Axiom™ 2.0 Assay 384HT Array Format Automated Workflow

USER GUIDE

on the Biomek™ i7 Automated Workstation

for use with:
Axiom™ 384HT Array Plates
Axiom™ 2.0 384HT Reagent Kit
Beckman Coulter™ Biomek™ i7 Automated Workstation

Catalog Number 902245
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ThermoFisher SCIENTIFIC

Thermo Fisher Scientific Baltics UAB |
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Vilnius, Lithuania

Products manufactured at this site:

- Axiom™ 2.0 384HT Reagent Kit

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

Products manufactured at this site:

- Axiom™ myDesign™ Array Plate
- Axiom™ 384HT Array Plate

Revision history: MAN0017555 E00 (English)

Revision	Date	Description
E00	7 October 2024	• Remove references to Cat. No. 905424 and Cat. No. 952433 as these products have been discontinued. Deleted associated appendices, "Target preparation with the Biomek i7 Automated Workstation (method version 1.1, v2 kit" and "Target preparation with the Biomek i7 Automated Workstation (method version 1.0" • Added information for GeneTitan™ MC Fast Scan Instrument. • Added E-Gel™ Power Snap Plus Electrophoresis Device and iBright™ CL750 Imaging System.
D.0	20 April 2022	• Added new chapter, Chapter 4, "Target preparation with the Biomek™ i7 Automated Workstation (method version 1.1, AB kit)", for target preparation with the new Axiom™ 384HT Consumables Kit for Biomek™ i7-AB. • Moved "Target preparation with the Biomek i7 Automated Workstation (method version 1.1, v2 kit" to appendices. • Added information to explain the consumable kit and Biomek™ i7 method differences for target preparation.
C.0	29 January 2021	Updated to add chapter for the Biomek method version 1.1.
B.0	12 September 2018	Updated to reflect that Axiom Reference gDNA 103 has been removed from module 1 of the reagent kit and has been made available for purchase separately.
A.0	11 July 2018	New publication.

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

Contents

Product information

About the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow

The Axiom™ 2.0 Assay 384HT Array Format Automated Workflow is a workflow for high-throughput microarray genotyping. The workflow includes:

- Automated DNA target preparation on the Beckman Coulter™ Biomek™ i7 Automated Workstation: DNA amplification, fragmentation, purification, 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 imaging.
- 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 or Axiom™ Analysis Suite v2.0 or later.

Traceability of samples is enabled with the use of barcoded consumables.

The Axiom™ 2.0 384HT Reagent Kit provides all necessary reagents for target preparation in volumes that are optimized for processing on the Biomek™ i7.

About the Axiom™ Genotyping Solution

The Axiom™ 2.0 Assay 384HT 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 to genotype approximately 65,000 variants (of single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphisms (indels) from diploid species or 43,000 variants from polyploid species, with a processing throughput of greater than 3,000 samples per week.

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

- For human disease research applications, Thermo Fisher Scientific conducted an empirical screen of genomic content from dbSNP (**ncbi.nlm.nih.gov/projects/SNP/**). The screen included markers from HapMap and the 1,000 Genomes Project and other sources, using HapMap phase 3 samples and/or the original 270 HapMap samples. All this information has gone into creating a proprietary database of verified markers that can be interrogated using the Axiom™ 2.0 Assay.
- For agriculture applications, the Axiom™ Genotyping Solution can genotype samples using DNA extracted from leaves and seeds, playing an important role in genotype-trait association studies and marker-assisted selection in both plant and animal breeding programs.
- For molecular breeding programs, where turn-around time, accuracy, and ease-of-use are all important, the Axiom™ Genotyping Solution is ideal for high-throughput screening.

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

Overview of the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow on the Biomek™ i7 Automated Workstation

	Genomic DNA preparation	
Day 1	Chapter 2, Genomic DNA preparation	
	▼	
	Target preparation	
Day 1	Stage 1: Amplify the genomic DNA (Biomek™ i7)	23-hour of Amplification Plate at 37°C. Optional stopping point.
	▼	
Day 2	"Stage 2A: Fragment and precipitate the DNA" on page 66 (Biomek™ i7)	Overnight precipitation at –20°C.
	▼	
Day 3	Stage 2B: Centrifuge and dry pellets (Off-deck)	Optional stopping point.
	▼	
Day 3	Stage 3: Prepare the resuspension and hybridization reagent mixture (Biomek™ i7 and off-deck)	
	▼	
Day 3	Stage 4: Reassemble Hybridization Ready Plate and perform sample QC (Biomek™ i7)	Optional stopping point.
	▼	
Day 3	Stage 5: Denature sample and transfer to hybridization tray (Biomek™ i7 and off-deck)	23.5 to 24-hour array hybridization in the GeneTitan™ MC Instrument.
	▼	
Day 4	Stage 6: Prepare GeneTitan™ reagents (Biomek™ i7)	
	Array processing	
Day 5	Process array plates with the GeneTitan™ instrument (page 119) *Array processing is completed with the GeneTitan™ MC Instrument and GeneChip™ Command Console™ software v4.3 or later.*	Fluidics: 5 hours Scan: ~5.5 hours

Multiplate workflows

Thermo Fisher Scientific supports high-throughput workflows that allow you to run a set of samples and array plates through the protocol using a minimum number of personnel 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 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.

Contact your local support representative for more information.

GeneTitan™ reagent tray barcodes

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

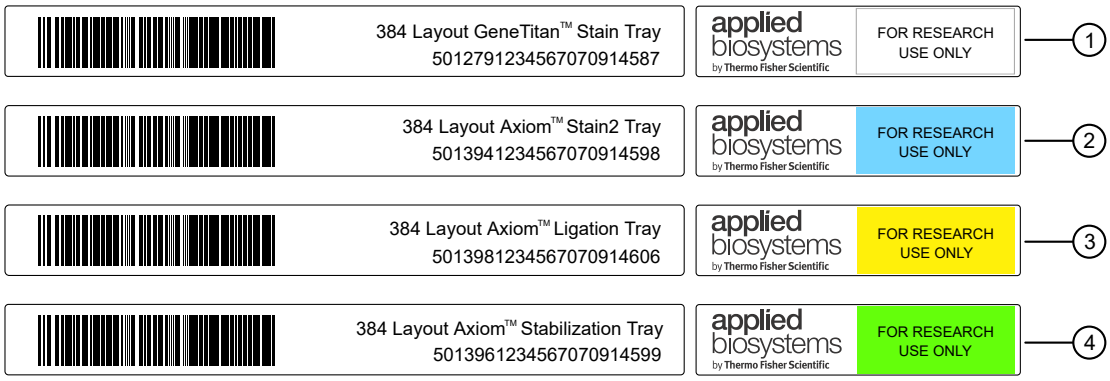


Figure 1 GeneTitan™ reagent tray barcodes and color-coded labels.

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

The unique barcodes along with the GeneChip™ Command Console™ v3 or later software prevents users from making errors when placing the trays in the GeneTitan™ MC Instrument during stage 3 of the array processing (“Stage 3—Ligate, wash, stain, and scan” on page 134).

It is critical to place the trays in the correct position on the Biomek™ i7 deck and then scan the labware barcodes with the handheld barcode scanner exactly as shown in the Biomek™ i7 deck setup window.

After the trays have been prepared on the Biomek™ i7 deck, ensure that the trays are placed in the appropriate drawer location in the GeneTitan™ MC Instrument. Failure to place the proper tray in the correct location results in an error and the GeneTitan™ MC Instrument will not proceed with the processing of the trays. See “Proper tray alignment and placement” on page 143 for detailed instruction.

GeneChip™ Command Console™ v4.3 or later also offers the facility for queuing a second plate for scanning before the first scan is complete. The software automatically moves the second plate into the scanner when the first plate has completed scanning. See “Queue a second plate for scanning” on page 133 for instructions.

2 Genomic DNA preparation

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

Sources of genomic DNA

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

Source	Sample type
Human	• Blood • Saliva • Cell line • WGA preamplified DNA: Genomic DNA amplified with the REPLI-g™ Kit (a whole–genome amplification kit, QIAGEN™, Cat. No. 150025) has been tested successfully with the Axiom™ 2.0 Assay. The REPLI-g™ Kit was used to amplify 20-ng genomic DNA, and the resulting yields quantitated by a PicoGreen™ assay. The amplified products (either 100 ng or 200 ng amplified DNA as required according to the Axiom™ array type) were used (without purification) as the input DNA sample in the subsequent steps in the Axiom™ 2.0 Assay. The stability of this amplified product to storage and repeated cycles of freeze/thaw has not been evaluated by Thermo Fisher Scientific.
Animal[1]	• Blood • Semen • Nasal swabs • Hair bulbs • Ear punch tissue

(continued)

Source	Sample type
Plant	• Seeds • Leaves

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

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

General requirements

- Starting DNA must be double-stranded for accurate concentration determination.
- DNA must be of high purity. DNA must be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (that is, EDTA). The gDNA extraction and purification method must create DNA that is salt-free because high concentrations of particular salts can also inhibit enzyme reactions. Assess DNA purity by measuring the OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios. The OD_{260}/OD_{280} ratio should be between 1.8 and 2.0 and the OD_{260}/OD_{230} ratio should be greater than 1.5. We recommend that DNA samples that do not meet these criteria be cleaned up as described in “Clean up genomic DNA” on page 17.
- DNA must not be degraded. The average size of gDNA can be evaluated on a 1% agarose gel using an appropriate size standard control. Approximately 90% of the DNA must be greater than 10 Kb in size. Control DNA can be run on the same gel for comparison.

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

Special requirements

Preamplification area

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

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

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

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

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

Equipment and reagents required

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

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

Guidelines for preparing the gDNA Sample Plate for gel analysis

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

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

Run a 48-lane 1% agarose E-Gel™

1. Insert the E-Gel™ *48* Agarose Gels, 1% into the electrophoresis unit.
2. Remove 2 combs.
3. Load 20 µL of gDNA samples onto the E-Gel™ *48* Agarose Gels, 1%.
4. If needed, load 15 µL of diluted E-Gel™ 96 High Range DNA Marker (1:3 dilution or ~0.34X from stock) into all marker wells.
5. Fill all empty wells with water.
6. Run the gel for ~27 minutes.

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

E-Gel™ results

The following figure shows gel images of intact gDNA (that is appropriate for use in the Axiom™ 2.0 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 Assay before starting any large-scale genotyping projects.

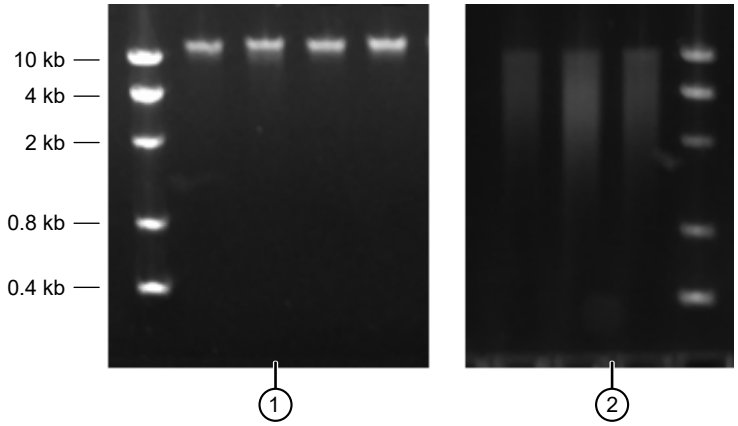


Figure 2 Gel images with intact gDNA and degraded gDNA.

① Intact samples ② Degraded samples

Genomic DNA extraction and purification methods

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

Clean up genomic DNA

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

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

Genomic DNA preparation

This step must be done before proceeding with the DNA amplification stages for automated target preparation.

The genomic DNA (gDNA) you process using the Axiom™ 2.0 Assay must meet the general requirements that are listed earlier in this chapter. The amount of gDNA depends on which Axiom™ array is used in the downstream protocol. “Genomic DNA input requirements” on page 18 details the sample input requirements for Axiom™ 2.0 Assay 384HT Array Format Automated Workflow.

Genomic DNA input requirements

Sample type	Volume per well	Input mass per well	gDNA concentration
Human	8.7 µL	100 ng	11.5 ng/µL
Diploid plants and animals	8.7 µL	150 ng	17.2 ng/µL
Polyploid plants and animals	8.7 µL	200 ng	23 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	384 deepwell plate • Axygen™ 384-well clear V-bottom 240 µL polypropylene deepwell nontreated plate, sterile (Cat. No. P-384-240SQ-C-S). **Note:** The 384 deepwell plate is used to store the gDNA samples and are processed in the target preparation method using the Biomek™ i7. To avoid error, see Chapter 4, "Target preparation with the Biomek™ i7 Automated Workstation (method version 1.1, AB kit)" for the correct deepwell plate to use. ⚠ **CAUTION!** Using the wrong 384 deepwell plate type will result in physical interference into the Thermoshake adapter and prevent the run from proceeding.
1	Plate centrifuge
1	Plate spectrophotometer Required only if no OD measurements are available for samples.
1	Vortexer

Reagents

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

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

Thaw samples and control

Thaw the following components to room temperature.

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

To thaw, either:

- Place items on the bench top for 60 minutes.

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

Quantitate and dilute gDNA

1. Gently vortex (50% maximum), then centrifuge the gDNA and control DNA.
2. *Recommendation*: Quantitate each sample (for example, using the Quant-iT™ PicoGreen™ dsDNA Assay Kit).
3. Using reduced EDTA TE buffer, dilute each sample to a concentration of:
   - 11.5 ng/µL for human DNA samples
   - 17.2 ng/µL for diploid plant and animal DNA samples
   - 23 ng/µL for polyploid plant and animal DNA samples
4. Seal, vortex, then centrifuge.

Aliquot the diluted samples and the control

The samples and control are placed in a 384 deepwell 250-µL plate for target preparation.

1. Aliquot 8.7 µL of each diluted gDNA sample to the deepwell plate (this amount is the equivalent of 100–200 ng of gDNA, as required by the sample type).
2. Aliquot 8.7 µL of the control DNA to the deepwell plate.

   Note: We recommend including at least 1 positive control on each plate.
3. Seal, then centrifuge.

Freeze or proceed

Do one of the following:

- Store the gDNA Sample Plate at –20°C.
- Proceed to DNA amplification for automated target preparation (see Chapter 4, “Target preparation with the Biomek™ i7 Automated Workstation (method version 1.1, AB kit)”).

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

GeneTitan™ Array Plate Registration file

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

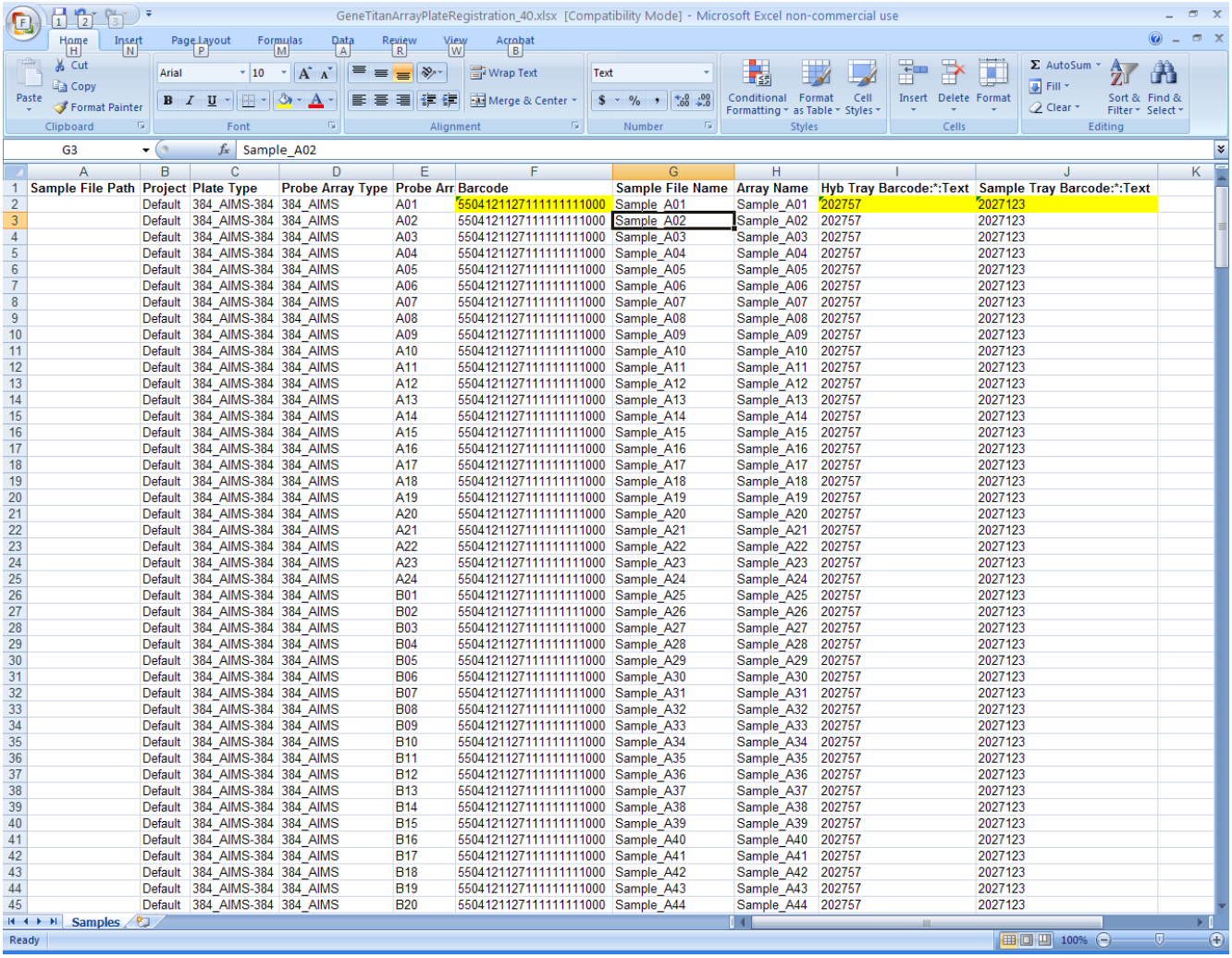
Create and save a GeneTitan™ Array Plate Registration file

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

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

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

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



GeneTitanArrayPlateRegistration_40.xlsx [Compatibility Mode] - Microsoft Excel non-commercial use

G3: Sample_A02

	A	B	C	D	E	F	G	H	I	J
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Arr	Barcode	Sample File Name	Array Name	Hyb Tray Barcode:*:Text	Sample Tray Barcode:*:Text
2		Default	384_AIMS-384	384_AIMS	A01	55041211271111111111000	Sample_A01	Sample_A01	202757	2027123
3		Default	384_AIMS-384	384_AIMS	A02	55041211271111111111000	Sample_A02	Sample_A02	202757	2027123
4		Default	384_AIMS-384	384_AIMS	A03	55041211271111111111000	Sample_A03	Sample_A03	202757	2027123
5		Default	384_AIMS-384	384_AIMS	A04	55041211271111111111000	Sample_A04	Sample_A04	202757	2027123
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10		Default	384_AIMS-384	384_AIMS	A09	55041211271111111111000	Sample_A09	Sample_A09	202757	2027123
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25		Default	384_AIMS-384	384_AIMS	A24	55041211271111111111000	Sample_A24	Sample_A24	202757	2027123
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39		Default	384_AIMS-384	384_AIMS	B14	55041211271111111111000	Sample_A38	Sample_A38	202757	2027123
40		Default	384_AIMS-384	384_AIMS	B15	55041211271111111111000	Sample_A39	Sample_A39	202757	2027123
41		Default	384_AIMS-384	384_AIMS	B16	55041211271111111111000	Sample_A40	Sample_A40	202757	2027123
42		Default	384_AIMS-384	384_AIMS	B17	55041211271111111111000	Sample_A41	Sample_A41	202757	2027123
43		Default	384_AIMS-384	384_AIMS	B18	55041211271111111111000	Sample_A42	Sample_A42	202757	2027123
44		Default	384_AIMS-384	384_AIMS	B19	55041211271111111111000	Sample_A43	Sample_A43	202757	2027123
45		Default	384_AIMS-384	384_AIMS	B20	55041211271111111111000	Sample_A44	Sample_A44	202757	2027123

Samples

Ready

100%

Figure 3 Example of a GeneTitan™ Array Plate Registration file for Axiom™ 384HT Array Plate.

3 Set up the Biomek™ i7 Automated Workstation

This chapter contains information describing the procedures, equipment, and materials required for running the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow. To help ensure operator safety and assay performance, operators must be familiar with this content before starting target preparation described in Chapter 4, "Target preparation with the Biomek™ i7 Automated Workstation (method version 1.1, AB kit)". Additional information for the Biomek™ i7 Automated Workstation is in the user documents from Beckman Coulter™. See Appendix H, "Documentation and support" for details.

Equipment required

Applied Biosystems™ equipment required

✓	Item	Cat. No.
☐	GeneTitan™ Multi-Channel Instrument[1]	Contact Thermo Fisher Scientific
☐	Digital Scanner/Barcode Reader (to enable barcode tracking)	74-0016
	External Barcode Reader Holder (to enable barcode tracking)	00-0093

[1] For a complete list of all equipment and supplies required for GeneTitan™ Multi-Channel Instrument installation and operation, consult the *GeneTitan™ Multi-Channel Instrument Site Preparation Guide* (Pub. No. MAN0025571)

Oven requirements

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

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

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

✓	Item	Source
☐	Thermo Scientific™ Heratherm™ Advanced Protocol Microbiological Incubator, capacity 66 L • 120V, 60 Hz • 230V, 50/60 Hz	• 51028066 • 51028133
☐	BINDER™ ED 56 Drying and Heating Chamber • ED056UL-120V Voltage: 120 V 1~60 Hz • ED056-230V Voltage: 230 V 1~50/60 Hz	• BINDER™ 9010-0334 • BINDER™ 9010-0333
☐	BINDER™ BD 56 Standard-Incubator with natural convection • BD056UL-120V Voltage: 120 V 1~60 Hz • BD056-230V Voltage: 230 V 1~50/60 Hz	• BINDER™ 9010-0324 • BINDER™ 9010-0323
☐	Optional—for low throughput of 3 or fewer array plates per week: • GeneChip™ Hybridization Oven 645[1]	• 00-0331

[1] The GeneChip™ Hybridization Oven 640 is currently not supported with the Axiom™ 2.0 Assay. However, to use it in the workflow contact your field service engineer (FSE) or Thermo Fisher Scientific Technical Support regarding the compatibility of this oven with the Axiom™ 2.0 Assay.

Spectrophotometer

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

Quantity	Item	Source
1	Multiskan™ Sky Microplate Spectrophotometer	51119600

Thermal cycler

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

We have verified the performance of this assay using the following off-deck thermal cyclers in their 384-well block configurations:

- ProFlex™ 2 × 384-well PCR System (Cat. No. 4484077)
- GeneAmp™ PCR System 9700 with dual 384-well sample block module (gold, silver, or aluminum)

The performance of this assay has not been verified with other thermal cyclers. Use of other thermal cyclers can result in assay failure and may violate the Axiom™ Array and Reagent Replacement policy.

Shakers

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

We recommend using one of the following shakers.

Item	Source
Thermo Scientific™ Digital Microplate Shaker	88882005 or 88882006
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific™ 88880023 or 88880024
Jitterbug™	Boekel Scientific™: 130000 (115V), 130000-2 (230V)

Plate centrifuge

One plate centrifuge is required for the Axiom™ 2.0 Assay. The plate centrifuges listed in Table 1 are recommended for the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow. When centrifuging and drying pellets, the centrifuge must be able to centrifuge plates at:

- rcf: 3,200 × *g* with an appropriate rotor/bucket combination (4,000 rpm for the Eppendorf™ Centrifuge 5810 R that is described in Table 1)
- Temperature: 4°C

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

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

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

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

Table 1 Plate centrifuge recommendations for the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow.

✓	Item	Source
☐	Thermo Scientific™ Sorvall™ X4R Pro-MD Centrifuge (refrigerated), with:	• 75009520(240 V, 50 Hz) • 75009521 (120 V) • 75009620 (220 V, 60 Hz)
	☐ TX-1000 Swinging Bucket Rotor Body	75003017 (rotor)
	• ☐ Adapter for TX-1000 Swinging Bucket Rotor • ☐ Buckets for TX-1000 Rotor	• 75007303 (adapter, pack of 4) • 75003001 (buckets, set of 4)
	Eppendorf™ Centrifuge 5810 R, with: ☐ Rotor A-4-81, with 4 MTP/Flex buckets	022625551 (230 V, 50–60 Hz) 022625501 (120 V, 50–60 Hz, 15 A) 022625101 (120 V, 50–60 Hz, 20 A) 022638807 (rotor)

Other equipment 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	Supplier	Cat. No.
Common laboratory equipment			
☐	Freezer, –20°C	**MLS**	—
☐	Refrigerator, 2°C to 8°C	**MLS**	—
☐	Vortexer	**MLS**	—
☐	Mini microcentrifuge, for 2 mL tubes.	**MLS**	—
☐	Bel-Art Cryo-Safe Mini Cooler, –15°C. Equivalent items from other manufacturers are acceptable.	**MLS**	—
☐	Ice bucket, 4 to 9 liters	**MLS**	—

Required materials

This section lists the labware, reagents, and consumables required for target preparation.

- "Images of labware used on the Biomek™ i7 Automated Workstation deck" on page 29
- "Axiom™ 2.0 384HT Reagent Kit" on page 36
- "Consumables required for target preparation" on page 37
- "Pipette tips" on page 38
- "Axiom™ 384HT GeneTitan™ High Volume Consumables Kit" on page 39

Images of labware used on the Biomek™ i7 Automated Workstation 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.

Table 2 Labware images and ordering information.

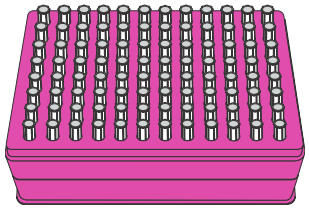
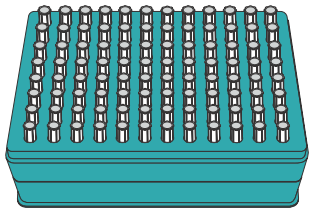
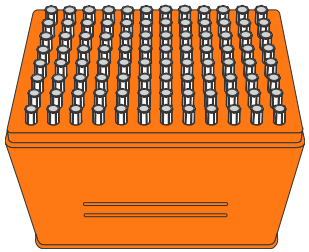
Labware	Source	Image	Name in the Biomek™ software method v1.1[1]
Pipette tips, i-Series 50 µL filter tips (pink)	Beckman Coulter™, B85888		**BC50F**
Pipette tips, i-Series 190 µL filter tips (green)	Beckman Coulter™, B85911		**BC190F**
Pipette tips, i-Series 1,025 µL filter tips (orange)	Beckman Coulter™, B85955		**BC1025F**

Table 2 Labware images and ordering information. *(continued)*

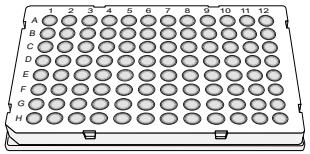
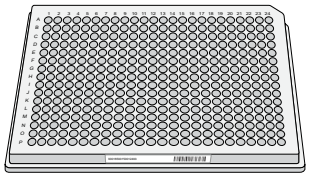
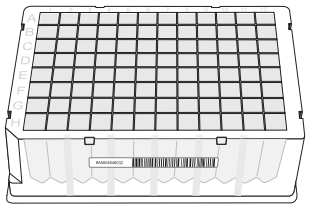
Labware	Source	Image	Name in the Biomek™ software method v1.1[1]
96 PCR plate	Available as a component of the Axiom™ 384HT Consumables Kit for Biomek™ i7-AB, Cat. No. 952472 Order separately: Blue plate: Bio-Rad™, HSP9631 White plate: Bio-Rad™ HSP9601 **Note:** The Bio-Rad™Hard-Shell™ 96-Well PCR Plate, low profile, full skirted is available in several different colors. Any color plate can be substituted and used successfully in this assay.		**BioRad_96_Hardshell**
384 PCR plate	Available as a component of the Axiom™ 384HT Consumables Kit for Biomek™ i7-AB, Cat. No. 952472 Order separately[2]: , AB0937 or 952435 (10-pack)		**AbGene384PCR**
96 deepwell 2-mL plate	Available as a component of the Axiom™ 384HT Consumables Kit for Biomek™ i7-AB, Cat. No. 952472 Order separately[2]: AB0932		**AbGene_96DeepSquare**

Table 2 Labware images and ordering information. *(continued)*

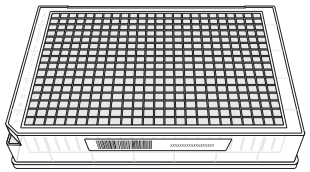
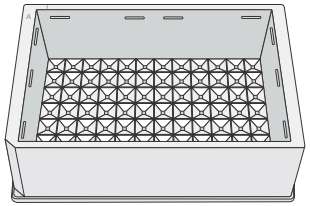
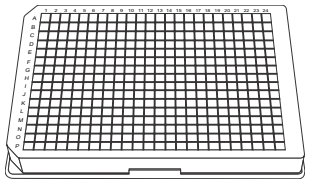
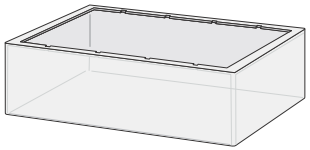
Labware	Source	Image	Name in the Biomek™ software method v1.1[1]
384 deepwell 250-µL plate	Available as a component of the Axiom™ 384HT Consumables Kit for Biomek™ i7-AB, Cat. No. 952472 Order separately[2]: Fisher Scientific™, AB1178 Barcoded plates in the consumables kit are used for the sample plate and non-barcoded plates in the consumables kit are used everywhere else.		**Axygen384ConePPDeep**
Deep 96 full reservoir	Available as a component of the Axiom™ 384HT Consumables Kit for Biomek™ i7-AB, Cat. No. 952472 Order separately: Thomas Scientific™, 1149J12		**Deep96FullReservoir_EK**
384 UV plate	Available as a component of the Axiom™ 384HT Consumables Kit for QC Cat. No. 902289 Order separately: Thomas Scientific™, 1184Q88		**Greiner384UV**
Frame for Modular Reservoirs	Beckman Coulter™, 372795 Fisher Scientific™, NC9337243		**ModularReservoir**

Table 2 Labware images and ordering information. *(continued)*

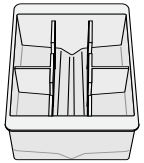
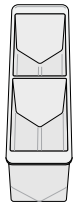
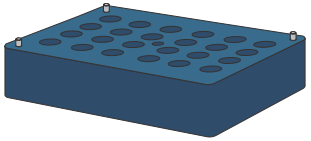
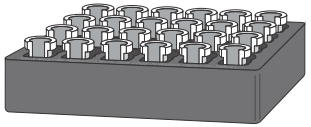
Labware	Source	Image	Name in the Biomek™ software method v1.1[1]
Half Reservoir, 75 mL	Beckman Coulter™, 372786 Fisher Scientific™, NC9226465		N/A
Quarter Reservoir, 40 mL	Beckman Coulter™, 372790 Fisher Scientific™, NC9168864		N/A
Quarter Reservoir, Divided by Width, 19 mL per section	Beckman Coulter™, 372792 Fisher Scientific™, NC9209505		N/A
Reagent block	Tube block adapter for peltier, 24 positions Beckman Coulter™, A83054		**BCTubeBlock_2mlTubes**
24-Position Tube Rack	Beckman Coulter™, 373661 Insert, 11 mm, for 1.5-mL Microfuge Tubes, 373696 At least two inserts in positions A1 and B1 are required. Additional inserts can be present.		**RT_Tube_Rack**

Table 2 Labware images and ordering information. *(continued)*

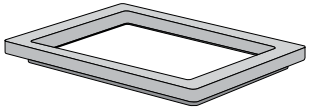
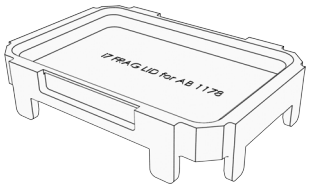
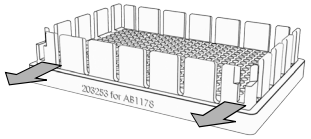
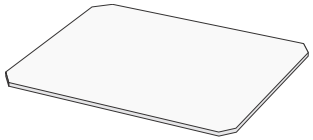
Labware	Source	Image	Name in the Biomek™ software method v1.1[1]
384-Well PCR Plate Collar	Available as a component of the Axiom™ 384HT Starter Kit for Biomek™ i7-AB, 952469 Order separately: 952400		N/A
Plastic plate lid	Available as a component of the Axiom™ 384HT Starter Kit for Biomek™ i7-AB, 952469 or 952471		**PlasticPlateLid**
384 deep-well adapter	Available as a component of the Axiom™ 384HT Starter Kit for Biomek™ i7-AB, 952469 or 952471		**SPelt_384_DeepWell_Adapter**
Microseal™ 'P' Reusable Replacement PCR Plate Sealing Pads	Bio-Rad™, MSP1003 Replace pad according to the manufacturer's recommendations.		N/A

Table 2 Labware images and ordering information. *(continued)*

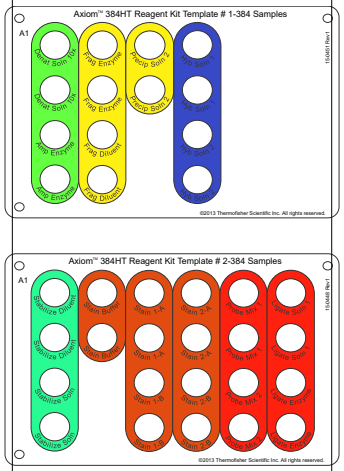
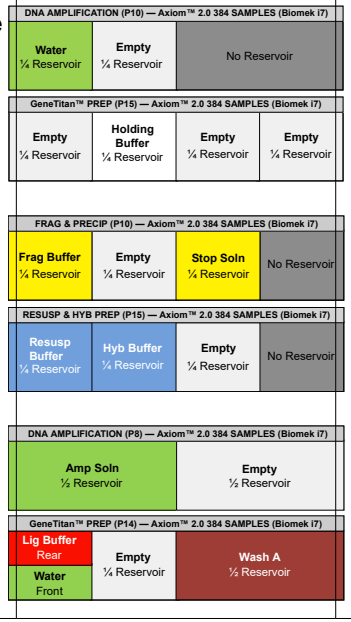
Labware	Source	Image	Name in the Biomek™ software method v1.1[1]
Reagent block templates	Axiom™ 2.0 384HT Reagent Kit Templates for reagent block Thermo Fisher Scientific, Part No. 15-0448, Part No. 15-0451 Available as a component of the Axiom™ 384HT Starter Kit for Biomek™ i7-AB, 952469		N/A

Table 2 Labware images and ordering information. *(continued)*

Labware	Source	Image	Name in the Biomek™ software method v1.1[1]
Reservoir frame stickers	Available as a component of the Axiom™ 384HT Starter Kit for Biomek™ i7-AB, 952469		N/A

[1] The names of the labware in this column may appear different than those in the “Source” column; however, it does not affect the functionality of the methods. Use the consumables and accessories described in the “Source” column to run method v1.1.

[2] When ordered separately, this item does not have a barcode. A custom barcode is required and must be applied before running the method.

Axiom™ 2.0 384HT Reagent Kit

Each Axiom™ 2.0 384HT Reagent Kit, Cat. No. 902245, is sufficient for one Axiom™ 384HT Array Plate.

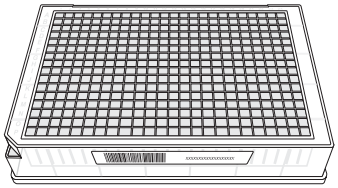
Note: Axiom™ 2.0 384HT Reagent Kit only states "Axiom™ 2.0" on Module 1.

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-B	Axiom™ Water	
Axiom™ Stain 1-A	Axiom™ Hold Buffer	

Consumables required for target preparation

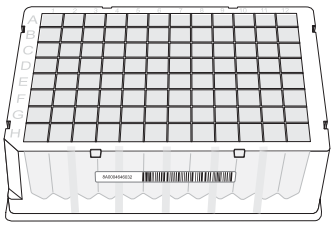
IMPORTANT! The 384 deepwell plate used in the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow on the Biomek™ i7, method v1.1, is the Abgene™ AB1178 deepwell plate.

Axiom™ 384HT Consumables Kit for Biomek™ i7-AB
384 deepwell 250-µL plate (Abgene™ AB1178 deepwell plate)



IMPORTANT! The 96 deepwell plate used in the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow on the Biomek™ i7, method v1.1, is the Abgene™ deepwell plate.

Axiom™ 384HT Consumables Kit for Biomek™ i7-AB
96 deepwell 2-mL plate (Abgene™ deepwell plate)



CAUTION! Using the wrong type of 96 deepwell plate results in liquid transfer error such as insufficient volume being transferred or dispense tip bottoming out in the wells. Such liquid transfer errors cause instrument interruption resulting in potential assay failure.

Table 3 Consumables required for target preparation on the Biomek™ i7 Automated Workstation, Biomek™ method 1.1.

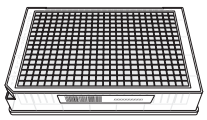
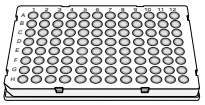
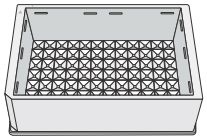
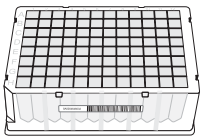
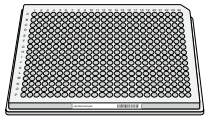
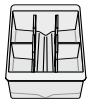
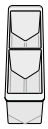
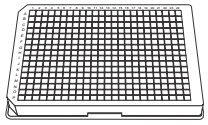
Contents	Image	per run with QC	per run without QC
Available in the Axiom™ 384HT Consumables Kit for Biomek™ i7-AB (Cat. No. 952472)[1]			
384 deepwell 250-µL plate (With barcode. This is the gDNA-containing sample plate.)		1	1
384 deepwell 250-µL plate (Without barcode.)		5	3
96 PCR plate		2	2
Deep 96 full reservoir		7	5
96 deepwell 2-mL plate		5	5

Table 3 Consumables required for target preparation on the Biomek i7 Automated Workstation, Biomek method 1.1. *(continued)*

Contents	Image	per run with QC	per run without QC
384 PCR plate		1	1
Additional consumables required for target preparation[2]			
Half Reservoir		3	3
Quarter Reservoir		13	13
Quarter Reservoir, Divided by Width		1	1
384 UV plate		1	0

[1] Contains sufficient quantities to process five 384-reaction runs.
[2] See Table 2 for ordering information.

Pipette tips

The following table provides the pipette tip usage for one full run of the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow on the Biomek™ i7 workstation.

Step	i-Series 50 µL filter tips (pink), BC50F	i-Series 190 µL filter tips (green), BC190F	i-Series 1,025 µL filter tips (orange), BC1025F
DNA amplification	—	480 tips	35 tips
Fragmentation and precipitation	—	488 tips	26 tips
Resuspension and hybridization preparation	—	384 tips	21 tips
Plate reassembly and QC	384 tips	96 tips	—
Plate reassembly (without QC)	*384 tips*	—	—
Transfer samples to hybridization tray	384 tips	—	—

(continued)

Step	i-Series 50 µL filter tips (pink), BC50F	i-Series 190 µL filter tips (green), BC190F	i-Series 1,025 µL filter tips (orange), BC1025F
GeneTitan™ reagent preparation	—	170 tips	74 tips
Totals: with QC option	**768 tips**	**1,618 tips**	**156 tips**
without QC option	**768 tips**	**1,522 tips**	**156 tips**

Axiom™ 384HT GeneTitan™ High Volume Consumables Kit

Each Axiom™ 384HT GeneTitan™ High Volume Consumables Kit is sufficient to process 5 . These trays are required for processing Axiom™ 384HT 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.

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

Table 4 Axiom™ 384HT GeneTitan™ High Volume Consumables Kit (Cat. No. 902629).

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

[1] See Table 5 for detailed descriptions of each component.

Table 5 Axiom™ GeneTitan™ tray consumables (from the Axiom™ 384HT GeneTitan™ High Volume Consumables Kit (Cat. No. 902629).

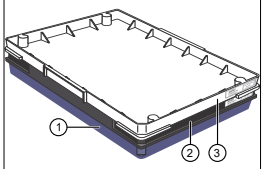
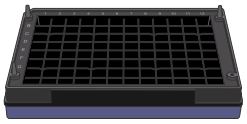
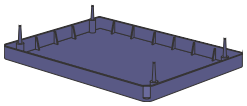
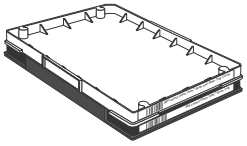
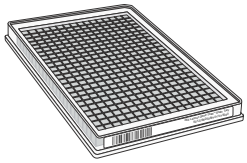
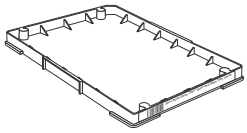
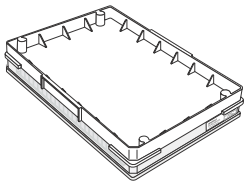
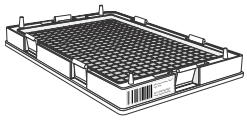
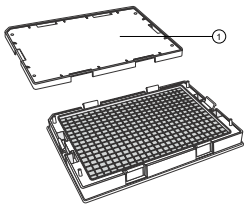
Item	Part No.	Image	Details
384-Layout GeneTitan™ Scan Tray[1]		 ① Scan tray protective base ② 384-Layout GeneTitan™ Scan Tray ③ Barcoded scan tray cover	The Axiom™ scan tray package includes the following: • The GeneTitan™ scan tray includes a scan tray cover. The tray cover should be used to cover the scan tray before placing the tray in the GeneTitan™ MC Instrument. • The scan tray must be protected at all times from damage or exposure to dust. The scan tray must be in the blue plate cover at all times except when loaded into the GeneTitan™ MC Instrument. • The blue scan tray protective base in the package is used to protect the bottom of the scan tray glass from damage. Remove the protective base from the scan tray before loading the scan tray with the scan tray cover in the GeneTitan™ MC Instrument.
384-Layout GeneTitan™ Scan Tray on blue base			This combination of the GeneTitan™ scan tray on the protective blue base is to be placed on the Biomek™ i7 deck during the GeneTitan™ reagent preparation method.
Blue scan tray protective base	202096		The blue scan tray protective base in the package is used to protect the bottom of the scan tray glass from damage. The blue scan tray protective base is distinct from the blue array plate protective base and must not be used with the array plate. Remove the protective base from the scan tray before loading in the GeneTitan™ MC Instrument
384-Layout GeneTitan™ Scan Tray with cover	Scan tray 501280 Cover 501315		The GeneTitan™ scan tray must be loaded with the scan tray cover into the GeneTitan™ MC Instrument. **Do not** load the scan tray with the protective base.

Table 5 Axiom GeneTitan tray consumables (from the Axiom 384HT GeneTitan High Volume Consumables Kit (Cat. No. 902629). *(continued)*

Item	Part No.	Image	Details
384 Layout GeneTitan™ Stain Trays	501279 - Stain 1 501394 - Stain 2 501398 - Ligation 501396 - Stabilization	  ① Stain 1 tray ② Stain 2 tray ③ Ligation tray ④ Stabilization tray	The stain trays are packaged in zip-top bags to keep them free of dust. Each stain tray is uniquely barcoded. **IMPORTANT!** Each stain tray is labeled with a name and an individual barcode. Ensure that you always use the appropriate tray with the correct reagent on the Biomek™ i7 deck. Failure to do so can result in the wrong stain in the wrong location on the GeneTitan™ MC Instrument and assay failure. When transferring the trays to the GeneTitan™ MC Instrument, ensure that the trays are placed in the proper location in the drawer. Failure to do so results in an error and the GeneTitan™ MC Instrument will not proceed with processing trays.
384-Layout GeneTitan™ Scan and Stain Tray Cover	501315		The 384-Layout GeneTitan™ Scan and Stain Tray Covers are provided to prevent evaporation of the GeneTitan™ reagents in stain trays and the array holding buffer in the scan tray. The GeneTitan™ scan and stain tray covers are barcoded.
Stain tray cover, shown on top of the stain tray	Cover 501315		The stain trays must be placed in the GeneTitan™ MC Instrument with the stain tray cover.
384-Layout GeneTitan™ Hybridization Tray	501278	  ① Hybridization tray cover to be discarded.	The 384-Layout GeneTitan™ Hybridization Trays are packaged in white pouches with the label "384 Layout GeneTitan™ Hyb Tray" ref# 501278 (pouch)/902278 (box) The hybridization trays are packaged with a protective cover that should be discarded before use. 384 hybridization tray cover, Part No. 203006

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

Guidelines for use

Biomek™ i7 light curtain

For your safety, the Biomek™ i7 is equipped with a light curtain that covers the front of the instrument and sides that are protected by plexiglass. The light curtain senses when an object, such as a hand or an arm, enters the space surrounding the deck. Any penetration from outside the deck breaks the light curtain and all movement on the workstation halts. Activity is resumed when the operator either clicks **OK** to continue the activity that was taking place, or aborts the activity by clicking **Stop** on the toolbar. Incubation timers are not interrupted unless the method is aborted.

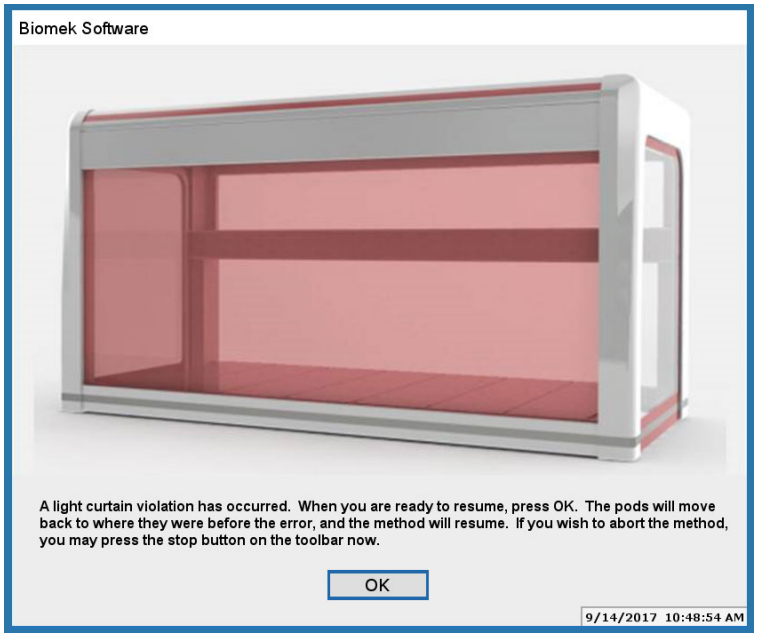


Figure 4 A message window is displayed when the light curtain has been penetrated.

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 help 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 5.
  - Vortex PCR plates for 2 seconds in each of the 5 sectors.

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

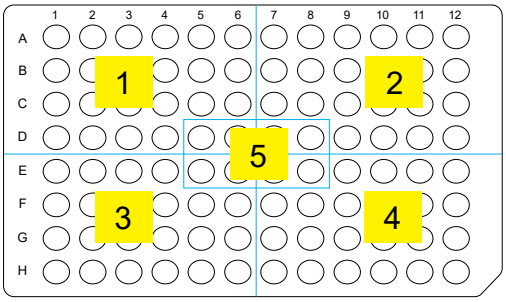


Figure 5 The 5 plate sectors.

- **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 Biomek™ i7

Observe the following guidelines when running the assay on the Biomek™ i7.

- When barcode tracking preference is enabled, scan the barcode label on labware after it has been placed onto the instrument deck position to prevent error.
- Position all plates and the chilled reagent 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.
- Remove caps from the reagent tubes and press tubes into the 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.
- Always discard the used multichannel pipette tips.
- Always store the reagent block at 4°C.

Set up the instrument for the first time

Thermoshake plate adapter installation

The Thermoshake 384 deepwell plate adapter that is used with the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow method is designed specifically to fit the 384 deepwell 250-µL plate. Proper installation is critical to ensuring even heating of the samples and proper liquid transfer in the fragmentation step.

The Thermoshake 384 Deepwell Plate Adapter is a component of the Axiom™ 384HT Starter Kit for Biomek™ i7-AB (Cat. No. 952469) or the Axiom™ 384HT Upgrade Kit for Biomek™ i7-AB (Cat. No. 952471).

Install the Thermoshake plate adapter

1. Ensure that the 2 notched corners are oriented facing the front of the Thermoshake 384 Deepwell Plate Adapter.

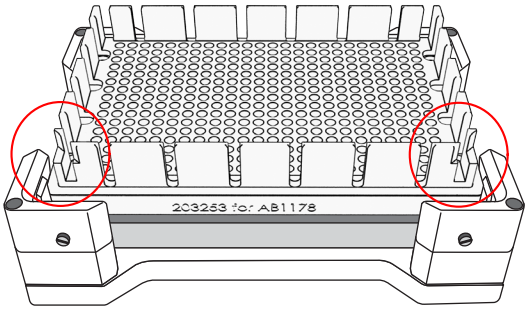


Figure 6 Thermoshake 384 Deepwell Plate Adapter orientation on the Biomek™ i7 deck.

2. Press down firmly to ensure that adapter is evenly seated on the shaker and that the bottom surface of the Thermoshake adapter is flush on the top surface of the shaker.

Place the reservoir labels on the reservoir frames

The reservoir labels are stick-on labels for the modular reagent reservoir frames for the different stages of the automated workflow. These labels are color-coded to match the colors that are on the caps of the reagent tubes in the Axiom™ 2.0 384HT Reagent Kit. Using these labels helps ensure the proper placement of reservoirs and reagents for each step.

There are three reservoir holders that are used in the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow method. Each of the reservoir holders require templates on two sides for a total of six templates.

Remove the protective surface from the back of the label and place on the reservoir frames as directed in the following figures.

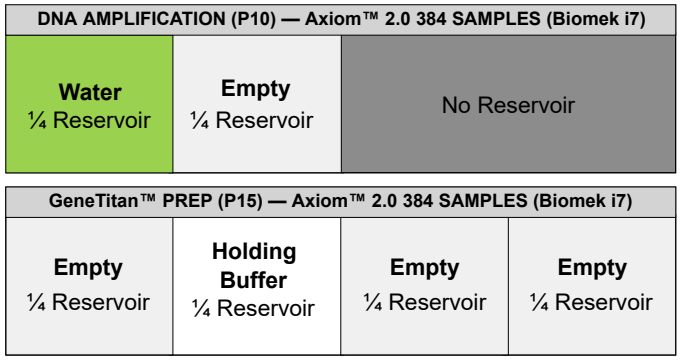


Figure 7 Reservoir frame 1, two sides.

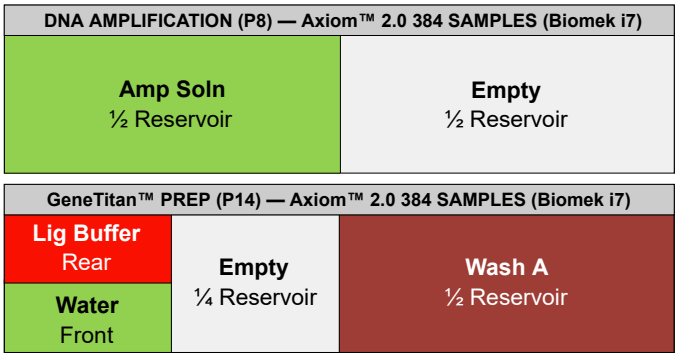


Figure 8 Reservoir frame 2, two sides.

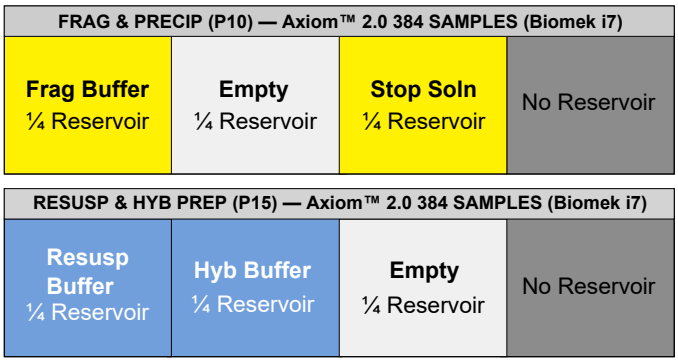
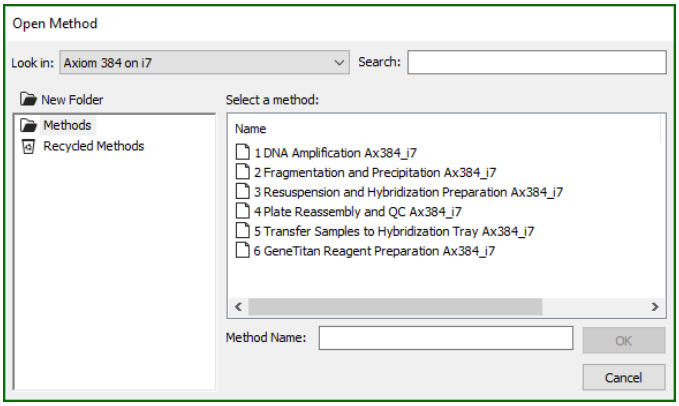


Figure 9 Reservoir frame 3, two sides.

Set the method preferences

IMPORTANT! The method preferences must be selected before starting the method. These settings are not prompted for at runtime. Typically, the Thermo Fisher Scientific field application scientist (FAS) sets the method preferences during method installation.

1. Open the Biomek™ Software using one of the following methods:
   - Double-click the Biomek 5 icon on the desktop.
   - In the Windows Search box, enter **Biomek 5**.
2. Click **File** ▸ **Open**.
3. For **Look in** of the **Open Method** window, select **Axiom 384 on i7**.



4. Click to select a method, then click **OK**.

5. In the Method View panel, click the line that reads **Click Here to Select Method Preferences** (Figure 10).

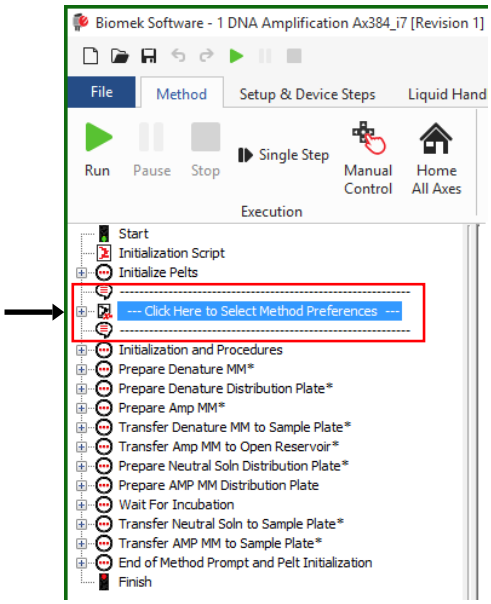
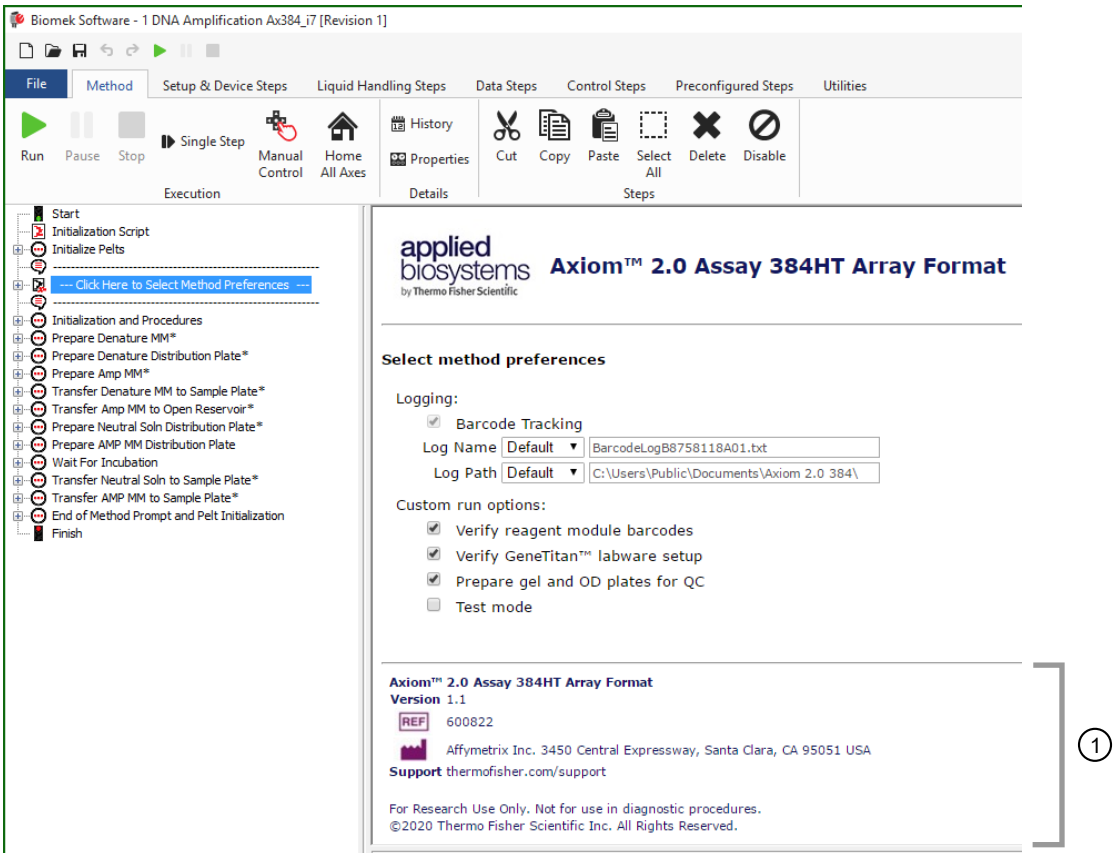


Figure 10 Click to select method preferences.

6. Click to select the method preferences.



① Method v1.1 information.

Logging options.

- **Barcode Tracking**: This feature is enabled by default. It is highly recommended to use the barcode tracking feature to support accuracy in processing the samples. Consult with a FAS if it is necessary to disable the barcode tracking feature. When selected the barcode tracking feature performs the following actions:
  - Prompts you to enter the barcode information on sample plates, Axiom™ reagent modules, and GeneTitan™ consumables.
  - Records barcode information into a log file.
  - Helps ensure that the correct amplification time for the samples has elapsed (minimum of 22 hours) before starting the fragmentation and precipitation step.
  - Helps ensure that the splitting and reassembly of samples are correctly associated to the starting sample plate.

Note: If using custom barcodes for plates, only use alpha-numeric, hyphen, or underscore characters.

Note: The barcode tracking feature is intended to track samples that go through the entire target preparation process on one instrument.

- **Log Name**: The log name is the name of the barcode log file. The default name is BarcodeLog[InstSN].txt, where "InstSN" is the serial number of the instrument. Naming of the log file is possible by selecting **Other** in the dropdown list for the setting. If using a custom log name, ensure that the name ends with ".txt" otherwise the file type will not be associated correctly. After entering a custom name, click the **Apply** to the right of the log name field.
- **Log Path**: The log path is the barcode log file location. The log path is: `C:\Users\Public\Documents\Axiom 2.0 384\`

Custom run options.

- **Verify Reagent Module barcodes**: When selected this feature helps ensure that the correct Axiom™ reagent is used in the specified target preparation step.
- **Verify GeneTitan Labware Setup**: When selected, this feature helps ensure that the GeneTitan™ reagents that are prepared on the Biomek™ i7 are correctly associated with the GeneTitan™ plate type.
- **Prepare gel and OD plates for QC**: the workstation prepares two plates of resuspended samples that are properly diluted for the fragmentation gel QC and OD quantification process control checks.

  See Appendix B, “Fragmentation quality control gel protocol” and Appendix C, “Sample quantification after resuspension” for instructions and result assessment guidelines.

  IMPORTANT! For troubleshooting and support purposes, we strongly recommend that you perform the gel QC and OD quantification process controls after resuspension.
- **Test Mode**: Select this option to skip all the incubation timers in a step. If selected, a prompt is displayed asking you to ensure that you want to run a step in test mode. Use this option to perform runs using deionized water only, not actual reagents or samples.

CAUTION! Never process samples in test mode. Processing samples in test mode results in assay failure and loss of samples and reagents.

Requirements for plate barcode tracking

To use the barcode tracking feature in the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow method, each plate in the assay that contains sample must be labeled with a scannable barcode. User-generated barcode labels can be of any length, but must only use alpha-numeric, hyphen, or underscore characters. The following is a list of plates requiring barcodes.

IMPORTANT! Using the wrong deepwell plate–Thermoshake adapter combination will result in physical interference on the Biomek™ i7 deck.

Plate type	Labware type (method version 1.0)	Labware type (method version 1.1)
gDNA samples	384 deepwell 250-µL plate: Abgene™ 384-Well 250 µL Polypropylene Storage Plate (Cat. No. AB1178), To avoid error, see Chapter 4, "Target preparation with the Biomek™ i7 Automated Workstation (method version 1.1, AB kit)" for the correct deepwell plate to use.	
Precipitation, Q1	96 deepwell 2,000-µL plate	96 deepwell 2-mL plate
Precipitation, Q2	96 deepwell 2,000-µL plate	96 deepwell 2-mL plate
Precipitation, Q3	96 deepwell 2,000-µL plate	96 deepwell 2-mL plate
Precipitation, Q4	96 deepwell 2,000-µL plate	96 deepwell 2-mL plate
HybRxn	384 PCR plate	
Five 384-layout stain trays	• 2 x 384-Layout GeneTitan™ Stain Tray (Stain 1) • 384-Layout Axiom™ Stain 2 Tray • 384-Layout Axiom™ Ligation Tray • 384-Layout Axiom™ Stabilization Tray	
GeneTitan™ hybridization tray	384-Layout GeneTitan™ Hybridization Tray	
GeneTitan™ scan tray	384-Layout GeneTitan™ Scan Tray	

Sample barcode tracking

The Axiom™ 2.0 Assay 384HT Array Format Automated Workflow method enables sample plate and reagent module tracking by using barcodes. The barcode tracking option is accessed in the Method Preferences section of the target preparation step of the method.

Barcode entry

Barcodes are easily entered into the method by scanning with a hand-held barcode scanner during deck setup. Each deck setup prompt has editable fields over each labware location requiring barcode entry. To identify a barcode field, find any labware position with an input field that is highlighted in yellow. If either the **Barcode Tracking** or **Verify GeneTitan labware setup** option is enabled, fields that

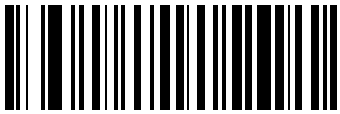
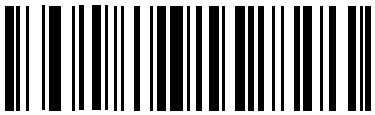
are designated as LW BARCODE are used with plates containing samples and with stain trays. Fields for reagents are indicated by the module number as MODULE or MOD plus the number of the module.

IMPORTANT! To prevent errors, scan the barcodes *after* the labware has been placed on the proper deck position.

The TAB key can be used to jump between input fields when entering barcodes manually.

If using the Zebra™ DS9808 Digital Scanner, we recommend configuring the barcode scanner to speed up barcode entry during deck setup by sending the TAB key automatically after a barcode is scanned. To configure the scanner, sequentially scan the configuration barcodes that are shown in Table 6.

Table 6 Zebra™ DS9808 Digital Scanner configuration barcodes.

Step	Command	Barcode
1	Begin new rule	
2	Send all data that remains	
3	Send tab key	
4	Save rule	

Barcode entries are added to a single text file found at the following location: `c:\Users\Public\Documents\Axiom 2.0 384`. The method automatically generates a log file and is named **BarcodeLog[InstrumentNumber].txt**. To view, open the log file in any text editor. Each method step that is recorded has a start and complete status entry. Log files can be changed to a more readable format.

Note: For troubleshooting information on barcode verification errors, see “Runtime errors” on page 172.

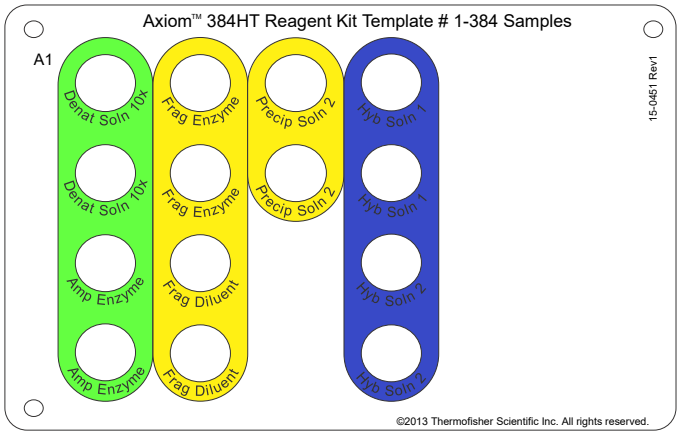
View log files in Microsoft™ Excel™

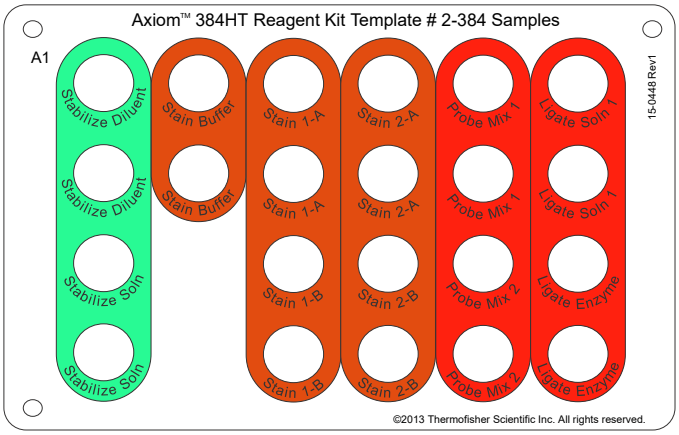
1. Open the log file in Microsoft™ Excel™, then highlight column A.
2. Select **Data ▸ Text to Column**.
3. In the **Convert Text to Columns Wizard** select **Delimited**, then click **Next**.
4. Select **Comma**, then click **Next**.
5. Click **Finish**.

Set up the Biomek™ i7 prerun

Assemble the reagent block templates with the reagent block

The Axiom™ 384HT Reagent Kit Templates fit precisely onto the top of the chilled reagent block, and are held in place by the metal posts on the block. Using these templates helps ensure the proper placement of reagent tubes onto the block for each method. One template is used for the DNA amplification, fragmentation, precipitation, and resuspension and hybridization preparation steps. The second template is used for the GeneTitan™ reagent preparation step. The Axiom™ 384HT Reagent Kit Templates are available as a component of the Axiom™ 384HT Starter Kit for Biomek™ i7-AB (Cat. No. 952469).





Assemble the plastic plate lid with the Microseal™ 'P' Pad plate seal

The plastic plate lid is used on the Fragmentation Plate and must be assembled with a reusable Microseal™ 'P' Pad plate seal before use. Proper placement of the pad helps ensure even coverage when placed on the Fragmentation Plate. To assemble, remove the protective backing on the pad and place on the center of the underside of the plate lid. Press down across to enable even adhesion of the pad to the plate lid.

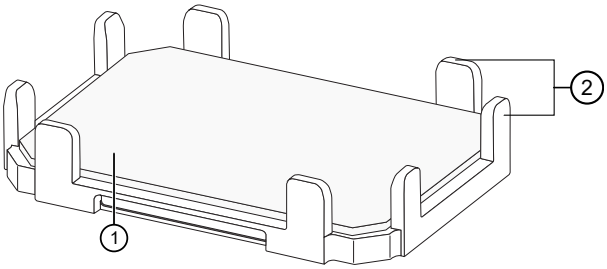


Figure 11 Plastic plate lid with the Microseal™ 'P' Pad plate seal.

① Microseal™ 'P' Pad
② Fence of the plastic plate lid facing up

Perform the prerun checklist

The following actions are required before the first operation of the Biomek™ i7 Automated Workstation 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 Biomek™ workstation.

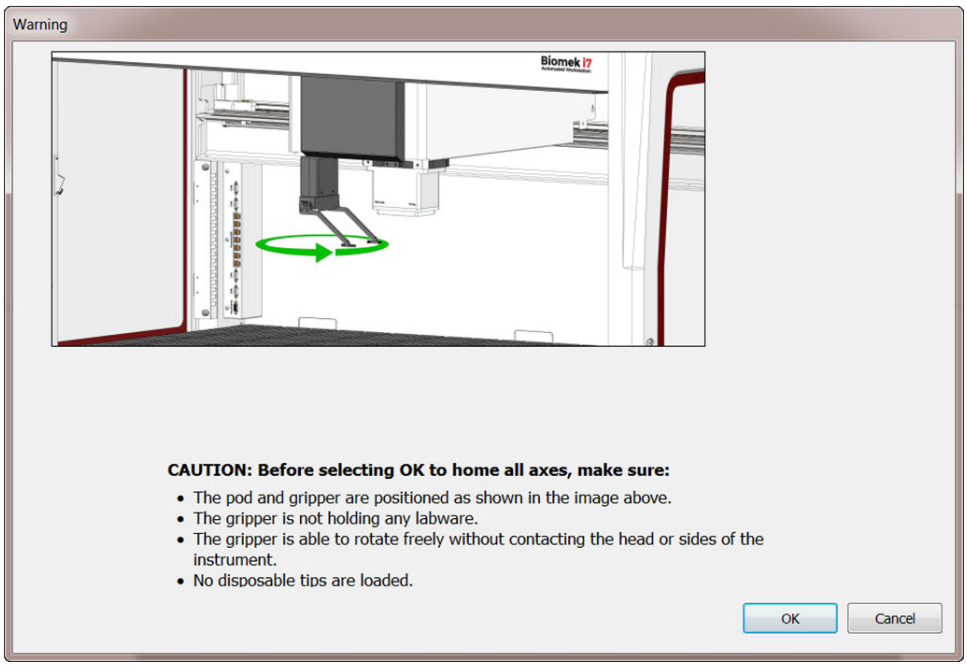
Power on the Biomek™ i7 Automated Workstation

1. Power on the workstation using the switch that is on the right side of the instrument.
2. If desired, power on the LED deck light by pressing the round button on the right side of the Biomek™ i7.

3. Ensure that all the peripherals are powered on.
   - INHECO Peltier Controllers—Power on from the power strip that is in the middle of the four Peltier controllers.
   - Computer and monitor.
4. Open the Biomek Software using one of the following methods:
   - Double-click the Biomek icon on the desktop.
   - In the Windows Search box, enter **Biomek 5**.

Home all axes

1. In the Biomek™ Software window, click **Method** ▸ **Home All Axes**.



2. Ensure that all conditions in the warning message are met, then click **OK**.
   The Biomek™ i7 homes the pod, gripper, and head. When complete, the status light remains blue indicating the workstation is ready for use.

4 Target preparation with the Biomek™ i7 Automated Workstation (method version 1.1, AB kit)

This chapter is for users running the Beckman Coulter™ Biomek™ i7 software on a Windows™ 10 operating system.

Note: This chapter provides target preparation instructions when running the Axiom™ 2.0 384HT Array Format Biomek™ method version 1.1 with the Axiom™ 384HT Consumables Kit for Biomek™ i7-AB, Cat. No. 952472.

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

CAUTION! Using the wrong 384 deepwell plate type will result in physical interference into the Thermoshake adapter and prevent the run from proceeding.

CAUTION! Using the wrong type of 96 deepwell plate results in liquid transfer error such as insufficient volume being transferred or dispense tip bottoming out in the wells. Such liquid transfer errors cause instrument interruption resulting in potential assay failure.

Stage 1: Amplify the genomic DNA

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

Time required

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

Activity	Time
Hands-on time[1]	15 minutes
Biomek™ i7	37 minutes

[1] Hands-on time is the estimated time required by the user for handling samples and reagents and setting up the i7 deck and it does not include sample and reagent thawing time.

Input and output plates

Plate	Description	Volume	Deck position
Input plate	384 deepwell 250-µL plate (gDNA Sample Plate) AB1178	8.7 µL	Peltier1
Output plate	384 deepwell 250-µL plate (Amplification Plate) AB1178	174.1 µL	Peltier1

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
As required	Adhesive seals for plates
As required	Laboratory tissues
1	Mini microcentrifuge (microcentrifuge with microtube rotor)
1	Oven (must maintain a constant temperature of 37°C for at least 24 hours with a temperature accuracy of ±1°C) • >3 array plates per week—we recommend using the BINDER™ ED 56 or Heratherm™ Advanced Protocol Microbiological Incubator, capacity 66 L • 3 array plates per week—okay to use the GeneChip™ Hybridization Oven 645, the BINDER™ ED 56, or Heratherm™ Advanced Protocol Microbiological Incubator, capacity 66 L
1	Vortexer
1	Plate centrifuge (set to room temperature)

(continued)

Quantity	Item
Biomek™ i7 Automated Workstation labware	
• 5 boxes • 1 box	Pipette tips • i-Series 190 µL filter tips (green) • i-Series 1,025 µL filter tips (orange)
2	Deep 96 full reservoir
1	96 PCR plate
2	384 deepwell 250-µL plate (AB1178)
2	Half Reservoir
2	Quarter Reservoir
1	Reagent block, chilled to 4°C
Sample Plate	One plate of genomic DNA samples prepared in Chapter 2, “Genomic DNA preparation” in 384 deepwell 250-µL plate (AB1178)

Reagent and sample plate handling

Thaw and prepare reagents and sample plate according to the following table.

Module	Quantity	Reagent and cap color	Place at room temperature	Deck loading instructions
Reagents from the Axiom™ 2.0 384HT Reagent Kit				
Module 1 –20°C	2	Axiom™ 2.0 Denat Soln 10X	✓	Vortex and centrifuge. Place in a chilled reagent block.
	2	Axiom™ 2.0 Neutral Soln	✓Thaw in room temperature water bath.	Vortex for 30 seconds. Pour in reservoir.
	2	Axiom™ 2.0 Amp Soln	✓Thaw in room temperature water bath.	Vortex for 30 seconds. Pour in reservoir.
	1	Axiom™ Water	✓Thaw in room temperature water bath.	Vortex for 30 seconds. Pour in reservoir.
	2	Axiom™ 2.0 Amp Enzyme	⊗Do not thaw. Keep at –20°C until ready to use.	*Immediately before use:* Gently flick tube 3 times, then centrifuge. Place in the chilled reagent block.
Note: Estimated reagent thawing time is 1 hour.				
Sample Plate				
Thaw the Sample Plate at room temperature, then centrifuge. **Note:** Do not place a frozen Sample Plate directly on the Biomek™ i7 workstation deck.				

Perform the pre-run checklist

The Biomek actions in the pre-run checklist are the same as described under “Perform the prerun checklist” on page 53. See this section for details. Some or all these steps are required depending on the current state of the Biomek™ i7 Automated Workstation.

1. Preheat the oven to 37°C.
2. Power on the Biomek™ i7 and all peripherals.
3. Check the waste container, then empty as required.
4. Open the Biomek™ Software using one of the following methods:
   - Double-click the Biomek™ icon on the desktop.
   - In the Windows Search box, enter **Biomek 5**.
5. Home all axes.

Run the DNA amplification step

1. Click **File** ▸ **Open**.
2. For **Look in** of the **Open Method** window, select **Axiom 384 on i7**.
3. Select **1 DNA Amplification Ax384_i7**, then click **OK**.
4. At the top of the **Biomek Software** window, click ▶ **Run** to start the DNA amplification step. The deck layout for the DNA amplification step is displayed.

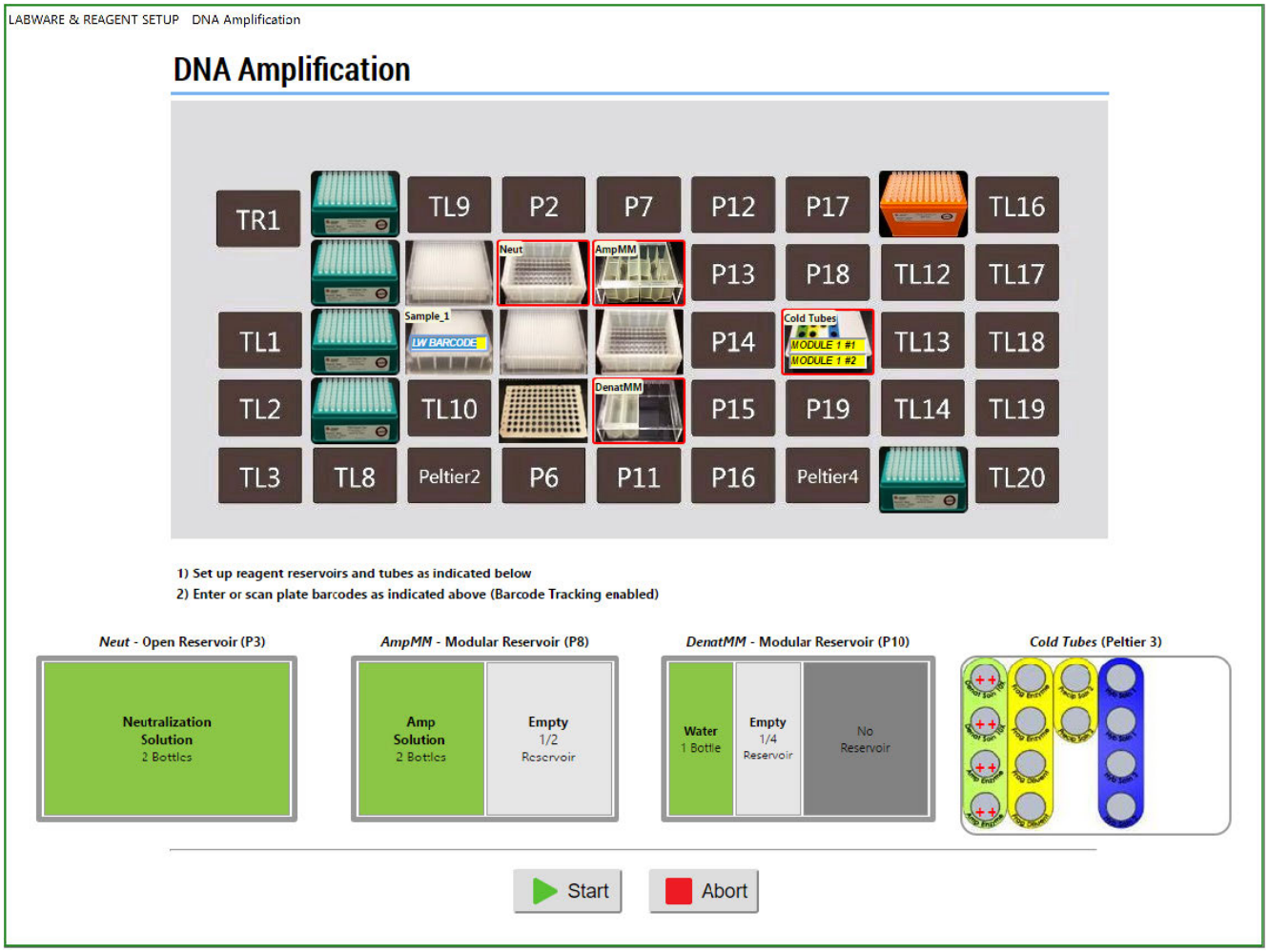


Figure 12 DNA amplification step deck layout.

5. Place the labware and reagents on the deck as directed in the following figures and table:
   - Figure 12—Deck layout.
   - Figure 13—Deck positions on the Biomek™ i7.
   - Table 7—Table detailing the labware, reagent, and modular reservoir placement for the deck layout.

- Figure 14—Reagent block.

Figure 13 Deck positions on the Biomek™ i7 Automated Workstation.

Table 7 Labware and reagent locations on the deck for the DNA amplification method.

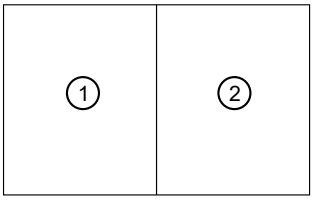
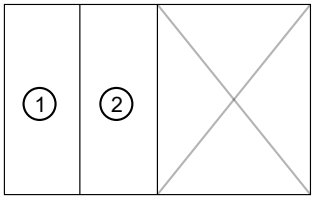
Deck position	Labware	Scan[1]	Reagent or sample
TL4	i-Series 190 µL filter tips (green)		
TL5	i-Series 190 µL filter tips (green)		
TL6	i-Series 190 µL filter tips (green)		
TL7	i-Series 190 µL filter tips (green)		
P1	384 deepwell 250-µL plate		
Peltier1	Abgene™ 384-Well 250 µL Polypropylene Storage Plate (AB1178) over the 384 deep-well adapter		gDNA Sample Plate
P3	Deep 96 full reservoir		
P4	384 deepwell 250-µL plate (AB1178)		
P5	96 PCR plate		
P8	Reservoirs in frame: • Half Reservoir (1) • Half Reservoir (2)		 ① Pour 2 bottles Axiom™ 2.0 Amp Soln into reservoir 1. ② Leave reservoir 2 empty.
P9	Deep 96 full reservoir		

Table 7 Labware and reagent locations on the deck for the DNA amplification method. *(continued)*

Deck position	Labware	Scan[1]	Reagent or sample
P10	Reservoirs in frame: • Quarter Reservoir (1) • Quarter Reservoir (2)		 ① Pour 1 bottle Axiom™ Water into reservoir 1. ② Leave reservoir 2 empty.
Peltier3	Reagent block, chilled to 4°C		See Figure 14.
TL11	i-Series 1,025 µL filter tips (orange)		
TL15	i-Series 190 µL filter tips (green)		

[1] Scan labware or reagent module barcode. Do not scan the image in this table.

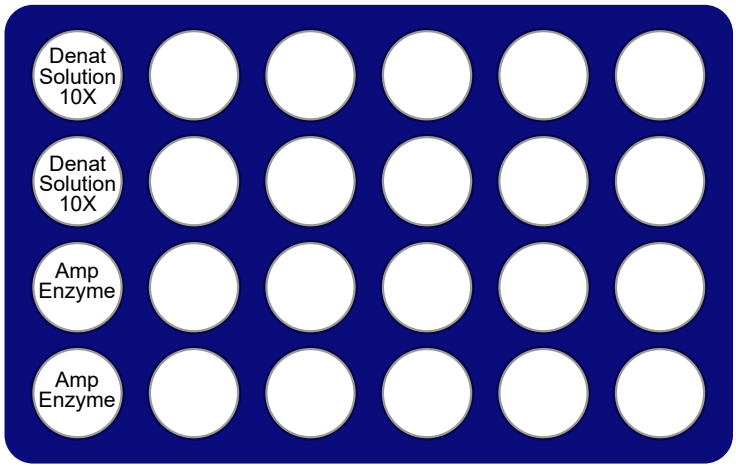
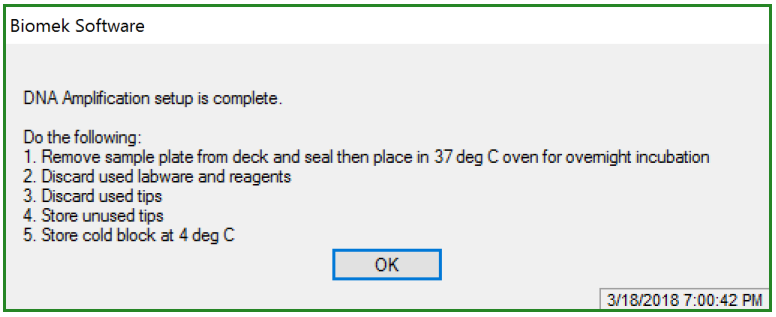


Figure 14 Placement of reagents on chilled reagent block for DNA amplification step.

IMPORTANT! Flick and centrifuge the Axiom™ 2.0 Amp Enzyme before placing in the chilled reagent block.

6. Check the deck layout to ensure that all labware and reagents are in the proper locations.
7. Scan the labware and reagent barcodes.
8. Click **Start**.
   The DNA amplification step on the Biomek™ i7 Automated Workstation starts. When complete, a message window appears.



9. Complete the following:
   a. Remove the Sample Plate from the Peltier1 deck position.
   b. Blot the top of the Sample Plate with a laboratory tissue to remove any droplets that are present.
   c. Tightly seal the Sample Plate.
   d. Centrifuge the Sample Plate at 1,000 rpm for 30 seconds.
   e. Place the Sample Plate in a preheated oven, then incubate at 37°C for 23 ±1 hour.

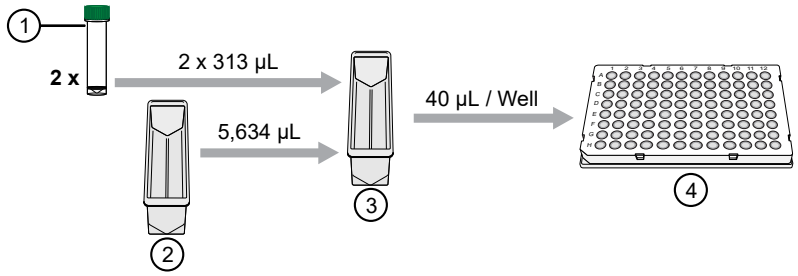
      Note: If using a GeneChip™ Hybridization Oven 645, place the plate on the bottom of the oven. Do not rotate the plates.

   f. Store the unused BC1025F tips from deck position TL11.
   g. Discard the tubes from the cold block at the Peltier3 deck position. Store the cold block at 4°C.
   h. Discard the remaining labware and tips on the deck. Store the reservoir frames. Click **OK** when done.

10. After 22 to 24 hours of incubation, do one of the following:
    - Proceed directly to "Stage 2A: Fragment and precipitate the DNA" on page 66.
    - Tightly seal and store the amplified samples at –20°C.

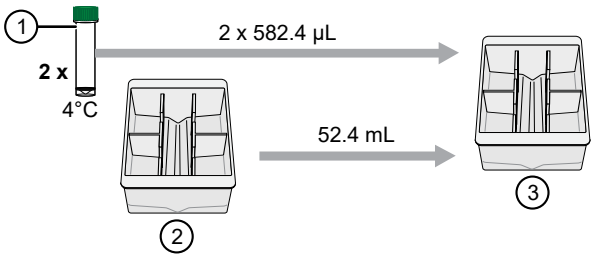
Workflow for Stage 1: Amplification

Prepare Denaturation Master Mix and Distribution Plate



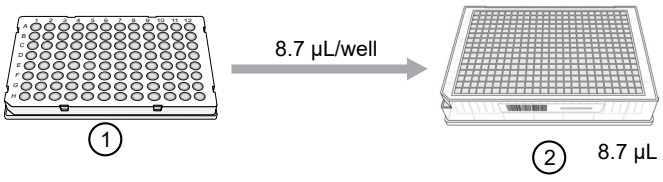
① Axiom™ 2.0 Denat Soln 10X (Peltier 3)
② Axiom™ Water (P10)
③ Denat MM (P10)
④ DenatDist-1 (P5)

Prepare Amplification Master Mix



① Axiom™ 2.0 Amp Enzyme (Peltier 3)
② Axiom™ 2.0 Amp Soln (P8)
③ Amp MM (P8)

Transfer Denaturation Master Mix to Sample Plate



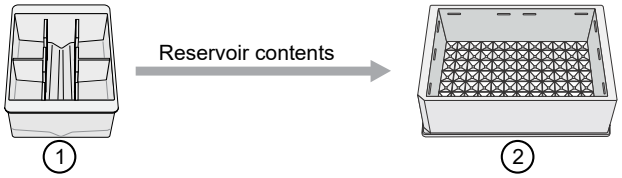
① DenatDist-1 (P5)
② Sample Plate (Peltier 1)

Incubate Sample Plate

Incubate Sample Plate on deck for 10 minutes at 22°C.

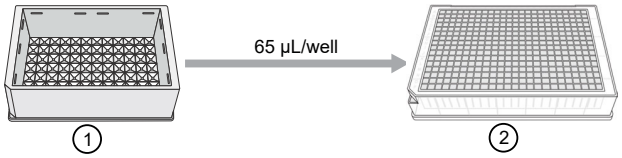
These steps occur during the 10 minute incubation.

Transfer Amplification Master Mix to open reservoir



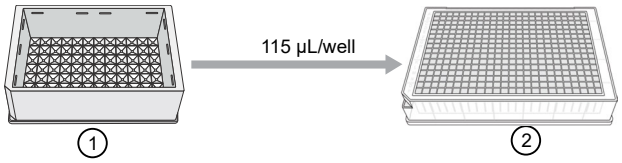
① Amp MM (P8)
② AmpMM-Full (P9)

Transfer Axiom™ 2.0 Neutral Soln to Neutralization Distribution Plate



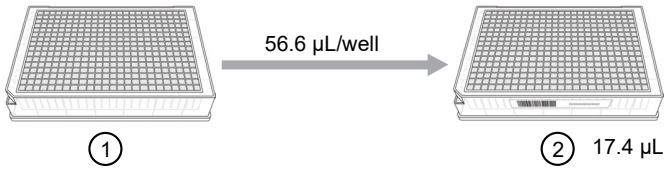
① Axiom™ 2.0 Neutral Soln (P3)
② NeutDist_1 (P1)

Prepare Amplification Master Mix Distribution Plate



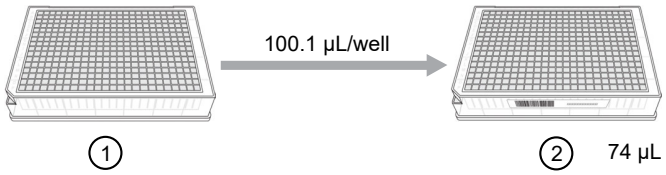
① AmpMM (P9)
② AmpMM_1 (P4)

Transfer Neutralization Solution to Sample Plate



① NeutDist_1 (P1)
② Sample Plate (Peltier 1)

Transfer Amplification Master Mix to Sample Plate



① AmpMM_1 (P4)
② Sample Plate (Peltier 1)

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

Stage 2A: Fragment and precipitate the DNA

During this stage, the 384 deepwell 250-µL plate of fragmented samples is split into four 96 deepwell 2-mL plates for precipitation. To avoid mislabeling samples, be careful to preserve the order of the four plates and avoid interchanging these plates with those derived from another 384-well sample plate.

An overnight incubation is required at the end of the stage.

Time required

Activity	Time
Hands-on time[1]	20 minutes
Biomek™ i7	1.5 hours

[1] Hands-on time is the estimated time required by the user for handling samples and reagents and setting up the i7 deck and does not include sample and reagent thawing time.

Input and output plates

Plate	Description	Volume	Deck position
Input plate	384 deepwell 250-µL plate (Amplification Plate)	174.1 µL	Peltier1
Output plate Q1	96 deepwell 2-mL plate	572.6 µL	P12
Output plate Q2	96 deepwell 2-mL plate	572.6 µL	P17
Output plate Q3	96 deepwell 2-mL plate	572.6 µL	P13
Output plate Q4	96 deepwell 2-mL plate	572.6 µL	P18

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
As required	Adhesive seals for plates
1	Ice bucket, filled with ice
As required	Laboratory tissue
1	Mini microcentrifuge (minifuge with microtube rotor)
1	Plate centrifuge
1	Vortexer (for plates and microtubes)

(continued)

Quantity	Item
Biomek™ i7 Automated Workstation	
• 6 boxes • 1 box	Pipette tips: • i-Series 190 µL filter tips (green) • i-Series 1,025 µL filter tips (orange)
2	Deep 96 full reservoir
1	96 PCR plate
1	384 deepwell 250-µL plate (AB1178)
4	96 deepwell 2-mL plate (Abgene™ deepwell plate)
1	Plastic plate lid (FRAG LID)
1	Reagent block, chilled to 4°C
3	Quarter Reservoir
Sample Plate	One plate of amplified DNA from Stage 1 in a 384 deepwell 250-µL plate (AB1178)

Reagent and sample plate handling

Thaw and prepare reagents and sample plate according to the following table.

Module	Qty	Reagent and cap color	Thaw, then place on ice	Place on ice	Place at room temp	Deck loading instructions
Reagents from the Axiom™ 2.0 384HT Reagent Kit						
Module 2-1 –20°C	2	Axiom™ Frag Enzyme	⊗ Do not thaw. Keep at –20°C until ready to use.			*Immediately before use:* Gently flick tube 3 times, centrifuge. Place in chilled reagent block.
	2	Axiom™ 10X Frag Buffer	✓ Thaw in a small water bath[1]			Vortex. Pour in reservoir.
	2	Axiom™ Precip Soln 2	✓			Vortex, then centrifuge. Place in chilled reagent block.
Module 2-2 2–8°C	2	Axiom™ Precip Soln 1		✓		Vortex. Pour in reservoir.

(continued)

Module	Qty	Reagent and cap color	Thaw, then place on ice	Place on ice	Place at room temp	Deck loading instructions
Module 2-2 2–8°C	2	Axiom™ Frag Diluent		✓		Vortex, then centrifuge. Place in chilled reagent block.
	2	Axiom™ Frag Rxn Stop			✓	Vortex. Pour in reservoir.
Note: Estimated reagent thawing time is 30 minutes.						
Amplified Sample Plate						
Follow reagent handling steps only if the Amplified Sample Plate was frozen after the DNA amplification step.			Place the deep-well plate in a small water bath[1] for 1 hour until all wells have thawed.	Centrifuge at 1,000 rpm for 30 seconds.		

[1] For example, on the benchtop at room temperature, pour ultra-pure water into a small tray.

Perform the pre-run checklist

The following actions are the same as described under “Perform the prerun checklist” on page 53. See this section for details. Some or all these steps are required depending on the current state of the Biomek™ i7 Automated Workstation.

1. Power on the Biomek™ i7 and all peripherals.
2. Check the waste container, then empty as required.
3. Open the Biomek™ Software using one of the following methods:
   - Double-click the Biomek™ icon on the desktop.
   - In the Windows Search box, enter **Biomek 5**.
4. Home all axes.

Run the fragmentation and precipitation step

1. Click **File** ▸ **Open**.
2. For **Look in** of the **Open Method** window, select **Axiom 384 on i7**.
3. Select **2 Fragmentation and Precipitation Ax384_i7**, then click **OK**.
4. At the top of the **Biomek Software** window, click ▶ **Run** to start the fragmentation and precipitation step.
   The deck layout for the DNA fragmentation and precipitation step is displayed.

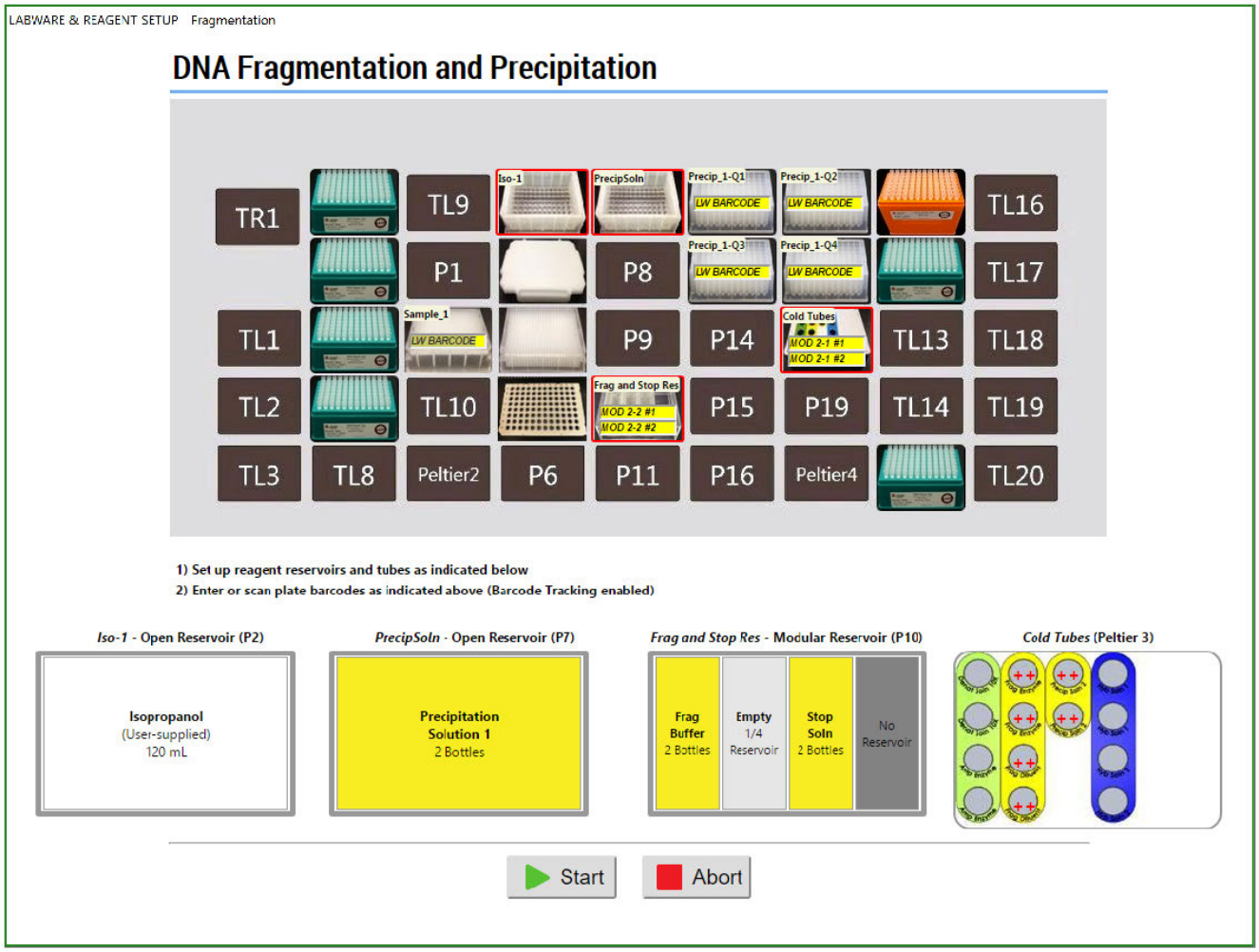


Figure 15 DNA fragmentation and precipitation step deck layout.

5. Place the labware and reagents on the deck as directed in the following figures and table: deck layout.
   - Figure 15—Deck layout.
   - Figure 16—Deck positions on the Biomek™ i7.
   - Table 8—Table detailing the labware, reagent, and modular reservoir placement for the deck layout.

- Figure 17—Reagent block.

Figure 16 Deck positions on the Biomek™ i7 Automated Workstation.

Table 8 Labware and reagent locations on the deck for the fragmentation and precipitation step.

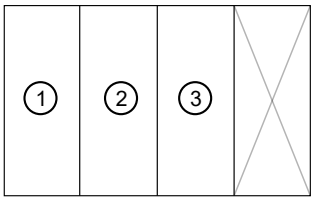
Deck position	Labware	Scan[1]	Reagent or sample
IMPORTANT! • Remove the seal from the amplified sample plate before placing on the deck. • Ensure that the P-pad that is attached to the plastic plate lid is cleaned with 70% ethanol before and after use.			
TL4	i-Series 190 µL filter tips (green)		
TL5	i-Series 190 µL filter tips (green)		
TL6	i-Series 190 µL filter tips (green)		
TL7	i-Series 190 µL filter tips (green)		
Peltier1	384 deepwell 250-µL plate over the 384 deep-well adapter (AB1178)	GENERIC BARCODE	Amplified DNA Sample Plate from stage 1
P2	Deep 96 full reservoir		Isopropanol 120 mL
P3	Plastic plate lid (FRAG LID)		
P4	384 deepwell 250-µL plate (AB1178)		
P5	96 PCR plate		
P7	Deep 96 full reservoir		Axiom™ Precip Soln 1, 2 bottles
P10	Reservoirs in frame: • Quarter Reservoir (1) • Quarter Reservoir (2) • Quarter Reservoir (3)	GENERIC BARCODE	 ① Pour 2 bottles Axiom™ 10X Frag Buffer into reservoir 1. ② Leave reservoir 2 empty. ③ Pour 2 bottles Axiom™ Frag Rxn Stop into reservoir 3.

Table 8 Labware and reagent locations on the deck for the fragmentation and precipitation step. *(continued)*

Deck position	Labware	Scan[1]	Reagent or sample
PL12	96 deepwell 2-mL plate Tip: Label the plate "Q1" for easier visualization during deck loading.	GENERIC BARCODE	Precipitation Plate, quadrant 1
PL13	96 deepwell 2-mL plate Tip: Label the plate "Q3" for easier visualization during deck loading.	GENERIC BARCODE	Precipitation Plate, quadrant 3
PL17	96 deepwell 2-mL plate Tip: Label the plate "Q2" for easier visualization during deck loading.	GENERIC BARCODE	Precipitation Plate, quadrant 2
PL18	96 deepwell 2-mL plate Tip: Label the plate "Q4" for easier visualization during deck loading.	GENERIC BARCODE	Precipitation Plate, quadrant 4
Peltier3	Reagent block, chilled to 4°C	GENERIC BARCODE	See Figure 17.
TL11	i-Series 1,025 µL filter tips (orange)		
TL12	i-Series 190 µL filter tips (green)		
TL15	i-Series 190 µL filter tips (green)		

[1] Scan labware or reagent module barcode. Do not scan the image in this table.

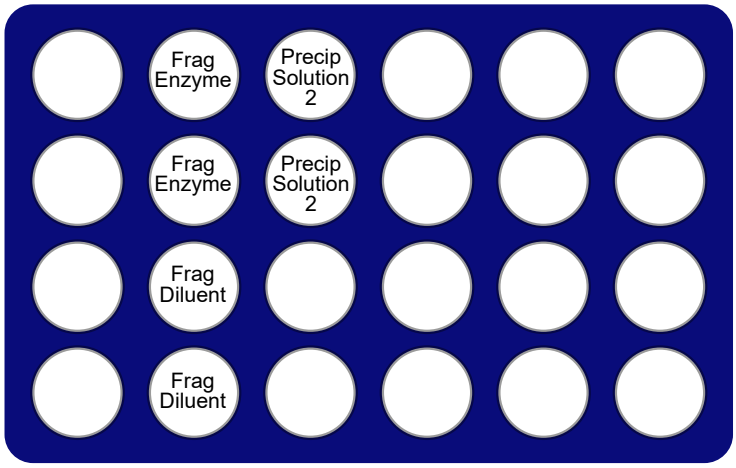


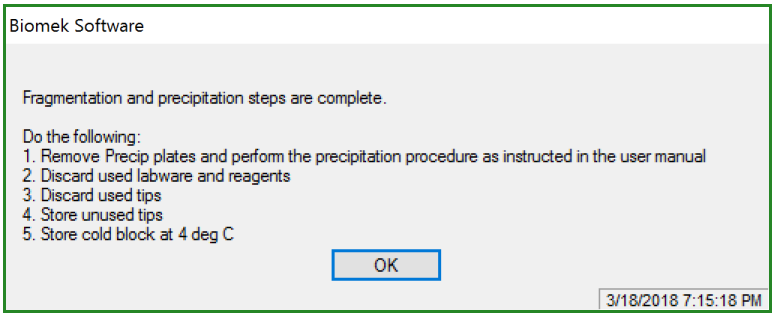
Figure 17 Placement of reagents on chilled reagent block for the fragmentation and precipitation step.

IMPORTANT! Flick and centrifuge the Axiom™ Frag Enzyme before placing in the block.

6. Check the deck layout to ensure that the labware, reagents, and samples are in the proper locations.

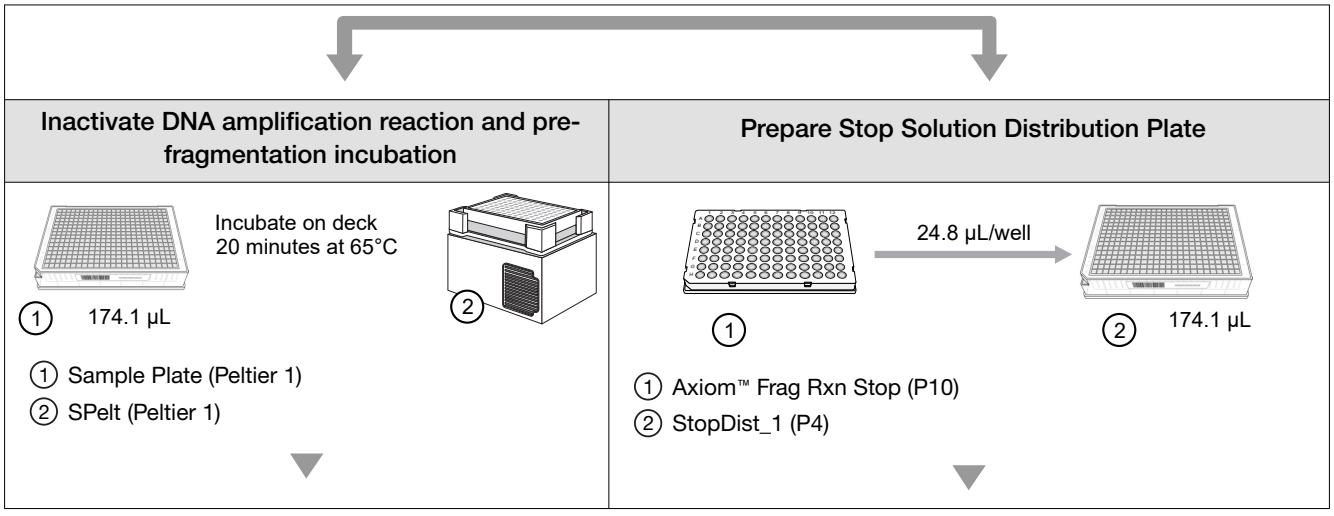
7. Scan the labware and reagent barcodes.

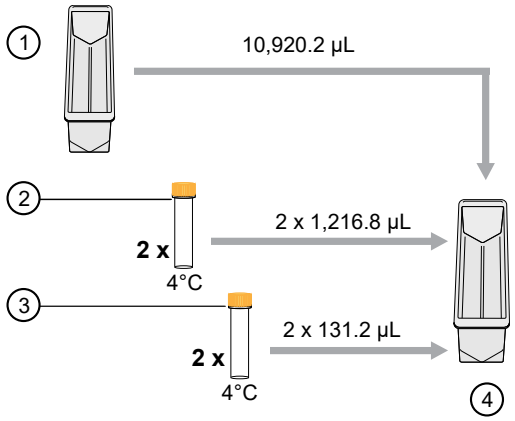
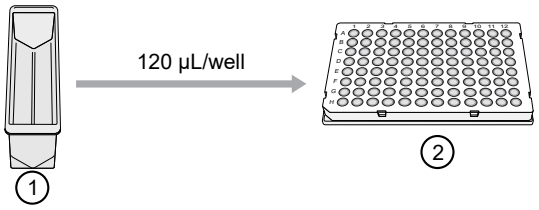
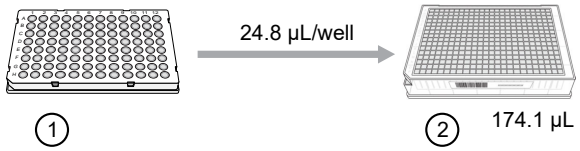
8. Click **Start** to continue.
   The fragmentation and precipitation step on the Biomek™ i7 Automated Workstation starts. When complete, a message window appears.



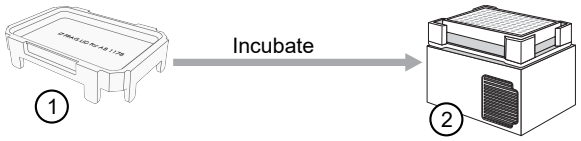
9. Complete the following:
   a. Remove the 4 precipitation plates that are found at deck positions P12, P13, P17, and P18.
   b. Blot the top of all 4 precipitation plates with a laboratory tissue, then seal each plate tightly.
   c. Place all 4 precipitation plates in a –20°C freezer overnight to precipitate the samples.
   d. Store the unused BC1025F tips from deck position TL11 and unused BC190F tips from deck position TL12.
   e. Discard the tubes from the cold block at the Peltier3 deck position. Store the cold block at 4°C.
   f. Discard the remaining labware and tips on the deck. Store the reservoir frames. Click **OK** when done.

Workflow for Stage 2: Fragmentation and precipitation



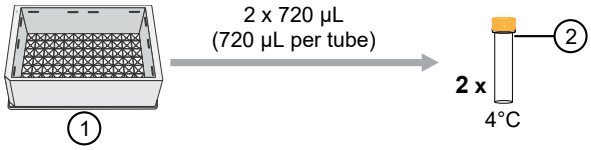
	Prepare Fragmentation Master Mix
	 ① Axiom™ 10X Frag Buffer (P10) ② Axiom™ Frag Diluent (Peltier 3) ③ Axiom™ Frag Enzyme (Peltier 3) ④ Frag MM (P10)
	Prepare Fragmentation Master Mix Distribution Plate
	 ① Frag MM (P10) ② FragDist-1 (P5)
	Transfer Fragmentation Master Mix to Sample Plate
Incubate on deck 20 minutes at 39.5°C	 ① FragDist-1 (P5) ② Sample Plate (Peltier 1)

Move plate lid onto Sample Plate



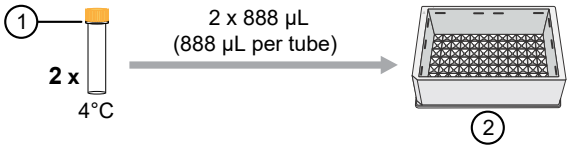
① Plate lid (P3)
② Shaking peltier + Sample Plate (Peltier 1)

Dilute Axiom™ Precip Soln 2



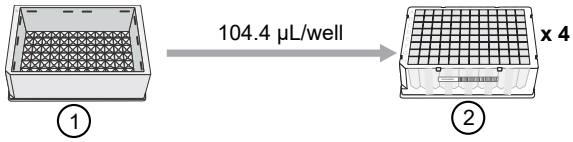
① Axiom™ Precip Soln 1 (P7)
② Axiom™ Precip Soln 2 (Peltier 3)

Prepare Precipitation Master Mix



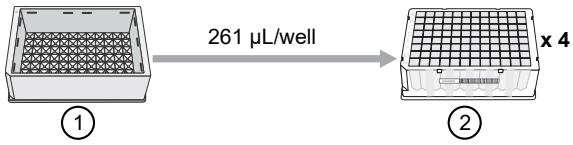
① Axiom™ Precip Soln 2 (Peltier 3)
② Axiom™ Precip Soln 1 (P7)

Transfer Precipitation Master Mix to precipitation plates

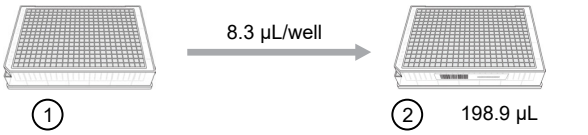
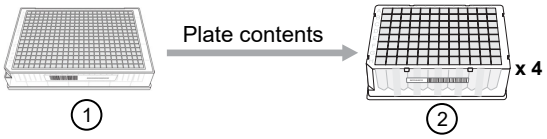
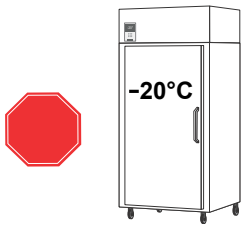


① Precip MM (P7)
② Precipitation plates (P12, P13, P17, P18)

Transfer isopropanol to precipitation plates



① Iso-1 (P2)
② Precipitation plates (P12, P13, P17, P18)

Move plate lid to deck position P8	
 ① Plate Lid (Peltier 1) ② Deck position P8	
Transfer Stop Solution to Sample Plate	
 ① StopDist_1 (P4) ② Sample Plate (Peltier 1)	
Transfer samples to precipitation plates	
 ① Sample Plate (Peltier 1) ② Precipitation plates (P12, P13, P17, P18)	
Offline precipitation	
Incubate precipitation plates containing samples at –20°C freezer overnight. 	

Stage 2B: Centrifuge and dry pellets

Time required

Activity	Time
Hands-on time	15 minutes
Biomek™ i7	Not required
Off deck equipment • Centrifugation • Drying	 • 40 minutes • 25 minutes

Input and output plates

Plate	Description	Volume	Deck position
Input plate Q1	96 deepwell 2-mL plate	572.6 µL	off-deck
Input plate Q2	96 deepwell 2-mL plate	572.6 µL	off-deck
Input plate Q3	96 deepwell 2-mL plate	572.6 µL	off-deck
Input plate Q4	96 deepwell 2-mL plate	572.6 µL	off-deck
Output plate Q1	96 deepwell 2-mL plate	Pelleted sample	off-deck
Output plate Q2	96 deepwell 2-mL plate	Pelleted sample	off-deck
Output plate Q3	96 deepwell 2-mL plate	Pelleted sample	off-deck
Output plate Q4	96 deepwell 2-mL plate	Pelleted sample	off-deck

Equipment and consumables required

Quantity	Item
As required	Adhesive seals for plates
As required	Laboratory tissue
1	Plate centrifuge, precooled to 4°C
1	Oven, preheated to 37°C Use an oven that can sustain a constant temperature of 37°C and has a temperature accuracy of ±1°C. We recommend the BINDER™ ED 56 or Heratherm™ Advanced Protocol Microbiological Incubator, capacity 66 L.
Sample plates	Four plates of precipitated samples from Stage 2 in 96 deepwell 2-mL plates

Note: Keep the centrifuge ready at 4°C.

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

Centrifuge and dry the pellets

1. Centrifuge the four 96-well precipitation plates for 40 minutes at 4°C at 3,200 x *g*.

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

2. Immediately after the 40-minute centrifugation time frame, empty the liquid from each plate using the following procedure:
   a. Remove the seal.
   b. Invert the plates over a waste container, then allow the liquid to drain.
   c. As the plate is inverted, gently press the plates on a pile of laboratory tissues on a bench, then allow them to drain for five minutes. Transfer the plates to a new pile of laboratory tissues twice during the 5-minute time frame.
3. Turn the plates 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 the plates.

4. Do one of the following:
   - Proceed directly to "Stage 3: Prepare the resuspension and hybridization reagent mixture" on page 78, even if some droplets of liquid remain. Leave the sample plate at room temperature. It is helpful to start preparing reagents for stage 3 during centrifuging and drying pellets.
   - Store the plates for resuspension later in the same day. Tightly seal the plates.
     - If resuspension is carried in 4 hours, keep the plates at room temperature.
     - If resuspension is carried out in more than 4 hours, store the plates in a refrigerator (2–8°C).
   - To process the plates for resuspension on another day, tightly seal the plates and store at –20°C.

Stage 3: Prepare the resuspension and hybridization reagent mixture

Note: In this stage, a resuspension and hybridization reagent mixture is prepared and delivered to the four plates of pelleted DNA by the Biomek™ i7 Automated Workstation. The samples are then resuspended by shaking off deck.

Time required

Activity	Time
Hands-on time[1]	10 minutes
Biomek™ i7	17 minutes
Off deck equipment—shaking	15 minutes

[1] Hands-on time is the estimated time required by the user for handling samples and reagents and setting up the Biomek™ i7 deck and does not include sample and reagent thawing time.

Input and output plates

Plate	Description	Volume	Deck position
Input plate Q1	96 deepwell 2-mL plate	Pelleted sample	P12
Input plate Q2	96 deepwell 2-mL plate	Pelleted sample	P17
Input plate Q3	96 deepwell 2-mL plate	Pelleted sample	P13
Input plate Q4	96 deepwell 2-mL plate	Pelleted sample	P18
Output plate Q1	96 deepwell 2-mL plate	50 µL	P12
Output plate Q2	96 deepwell 2-mL plate	50 µL	P17
Output plate Q3	96 deepwell 2-mL plate	50 µL	P13
Output plate Q4	96 deepwell 2-mL plate	50 µL	P18

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
As required	Adhesive seals for plates
1	Mini microcentrifuge (minifuge with microtube rotor)
1	Plate centrifuge at room temperature

(continued)

Quantity	Item
1	Shaker, either: • Thermo Scientific™ Digital Microplate Shaker, 88882005 or 88882006 • Thermo Scientific™ Compact Digital Microplate Shaker, 88880023 or 88880024 • Boekel™ Jitterbug™
1	Vortexer
Biomek™ i7 Automated Workstation	
• 4 boxes • 1 box	Pipette tips: • i-Series 190 µL filter tips (green) • i-Series 1,025 µL filter tips (orange)
1	Reagent block, chilled to 4°C
1	96 deepwell 2-mL plate (Abgene™ deepwell plate)
3	Quarter Reservoir
1	384 deep-well adapter

Reagent handling

Thaw and prepare reagents according to the following table.

Module	Quantity	Reagent and cap color	Place at room temperature	Deck loading instructions
Reagents from the Axiom™ 2.0 384HT Reagent Kit				
Module 2-1 –20°C	2	Axiom™ Hyb Buffer	✓	Vortex. Pour in reservoir.
	2	Axiom™ Hyb Soln 1	✓	Vortex, then centrifuge. Place in chilled reagent block.
Module 2-2 2–8°C	2	Axiom™ Resusp Buffer	✓	Vortex. Pour in reservoir.
	2	Axiom™ Hyb Soln 2	✓	Vortex, then centrifuge. Place in chilled reagent block.
Note: Estimated reagent thawing time is 1 hour.				

IMPORTANT! The resuspension reagents must be at room temperature for *1 hour* before proceeding with this step. Failure to equilibrate to room temperature causes incomplete resuspension of pellets and compromises results.

Prepare pelleted DNA

Guidelines for pelleted DNA plates

IMPORTANT! DNA pellets must be at room temperature before resuspension.

Plates of fresh pelleted DNA

- If proceeding with the resuspension and hybridization protocol in 4 hours, plates with fresh pellets can be kept at room temperature.
- If plates with fresh pellets are not processed in 4 hours but are processed the same day, these plates can be transferred to a refrigerator (2–8°C). However, it is critical to equilibrate the plate to room temperature for at least 30 minutes before proceeding with the resuspension and hybridization protocol.

Plates of frozen pelleted DNA

Plates with frozen pellets (for example, on day 5 of the 8-plate workflow) must be equilibrated to room temperature for at least 1.5 hours before proceeding with resuspension and hybridization.

Perform the pre-run checklist

The following actions are the same as described under "Perform the prerun checklist" on page 53. See this section for details. Some or all these steps are required depending on the current state of the Biomek™ i7 Automated Workstation.

1. Power on the Biomek™ i7 and all peripherals.
2. Check the waste container, then empty as required.
3. Open the Biomek™ Software using one of the following methods:
   - Double-click the Biomek™ icon on the desktop.
   - In the Windows Search box, enter **Biomek 5**.
4. Home all axes.

Run the resuspension and hybridization preparation step

1. Click **File** ▸ **Open**.
2. For **Look in** of the **Open Method** window, select **Axiom 384 on i7**.
3. Select **3 Resuspension and Hybridization Preparation Ax384_i7**, then click **OK**.
4. At the top of the Biomek Software window, click **Run** to start the resuspension and hybridization step.
   The deck layout for the resuspension and hybridization preparation step is displayed.

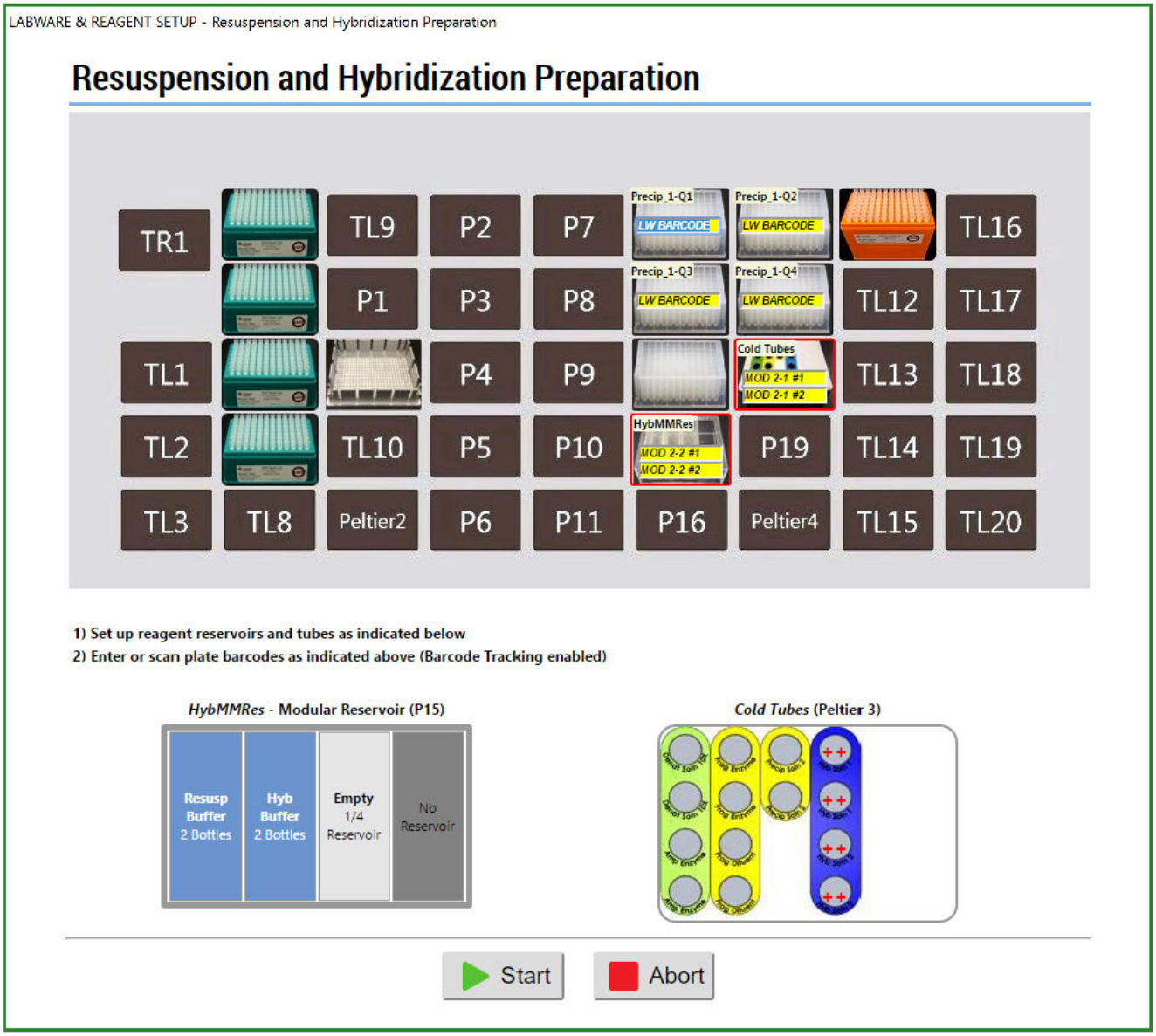


Figure 18 Resuspension and hybridization preparation step deck layout.

5. Place the labware and reagents on the deck as directed in the following figures and table: deck layout.

   - Figure 18—Deck layout.
   - Figure 19—Deck positions on the Biomek™ i7.
   - Table 9—Table detailing the labware, reagent, and modular reservoir placement for the deck layout.

- Figure 20—Reagent block.

Figure 19 Deck positions on the Biomek™ i7 Automated Workstation.

Table 9 Labware and reagent locations on the deck for the resuspension and hybridization preparation step.

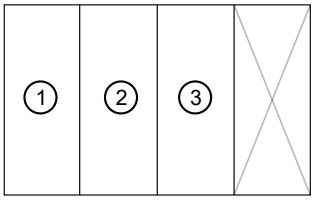
Deck position	Labware	Scan[1]	Reagent or sample
TL4	i-Series 190 µL filter tips (green)		
TL5	i-Series 190 µL filter tips (green)		
TL6	i-Series 190 µL filter tips (green)		
TL7	i-Series 190 µL filter tips (green)		
Peltier1	384 deep-well adapter (no other labware)		
P12	96 deepwell 2-mL plate (Use plate labeled "Q1" from stage 2)		Pelleted samples, quadrant 1
P13	96 deepwell 2-mL plate (Use plate labeled "Q3" from stage 2)		Pelleted samples, quadrant 3
P14	96 deepwell 2-mL plate		
P15	Reservoirs in frame: • Quarter Reservoir (1) • Quarter Reservoir (2) • Quarter Reservoir (3)		 ① Pour 2 bottles Axiom™ Resusp Buffer into reservoir 1. ② Pour 2 bottles Axiom™ Hyb Buffer into reservoir 2. ③ Leave reservoir 3 empty.
P17	96 deepwell 2-mL plate (Use plate labeled "Q2" from stage 2)		Pelleted samples, quadrant 2
P18	96 deepwell 2-mL plate (Use plate labeled "Q4" from stage 2)		Pelleted samples, quadrant 4

Table 9 Labware and reagent locations on the deck for the resuspension and hybridization preparation step. *(continued)*

Deck position	Labware	Scan[1]	Reagent or sample
Peltier3	Reagent block, chilled to 4°C		See Figure 20.
TL11	i-Series 1,025 µL filter tips (orange)		

[1] Scan labware or reagent module barcode. Do not scan the image in this table.

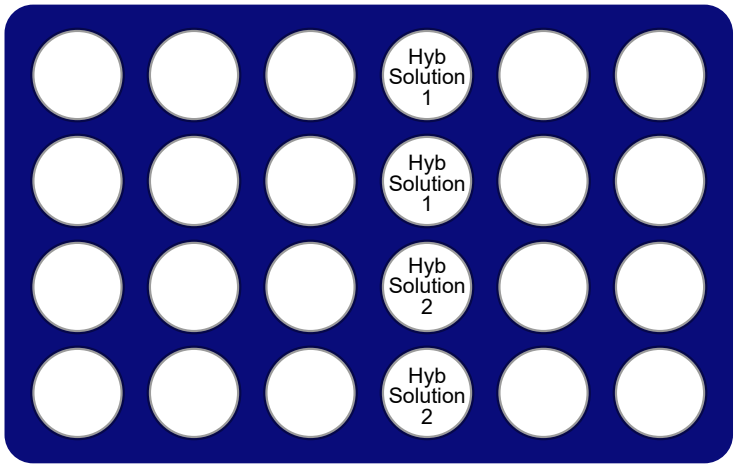
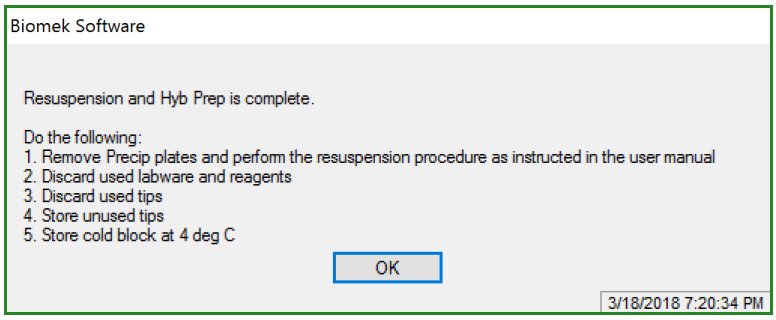


Figure 20 Placement of reagents on chilled reagent block for the resuspension and hybridization preparation step.

6. Check the deck layout to ensure that the labware, reagents, and samples are in the proper locations.

7. Scan the labware and reagent barcodes.

8. Click **Start** to continue.
   The resuspension and hybridization preparation step on the Biomek™ i7 Automated Workstation starts. When complete, a message window appears.



9. Complete the following tasks:
   a. Remove the 4 precipitation plates from deck positions P12, P13, P17, and P18.
   b. Blot the top of the precipitation plates with a laboratory tissue to remove any droplets present.
   c. Tightly seal the precipitation plates.

d. Follow the instructions in "Complete resuspension by off deck shaking" on page 84 to carry out the resuspension by off deck shaking.

e. Store the unused BC1025F tips from deck position TL11.

f. Discard the tubes from the cold block at the Peltier3 deck position. Store the cold block at 4°C.

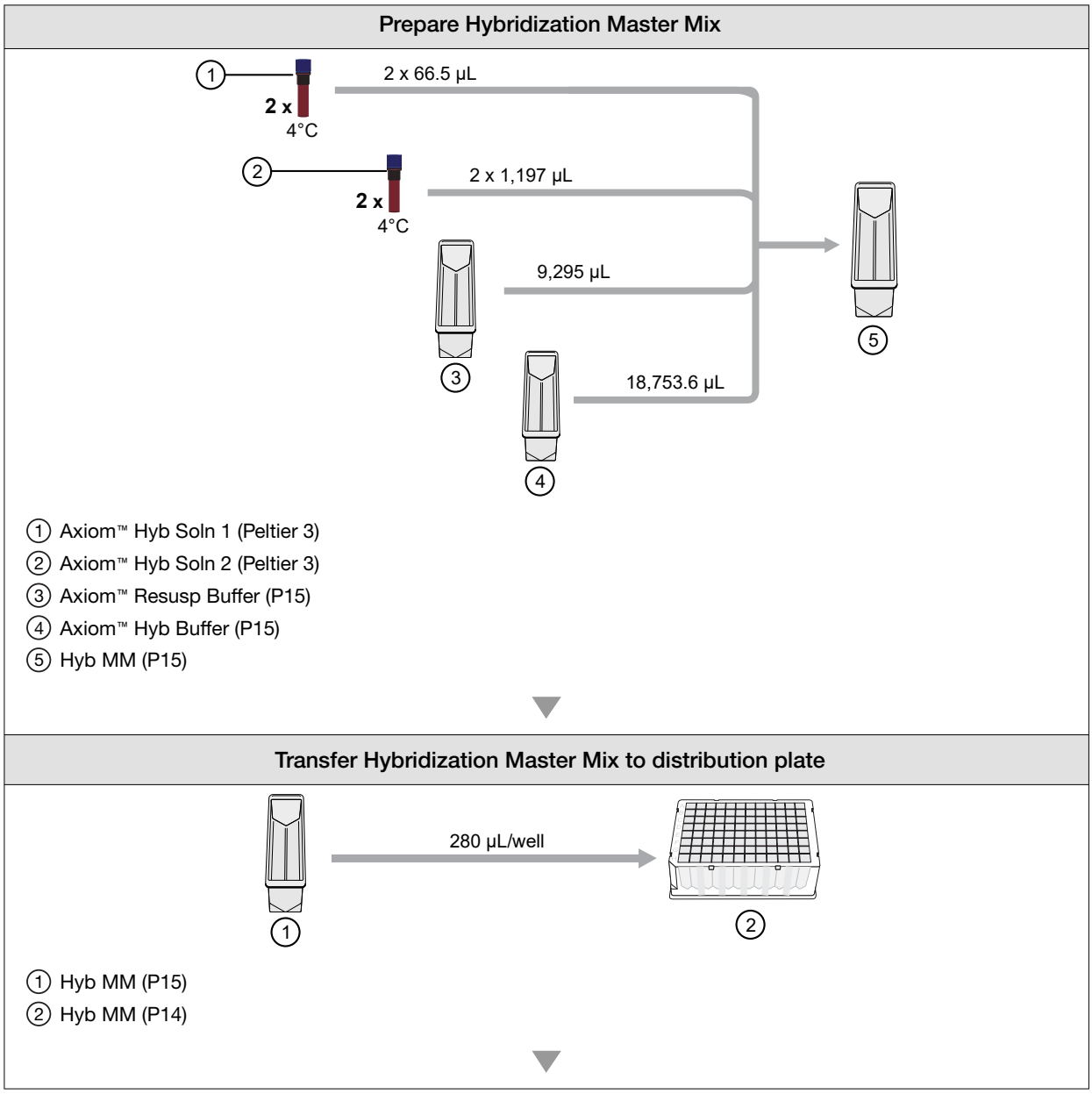
g. Discard the remaining labware and tips on the deck. Store the reservoir frames. Click **OK** when done.

Complete resuspension by off deck shaking

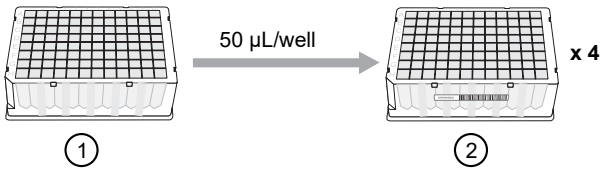
On completion of the on-deck method to prepare the resuspension and hybridization mixture and deliver this mixture to the four 96 deepwell 2-mL plate containing the samples, resuspension is carried out by shaking off deck using the following steps:

1. Seal the 4 plates tightly. Check to see that blue pellets are visible at the bottom of the wells.
2. Centrifuge the 4 plates at room temperature for 30 seconds.
3. Place all 4 plates onto the shaker and shake the plates at 900 rpm for 15 minutes.
4. Inspect the 4 plates from the bottom. If the pellets are not dissolved, repeat the shaking step.
5. Centrifuge the 4 plates at room temperature for 30 seconds.
6. Proceed to "Stage 4: Reassemble Hybridization Ready Plate and perform sample QC" on page 86.

Workflow for Stage 3: Resuspension and hybridization preparation



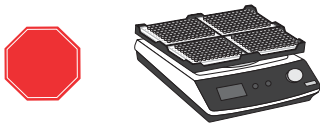
Transfer Hybridization Master Mix to precipitation plates



Final volume = 50 µL/well

① Hyb MM (P14)
② Precipitation plates containing dried pellets (P12, P13, P17, P18)

Off-line resuspension



Shake the four sample plates on an off-deck shaker for 15 minutes.

Stage 4: Reassemble Hybridization Ready Plate and perform sample QC

Time required

Activity	Time
Hands-on time[1]	15 minutes
Biomek™ i7	19 minutes (with sample QC) 5 minutes (without sample QC)
Off deck equipment (if performing sample QC)	30 minutes

[1] Hands-on time is the estimated time required by the user for handling samples and reagents and setting up the Biomek™ i7 deck and does not include sample and reagent thawing time.

Input and output plates

Plate	Description	Volume	Deck position
Input plate Q1	96 deepwell 2-mL plate	50 µL	P12
Input plate Q2	96 deepwell 2-mL plate	50 µL	P17
Input plate Q3	96 deepwell 2-mL plate	50 µL	P13
Input plate Q4	96 deepwell 2-mL plate	50 µL	P18

(continued)

Plate	Description	Volume	Deck position
Output plate	384 PCR plate	45 µL	P9
Output plate (if QC selected)	384 UV plate	30 µL	P5
Output plate (if QC selected)	384 deepwell 250-µL plate (AB1178)	62 µL	P11

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
As required	Adhesive seals for plates
1	Laboratory tissue
Biomek™ i7 Automated Workstation	
• 4 boxes • 1 box	Pipette tips: • i-Series 50 µL filter tips (pink) • i-Series 190 µL filter tips (green)[1]
2	Deep 96 full reservoir[1]
1	384 deepwell 250-µL plate[1] (AB1178)
1	384 deepwell 250-µL plate[1] (AB1178)
1	384 PCR plate
1	384 UV plate[1]
Sample Plate	Four plates of resuspended DNA from Stage 3 in 96 deepwell 2,000-µL plate
1	Plate collar
1	384 deep-well adapter

[1] QC labware. Not required if QC was disabled in method preferences.

Reagent handling

Prepare reagents according to the following table.

Quantity	Reagent	Handling
User supplied reagents		
75 mL	Nuclease-free water, ultrapure MB grade[1] (Thermo Fisher Scientific, Cat. No. 71786; for OD and gel plate preparation)	Add 75 mL of nuclease-free water to the water reservoir (Deep 96 full reservoir)
35 mL	Gel diluent[1] (see Appendix B, "Fragmentation quality control gel protocol" for dilution instructions)	Add 35 mL of diluted loading dye to the dye reservoir (Deep 96 full reservoir).

[1] QC user-supplied reagents. Not required if QC is disabled in method preferences.

Perform the pre-run checklist

The following actions are the same as described under "Perform the prerun checklist" on page 53. See this section for details. Some or all these steps are required depending on the current state of the Biomek™ i7 Automated Workstation.

1. Power on the Biomek™ i7 and all peripherals.
2. Check the waste container, then empty as required.
3. Open the Biomek™ Software using one of the following methods:
   - Double-click the Biomek™ icon on the desktop.
   - In the Windows Search box, enter **Biomek 5**.
4. Home all axes.

Run the plate reassembly and QC step

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

- A gel to view fragmentation.
- An OD quantification of each resuspended sample to check DNA yield.

  The Biomek™ i7 Automated Workstation can be set to prepare fragmentation and OD plates that are ready for processing. These process controls must be selected as a run preference before starting a run. See "Set the method preferences" on page 46.

1. Click **File** ▸ **Open**.
2. For **Look in** of the **Open Method** window, select **Axiom 384 on i7**.
3. Select **4 Plate Reassembly and QC Ax384_i7**, then click **OK**.
4. At the top of the Biomek Software window, click **Run** to start the plate reassembly and QC step.

   The deck layout for the plate reassembly and QC step is displayed.

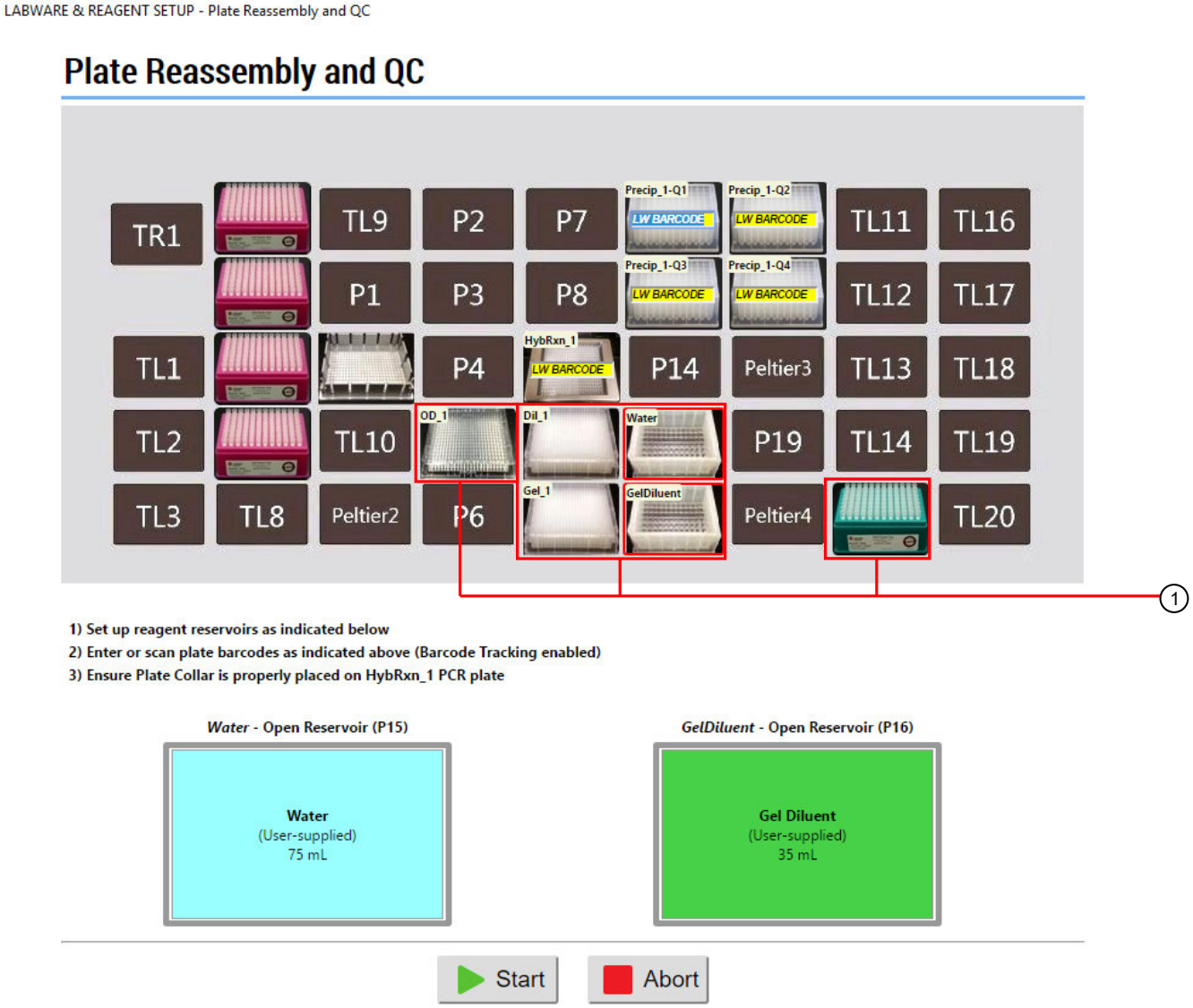


Figure 21 Plate reassembly and QC step deck layout.

① If **Prepare gel and OD plates for QC** is not enabled, these plates and tips do not appear in the deck layout. See "Set the method preferences" on page 46 for more information.

5. Place the labware and reagents on the deck as directed in the following figures and table: deck layout.
   - Figure 21—Deck layout.
   - Figure 22—Deck positions on the Biomek™ i7 Automated Workstation.
   - Table 10—Table detailing the labware, reagent, and modular reservoir placement for the deck layout.

IMPORTANT! In this stage of the assay, the four 96 deepwell 2-mL plates of resuspended samples are reassembled into one 384 PCR plate. To avoid mislabeling samples, it is critical that the four 96 deepwell 2-mL plates of resuspended samples are:

- All derived from one original 384-well genomic DNA sample plate and not mixed with those of another plate being processed.
- Placed on the Biomek™ i7 Automated Workstation deck in the same order as used in "Stage 3: Prepare the resuspension and hybridization reagent mixture" on page 78.

The barcode tracking feature of the method (see "Sample barcode tracking" on page 50) helps in associating and maintaining the order of the four plates of resuspended samples.

Figure 22 Deck positions on the Biomek™ i7 Automated Workstation

Table 10 Labware and reagent locations on the deck for the plate reassembly and QC step

Deck position	Labware	Scan[1]	Reagent or sample
TL4	i-Series 50 µL filter tips (pink)		
TL5	i-Series 50 µL filter tips (pink)		
TL6	i-Series 50 µL filter tips (pink)		
TL7	i-Series 50 µL filter tips (pink)		
Peltier1	384 deep-well adapter (no other labware)		
P5	384 UV plate[2]		
P9	384 PCR plate with plate collar	GENERIC BARCODE	Hybridization reaction plate
P10	384 deepwell 250-µL plate[2] (AB1178)		
P11	384 deepwell 250-µL plate[2] (AB1178)		
P12	96 deepwell 2-mL plate (Use plate labeled "Q1" from stage 3)	GENERIC BARCODE	Resuspended samples, quadrant 1
P13	96 deepwell 2-mL plate (Use plate labeled "Q3" from stage 3)	GENERIC BARCODE	Resuspended samples, quadrant 3
P15	Deep 96 full reservoir[2]		Nuclease-free water, ultrapure MB grade
P16	Deep 96 full reservoir[2]		Gel diluent

Table 10 Labware and reagent locations on the deck for the plate reassembly and QC step *(continued)*

Deck position	Labware	Scan[1]	Reagent or sample
P17	96 deepwell 2-mL plate (Use plate labeled "Q2" from stage 3)	GENERIC BARCODE	Resuspended samples, quadrant 2
P18	96 deepwell 2-mL plate (Use plate labeled "Q4" from stage 3)	GENERIC BARCODE	Resuspended samples, quadrant 4
TL15	i-Series 190 µL filter tips (green)[2]		

[1] Scan labware or reagent module barcode. Do not scan the image in this table.

[2] QC labware. Not required if QC was disabled in method preferences.

6. Check the deck layout to ensure that the labware, reagents, and samples are in the proper locations.

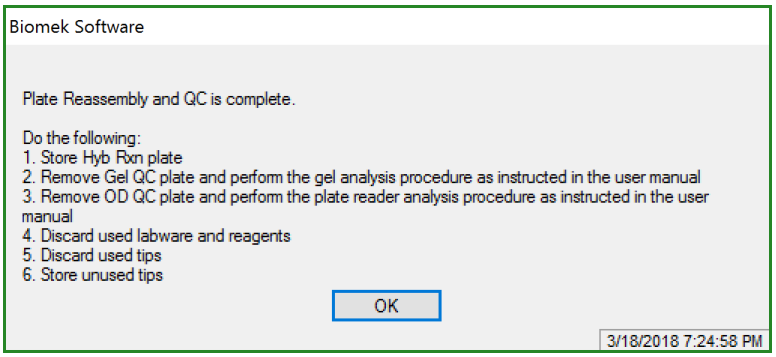
7. Place the 384 PCR plate at deck position P9, scan the labware barcode, then place the plate collar on top of the 384 PCR plate.

   IMPORTANT! Ensure that the plate collar is cleaned with 70% ethanol before and after use.

8. Scan the remaining labware barcodes.

9. Click **Start** to continue.

   The Plate reassembly and QC step on the Biomek™ i7 Automated Workstation starts. When complete, a message window appears.



10. Complete the following tasks:

    a. Remove and tightly seal the Hyb Rxn Plate from deck position P9. This plate contains the hybridization ready samples. Store at –20°C if you will not proceed to “Stage 5: Denature sample and transfer to hybridization tray” on page 97 following assessment of the QC results.

    b. Perform the gel assessment of the fragmentation pattern using the Gel QC Plate from deck position P11 as described in Appendix B, “Fragmentation quality control gel protocol”. (Skip this step if not preparing QC plates.)

    c. Perform the OD quantification on the OD QC Plate from deck position P5, then evaluate the results as described in Appendix C, “Sample quantification after resuspension”. (Skip this step if not preparing QC plates.)

d. (Optional) Seal the Dilution (Dil_1) Plate if repeating any QC analysis is required.

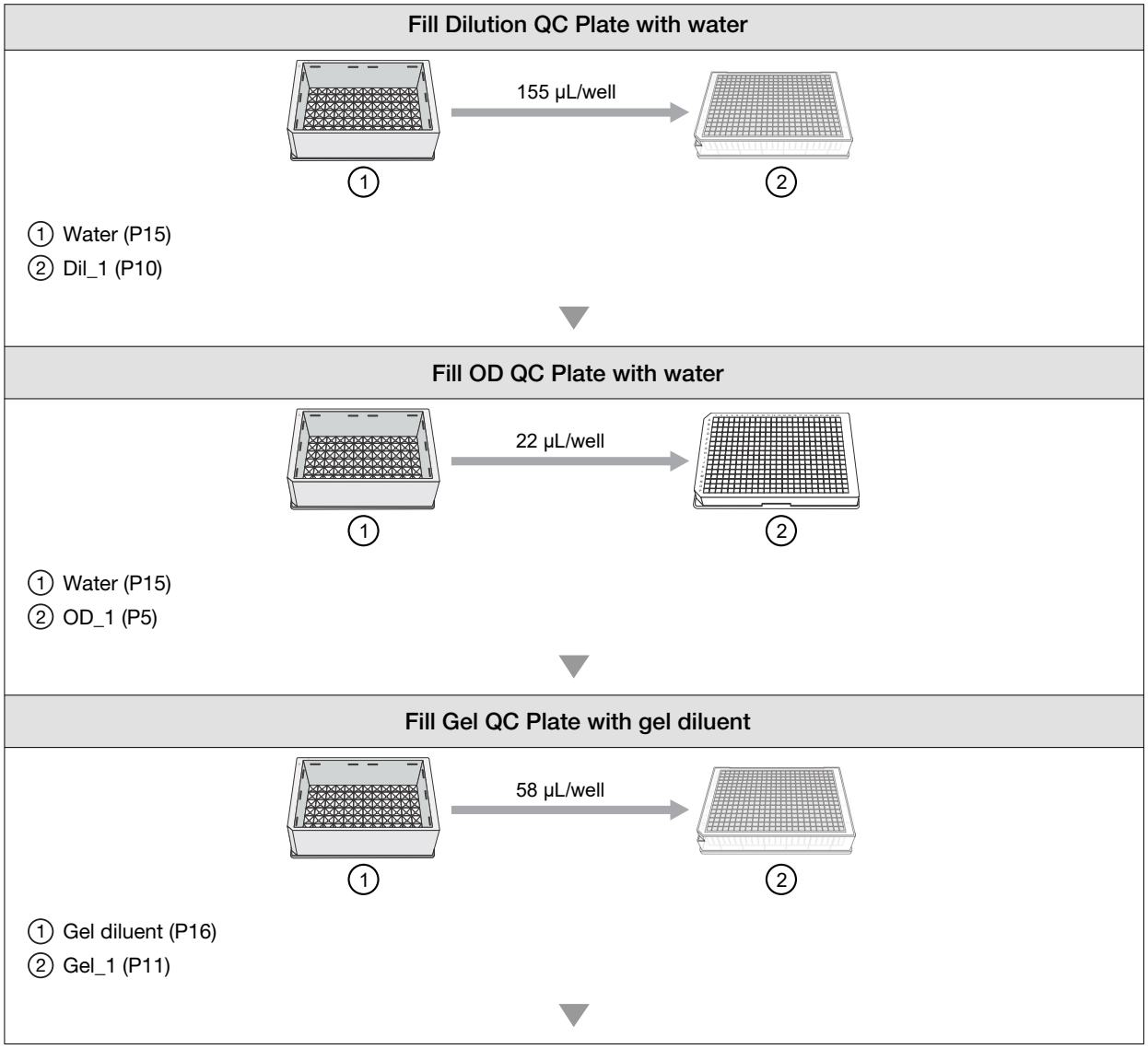
e. Discard the remaining labware and tips on the deck. Click **OK** when done.

11. If the gel and OD quantification results are acceptable, do one of the following:

- Proceed to "Stage 5: Denature sample and transfer to hybridization tray" on page 97 if the GeneTitan™ MC Instrument is available for hybridization.
- Store the Hyb Rxn Plate of hybridization ready samples at –20°C.

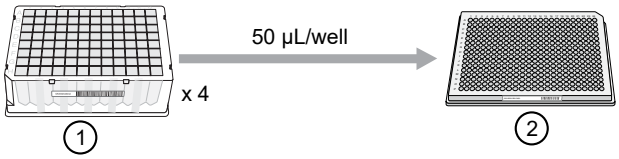
Workflows for Stage 4: Hybridization ready plate reassembly and sample QC

Workflow for Stage 4: Plate reassembly (QC enabled)



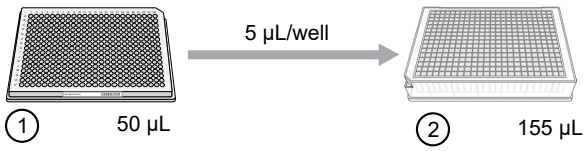
x 4

Transfer hybridization ready samples to Hyb Rxn Plate



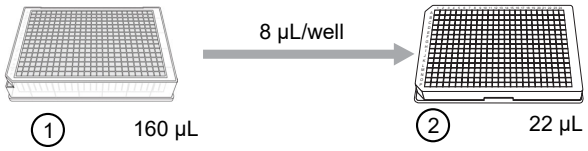
① Precipitation plate x 4 (P12, P13, P17, P18)
② HybRxn_1 (P9)

Transfer hybridization ready samples to Dilution QC Plate



① HybRxn_1 (P9)
② Dil_1 (P10)

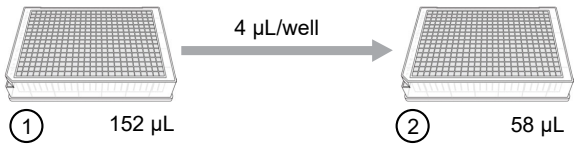
Transfer diluted samples to OD QC Plate



Final volume = 30 µL/well

① Dil_1 (P10)
② OD_1 (P5)

Transfer diluted samples to Gel QC Plate



Final volume = 62 µL/well

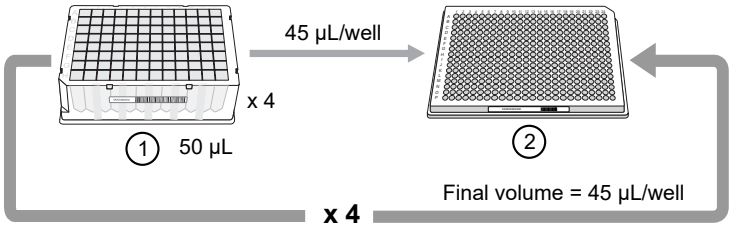
① Dil_1 (P10)
② Gel_1 (P11)

Next steps
• QC plates—perform off-line analysis with QC plates. • Hyb reaction plate—proceed to off-line denaturation or store samples.

Workflow for Stage 4: Plate reassembly (QC disabled)

Continued from off-line resuspension

Transfer hybridization ready samples to Hyb Rxn Plate



① Precipitation plate × 4 (P12, P13, P17, P18)
② HybRxn_1 (P9)

Proceed to off-line denaturation or store samples

Stage 5: Denature sample and transfer to hybridization tray

Note: Before denaturing samples see Chapter 5, "Process array plates with the GeneTitan™ instrument" ("Stage 2—Hybridize plates in the GeneTitan™ MC Instrument" on page 122 to ensure that the GeneTitan™ MC is ready for use.

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

Time required

Activity	Time
Hands-on time[1]	5 minutes
Biomek™ i7	3 minutes
Off deck equipment—denaturation in a thermal cycler	15 minutes

[1] Hands-on time is the estimated time required by the user for handling samples and setting up the Biomek™ i7 deck and does not include sample and reagent thawing time.

Input and output plates

Plate	Description	Volume	Deck position
Input plate	384 PCR plate	45 µL	P9
Output plate	384 hybridization tray	35 µL	P10

Equipment and labware required

Quantity	Item
As required	Laboratory tissue
1	Thermal cycler: ProFlex™ 2 × 384-well PCR System
Biomek™ i7 Automated Workstation	
4 boxes	Pipette tips, i-Series 190 µL filter tips (green)
1	Plate collar
1	384 deep-well adapter
1	Hybridization tray (Part No. 501278) from the Axiom™ 384HT GeneTitan™ Consumables Kit (Cat. No. 902234)
Sample plate	One plate of hybridization ready samples from Stage 4 in a 384 PCR plate

Thermal cycler requirements

The recommended thermal cycler is the ProFlex™ 2 × 384-well PCR System (Cat. No. 4484077). The thermal cycler must be programmed with the **Axiom 2.0 Denature** protocol of:

1. 95°C × 10 minutes
2. 48°C × 3 minutes
3. 48°C hold

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

---

IMPORTANT! Evaporation during denaturation can negatively affect assay performance. Use the thermal cycler, consumables, and sealing film that is recommended in the site preparation guide to eliminate condensation and evaporation. Always use the heated lid option when programming protocols.

Perform the pre-run checklist

The following actions are the same as described under "Perform the prerun checklist" on page 53. See this section for details. Some or all these steps are required depending on the current state of the Biomek™ i7 Automated Workstation.

1. Power on the Biomek™ i7 and all peripherals.
2. Check the waste container, then empty as required.
3. Open the Biomek™ Software using one of the following methods:
   - Double-click the Biomek™ icon on the desktop.
   - In the Windows Search box, enter **Biomek 5**.
4. Home all axes.

Prepare the array plate and the Hyb Ready Sample Plate (if stored at –20°C)

Prepare the Hyb Ready Sample Plate that was stored at –20°C

1. Warm up the Hyb Ready Sample Plate at room temperature for five minutes. It is not necessary to equilibrate the plate for longer lengths of time.
2. Make sure the Hyb Ready Sample Plate is sealed well. If the plate is not sealed well:
   a. 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 Hyb Ready Sample Plate briefly, then centrifuge at 1,000 rpm for 30 seconds.
4. Place the Hyb Ready Sample Plate at room temperature.

Prepare the array plate

Warm up the array plate on the benchtop before setting up hybridization on the GeneTitan™ MC Instrument.

1. Equilibrate the array plate in the sealed pouch at room temperature for at least 25 minutes.
2. 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 as described in Appendix D, "Register samples in GeneChip™ Command Console™".

WARNING! Do not remove the array plate from the protective base or touch the surface of any 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 122 and Appendix D, "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 Hyb Ready Sample Plate with an off deck thermal cycler

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

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

3. Close the lid, then start the **Axiom 2.0 Denature** protocol.

Run the transfer denatured samples to hybridization tray step

As the hybridization ready samples are denaturing, set up the Biomek™ i7 deck for the **Transfer Denatured Samples to Hybridization Tray** step.

1. Click **File** ▸ **Open**.

2. For **Look in** of the **Open Method** window, select **Axiom 384 on i7**.

3. Select **5 Transfer Samples to Hybridization Tray Ax384_i7**, then click **OK**.

4. At the top of the Biomek Software window, click ▶ **Run** to start the transfer denatured samples to hybridization tray step.
The deck layout for the transfer denatured samples to hybridization tray step is displayed.

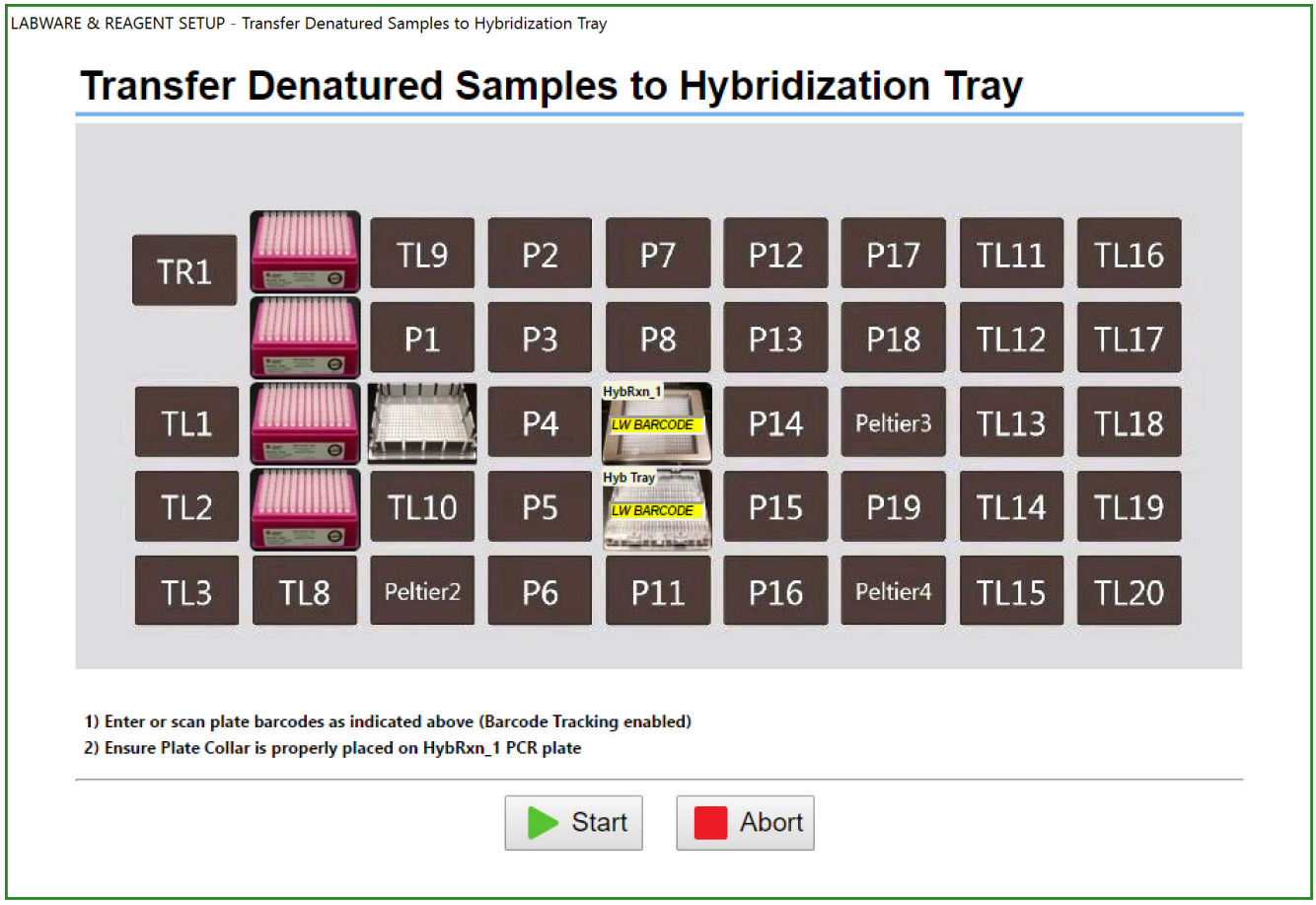


Figure 23 Transfer denatured samples to hybridization tray deck layout

5. Place the labware and reagents on the deck as directed in the following figures and table:
   - Figure 23—Deck layout.
   - Figure 24—Deck positions on the Biomek™ i7.

- Table 11—Table detailing the labware, reagents, and modular reservoir placement for the deck layout.

IMPORTANT!

- Write only on the proper location of the hybridization tray (on the edge in front of wells A1 and F1) as illustrated in Figure 49. Do not write on any other side, as the writing can interfere with sensors inside the GeneTitan™ MC Instrument and result in experiment failure.
- Ensure to remove the hybridization tray cover before loading the hybridization tray onto the deck.

Figure 24 Deck positions on the Biomek™ i7 Automated Workstation

Table 11 Labware and reagent locations on the deck for the transfer denatured samples to hybridization tray step

Deck position	Labware	Scan[1]	Reagent or sample
TL4	i-Series 50 µL filter tips (pink)		
TL5	i-Series 50 µL filter tips (pink)		
TL6	i-Series 50 µL filter tips (pink)		
TL7	i-Series 50 µL filter tips (pink)		
Peltier1	384 deep-well adapter (no other labware)		
P9	384 PCR plate from stage 4 with plate collar	GENERIC BARCODE	Hybridization ready samples
P10	Hybridization tray	GENERIC BARCODE	Hybridization tray for denatured samples

[1] Scan labware or reagent module barcode. Do not scan the image in this table.

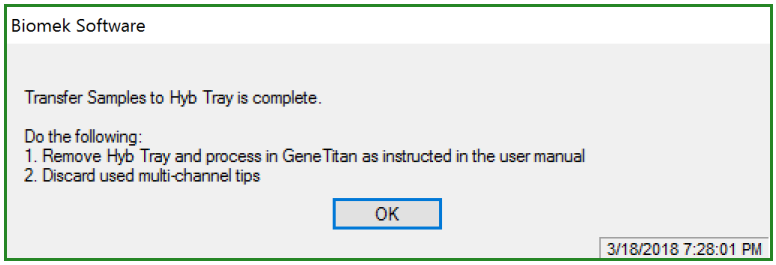
6. After setting pipette tips and hybridization tray on deck, return to the thermal cycler, then retrieve the denatured hybridization ready plate immediately after the **Axiom 2.0 Denature** protocol has completed.

IMPORTANT! Avoid letting the samples sit at room temperature for an extended amount of time after denaturation. Do not remove the denatured hybridization ready plate from the thermal cycler unless both the GeneTitan™ MC Instrument and Biomek™ i7 Automated Workstation are ready.

7. Remove seal from the denatured hybridization ready plate, then place the plate on the deck at position P9.

8. Scan labware barcodes, then place the plate collar on the Hyb Reaction (HybRxn_1) Plate.

9. Click **Start** immediately after the denatured hybridization ready sample plate is placed on the deck. The method begins. After the step is complete, a message window appears.



10. Immediately load the hybridization tray plate from deck position P10 into the GeneTitan™ MC Instrument.

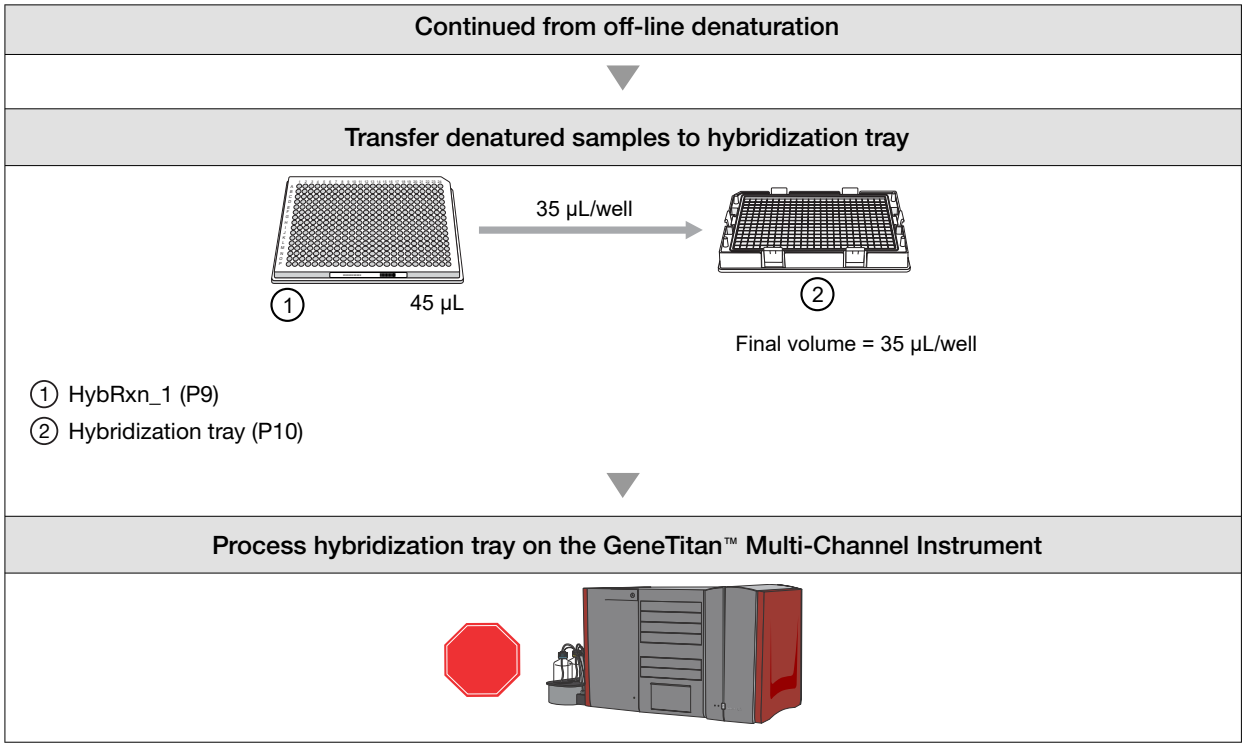
11. Discard the remaining labware and tips on the deck. Click **OK** when done.

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

1. Immediately on completion of the Transfer Denatured Samples to Hyb Tray step on the Biomek™ i7, 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 122.

2. Near the end of the 23.5 to 24-hour hybridization in the GeneTitan™ MC Instrument, proceed to “Stage 6: Prepare GeneTitan™ reagents” on page 105.

Workflow for Stage 5: Sample denaturation and transfer to hybridization tray



Stage 6: Prepare GeneTitan™ reagents

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

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

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

Time required

Activity	Time
Hands-on time[1]	30 minutes
Biomek™ i7	54 minutes

[1] Hands-on time is the estimated time required by the user for handling reagents and setting up the Biomek™ i7 deck and does not include reagent thawing time.

Materials, labware, and reagents required

Equipment and labware required

Quantity	Item
As required	Laboratory tissue
1	Mini microcentrifuge (microcentrifuge with microtube rotor)
1	Vortexer
Biomek™ i7 Automated Workstation	
• 1 • 5 • 5	Items required from the Axiom™ 384HT GeneTitan™ Consumables Kit (Cat. No. 902234) • Scan tray with cover and protective base • Stain trays • Covers for stain trays
• 2 boxes • 1 box	Pipette tips: • i-Series 190 µL filter tips (green) • i-Series 1,025 µL filter tips (orange)
1	Reagent block, chilled to 4°C
1	Quarter Reservoir, Divided by Width

(continued)

Quantity	Item
5	Quarter Reservoir
1	Half Reservoir
1	Deep 96 full reservoir
1	24-position tube rack with 2 inserts (room temperature rack)

Reagent handling

Prepare reagents according to the following table.

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

(continued)

Module	Qty	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	2	Axiom™ Stain 1-B[2]		✓		Gently flick tube 3 times, then centrifuge. Place in chilled reagent block.
	2	Axiom™ Stain 2-A[2]		✓		Gently flick tube 3 times, then centrifuge. Place in chilled reagent block.
	2	Axiom™ Stain 2-B[2]		✓		Gently flick tube 3 times, then centrifuge. Place in chilled reagent block.
	2	Axiom™ Stabilize Diluent		✓		Vortex, then centrifuge[1]. Place in chilled reagent block.
	1	Axiom™ Water		✓		Pour in reservoir.
	2	Axiom™ Hold Buffer[2]			✓	Vortex for 30 seconds. Pour in reservoir.
Estimated reagent thawing time is 1 hour.						

[1] Check for precipitate.
[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 pre-run checklist

The following actions are the same as described under “Perform the prerun checklist” on page 53. See this section for details. Some or all these steps are required depending on the current state of the Biomek™ i7 Automated Workstation.

1. Power on the Biomek™ i7 and all peripherals.
2. Check the waste container, then empty as required.
3. Open the Biomek™ Software using one of the following methods:
   - Double-click the Biomek™ icon on the desktop.
   - In the Windows Search box, enter **Biomek 5**.
4. Home all axes.

Run the GeneTitan™ reagent preparation step

1. Click **File** ▸ **Open**.
2. For **Look in** of the **Open Method** window, select **Axiom 384 on i7**.

3. Select **6 GeneTitan Reagent Preparation Ax384_i7**, then click **OK**.

4. At the top of the **Biomek Software** window, click ▶ **Run** to start the GeneTitan™ reagent preparation step.
   The deck layout for the GeneTitan™ reagent preparation step is displayed.

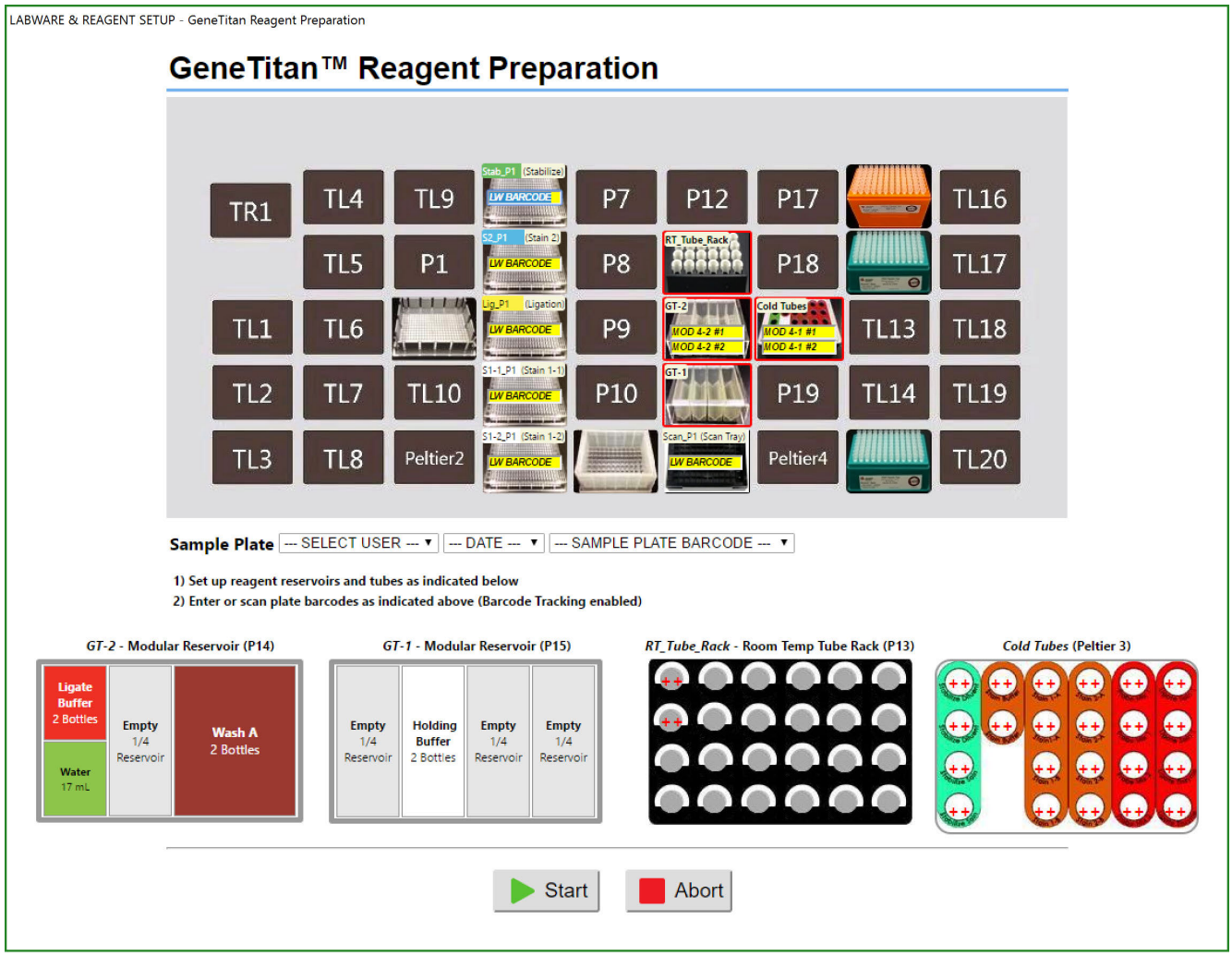


Figure 25 GeneTitan™ reagent preparation deck layout.

5. Place the labware and reagents on the deck as directed in the following figures and table: deck layout.

   - Figure 25—Deck layout.
   - Figure 26—Deck positions on the Biomek™ i7.
   - Table 12—Table detailing the labware, reagent, and modular reservoir placement for the deck layout.

Figure 26 Deck positions on the Biomek™ i7 Automated Workstation.

Table 12 Labware and reagent locations on the deck for the GeneTitan™ reagent preparation step.

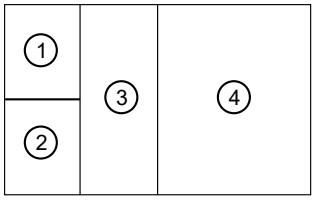
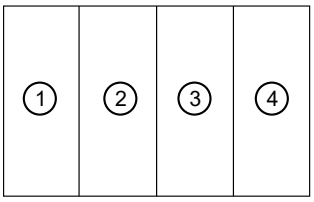
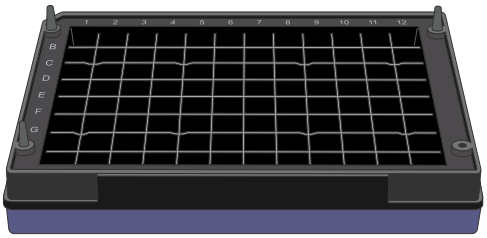
Deck position	Labware	Scan[1]	Reagent or sample
Peltier1	384 deep-well adapter (no other labware)		
P2	Axiom™ Stabilization Tray (green label)		
P3	Axiom™ Stain2 Tray (blue label)		
P4	Axiom™ Ligation Tray (yellow label)		
P5	GeneTitan™ Stain Tray (white label)		
P6	GeneTitan™ Stain Tray (white label)		
P11	Deep 96 full reservoir		
P13	24-Position Tube Rack		See Figure 28.
P14	Reservoirs in frame: • Quarter divided by width reservoir (1 and 2) • Quarter reservoir (3) • Half reservoir (4)		 ① Pour 2 bottles Axiom™ Ligate Buffer into reservoir 1. ② Pour 17 mL Axiom™ Water into reservoir 2. ③ Leave reservoir 3 empty. ④ Pour 2 bottles of Axiom™ Wash A into reservoir 4.

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

Deck position	Labware	Scan[1]	Reagent or sample
P15	Reservoirs in frame: • Quarter reservoir (1) • Quarter reservoir (2) • Quarter reservoir (3) • Quarter reservoir (4)		 ① Leave reservoir 1 empty. ② Pour 2 bottles of Axiom™ Hold Buffer into reservoir 2. ③ Leave reservoir 3 empty. ④ Leave reservoir 4 empty.
P16	GeneTitan™ Scan tray on blue protective base 		
Peltier3	Reagent block, chilled to 4°C		See Figure 27.
TL11	Pipette tips, i-Series 1,025 µL filter tips (orange)		
TL12	Pipette tips, i-Series 190 µL filter tips (green)		
TL15	Pipette tips, i-Series 190 µL filter tips (green)		

[1] Scan labware or reagent module barcode. Do not scan the image in this table.

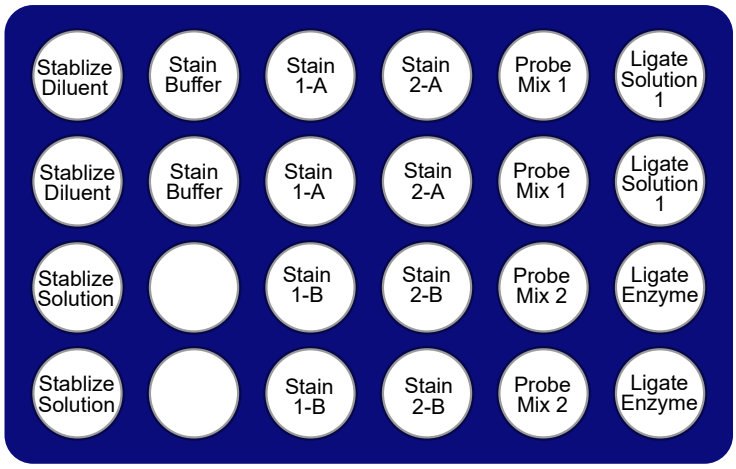


Figure 27 Reagent cold block for GeneTitan™ reagent preparation.

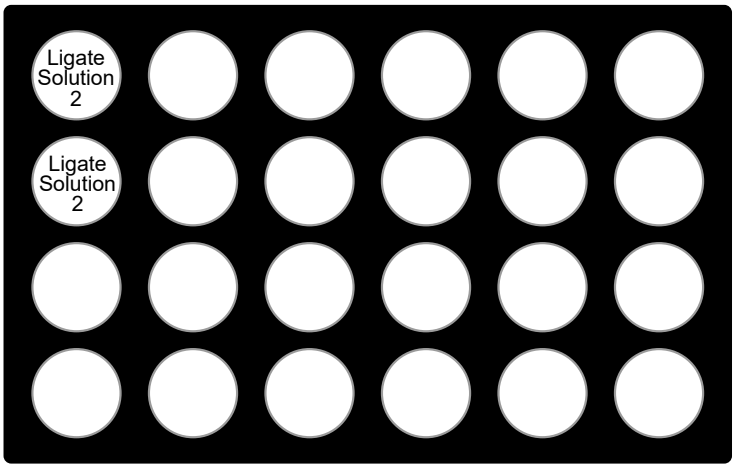


Figure 28 Placement on reagents on 24-position tube rack for GeneTitan™ reagent preparation.

6. Check the deck layout to ensure that the labware, reagents, and samples are in the proper locations.

7. For **Sample Plate**, make the appropriate selections from the dropdown lists under the deck setup image.

   Note: The **Date** dropdown list indicates the date that the "Transfer Samples to Hybridization Tray" step was run and the **Sample Plate Barcode** dropdown list indicates the barcode of the sample plate that is used during the amplification step (Sample_1).

8. Scan the labware and reagent barcodes.

9. Click **Start** to continue.
   The GeneTitan reagent preparation step on the Biomek™ i7 Automated Workstation starts.

10. Prepare the GeneTitan™ MC Instrument.
    During the reagent tray preparation on the Biomek™ i7 Automated Workstation, ensure that the GeneTitan™ MC Instrument is ready for use by following the instructions that are given in “Stage 3—Ligate, wash, stain, and scan” on page 134, and summarized here.
    a. Fill the Wash A, Wash B, and Rinse bottles with Axiom™ Wash Buffer A, Axiom™ Wash Buffer B, and Axiom™ Water from Module 3 respectively.
    b. Empty the Waste bottle.
    c. Empty the trash bin.
    d. 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.

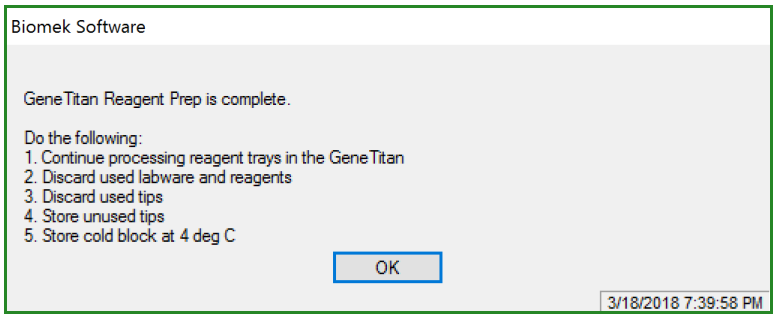
11. When message window appears indicating that the GeneTitan reagent preparation step is complete, *do not* click **OK** yet.

12. Remove the 5 stain trays with reagents and the scan tray with Axiom™ Hold Buffer from the deck, then examine each tray to ensure that:
    - All the wells have been filled. If any wells do not contain reagents, then manually add reagents to these wells.
    - There are no air bubbles present. Puncture any air bubbles that you see using a pipette tip.

13. Cover the 5 stain trays and the scan tray with lids. If needed, see Figure 48 for information on proper placement of the stain tray lids.

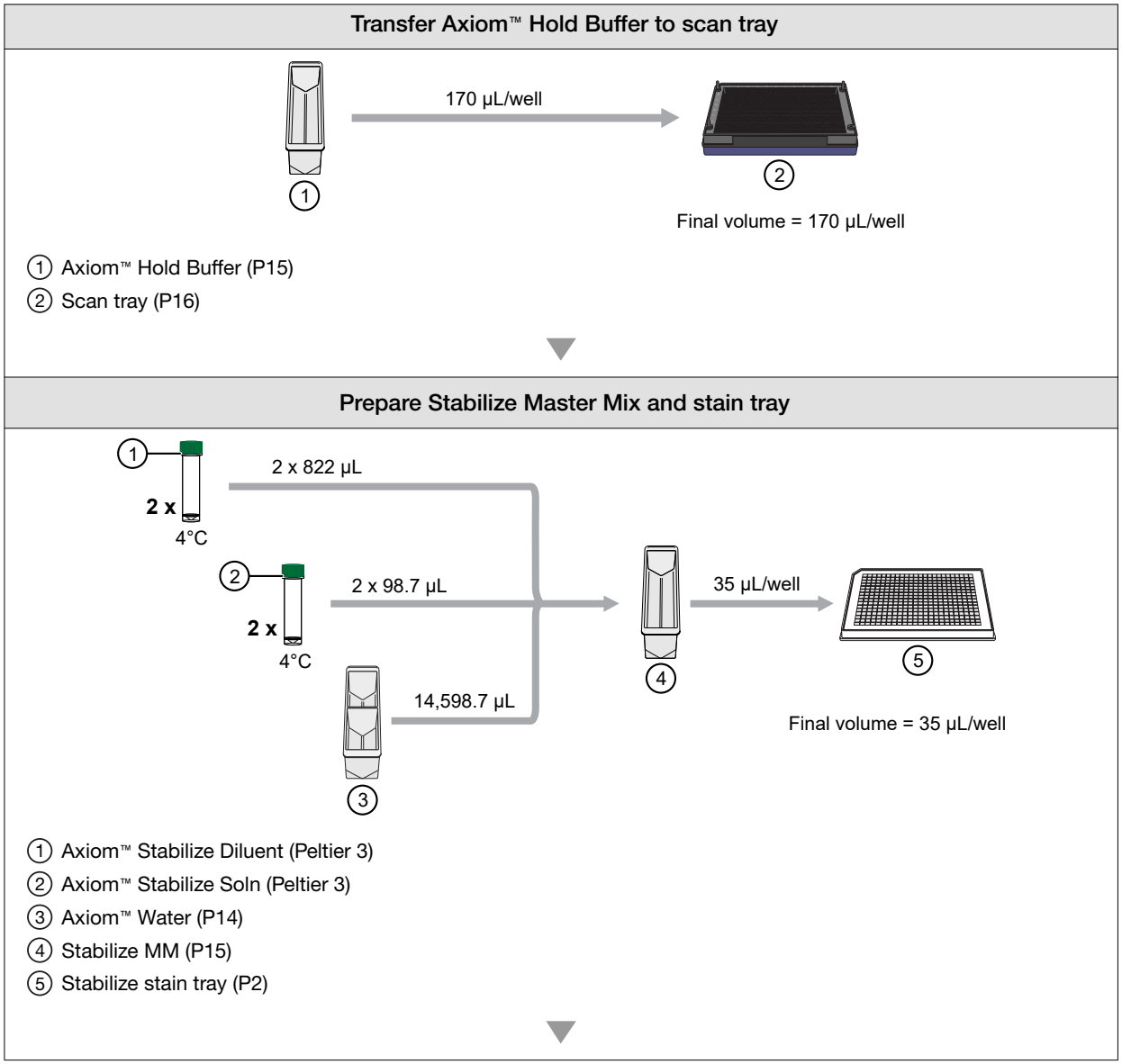
14. Immediately transfer the 5 stain trays and the scan tray to the GeneTitan™ MC Instrument and load. See "Stage 3—Ligate, wash, stain, and scan" on page 134 to continue the process on the GeneTitan™ MC Instrument.

15. Return to the Biomek™ i7 Automated Workstation, then clear the deck as instructed in the message window.

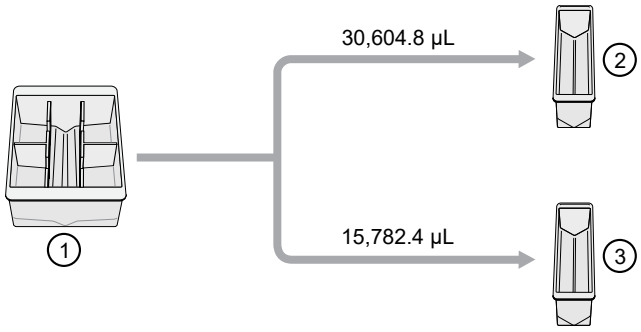


16. Complete the following tasks.
    a. Store the unused BC1025F tips from deck position TL11 and unused BC190F tips from deck position TL12.
    b. Discard the tubes from the cold block at the Peltier3 deck position. Store the cold block at 4°C.
    c. Discard the tubes from the 24-Position Tube Rack at deck position P13. Store the 24-Position Tube Rack.
    d. Discard the remaining labware and tips on the deck. Store the reservoir frames. Click **OK** when done.

Workflow for Stage 6: GeneTitan™ reagent preparation

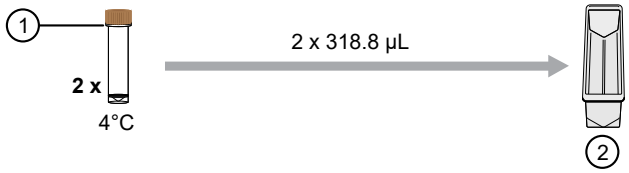


Transfer Axiom™ Wash A to Stain 1 & 2 Master Mix reservoirs



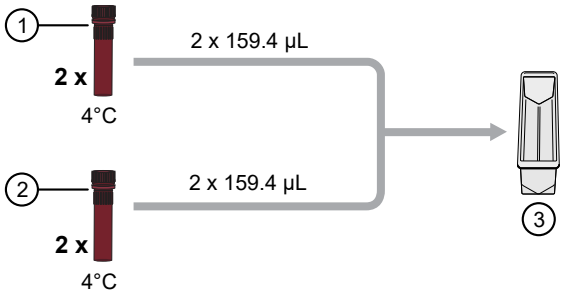
① Axiom™ Wash A (P14)
② Stain 1 MM (P14)
③ Stain 2 MM (P15)

Transfer Axiom™ Stain Buffer to Stain 1 Master Mix reservoir



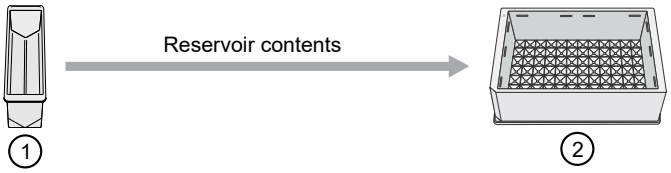
① Axiom™ Stain Buffer (Peltier 3)
② Stain 1 MM (P14)

Transfer Axiom™ Stain 1-A & Axiom™ Stain 1-B to Stain 1 Master Mix reservoir



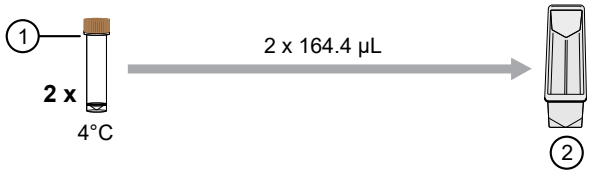
① Axiom™ Stain 1-A (Peltier 3)
② Axiom™ Stain 1-B (Peltier 3)
③ Stain 1 MM (P14)

Transfer Stain 1 Master Mix to Stain 1 reservoir



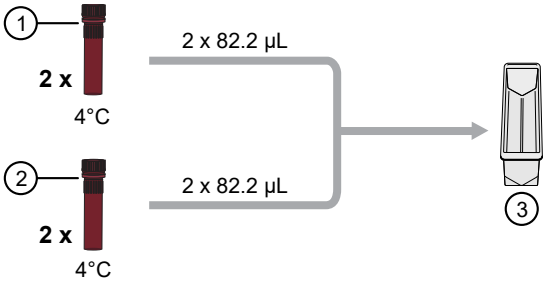
① Stain 1 MM (P14)
② Stain1 (P11)

Transfer Axiom™ Stain Buffer to Stain 2 Master Mix reservoir



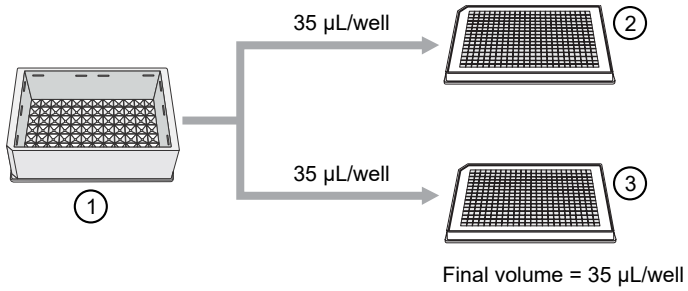
① Axiom™ Stain Buffer (Peltier 3)
② Stain 2 MM (P15)

Transfer Axiom™ Stain 2-A & Axiom™ Stain 2-B to Stain 2 Master Mix reservoir



① Axiom™ Stain 2-A (Peltier 3)
② Axiom™ Stain 2-B (Peltier 3)
③ Stain 2 MM (P15)

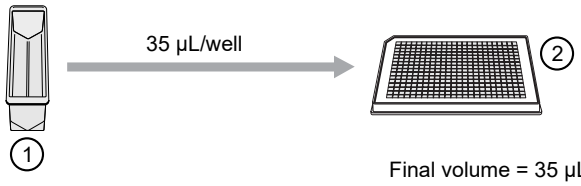
Prepare Stain 1 stain trays



Final volume = 35 µL/well

① Stain1 (P11)
② Stain 1-1 stain tray (P5)
③ Stain 1-2 stain tray (P6)

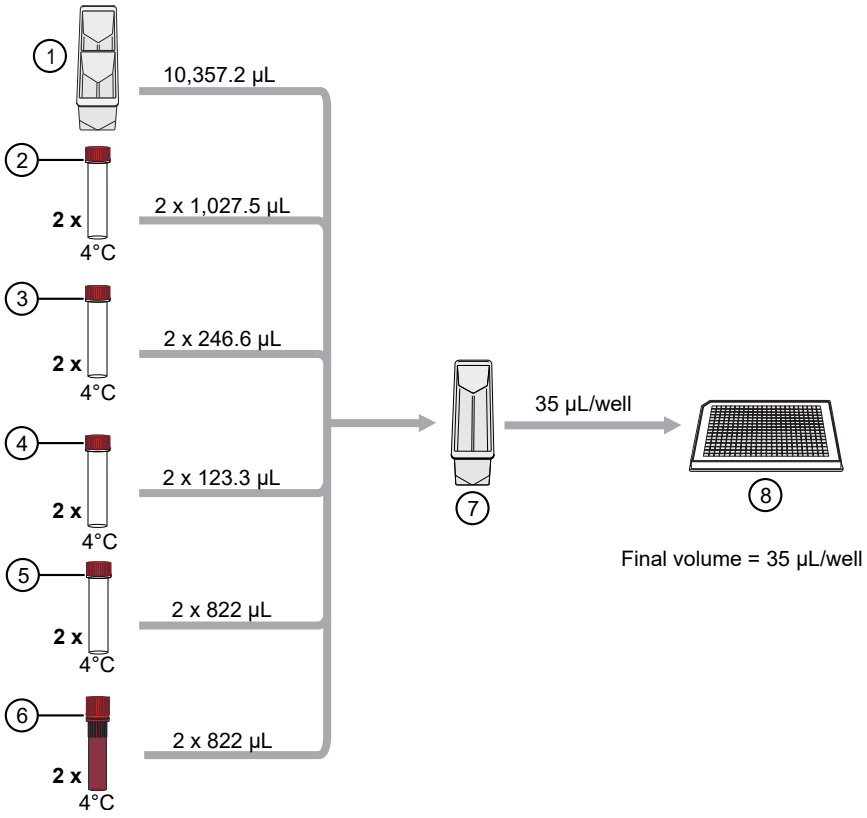
Prepare Stain 2 stain tray



Final volume = 35 µL/well

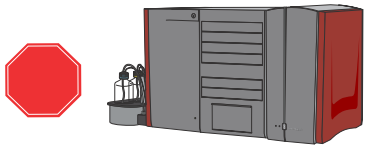
① Stain 2 MM (P15)
② Stain 2 stain tray (P3)

Prepare Ligate Master Mix & stain tray



1. Axiom™ Ligate Buffer (Peltier 3)
2. Axiom™ Ligate Soln 1 (Peltier 3)
3. Axiom™ Ligate Soln 2 (P11)
4. Axiom™ Ligate Enzyme (Peltier 3)
5. Axiom™ Probe Mix 1 (Peltier 3)
6. Axiom™ Probe Mix 2 (Peltier 3)
7. Ligate MM (P15)
8. Ligate stain tray (P4)

Stop



Place stain and scan trays in the GeneTitan™ Multi-Channel Instrument.

5 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.

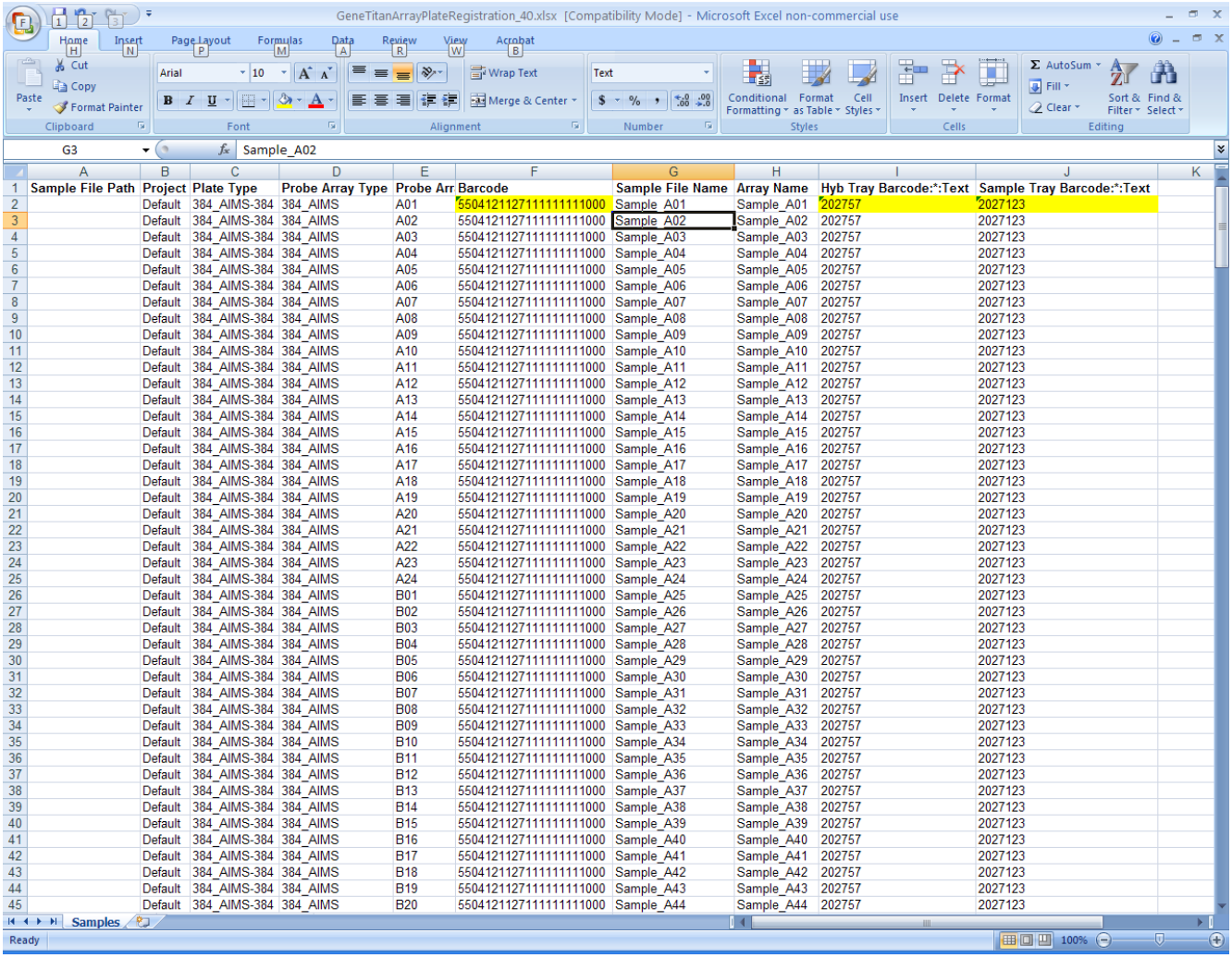
Stage 1—Create and upload a GeneTitan™ Array Plate Registration file

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 122. 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 29.
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	I	J	K
1	Sample File Path	Project	Plate Type	Probe Array Type	Probe Arr	Barcode	Sample File Name	Array Name	Hyb Tray Barcode:*:Text	Sample Tray Barcode:*:Text	
2		Default	384_AIMS-384	384_AIMS	A01	55041211271111111111000	Sample_A01	Sample_A01	202757	2027123	
3		Default	384_AIMS-384	384_AIMS	A02	55041211271111111111000	Sample_A02	Sample_A02	202757	2027123	
4		Default	384_AIMS-384	384_AIMS	A03	55041211271111111111000	Sample_A03	Sample_A03	202757	2027123	
5		Default	384_AIMS-384	384_AIMS	A04	55041211271111111111000	Sample_A04	Sample_A04	202757	2027123	
6		Default	384_AIMS-384	384_AIMS	A05	55041211271111111111000	Sample_A05	Sample_A05	202757	2027123	
7		Default	384_AIMS-384	384_AIMS	A06	55041211271111111111000	Sample_A06	Sample_A06	202757	2027123	
8		Default	384_AIMS-384	384_AIMS	A07	55041211271111111111000	Sample_A07	Sample_A07	202757	2027123	
9		Default	384_AIMS-384	384_AIMS	A08	55041211271111111111000	Sample_A08	Sample_A08	202757	2027123	
10		Default	384_AIMS-384	384_AIMS	A09	55041211271111111111000	Sample_A09	Sample_A09	202757	2027123	
11		Default	384_AIMS-384	384_AIMS	A10	55041211271111111111000	Sample_A10	Sample_A10	202757	2027123	
12		Default	384_AIMS-384	384_AIMS	A11	55041211271111111111000	Sample_A11	Sample_A11	202757	2027123	
13		Default	384_AIMS-384	384_AIMS	A12	55041211271111111111000	Sample_A12	Sample_A12	202757	2027123	
14		Default	384_AIMS-384	384_AIMS	A13	55041211271111111111000	Sample_A13	Sample_A13	202757	2027123	
15		Default	384_AIMS-384	384_AIMS	A14	55041211271111111111000	Sample_A14	Sample_A14	202757	2027123	
16		Default	384_AIMS-384	384_AIMS	A15	55041211271111111111000	Sample_A15	Sample_A15	202757	2027123	
17		Default	384_AIMS-384	384_AIMS	A16	55041211271111111111000	Sample_A16	Sample_A16	202757	2027123	
18		Default	384_AIMS-384	384_AIMS	A17	55041211271111111111000	Sample_A17	Sample_A17	202757	2027123	
19		Default	384_AIMS-384	384_AIMS	A18	55041211271111111111000	Sample_A18	Sample_A18	202757	2027123	
20		Default	384_AIMS-384	384_AIMS	A19	55041211271111111111000	Sample_A19	Sample_A19	202757	2027123	
21		Default	384_AIMS-384	384_AIMS	A20	55041211271111111111000	Sample_A20	Sample_A20	202757	2027123	
22		Default	384_AIMS-384	384_AIMS	A21	55041211271111111111000	Sample_A21	Sample_A21	202757	2027123	
23		Default	384_AIMS-384	384_AIMS	A22	55041211271111111111000	Sample_A22	Sample_A22	202757	2027123	
24		Default	384_AIMS-384	384_AIMS	A23	55041211271111111111000	Sample_A23	Sample_A23	202757	2027123	
25		Default	384_AIMS-384	384_AIMS	A24	55041211271111111111000	Sample_A24	Sample_A24	202757	2027123	
26		Default	384_AIMS-384	384_AIMS	B01	55041211271111111111000	Sample_A25	Sample_A25	202757	2027123	
27		Default	384_AIMS-384	384_AIMS	B02	55041211271111111111000	Sample_A26	Sample_A26	202757	2027123	
28		Default	384_AIMS-384	384_AIMS	B03	55041211271111111111000	Sample_A27	Sample_A27	202757	2027123	
29		Default	384_AIMS-384	384_AIMS	B04	55041211271111111111000	Sample_A28	Sample_A28	202757	2027123	
30		Default	384_AIMS-384	384_AIMS	B05	55041211271111111111000	Sample_A29	Sample_A29	202757	2027123	
31		Default	384_AIMS-384	384_AIMS	B06	55041211271111111111000	Sample_A30	Sample_A30	202757	2027123	
32		Default	384_AIMS-384	384_AIMS	B07	55041211271111111111000	Sample_A31	Sample_A31	202757	2027123	
33		Default	384_AIMS-384	384_AIMS	B08	55041211271111111111000	Sample_A32	Sample_A32	202757	2027123	
34		Default	384_AIMS-384	384_AIMS	B09	55041211271111111111000	Sample_A33	Sample_A33	202757	2027123	
35		Default	384_AIMS-384	384_AIMS	B10	55041211271111111111000	Sample_A34	Sample_A34	202757	2027123	
36		Default	384_AIMS-384	384_AIMS	B11	55041211271111111111000	Sample_A35	Sample_A35	202757	2027123	
37		Default	384_AIMS-384	384_AIMS	B12	55041211271111111111000	Sample_A36	Sample_A36	202757	2027123	
38		Default	384_AIMS-384	384_AIMS	B13	55041211271111111111000	Sample_A37	Sample_A37	202757	2027123	
39		Default	384_AIMS-384	384_AIMS	B14	55041211271111111111000	Sample_A38	Sample_A38	202757	2027123	
40		Default	384_AIMS-384	384_AIMS	B15	55041211271111111111000	Sample_A39	Sample_A39	202757	2027123	
41		Default	384_AIMS-384	384_AIMS	B16	55041211271111111111000	Sample_A40	Sample_A40	202757	2027123	
42		Default	384_AIMS-384	384_AIMS	B17	55041211271111111111000	Sample_A41	Sample_A41	202757	2027123	
43		Default	384_AIMS-384	384_AIMS	B18	55041211271111111111000	Sample_A42	Sample_A42	202757	2027123	
44		Default	384_AIMS-384	384_AIMS	B19	55041211271111111111000	Sample_A43	Sample_A43	202757	2027123	
45		Default	384_AIMS-384	384_AIMS	B20	55041211271111111111000	Sample_A44	Sample_A44	202757	2027123	

Figure 29 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 384HT Reagent Kit are required for the hybridization step.

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

Materials required

- Hybridization tray containing denatured samples.

  Note: The denatured samples must be transferred to the hybridization tray only after the GeneTitan™ MC Instrument is ready for loading, described in the "Load an array plate and hybridization tray into the GeneTitan™ MC Instrument" on page 127.

- A human or non-human Axiom™ 384HT Array Plate or an Axiom™ myDesign™ Array Plate (384HT format) 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 120.

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

Set up the GeneTitan™ MC Instrument

1. 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 141.

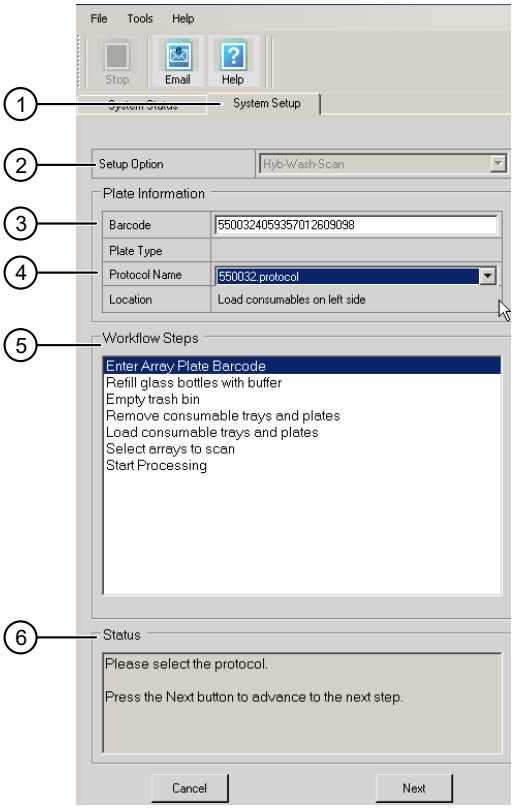


Figure 30 System Setup tab

① **System Setup** tab.

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

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

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

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

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

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

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

   Other options available are described under “Setup options for array plate processing” on page 149.

3. Click **Next**.

Note: A message is displayed when there is insufficient disk space. Delete or move .dat files to another location to free up sufficient disk space for the data that are generated by 8 Axiom™ 384HT Array Plates. One 384HT-array plate requires ~27 GB.

---

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

   The first six 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** dropdown list, then click **Next**.

6. Refill the bottles with Module 3 reagents.

   - Wash A: fill with Axiom™ Wash Buffer A—keep at 2 L full.
   - Wash B: fill with Axiom™ Wash Buffer B—use all 600 mL of Wash B from the reagent kit per Axiom™ 384HT Array Plate. Fill to 1-L mark when processing two plates on the same day.
   - Rinse: fill with Axiom™ Water—keep at 1 L full.

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

   All 600 mL of the Wash Buffer B from the Axiom™ 2.0 384HT Reagent Kit must be emptied into the GeneTitan™ Wash B bottle when setting up the system.
   - Using all the Wash B contents from the reagent kit helps ensures that the GeneTitan™ Wash B bottle is filled to more than the minimum requisite 35% of bottle volume.
   - If you intend to load two array plates on the same day, fill the Wash B bottle to the 1-L mark (use both bottles from the Axiom™ 2.0 384HT Reagent Kit).

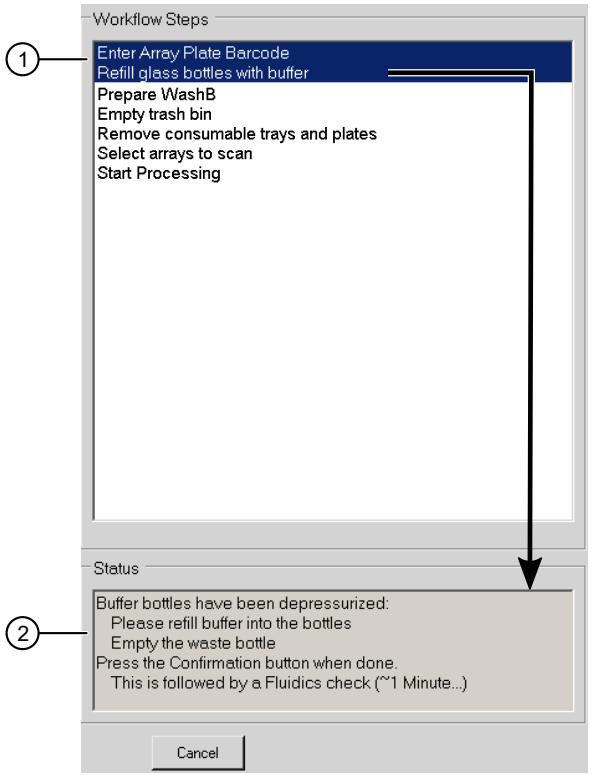
   Do not overfill the bottles.
   - The maximum volume for the Wash B and Rinse bottles is 1 L. Fill to the 1-L mark only.
   - The maximum volume for the Wash A bottle is 2 L.

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

   ---

7. Empty the waste bottle.

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



① **Workflow Step**

② Specific instructions for the current workflow step 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 blue confirmation button to continue.
10. Remove consumable trays and plates.
    a. Remove used trays and plates when drawers open.
    b. If no consumables to remove, the **Status** window reads "Drawers are empty".
    c. Press the blue confirmation button on the GeneTitan™ Instrument to continue.
11. Continue to “Load an array plate and hybridization tray into the GeneTitan™ MC Instrument” on page 127 when prompted by the GCC software.

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

The steps in "Set up the GeneTitan™ MC Instrument" on page 123 must be completed before starting the following procedure.

1. When drawer 6 opens, load the array plate and hybridization tray in the following manner:
   a. Examine the wells of the hybridization tray for bubbles, then puncture any bubbles with a pipette tip.

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

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

   c. Remove the array plate and protective blue base from its package. For more information on the array packaging, see "Array plate packaging" on page 143.

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

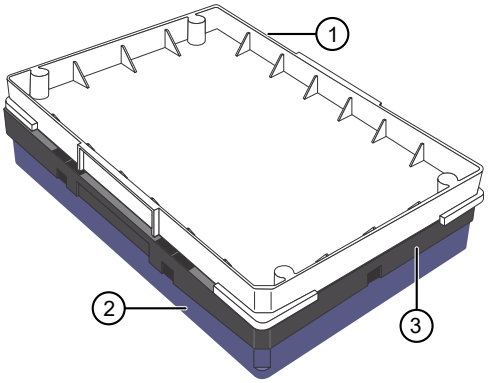


Figure 31 Array plate components, as shipped.

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

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

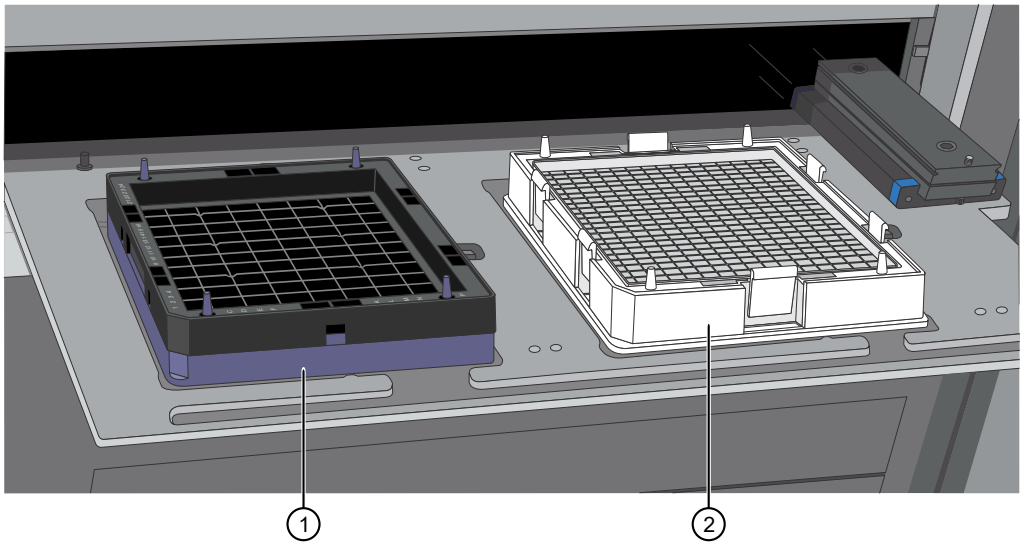


Figure 32 Array plate on protective blue base and the hybridization tray properly loaded into drawer 6.

① Array plate on protective base
② Hybridization tray

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

CAUTION! The notched corner of each plate, cover, and tray must be aligned. When loading onto the GeneTitan™ MC Instrument, the notched edge plates, covers, and trays must be aligned as indicated by the Tray Alignment guide in the drawer.

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

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

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

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

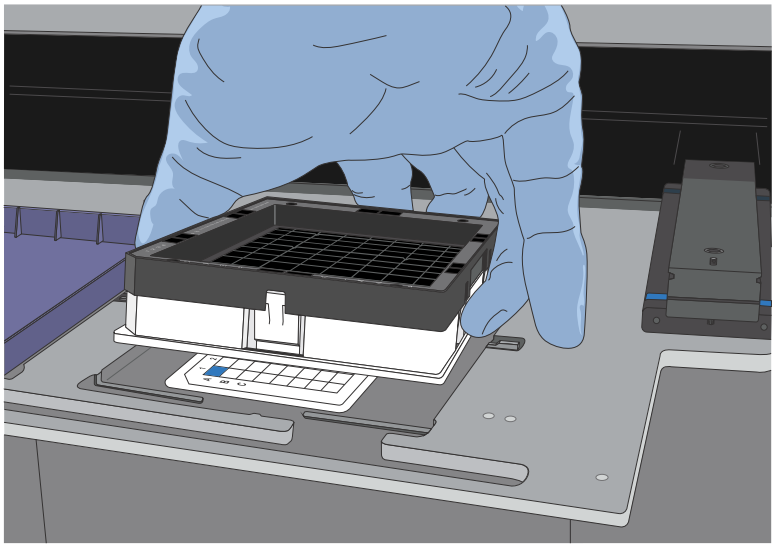
2. Select the arrays to scan. By default, all arrays are selected.

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

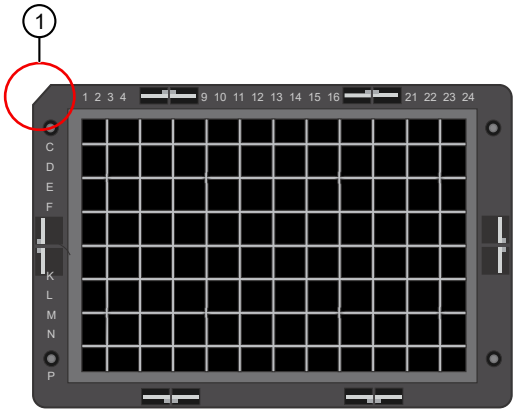
4. Press **OK**, then wait for the drawer to open completely before retrieving the array plate and hybridization tray combination for manual clamping and inspection.
   After clamping is complete in the instrument, drawer 6 opens and the **Ensure Clamping** dialog appears. Do not click **OK** yet. The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

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

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

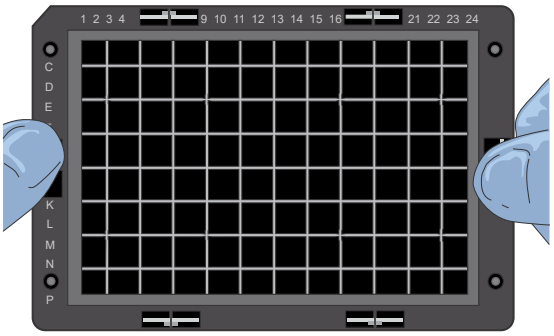


b. Place the plate stack on a flat surface of the table or the lab bench. Position the plate stack to match the orientation as shown in the picture.

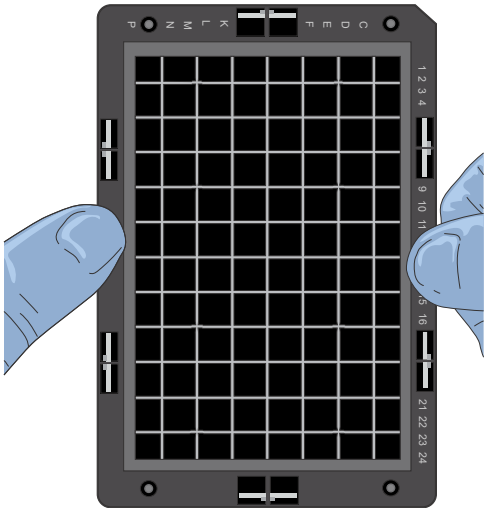


① Chamfer/notched corner

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



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



6. Ensure the clamping of the plate stack to check that the array plate is securely fastened to the hybridization tray. Using your thumbs, press the array plate downward following the positions that are specified in Figure 33. No clicking sound indicates proper clamping.

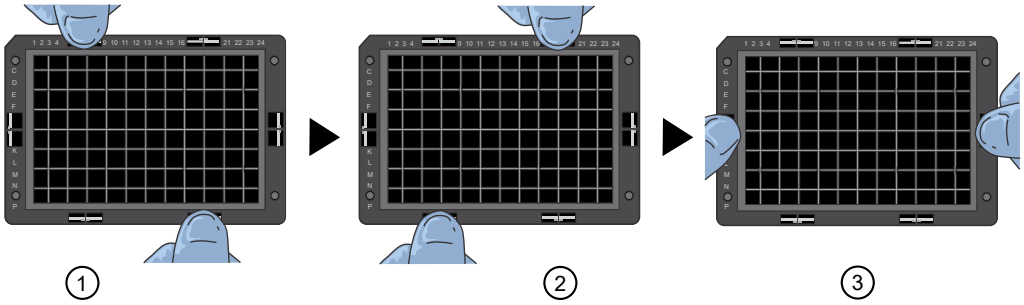


Figure 33 Clamping verification steps.

7. Inspect the array plate for bubbles.
   a. **Keeping the plate stack level**, inspect the bottom for bubbles under the arrays—*do not* tilt or invert the plates.
   b. If bubbles are present, gently tap the plate until the bubbles move out from under the arrays—*do not* unclamp the plate stack.
8. Return the plate stack to the drawer with the notched corner facing you, then press the blue confirmation button on the GeneTitan™ Instrument to proceed.
9. A message is displayed if plate orientation is not correct or if the hybridization tray barcode cannot be read. If this message appears, complete one or both of the following actions.
   - Check the loading of the array plate and click **OK**.
   - Click **Skip** if the instrument continues to have problems reading the barcode and after ensuring that the correct trays have been placed in the proper orientation.
10. Continue to “Load a second array plate and hybridization tray” on page 132.

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 34). You cannot load another hybridization tray before or after this time.

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

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

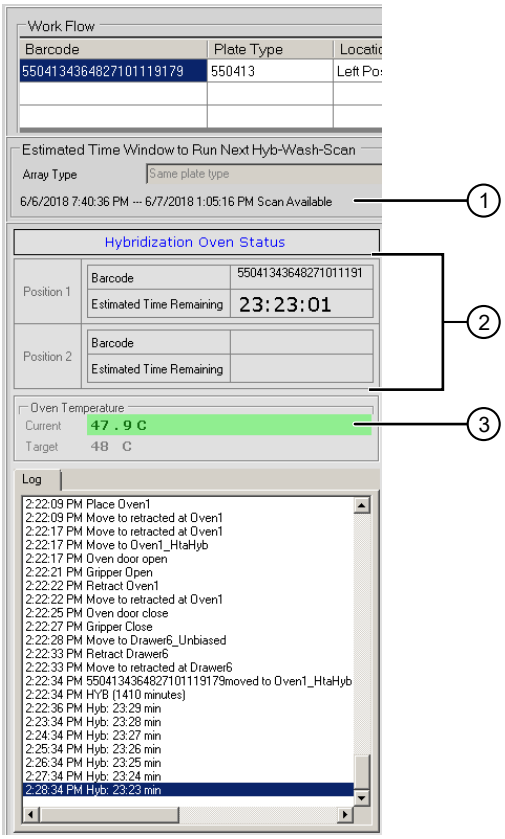


Figure 34 When to load a second array plate and hybridization tray based on oven status information.

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.

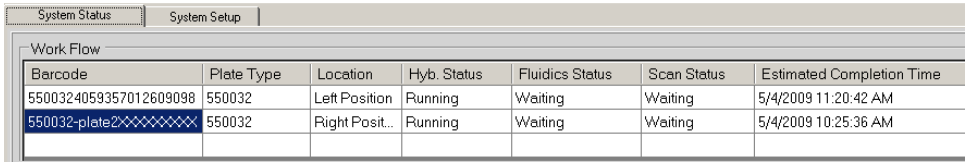


Figure 35 Example of the Work Flow pane when 2 plates are loaded and are in the hybridization oven.

Queue a second plate for scanning

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

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

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

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

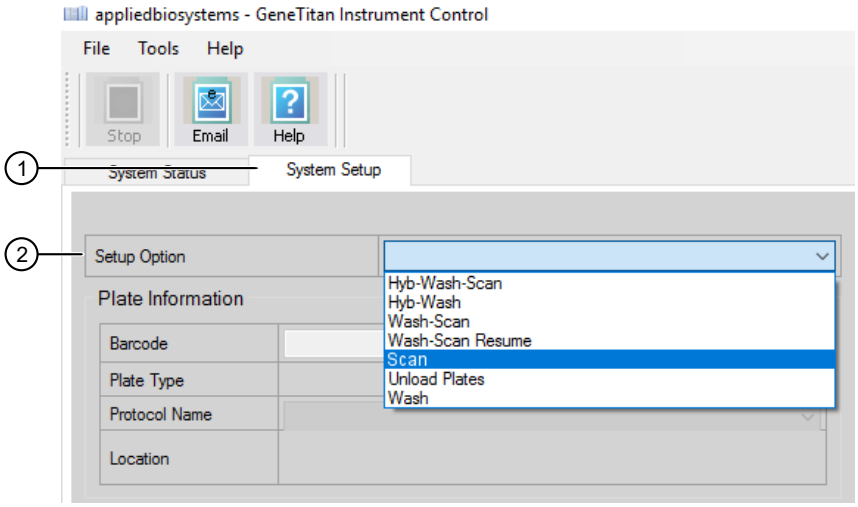


Figure 36 Scan setup option to process a second array plate.

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

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

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

5. Follow the instructions in the **Status** pane and empty the trash bin if needed, then press the blue confirmation button on the instrument to continue.

6. Place the array plate on top of a scan tray in the correct orientation such that notched corner of the array plate and scan tray are aligned.

7. Load the array plate/scan tray combination in drawer 2 of the GeneTitan™ MC Instrument, on the left or right side, as instructed in the **Status** pane. Ensure that the array plate/scan tray combination is loaded in the correct orientation in the drawer. If needed, see Figure 41 for further information on the proper alignment and loading of plates, covers, and trays in the GeneTitan™ MC Instrument.

8. When ready, press the blue confirmation button on the instrument.

9. Select the arrays to scan in the **Array Selection** section in the upper right corner of the window, then click **Next**.

10. In the **Start Processing** confirmation message, click **OK** to continue.
    The second queued plate runs after the first scan finishes and the scanner is available.

Stage 3—Ligate, wash, stain, and scan

The GeneTitan™ tray loading process

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

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

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

   b. Empty the trash bin.

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

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

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

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

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

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

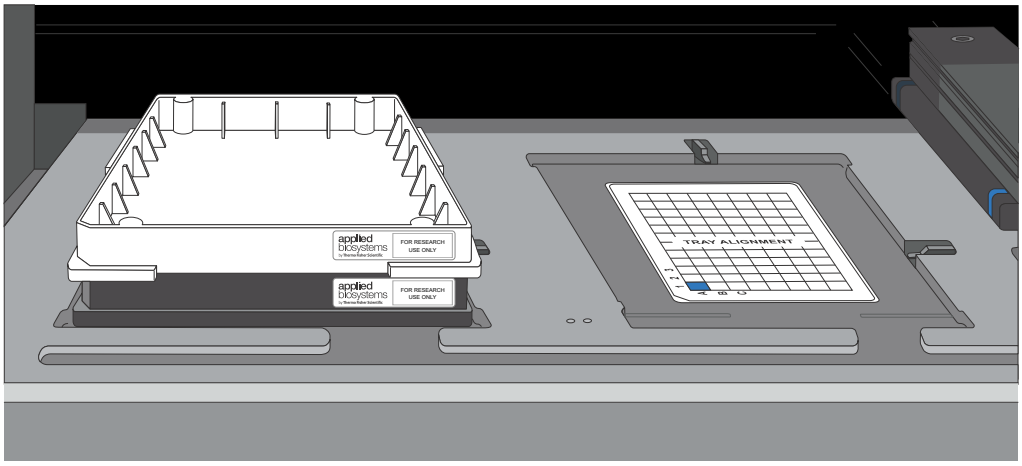


Figure 37 Drawer 2: Scan tray with cover on left side.

b. When drawer 3 opens:

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

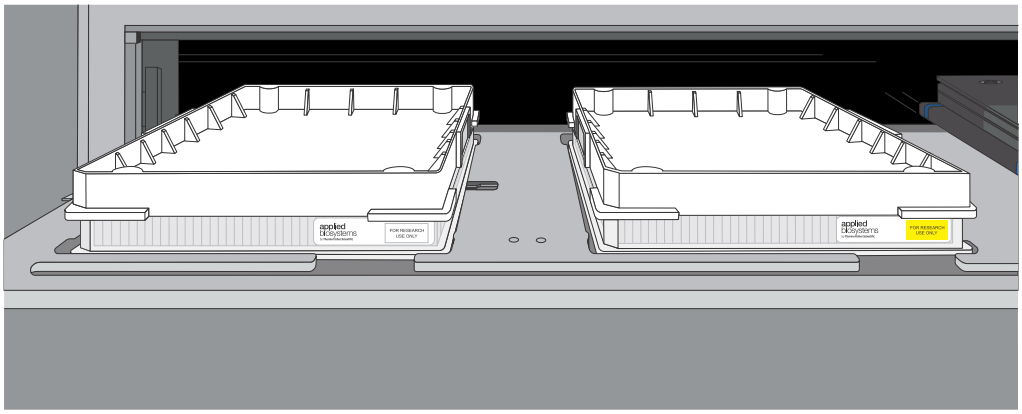


Figure 38 Drawer 3 trays.

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

c. When drawer 4 opens:

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

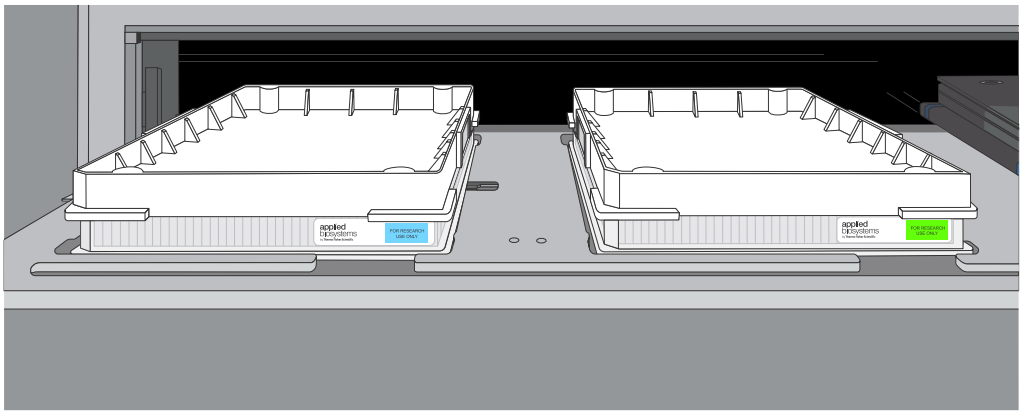


Figure 39 Drawer 4 trays.

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

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

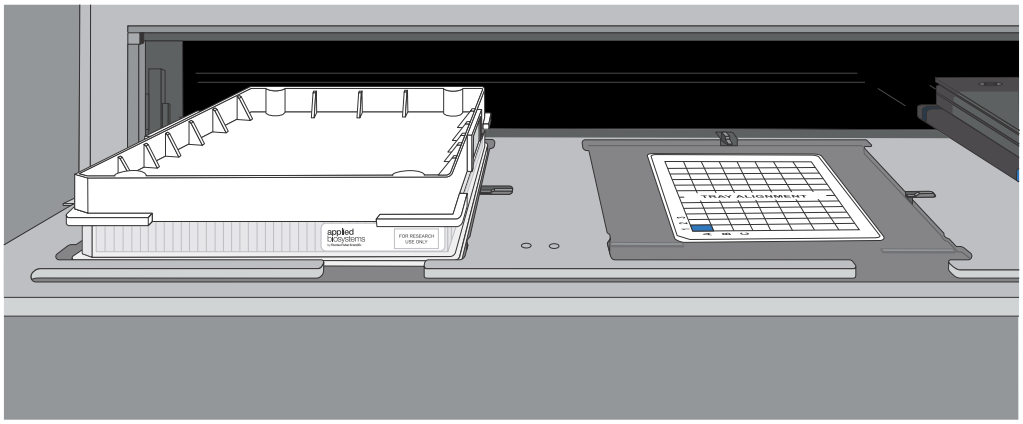


Figure 40 Drawer 5: Stain 1 tray with cover (white label) on left side.

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

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

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

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

7. When drawer 6 opens:
   a. Remove the blue cover from the previous array plate.

   b. Load a new array plate and new blue base on the left side of the drawer, then load a new hybridization tray on the right side of the drawer.

   c. Press the confirmation button.

   d. Click **OK** to continue.

   e. When drawer 6 opens, ensure that the plate stack is securely clamped. For clamping procedure review, see step 5 on page 129.

   f. Press the blue confirmation button.

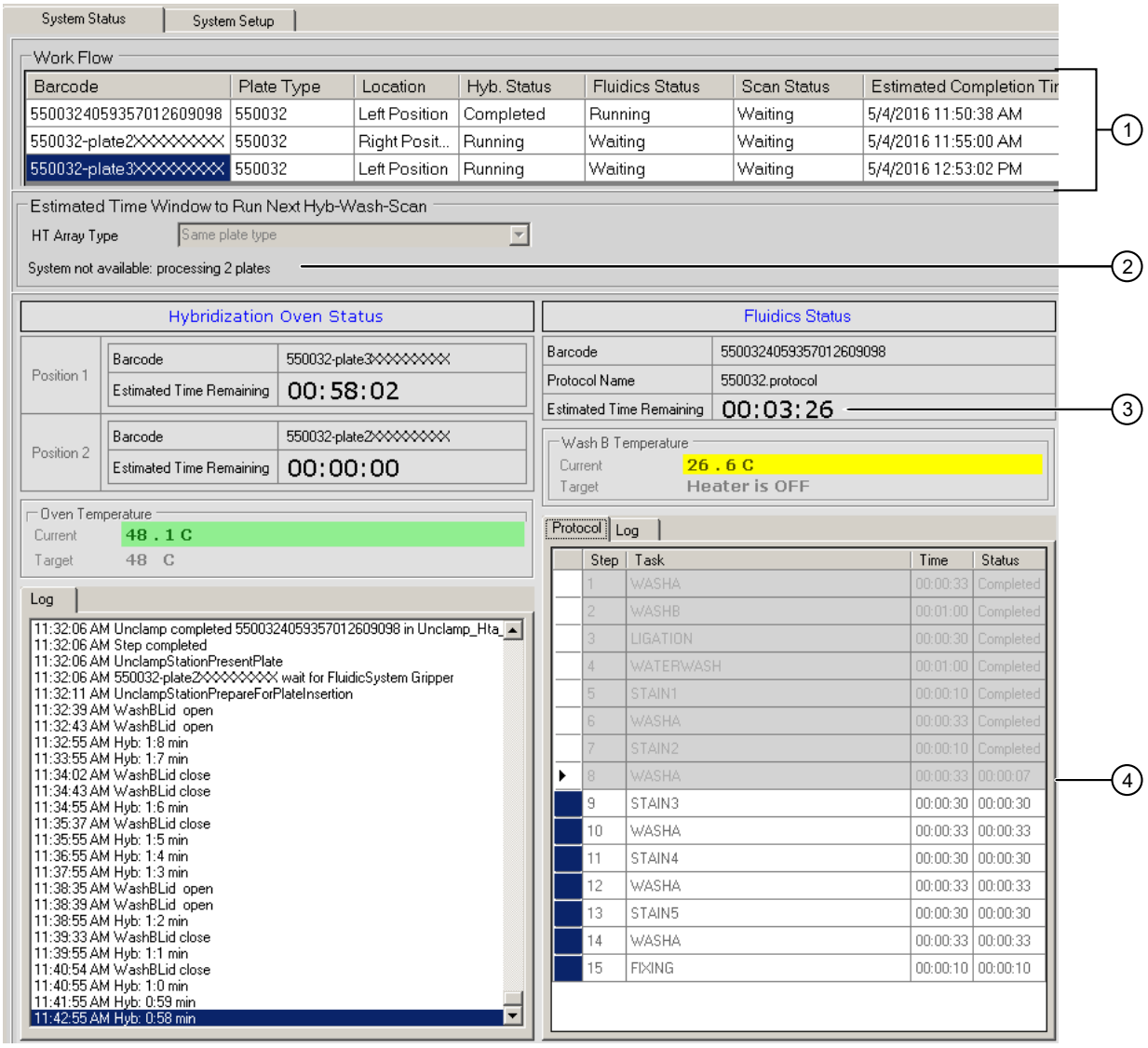
GeneTitan™ MC Instrument internal array plate activity

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

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

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

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



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

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

③ **Estimated Time Remaining** is displayed for the current process. 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.

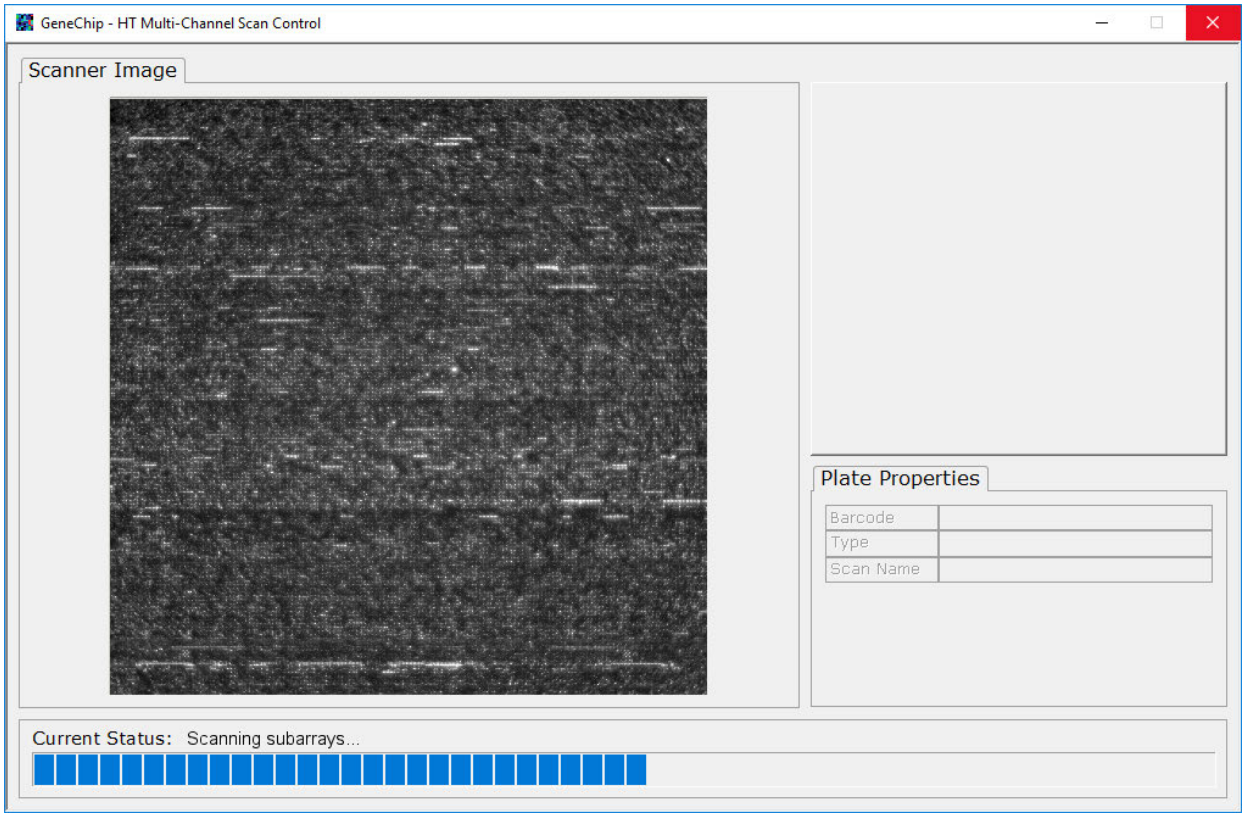
Scan workflow

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.

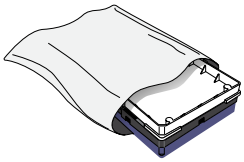
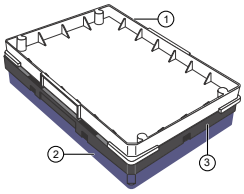
1. From the **System Setup** window, open the **Setup Options** dropdown list, then select **Unload Plates**.
2. Unload all the consumables as prompted.
3. Power off the GeneTitan™ MC Instrument by opening **Tools** ▸ **Shutdown**.
4. Exit the GCC software if it does not close automatically.

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

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 Assay 384HT 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	Part No.	Image	Details
Axiom™ 384HT Array Plate or custom Axiom™ 384HT myDesign™ Array Plate	All array plates have the Part No. 202091 etched on the plastic	  ① Shipping cover (to be discarded) ② Array plate protective base ③ Array plate	The array plate package includes the following: • White plastic cover: The function of the white plastic cover for the array plate is to protect the array plate during transport. You can discard this after removing the array plate. • Array plate: The array plate must be protected at all times from damage or exposure to dust. The array plate must be in the blue array plate protective base at all times. • Protective base: The blue array plate protective base in the package must be used to protect the array plate from damage. • Desiccant pack: The desiccant pack can be discarded after the array plate is removed from the pouch.

Proper tray alignment and placement

Proper alignment and loading of plates, covers, and trays is critical when using the GeneTitan™ MC Instrument. Each plate, cover, and tray has one notched corner. The notched corner of plates, trays, covers, and bases must be in vertical alignment with each other and placed in position A1 per the Tray Alignment guide inside each GeneTitan™ MC Instrument drawer.

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

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

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

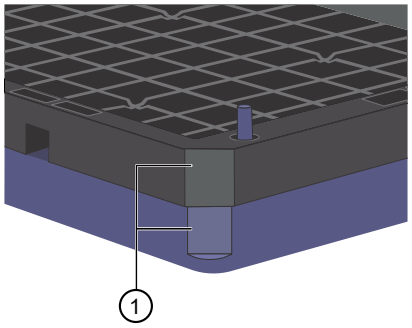


Figure 41 Notched corners aligned.

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

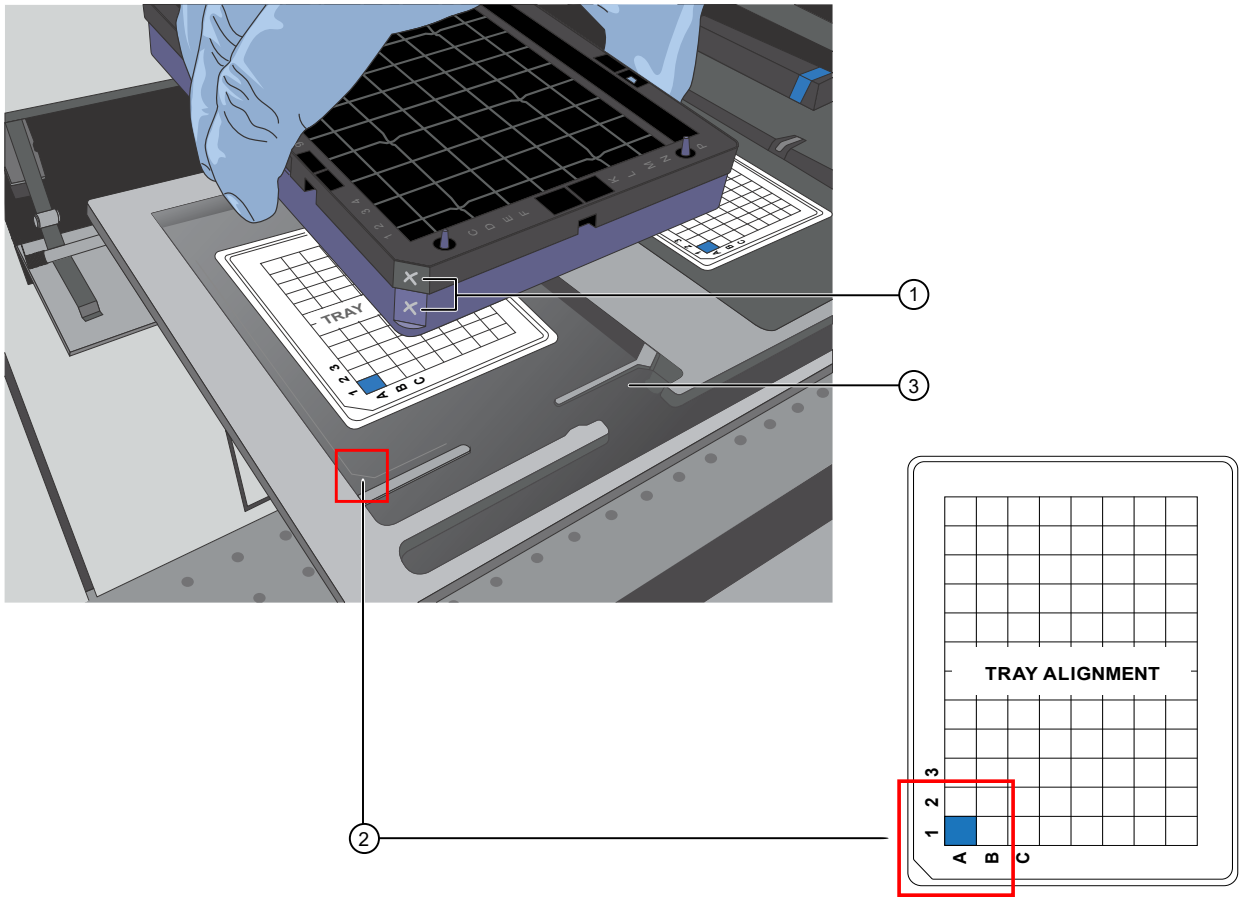


Figure 42 Notched corners marked and aligned with tray alignment guide.

① The notched corners of array plate and base that are marked with permanent marker.

② The notched corner of all plates, bases, and covers and must be seated in the front left corner of the drawer, as indicated in the Tray Alignment guide.

③ Plates and trays must be seated in this groove.

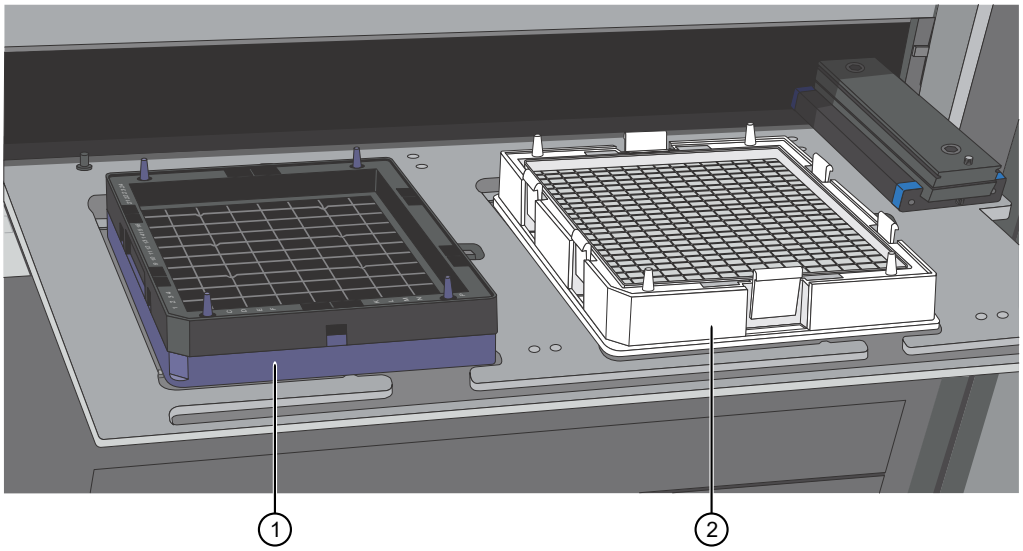


Figure 43 Array plate with blue protective base and the hybridization tray aligned properly loaded into drawer 6.

① Array plate on blue protective base
② Hybridization tray

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.

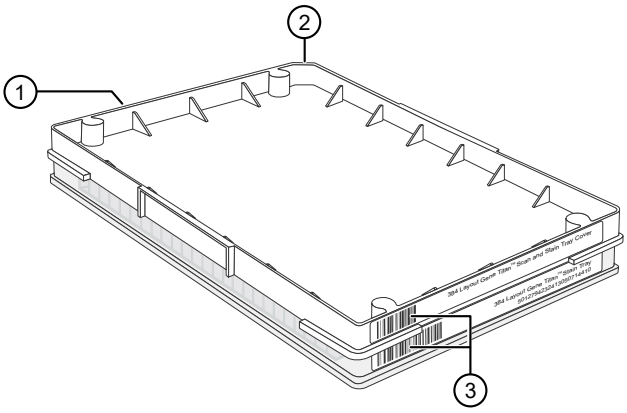


Figure 44 Example shows consumables that must be rotated and loaded on the drawer so that the barcodes face into the instrument.

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.

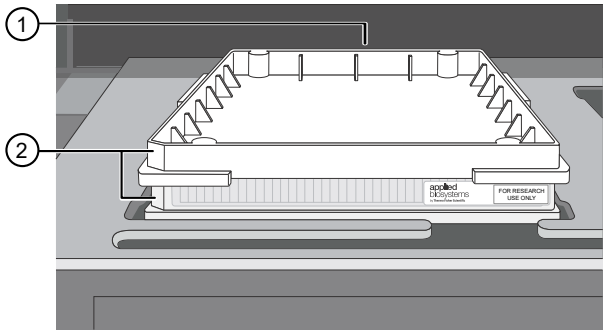


Figure 45 Example of properly loaded GeneTitan™ tray consumables. A GeneTitan™ stain tray and the stain tray cover are shown in this example.

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

Drawer tabs in the GeneTitan™ MC Instrument

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

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

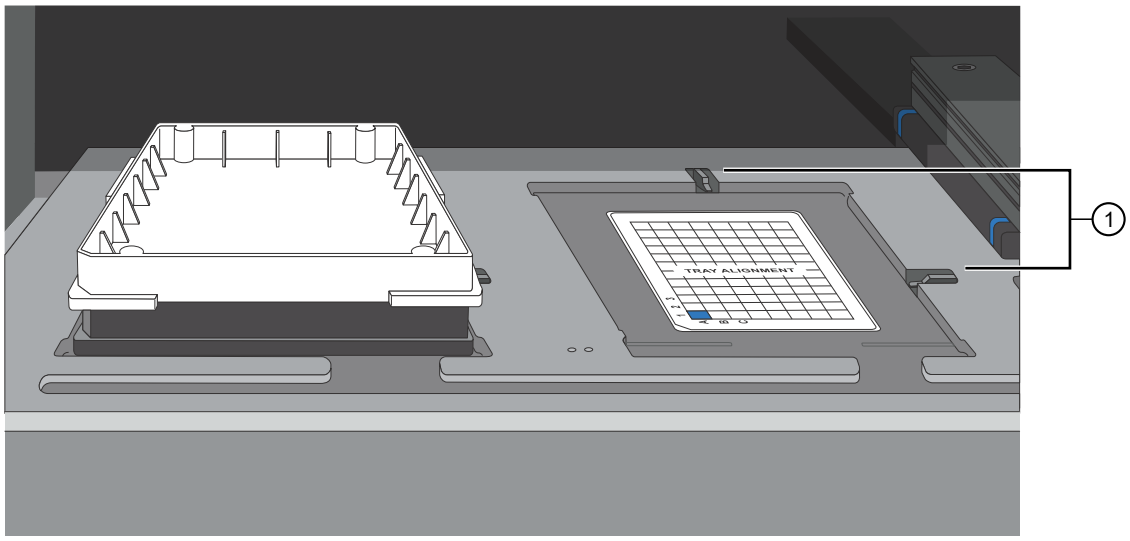


Figure 46 Location of drawer tabs, or fingers.

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

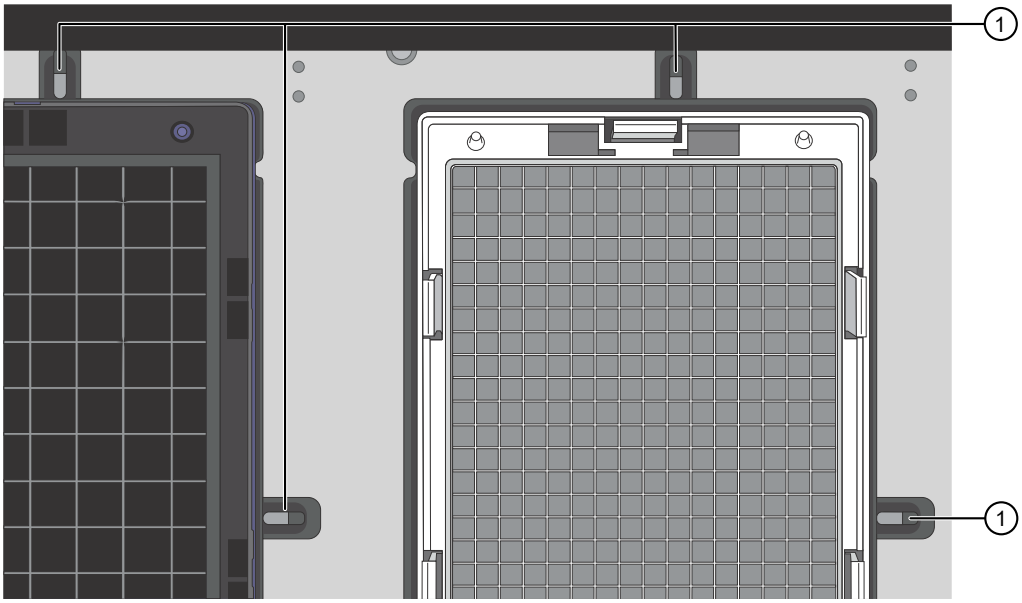


Figure 47 Place trays on the drawer when the tabs, or fingers, are retracted.

① Fingers retracted

Stain trays and covers

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

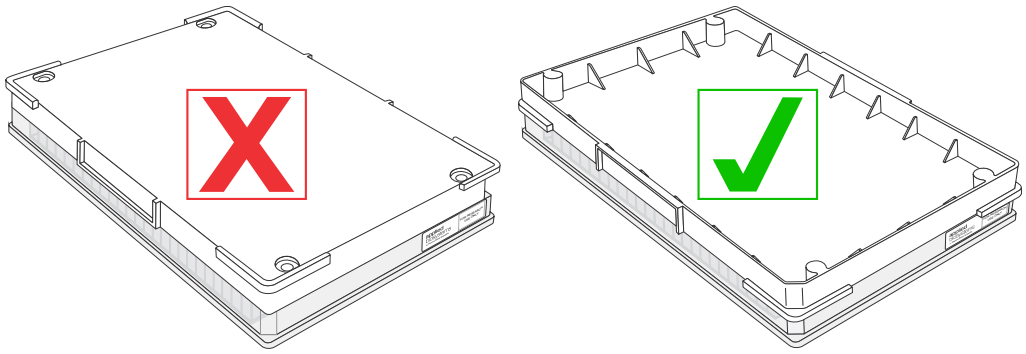


Figure 48 Placement of covers on trays.

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 support proper placement of lids onto stain trays, and trays onto the GeneTitan™ MC Instrument, you can also mark the notched corner of the trays and lids.

Label a GeneTitan™ 384 Layout Hybridization Tray

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

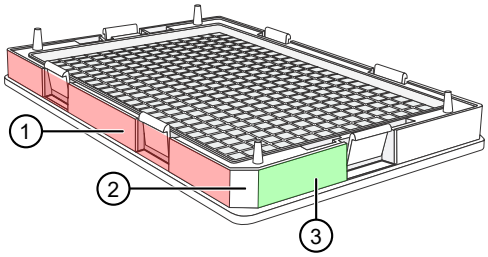


Figure 49 Correct area to label a GeneTitan™ 384 Layout Hybridization Tray.

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

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

Label a GeneTitan™ 384 Layout Reagent Tray

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

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

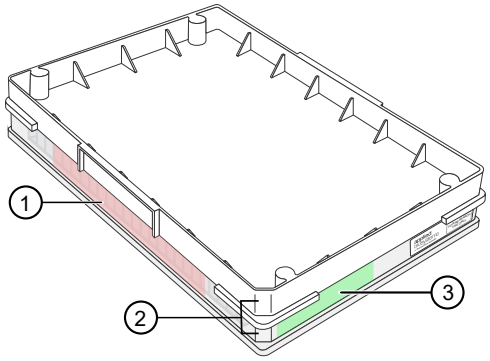


Figure 50 Correct area to label a GeneTitan™ 384 Layout Reagent Tray.

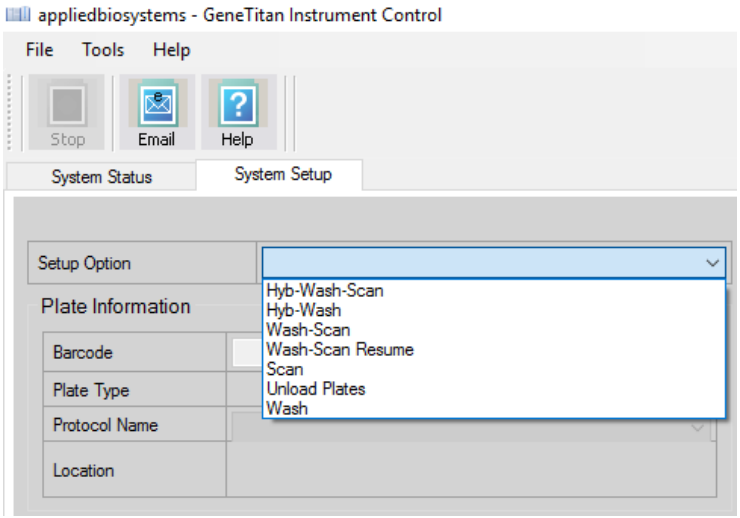
① Do not label the reagent tray on the long side.
② Notched corners of the reagent tray and cover must align and face the front.
③ Label the reagent tray here.

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 384 samples = 23.5 hours
- **Wash**: Samples on arrays are ligated, washed, stained, and stabilized.
  - Time that is required for 384 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 384 samples = ~5.5 hours

Hyb-Wash

When the **Hyb-Wash** setup option is selected, processing stops after the array has gone through fluidics processing. Use this option if an array plate cannot be scanned on the same GeneTitan™ MC Instrument as the one used for hybridization and fluidics processing.

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

      No lid is required. Do not tilt or invert the plate stack. If tilted or inverted, the Hold Buffer spills out of the tray. To prevent liquid spillage, keep the plate level when handling it. Do not touch the bottom optical surface of the scan tray.

   b. Store at 4°C.

   c. Scan the array plate in 3 days or less.

2. When ready to scan, prepare the array plate following these steps:
   a. Protect the plate from light.

   b. Bring the plate to room temperature for approximately 50 minutes.

   c. Remove the aluminum foil, then load the plate onto the GeneTitan™ MC Instrument.

Wash-Scan

Note: The **Wash-Scan** option is available in GCC version 6.1 or later.

Use the **Wash-Scan** option if:

- The array plate was hybridized in an oven separate from the GeneTitan™ MC Instrument.
- To bypass the hybridization step and perform only the wash/stain and scan steps.

Note: If the **Wash-Scan** option is selected, it usually takes 25–30 minutes to warm up the Wash B.

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 for the following circumstances.

- To rescan an entire array plate or specific arrays on a plate that failed to scan for reasons such as bubbles or gridding failure.
- To scan a plate that has already been hybridized, stained and washed on a different GeneTitan™ MC Instrument.

Unload Plates

Use the **Unload Plates** option to unload plates and trays from the instrument when processing is complete or has been aborted.

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.

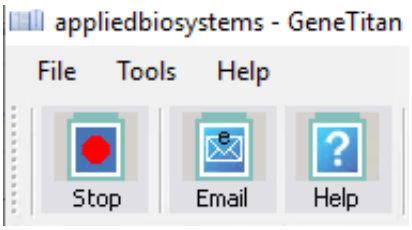
The operator can initiate an abort on 1 plate and the gripper can continue to process other plates in the instrument.

Abort a process

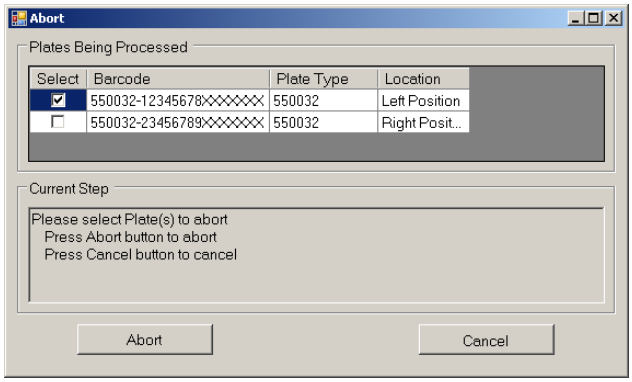
Use the following procedure to abort a process underway in the GeneTitan™ MC Instrument.

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.

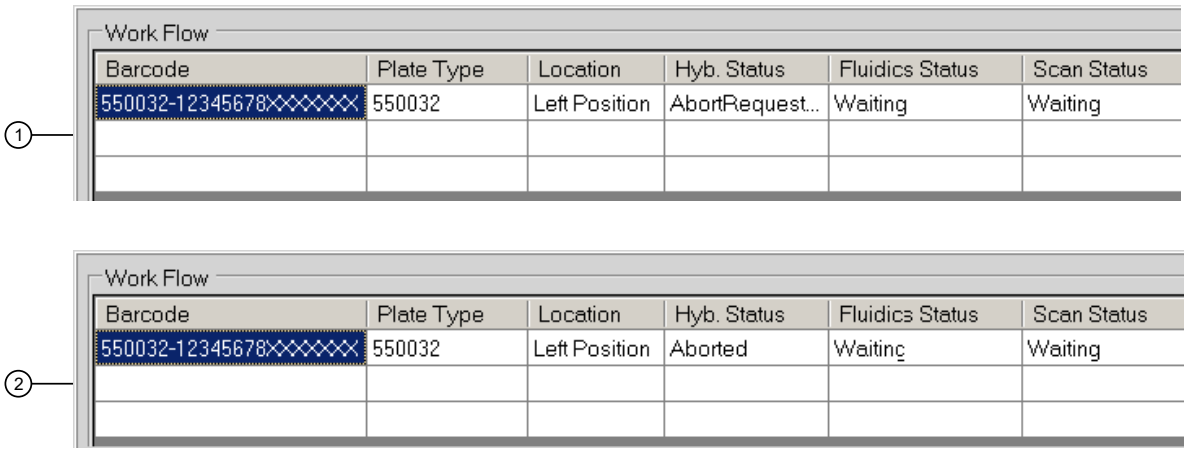


Figure 51 The Work Flow pane.

① Shows that the abort has been requested.
② Shows that the abort has been completed.

5. After the abort process is completed, do one of the following to retrieve the array plate and related consumables.

   - In the **Setup Option** list, select **Unload Plates**.
   - Start to load a new array plate.

Email notifications from the GeneTitan™ MC Instrument

You can configure the GeneChip™ Command Console™ software to send email notifications about the GeneTitan™ MC Instrument status. It is critical that you know when the instrument requires attention for sample handling or troubleshooting. Rapid notification can lessen the risk of sample loss.

The system can notify you when a process starts, completes, aborts, or encounters an error.

For instructions on setting up notifications, see the *GeneChip™ Command Console™ User Guide*.

GeneTitan™ MC Instrument lamp

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

The GeneTitan™ Instrument Control software provides a timer that indicates the remaining useful life of the bulb and notifies you when it requires replacement. It is important to adhere to the warnings specified in the *GeneTitan™ Multi-Channel Instrument User Guide*.

See the user guide for the Lambda LS and Smart controller system. Never manually switch the lamp and the controller on or off. The GeneTitan™ Instrument Control software manages the lamp activity and switches the lamp on and off as required. It takes 10 minutes to warm up the lamp. In idle mode, the lamp remains on for 2 hours before it is automatically switched off if there are no more plates being transferred from the fluidics to the imaging station. This automatic switching is by design and is intended behavior. Do not try to save the lamp life by powering off the switch on the lamp.

Note: The power switch on the shutter box must always be ON. The OPEN/CLOSE switch on the shutter box must always be at the AUTO position.

B

Fragmentation quality control gel protocol

Equipment required

"MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Gel Imager	**MLS**
Pipette, multichannel or single-channel P20	**MLS**
Plate centrifuge	**MLS**
Vortexer	**MLS**

E-Gel™ and reagents required

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

Item	Source
E-Gel™ Power Snap Plus Electrophoresis Device	G9110
iBright™ CL750 Imaging System	A44116
Invitrogen™ E-Gel™ *48* Agarose Gels, 4%	G800804
Applied Biosystems™ 25 bp DNA Ladder, or a similar product prepared as instructed by the manufacturer	931343
Invitrogen™ TrackIt™ Cyan/Orange Loading Buffer	10482028
Invitrogen™ UltraPure™ DNase/RNase-Free Distilled Water	10977023

Consumables required

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

Item	Source
Adhesive film—use one of the following: • Applied Biosystems™ MicroAmp™ Clear Adhesive Film • Microseal™ 'B' PCR Plate Sealing Film	 • 4306311 • Bio-Rad™, MSB1001
Pipette tips	Same brand as pipettor

Prepare the gel diluent

A 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer can be used in "Stage 4: Reassemble Hybridization Ready Plate and perform sample QC" on page 86.

1. Add 500 µL of TrackIt™ Cyan/Orange Loading Buffer to 49.5-mL nuclease-free water. Total volume 50 mL.
2. Mix well.
3. Store at room temperature.

Run the fragmentation QC gel

Running one 48 lane E-Gel to sample a 384-well plate is recommended. A suggested sampling pattern is to load the gel with the following wells from the 384-well Gel QC Plate:

- Row A—Odd-numbered columns
- Row E—Even-numbered columns
- Row J—Odd-numbered columns
- Row N—Even-numbered columns

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 produced during "Stage 4: Reassemble Hybridization Ready Plate and perform sample QC" on page 86.
2. Vortex the plate for 1 second each corner and 1 second in the center at the maximum setting, then centrifuge at 1,000 rpm for 30 seconds.
3. Power on the electrophoresis unit.
4. Place the E-Gel™ *48* Agarose Gel onto the electrophoresis unit.
5. Remove 2 combs from the gel.

6. Load 15 µL of samples from user-selected wells of the Gel QC Plate onto the gel.
7. Load 15 µL of 25 bp DNA ladder into the marker wells (M).
8. Load 15-µL nuclease-free water into any unused wells.
9. Run the gel for 19 minutes.
10. Capture a gel image.

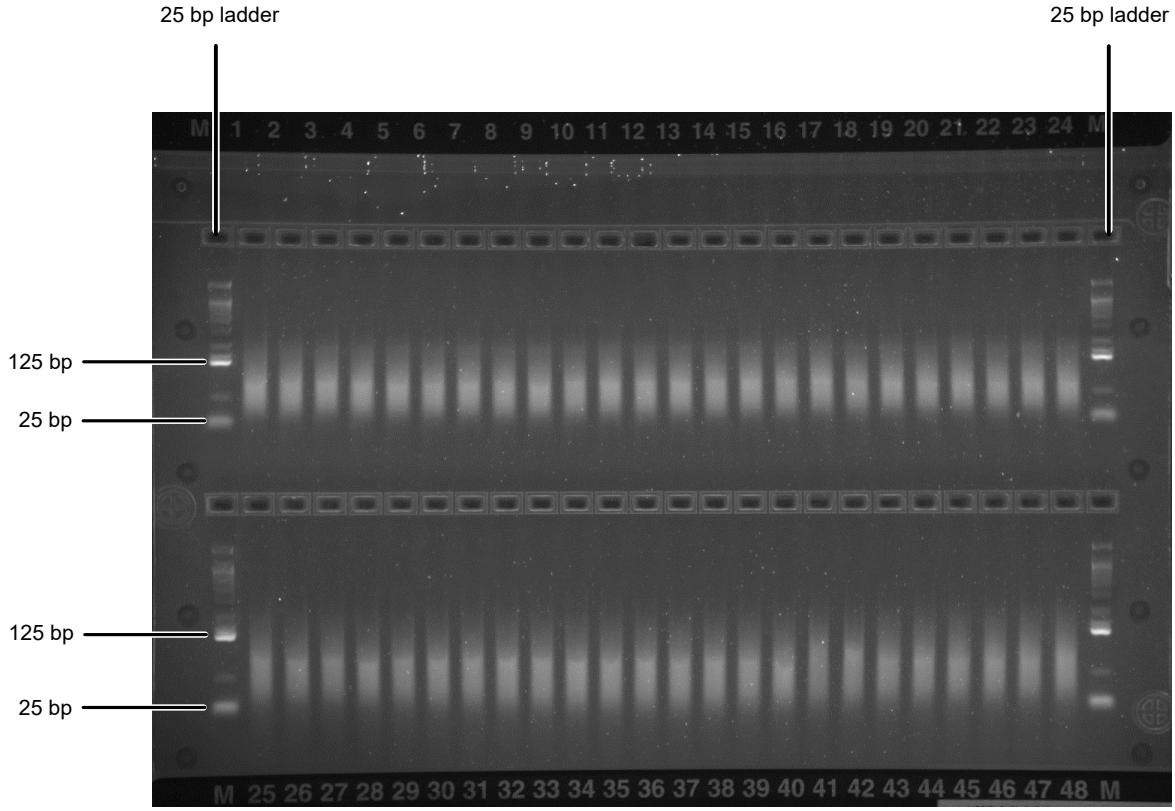


Figure 52 Fragments fall between 125 bp and 25 bp on a successful gel image.

Sample quantification after resuspension

Equipment required

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

Quantity	Item	Source
1	Multiskan™ SkyHigh Microplate Spectrophotometer	A51119500C

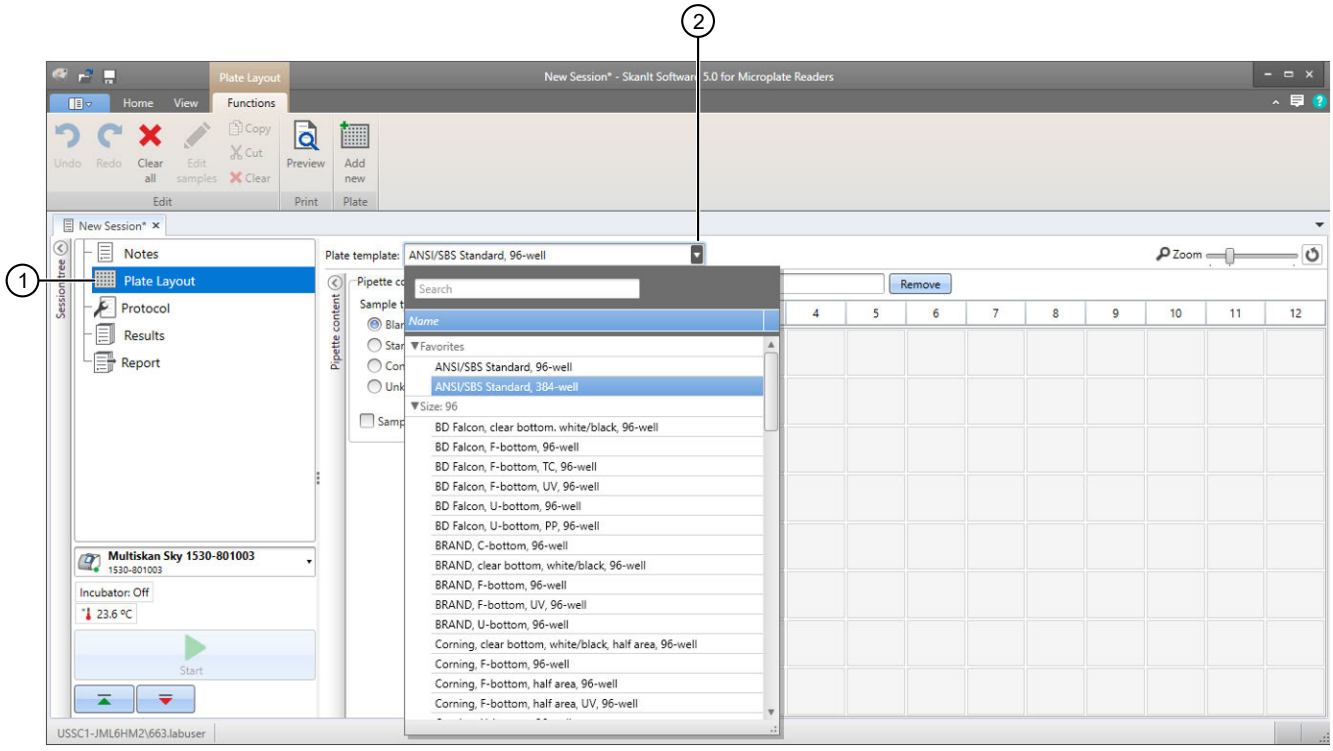
Quantify the diluted samples

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.

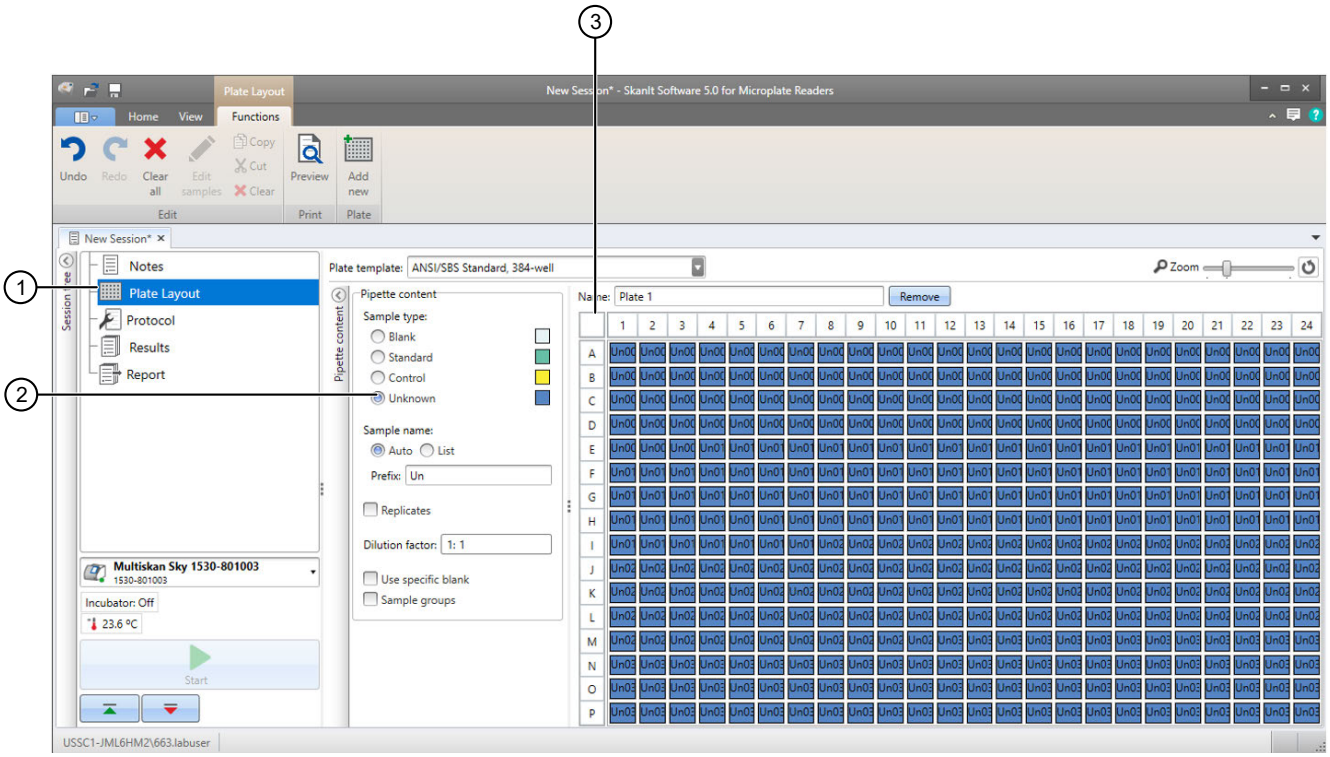
For OD quantification, readings must be taken at wavelengths of 260 nm, 280 nm, and 320 nm. See “Plate reader guidelines for sample quantification” on page 167.

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

1. Launch the SkanIt™ software, then click **New session**.
2. In **New Session** pane, click **Plate Layout** then select **ANSI/SBS Standard, 384-well** from the **Plate template** dropdown list.



① Plate Layout.
② **Plate template** dropdown list box.



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

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

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

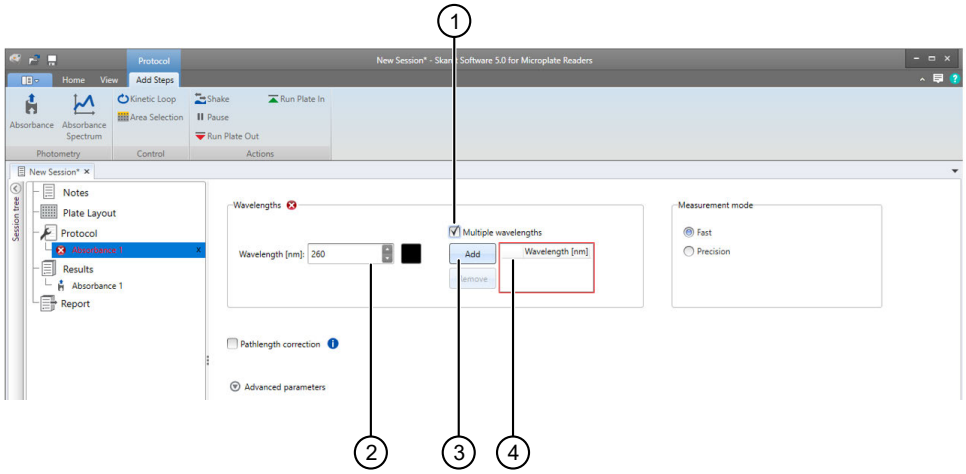


Figure 53 Assign wavelengths.

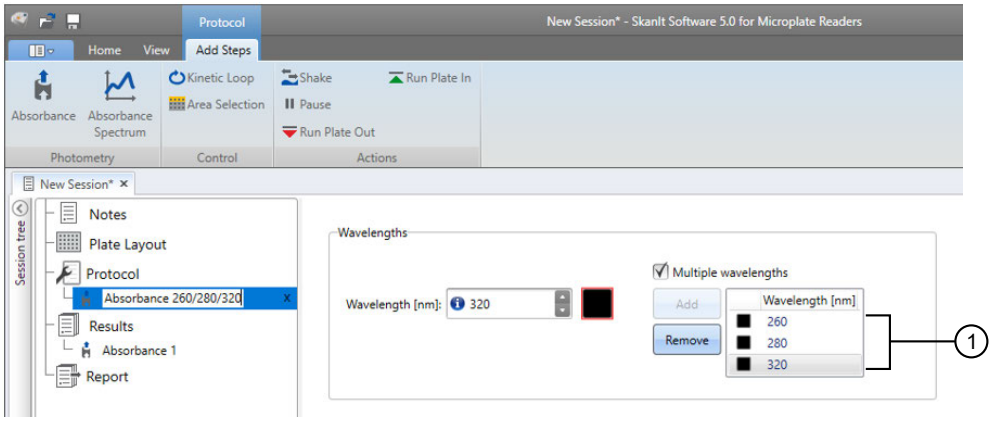
① **Multiple wavelengths** checkbox.
② **Wavelength [nm]** field.
③ **Add**.
④ **Wavelengths [nm]** box.

a. Check the **Multiple wavelengths** checkbox.

b. Enter "260" in the **Wavelength [nm]** field, then click **Add**.

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

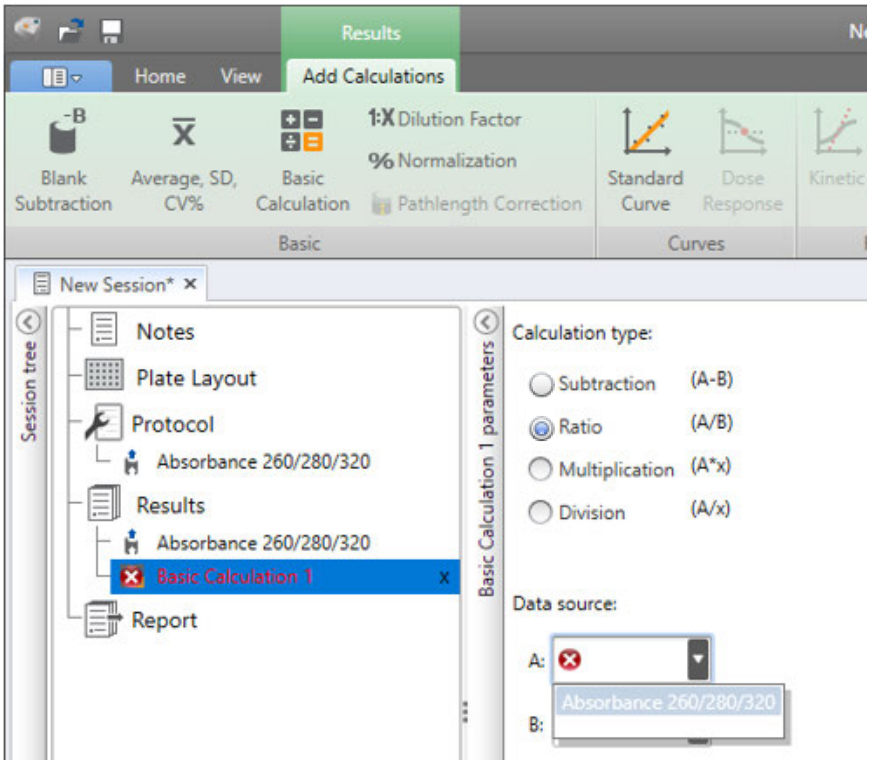
7. Repeat step 6 to add 280 nm and 320-nm wavelengths. When complete, 260, 280, and 320 appears in the **Wavelengths [nm]** box.



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

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

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

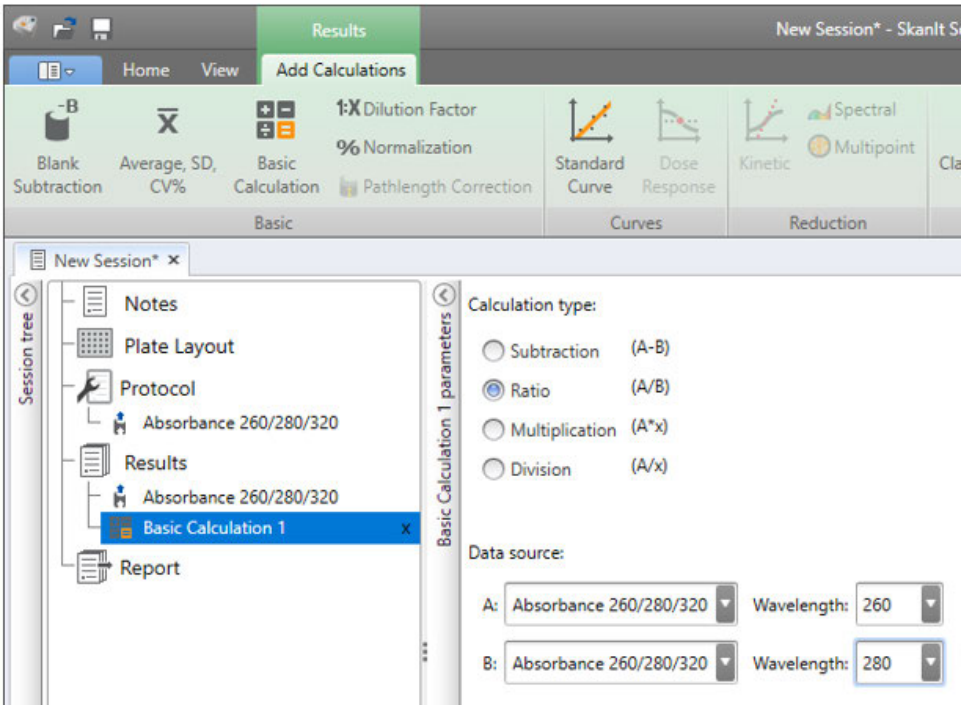


a. Under **Results** in the session tree pane, click **Absorance 260/280/320**.

b. Click **Basic Calculation** to calculate 260 nm/280 nm ratio for each sample, then select **Ratio (A/B)**.

c. Define the **Data source**:

- For **A**, select **Absorbance 260/280/320** and for **Wavelength** select **260**.
- For **B**, select **Absorbance 260/280/320** and for **Wavelength** select **280**.



10. Click **Basic Calculations 1** in the left pane, then rename it "***Ratio 260/280***".

11. Define the calculation for the DNA yields in each well.

    a. Click the **Custom Formula** button under the menu bar, then click the **Define Variables** button in the middle pane.

    b. In the **Define Variables** window, define a variable that is named "A260".

    1. In the **Variable Name** field, enter "***A260***".
    2. In the **Source Steps** dropdown list, select **Absorbance 260/280/320**.
    3. For **Wavelengths**, select **260**, then click **Add**.

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

c. Define a variable named "A320".

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

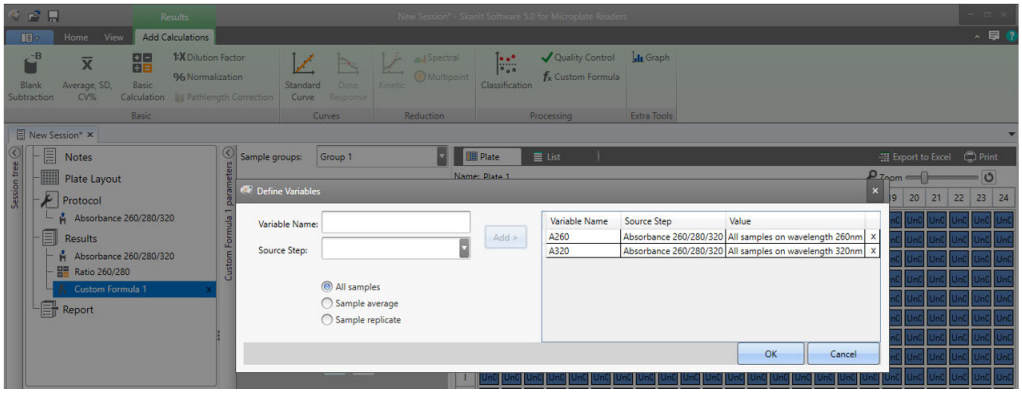
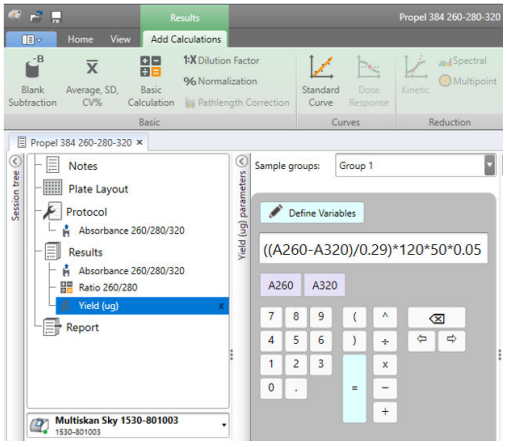


Figure 54 Define Variables window with 2 new variables added.

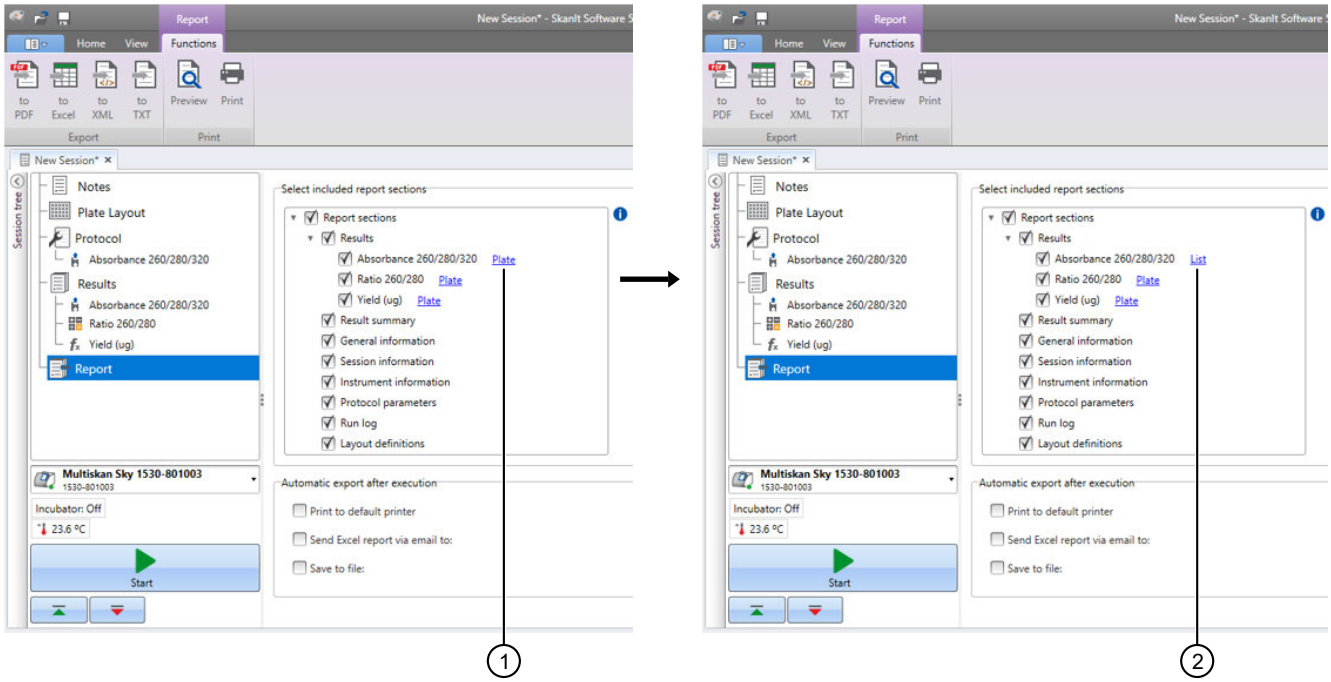
d. In the Custom Formula screen, enter the following DNA yield equation. Use the purple **A260** and **A320** buttons to enter them into the equation.

- *((A260-A320)/0.29)*120*50*0.05*

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



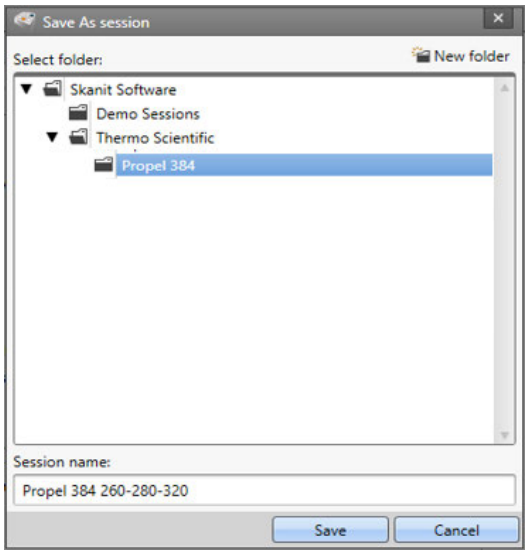
f. Click **Report** in the left pane. Results of the calculation are provided in either Plate or List format. Click **Plate** to change the results to **List** format.



① **Plate** format.
② **List** format.

12. Click the **Home** tab, then click **Save**.

13. In the **Save As session** window, select or create a folder to save it to, and then enter a **Session name**.



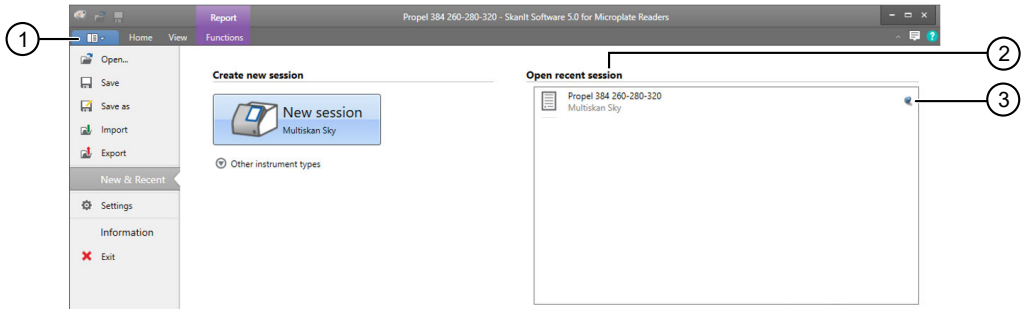
14. Click **Save**.
The session is available to be used to read the Axiom™ 384-well OD QC Plates.

Use a Multiskan™ Sky session

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

1. In the SkanIt™ software, click the file icon tab to the left of the **Home** tab in the upper left of the window.

2. Open a session using one of the following methods.
   - Click **Open** and navigate to the filepath of the session.
   - Click on the session name in the **Open recent session** section on the right side of the window. The session can be pinned to the Open recent session window by clicking on the pin icon on the right.



① File icon tab
② Saved session list
③ Pin

OD yield evaluation guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow. If the median yield for the plate is <525 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}-A_{320} value of approximately 0.53.

Plate reader guidelines for sample quantification

When performing sample quantification, the plate reader must be calibrated to help 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 QC Plate).
- V = 50 (the volume of the sample in µL after the resuspension step).
- E = 0.05 (the extinction coefficient of duplex DNA at 260 nm).
- P = the optical path length for the plate type and plate reader used.

If your plate reader does not record the 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.

D

Register samples in GeneChip™ Command Console™

GeneTitan™ Array Plate Registration file

A GeneTitan™ Array Plate Registration file is a Microsoft™ Excel™ spreadsheet that includes information on the samples that you are processing on a single array plate. This information includes the array plate format, the array plate barcode, and the sample file names for tracking the samples that are loaded onto a particular array plate.

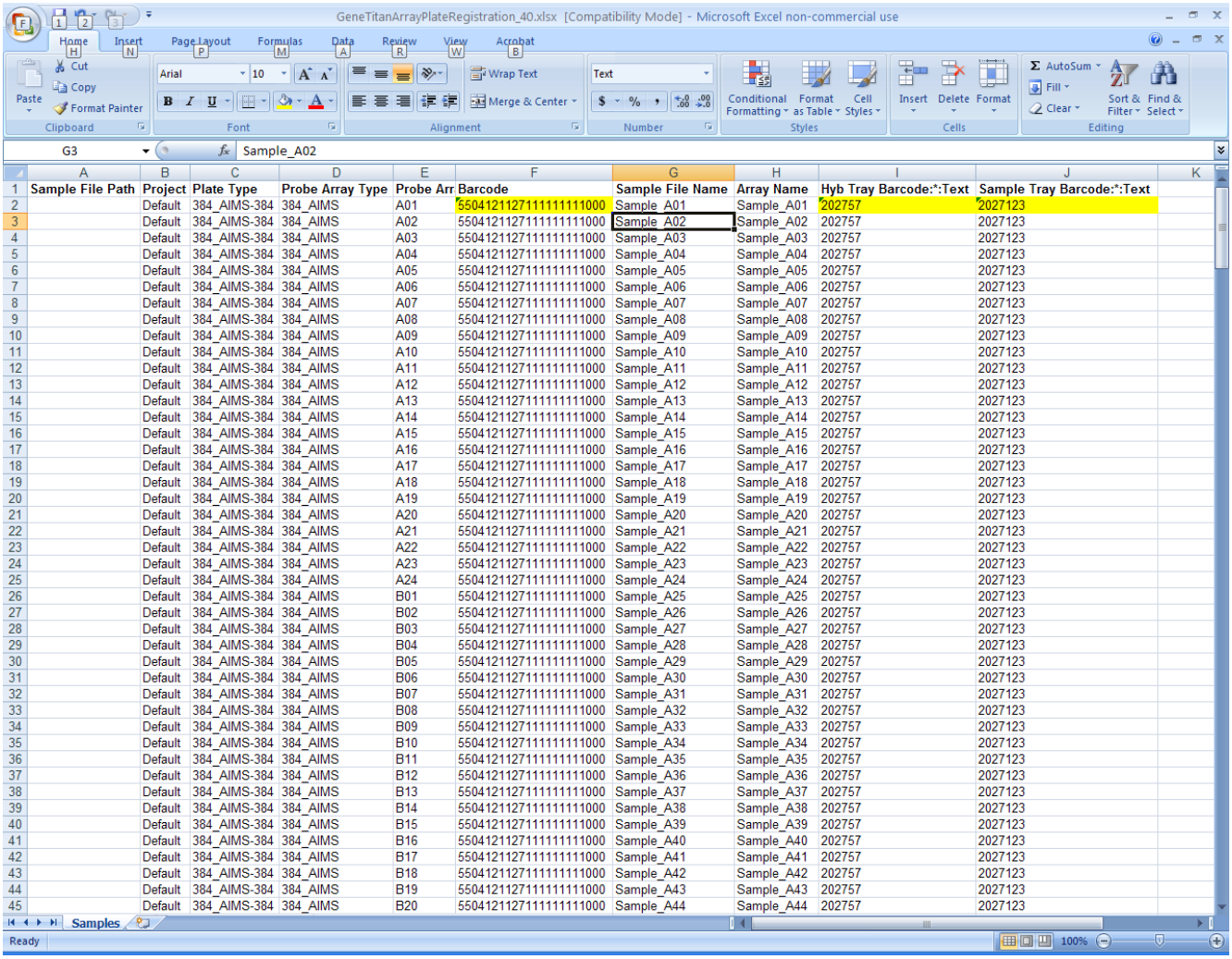
Note: The GeneTitan™ Array Plate Registration file uses the *.xls Microsoft™ Excel™ file extension. Do not use the *.xlsx file extension.

Create a GeneTitan™ Array Plate Registration file

1. In GCC Portal, click **Samples** ▸ **GeneTitan Array Plate Registration**.

2. Create a new template in GCC that includes fields required for sample traceability.
3. Select the array plate to be processed on the GeneTitan™ MC Instrument.
4. Select the newly created template that contains the fields that are required for traceability.
5. Select the **GeneTitan Array Plate Type** from the dropdown list.
6. Select the project where the sample registration data and all associated data files are saved.
7. Click **Download**.

8. Click the Microsoft™ Excel™ icon to open the spreadsheet.



Sample File Path	Project	Plate Type	Probe Array Type	Probe Arr	Barcode	Sample File Name	Array Name	Hyb Tray Barcode:*:Text	Sample Tray Barcode:*:Text
	Default	384_AIMS-384	384_AIMS	A01	55041211271111111111000	Sample_A01	Sample_A01	202757	2027123
	Default	384_AIMS-384	384_AIMS	A02	55041211271111111111000	Sample_A02	Sample_A02	202757	2027123
	Default	384_AIMS-384	384_AIMS	A03	55041211271111111111000	Sample_A03	Sample_A03	202757	2027123
	Default	384_AIMS-384	384_AIMS	A04	55041211271111111111000	Sample_A04	Sample_A04	202757	2027123
	Default	384_AIMS-384	384_AIMS	A05	55041211271111111111000	Sample_A05	Sample_A05	202757	2027123
	Default	384_AIMS-384	384_AIMS	A06	55041211271111111111000	Sample_A06	Sample_A06	202757	2027123
	Default	384_AIMS-384	384_AIMS	A07	55041211271111111111000	Sample_A07	Sample_A07	202757	2027123
	Default	384_AIMS-384	384_AIMS	A08	55041211271111111111000	Sample_A08	Sample_A08	202757	2027123
	Default	384_AIMS-384	384_AIMS	A09	55041211271111111111000	Sample_A09	Sample_A09	202757	2027123
	Default	384_AIMS-384	384_AIMS	A10	55041211271111111111000	Sample_A10	Sample_A10	202757	2027123
	Default	384_AIMS-384	384_AIMS	A11	55041211271111111111000	Sample_A11	Sample_A11	202757	2027123
	Default	384_AIMS-384	384_AIMS	A12	55041211271111111111000	Sample_A12	Sample_A12	202757	2027123
	Default	384_AIMS-384	384_AIMS	A13	55041211271111111111000	Sample_A13	Sample_A13	202757	2027123
	Default	384_AIMS-384	384_AIMS	A14	55041211271111111111000	Sample_A14	Sample_A14	202757	2027123
	Default	384_AIMS-384	384_AIMS	A15	55041211271111111111000	Sample_A15	Sample_A15	202757	2027123
	Default	384_AIMS-384	384_AIMS	A16	55041211271111111111000	Sample_A16	Sample_A16	202757	2027123
	Default	384_AIMS-384	384_AIMS	A17	55041211271111111111000	Sample_A17	Sample_A17	202757	2027123
	Default	384_AIMS-384	384_AIMS	A18	55041211271111111111000	Sample_A18	Sample_A18	202757	2027123
	Default	384_AIMS-384	384_AIMS	A19	55041211271111111111000	Sample_A19	Sample_A19	202757	2027123
	Default	384_AIMS-384	384_AIMS	A20	55041211271111111111000	Sample_A20	Sample_A20	202757	2027123
	Default	384_AIMS-384	384_AIMS	A21	55041211271111111111000	Sample_A21	Sample_A21	202757	2027123
	Default	384_AIMS-384	384_AIMS	A22	55041211271111111111000	Sample_A22	Sample_A22	202757	2027123
	Default	384_AIMS-384	384_AIMS	A23	55041211271111111111000	Sample_A23	Sample_A23	202757	2027123
	Default	384_AIMS-384	384_AIMS	A24	55041211271111111111000	Sample_A24	Sample_A24	202757	2027123
	Default	384_AIMS-384	384_AIMS	B01	55041211271111111111000	Sample_A25	Sample_A25	202757	2027123
	Default	384_AIMS-384	384_AIMS	B02	55041211271111111111000	Sample_A26	Sample_A26	202757	2027123
	Default	384_AIMS-384	384_AIMS	B03	55041211271111111111000	Sample_A27	Sample_A27	202757	2027123
	Default	384_AIMS-384	384_AIMS	B04	55041211271111111111000	Sample_A28	Sample_A28	202757	2027123
	Default	384_AIMS-384	384_AIMS	B05	55041211271111111111000	Sample_A29	Sample_A29	202757	2027123
	Default	384_AIMS-384	384_AIMS	B06	55041211271111111111000	Sample_A30	Sample_A30	202757	2027123
	Default	384_AIMS-384	384_AIMS	B07	55041211271111111111000	Sample_A31	Sample_A31	202757	2027123
	Default	384_AIMS-384	384_AIMS	B08	55041211271111111111000	Sample_A32	Sample_A32	202757	2027123
	Default	384_AIMS-384	384_AIMS	B09	55041211271111111111000	Sample_A33	Sample_A33	202757	2027123
	Default	384_AIMS-384	384_AIMS	B10	55041211271111111111000	Sample_A34	Sample_A34	202757	2027123
	Default	384_AIMS-384	384_AIMS	B11	55041211271111111111000	Sample_A35	Sample_A35	202757	2027123
	Default	384_AIMS-384	384_AIMS	B12	55041211271111111111000	Sample_A36	Sample_A36	202757	2027123
	Default	384_AIMS-384	384_AIMS	B13	55041211271111111111000	Sample_A37	Sample_A37	202757	2027123
	Default	384_AIMS-384	384_AIMS	B14	55041211271111111111000	Sample_A38	Sample_A38	202757	2027123
	Default	384_AIMS-384	384_AIMS	B15	55041211271111111111000	Sample_A39	Sample_A39	202757	2027123
	Default	384_AIMS-384	384_AIMS	B16	55041211271111111111000	Sample_A40	Sample_A40	202757	2027123
	Default	384_AIMS-384	384_AIMS	B17	55041211271111111111000	Sample_A41	Sample_A41	202757	2027123
	Default	384_AIMS-384	384_AIMS	B18	55041211271111111111000	Sample_A42	Sample_A42	202757	2027123
	Default	384_AIMS-384	384_AIMS	B19	55041211271111111111000	Sample_A43	Sample_A43	202757	2027123
	Default	384_AIMS-384	384_AIMS	B20	55041211271111111111000	Sample_A44	Sample_A44	202757	2027123

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.

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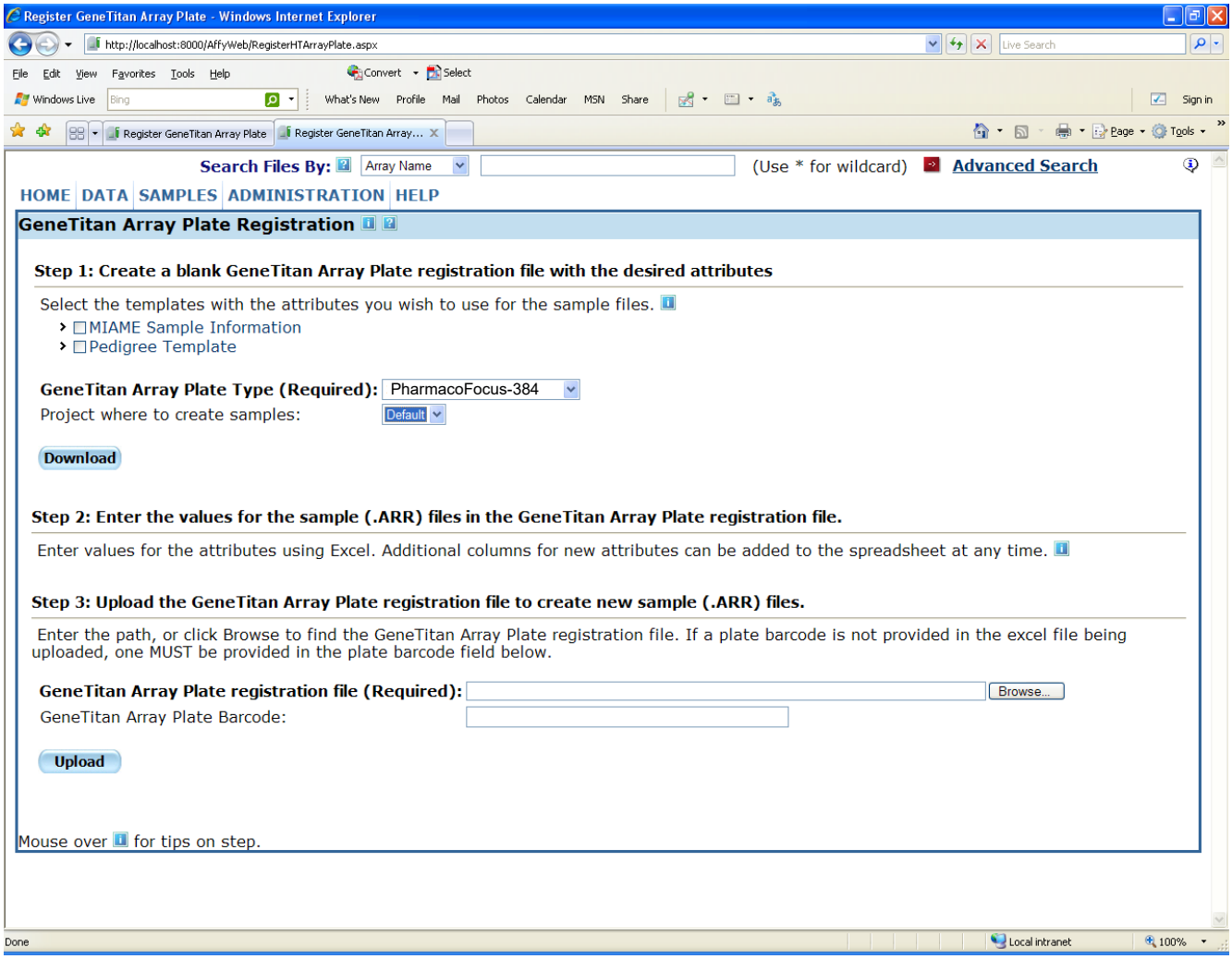
10. Complete one of the following:
    - If you are ready to load the array plate onto the GeneTitan™ MC Instrument, scan the array plate barcode into column F, then proceed to step 11.
    - If you are not ready to load the array plate onto the GeneTitan™ MC Instrument, proceed to step 11.

11. Follow these steps to save the file:
    a. Click **File** ▸ **Save As**.

    b. Enter a name for the array plate registration file.

c. Click **Save**.

12. Follow these steps when you are ready to load the array plate onto the GeneTitan™ MC Instrument.
    a. Click **Browse**, navigate to the GeneTitan™ Array Plate Registration file, then click **Open**.
    b. Scan the array plate barcode, if it has not already been scanned, and save the registration file.
    c. Click **Upload**, wait for the information to load, then click **Save** found at the bottom of the next window that is displayed.



E

Troubleshooting

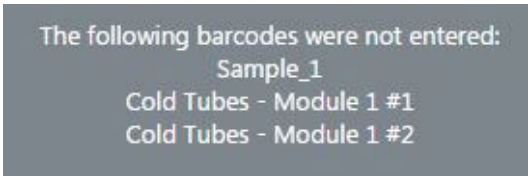
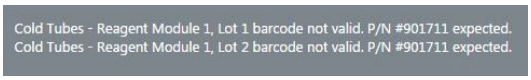
Biomek™ i7 Automated Workstation

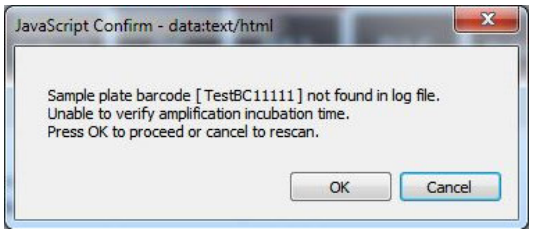
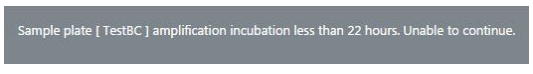
If a hardware problem is encountered when running the Axiom™ target preparation methods on the Biomek™ i7 Automated Workstation, you can do the following:

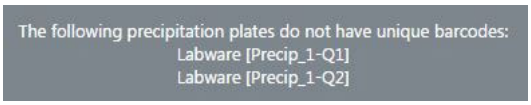
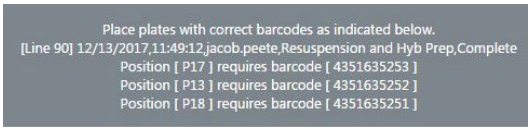
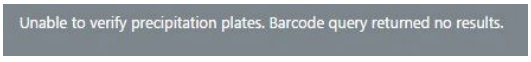
- See these documents:
  - *Biomek™ i-Series Automated Workstation Hardware Reference Manual*, Beckman Coulter™ Pub. No. B54474AA
  - *Biomek™ i-Series Automated Workstation Instructions for Use*, Beckman Coulter™ Pub. No. B54473AA
- For information on recovering a run, contact your Thermo Fisher Scientific field application scientist.
- For additional information on Biomek™ i7 Automated Workstation hardware, error messages, or to request service, contact Beckman Coulter™. Be sure to have the serial number of your workstation available.

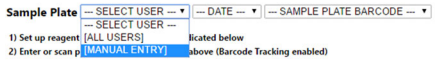
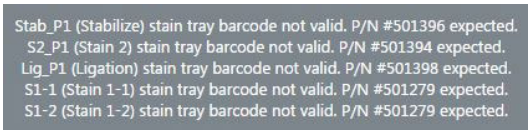
Runtime errors

Note: If an error persists after following the troubleshooting guidelines, contact your field application scientist.

Observation	Possible cause	Recommended action
Barcode not entered 	A barcode has not been entered in one or more fields.	Enter missing barcodes to proceed.
Reagent barcodes are not correct  This error can occur when the **Verify Reagent Module Barcodes** option is enabled.	Wrong reagent module part numbers were entered.	Ensure that the correct reagent module bags are being scanned and try again.
Duplicate sample plate barcode **Details:** One or more records in the log files were found to contain barcodes from the current plate set.  This error can occur at the DNA amplification, fragmentation and precipitation, resuspension and hybridization preparation, plate reassembly and QC, transfer samples to hybridization tray, and GeneTitan™ reagent preparation steps.	The same custom barcodes were used more than once.	Ensure that a unique sample plate is being entered. If the original barcode is not correct, press **Cancel** to enter in the correct barcode. If you decide to keep the duplicate barcode, press **OK** to proceed with the run. **Note:** By selecting to keep the duplicate barcode, the log file is archived and saved in the **Archive** folder. The current log indicates *(Duplicate Plate Set) Record removed by user* plus the date, and continues appending future runs to the current log file.

Observation	Possible cause	Recommended action
Duplicate sample plate barcode **Details:** One or more records in the log files were found to contain barcodes from the current plate set.  This error can occur at the DNA amplification, fragmentation and precipitation, resuspension and hybridization preparation, plate reassembly and QC, transfer samples to hybridization tray, and GeneTitan™ reagent preparation steps. *(continued)*	A run was reprocessed. For example, when recovering from an aborted run.	If the original barcode is not correct, press **Cancel** to enter in the correct barcode.
		If you decide to keep the duplicate barcode, press **OK** to proceed with the run. **Note:** By selecting to keep the duplicate barcode, the log file is archived and saved in the **Archive** folder. The current log indicates *(Duplicate Plate Set) Record removed by user* plus the date, and continues appending future runs to the current log file.
Sample plate barcode not found **Details:** No record of the input sample plate barcode was in the log file.  This error can occur at the fragmentation and precipitation step.	The sample plate was processed on a different instrument.	Confirm that the correct sample plate barcode has been entered, otherwise, press **Cancel** to edit the barcode.
		If the sample plate was amplified on a different instrument, press **Cancel**, and process it on the instrument where the sample was originally processed.
		If you decide to use the unfound sample plate barcode, press **OK** to proceed with the run.
	A sample plate that was never amplified was used.	Confirm that the correct sample plate barcode has been entered, otherwise, press **Cancel** to edit the barcode.
		If you decide to use the unfound sample plate barcode, press **OK** to proceed with the run.
DNA amplification less than 22 hours  This error can occur at the fragmentation and precipitation step.	Fragmentation and precipitation is attempted for the sample plate that has not completed the minimum 22 hours of amplification incubation.	Select **Abort** and wait for the amplification incubation to finish (at least 22 hours).

Observation	Possible cause	Recommended action
Precipitation barcodes are identical on one or more of the plates  In the example above, plates in the Precip_1_Q1 and Precip_1_Q2 labware positions are identical. This error can occur at the fragmentation and precipitation step.	A plate is accidentally double-scanned.	Verify that the precipitation plate barcodes are all unique.
		If all 4 barcodes are visually unique but the error persists, try scanning all 4 into Notepad (or any text editor). Use the information in the text editor to confirm that the scanned barcodes match what is seen visually. If not, enter the barcode manually throughout this and subsequent steps then proceed with the run
	Custom barcodes are used more than once.	Verify that the precipitation plate barcodes are all unique.
		If all 4 barcodes are visually unique but the error persists, try scanning all 4 into Notepad (or any text editor). Use the information in the text editor to confirm that the scanned barcodes match what is seen visually. If not, enter the barcode manually throughout this and subsequent steps then proceed with the run
The precipitation plates do not match the order identified in the fragmentation and precipitation step  This error can occur at the resuspension and hybridization preparation, and plate reassembly and QC steps.	Deck is loaded with precipitation plates from multiple fragmentation and precipitation runs.	Gather and confirm that all 4 precipitation plates are associated with the desired DNA Amplification Sample_1 plate.
		Follow the instructions in the error message to place the correct plates on the deck then proceed with the run.
	The plates were from the same fragmentation and precipitation run, but were placed in one or more incorrect positions on the deck.	Follow the instructions in the error message to place the plate in the correct position on the deck, then proceed with the run.
Unable to verify precipitation plates **Details:** No record of any of the scanned precipitation plate barcodes was found in the log file.  This error can occur at the resuspension and hybridization preparation, and plate reassembly and QC steps.	Plates that were processed on a different instrument are being used.	The only option is to select **Abort** to abort the run. 1. If the plates were processed on a different instrument, run the plates on the instrument where the samples were originally processed. 2. Ensure that the correct precipitation plate barcodes are entered. Make necessary corrections, then proceed with the run.

Observation	Possible cause	Recommended action
Sample plate barcode shows listed as [Unknown]  This error can occur at the GeneTitan reagent preparation step.	The preceding transfer samples to hybridization tray step was run with a sample barcode which could not be found in the log file.	In the first drop-down list, select **Manual Entry**, and type in a sample plate barcode. 
Stain tray barcodes invalid **Details:** Part number on the stain trays are not correct.  This error can occur when **Verify GeneTitan labware** setup option is enabled.	Placement of one or more stain trays is not correct.	Verify the deck layout and ensure that the stain trays are in the correct position.
	The stain tray type being used is not correct.	Verify that the deck layout is correct and ensure that the correct stain tray type is being used in each position.
Peltier is not reaching the target temperature **Details:** This error is detected visually when the temperature display on the Peltier controller does not match the target temperature.	Peltier does not have sufficient cooling fluid.	See the INHECO™ user guide for instructions on checking and filling the cooling fluid.
		Contact Beckman Coulter™ technical support.

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

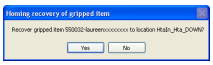
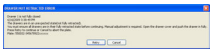
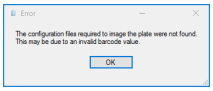
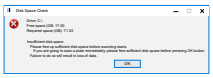
- `C:\Affymetrix\GeneChipHTScanControlMC\Log` - collect all dated directories and contents from the time the GeneTitan™ application was started.
- `C:\Affymetrix\GeneChipHTScanControlMC\RunLog` - collect all dated directories and contents from the time the GeneTitan™ application was started.

Troubleshooting the GeneTitan™ MC Instrument

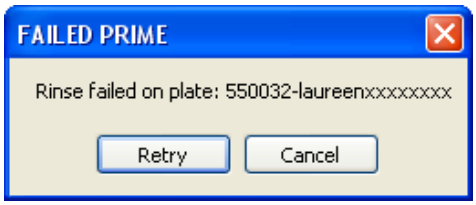
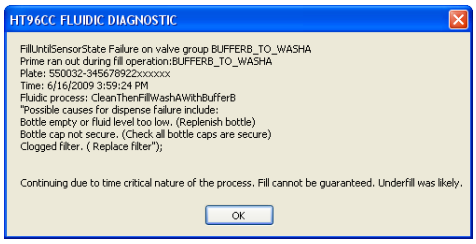
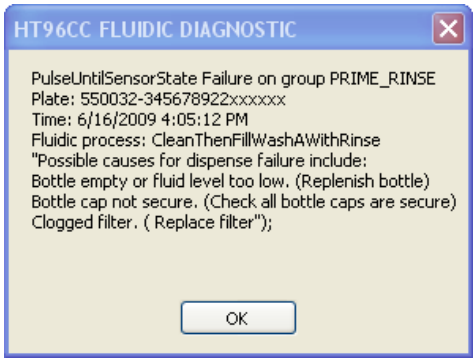
This section provides instructions on how to identify and solve simple problems with the GeneTitan™ MC Instrument. If a problem or error occurs that is not listed in this chapter, contact Thermo Fisher Scientific Technical Support for help.

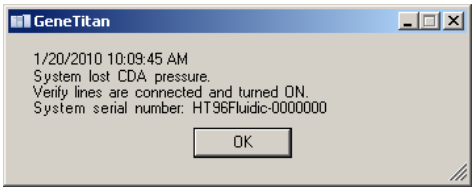
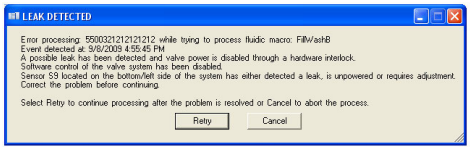
For software errors that do not involve hardware crashes, the most common solution is to close or exit the application, then restart it. If the same error occurs, close the application and power off the computer, then restart. If the error still occurs, power off the GeneTitan™ MC Instrument, then restart.

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.
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 151.

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.
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 384HT Reagent Kit be emptied into the GeneTitan™ Wash B bottle when setting up the system to process a plate. Using all 600 mL of the Axiom™ Wash Buffer B helps ensure that the bottle is filled to more than the requisite 35% of Wash B bottle volume.
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 182.

Observation	Possible cause	Recommended action
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.
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 182.

F

GeneTitan™ Multi-Channel Instrument care

Overview

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

- The GeneTitan™ Multi-Channel (MC) Instrument must be positioned on a sturdy level bench away from extremes in temperature and away from moving air.
- Always run a Shutdown protocol when the instrument is off or unused overnight or longer to prevent salt crystals from forming in the fluidics system.
- Always use deionized (DI) water to prevent contamination of the lines. Swap out old buffers with freshly prepared buffer at each system startup.

IMPORTANT! Before performing maintenance power off the instrument to avoid injury if an electrical malfunction occurs.

Maintenance

The GeneTitan™ family of instruments requires little in the way of customer maintenance. The instruments must be kept clean and free of dust. Dust buildup can degrade performance. Wipe the exterior surfaces clean using a mild dish detergent solution in water. Do not use ammonia-based cleaners or organic solvents such as alcohol or acetone to clean the system because they can damage the exterior surfaces.

The following tasks must be performed regularly to help ensure that the imaging device remains in working order.

Monthly

Wipe down the outer surface of the imaging device with a dry cloth.

Every 6 months

1. Replace the cooling fan air filters at the rear of the instrument.
2. Replace the Micropore™ filters in the Wash A, Wash B, and Rinse bottles. If you run 4-8 plates/week, then replace the Micropore™ filters more frequently.

Outer enclosure fan filters

Cleaning schedule

The GeneTitan™ fan filter cartridge must be cleaned at least every 90 days of service. Note that in some service locations, the presence of excessive dust or particulate matter can require cleaning the cartridge more often than 90 days.

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

Figure 55 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.

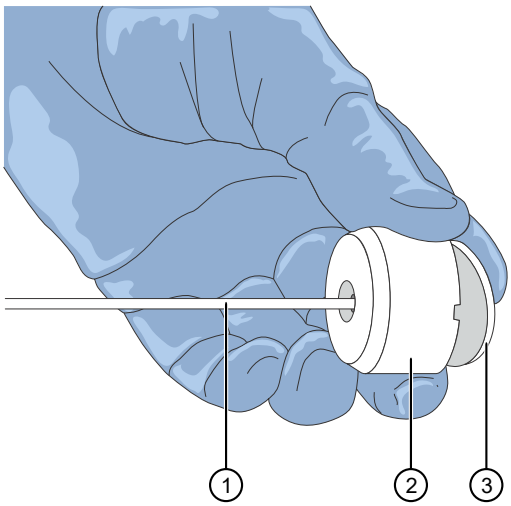


Figure 56 Components of the reagent buffer supply line and filter.

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 56).
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 186, and “Replace the xenon lamp” on page 187. After changing the lamp, you must manually reset the lamp life clock.

Remove the xenon lamp

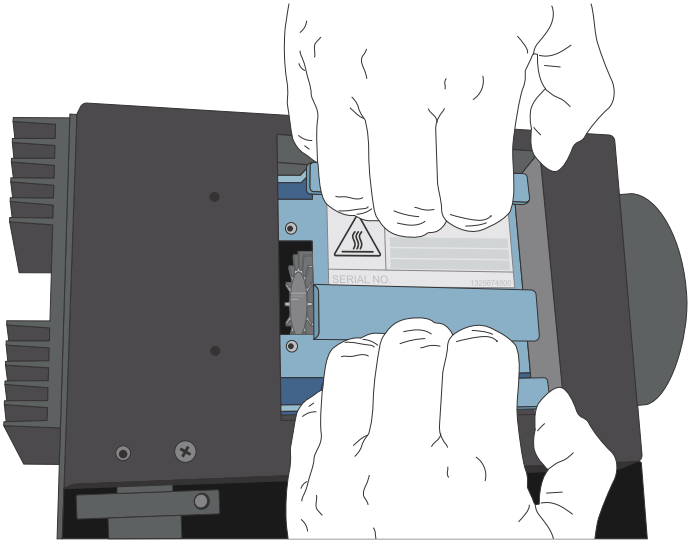
WARNING! Power off the lamp using the switch in the rear of the unit and then disconnect the power cord. Allow to cool before attempting to replace the lamp.

1. Unscrew the 4 retaining bolts with your fingers.

① Remove these 4 bolts.

2. Remove, then set aside the warning cover to reveal the xenon lamp that is contained inside.

3. Place a hand on each side of the blue plastic flange, then lift out the lamp in a vertical motion. Both hands must be used to remove the lamp. Apply equal pressure on each side of the lamp and gently lift.

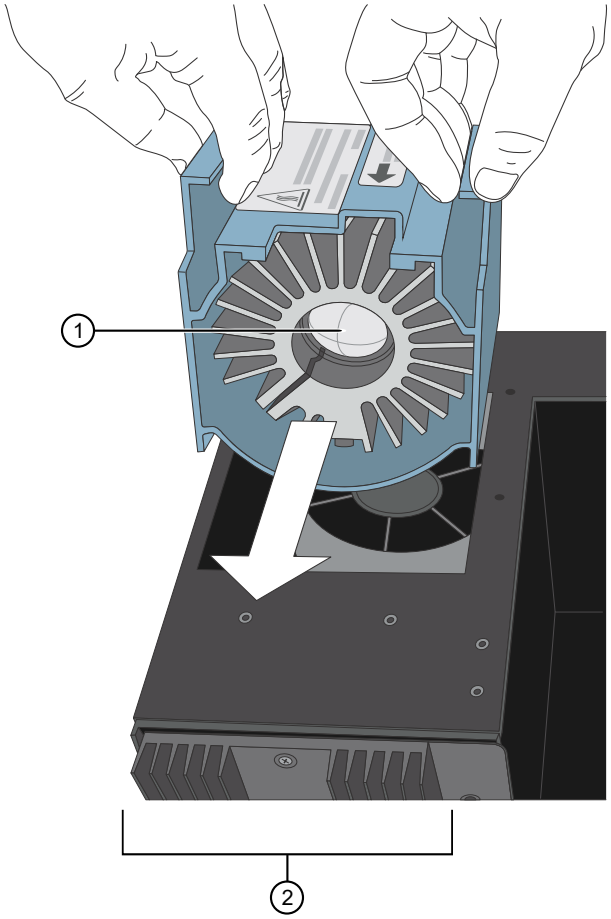


Replace the xenon lamp

A new Cermax™ Xenon Arc Lamp (Cat. No. 01-0740) is required for this procedure.

IMPORTANT! Ensure that you install the lamp in the correct orientation.

1. Hold the lamp by the blue plastic flanges. Ensure that the lamp bulb faces inward toward the rear heat sink on the unit, and then vertically lower the lamp to install.



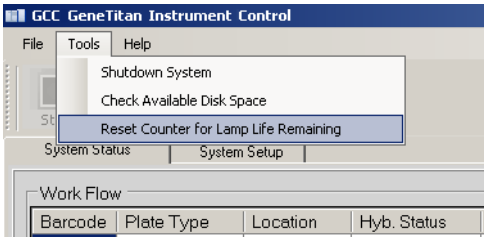
① Xenon bulb faces away from the fan and towards the heat sink.
② Heat sink on the Lambda LS unit.

2. Replace the warning cover, then hand tighten the bolts.

Reset the lamp life counter

Using the **GCC GeneTitan Instrument Control** module accessed from the **Launcher** window, you must alert the software that the lamp has been replaced so that the hours of the lamp counter are reset. This menu option is only available when the system is not processing any plates.

1. Select **Tools** ▸ **Reset Counter for Lamp Life Remaining**.



2. Click **Yes** in the message window to reset the counter.

G

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

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

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

H Documentation and support

Related documentation

Document	Publication number	Description
Axiom™ 2.0 Assay 384HT Array Format Automated Workflow Site Preparation Guide—Biomek™ i7	MAN0017556	Provides guidance on reagents, instruments, and supplies required to run the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow on the Biomek™ i7.
Axiom™ 2.0 Assay 384HT Array Format Automated Workflow Quick Reference—Biomek™ i7 Method v1.1	MAN0019790	An abbreviated reference for the target preparation step of the Axiom™ 2.0 Assay 384HT Array Format Automated Workflow on the Biomek™ i7, running the Biomek™ method v1.1. This document is for experienced users.
Axiom™ 384HT and Mini 96 gDNA Sample Preparation Quick Reference	MAN0017719	An abbreviated reference on the genomic DNA sample preparation protocol. This document is for experienced users.
GeneTitan™ MC Protocol for Axiom™ 384HT Array Plate Processing Quick Reference	MAN0017596	An abbreviated reference for processing Axiom™ 384HT Array Plates with the GeneTitan™ MC Instrument. This document is for experienced users.
GeneTitan™ Multi-Channel Instrument User Guide	MAN0027694	The GeneTitan™ Multi-Channel (MC) Instrument automates array processing from target hybridization to data generation by combining a hybridization oven, fluidics processing, and state-of-the-art imaging device into a single benchtop instrument. This document detailing the use, care, and maintenance for the GeneTitan™ MC.
GeneTitan™ Multi-Channel Instrument Site Preparation Guide	MAN0025571	Provides guidance on creating and maintaining the proper environment required for the GeneTitan™ MC Instrument.
Recommended Alternative Microarray Consumables Quick Reference	MAN0019853	A quick reference document identifying recommended alternative replacement consumables for use in microarray assays.

(continued)

Document	Publication number	Description
Software and analysis		
GeneChip™ Command Console™ User Guide	MAN0027771	This user guide provides instructions on using Applied Biosystems™ GeneChip™ Command Console™ software (GCC) used to control GeneChip™ instrument systems. GeneChip™ Command Console™ software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip™ arrays.
Axiom™ Analysis Suite User Guide	MAN0027928	Axiom™ Analysis Suite advances genotyping data analysis with a single-source software package to enable complete genotyping analysis of all Axiom™ arrays. This document provides instructions on using the software to automate the Best Practices Workflow to deliver accurate results in a single step for export in PLINK, VCF, or TXT formats.
Axiom™ Genotyping Solution Data Analysis User Guide	MAN0018363	This guide provides information and instructions for analyzing Axiom™ genotyping array data. It includes the use of Axiom™ Analysis Suite, Applied Biosystems™ Analysis Power Tools and SNPolisher R package to perform quality control analysis (QC) for samples and plates, SNP filtering before downstream analysis, and advanced genotyping methods.
Beckman Coulter™ and other third-party documentation		
Biomek™ i-Series Automated Workstation Hardware Reference Manual	B54474AA	Reference documents related to using and troubleshooting the Biomek™ i7 Automated Workstation. After installing the Biomek™ Software, these documents are found by clicking **Start ▸ All Programs ▸ Beckman Coulter Manuals**.
Biomek™ i-Series Automated Workstation Instructions for Use	B54473AA	
Biomek™ i-Series Automated Workstation Tutorials	B54475AA	
Zebra™ DS9808 Digital Scanner Product Reference Guide (Barcode Scanner)	72E-112999-11	Reference document for using the Zebra™ DS9808 Digital Scanner. A PDF version is available from the Zebra website.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.

References

Hindorff LA, Junkins HA, Mehta JP, and Manolio TA. A Catalog of Published Genome-Wide Association Studies. Available at: www.genome.gov/gwastudies. Accessed 09/28/2009.www.genome.gov/gwastudies. Accessed 09/28/2009.

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