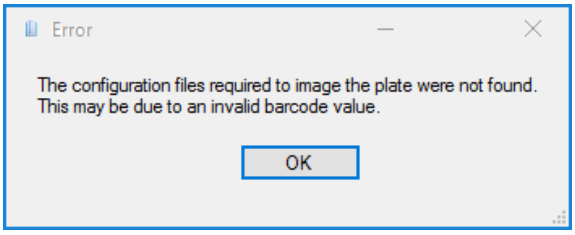
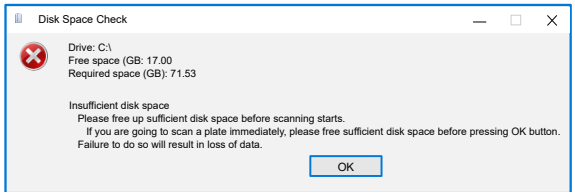
This section provides instructions on how to identify and solve simple problems with the GeneTitan™ MC Instrument. If a problem or error occurs that is not listed in this chapter, contact Thermo Fisher Scientific Technical Support for help.

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.

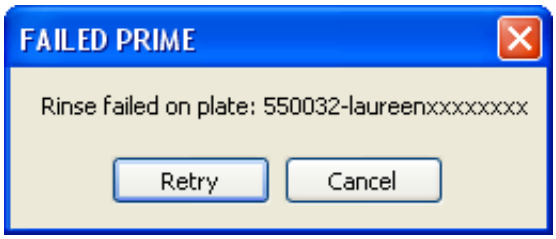
Observation	Possible cause	Recommended action
Plate trapped in the GeneTitan™ MC Instrument	• Plate (or plate with lid) not properly loaded in drawer. • Notched edge of lid and plate not aligned. • Gripper failed to retrieve plate. • System requires adjustment.	1. Restart the GeneTitan™ MC Instrument 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.

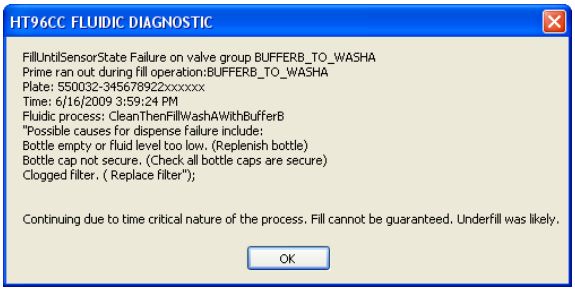
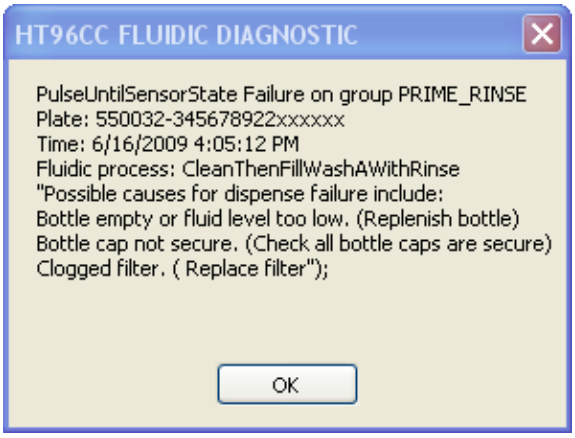
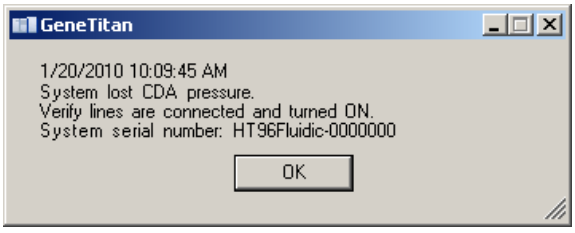
Observation	Possible cause	Recommended action
Hybridization aborted	• System-initiated abort: power loss. • User-initiated abort: – User error – Other	If the array plate and hybridization tray are still clamped, contact your local field service engineer with information on the workstation model.
		If the plate stack is moved to drawer 1: 1. Remove the plate stack and finish hybridization offline. 2. Return the hybridized array plate stack to the GeneTitan™ MC Instrument and finish processing using the **Wash/Scan** process.
Fluidics aborted	• System-initiated abort: power loss. • User-initiated abort: incorrect protocol selected.	Follow the recommendations and instructions under "Wash-Scan Resume" on page 214.

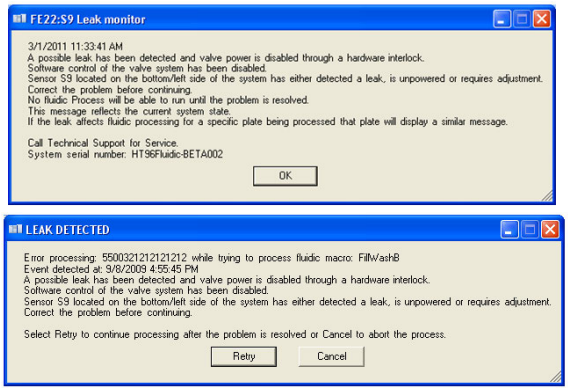
Observation	Possible cause	Recommended action
Homing recovery of gripped item 	Indicates that an item is in the gripper, and normal startup of the GeneTitan™ MC Instrument is not possible. The item must be removed from the instrument before you can start processing array plates.	Recommendation: click **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™ 2.0 Plus Reagent Kit 96F be emptied into the GeneTitan™ Wash B bottle when setting up the system to process a plate. Using all 600 mL of the Axiom™ Wash Buffer B ensures that the bottle is filled to more than the requisite 35% of Wash B bottle volume.

Observation	Possible cause	Recommended action
Fluidics diagnostic dispense error  BUFFER*x* = Buffer bottle A, B, or Rinse. WASH*x* = Wash A or B reservoir in the fluidics station.  	Reagent bottle is empty or too low.	Replenish fluid level in the Rinse or Wash Bottle B to the 1-L mark. Do not overfill. **IMPORTANT!** Only replenish bottles when prompted by the UI. Replenishing during fluidic processing can cause system malfunction including overflowing inside the system and more problems. The only thing to do when a plate is running is to ensure that bottle caps are secure.
		Replenish fluid level in Wash Bottle A to 2 L.
	GeneTitan™ reagent bottle cap is loose.	Fasten the bottle cap.
	The GeneTitan™ reagent bottle filter is clogged.	Replace the filter. See “Bottle filter replacement” on page 242.
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 242.

GeneTitan™ Multi-Channel Instrument care

Overview

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

- The GeneTitan™ Multi-Channel (MC) Instrument must be positioned on a sturdy level bench away from extremes in temperature and away from moving air.
- Always run a Shutdown protocol when the instrument is off or unused overnight or longer to prevent salt crystals from forming in the fluidics system.
- Always use deionized (DI) water to prevent contamination of the lines. Swap out old buffers with freshly prepared buffer at each system startup.

IMPORTANT! Before performing maintenance power off the instrument to avoid injury if an electrical malfunction occurs.

Maintenance

The GeneTitan™ family of instruments requires little in the way of customer maintenance. The instruments must be kept clean and free of dust. Dust buildup can degrade performance. Wipe the exterior surfaces clean using a mild dish detergent solution in water. Do not use ammonia-based cleaners or organic solvents such as alcohol or acetone to clean the system because they can damage the exterior surfaces.

The following tasks must be performed regularly to ensure that the imaging device remains in working order.

Monthly

Wipe down the outer surface of the imaging device with a dry cloth.

Every 6 months

1. Replace the cooling fan air filters at the rear of the instrument.
2. Replace the Micropore™ filters in the Wash A, Wash B, and Rinse bottles. If you run 4-8 plates/week, then replace the Micropore™ filters more frequently.

Outer enclosure fan filters

Cleaning schedule

The GeneTitan™ fan filter cartridge must be cleaned at least every 90 days of service. Note that in some service locations, the presence of excessive dust or particulate matter can require cleaning the cartridge more often than 90 days.

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

Figure 60 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.

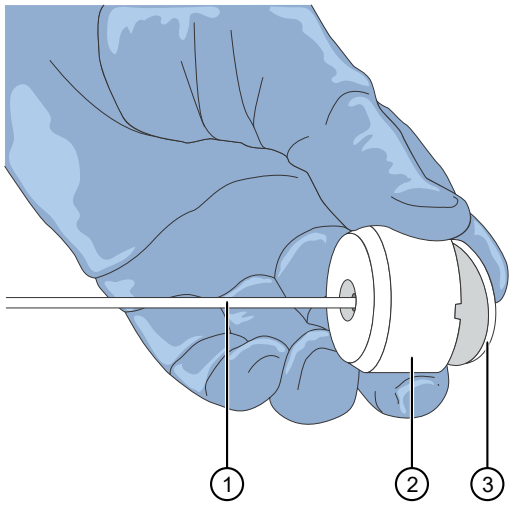


Figure 61 Components of the reagent buffer supply line and filter.

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 61).

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.

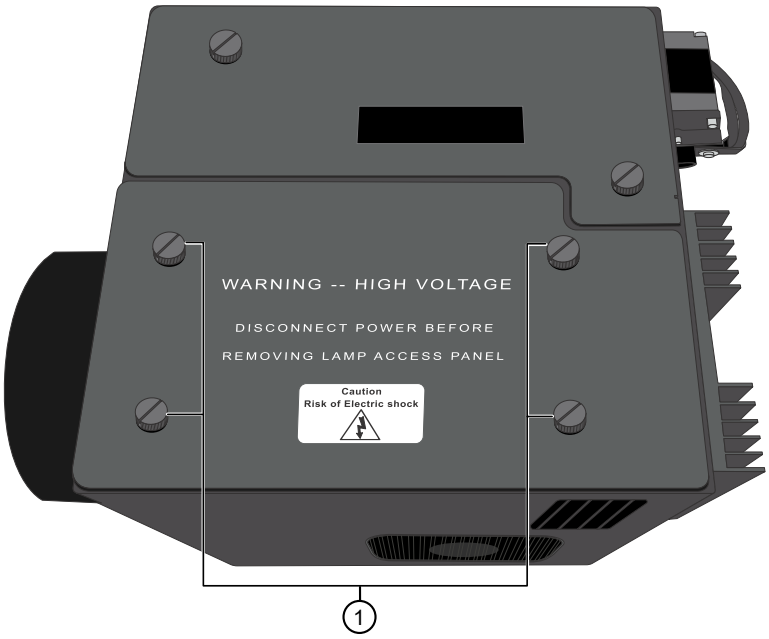
Imaging Device Status	
Barcode	
Estimated Time Remaining	
Scanner Status	Offline: scanning is not available.

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 245, and “Replace the xenon lamp” on page 246. 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.

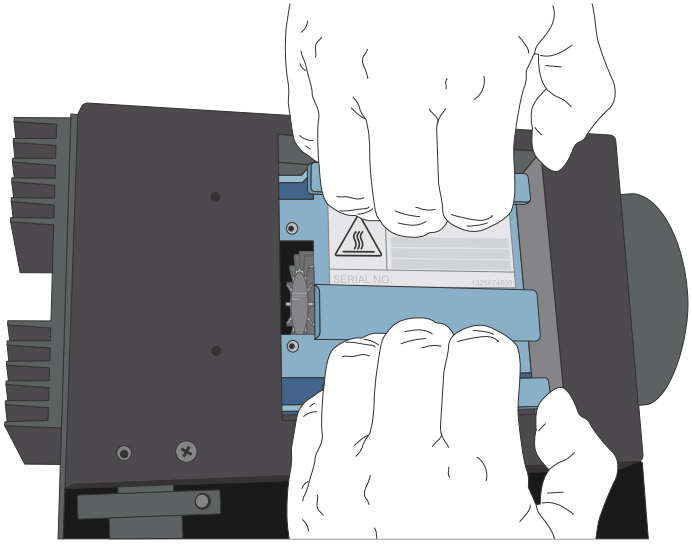
1. Unscrew the 4 retaining bolts with your fingers.



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.

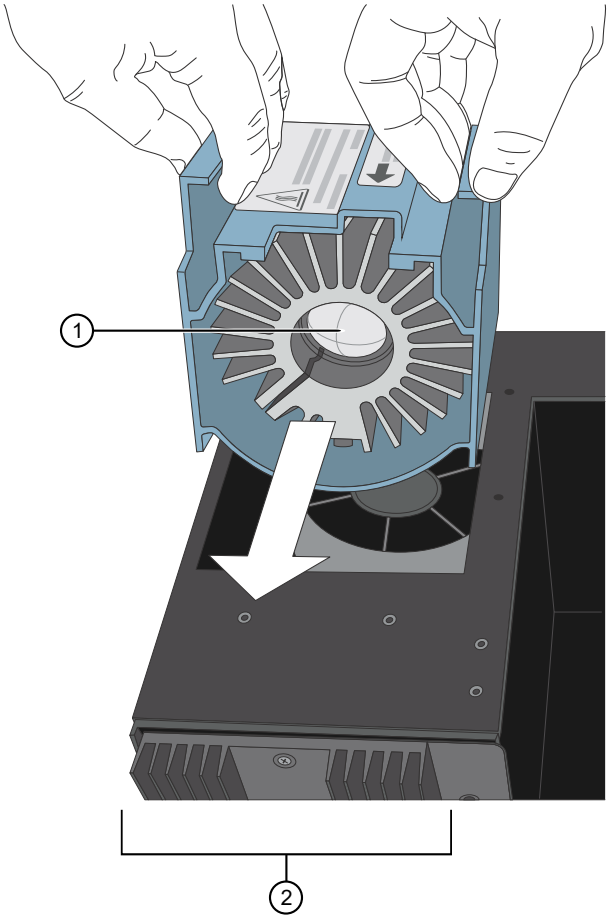


Replace the xenon lamp

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.



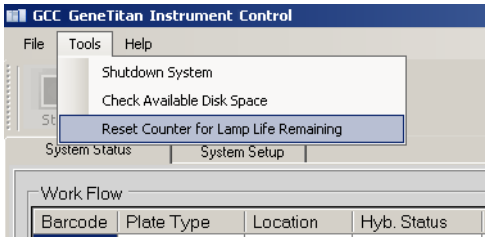
① Xenon bulb faces away from the fan and towards the heat sink.
② Heat sink on the Lambda LS unit.

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.

H

Routine care for the Applied Biosystems™ NIMBUS® Target Preparation Instrument

Regular care and cleaning of the NIMBUS® Target Preparation Instrument is recommended to ensure successful runs, avoid stoppage during a run, and avoid premature damage to the equipment.

O-ring care

The O-rings on the head of the NIMBUS® Target Preparation Instrument allow the instrument to pick up and manipulate the pipette tips that are loaded on the deck. When the head is left in a squeezed position for an extended amount of time, the O-rings wear out and the head is stressed.

WARNING! Do Not leave tips on the head or leave the head in a squeezed position for extended lengths of time. This practice can lead to premature O-ring wear and poor system performance.

On successfully completing a method, the head is normally parked with the O-rings in a relaxed position. If a method is interrupted or stopped, it is possible that the head can stand still with the O-rings in the squeezed position (Figure 62). For instances when the O-rings remain in the squeezed position, try initializing the instrument.

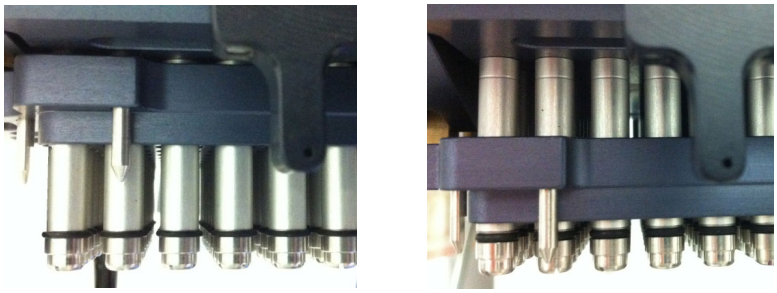


Figure 62 O-rings shown in a squeezed and relaxed state.

① "Squeezed" state.
② O-rings relaxed.

To help preserve the longevity of the O-rings, remember the following:

- Never power off the instrument with any tips that are loaded on the head.
- Never leave the instrument with the head in the squeezed position.
- Initializing the instrument usually resolves the head remaining in the squeezed position leaving the head in a relaxed position.

Tip isolator

The tip isolator is the frame that is fastened in deck position 11. For several steps in the method, a square deepwell plate is placed under the tip isolator frame. During a run, tips that are placed in this position are designated for stamping reagents from one plate to another. During the deck cleanup step of a method, the used tips can accidentally touch against the inner dividing walls of the tip isolator frame. Cross-contamination can occur when new tips are placed in deck position 11 for subsequent methods or runs.

Clean the tip isolator

We recommend cleaning the inner dividing walls of the tip isolator frame after every run.

1. Spray a laboratory tissue with 70% ethanol.
2. Wipe the surfaces of the inner dividing walls of the tip isolator frame.

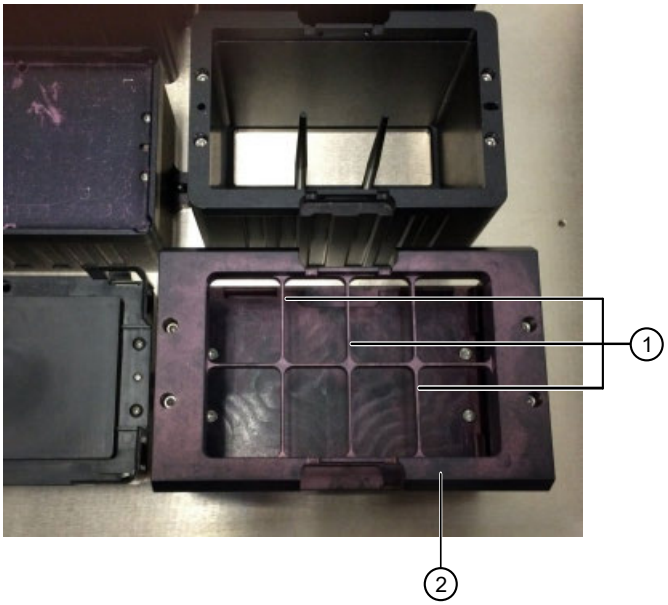


Figure 63 Tip Isolator on NIMBUS® Target Preparation Instrument deck.

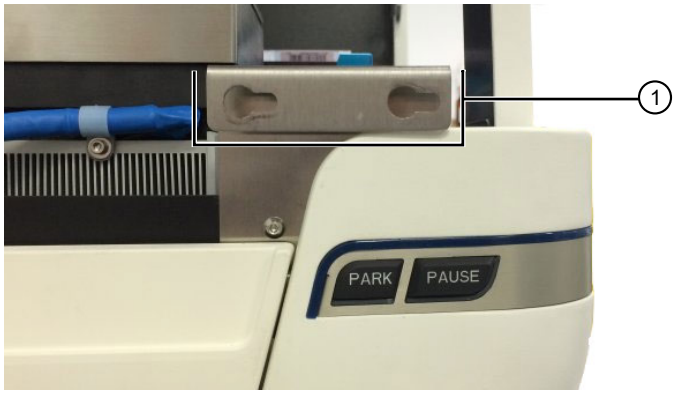
① Inner dividing walls of the Tip Isolator frame.
② Tip Isolator frame in deck position 11.

Trash chute

Assemble the trash chute

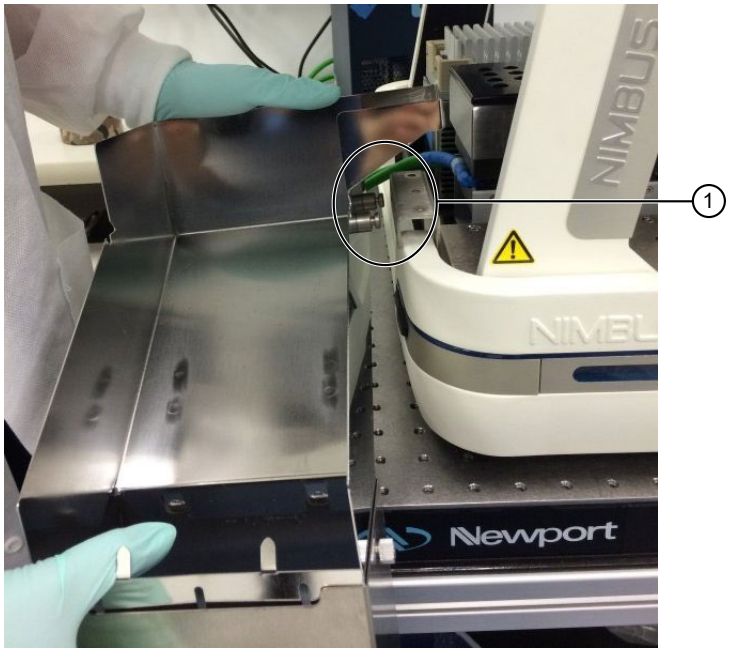
This section provides instructions for the proper assembly of the trash chute and trash chute cover to the NIMBUS® Instrument. Use these instructions to attach properly or remove the trash chute if needed for cleaning or other purposes. To remove the trash chute, follow these instructions in reverse.

1. Find the trash chute mounting bracket on the left side of the NIMBUS® Instrument.



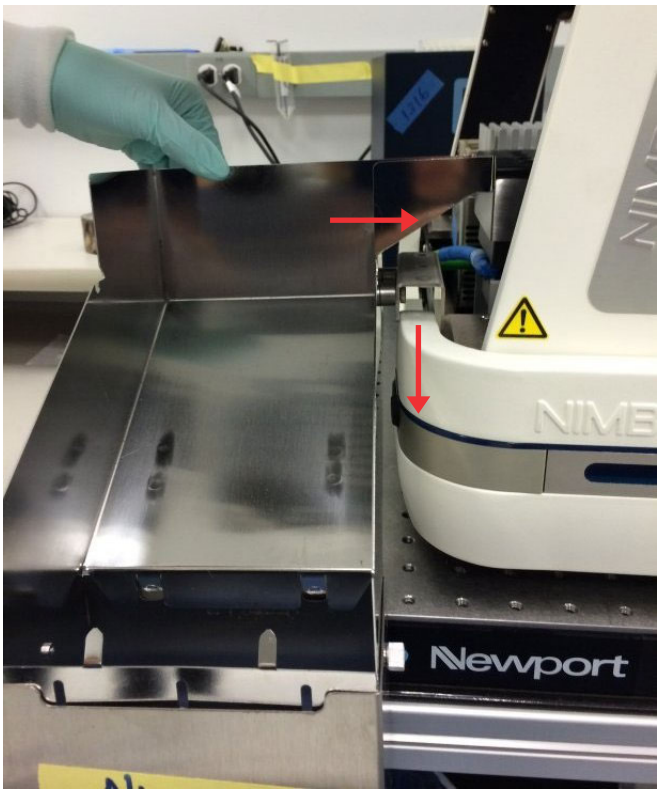
① Trash chute mounting bracket

2. Hold the trash chute with both hands, then align the 2 securing pins on the right side of the trash chute with the 2 holes in the trash chute mounting bracket.



① Align the securing pins with holes in trash chute mounting bracket.

3. Insert the securing pins into the holes in the trash chute mounting bracket, then pull the trash chute forward toward the front of the NIMBUS® Instrument. This action holds the trash chute in place.

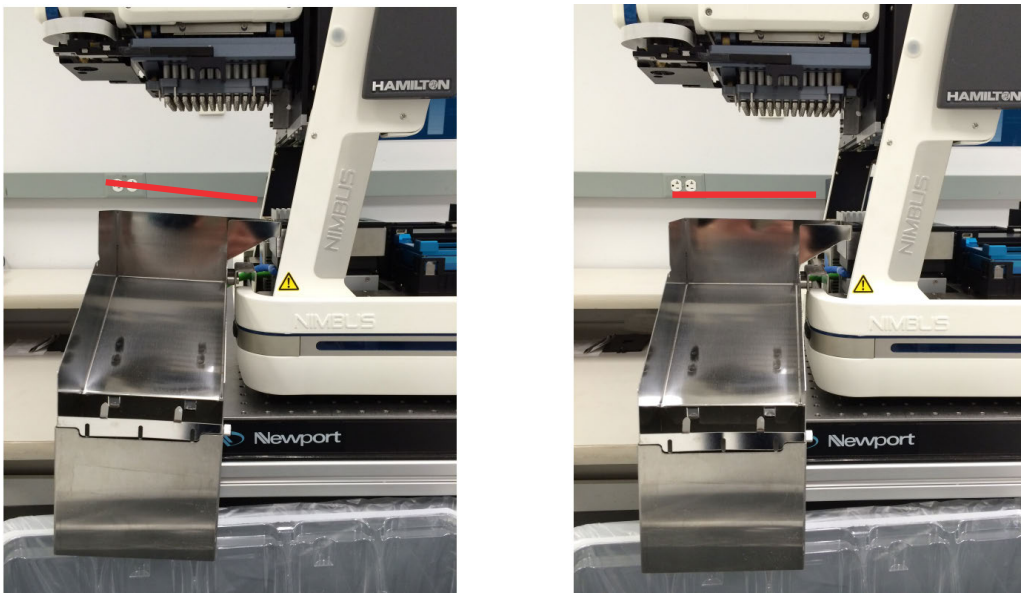


4. After successfully attaching the trash chute to the NIMBUS® Instrument, turn the leveling foot, on the underside of the trash chute, to support, then level the trash chute.

① Leveling foot

5. Ensure that the trash chute is level.

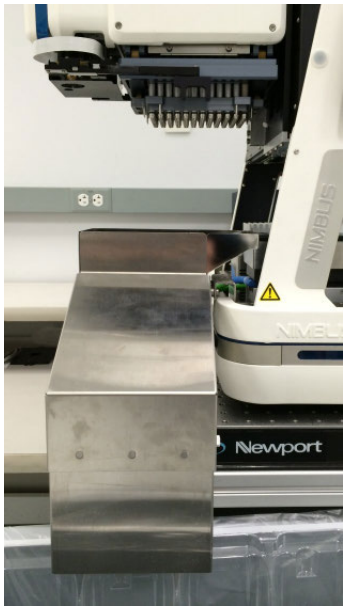
IMPORTANT! Ensure that the trash chute is level. Trash chutes that are not level result in problems with disposal of tips, plates, or other materials from the deck.



① ②

① Example of a trash chute that is not level.
② Example of a trash chute that is properly attached and leveled.

6. After the trash chute has been installed, then leveled, attach the trash chute cover.



Clean the trash chute

As you run the assay and complete the various methods, the NIMBUS® Instrument discards all used tips and a 1.2-mL square plate down the trash chute. As this happens, it is common for the trash chute to develop a sticky buildup from the liquids that remained on the disposed materials. A trash chute with heavy buildup can result in disposed plates and tips to get lodged or stuck. It also increases the likelihood for tips to bounce off the chute and onto the deck as they are ejected from the head. When a blockage in the chute occurs, disposed materials can no longer freely fall onto the waste container. Instead, disposed materials build up in the chute and eventually spill over onto the deck or the track of the robotic arm.

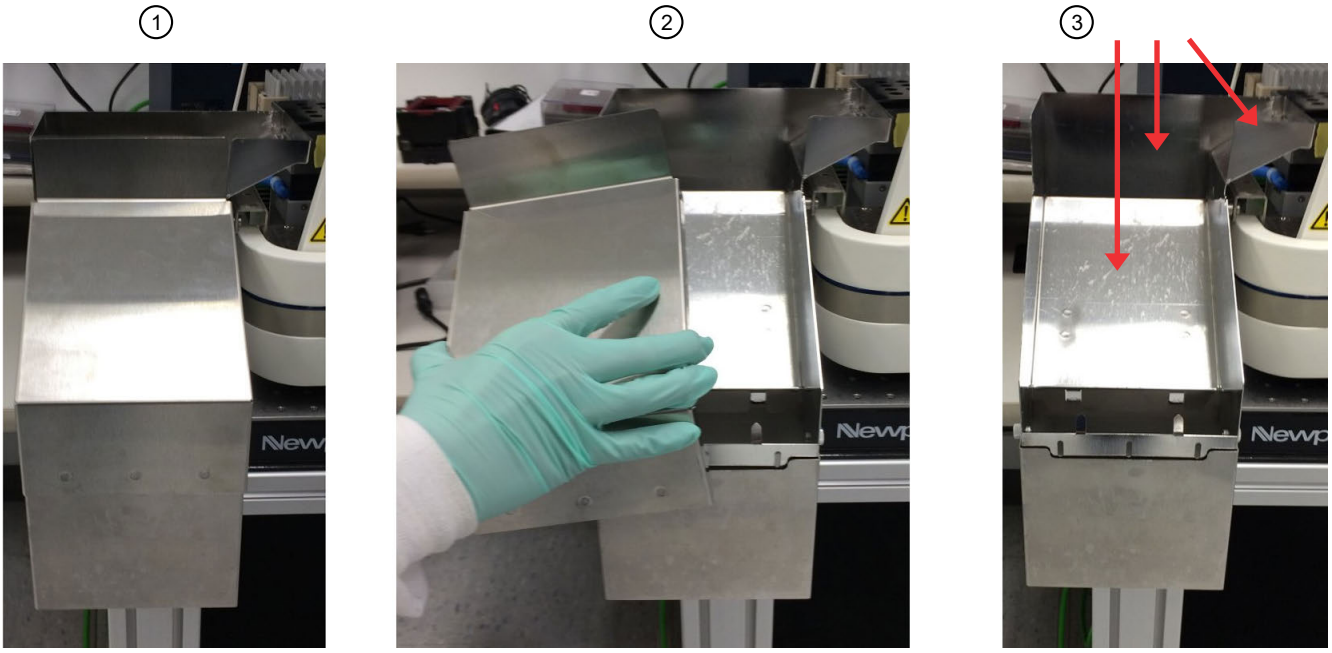
To avoid problems with items getting stuck in the trash chute, we recommend a light cleaning after each stage of the assay. A deep cleaning of the trash chute is recommended periodically to remove accumulated build-up on the trash chute that was not successfully removed with the recommended light cleaning procedure.

Perform a light cleaning

1. Remove the trash chute cover.
2. Clean the inner surface of the trash chute by wiping down the area with ultra-pure water and laboratory wipes. Follow this cleaning with a 70% ethanol wipe-down to disinfect the surface.

   Note: Do not use bleach to clean trash chute or trash chute cover.

3. Replace cover.



① Trash chute with cover.
② Remove cover.
③ Surfaces of the trash chute to clean.

Perform a deep cleaning of the trash chute

1. Remove the trash chute cover.
2. Remove the trash chute from the NIMBUS® Instrument by first sliding the chute towards the back of the instrument, then pulling it away from the machine. If needed, review detailed instructions. (See “Assemble the trash chute” on page 251.)

   Note: Do not use bleach to clean trash chute or trash chute cover.
3. In a warm bath of soapy DI water, clean the entire trash chute and trash chute cover removing any buildup present.
4. Rinse with DI water. Wipe down with 70% ethanol.
5. Dry the trash chute and trash chute cover.
6. Reconnect the trash chute to the NIMBUS® Instrument, as instructed in “Assemble the trash chute” on page 251.

Thermoshake device maintenance

The INHECO™ Thermoshake device must be maintained with periodic checking and refilling of cooling fluids to ensure proper function and avoid damages. See the INHECO™ user guide for detailed instructions.

Additional cooling fluid can be ordered from INHECO™ (Cat. No. 3800053).

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

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.
CAUTION! Hot surface.

(continued)

Symbol and description
 **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.

Location of safety labels

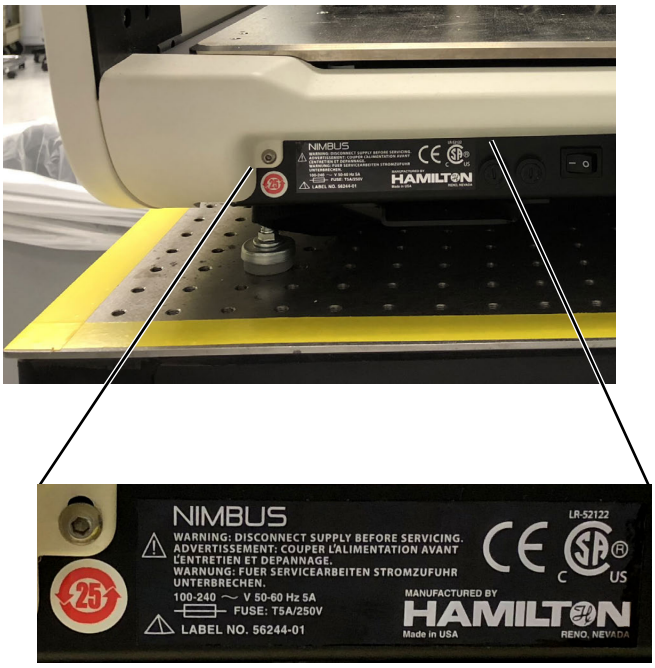
Label and location


Figure 64 Warning label on the right side of the unit to the left of the power switch.

(continued)

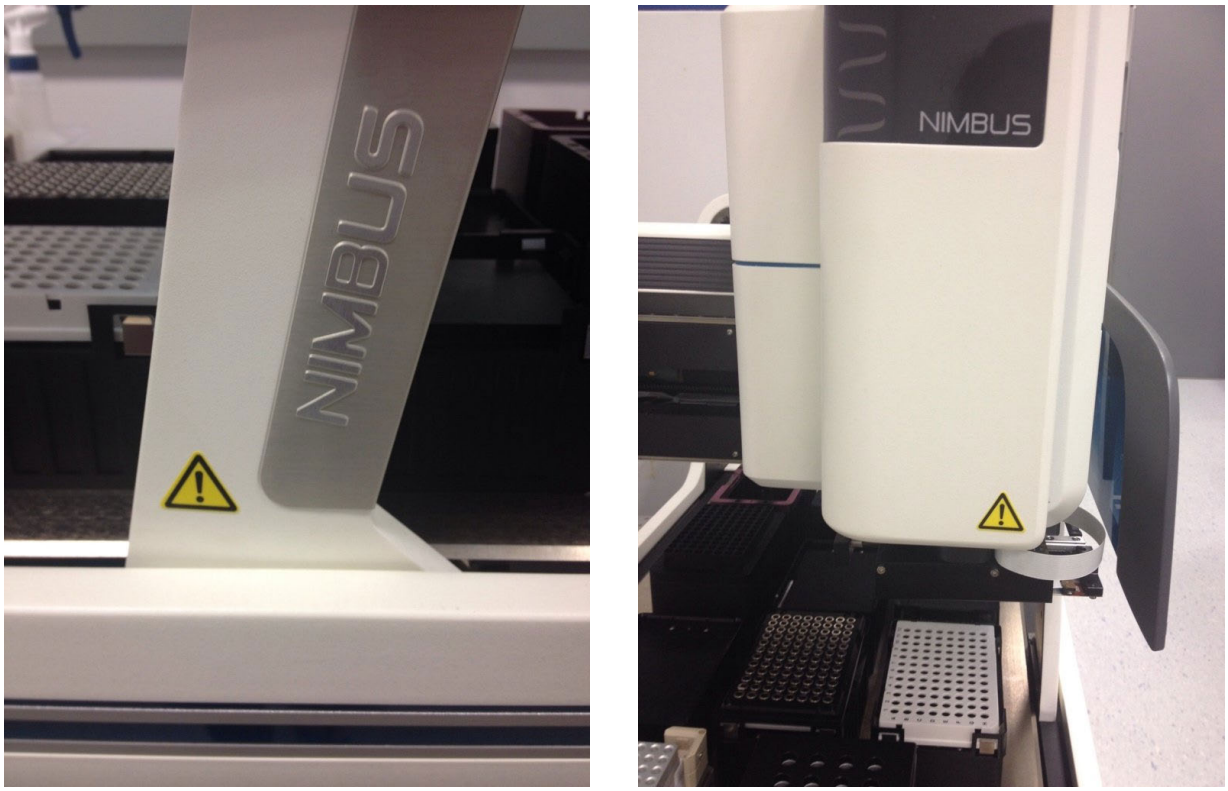
Label and location


Figure 65 Pinch points.

① Gantry arm
② NIMBUS® Instrument head

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Earth (ground) terminal
	Protective conductor terminal (main ground)

(continued)

Symbols and descriptions	
⎓	Direct current
~	Alternating current
≂	Both direct and alternating current

Conformity symbols

Conformity mark	Description
C SP US	Indicates conformity with safety requirements for Canada and U.S.A.
25	Indicates conformity with China RoHS requirements.
CE	Indicates conformity with European Union requirements.
	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.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Physical injury

CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

AVERTISSEMENT ! Installation des fusibles. Avant d'installer l'instrument, vérifier que les fusibles sont correctement insérés et que leur tension correspond à celle fournie par le circuit d'alimentation. Ne remplacer les fusibles que par des modèles du type et de la puissance spécifiés pour l'appareil. L'utilisation de fusibles inadaptés peut endommager le circuit électrique de l'instrument et provoquer un incendie.

WARNING! Voltage Selector Switch. Before installing the instrument, verify that the voltage selector switch is set for the supply voltage. This will prevent damage to the instrument, reduce risk of fire, and enable proper operation.

AVERTISSEMENT ! Sélecteur de tension. Avant d'installer l'instrument, vérifier que le sélecteur de tension est adapté à la tension d'alimentation. Cela permet d'éviter d'endommager l'instrument, de réduire le risque d'incendie et d'assurer son bon fonctionnement.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

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 and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures: • Reorient or relocate the receiving antenna. • Increase the separation between the equipment and receiver. • Connect the equipment into an outlet on a circuit different from that to which the receiver is connected. • Consult the dealer or an experienced radio/TV technician for help.

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	“China RoHS” Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical 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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

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
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Publication number	Description
Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow Site Preparation Guide—Applied Biosystems™ NIMBUS® Target Preparation Instrument	MAN0018332	Provides guidance on reagents, instruments, and supplies required to run the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow on the Applied Biosystems™ NIMBUS® Target Preparation Instrument.
Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow Quick Reference—Applied Biosystems™ NIMBUS® Target Preparation Instrument	MAN0018333	An abbreviated reference for the target preparation step of the Axiom™ 2.0 Plus Assay 96-Array Format Automated Workflow on the Applied Biosystems™ NIMBUS® Target Preparation Instrument. This quick reference document is for experienced users.
Axiom™ 2.0 gDNA Sample Preparation Quick Reference	MAN0017720	An abbreviated reference on preparing the genomic DNA sample.
Recommended Alternative Microarray Consumables Quick Reference	MAN0019853	A quick reference document identifying recommended alternative replacement consumables for use in microarray assays.
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.
GeneTitan™ Multi-Channel Instrument User Guide	MAN0027694	The GeneTitan™ Multi-Channel Instrument automates array processing from target hybridization to data generation by combining a hybridization oven, fluidics processing, and state-of-the art imaging device into a single benchtop instrument. This document details the use, care, and maintenance for the GeneTitan™ Multi-Channel Instrument.
GeneTitan™ Multi-Channel Instrument Site Preparation Guide	MAN0025571	Provides guidance on creating and maintaining the proper environment required for the GeneTitan™ Multi-Channel Instrument.

(continued)

Document	Publication number	Description
Analysis and software		
Axiom™ Genotyping Solution Data Analysis User Guide	MAN0018363	This guide provides information and instructions for analyzing Axiom™ genotyping array data. It includes the use of Axiom™ Analysis Suite, Applied Biosystems™ Analysis Power Tools (APT) and SNPolisher™ Package to perform quality control analysis (QC) for samples and plates, SNP filtering before downstream analysis, and advanced genotyping methods.
GeneChip™ Command Console™ User Guide	MAN0027771	This user guide provides instructions about using GeneChip™ Command Console™ (GCC) used to control GeneChip™ instrument systems. GeneChip™ Command Console™ software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip™ arrays.
Axiom™ Analysis Suite User Guide	MAN0027928	This user guide provides instructions about using Axiom™ Analysis Suite—a single-source software package to enable complete genotyping analysis of all Axiom™ arrays.
NIMBUS® Target Preparation Instrument documents		
Microlab NIMBUS® User Guide	62965-01	The Hamilton™ Company user guide for the NIMBUS® Instrument. This document is shipped within the deck hardware kit.

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