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CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow

USER GUIDE

for use with:
CytoScan™ HT-CMA 96-Array Plate
Applied Biosystems™ HT Target Prep Reagent Kit 96F

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ThermoFisher SCIENTIFIC

Manufacturer:
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Products:
Applied Biosystems™ HT Target Prep Reagent Kit 96F

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

Products:
CytoScan™ HT-CMA 96-Array Plate

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

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Revision	Date	Description
B.0	13 Oct 2020	• Updated reagent kit name to Applied Biosystems™ HT Target Prep Reagent Kit 96F. • Added missing steps to Prepare the GeneTitan™ MC Instrument. • Clarified user activities in Overview of the 3-plate workflow for manual target preparation using an overnight precipitation step. • Changed time to 50 minutes to bring plate to room temperature in Hyb-Wash. • Changed the median OD yield of DNA for the plate to <1,000 ng in OD yield evaluation guidelines. • Standardized plate names throughout.
A.0	26 January 2020	New document.

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Contents

1 Overview

Cytogenetics studies are performed to identify structural changes in DNA, such as copy number changes. Individuals typically have 2 copies of the genome in each of their cells: 1 inherited from the mother, and 1 inherited from the father. Chromosomal abnormalities are common in several disease states such as:

- Deletions—When 1 or both copies of a particular chromosome region are lost.
- Gains—When a chromosome or chromosomal region is duplicated or multiplied.
- Uniparental disomies (UPDs)—When 2 copies of a chromosome or chromosomal region are present, but both have been inherited from a single parent.

The Applied Biosystems™ CytoScan™ HT-CMA Assay has been optimized for the detection of these chromosomal abnormalities and does so in a high throughput format. The assay is used with genomic DNA from blood or amniotic fluid samples. The CytoScan™ HT-CMA Assay includes arrays and reagents in either a 96- or 24-sample format for use on the GeneTitan™ Multi-Channel Instrument. The high-throughput nature of CytoScan™ HT-CMA teamed with the intuitive Reproductive Health Research Analysis Software (RHAS) enables you to perform high-resolution genome-wide copy number analysis faster and easier than before. The combined high-resolution DNA copy number data and the ability to screen a panel of single nucleotide variants (SNVs) on a single array makes the CytoScan™ HT-CMA Assay the new standard for high throughput cytogenetics analysis.

Introduction

The CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow is available as a bundled kit that includes the arrays, reagents, and consumables needed for processing one 96-array format plate.

The CytoScan™ HT-CMA Assay interrogates biallelic and multiallelic SNPs, indels, and copy number variation (CNV) in a single-assay workflow. Starting with genomic DNA, the samples are processed by performing a manual target preparation protocol followed by automated processing of the array plates on the GeneTitan™ Multi-Channel (MC) Instrument.

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

Array plates are processed on a GeneTitan™ MC Instrument controlled by Applied Biosystems™ GeneChip™ Command Console™ 4.3 or later. The resulting CEL files are analyzed with the Reproductive Health Research Analysis Software and the Chromosome Analysis Suite.

For a list of the equipment and supplies required to run the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow, see the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018215.

For more information, see "Related documentation" on page 191.

Assay highlights

This user guide includes an option for a 3-hour DNA target precipitation step to enable a faster assay turnaround time, going from sample to CEL file generation within 72 hours. The 3-hour precipitation step shortens "Stage 2: Fragment and precipitate the DNA" on page 47 to enable the operator to advance through "Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC" on page 57, "Stage 4: Denature and hybridize the sample plate" on page 72, and the start of "Stage 5: Prepare GeneTitan™ reagents and trays" on page 78, to start hybridization on the GeneTitan™ MC Instrument, on day 2 of the assay workflow. Note that this workflow option requires approximately 9–10 hours to complete the combined day 2 activities, from fragmentation to initiation of hybridization on the GeneTitan™ MC Instrument.

In addition, this user guide presents a recommended workflow to support the processing of 3 plates per week using the shortened DNA precipitation step.

The standard CytoScan™ HT-CMA Assay workflow, in which the DNA is precipitated overnight, provides a convenient stopping point to support single operator assay execution of 1 plate in an eight-hour workday. This user guide also presents a recommended workflow to support the processing of 3 plates per week using the standard overnight DNA precipitation.

The user guide provides equipment requirements and operator assumptions to support these workflows. See Chapter 6, "Three-plate workflow for CytoScan™ HT-CMA Array Plates using an overnight DNA precipitation step" and Chapter 7, "Three-plate workflow for CytoScan™ HT-CMA Array Plates using a 3-hour DNA precipitation step".

Multiplate workflows

Thermo Fisher Scientific supports workflows to run a set of samples and array plates through the protocol using a minimum number of personnel in a 40-hour week. The timing of steps is critical because of the following limits:

- Incubation for DNA amplification is 22–24 hours.
- Hybridization in the GeneTitan™ MC Instrument is 23.5–24 hours.
- Reagent trays for washing, staining, and imaging must be prepared as hybridization finishes.
- A second hybridization tray and array plate can be loaded into the GeneTitan™ MC Instrument only during specific windows.

These limitations require careful timing. Using the manual target-preparation protocol, workflows are available for processing 3 array plates per week.

- Chapter 6, “Three-plate workflow for CytoScan™ HT-CMA Array Plates using an overnight DNA precipitation step”
- Chapter 7, “Three-plate workflow for CytoScan™ HT-CMA Array Plates using a 3-hour DNA precipitation step”

Overview of the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow

	Genomic DNA preparation	
Day 1	Chapter 2, Genomic DNA preparation	
	▼	
	Target preparation	
Day 1	Stage 1: Amplify the genomic DNA	23 ±1-hour amplification at 37°C. Optional stopping point.
	▼	
Day 2	Stage 2: Fragment and precipitate the DNA	16- to 24-hour incubation of Precipitation Plate (or optional 3-hour precipitation step) at –20°C.
	▼	
Day 3	Stage 3A: Centrifuge the Precipitation Plate and dry the DNA pellets	Optional stopping point.
	▼	
Day 3	Stage 3B: Resuspend the pellets and prepare for hybridization	
	▼	
Day 3	Stage 3C: Perform quantification and fragmentation QC checks	Optional stopping point.
	▼	
Day 3	Stage 4: Denature and hybridize the sample plate	23.5- to 24-hour array hybridization in the GeneTitan™ MC Instrument.
	▼	
Day 4	Stage 5: Prepare GeneTitan™ reagents and trays	
	Array processing	
Day 5	Chapter 5, Process array plates with the GeneTitan™ Multi-Channel Instrument *Array processing is completed with the GeneTitan™ MC Instrument and GeneChip™ Command Console™ software v4.3 or later.*	Fluidics: 5 hours Scan: ~7.5 hours

2 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.

For this protocol, you use the Applied Biosystems™ HT Target Prep Reagent Kit 96F (Cat. No. 906024). Genomic DNA Standard (Ref 103) (Cat. No. 951957), available separately, meets the DNA requirements that are outlined in this chapter and can be used as a control. The size and purity of sample gDNA can be compared with the size and purity of the control DNA to evaluate sample quality. Routinely use the control DNA as an experimental positive control and for troubleshooting purposes.

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

Sources of genomic DNA

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

- Whole blood
- Cell lines
- Amniotic fluid

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

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

General requirements

- Starting DNA must be double-stranded for accurate concentration determination.
- DNA must be of high purity. DNA must be free of DNA polymerase inhibitors. Examples of inhibitors include high concentrations of heme (from blood) and high concentrations of chelating agents (that is, EDTA). The gDNA extraction/ purification method must create DNA that is salt-free, because high concentrations of particular salts can inhibit enzyme reactions. DNA purity indicated by OD_{260}/OD_{280} and OD_{260}/OD_{230} ratios. The OD_{260}/OD_{280} ratio should be between 1.8 and 2.0 and the OD_{260}/OD_{230} ratio should be greater than 1.5. We recommend that DNA samples that do not meet these criteria be cleaned up as described in "Genomic DNA cleanup" on page 18.
- 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.
- We recommend that the assay plate contain at least 90 samples for accurate determination of SMN1 carrier status on the CytoScan™ HT-CMA 96-Array Plate. For questions regarding this recommendation contact Thermo Fisher Scientific technical support.

Note: DNA size integrity is important for successful assay performance. We recommend evaluating gDNA by gel electrophoresis.

Special requirements

Preamplification area

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

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

Ideally, the preamplification area would be separate from the amplification staging area described in Chapter 3, "Assay preparation". However, these areas can 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

Item	Source
Mother E-Base™ Device	EBM03
Daughter E-Base™ Device (optional for running multiple gels in parallel)	EBD03
E-Gel™ *48* Agarose Gels, 1%	G800801
*Redi*Load™ Loading Buffer	750026
E-Gel™ 96 High Range DNA Marker	12352019

Guidelines for gDNA Sample Plate preparation

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

- Loading a DNA mass of 10 ng to 20 ng per well is recommended. If lower amounts are loaded, omission of the loading dye is recommended 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, and 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. Power on the E-Base™ Device.
   An LED will be illuminated red to indicate that the power is on.
2. Push the **Power/Prg** button to ensure that the gel base is in **EG** mode, not EP mode.
3. Insert the E-Gel™ *48* Agarose Gels, 1% into the slot.
4. Remove 2 combs.
5. Load 20 µL of gDNA samples onto the E-Gel™ *48* Agarose Gels, 1%.
6. Load 15 µL of diluted E-Gel™ 96 High Range DNA Marker into all marker wells, if needed. Use a 1:3 dilution or ~0.34 X from stock.
7. Fill all empty wells with water.

8. Adjust the run time to ~27 minutes.

9. Push the **Power/Prg** button again. It will change from red to green.

When run time is reached and 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 CytoScan™ HT-CMA Assay) and degraded gDNA samples. For gDNA that is degraded, perform a test experiment to investigate the performance of their samples in the CytoScan™ HT-CMA Assay before starting any large-scale genotyping projects.

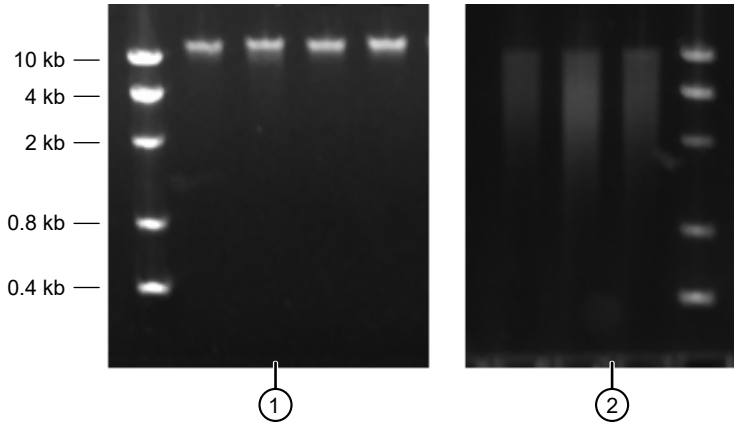


Figure 1 Gel images with intact gDNA and degraded gDNA

① Intact samples ② Degraded samples

Genomic DNA extraction/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.

Genomic DNA cleanup

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

1. Add 0.5 volumes of 7.5 M NH_4OAc and 2.5 volumes of absolute ethanol (stored at –20°C) to the 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 the supernatant, then wash the 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.
7. Resuspend the pellet in Low 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.

The genomic DNA (gDNA) you process using the CytoScan™ HT-CMA Assay must meet the general requirements that are listed earlier in this chapter. The amount of gDNA is 100 ng for the CytoScan™ HT-CMA whole-genome amplification step.

Genomic DNA input requirements

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

Time required

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

Equipment, consumables, and reagents required

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

Equipment and consumables required

Quantity	Item
As needed	Adhesive seals for plates
1	Ice bucket, filled with ice
1 each	Pipettes: single channel P10 or P20 Optional: multichannel P10 or P20
As needed	Pipette tips
1	Eppendorf™ DeepWell™ Plate 96, 13-864-302
1	96-well PCR plate: • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (HSS9641) for the following thermal cyclers: – Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block) – Applied Biosystems™ Veriti™ Thermal Cycler – Applied Biosystems™ ProFlex™ PCR System • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (HSP9631 or HSP9601) for the Eppendorf™Mastercycler™ pro S thermal cycler.
1	Plate centrifuge
1	Plate spectrophotometer (required only if no OD measurements available for samples)
1	Vortexer

Reagents required

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

Thaw samples and control

Thaw the following components to room temperature:

- gDNA samples
- Genomic DNA Standard (Ref 103)

To thaw, do one of the following:

- Place the items on the benchtop for 60 minutes.
- Thaw the items in a water bath.
  a. Fill a small plastic dish with ultra-pure water. Do not overfill, to prevent the level of the water overflowing when the sample tubes or plates are placed in the bath.
  b. Thaw the sealed gDNA Sample Plate for 30 minutes.
  c. Remove the gDNA Sample Plate and the control DNA tube from the water bath and wipe them dry with lab wipes. Ensure that the outside is dry before opening the gDNA Sample Plate or tube to minimize contamination.

Quantify and dilute gDNA

1. Gently vortex the gDNA samples and Genomic DNA Standard (Ref 103) at 50% maximum speed, then centrifuge them.
2. *Recommendation*: Quantify each sample. Use the Quant-iT™ PicoGreen™ dsDNA Assay Kit.
3. Using Low EDTA TE Buffer, dilute each sample to a concentration of 5 ng/µL.
4. Seal, vortex, then centrifuge the samples.

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

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

The samples and control will now be placed in a deep-well plate for amplification. Ensure that the gDNA is well mixed before transferring it to the plate.

Aliquot diluted samples and the Genomic DNA Standard (Ref 103) control to the deep-well plate

1. Aliquot 20 µL of each diluted gDNA sample to all 96 wells in the Eppendorf™ DeepWell™ Plate 96.
2. Pipet 20 µL of Genomic DNA Standard (Ref 103) control. We recommend including at least 1 positive control on each plate.
3. Seal, then centrifuge.

Freeze or proceed

Do one of the following:

- Store the sample plates at –20°C.
- Proceed to DNA amplification for manual target preparation. See “Stage 1: Amplify the genomic DNA” on page 38.

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

GeneTitan™ Array Plate Registration file

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. We recommend that you create, but not upload, this file at the same time that you prepare your plate of genomic DNA. When samples are ready for hybridization, scan the array plate barcode into the registration file and upload the file to GeneChip™ Command Console™ (GCC), which is the software that controls the GeneTitan™ instrument.

Each array plate has a barcode for tracking, and each row letter and column number identifies an individual array. The GeneTitan™ Array Plate Registration file is where you enter the sample information for individual arrays of the array plate to be run. GeneTitan™ Array Plate Registration files contain information that is critical for the following:

- Generating data files during imaging.
- Tracking the experimental results for each sample on an array plate.

Create and save a GeneTitan™ Array Plate Registration file

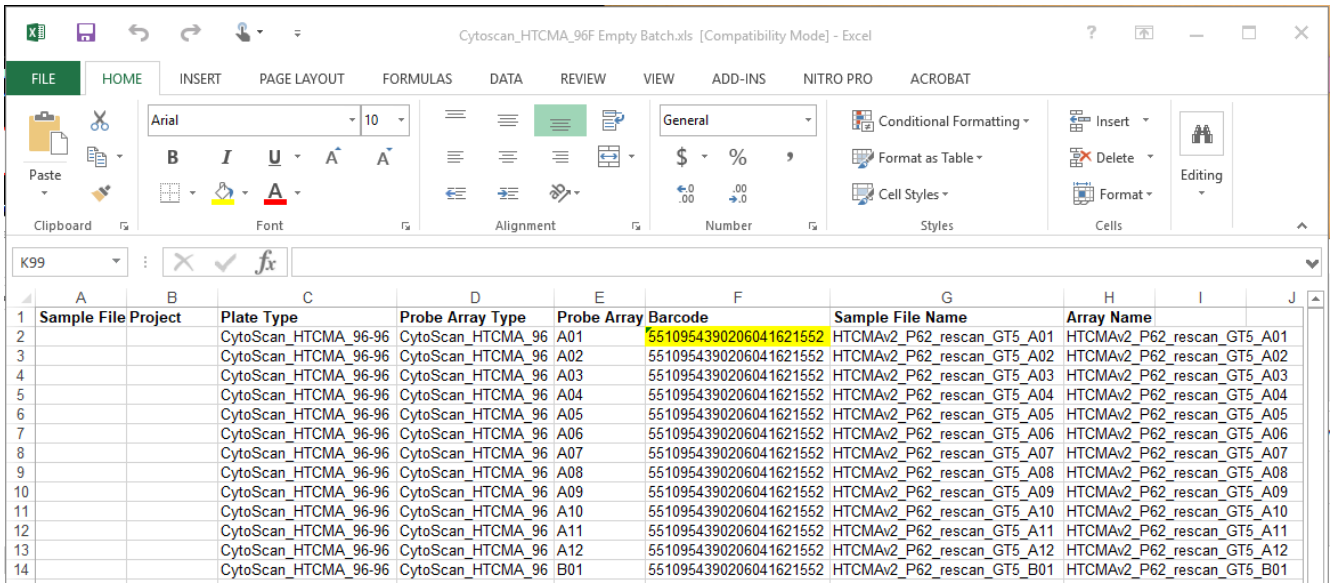
This procedure creates and saves a GeneTitan™ Array Plate Registration file but does not upload the file to GeneChip™ Command Console™. The array plate barcode is scanned, and the GeneTitan™ Array Plate Registration file is uploaded, when you are ready to load the plate and samples onto the GeneTitan™ 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 the 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 further information on the GeneTitan™ Array Plate Registration file, see the *GeneChip™ Command Console™ User Guide* (Pub. No. 702569).

7. Save the file. Do not upload the file at this point.

See details for the array plate scanning step, and the GeneTitan™ Array Plate Registration file uploading steps, in Chapter 5, “Process array plates with the GeneTitan™ Multi-Channel Instrument”.



	A	B	C	D	E	F	G	H	I	J
1	Sample File	Project	Plate Type	Probe Array Type	Probe Array	Barcode	Sample File Name	Array Name		
2			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A01	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A01	HTCMAv2_P62_rescan_GT5_A01		
3			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A02	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A02	HTCMAv2_P62_rescan_GT5_A02		
4			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A03	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A03	HTCMAv2_P62_rescan_GT5_A03		
5			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A04	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A04	HTCMAv2_P62_rescan_GT5_A04		
6			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A05	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A05	HTCMAv2_P62_rescan_GT5_A05		
7			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A06	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A06	HTCMAv2_P62_rescan_GT5_A06		
8			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A07	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A07	HTCMAv2_P62_rescan_GT5_A07		
9			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A08	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A08	HTCMAv2_P62_rescan_GT5_A08		
10			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A09	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A09	HTCMAv2_P62_rescan_GT5_A09		
11			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A10	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A10	HTCMAv2_P62_rescan_GT5_A10		
12			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A11	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A11	HTCMAv2_P62_rescan_GT5_A11		
13			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A12	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A12	HTCMAv2_P62_rescan_GT5_A12		
14			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	B01	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_B01	HTCMAv2_P62_rescan_GT5_B01		

Figure 2 Example of A CytoScan™ HT-CMA 96-Array Plate GeneTitan™ Array Plate Registration file.

3 Assay preparation

This chapter describes the procedures, equipment, and materials required for running the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow. To ensure operator safety and assay performance, operators must be familiar with this content before starting target preparation.

The manual assay format allows the user to run the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow for 96 samples using one HT Target Prep Reagent Kit 96F. This section provides information on procedures that are performed multiple times during manual target preparation and on steps that are critical to the success of manual target preparation. It is essential that you familiarize yourself with the information in this section prior to running the CytoScan™ Assay.

The manual assay requires the use of disposable reservoirs with a “trough-within-a-trough” design, which maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent.

A list of all equipment and resources required for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow is provided in the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018215.

Requirements and recommendations

This section describes requirements and recommendations for facilities and equipment needed to perform the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow.

Room temperature

When referred to in the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow, room temperature is 18–25°C.

Special requirements

Amplification staging area

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

The amplification staging area requires a dedicated set of pipettes and plasticware. If no dedicated area is available, use a dedicated bench or a dedicated biosafety hood and dedicated pipettes. If no dedicated bench or biosafety hood is available, a dedicated set of pipettes is recommended.

Fume hood

At specific steps in the protocol, we recommend the use of adequate local or general ventilation to keep airborne concentrations low. A fume hood is suggested to achieve the desired concentration. A fume hood is recommended for several steps of this assay.

Control requirement

A negative control is not required for this assay. Genomic DNA Standard (Ref 103) is used as a positive control.

Plate requirements and recommendations

The following types of plates are required for performing manual target preparation. See *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018215, for supplier information.

- 2-mL Eppendorf™ DeepWell™ Plate 96
- 96-well PCR plate:
  - Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641) for the following thermal cyclers:
    - Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block)
    - Applied Biosystems™ Veriti™ Thermal Cycler
    - Applied Biosystems™ ProFlex™ PCR System
  - Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (Cat. No. HSP9631 or HSP9601) for the following thermal cycler:
    - Eppendorf™ Mastercycler™ pro S
- Greiner Bio-One™ UV-Star™ 96-Well UV Spectroscopy Microplate, 370 µL/well (Cat. No. 07-000-407)

Thermal cycler recommendations and protocol

The following thermal cyclers are recommended for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow.

- Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block)
- Applied Biosystems™ Veriti™ Thermal Cycler
- Applied Biosystems™ ProFlex™ PCR System
- Eppendorf™ Mastercycler™ pro S

Note:
To run the 3-plate/week manual target-preparation workflow, 1 verified thermal cycler is required.

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

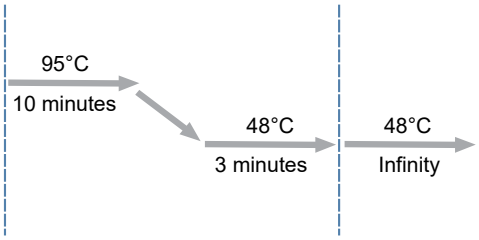


Figure 3 CytoScan HT-CMA Denature protocol (Stage 4).

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

Thermal cycler consumables

The following table lists the consumables for each thermal cycler model.

Thermal cycler	PCR plate type	Seal[1]
Applied Biosystems™ GeneAmp™ PCR System 9700 (with gold-plated or silver block)	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted, HSS9641	MicroAmp™ Clear Adhesive Film, 4306311
Applied Biosystems™ Veriti™ Thermal Cycler	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted, HSS9641	
Applied Biosystems™ ProFlex™ PCR System	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted, HSS9641	
Eppendorf™ Mastercycler™ pro S	Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted, HSP9631or HSP9601	

[1] Microseal™ 'B' PCR Plate Sealing Film (Bio-Rad™, MSB1001) can be used instead of MicroAmp™ Clear Adhesive Film for the Bio-Rad™ and Applied Biosystems™ thermal cyclers.

Oven recommendations

The following ovens are recommended. See the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide* (Pub. No. MAN0018215) for supplier information.

- BINDER™ ED 56 drying oven
- GeneChip™ Hybridization Oven 645

  The GeneChip™ Hybridization Oven 640 is currently not supported with the CytoScan™ HT-CMA Assay. If you intend to use the GeneChip™ Hybridization Oven 640 in the workflow, first contact your field service engineer (FSE) or Thermo Fisher Scientific Technical Support regarding the compatibility of this oven with the CytoScan™ HT-CMA Assay.
  - If using a GeneChip™ Hybridization Oven, set the rotation speed to 15 rpm to aid in even heat distribution.
  - For the GeneChip™ Hybridization Oven, place plates in the bottom of the oven. To avoid interfering with the rotation apparatus, do not stack plates in the oven.

Plate centrifuge

One plate centrifuge is required for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow. See the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018215, for an appropriate plate centrifuge. When centrifuging and drying pellets, the centrifuge must meet the following requirements:

- Rcf: 3,200 × *g* (4,000 rpm for the Eppendorf™ 5810R with the rotor configuration that is described in the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018215).
- Temperature: 4°C and room temperature.

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

Plate shakers

We recommend using one of the following shakers.

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

Equipment care and calibration

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

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

Procedures

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

Guidelines for handling plates and tubes

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

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

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

  Blot-dry: Before sealing plates, check the top of the plate to ensure that there are no droplets. If droplets are present, blot-dry the top of the plate before sealing to ensure a tight seal.
  - To remove droplets before sealing, overlay a sheet of laboratory tissue across the top of the plate and gently pat down to dry.
  - Lift the sheet off the plate and discard. Ensure that the top of the plate is dry and seal the plate as usual.
- **Vortex reagent vials**:
  - Vortex 3 times for 1 second each time at the maximum setting.

- **Vortex plates**:
  - Vortex deep-well plates for 5 seconds in each of the 5 sectors. See the sectors in Figure 4.
  - Vortex PCR plates such as the Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted and the Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted for 2 seconds in each of the 5 sectors.

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

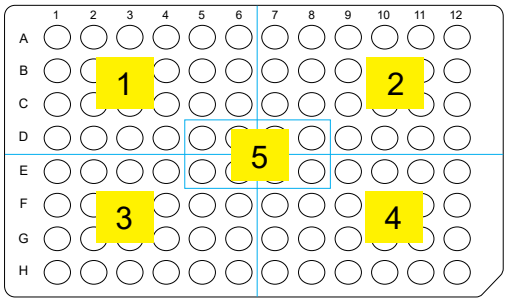


Figure 4 The 5 plate sectors.

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

Sample quantification

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

See Chapter 2, “Genomic DNA preparation”.

About the reagents and master mix preparations

- About the HT Target Prep Reagent Kit 96F components:
  - Caps on the vials are color-coded according to assay stage.
  - Properly store all enzyme reagents, especially enzyme-containing vials. Improper storage methods can profoundly affect activity.
- About reagents from other suppliers:
  - Use only fresh reagents from the recommended vendors to help eliminate changes in pH or the salt concentration of buffers.
  - Consult the appropriate SDS for reagent storage and handling requirements.

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

    The volume of master mixes prepared are designed to provide consistent handling of reagents and consistent assay results. The percent overage of different master mixes can differ, depending on the reagent volumes involved.
- About reagents at the laboratory bench:
  - Properly chill essential equipment such as reagent coolers before use.
  - Ensure that the enzymes are kept at –20°C until needed. When removed from the freezer, immediately place in a cooler that has been chilled to –20°C.

Pipette recommendations

- Use a pipette of appropriate size for the volume of liquid being transferred.

Pipette size	Recommended volume range
Single channel P20 / 8- or 12-channel P20	1-20 µL
Single channel P200 / 8- or 12-channel P200	20-200 µL
Single channel P1000 / 12-channel P1200	200-1,000 µL

- Always use pipettes that have been calibrated.
- Proficiency with the use of single and multichannel pipettes is essential. To familiarize yourself with the use of multichannel pipettes, we strongly recommend practicing several times before processing actual samples. Use water and reagent reservoirs to get a feel for aspirating and dispensing solutions to multiple wells simultaneously.

Single-channel and serological pipettes

Use single-channel pipettes for preparing master mixes and for puncturing bubbles in GeneTitan™ trays. The single-channel pipettes are not used for working with plates or trays.

- Use single-channel pipettes for volumes less than or equal to 2 mL. For volumes between 1 mL and 2 mL, add the reagent in 2 portions with a fresh tip for each portion.
- Use serological pipettes for volumes greater than 2 mL.
- Usually, 25-mL or 50-mL serological pipettes do not fit into the mouths of the reagent bottles. Multiple transfers using 5-mL or 10-mL serological pipettes are recommended.

Multichannel pipettes

Use 12-channel pipettes when adding master mix or transferring samples to plates and GeneTitan™ trays.

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

Matrix™ Reagent Reservoirs

The CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow requires the use of disposable reservoirs with a "trough within a trough" design. This design maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent.

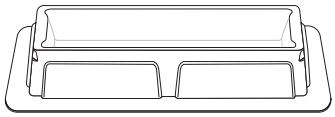


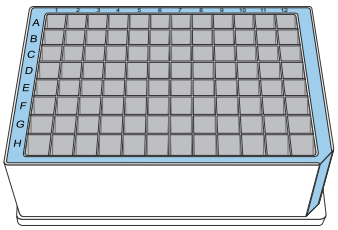
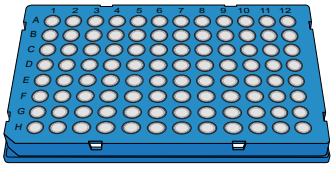
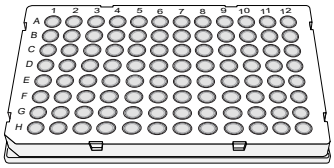
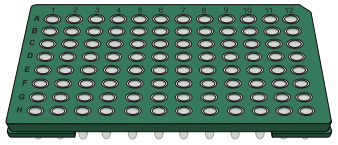
Figure 5 Dispense reagents from Matrix™ 25-mL reagent reservoirs.

Required materials

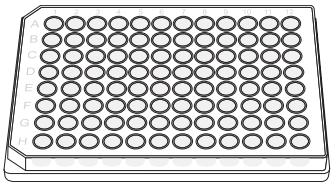
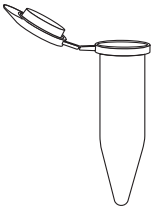
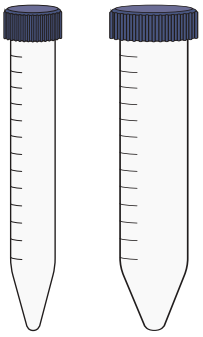
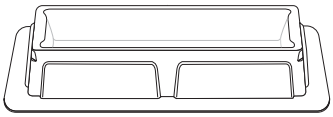
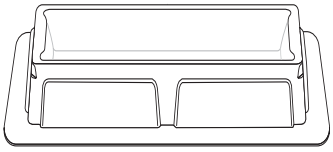
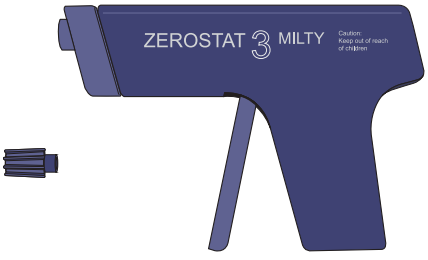
Labware consumables required

The following table describes the labware consumables required, including ordering information and images.

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.

Labware	Supplier and Cat. No.	Image
Eppendorf™ DeepWell™ Plate 96, 2,000 µL	Fisher Scientific™, 13-864-302	
Hard-Shell™ 96-Well PCR Plate, low profile, full skirted[1]	Bio-Rad™, HSP9631 (blue) or HSP9601 (white)	 
96 half-skirt plate also called Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9601) [1]	Bio-Rad™, HSS9641	

(continued)

Labware	Supplier and Cat. No.	Image
Greiner Bio-One™ UV-Star™ 96-Well UV Spectroscopy Microplate	Fisher Scientific™, 07-000-407	
1.7-mL microcentrifuge tubes, DNAse and RNAse-free	**MLS**	
15-mL and 50-mL conical-bottom centrifuge tubes, polypropylene	**MLS**	
Matrix™ Reagent Reservoir, 25 mL	8093	
Matrix™ Reagent Reservoir, 100 mL	8085	
GeneTitan™ ZeroStat AntiStatic Gun and Ion-Indicator Cap	74-0014	

(continued)

Labware	Supplier and Cat. No.	Image
96-well metal chamber Cooling Chamber for 0.2-mL tubes, 96 holes (four for 1.5-mL and six for 0.5-mL tubes), Dim.: 6 1/8”L x 3 1/8”W x 1” H	Diversified Biotech™, R-1007-1	
Adhesive film	Use one of the following: • MicroAmp™ Clear Adhesive Film, 4306311 • Microseal™ 'B' PCR Plate Sealing Film, Bio-Rad™, MSB1001	

[1] See “Thermal cycler consumables” on page 26 for the PCR plate type recommended for your specific thermal cycler.

Axiom™ GeneTitan™ Consumables Kit

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

Table 1 Axiom™ GeneTitan™ Consumables Kit (Cat. No. 901606).

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

[1] See Table 2 for detailed descriptions of each component.

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

Table 2 Axiom™ GeneTitan™ tray consumables (from the Axiom™ GeneTitan™ Consumables Kit (Cat. No. 901606).

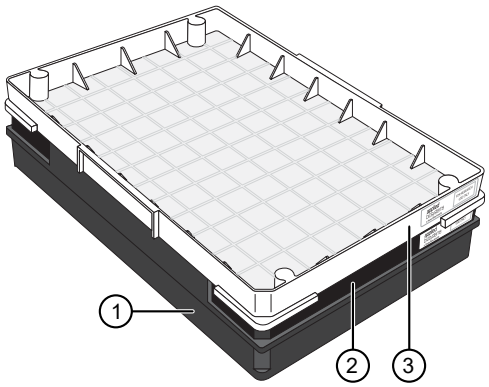
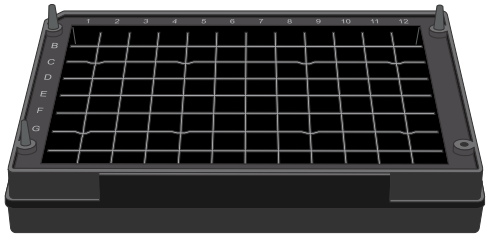
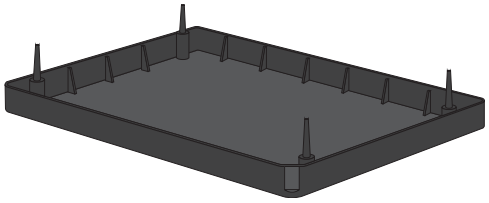
Item	Part No.	Image	Details
Scan tray	900746 Box 501006 Pouch	 ① Scan tray protective base ② 96-layout scan tray ③ Scan tray cover	The scan tray package includes the scan tray, a cover, and a black protective base. • Cover the scan tray with the scan tray cover before placing the tray in the GeneTitan™ MC Instrument. • Protect the scan tray at all times from damage or exposure to dust. The scan tray must be in the black plate base at all times except when loaded into the GeneTitan™ MC Instrument. • Use the black scan tray protective base in the package to protect the bottom of the scan tray glass from damage. Remove the protective base from the scan tray before loading the scan tray with the scan tray cover in the GeneTitan™ MC Instrument.
GeneTitan™ scan tray on black base			Use this combination of the GeneTitan™ scan tray on the protective black base during the GeneTitan™ reagent preparation method.
Black scan tray protective base			Use the black scan tray protective base in the package to protect the bottom of the scan tray glass from damage. The black scan tray base is distinct from the blue array plate protective base and must not be used with the array plate. Remove the protective base from the scan tray before loading in the GeneTitan™ MC Instrument.

Table 2 Axiom GeneTitan tray consumables (from the Axiom GeneTitan Consumables Kit (Cat. No. 901606). *(continued)*

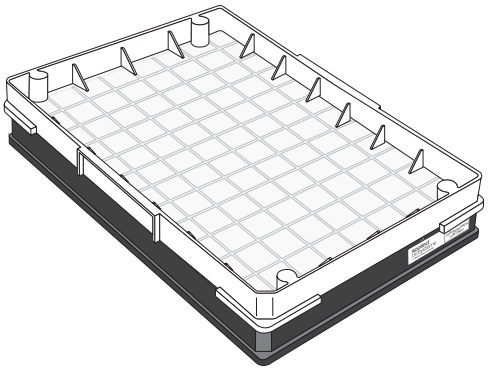
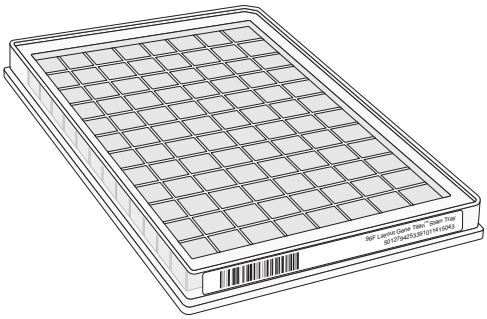
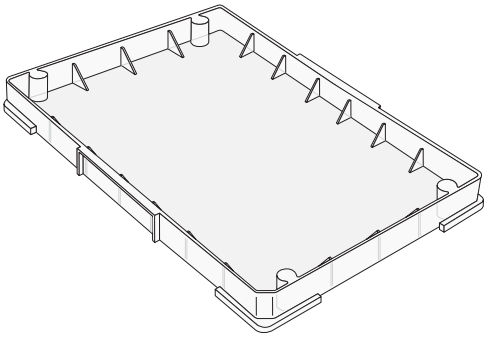
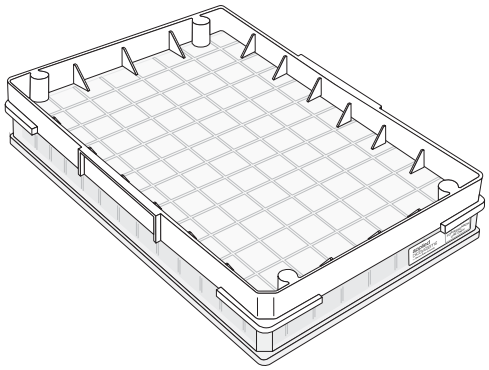
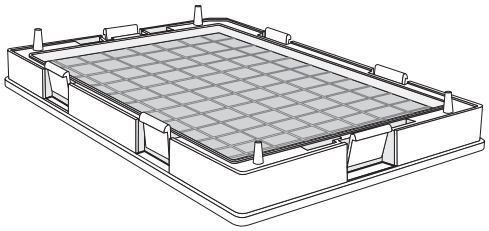
Item	Part No.	Image	Details
GeneTitan™ scan tray with cover			The GeneTitan™ scan tray must be loaded with the scan tray cover into the GeneTitan™ MC Instrument. *Do not* load the scan tray with the protective base.
96-layout GeneTitan™ stain trays	9016110 Kit (5 stain trays) 501025 Tray		The GeneTitan™ stain trays are packaged in zip-top bags to keep them free of dust. Each GeneTitan™ stain tray is uniquely barcoded. All trays must be deionized to remove static electricity before the prepare GeneTitan™ reagent plates step. See “Deionization of GeneTitan™ trays and covers” on page 147.
96-layout stain and scan tray cover	202757		The 96-layout scan and stain tray covers are provided to prevent any evaporation of the stains in stain trays and the array holding buffer in the scan tray. The GeneTitan™ scan and stain tray covers are barcoded. All stain and scan trays must be placed in the GeneTitan™ MC Instrument with the tray cover. All tray covers must be deionized to remove static electricity before placing the cover on the tray. See “Deionization of GeneTitan™ trays and covers” on page 147.

Table 2 Axiom GeneTitan tray consumables (from the Axiom GeneTitan Consumables Kit (Cat. No. 901606). *(continued)*

Item	Part No.	Image	Details
GeneTitan™ stain tray cover, shown on top of the stain tray	Tray 501025 Cover 202757		The GeneTitan™ stain trays must be placed in the GeneTitan™ MC Instrument with the GeneTitan™ stain tray cover. See "Label GeneTitan™ hybridization and reagent trays" on page 145.
96-layout hybridization tray	900747		After aliquoting the denatured hybridization ready samples into the hybridization tray, the tray should be immediately loaded into the GeneTitan™ MC Instrument with the barcode facing away from the operator, that is, the barcode should be on the back side.

HT Target Prep Reagent Kit 96F

Each HT Target Prep Reagent Kit 96F (Cat. No. 906024) is sufficient for 1 CytoScan™ HT-CMA 96-Array Plate.

Component and cap color		Storage
HT Target Prep Module 1		–25°C to –15°C
10X Denat Solution	Amp Solution	
Neutral Solution	Amp Enzyme	
Water		
HT Target Prep Module 2-1		–25°C to –15°C
Frag Enzyme	Hyb Buffer	
10X Frag Buffer	Hyb Solution 1	
Precip Solution 2		
HT Target Prep Module 2-2		2°C to 8°C
Frag Diluent	Resuspension Buffer	
Frag Reaction Stop	Hyb Solution 2	
Precip Solution 1		
HT Target Prep Module 3-1		–25°C to –15°C
Ligate Buffer	Probe Mix 1	
Ligate Enzyme	Stain Buffer	
Ligate Solution 1	Stabilize Solution	
HT Target Prep Module 3-2		2°C to 8°C
Ligate Solution 2	Stain 2-A	
Probe Mix 2	Stain 2-B	
Wash A	Stabilize Diluent	
Stain 1-A	Water	
Stain 1-B	Hold Buffer	
HT Target Prep Wash A		Room temperature
Wash Buffer A		
HT Target Prep Wash B		Room temperature
Wash Buffer B		
HT Target Prep Water		Room temperature
Water		

4 Target preparation

Manual target preparation for the CytoScan™ HT-CMA Assay enables you to perform target preparation to process 96 samples at a time without the use of automation equipment. Array handling and processing procedures still require the use of a GeneTitan™ MC Instrument. See Chapter 5, “Process array plates with the GeneTitan™ Multi-Channel Instrument”.

IMPORTANT! Read the instructions in Chapter 3, “Assay preparation” before performing manual target preparation.

For a list of equipment and resources required for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow, see the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018215.

Using the manual target preparation protocol, 3 array plates can be processed per workweek, for a total of 288 arrays. See Chapter 6, “Three-plate workflow for CytoScan™ HT-CMA Array Plates using an overnight DNA precipitation step” and Chapter 7, “Three-plate workflow for CytoScan™ HT-CMA Array Plates using a 3-hour DNA precipitation step”.

Stage 1: Amplify the genomic DNA

Before proceeding to DNA amplification, complete the gDNA preparation. See Chapter 2, "Genomic DNA preparation".

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

IMPORTANT! Amplification preparation must take place in a dedicated area such as a biosafety hood with dedicated pipettes, tips, and vortex.

Time required

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

Activity	Time
Thaw material	1 hour
Hands-on time	30 minutes
Incubation at 37°C	23 hours (±1 hour)
Total	**~24.5 hours**

Input required

The gDNA Sample Plate, with 20 µL of each gDNA diluted to a concentration of 5 ng/µL in an Eppendorf™ DeepWell™ Plate 96, 2,000 µL.

Materials, labware, and reagents required

Equipment and consumables required for Stage 1

Quantity	Item
Equipment and consumables	
As needed	Adhesive seals for 96-well plates
As needed	Laboratory tissues
1	Microcentrifuge tube holder
1	Cooler, chilled to –20°C
1	Fine-point permanent marker
1	50-mL tube holder
1	15-mL tube holder
1	Mini microcentrifuge (microfuge with microtube rotor)

(continued)

Quantity	Item
1 each	Rainin™ pipettes: • Single-channel P200 • Single-channel P1000 • Multichannel P20 • Multichannel P200 • Multichannel P1200
As needed	Pipette tips
As needed	Serological pipettes: • 5 mL (VWR Cat. No. 89130-896) • 10 mL (VWR Cat. No. 89130-898)
1	Pipet-Aid™ Pipette Controller
1	Plate centrifuge at room temperature
1	Oven, set at 37°C
1	50-mL conical tube
1	15-mL conical tube
1	Vortexer
1	Timer
3	Matrix™ Reagent Reservoir, 25 mL

Reagents required for Stage 1

Reagent and cap color	Module
From the Applied Biosystems™ HT Target Prep Reagent Kit 96F	
10X Denat Solution	HT Target Prep Module 1, –20°C (Part. No. 906011)
Neutral Solution	
Amp Solution	
Amp Enzyme	
Water	

Prepare for DNA amplification

1. Set the incubator or oven temperature to 37°C.

   We recommend using one of the following ovens:

   - BINDER™ ED 56
   - GeneChip™ Hybridization Oven 645, with rotation set to 15 rpm

2. Set the centrifuge to room temperature.

3. Thaw the sample plate on the benchtop at room temperature, then vortex it, centrifuge it briefly, and leave it at room temperature.

   IMPORTANT!

   - The gDNA samples must be brought to room temperature before proceeding with denaturation.
   - The gDNA samples must be 20-µL volume of each gDNA at a concentration of 5 ng/µL in an Eppendorf™ DeepWell™ Plate 96, 2,000 µL. See Chapter 2, “Genomic DNA preparation”.

4. Prepare reagents as shown in the following table.

Reagent and cap color	Treatment
10X Denat Solution	Thaw, vortex, and centrifuge, then keep at room temperature.
Neutral Solution	Thaw and vortex, then keep at room temperature.
Amp Solution	Thaw and vortex, then keep at room temperature.
Water	Thaw and vortex, then keep at room temperature.
Amp Enzyme[1]	Flick the tube 3 times, centrifuge it, then keep it in a –20°C cooler until ready to use. Just before using it, gently flick the tube 3 times to mix, then centrifuge it briefly.

[1] Leave at –20°C until ready to use.

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

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

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

6. Label 3 Matrix™ Reagent Reservoirs as indicated in the following table.

Label	Reservoir size	Temperature	Contents
D MM	25 mL	Leave reservoir at room temperature.	Denaturation Master Mix
N Soln	25 mL	Leave reservoir at room temperature.	Neutral Solution
Amp MM	25 mL	Leave reservoir at room temperature.	Amplification Master Mix

Prepare the Denaturation Master Mix

Carry out the following steps at room temperature.

1. To the 15-mL conical tube labeled “D MM”, add the amount of 10X Denat Solution shown in the following table, then dilute it with the amount of Water shown.

Reagent and cap color	Per sample	Master mix 96+
10X Denat Solution	2 µL	400 µL
Water	18 µL	3.6 mL
Total volume	**20 µL**	**4.0 mL**

2. Vortex the tube, then leave it at room temperature.

Add Denaturation Master Mix to samples

Carry out the following steps at room temperature.

1. Briefly centrifuge the sample plate.

   Note: The samples must be at room temperature for this step.

2. Gently pipet the Denaturation Master Mix using a P1000 or pour it into the reagent reservoir labeled “D MM”.

3. Carefully remove the seal from the Amplification Sample Plate. Discard the seal.

4. Using a P20 12-channel pipette, add 20 µL of Denaturation Master Mix to each sample. The total volume will be 40 µL/well.
   - Pipet directly into the liquid of each well.
   - Do not mix by pipetting up and down.
   - Change tips between each addition.

   Note: This plate is now the Denaturation Plate.

5. Seal and vortex the Denaturation Plate. After vortexing, start the timer for a 10-minute incubation.

6. Centrifuge the Denaturation Plate in a room-temperature centrifuge for 1 minute at 1,000 rpm.

   Note: The centrifuge time is included in the 10-minute incubation.

7. Visually examine the volume in each well. It should be 40 µL/well.
   a. Keep a record of wells that appear to have an unusually low or high volume. These samples might need to be repeated.
   b. Do *not* stop to measure volumes. Proceed without delay.

8. Complete the 10-minute incubation on the benchtop at room temperature.

   While completing the incubation at room temperature, prepare the Neutral Solution as described in “Prepare for DNA amplification” on page 40.

9. After incubation, immediately add the Neutral Solution. Follow the instructions in “Add Neutral Solution to samples” on page 42.

Add Neutral Solution to samples

Carry out the following steps at room temperature.

1. Pour the Neutral Solution into the reagent reservoir labeled “N Soln”.
2. Carefully remove the seal from the Denaturation Plate. Discard the seal.
3. Using a P200 12-channel pipette, add 130 µL of Neutral Solution to each sample. The total volume will be 170 µL/well.
   - Pipet down the wall of each well.
   - Change tips between each addition.

   Note: This plate is now the Neutralization Plate.

4. Seal the Neutralization Plate, vortex it, then briefly centrifuge it.
5. Visually examine the volume in each well. It should be ~170 µL/well.
   a. Keep a record of wells that appear to have an unusually low or high volume. These samples might need to be repeated.
   b. Do *not* stop to measure volumes.

6. Proceed immediately to Amplification Master Mix preparation. See “Add the Amplification Master Mix to samples” on page 43.

Prepare the Amplification Master Mix

Carry out the following steps at room temperature.

1. Pipet the amount of Amp Solution shown in the following table into the 50-mL tube labeled “Amp MM” at room temperature.

   Note: The Amp Solution is a viscous solution. Follow these steps to ensure that the reagent transfer is accurate:
   - Pipet slowly.
   - Allow bubbles that are generated from mixing to float to the top before pipetting.
   - Use a 10-mL serological pipette to transfer the Amp Solution into the Amp MM tube.

Reagent and cap color	Per sample	Master mix 96+
Amp Solution	225 µL	26.0 mL
Amp Enzyme	5 µL	578 µL
Total volume	**230 µL**	**26.58 mL**

2. Remove the Amp Enzyme from the freezer, then place it in a portable cooler at –20°C.
   a. Invert, then flick the Amp Enzyme tube 3 times. Briefly centrifuge the tube.
   b. Add the amount of Amp Enzyme shown to the Amp MM tube.
   c. Vortex the Amplification Master Mix well, invert the tube 2 times, then vortex it again.

Add the Amplification Master Mix to samples

1. *Slowly* pour the Amplification Master Mix into the reagent reservoir labeled “Amp MM”.
2. Carefully remove the seal from the Neutralization Plate. Discard the seal.
3. Using a P1200 12-channel pipette, slowly add 230 µL Amplification Master Mix to each well of the Neutralization Plate. The total volume will be 400 µL/well.
   - Pipet down the wall of the well.
   - Do not mix by pipetting up and down.
   - Change tips between each addition.

   Note: This plate is now the Amplification Plate.
4. Blot the top of the plate with a laboratory tissue. Seal the plate tightly, vortex it twice, then centrifuge it for 1 minute at 1,000 rpm.

   See “Guidelines for handling plates and tubes” on page 27.

5. Place the sealed Amplification Plate in an oven set at 37°C, then leave it undisturbed for 23 ±1 hours.

Note: If using a GeneChip™ Hybridization Oven, place the plate on the bottom of the oven. Do not turn on the oven rotation apparatus.

Freeze the plate or proceed

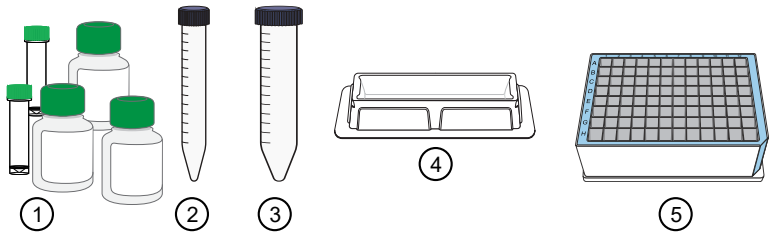
After the incubation finishes, do one of the following:

- Proceed to "Stage 2: Fragment and precipitate the DNA" on page 47.
- Store the Amplification Plate at –20°C.

Note: If freezing, do not perform the stop amplification reaction step before you store the Amplification Plate at –20°C. The stop amplification reaction step is performed after thawing the frozen plate. See "Prepare for fragmentation and precipitation" on page 48.

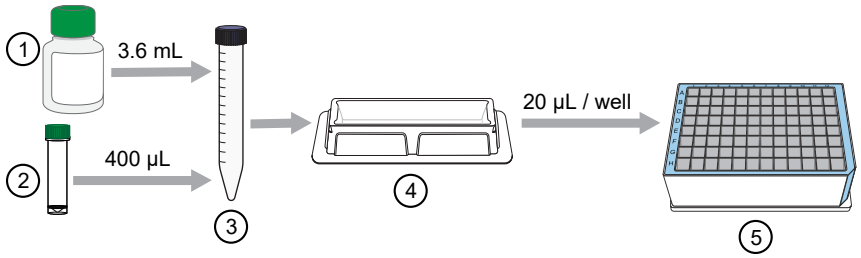
Workflow for Stage 1: Amplify the genomic DNA

Materials required



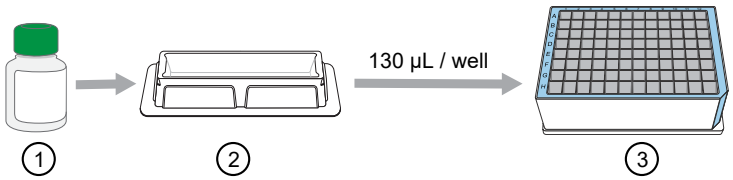
1. HT Target Prep Module 1 reagents
2. Quantity = 1
3. Quantity = 1
4. Quantity = 3
5. Quantity = 1, Amplification Sample Plate

Denaturation step



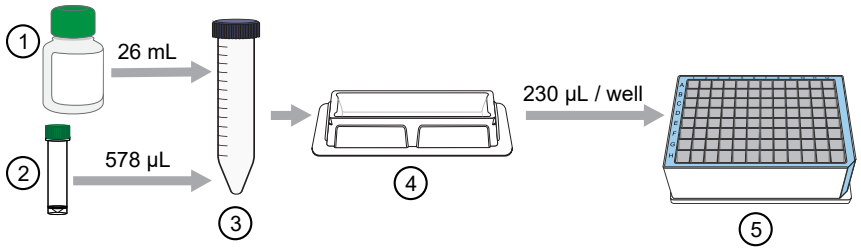
1. Water
2. 10X Denat Solution
3. Vortex, then briefly centrifuge.
4. Pour into reagent reservoir.
5. Denaturation Plate. Vortex, briefly centrifuge, then incubate at room temperature for 10 minutes.

Neutralization step



1. Neutral Solution
2. Pour into reagent reservoir.
3. Neutralization Plate. Vortex, then briefly centrifuge.

DNA amplification preparation



Final volume = 400 µL / well

1. Amp Solution
2. Amp Enzyme
3. Vortex, then invert tube to mix.
4. Pour into reagent reservoir.
5. Amplification Plate. Vortex for 30 seconds, then centrifuge briefly.

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


Stage 2: Fragment and precipitate the DNA

Time required

Total time: Approximately 2 hours, plus time for precipitation incubation at –20°C, for 3 hours or overnight.

Input required

The Amplification Plate from stage 1.

Materials, labware, and reagents required

Equipment and consumables required for Stage 2

Quantity	Item
As needed	Adhesive seals for 96-well plates
1	Freezer, set to –20°C (Designate a shelf where the precipitation plates can be left undisturbed.)
1	Cooler, chilled to –20°C
1	Ice bucket, filled with ice
1	Fine-point permanent marker
1 each	Rainin™ pipettes: • Single-channel P200 • Single-channel P1000 • Multichannel P20 • Multichannel P200 • Multichannel P1200
As needed	Pipette tips
As needed	Serological pipettes: • 5 mL • 10 mL
1	Pipet-Aid™ Pipette Controller
1	Plate centrifuge set at room temperature
1	Mini microcentrifuge (microfuge with microtube rotor)
2–3	Ovens • One set at 37°C • One set at 65°C

(continued)

Quantity	Item
1	15-mL conical tube and holder
3	Matrix™ Reagent Reservoir, 25 mL
1	Matrix™ Reagent Reservoir, 100 mL
1	Vortexer

Reagents required for Stage 2

Reagent and cap color	Module
From the HT Target Prep Reagent Kit 96F	
Frag Enzyme (leave at –20°C until ready to use)	HT Target Prep Module 2-1, –20°C (Part No. 906012)
10X Frag Buffer	
Precip Solution 2	
Frag Diluent	HT Target Prep Module 2-2 , 2–8°C (Part No. 906013)
Frag Reaction Stop	
Precip Solution 1	
User-supplied	
Isopropanol, 99.5%, 70 mL	—

Prepare for fragmentation and precipitation

Set oven and centrifuge temperatures

1. Set up 2 incubators or ovens. This should be done the night before they are used.
   a. Set one oven set at 37°C. Use an oven that can sustain a constant temperature of 37°C and has a temperature accuracy of ±1°C.
   b. Set one oven at 65°C.

Note: For the 3-hour precipitation workflow, 3 ovens are recommended. Set one oven at 37°C, one at 65°C, and one at 48°C in preparation for "Stage 4: Denature and hybridize the sample plate" on page 72.

2. Set the centrifuge to room temperature.

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

Thaw and prepare the amplified DNA samples and reagents

1. If the Amplification Plate is *not* frozen, skip to step 3.
2. If the Amplification Plate is frozen, thaw, then prepare it as described here.
   a. Place the deep-well plate in a small bath of room-temperature ultra-pure water.
   b. Leave the plate in the water bath for ~50 minutes, until all wells have thawed.
   c. Centrifuge the plate at 1,000 rpm for 30 seconds.

   Note: To avoid cross-contamination of wells during vortexing:
   - Remove the seal and blot the top of the plate with a laboratory tissue.
   - Tightly reseal the plate with a fresh seal.

   d. Vortex the plate for 30 seconds to mix the contents.
   e. Centrifuge the plate at 1,000 rpm for 30 seconds.
3. Prepare the following fragmentation reagents:
   a. 10X Frag Buffer
      - Thaw on the benchtop at room temperature, then place on ice.
      - Vortex before use.
   b. Frag Diluent
      - Place on ice.
      - Vortex, then centrifuge briefly before use.
   c. Frag Reaction Stop
      - Place on the benchtop to warm to room temperature.
      - Vortex before use.
   d. Frag Enzyme
      - Leave at –20°C until ready to use.
      - Immediately before use, gently flick the tube 3 times to mix, then centrifuge briefly.
4. Prepare the following precipitation reagents:
   a. Precip Solution 1
      - Place on the benchtop to warm to room temperature.
      - Vortex before use.
   b. Precip Solution 2
      - Thaw on the benchtop at room temperature and keep at room temperature.
      - Vortex, then centrifuge briefly before use.

c. Keep the isopropanol at room temperature.

Label tubes and reagent reservoirs

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

Label	Temperature	Contents
Frag MM	Place tube on ice	Fragmentation Master Mix

2. Label the 4 Matrix™ Reagent Reservoirs as indicated in the following table.

Label	Reservoir size	Temperature	Contents
Frag MM	25 mL	Room temperature	Fragmentation Master Mix
Stop	25 mL	Room temperature	Frag Rxn Stop
Precip MM	25 mL	Room temperature	Precipitation Master Mix
ISO	100 mL	Room temperature	Isopropanol

Incubate the samples in preheated ovens

Note: If the Amplification Plate is frozen, thaw the plate before beginning Stage 2. See instructions on thawing amplified DNA samples in the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow User Guide*, Pub. No. MAN0018214. If the Amplification Plate is not frozen, continue with the instructions in this section.

Stop the DNA amplification reaction

1. Place the Amplification Plate in the 65°C oven.
2. Incubate for 20 minutes.

Prepare for fragmentation

1. Remove the Amplification Plate from the 65°C oven, then check the seal. Press the seal, if needed.
2. Transfer the Amplification Plate to the 37°C oven.
3. Incubate for 45 minutes.

Prepare the Fragmentation Master Mix

Start making the Fragmentation Master Mix 5 minutes before completion of the 37°C incubation.

1. Transfer the Frag Enzyme to a –20°C portable cooler until ready to use.
2. Add the reagents in the order shown in the following table, to the 15-mL tube labeled “Frag MM”. Use appropriate single-channel pipettes.
   a. Just before the end of the 45-minute 37°C incubation, flick the Frag Enzyme tube 2 to 3 times, then centrifuge it.

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

IMPORTANT! Leave the Frag Enzyme at –20°C until ready to use.

Reagent and cap color	Per sample	Master mix 96+
10X Frag Buffer	45.7 µL	6.0 mL
Frag Diluent	10.3 µL	1.35 mL
Frag Enzyme	1.0 µL	131 µL
Total volume	**57 µL**	**7.48 mL**

3. Vortex the Frag MM tube twice, then place it on ice.
4. Slowly pour the Fragmentation Master Mix into the reagent reservoir labeled "Frag MM". Place the reservoir at room temperature.

Add Fragmentation Master Mix to samples

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

1. Carefully remove the Amplification Plate from the 37°C oven and place it on the benchtop at room temperature.

   Do *not* place the Amplification Plate on ice.
2. Carefully remove the seal from the Amplification Plate. Discard the seal.
3. Use a P200 12-channel pipette to add 57 µL of Fragmentation Master Mix to each reaction.
   - Pipet directly into the liquid of each well.
   - Do *not* mix by pipetting up and down.
   - Change tips after each addition.

   Note: This plate is now the Fragmentation Plate.
4. Seal the Fragmentation Plate, then vortex it twice.
5. Start the timer for 30 minutes.

   Note: Keep your timer in a safe place. If the timer accidentally stops, it is helpful to have noted when the incubation started.
6. Briefly centrifuge the Fragmentation Plate at room temperature.

7. Quickly transfer the plate to the 37°C oven, then incubate for 30 minutes.

CAUTION! Watch for the end of the 30-minute incubation. *Fragmentation is an exact 30-minute incubation step.* Longer or shorter incubation times can lead to poor performance of the assay.

8. Prepare the Frag Reaction Stop solution a few minutes before the end of the 30-minute incubation.
See “Add the Frag Reaction Stop solution to the Fragmentation Plate” on page 52.

Add the Frag Reaction Stop solution to the Fragmentation Plate

Carry out the following steps at room temperature.

1. A few minutes before the end of the 30-minute incubation, pour the Frag Reaction Stop solution into the reagent reservoir labeled "Stop".
2. At the end of the 30-minute fragmentation incubation, remove the Fragmentation Plate from the oven. Place it on the benchtop at room temperature.
3. Carefully remove the seal from the Fragmentation Plate. Discard the seal.
4. Using a P20 12-channel pipette, end the fragmentation reaction by adding 19 µL of Frag Reaction Stop to each reaction.
   - Do not mix by pipetting up and down.
   - Pipette directly into the liquid of each well.
   - Change tips after each addition.
   - Proceed immediately to the next step.
5. Seal the Fragmentation Plate, then vortex and briefly centrifuge it at 1,000 rpm.
6. Leave the Fragmentation Plate on the benchtop while you prepare the Precipitation Master Mix.

Prepare the Precipitation Master Mix

Carry out the following steps at room temperature.

1. Prepare the Precipitation Master Mix (Precip MM) by adding 218 µL of Precip Solution 2 directly to the Precip Solution 1 bottle.

Reagent and cap color	Per sample	Master mix 96+
Precip Solution 1	238 µL	26 mL
Precip Solution 2	2 µL	218 µL
Total volume	**240 µL**	**26.22 mL**

2. Vortex the Precip MM bottle. Place it on the benchtop at room temperature.

Add Precipitation Master Mix to samples

Carry out the following steps at room temperature.

1. Pour the Precipitation Master Mix into the reagent reservoir labeled “Precip MM”.
2. Carefully remove the seal from the Fragmentation Plate. Discard the seal.
3. Use a P1200 12-channel pipette to add 240 µL Precipitation Master Mix to each sample.
   - Rest the pipette tip against the wall of the well while delivering.
   - Do not mix by pipetting up and down.
   - Change tips after each addition.

   Note: This plate is now the Precipitation Plate.
4. Seal the Precipitation Plate, vortex it, then briefly centrifuge it.

Prepare and add isopropanol to the Precipitation Plate

1. Remove the Precipitation Plate from the centrifuge. Place it on the benchtop at room temperature.
2. Pour 65 mL of isopropanol into the 100-mL reagent reservoir labeled “ISO”.
3. Carefully remove the seal from the Precipitation Plate. Discard the seal.
4. Use a P1200 12-channel pipette to add 600-µL of isopropanol to each sample, then mix well by pipetting up and down 6–7 times.
   - Change the tips after each addition.
   - Observe the solution in the tips. It should look homogeneous after pipetting 6–7 times. If not, repeat mixing a few more times until the solution looks homogeneous.
   - Do not vortex the plate after adding the isopropanol to avoid cross-contamination of the samples.
5. Blot the top of the plate with laboratory tissue, then seal tightly with MicroAmp™ Clear Adhesive Film.

Freeze the Precipitation Plate

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

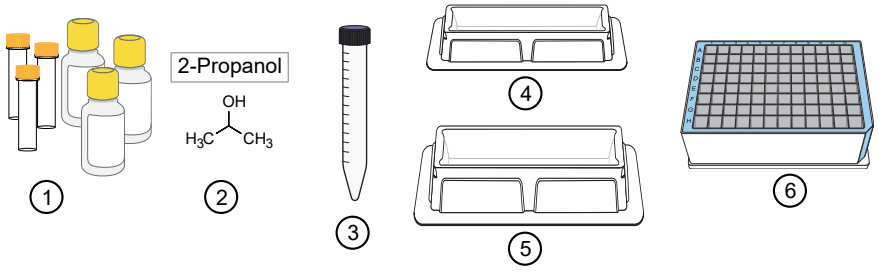
1. Carefully transfer the Precipitation Plate into the –20°C freezer.
2. Incubate the Precipitation Plate for the desired length of time.
   - Overnight (16–24 hours)
   - 3 hours

Note: The shortened 3-hour precipitation allows you to proceed to "Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC" on page 57 followed by "Stage 4: Denature and hybridize the sample plate" on page 72 on day 2 of the assay workflow.

IMPORTANT! The 3-hour DNA precipitation workflow extends the day 2 assay schedule. Approximately 9–10 hours are required to complete Stage 2 through Stage 4.

Workflow for Stage 2: Fragment and precipitate the DNA

Materials required

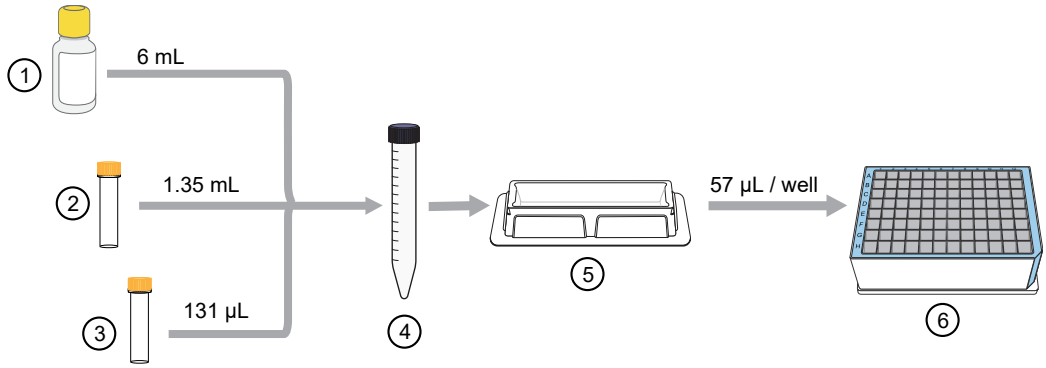


1. HT Target Prep Module 2-1 and HT Target Prep Module 2-2 reagents
2. 65 mL 2-Propanol
3. Quantity = 1
4. Quantity = 3 (25 mL)
5. Quantity = 1 (100 mL)
6. Quantity = 1, Amplification Plate

Incubate samples in preheated ovens

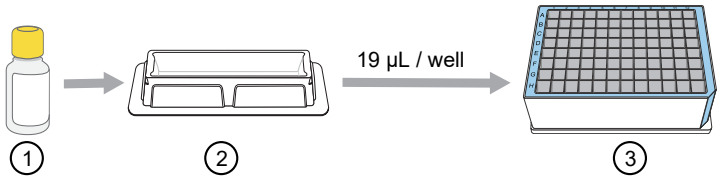
1. 20 minutes at 65°C
2. 45 minutes at 37°C

Fragmentation step



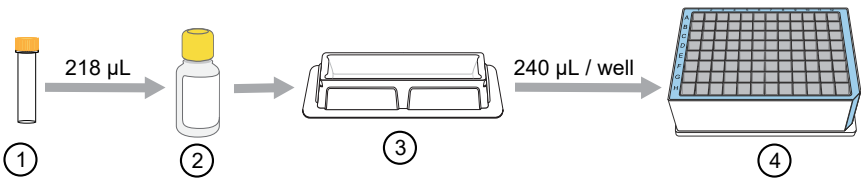
1. 10X Frag Buffer
2. Frag Diluent
3. Frag Enzyme
4. Vortex to mix.
5. Pour into reagent reservoir.
6. Fragmentation Plate. Vortex, briefly centrifuge, then incubate at 37°C for 30 minutes.

Stop fragmentation reaction



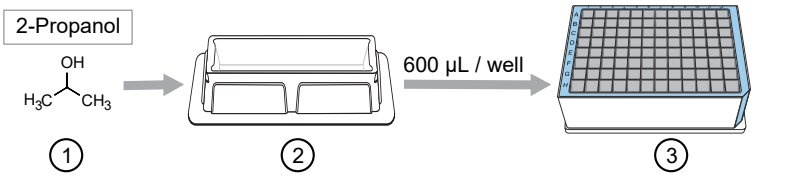
1. Frag Reaction Stop
2. Pour into reagent reservoir.
3. Fragmentation Plate. Vortex, then briefly centrifuge.

Precipitation preparation



1. Precip Solution 2
2. Precip Solution 1. Vortex well to mix.
3. Pour into reagent reservoir.
4. Precipitation Plate. Vortex for 30 seconds, then centrifuge briefly.

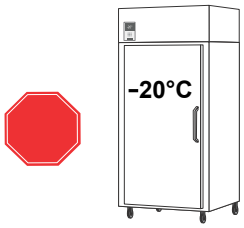
Add 2-Propanol



Final Volume: 1,326 µL/well

① 65 mL 2-Propanol
② 100-mL reagent reservoir
③ Precipitation Plate. Pipet up and down to mix. Do not vortex.

Incubate Precipitation Plate at –20°C overnight (or optional 3-hour precipitation step).



Stage 3A–3C: Centrifuge and dry, resuspension and hybridization preparation, and sample QC

CAUTION! Some steps in this stage must be performed under a fume hood.

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

Time required

Activity	Time
Centrifuge and dry plates	1 hour 20 minutes
Resuspension and hybridization mix preparation	25 minutes
Gel QC and OD	45 minutes
Total	**~2.5 hours**

Input required

The Precipitation Plate from Stage 2.

Materials, labware, and reagents required

Equipment and consumables required for Stages 3A, 3B, and 3C

Quantity	Item
As needed	Adhesive seals for 96-well plates
1	Fine-point permanent marker
1 each	Rainin™ pipettes: • Single-channel P20 • Single-channel P100 • Single-channel P1000 • Multichannel P20 • Multichannel P200
As needed	Pipette tips
2	Any 96-well PCR plate for making the dilutions: • QC Dilution Plate • Gel QC Plate

(continued)

Quantity	Item
1	Hyb-Ready Plate, 96-well PCR plate, one of the following: • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, low profile, full skirted (Cat. No. HSP9631 or HSP9601) for Eppendorf™ Mastercycler™ pro S • Bio-Rad™ Hard-Shell™ 96-Well PCR Plate, high profile, semi skirted (Cat. No. HSS9641) for Applied Biosystems™ GeneAmp™ PCR System 9700, Applied Biosystems™ Veriti™ Thermal Cycler, and Applied Biosystems™ ProFlex™ 96-well PCR System
1	OD plate: Greiner Bio-One™ 96-Well UV-Star™ Plate, Flat Bottom, Chimney Style, Clear, 370 µL/well
1	Oven, set at 37°C
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Fume hood
1	Plate centrifuge, set at 4°C
1	15-mL conical tube
1	10-mL serological pipette
1	Pipet-Aid™ Pipette Controller
1	Shaker
1	Vortexer
4	Matrix™ Reagent Reservoir, 25 mL

Reagents required for Stages 3A, 3B, and 3C

Reagent and cap color	Module
From the HT Target Prep Reagent Kit 96F	
Hyb Buffer	HT Target Prep Module 2-1, –20°C (Part No. 906012)
Hyb Solution 1	
Resuspension Buffer	HT Target Prep Module 2-2, 2–8°C (Part No.906013)
Hyb Solution 2	
Other reagents and gel required for QC steps (optional)	
Gel Diluent, 15 mL 100-fold dilution of TrackIt™ Cyan/Orange Loading Buffer See Appendix C, “Fragmentation quality control gel protocol”.	—
E-Gel™ *48* Agarose Gels, 4%	—

(continued)

Reagent and cap color	Module
25 bp DNA Ladder (Cat. No. 931343) or a similar product prepared as instructed by manufacturer.	—
Nuclease-free water, ultrapure MB Grade 15 mL (for OD QC Plate and Dilution QC Plate preparation)	—

Gels and related materials required

At the end of this stage, verifying the fragmentation reaction is highly recommended. See the appropriate section for required gel and related materials. See Appendix C, “Fragmentation quality control gel protocol”.

Stage 3A: Centrifuge the Precipitation Plate and dry the DNA pellets

Centrifuge the Precipitation Plate and dry the DNA pellets

1. Preheat the oven 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. If using a GeneChip™ Hybridization Oven 645, set the rotation speed to 15 rpm to distribute heat.

2. Transfer the Precipitation Plate from the –20°C freezer to a pre-chilled centrifuge.

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

   4,000 rpm for the Eppendorf 5810R centrifuge with the rotor configuration that is described in the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide*, Pub. No. MAN0018215.

Note: If you are processing 2 plates at the same time, you can centrifuge both plates at the same time.

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

4. Immediately after the 40-minute centrifugation time, empty the liquid from the plate using the following steps:

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

   a. Carefully remove the seal from the Precipitation Plate. Discard the seal.

   b. Invert the plate over a clean waste container. Allow the liquid to drain. Collect the liquid, then discard it according to local, state, and federal regulations.

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

5. Turn the plate right side up and place it 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 plate.
- Turn off the rotor during the 20 minutes drying time.

---

6. Seal the plate tightly.

7. Do one of the following:

   - Proceed directly to the next stage, even if some droplets of liquid remain. See "Stage 3B: Resuspend the pellets and prepare for hybridization" on page 62. Leave the sample plate at room temperature. It is helpful to start preparing reagents for the next stage while centrifuging and drying pellets.
   - Store the Precipitation Plate for resuspension later in the same day. Tightly seal the plate.
     - If resuspension is carried out within 4 hours, keep the plate at room temperature.
     - If resuspension will be carried out after more than 4 hours, store the plate in a refrigerator at 2–8°C.
   - If resuspension will be carried out on another day, store the tightly sealed plate at –20°C.

Stage 3B: Resuspend the pellets and prepare for hybridization

Prepare for resuspension and hybridization

Set the centrifuge to room temperature.

Prepare DNA pellets and warm the Resuspension Buffer

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

The equilibration of the plate of pelleted DNA and Resuspension Buffer to room temperature (18—25°C) is critical for the success of the CytoScan™ HT-CMA Assay target preparation. When the plate of DNA pellets or Resuspension Buffer is cooler than room temperature, pellets might not resuspend completely and can compromise assay performance.

Guidelines for the DNA pellet types

Observe the following guidelines on how to work with plates with fresh, cold, or frozen pellets.

- **Fresh Pellets**: If proceeding with the resuspension and hybridization preparation protocol within 4 hours, a plate with fresh pellets can be kept at room temperature.
- **Cold Pellets**: If processed during the same day, a plate with fresh pellets that are not processed within 4 hours can be transferred to a refrigerator and kept at 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 preparation protocol.
- **Frozen Pellets**: A plate with frozen pellets must be equilibrated at room temperature for at least 1.5 hours before proceeding with the resuspension and hybridization preparation protocol.

Guidelines for resuspension and hybridization reagents

The Resuspension Buffer needs at least 60 minutes to equilibrate to room temperature.

Thaw and prepare reagents

1. Thaw Hyb Solution 1 on the benchtop at room temperature.
2. Warm Resuspension Buffer, Hyb Buffer, and Hyb Solution 2 on the benchtop at room temperature for at least 1 hour.
3. Vortex the Resuspension Buffer and the Hyb Buffer. Keep at room temperature.
4. Vortex and briefly centrifuge the Hyb Solution 1 and Hyb Solution 2 before use.

Label tubes and reagent reservoirs

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

Label	Temperature	Contents
Hyb MM	Room temperature in fume hood	Hybridization Master Mix

2. Label the 2 Matrix™ Reagent Reservoirs as indicated in the following table.

Label	Reservoir size	Temperature	Contents
Resus	25 mL	Room temperature	Resuspension Buffer
Hyb MM	25 mL	Room temperature in fume hood	Hybridization Master Mix

Add the Resuspension Buffer to the DNA pellets

A plate stored at –20°C after drying the pellets must be allowed to sit at room temperature for 1.5 hours before carrying out resuspension.

Ensure that the Resuspension Buffer has equilibrated to room temperature before adding it to the dry pellets in step 3.

Carry out the following steps at room temperature.

1. Pour the Resuspension Buffer into the reagent reservoir labeled "Resus".
2. Carefully remove the seal from the Precipitation Plate. Discard the seal.
3. Use a P200 12-channel pipette to transfer 35 µL of Resuspension Buffer to each well of the Precipitation Plate.
   - Avoid touching the pellets with the pipette tips.
   - Change pipette tips after each addition.

Note: This plate is now the Resuspension Plate.

4. Seal the plate tightly.

Note: Blue pellets should be visible at the bottom of the wells.

Resuspend the DNA pellets

1. Place the sealed Resuspension Plate on one of the following shakers, then run for the time specified.
   - Thermo Scientific™ Compact Digital Microplate Shaker: 900 rpm for 10 minutes
   - Jitterbug™: Speed 7 for 10 minutes
2. Inspect the Resuspension Plate from the bottom. If the pellets are not dissolved, repeat step 1.
3. Centrifuge the plate briefly at 1,000 rpm.

Prepare the Hybridization Master Mix

CAUTION! Perform the remainder of Stage 3B under a fume hood.

1. While the Resuspension Plate is shaking, prepare the Hybridization Master Mix in the 15-mL tube labeled “Hyb MM”.
2. Add the reagents, in the order shown in the following table, to the Hyb MM tube. Use serological and single-channel pipettes.

Reagent and cap color	Per sample	Master mix 96+
Hyb Buffer	70.5 µL	7.8 mL
Hyb Solution 1	0.5 µL	55.6 µL
Hyb Solution 2	9 µL	1.0 mL
Total	**80 µL**	**8.86 mL**

3. Vortex the Hyb MM tube twice to mix.

Prepare the Hyb-Ready Plate

1. Select a 96-well plate that is compatible with the thermal cycler model that is used for sample denaturation.
   See “Thermal cycler consumables” on page 26.
2. Label the 96-well PCR plate “Hyb Ready [Plate ID]”.
3. Set a P200 12-channel pipette to 45 µL. This volume is slightly more than the volume of the sample in each well of the Resuspension Plate.
4. Using the P200 pipette, transfer the entire contents of each well of the Resuspension Plate to the corresponding wells of the Hyb-Ready Plate.

   Note: Change pipette tips after each addition.
5. Pour the Hybridization Master Mix into the reagent reservoir labeled “Hyb MM”.
6. Using a P200 12-channel pipette, add 80 µL of the Hybridization Master Mix to each well of the Hyb-Ready Plate.

   Note: Change pipette tips after each addition.
7. Seal the Hyb-Ready Plate, vortex it twice, then centrifuge it briefly.

Freeze or proceed

Do one of the following:

- Proceed to “Stage 3C: Perform quantification and fragmentation QC checks” on page 66. We strongly recommend performing the check.
- Proceed to “Stage 4: Denature and hybridize the sample plate” on page 72.
- Store the Hyb-Ready Plate at –20°C.

Stage 3C: Perform quantification and fragmentation QC checks

Before proceeding to "Stage 4: Denature and hybridize the sample plate" on page 72, we highly recommend that you perform quantification and fragmentation quality control checks.

Prepare for sample QC

Prepare the reagents

Obtain the following reagents for Sample QC.

- Nuclease-free water, 15 mL, for the water reservoir
- Gel diluent, 15 mL
  Gel diluent is a 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer. See "Dilute the TrackIt™ Cyan/Orange Loading Buffer" on page 166.
- 25 bp DNA Ladder, or similar product prepared as instructed by the manufacturer.
- Two E-Gel™ *48* Agarose Gels, 4%

Label the reagent reservoirs

Label 2 Matrix™ Reagent Reservoirs as indicated in the following table.

Label	Reservoir size	Temperature	Contents
H2O	25 mL	Room temperature	Nuclease-free Water
Gel Dil	25 mL	Room temperature	Diluted loading dye

Prepare sample QC plates

1. Label two 96-well PCR plates for making the dilutions.
   - Label one plate "QC Dil".
   - Label one plate "Gel QC".
2. Obtain 1 Greiner Bio-One™ 96-well UV-Star™ Plate.

Perform QC checks

Carry out the following steps at room temperature.

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

1. Prepare the Dilution QC Plate and OD QC Plate.
   a. Pour 15 mL of Nuclease-free Water into the reagent reservoir labeled "H20".
      The water is used to make the Dilution QC Plate and the OD QC Plate.
   b. Add 33 µL of Nuclease-free Water to each well of the Dilution QC Plate.

c. Add 90 µL of Nuclease-free Water to each well of the OD QC Plate (96-well UV-Star™ plate).

2. Prepare the Dilution QC Plate.
   a. Transfer 3 µL of the hybridization-ready sample from each well of the Hyb-Ready Plate to the corresponding well of the Dilution QC Plate.
      - Change pipette tips after each transfer.
   b. Seal, vortex, then briefly centrifuge the plate.
3. Prepare the OD QC Plate.
   a. Carefully remove the seal from the Dilution QC Plate. Discard the seal.
   b. Transfer 10 µL of each QC Dilution sample to the corresponding wells of the OD QC Plate and mix by pipetting up and down. Change pipette tips after each transfer.
   c. Mix by pipetting up and down.
      Final sample mass dilution is 120-fold.
      If needed, review the instructions on performing the sample quantification. See Appendix D, “Sample quantification after resuspension”.
4. Prepare the Gel QC Plate.
   a. Pour 15 mL of gel diluent into the reagent reservoir labeled “Gel Dil”.
   b. Add 120 µL of gel diluent to each well of the Gel QC Plate.
   c. Transfer 3 µL of each QC Dilution sample to the corresponding wells of the Gel QC Plate.
      - Change pipette tips after each transfer.
   d. Seal, vortex, then briefly centrifuge the plate.
5. Run the gel. See Appendix C, “Fragmentation quality control gel protocol”.

The Dilution QC Plate, OD QC Plate, and remaining Gel QC samples can be discarded after you have obtained satisfactory results from the gel and OD_{260} readings.

Freeze or proceed

Do one of the following:

- Proceed to “Stage 4: Denature and hybridize the sample plate” on page 72.
- Store the Hyb-Ready Plate at –20°C.

Workflow for Stage 3A: Centrifuge the Precipitation Plate and dry the pellets

Centrifuge Precipitation Plate to pellet DNA

- Speed: 3,200 × *g*
- Time: 40 minutes
- Temperature: 4°C

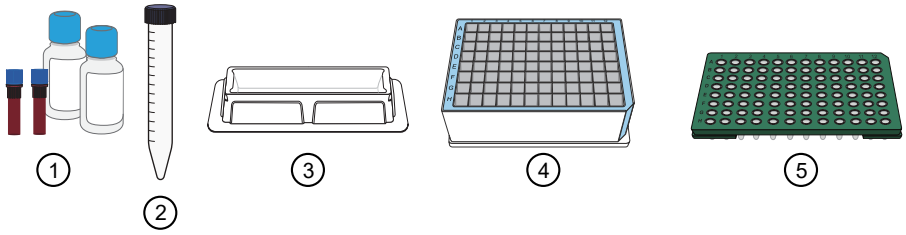
Dry DNA pellets

1. Decant liquid by inverting plate.
2. Blot-dry inverted plate for 5 minutes.
3. Incubate at 37°C for 20 minutes right-side up.

Continue to Resuspend the pellets

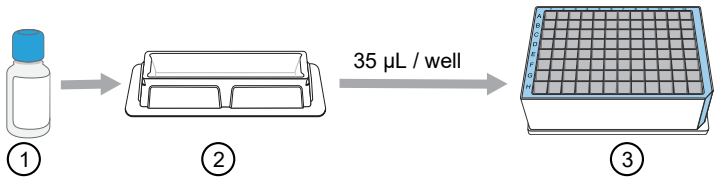
Workflow for Stage 3B: Resuspend the pellets and prepare for hybridization

Materials required



① HT Target Prep Module 2-1 and HT Target Prep Module 2-2 reagents
② Quantity = 1
③ Quantity = 2
④ Precipitation Plate (samples)
⑤ Hyb-Ready Plate: Quantity = 1 (Plate type dependent on thermal cycler used)

Add Resuspension Buffer

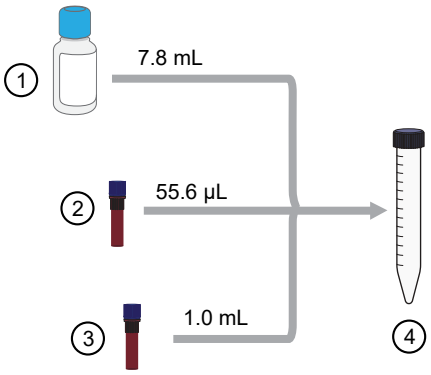


① Resuspension Buffer
② Pour into reagent reservoir.
③ Resuspension Plate. Resuspend pellets on plate shaker for 10 minutes. Briefly centrifuge.

Resuspend DNA pellet

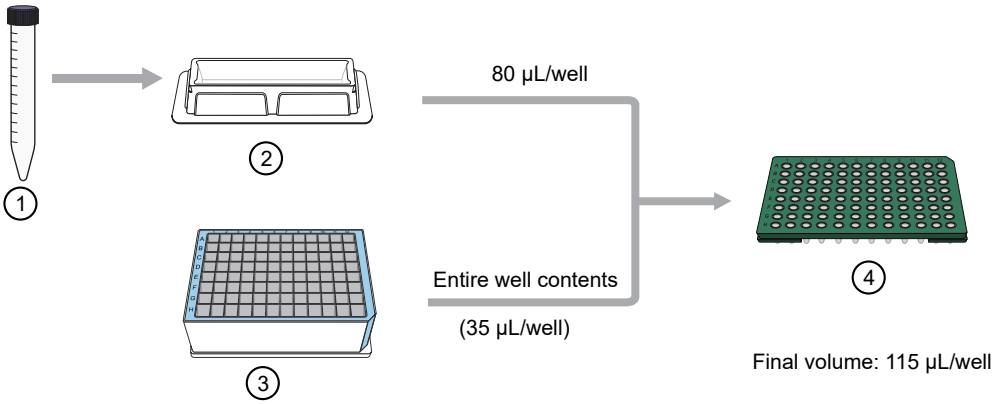
- Time: 10 minutes
- Jitterbug: Speed 7
- Thermo Scientific Titer Plate Shaker: 900 rpm

Prepare Hybridization Master Mix



① Hyb Buffer
② Hyb Solution 1
③ Hyb Solution 2
④ Hybridization Master Mix. Vortex to mix.

Prepare Hyb-Ready Plate



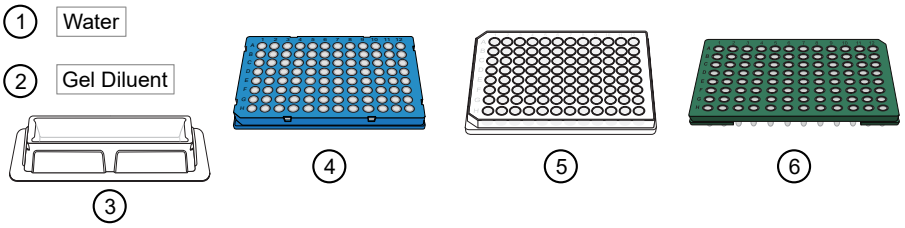
① Hybridization Master Mix.
② Pour Hybridization Master Mix into reagent reservoir.
③ Resuspension Plate with resuspended DNA.
④ Hyb-Ready Plate Vortex well. Briefly centrifuge.

Continue to Sample QC

Workflow for Stage 3C: Sample QC

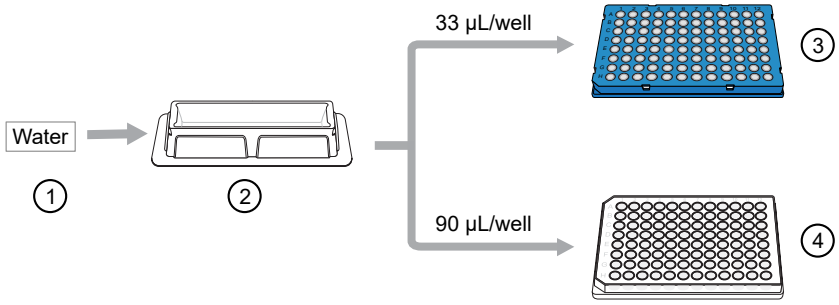
Note: This workflow shows the HSP9631 (blue plate) for this stage, however the HSP9601 (white plate) is acceptable.

Materials required



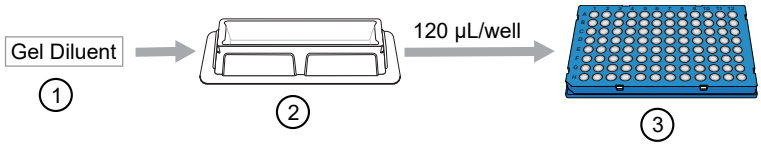
1. 15 mL water
2. 15 mL Gel diluent
3. Quantity = 2
4. Quantity = 2
5. Quantity = 1
6. Quantity = 1 (Hyb-Ready Plate)

Aliquot water to Dilution QC Plate and OD QC Plate



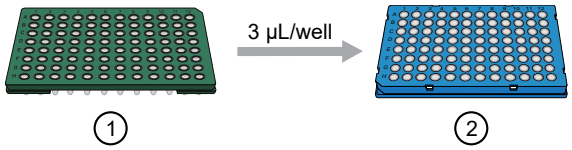
1. 15 mL water
2. Pour water into reagent reservoir.
3. Dilution QC Plate
4. OD QC Plate

Aliquot gel diluent to Gel QC Plate



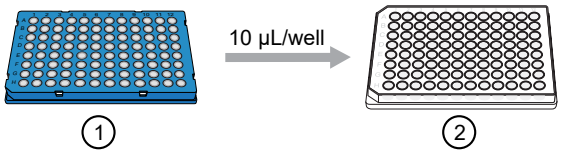
1. 15 mL Gel diluent
2. Pour gel diluent into reagent reservoir.
3. Gel QC Plate

Prepare Dilution QC Plate



1. Hyb-Ready Plate. Vortex, then centrifuge briefly.
2. Dilution QC Plate. Vortex, then centrifuge briefly.

Prepare OD QC Plate



1. Dilution QC Plate
2. OD QC Plate. Read Abs 260 nm, 280 nm, and 320 nm

Prepare Gel QC Plate

1. Dilution QC Plate
2. Gel QC Plate. Vortex, then briefly centrifuge. Load 20 µL into a 4% E-Gel™ Agarose Gel for analysis

Proceed to Stage 4, or store hybridization-ready samples at –20°C.

Stage 4: Denature and hybridize the sample plate

You proceed to Stage 4 in 1 of 2 ways:

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

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

Time required

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

Input required

The Hyb-Ready Plate from Stage 3B.

Materials, labware, and reagents required

Equipment and consumables required for Stage 4

Quantity	Item
Equipment and consumables	
1	GeneTitan™ MC Instrument
1	Thermal cycler (Approved thermal cycler, programmed with the **CytoScan HT-CMA Denature** protocol. See “Thermal cycler recommendations and protocol” on page 25.)
1	96-well metal chamber warmed in a 48°C oven[1].
1	Rainin™ P200 12-channel pipette
As needed	Pipette tips
Labware	
1	CytoScan™ HT-CMA 96-Array Plate, in a protective base
1	Hybridization tray[2]

[1] The 96-well metal chamber coming out of a 48°C oven is warm to the touch. Wear gloves or mitts if it feels too hot.

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

Reagents required for Stage 4

Reagent	Temperature	Treatment
From the HT Target Prep Reagent Kit 96F		
Wash Buffer A	Room temperature	Invert 2 or 3 times to mix before filling the GeneTitan™ Wash A bottle
Wash Buffer B		Invert 2 or 3 times to mix before filling the GeneTitan™ Wash B bottle
Water		None

Prepare for denaturation and hybridization

1. Preheat the 96-well metal chamber in a 48°C oven.
2. Allow the array plate to equilibrate to room temperature for at least 25 minutes.
   a. Leave the array plate in its pouch at room temperature for at least 25 minutes to allow the plate to come to room temperature before opening and loading on the GeneTitan™ MC Instrument.
   b. 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. See Appendix B, “Register samples in GeneChip™ Command Console™”.

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

3. Power on the thermal cycler, then prepare for the **CytoScan HT-CMA Denature** protocol to run with the heated-lid option selected.

Prepare hybridization-ready samples stored at –20°C

1. Warm the Hyb-Ready Plate at room temperature for 5 minutes.
   It is not necessary to equilibrate the plate for a longer length of time.
2. Ensure that the Hyb-Ready Plate is sealed well. If the plate is not sealed well, follow these steps.
   a. Centrifuge the plate, then carefully remove the old seal.
   b. If there is condensation on the top of the plate, blot dry gently with a laboratory tissue.
   c. Tightly reseal the plate with a fresh seal.
3. Vortex the Hyb-Ready Plate briefly, then centrifuge it at 1,000 rpm for 30 seconds.
4. Place the Hyb-Ready Plate at room temperature.

Prepare the GeneTitan™ MC Instrument

Before you denature the Hyb-Ready Plate, ensure that the GeneTitan™ MC Instrument is ready for use. If needed, review and follow the instructions that are provided in this document.

- Array processing: "Stage 2: Hybridize plates in the GeneTitan™ MC Instrument" on page 97
- Appendix B, "Register samples in GeneChip™ Command Console™"

The following is a brief summary of the steps to be performed.

1. Prepare the reagents from the HT Target Prep Reagent Kit 96F as described in "Reagents required for Stage 4" on page 73.
2. Launch the GeneChip™ Command Console™ software (GCC), then select **GCC GeneTitan Control**.
3. Upload your GeneTitan™ Array Plate Registration file.

   Note: If you do not upload your GeneTitan™ Array Plate Registration file after scanning the array plate barcode, the software assigns names to your sample.

4. Select the **System Setup** tab.

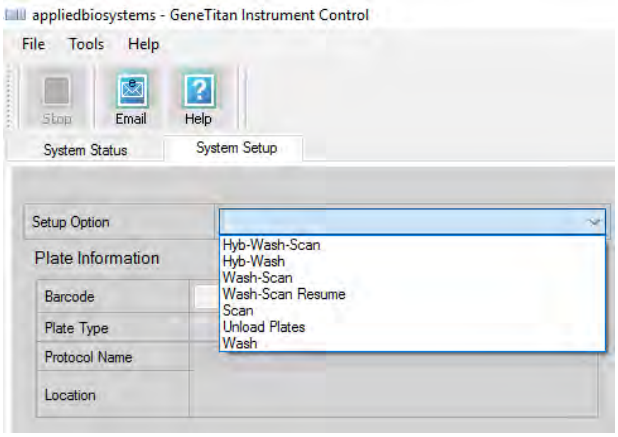


Figure 6 The GCC System Setup tab

5. Configure the software.
   a. For **Setup Option**, select **Hyb-Wash-Scan**.
   b. Click **Next**.
   c. Scan or manually enter the array plate barcode, then click **Next**.
   d. Select the protocol name, then click **Next**.
6. Fill the Wash A, Wash B, and Rinse bottles with Wash Buffer A, Wash Buffer B, and Water, respectively.
7. Empty the waste bottle.

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

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

10. When the drawers open, remove consumable trays and plates, then press the blue confirmation button to continue.
    If no consumables to remove, the **Status** pane reads "Drawers are empty".

11. When prompted by the GeneChip™ Command Console™ software, continue to "Load an array plate and hybridization tray into the GeneTitan™ MC Instrument (for Hyb-Wash-Scan or Hyb-Wash)" on page 102.

Denature the Hyb-Ready Plate

1. Ensure that the thermal cycler is powered on and the **CytoScan HT-CMA Denature** protocol with the heated-lid option has been selected.

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

3. Close the lid.

4. Start the **CytoScan HT-CMA Denature** protocol. See "Thermal cycler recommendations and protocol" on page 25.

Prepare the hybridization tray and load it into the GeneTitan™ MC Instrument

The following instructions assume familiarity with techniques and procedures that are associated with loading and operating the GeneTitan™ MC Instrument. If needed, review procedures before starting. See Appendix A, "Recommended techniques for GeneTitan™ MC Instrument operation".

CAUTION! Perform the next set of steps under a fume hood.

1. Remove the hybridization tray from the Axiom™ GeneTitan™ Consumables Kit.

2. Label the hybridization tray.

   IMPORTANT! It is critical that you write only on the proper location of the hybridization tray, on the edge in front of wells A1 and B1. Do *not* write on any other side, as the writing can interfere with sensors inside the GeneTitan™ MC Instrument and result in experiment failure.

3. Place the hybridization tray under the fume hood.

4. After the **CytoScan HT-CMA Denature** protocol has completed, remove the Hyb-Ready Plate from the thermal cycler and place it in a 96-well metal chamber that has been warmed in an oven at 48°C.

5. Move the metal chamber containing the denatured Hyb-Ready Plate to a fume hood.

6. Remove the seal from the Hyb-Ready Plate. Discard the seal.

7. Using a P200 12-channel pipette set at 105 µL, slowly transfer the denatured samples in the Hyb-Ready Plate into the corresponding wells of the hybridization tray. Dispense to the first stop to avoid creating bubbles.
   - Change pipette tips after each transfer. Discard the tip even if it shows some remaining volume.
   - Ensure that there are no air bubbles present in the hybridization tray. Puncture air bubbles with a clean pipette tip for each sample.
   - It is not necessary to spread the sample on the bottom of the hybridization tray wells. Sample distribution across wells occurs when the array plate is stacked together with the hybridization tray by the GeneTitan™ MC Instrument.

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

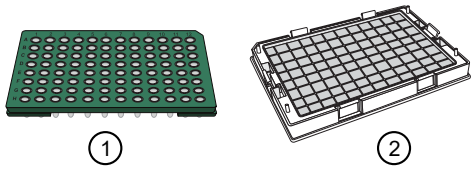
   IMPORTANT! After the GeneTitan™ MC Instrument has stacked the array plate and hybridization tray, the instrument extends the drawer. Manually check the stacking by gently pressing the 6 latching points to ensure that the 2 parts are clamped properly, and check underneath the arrays to ensure that there are no bubbles. If bubbles are found, gently tap the plate on top to eliminate the bubbles. Do *not* tip/tilt the array plate/hybridization tray stack as you are inspecting the bottom for bubbles.

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

9. Near the end of the 23.5- to 24-hour hybridization, proceed to Stage 5. See “Stage 5: Prepare GeneTitan™ reagents and trays” on page 78.

Workflow for Stage 4: Denature and hybridize the sample plate

Labware required

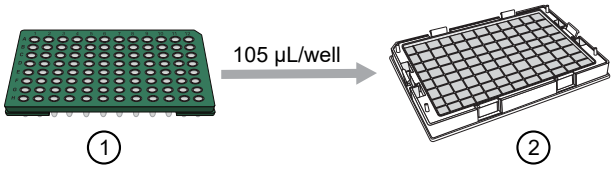


① Hyb-Ready Plate
② 384-Layout GeneTitan™ Hybridization Tray

Denature the hybridization-ready samples on the Denaturation Plate

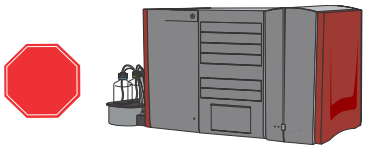
1. 10 minutes at 95°C.
2. 3 minutes at 48°C.

Prepare the hybridization tray



① Denatured hybridization-ready samples on the Denaturation Plate. Keep samples on a 48°C heat 96-well metal chamber.
② Hybridization tray with samples. If needed, pierce bubbles.

Process hybridization tray in the GeneTitan™ MC Instrument



Stage 5: Prepare GeneTitan™ reagents and trays

Start this stage when hybridization in the GeneTitan™ MC Instrument is ~1.5 hours before completion. Completing this stage before the end of hybridization enables the reagent trays to be loaded for the GeneTitan™ MC Instrument for array processing steps.

IMPORTANT! The reagent trays prepared in this step are for the continued processing of an array plate that:

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

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

Time required

Activity	Time
Prepare reagents (thaw and organize reagents)	~30 minutes
Hands-on time for GeneTitan™ reagent preparation	~50 minutes
Load reagent trays onto the GeneTitan™ MC Instrument	~10 minutes
Total	**~90 minutes**

Materials, labware, and reagents required

Reagent trays required for Stage 5

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

Tray type	Quantity	Tray designation	Master mix or reagent
Stain tray with cover	2	S1	Stain 1 Master Mix
Stain tray with cover	1	S2	Stain 2 Master Mix
Stain tray with cover	1	Stbl	Stabilization Master Mix
Stain tray with cover	1	Lig	Ligation Master Mix
Scan tray	1	Scan tray	Hold Buffer

Equipment and consumables required for Stage 5

Quantity	Item
Equipment and consumables	
1	GeneTitan™ MC Instrument
1	Microcentrifuge
1	Vortexer
1	GeneTitan™ ZeroStat AntiStatic Gun
1	Cooler for enzyme
1	Ice bucket, filled with ice
As needed	Laboratory tissues
As needed	Aluminum foil (optional)
1	Fine-point permanent marker
As needed	Serological pipettes: • 5 mL • 10 mL
1 each	Rainin™ pipettes: • Single-channel P200 • Single-channel P1000 • Multichannel P200
As needed	Pipette tips
1	Pipet-Aid™ Pipette Controller
5	Matrix™ Reagent Reservoir, 25 mL
3	15-mL conical tube
1	50-mL conical tube
GeneTitan™ labware	
1	Scan tray with cover and protective base[1]
5	Stain tray[1]
5	Cover for stain tray[1]

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

Reagents required and reagent handling for Stage 5

Prepare reagents according to the following table.

Module	Reagent and cap color	Thaw, then place on ice	Place on ice	Place at room temperature
HT Target Prep Module 3-1 –20°C	Ligate Buffer[1]			✓
	Ligate Enzyme[2]	⊗ Do not thaw. Keep at –20°C until ready to use.		
	Ligate Solution 1	✓		
	Probe Mix 1	✓		
	Stain Buffer	✓		
	Stabilize Solution	✓		
HT Target Prep Module 3-2 2°C to 8°C	Ligate Solution 2			✓
	Probe Mix 2[3]		✓	
	Wash A[1]			✓
	Stain 1-A[3]		✓	
	Stain 1-B[3]		✓	
	Stain 2-A[3]		✓	
	Stain 2-B[3]		✓	
	Stabilize Diluent[1]		✓	
	Water			✓
	Hold Buffer[3], [4]			✓
Estimated reagent thawing time is ~30 minutes.				

[1] Check for precipitate. If precipitate is present, repeat the vortex and centrifuge step.
[2] If needed, an extra tube of Ligate Enzyme is in HT Target Prep Module 4-1.
[3] These solutions are light sensitive. Keep tubes out of direct light for a prolonged length of time.
[4] Hold Buffer for preparing the second, third, and fourth plates is provided in HT Target Prep Module 4-2.

Guidelines for handling reagents with precipitates

When the HT Target Prep Module 3-2 is stored at 4°C, precipitation in the form of clear crystals can sometimes occur in the Wash A and Stabilize Diluent. Use the following procedures to ensure that any precipitate is returned to solution before use.

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

Prepare 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.

Prepare Stabilize Diluent

Use this procedure if crystals are observed in the Stabilize Diluent.

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

Prepare Ligate Buffer

White precipitate is sometimes observed when the 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. Place on the benchtop at room temperature for 30 minutes.
   This bottle can also be thawed in a dish with room temperature ultra-pure water.
2. Vortex for 30 seconds.
3. Examine the buffer for precipitate by looking into the top of the bottle.
4. If precipitate is still present, warm the bottle with your hands, then vortex again for 30 seconds. Repeat this step again if precipitate is still present.
5. Leave the Ligate Buffer on the benchtop until ready to use.

Prepare the remaining reagents

1. Leave the Ligate Enzyme at –20°C until ready to use.
2. Thaw the following reagents from the HT Target Prep Module 3-1 on the benchtop at room temperature. Vortex and centrifuge them briefly, then place them on ice.
   - Ligate Solution 1
   - Probe Mix 1
   - Stabilize Solution
   - Stain Buffer
3. Prepare the remaining reagents from HT Target Prep Module 3-2 as follows.
   a. Gently flick each tube 2 to 3 times to mix, then centrifuge them.
   b. Place the reagents on ice, except for the following. Leave them on the benchtop at room temperature.
      - Hold Buffer
      - Ligate Solution 2
      - Water
      - Wash A

Label master mix tubes and reagent reservoirs

1. Label side of each conical tube as indicated in the following table.

Tube size	Label	Contents
50 mL	S1	Stain 1 Master Mix
15 mL	S2	Stain 2 Master Mix
15 mL	Stbl	Stabilization Master Mix
15 mL	Lig	Ligation Master Mix

2. Place the 4 tubes on ice.
3. Label the 5 Matrix™ Reagent Reservoirs as indicated in the following table.

Label	Reservoir size	Contents
S1	25 mL	Stain 1 Master Mix
S2	25 mL	Stain 2 Master Mix
Stbl	25 mL	Stabilization Master Mix
Lig	25 mL	Ligation Master Mix
Hold	25 mL	Hold Buffer

Prepare the stain, ligation, and stabilization master mixes

Prepare the Stain 1 Master Mix

1. Use the appropriate serological and single-channel pipettes to add reagents to the 50-mL tube labeled “S1” in the order shown in the following table. This recipe provides enough for both S1 reagent trays.

Reagent and cap color	Per array	Master mix 96+
Wash A	201.6 µL	22.2 mL
Stain Buffer	4.2 µL	463 µL
Stain 1-A	2.1 µL	231 µL
Stain 1-B	2.1 µL	231 µL
Total	**210 µL (105 µL x 2)**	**23.13 mL**

2. Gently invert the tube 10 times to mix. Do not vortex.

3. Place the tube on ice and protect it from direct light. For example, cover it with aluminum foil or the ice bucket lid.

Prepare the Stain 2 Master Mix

1. Use the appropriate serological and single-channel pipettes to add reagents to the 15-mL tube labeled “S2” in the order shown in the following table.

Reagent and cap color	Per array	Master mix 96+
Wash A	100.8 µL	11.1 mL
Stain Buffer	2.1 µL	231 µL
Stain 2-A	1.05 µL	115.6 µL
Stain 2-B	1.05 µL	115.6 µL
Total	**105 µL**	**11.56 mL**

2. Gently invert the S2 tube 10 times to mix. Do not vortex.

3. Place on ice, then protect from direct light. For example, cover it with aluminum foil or the ice bucket lid.

Prepare the Stabilization Master Mix

1. Use the appropriate serological and single-channel pipettes to add reagents to the 15-mL tube labeled “Stbl” in the order shown in the following table.

Reagent and cap color	Per array	Master mix 96+
Water	93.19 µL	10.3 mL
Stabilize Diluent	10.50 µL	1.16 mL
Stabilize Solution	1.31 µL	144.8 µL
Total	**105 µL**	**11.61 mL**

2. Vortex the Stbl tube at high speed for 3 seconds.

3. Place it on ice.

Prepare the Ligation Master Mix—part 1

The Ligation Master Mix is prepared in 2 stages.

1. Place the 15-mL conical tube labeled “Lig” on ice.

2. Use the appropriate serological and single-channel pipettes to add reagents to the Lig tube in the order shown in the following table.

Reagent and cap color	Per array	Master mix 96+
Ligate Buffer	66.15 µL	7.3 mL
Ligate Solution 1	13.12 µL	1.45 mL
Ligate Solution 2	3.15 µL	348 µL
Total	**82.42 µL**	**9.10 mL**

3. Mix well by vortexing the Lig tube for 3 seconds.

4. Place the tube back on ice.

Prepare the Ligation Master Mix—part 2

The Ligation Master Mix is prepared in 2 stages.

1. Remove the Ligate Enzyme from the –20°C freezer. Place it in a cooler chilled to –20°C.
2. Use the appropriate single-channel pipettes to add reagents to the 15-mL tube labeled “Lig” in the order shown in the following table.

   Note: Gently flick the Ligate Enzyme tube 2-3 times, then centrifuge it briefly immediately before adding the enzyme to the master mix.

Reagent and cap color	Per array	Master mix 96+
Ligation Master Mix from stage 1 preparation	82.42 µL	9.10 mL
Probe Mix 1	10.5 µL	1.16 mL
Probe Mix 2	10.5 µL	1.16 mL
Ligate Enzyme	1.58 µL	174.4 µL
Total	**105 µL**	**11.59 mL**

3. Gently invert the Lig tube 10 times to mix. Do not vortex.
4. Place the tube on ice and protect it from direct light. For example, cover it with aluminum foil or the ice bucket lid.

Aliquot master mixes and Hold Buffer into trays

Label the GeneTitan™ reagent trays

When preparing the reagent trays to be loaded onto the GeneTitan™ MC Instrument, it is helpful to mark the front of each tray in a way that identifies its contents.

IMPORTANT! It is critical that you write only on the proper location of the stain/reagent trays, on the edge in front of wells A1 to F1. Do *not* write on any other side, because the writing can interfere with sensors inside the GeneTitan™ MC Instrument and result in experiment failure. To ensure proper placement of covers onto stain trays, and trays onto the GeneTitan™ MC Instrument, you can also mark the notched corner of the trays and covers. If needed, review reagent tray handling techniques before continuing. See Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation”.

1. Gather the scan tray and the stain trays and covers from the Axiom™ GeneTitan™ Consumables Kit.
2. Label 2 stain trays “S1”.
3. Label the remaining stain trays as shown below:
   - “S2”
   - “Stbl”
   - “Lig”

Deionize the GeneTitan™ reagent trays and covers

If needed, review the deionization procedure. See “Deionize GeneTitan™ trays and covers” on page 149.

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

About aliquoting reagents to GeneTitan™ trays

IMPORTANT!

- Deionize the stain trays and the covers before aliquoting master-mixes.
- Always aliquot reagents to the bottom of the reagent tray. Avoid touching the sides or the top of the wells with the pipette tips. Droplets close to or on the top of the well dividers can cause the cover to stick to the tray during GeneTitan™ MC Instrument processing.

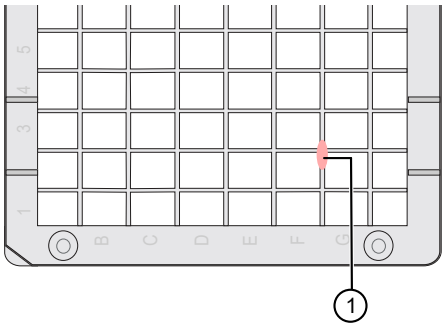
Place trays on the benchtop to pipet. If the trays will not be used immediately, protect them from light by covering with foil or placing them in a cabinet.

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

Aliquot the Stain 1 Master Mix

1. Place the reagent reservoir labeled “S1” on the benchtop at room temperature.
2. Pour the S1 Master Mix into the S1 reagent reservoir.
3. Load a P200 12-channel pipette with 12 new pipette tips, then aliquot 105 µL per well to both trays labeled “S1”.
   - Dispense to the first stop only to avoid creating bubbles.
   - It is not necessary to change pipette tips between additions of the Stain 1 Master Mix.
4. Visually inspect the S1 trays.
   a. If bubbles are present, puncture them with a pipette tip.

b. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.



(1) Example of a droplet of liquid that has splashed onto the well divider of a stain tray during reagent aliquoting. Ensure that no droplets of liquid rest on the well dividers.

IMPORTANT! Liquid on the top of the dividers can cause excessive evaporation or can form a seal that restricts the removal of the GeneTitan™ tray cover.

5. Place covers on the S1 trays. Orient the covers correctly on the trays with the notched corners together.

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

Aliquot the Stain 2 Master Mix

1. Place the reagent reservoir labeled “S2” on the benchtop at room temperature.

2. Pour the S2 Master Mix into the S2 reagent reservoir.

3. Load a P200 12-channel pipette with 12 new pipette tips, then aliquot 105 µL per well to the tray labeled “S2”.
   - Dispense to the first stop only to avoid creating bubbles.
   - It is not necessary to change pipette tips between additions of the Stain 2 Master Mix.

4. Visually inspect the S2 tray.
   a. If bubbles are present, puncture them with a pipette tip.

   b. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.

5. Place a cover on the S2 tray. Orient the cover correctly on the tray with the notched corners together.

6. Protect the tray from light if not immediately loading onto the GeneTitan™ MC Instrument.

Aliquot the Stabilization Master Mix

1. Place the reagent reservoir labeled “Stbl” on the benchtop at room temperature.
2. Pour the Stabilization Master Mix into the Stbl reagent reservoir labeled “Stbl”.
3. Load a P200 12-channel pipette with 12 new pipette tips, then aliquot 105 µL per well to the tray labeled “Stbl”.
   - Dispense to the first stop only to avoid creating bubbles.
   - It is not necessary to change pipette tips between additions of the Stabilization Master Mix.
4. Visually inspect the Stbl tray.
   a. If bubbles are present, puncture them with a pipette tip.
   b. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place a cover on the tray. Orient the cover correctly on the tray with the notched corners together.

Aliquot the Ligation Master Mix

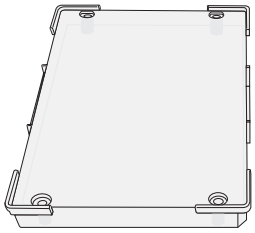
1. Place the reagent reservoir labeled “Lig” on the benchtop at room temperature.
2. Pour the Ligation Master Mix into the Lig reagent reservoir labeled “Lig”.
3. Load a P200 12-channel pipette with 12 new pipette tips, then aliquot 105 µL per well to the tray labeled “Lig”.
   - Dispense to the first stop only to avoid creating bubbles.
   - It is not necessary to change pipette tips between additions of the Ligation Master Mix.
4. Visually inspect the Lig tray.
   a. If bubbles are present, puncture them with a pipette tip.
   b. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
5. Place a cover on the Lig tray. Orient the cover correctly on the tray with the notched corners together.
6. Protect the tray from light if not immediately loading onto the GeneTitan™ MC Instrument.

Aliquot the Hold Buffer to the scan tray

CAUTION!

- Do not remove the scan tray from its protective black base until loading it onto the GeneTitan™ MC Instrument.
- To avoid scratching, do not touch the bottom of the tray with pipette tips.
- Dispense Hold Buffer to the first stop only.

1. Ensure that the Hold Buffer has equilibrated to room temperature.
2. Place the reagent reservoir labeled “Hold” on the benchtop at room temperature.
3. Vortex the Hold Buffer, then pour it into the Hold reservoir.
4. Remove the scan tray from its pouch.
5. Remove the scan tray cover, but leave the scan tray on its protective black base.
6. Prepare the barcoded scan tray cover (Part No. 202757) that came with the scan tray by deionizing it. See “Deionize GeneTitan™ trays and covers” on page 149.
7. Place the cover upside down to prevent dust or static from collecting on the bottom of the cover.



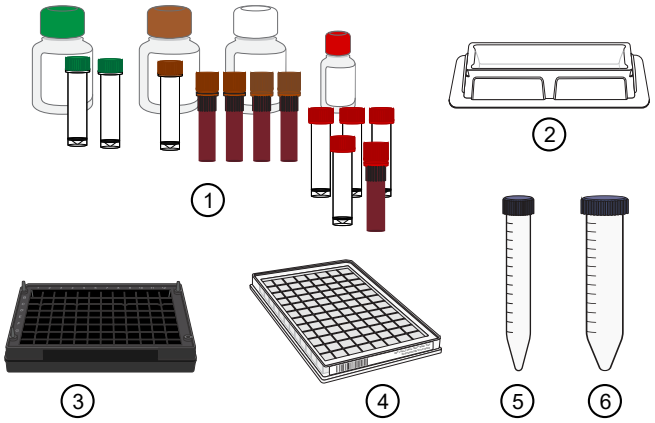
8. Use a 12-channel P200 pipette with new tips to aliquot 150 µL of Hold Buffer to *every* well of the 96-plate scan tray.
   - Dispense to the first stop and avoid touching the bottom of the tray.
   - Avoid touching the bottom of the tray with the pipette tips.
   - It is not necessary to change pipette tips between additions of the Hold Buffer.
9. If droplets of liquid have splashed onto the well dividers, place a laboratory tissue on top of the tray to blot, then remove.
10. Cover the tray by orienting the notched corner of the scan tray cover over the notched edge of the tray with the flat side of the cover against the scan tray.

    IMPORTANT! The scan tray has an open-bottom design, so it is important that all 96-wells of the scan tray receive 150 µL of Hold Buffer.

If needed, review instructions for loading reagent trays. See “Stage 3: Ligate, wash, stain, and scan” on page 109.

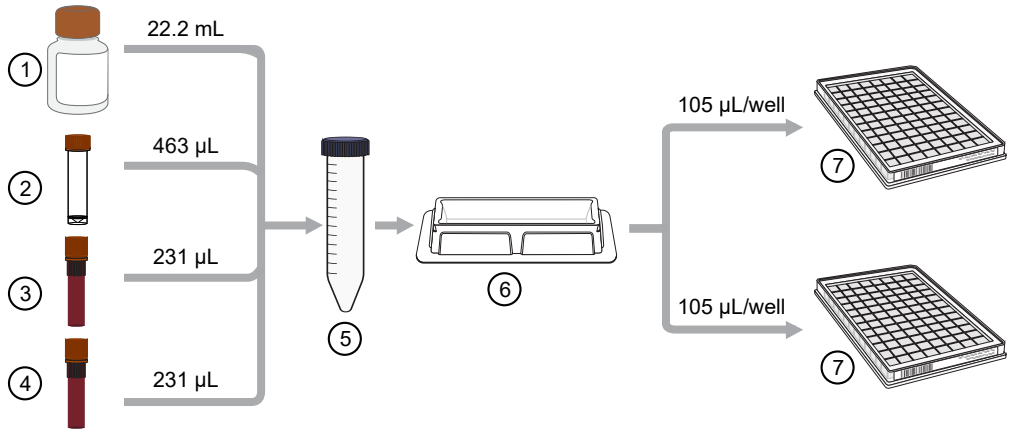
Workflow for Stage 5: Prepare GeneTitan™ reagents and trays

Materials required



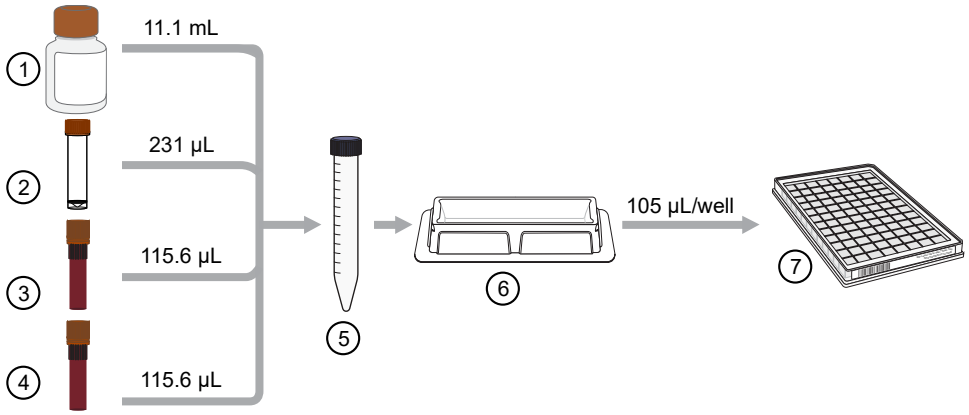
1. HT Target Prep Module 3-1 and HT Target Prep Module 3-2 reagents
2. Quantity = 5
3. Scan tray, quantity = 1
4. Stain tray, quantity = 5
5. Quantity = 3
6. Quantity = 1

Prepare two Stain 1 trays



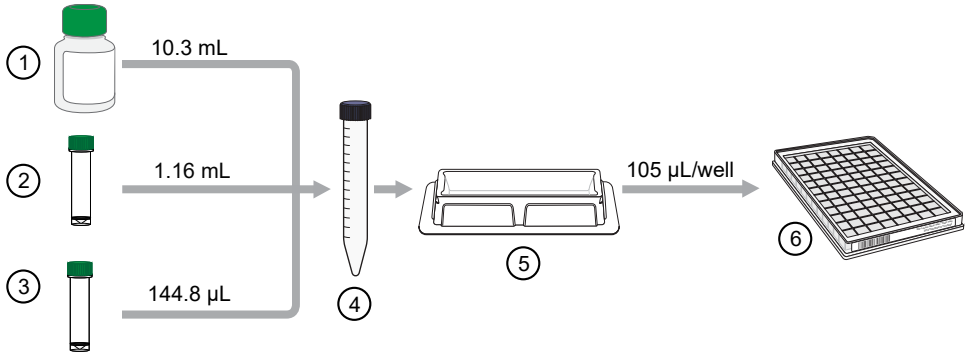
1. Wash A
2. Stain Buffer
3. Stain 1-A
4. Stain 1-B
5. Invert tube 10 times to mix. Do not vortex.
6. Pour into reagent reservoir.
7. Trays labeled “S1”. Ensure that there are no droplets on well dividers.

Prepare Stain 2 tray



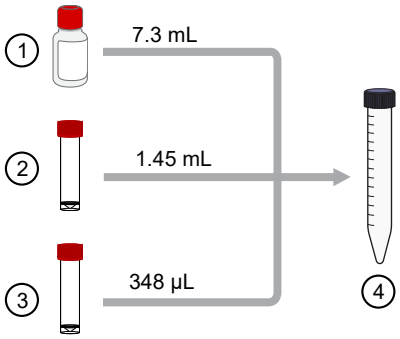
1. Wash A
2. Stain Buffer
3. Stain 2-A
4. Stain 2-B
5. Invert tube 10 times to mix. Do not vortex.
6. Pour into reagent reservoir.
7. Tray labeled “S2”. Ensure that there are no droplets on well dividers.

Prepare the stabilization tray



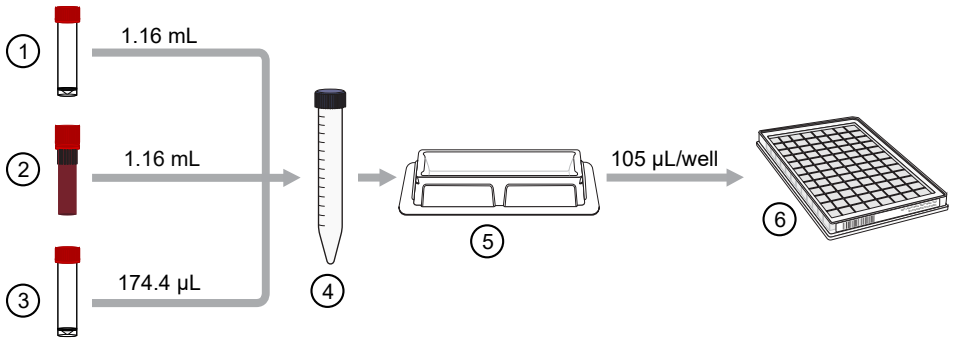
1. Water
2. Stabilize Diluent
3. Stabilize Solution
4. Vortex to mix.
5. Pour into reagent reservoir.
6. Tray labeled “Stbl”. Ensure that there are no droplets on well dividers.

Prepare the Ligation Master Mix, part 1



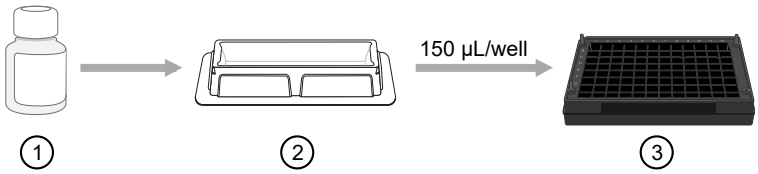
1. Ligate Buffer
2. Ligate Solution 1
3. Ligate Solution 2
4. Ligation Master Mix tube. Vortex to mix.

Prepare the Ligation Master Mix, part 2, then prepare the ligation tray

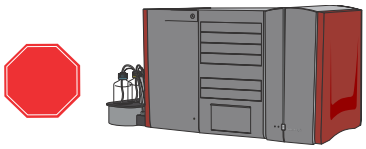


1. Probe Mix 1
2. Probe Mix 2
3. Ligate Enzyme
4. Add reagents to Ligation Master Mix tube from Part 1. Invert tube 10X to mix. Do not vortex.
5. Pour into reagent reservoir.
6. Tray labeled “Lig”. Confirm that there are no droplets on well dividers.

Aliquot the Hold Buffer to the scan tray



1. Hold Buffer
2. Pour into reagent reservoir.
3. Confirm that there are no droplets on well dividers.

Process array plates on the GeneTitan™ MC Instrument


5 Process array plates with the GeneTitan™ Multi-Channel 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.

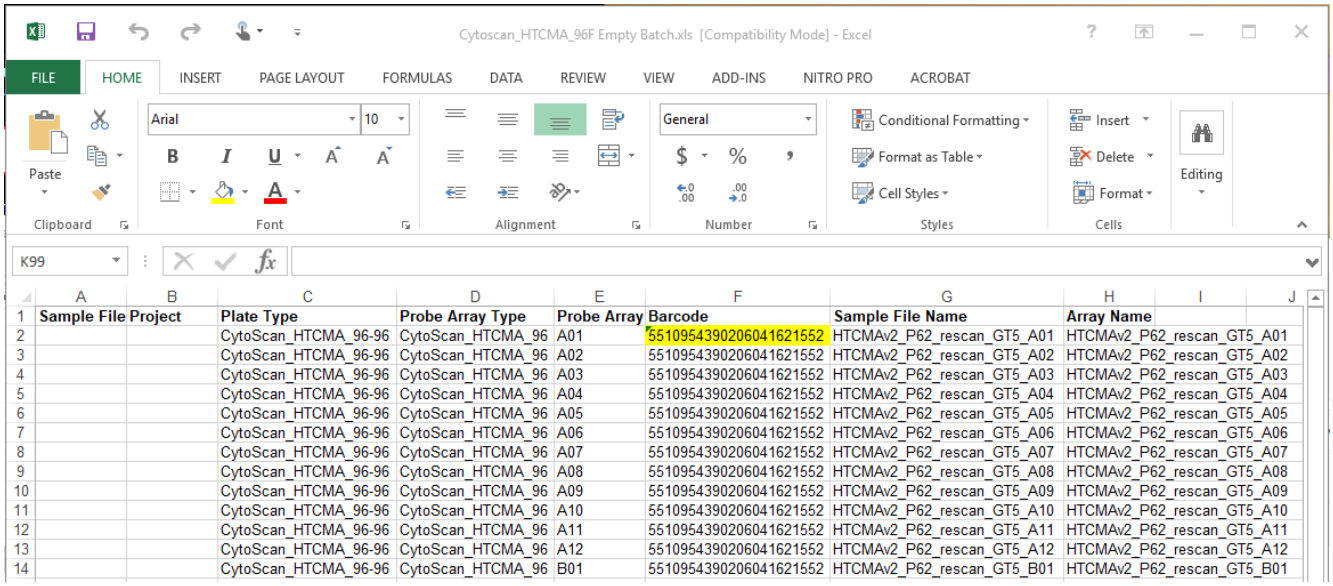
Stage 1: Create and upload a GeneTitan™ Array Plate Registration file

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 97. 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, select the **GeneTitan Array 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* (Pub. No. 702569).
6. Scan the array plate barcode into the yellow **Barcode** field, column **F** of the GeneTitan™ Array Plate Registration file.
   See Figure 7.
7. Save the file.
8. Return to the GCC Portal GeneTitan™ Array Plate Registration page.
   a. Click **Browse**, navigate to the array plate registration file, then click **Open**.
   b. Under **Step 3**, click **Upload**, wait for the information to load, then click **Save** found at the bottom of the next window that is displayed.



	A	B	C	D	E	F	G	H	I	J
1	**Sample File**	**Project**	**Plate Type**	**Probe Array Type**	**Probe Array**	**Barcode**	**Sample File Name**	**Array Name**		
2			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A01	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A01	HTCMAv2_P62_rescan_GT5_A01		
3			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A02	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A02	HTCMAv2_P62_rescan_GT5_A02		
4			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A03	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A03	HTCMAv2_P62_rescan_GT5_A03		
5			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A04	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A04	HTCMAv2_P62_rescan_GT5_A04		
6			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A05	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A05	HTCMAv2_P62_rescan_GT5_A05		
7			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A06	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A06	HTCMAv2_P62_rescan_GT5_A06		
8			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A07	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A07	HTCMAv2_P62_rescan_GT5_A07		
9			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A08	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A08	HTCMAv2_P62_rescan_GT5_A08		
10			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A09	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A09	HTCMAv2_P62_rescan_GT5_A09		
11			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A10	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A10	HTCMAv2_P62_rescan_GT5_A10		
12			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A11	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A11	HTCMAv2_P62_rescan_GT5_A11		
13			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A12	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A12	HTCMAv2_P62_rescan_GT5_A12		
14			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	B01	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_B01	HTCMAv2_P62_rescan_GT5_B01		

Figure 7 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 HT Target Prep Reagent Kit 96F are required for the hybridization step.

Reagent	Module	Storage
Wash Buffer A (both bottles, 1 L)	HT Target Prep Wash A (Part No. 906022)	Room temperature
Wash Buffer B	HT Target Prep Wash B (Part No. 906023)	
Water	HT Target Prep Water (Part No. 906020)	

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.

- A CytoScan™ HT-CMA 96-Array Plate is required for this step. Before inserting this plate into the GeneTitan™ MC Instrument for hybridization, the array plate must be at room temperature.

Warm array plate to room temperature

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

1. Remove the array plate packaging from the 4°C refrigerated storage.
2. Open the array plate box, then remove the pouch containing the array plate and protective base. Do not open the pouch.
3. Equilibrate the unopened pouch on the bench for at least 25 minutes.
4. At the end of the array warm-up time, open the pouch, then scan the array plate barcode into the batch registration file.
   See “Stage 1: Create and upload a GeneTitan™ Array Plate Registration file” on page 95.

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

Set up the instrument

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

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

IMPORTANT! Do not close the scanner application by right-clicking and selecting the **Close** option. This method causes the scanner application to exit abnormally and delay in processing the next plate. The correct way to close the application is described in “Shut down the GeneTitan™ MC Instrument” on page 115.

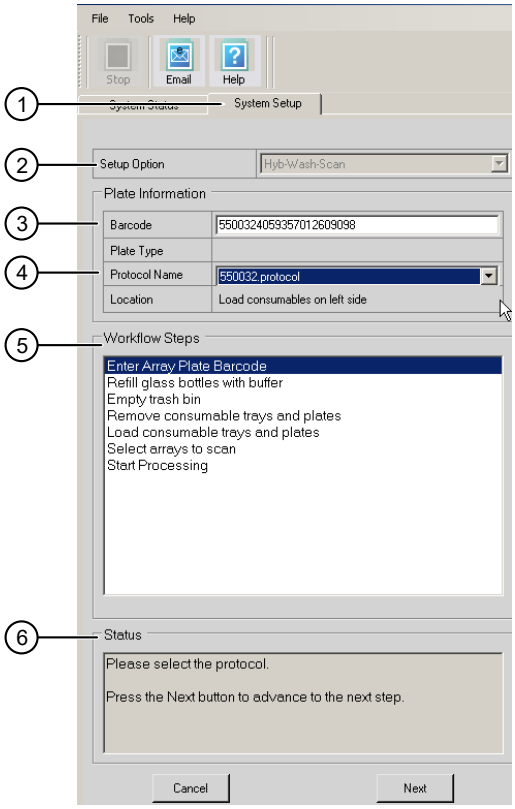


Figure 8 System Setup tab.

① **System Setup** tab.
② **Setup Option**: Access a list of the various options available for processing array plates.
③ **Barcode**: The array plate barcode. The barcode can be scanned or entered manually.
④ **Protocol Name**: The list of protocols that are displayed is based on the first 6 digits of the array plate barcode. Only the protocols that are valid for the array plate type that is loaded are displayed.
⑤ **Workflow Steps**: This field displays an overview of the user actions that are required to process an array plate that is based on the **Setup Option** selected.
⑥ **Status**: This field displays the actions that must be performed to prepare or unload the GeneTitan™ MC Instrument for the selected **Setup Option**.
After each action, click **Next** or press the blinking blue confirmation button on the GeneTitan™ MC Instrument to continue.

2. Select **Hyb-Wash-Scan** from the **Setup Option** list.
Other options available are described under "Setup options for array plate processing" on page 150.

3. Click **Next**.

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

---

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

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

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

   ---

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

6. Refill the bottles with the following reagents.

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

   IMPORTANT! Always ensure that the GeneTitan™ bottles containing Wash Buffer A and Water 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 HT Target Prep Reagent Kit 96F must be emptied into the GeneTitan™ Wash B bottle when setting up the system.

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

   Do not overfill the bottles.

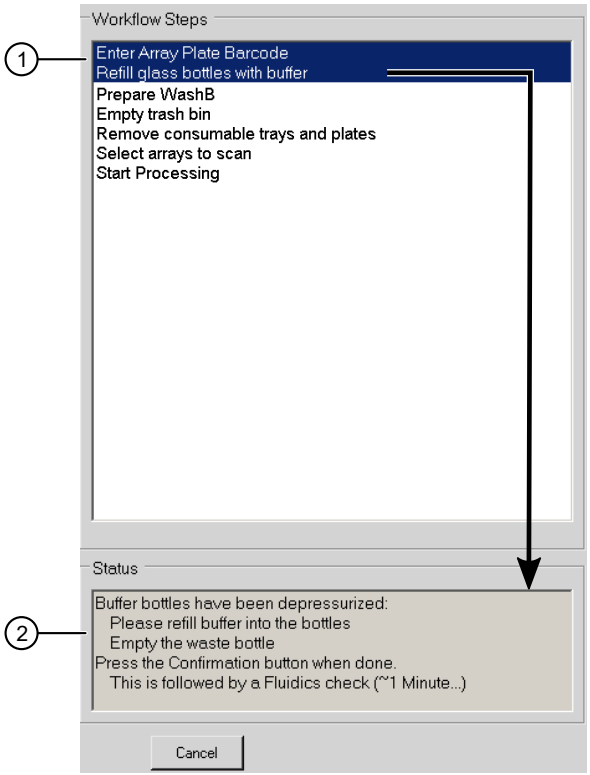
   - The maximum volume for the Wash B and Rinse bottles is 1 L. Fill to the 1-L mark only.
   - The maximum volume for the Wash A bottle is 2 L.

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

7. Empty the waste bottle.

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

   A fluidics check is run (~1 minute).



① **Workflow Step**
② Specific instructions for the current workflow step are displayed in the **Status** pane.

9. Empty the trash bin.
   a. Open the trash bin and empty.
   b. If already empty, the trash bin remains locked and the **Status** pane reads "Trash bin is empty".
   c. Press the blue confirmation button to continue.
10. Remove consumable trays and plates.
   a. Remove used trays and plates when drawers open.
   b. If no consumables are in the drawers to remove, the **Status** window reads "Drawers are empty".
   c. Press the blue confirmation button to continue.
11. Continue to “Load an array plate and hybridization tray into the GeneTitan™ MC Instrument (for Hyb-Wash-Scan or Hyb-Wash)” on page 102 when prompted by the GCC software.

Load an array plate and hybridization tray into the GeneTitan™ MC Instrument (for Hyb-Wash-Scan or Hyb-Wash)

1. When drawer 6 opens, load the array plate and hybridization tray in the following manner:
   a. Examine the wells of the hybridization tray for bubbles, then puncture any bubbles with a 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 140.

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

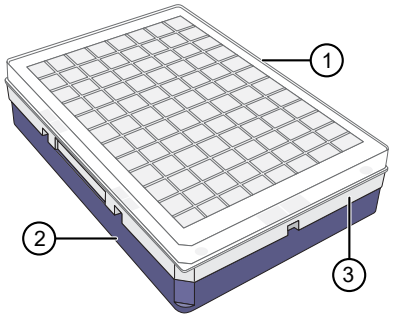


Figure 9 Array plate components, as shipped.

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

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

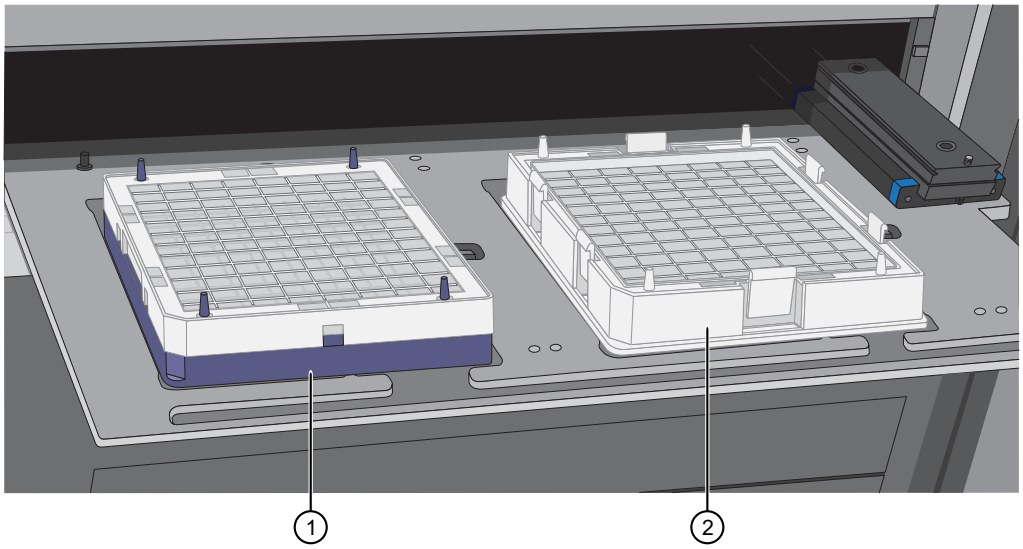


Figure 10 Array plate on protective blue base and the hybridization tray properly loaded into drawer 6.

① Array plate on protective base.
② Hybridization tray.

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

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

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

e. Press the 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 clamping the array plate onto the hybridization tray. A **Clamping in Progress** dialog appears.

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

5. Verify the plate clamping step to ensure that the array plate is securely fastened to the hybridization tray. Using your thumbs, press the array plate downward following the positions that are specified in Figure 11. *No clicking sound indicates proper clamping.*

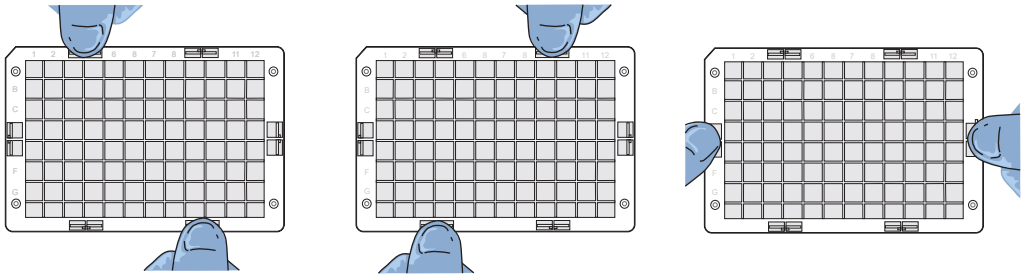


Figure 11 Clamping verification steps.

6. Inspect the array plate for bubbles.
   a. **Keeping the plate stack level**, inspect the bottom for bubbles under the arrays—*do not* tilt or invert the plates.
   b. If bubbles are present, gently tap the plate until the bubbles move out from under the arrays—*do not* unclamp the plate stack.

7. Return the plate stack to the drawer with the notched corner facing you, then press the blue confirmation button on the GeneTitan™ Instrument to proceed.

8. 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.

9. Continue to “Load a second array plate and hybridization tray” on page 106.

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 12). 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 which results in disruption of the multi-plate plate workflow.

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

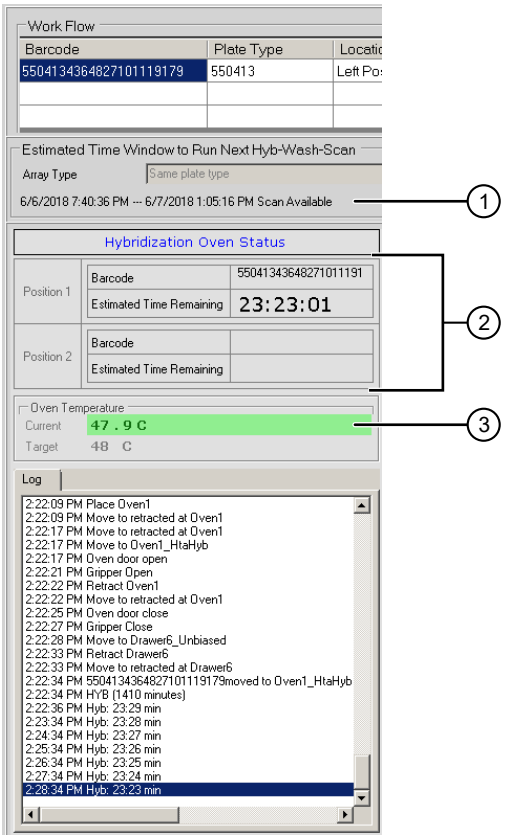


Figure 12 When to load a second array plate and hybridization tray based on oven status information.

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 blue 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.

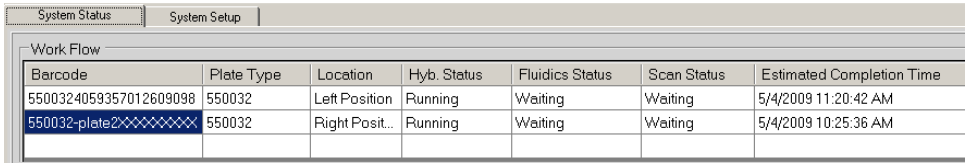


Figure 13 Example of the Work Flow pane when 2 plates are loaded and are in the hybridization oven.

Queue a second plate for scanning

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

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

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

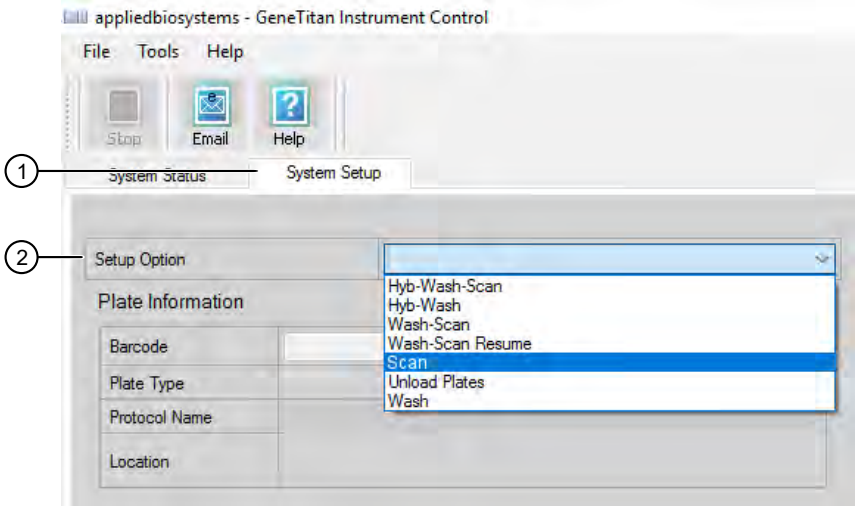


Figure 14 Scan setup option to process a second array plate.

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

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

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

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

6. Place the array plate on top of a scan tray in the correct orientation, with the notched corner of the array plate and scan tray aligned.

7. Load the array plate/scan tray stack in drawer 2 of the GeneTitan™ MC Instrument, on the left or right side, as instructed in the **Status** pane. Ensure that the array plate/scan tray stack is loaded in the correct orientation in the drawer.

   For further information on the proper alignment and loading of plates, covers, and trays in the GeneTitan™ MC Instrument, see “Proper tray alignment and placement” on page 140.

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 becomes available.

Stage 3: Ligate, wash, stain, and scan

The GeneTitan™ tray loading process

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

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

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

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

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

Load trays in the 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, review of proper loading techniques. (See Appendix A, “Recommended techniques for GeneTitan™ MC Instrument operation”.)

a. When drawer 2 opens:

- Left side: Scan tray with cover. Remove the protective black base from the scan tray immediately before loading. Do not load the protective black base.
- Press the blue confirmation button on the GeneTitan™ Instrument to continue.

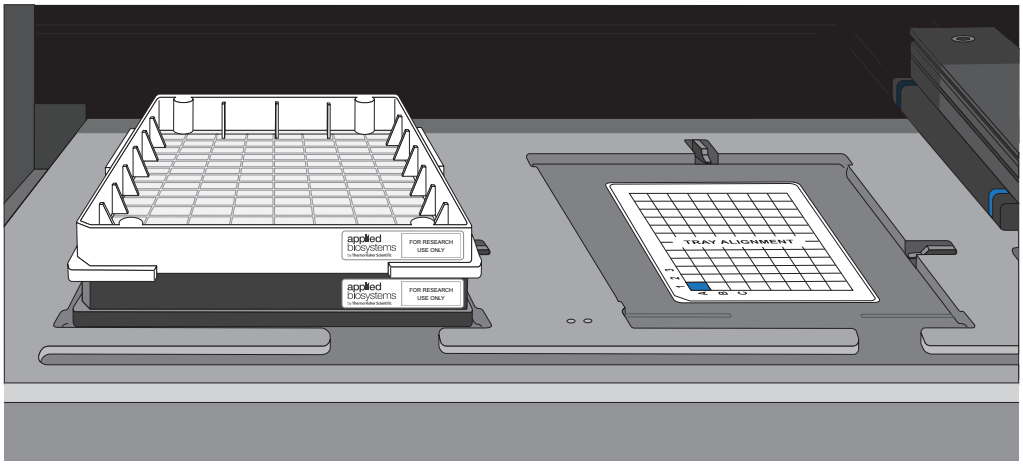


Figure 15 Drawer 2, left side: Scan tray with cover.

b. When drawer 3 opens:

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

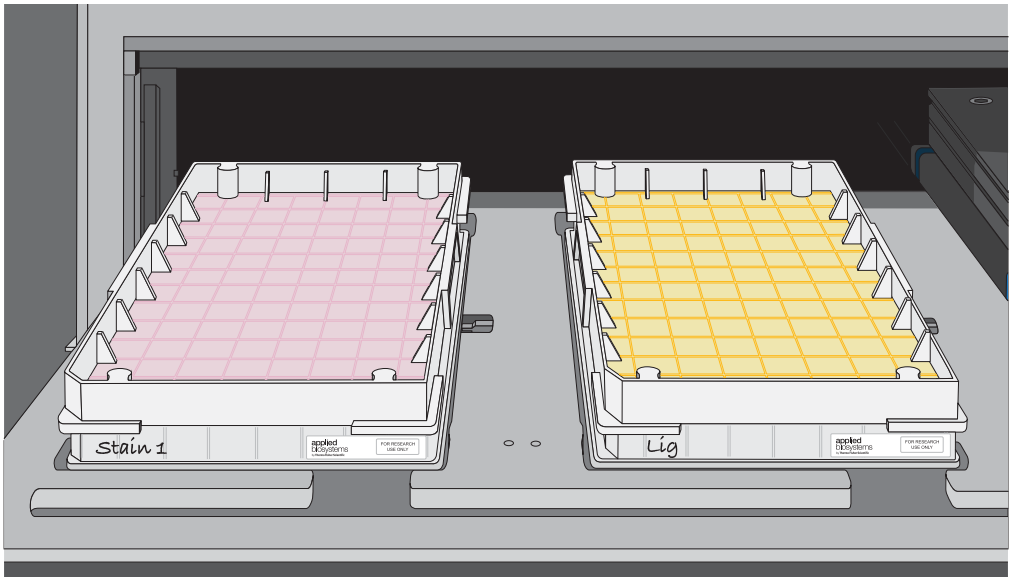


Figure 16 Drawer 3, left side: Stain 1 tray with cover. Drawer 3, right side: Ligation tray with cover.

c. When drawer 4 opens:
   - Left side: Stain 2 tray with cover.
   - Right side: Stabilization tray with Stabilize Solution and cover.
   - Press the blue confirmation button on the GeneTitan™ Instrument to continue.

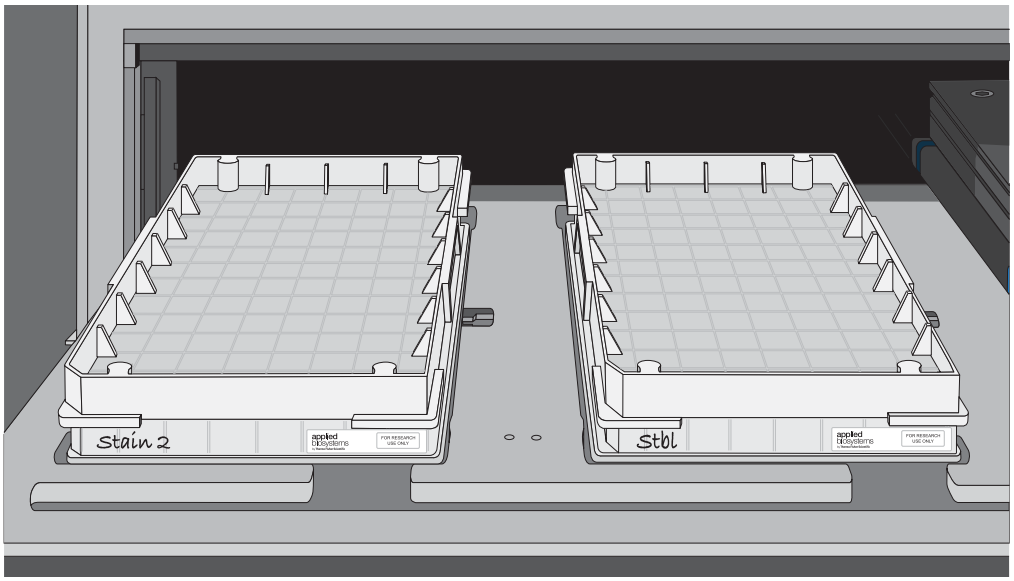


Figure 17 Drawer 4, left side: Stain 2 tray with cover. Drawer 4 right side: Stabilization tray with Stabilize Solution.

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

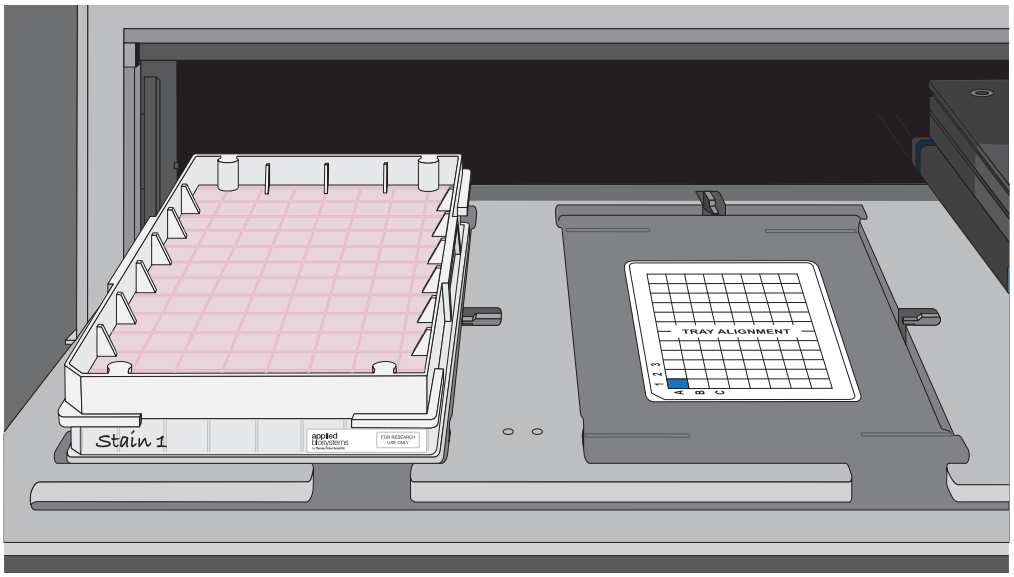


Figure 18 Drawer 5, left side: Stain 1 tray with cover.

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

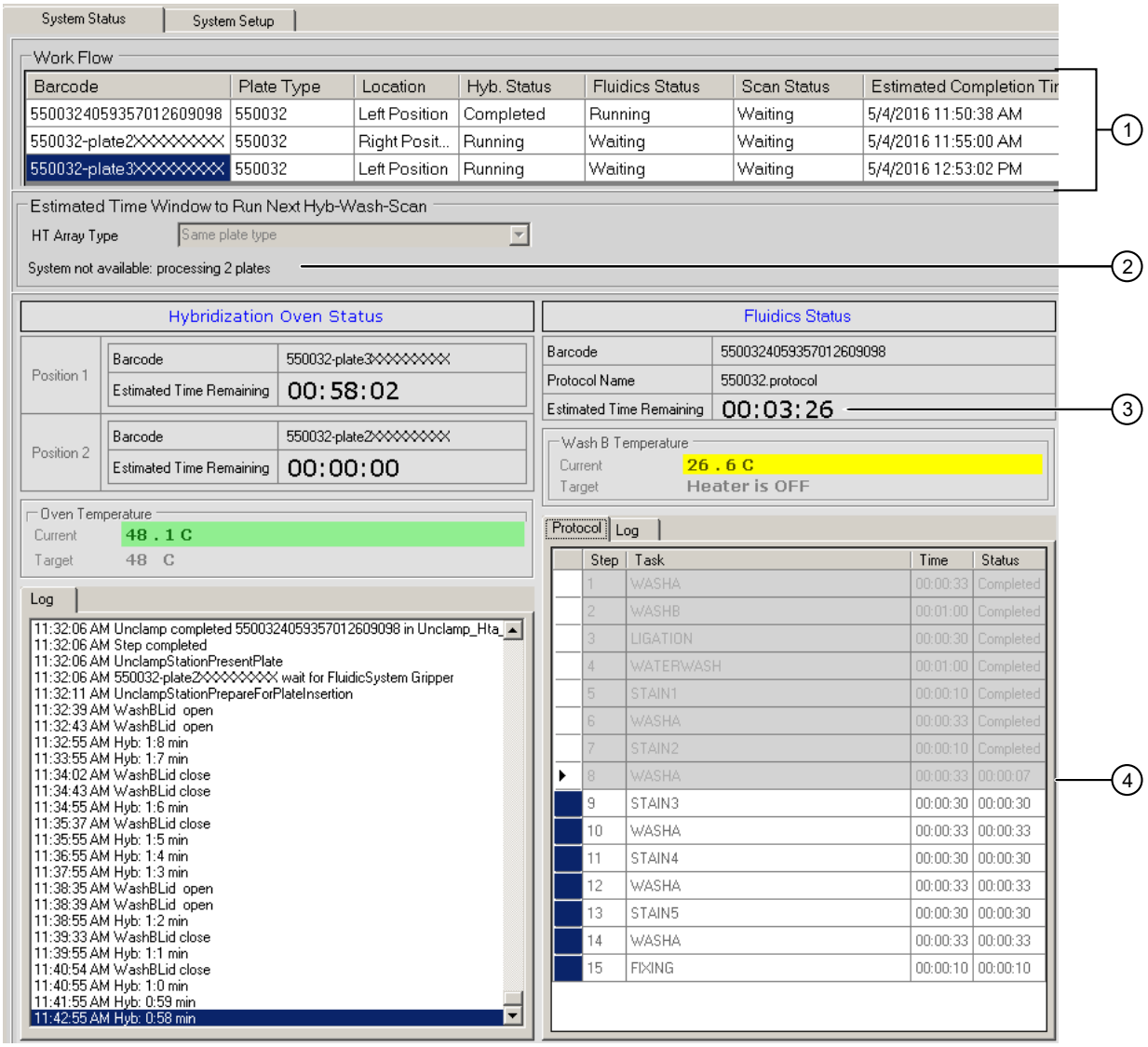
GeneTitan™ MC Instrument internal array plate activity

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. If needed the **Estimated Time Remaining** for fluidics is adjusted. Changes can be due to process interruptions such as a drawer being opened.

④ The step that is currently executing in fluidics.

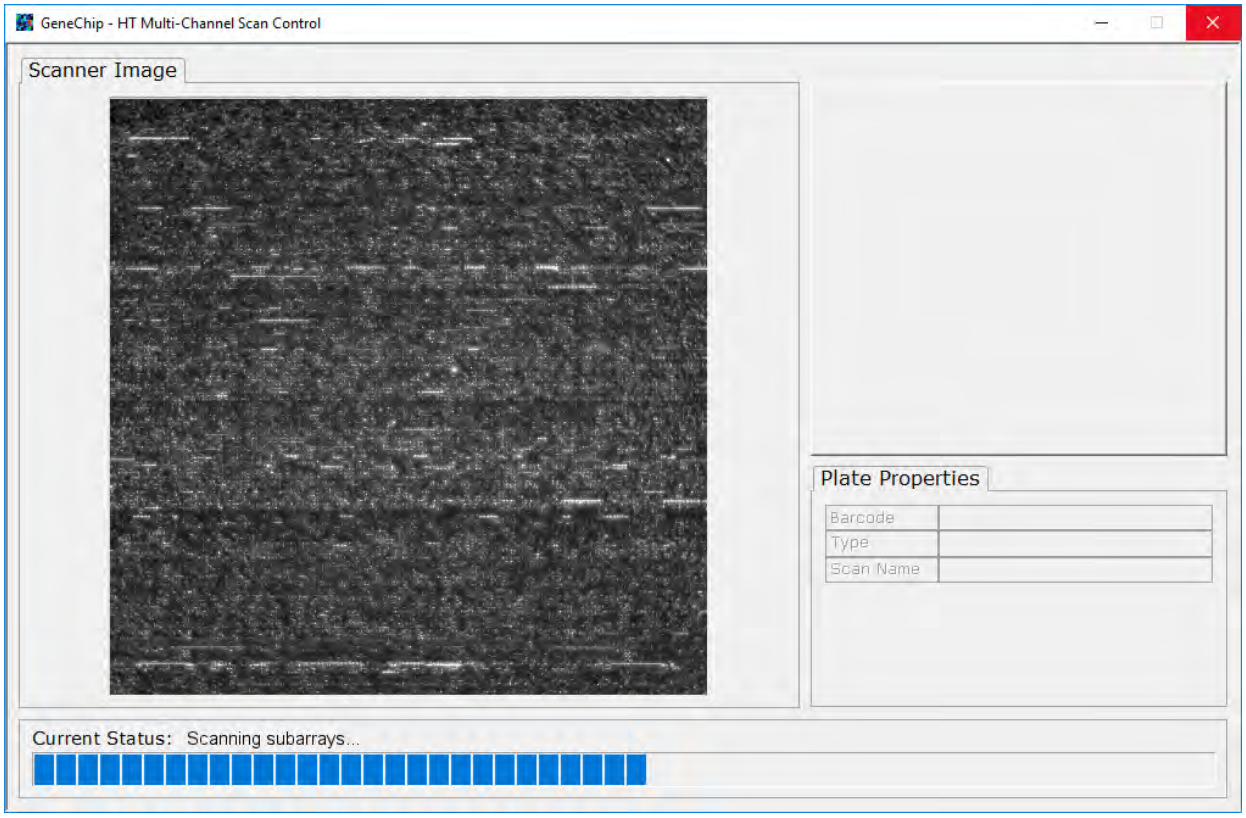
Continue the scan workflow

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 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.

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6 Three-plate workflow for CytoScan™ HT-CMA Array Plates using an overnight DNA precipitation step

When using the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using the overnight DNA precipitation step, 1 to 2 people can process up to 3 CytoScan™ HT-CMA 96-array format plates in one 40-hour work week. This chapter describes the timing of the steps for each sample and array plate that are required to perform this workflow.

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

For detailed instructions on the manual target preparation protocol and array plate processing, see the following chapters:

- Chapter 4, “Target preparation”
- Chapter 5, “Process array plates with the GeneTitan™ Multi-Channel Instrument”

Overview of the 3-plate workflow for manual target preparation using an overnight precipitation step

The figure and table below show the timing and duration of the hands-on processing necessary for performing the 3-plate workflow.

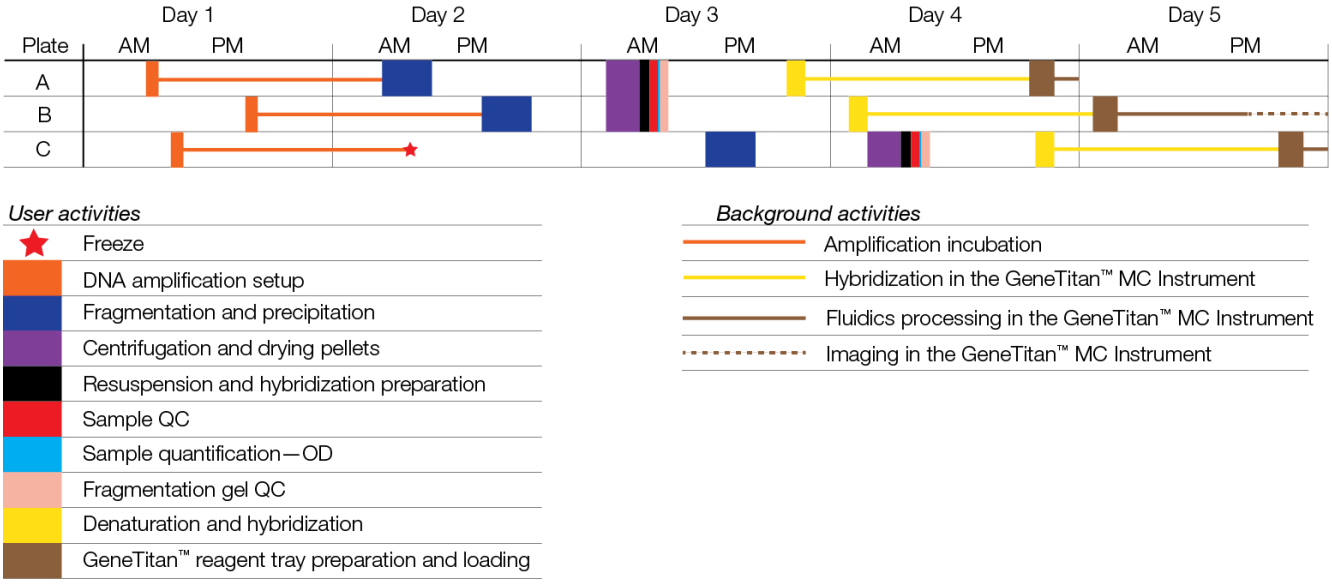


Figure 19 Full-week target preparation and array processing activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using an overnight precipitation step.

The 3 plates are referred to as plates A, B, and C in the manual target preparation and in the GeneTitan™ MC Instrument array processing steps.

To process 3 plates during a 40-hour week, the steps must be performed in the order and with the timing that is described in this chapter. It is helpful to have 2 people working together on day 3 and day 4.

Table 3 Daily steps for manual target preparation workflow

Day	Activities	Plates
1	Amplify 3 plates of genomic DNA.	A, B, C
2	• Fragment and precipitate 2 plates amplified on day 1. • Freeze 1 plate of amplified DNA for fragmentation and precipitation on day 3.	• A, B • C
3	• Fragment and precipitate 1 plate amplified on day 1. • Centrifuge, dry, resuspend, prepare hybridization, and QC 2 plates precipitated on day 2. • Denature and begin hybridization for 1 plate of hybridization-ready samples on the GeneTitan™ MC Instrument. • Freeze 1 Hyb-Ready Plate.	• C • A, B • A • B

Table 3 Daily steps for manual target preparation workflow *(continued)*

Day	Activities	Plates
4	• Centrifuge, dry, resuspend, prepare hybridization, and QC 1 plate precipitated on day 3. • Denature and begin hybridization for 2 plates of hybridization-ready samples on the GeneTitan™ MC Instrument. • Prepare GeneTitan™ reagents and load GeneTitan™ reagent trays.	• C • B, C • A
5	Prepare GeneTitan™ reagents and load GeneTitan™ reagent trays.	B, C

The timing of these steps is critical because of constraints on both the target preparation, done on the lab bench, and the array processing, done using the GeneTitan™ MC Instrument. These constraints are described in more detail in the following sections:

- "Timing considerations for manual target preparation" on page 118
- "Timing considerations for processing arrays on the GeneTitan™ MC Instrument" on page 119

Timing considerations for manual target preparation

The GeneTitan™ reagent trays for array processing cannot be loaded into the GeneTitan™ MC Instrument until the array plate has finished hybridization. Do not prepare the reagent trays more than 1.5 hours before hybridization finishes. The GeneTitan™ reagent trays cannot be prepared ahead of time and stored.

Table 4 Time required for manual target preparation steps.

Stage	Hands-on time	Total preparation time[1]	Incubation/hybridization/ processing
"Stage 1: Amplify the genomic DNA" on page 38	30 minutes	1.5 hours	23 ±1 hours
"Stage 2: Fragment and precipitate the DNA" on page 47	2 hours	2 hours	Overnight precipitation
"Stage 3A: Centrifuge the Precipitation Plate and dry the DNA pellets" on page 60	30 minutes	1 hour 20 minutes	N/A
"Stage 3B: Resuspend the pellets and prepare for hybridization" on page 62	25 minutes	25 minutes	N/A
"Stage 3C: Perform quantification and fragmentation QC checks" on page 66	45 minutes	45 minutes	N/A
"Stage 4: Denature and hybridize the sample plate" on page 72	25 minutes	45 minutes	23.5-hour to 24-hour hybridization
"Stage 5: Prepare GeneTitan™ reagents and trays" on page 78	1 hour	1.5 hours	Additional time for processing: 96 arrays: 12.5 hours

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

Timing considerations for processing arrays on the GeneTitan™ MC Instrument

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

The hybridization time for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow on the GeneTitan™ MC Instrument is 23.5–24 hours. This time range provides a 30-minute window during which the instrument control software prompts you to load the reagents that are required for washing and staining.

Table 5 Time required for array plate processing steps on the GeneTitan™ MC Instrument.

Step	Time required
Hybridization of 2 plates in 1 day • First plate loaded at 9:30 a.m. • Second plate loaded at 5:00 p.m.	23.5 hours per plate
Loading reagent trays	10 minutes
Fluidics	5 hours per plate
Scanning[1]	Up to 7.5 hours depending on array format

[1] For labs that run several array plate formats, scanning times can vary.

Oven temperatures for the 3-plate workflow

Multiple ovens are required for manual target preparation. If you are running the 3 plate/week workflow, 3 ovens are recommended. Table 6 lists the different temperatures that are required for each step. Though only 2 ovens are strictly required, we recommend maintaining separate 37°C ovens for the amplification and fragmentation stages. The additional oven helps to avoid confusion of plates and to minimize excess opening and closing of oven doors during incubation. Table 7 provides a list of suggested settings for 3 ovens when performing the 3 plate/week workflow.

Table 6 Oven temperatures needed for each step of the workflow.

Workflow step	Oven temperature
Amplification	37°C
Stopping amplification	65°C
Pre-fragmentation incubation	37°C
Fragmentation incubation	37°C
Drying	37°C
Hybridization[1]	48°C

[1] For preheating of the 96-well metal chamber for hybridization transfer.

Table 7 Suggested oven settings for the 3 plate/week manual target preparation workflow.

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

Thermal cycler requirements for the 3-plate workflow

One thermal cycler is required to conduct the 3-plate workflow. See the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide* (Pub. No. MAN0018215) for a list of verified thermal cyclers.

Thaw frozen plates of amplified DNA

1. Place the deep-well plate in a small water bath.
   For example, pour ultra-pure water into a small tray. Place the frozen plate on the water in the tray.
2. Leave the plate in the water bath for ~1 hour until all wells have thawed, especially the middle wells.
3. Centrifuge at 1,000 rpm for 30 seconds.
4. Avoid cross-contamination of wells during vortexing.
   a. Remove the seal, then blot the top of the plate with a laboratory tissue.
   b. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 seconds to mix. See “Guidelines for handling plates and tubes” on page 27.
6. Centrifuge at 1,000 rpm for 30 seconds.

Manual target preparation and array processing for the 3-plate workflow using an overnight DNA precipitation step

Day 1 activities

- On this day, you start whole-genome amplification of the 3 plates. Each plate must incubate 23 ±1 hours after amplification starts.
- All amplifications are set up on day 1 to allow for a 23 ±1-hour amplification incubation for each plate. This schedule helps to minimize movement between preamplification and post-amplification areas.

Reagent and plate handling

- Start thawing the amplification reagents, particularly the Amp Solution, 60 minutes before the start of each reaction.

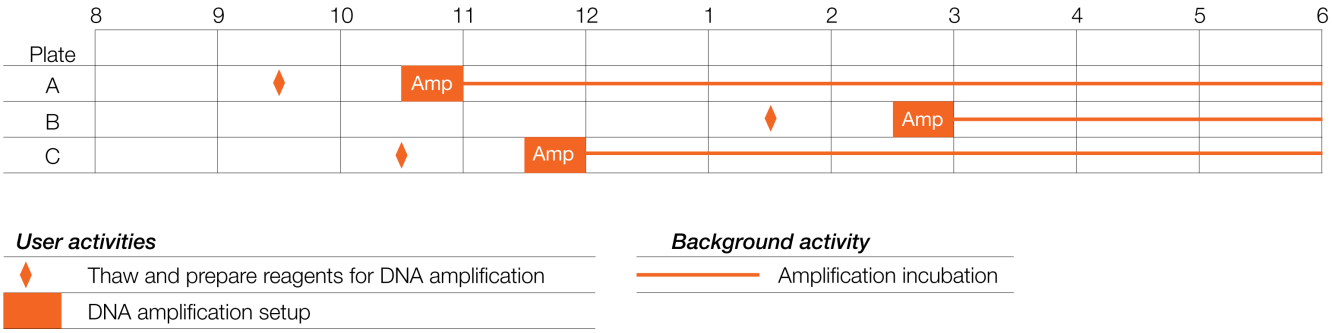
IMPORTANT! Amplification preparation must take place in an Amplification Staging Room or dedicated area with a biosafety hood, dedicated pipettes, tips, vortexer, and other equipment.

Table 8 Day 1 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using overnight precipitation.

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

[1] Approximate start time indicates start of thawing of reagents.

Day 1 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using overnight precipitation



Day 2 activities

- Fragment (including the 65°C stop amplification reaction step) and precipitate plates A and B. Precipitation is carried out at –20°C overnight.
- Freeze plate C at –20°C at the end of the 23-hour amplification incubation.

Reagent and plate handling

- Start thawing the fragmentation reagents 30 minutes before the start of each fragmentation step.

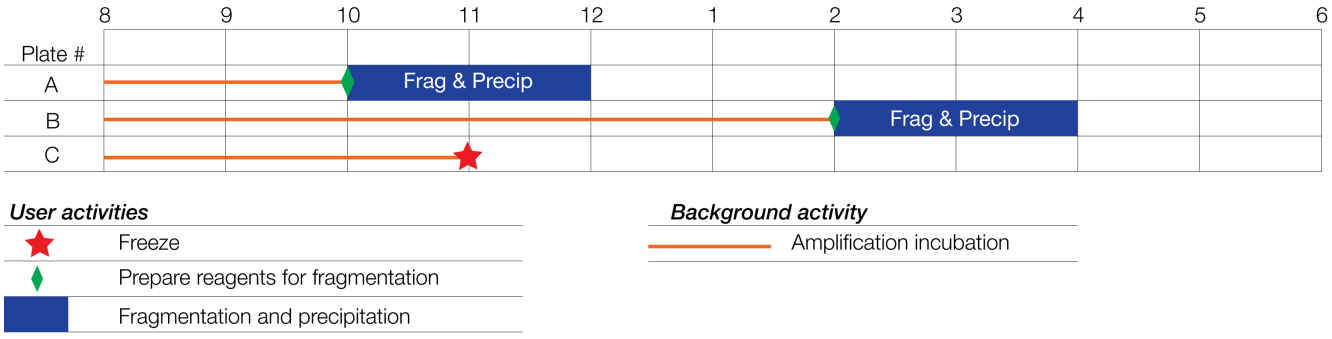
IMPORTANT! Store plate C at –20°C immediately after the end of the 23-hour amplification reaction without performing the 65°C stop amplification reaction step.

Table 9 Day 2 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using overnight precipitation.

Activity	Plate	Approximate times	
		Start time[1]	End time
Fragment and precipitate.	A	10:00 a.m.	12:00 p.m.
Freeze at –20°C at end of 23-hour DNA amplification.	C	11:00 a.m.	—
Fragment and precipitate.	B	2:00 p.m.	4:00 p.m.

[1] Approximate start time indicates start of thawing of reagents.

Day 2 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using overnight precipitation



Day 3 activities

- Centrifuge, dry, resuspend, prepare hybridization, and QC plates A and B.
- Store hybridization-ready plate B at –20°C.
- Thaw plate C. See “Thaw frozen plates of amplified DNA” on page 120.
- Fragment (including the 65°C stop amplification reaction step) and precipitate plate C. Precipitation is carried out at –20°C overnight.
- Perform denaturation and hybridization for plate A.
- Transfer the denatured hybridization samples to the hybridization tray for plate A.
- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization for plate A.

Reagent and plate handling

- Start preparing the resuspension reagents at least 60 minutes before the start of the resuspension step.
- Start thawing the fragmentation reagents 30 minutes before the start of the fragmentation step.
- Before opening the array plate pouch or removing the array plate, warm the array plate to room temperature for at least 25 minutes.
- Warm the hybridization-ready sample plate at room temperature for at least 5 minutes before the denature step.

CAUTION! Hybridization tray preparation must take place under a running fume hood.

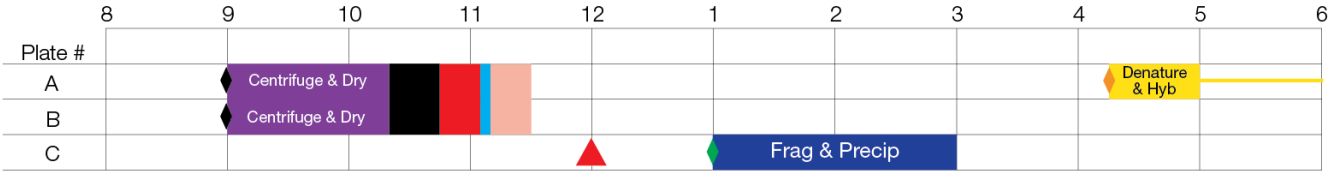
IMPORTANT! Amplified plates that are frozen must be thawed and thoroughly mixed. See “Thaw frozen plates of amplified DNA” on page 120.

Table 10 Day 3 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using overnight precipitation.

Activity	Plate	Approximate times	
		Start time[1]	End time
Centrifuge and dry.	A, B	9:00 a.m.	10:20 a.m.
Resuspension and hybridization preparation.	A, B	10:20 a.m.	10:45 a.m.
Sample QC.	A, B	10:45 a.m.	11:05 a.m.
Sample quantification (OD)[2]	A, B	11:05 a.m.	11:10 a.m.
Fragmentation gel QC.	A, B	11:05 a.m.	11:30 a.m.
Store Hyb-Ready Plate at –20°C.	B	11:10 a.m.	—
Thaw plate C.	C	12:00 p.m.	1:00 p.m.
Fragment and precipitate.	C	1:00 p.m.	3:00 p.m.
Denaturation and hybridization.	A	4:15 p.m.	5:00 p.m. on day 4[3]

[1] Approximate start time indicates start of thawing of reagents.
[2] Sample quantification runs concurrently with Fragmentation gel QC run. Load the Gel QC Plate first, then read the OD QC Plate.
[3] 45 minutes setup, 23.5–24 hours hybridization.

Day 3 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using overnight precipitation



User activities	
♦	Prepare reagents for resuspension and hyb preparation
♦	Prepare reagents for fragmentation
♦	Warm array plate to room temperature
▲	Thaw Amplification Plate
	Fragmentation and precipitation
	Centrifugation and drying pellets
	Resuspension and hybridization preparation
	Sample QC
	Sample quantification—OD
	Fragmentation gel QC
	Denaturation and hybridization

Background activity	
—	Hybridization in the GeneTitan™ MC Instrument

Day 4 activities

- Perform denaturation and hybridization for plates B and C.
- Transfer the denatured hybridization samples to the hybridization tray for plates B and C.
- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization for plates B and C.
- Centrifuge, dry, resuspend, prepare hybridization, and QC plate C.
- GeneTitan™ reagent trays preparation and loading for plate A.

Reagent and plate handling

- Before opening the array plate pouch or removing the array plate, warm the array plate to room temperature for at least 25 minutes.
- Load the array plate and hybridization tray into the GeneTitan™ MC Instrument.
- Warm the hybridization-ready sample plate at room temperature for at least 5 minutes before the denaturation step.
- Start preparing the resuspension reagents at least 60 minutes before the start of the resuspension step.
- Start preparing the reagents at least 30 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.

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

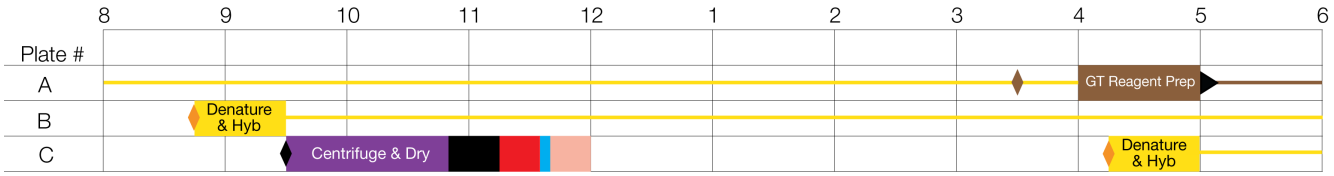
IMPORTANT! The GeneTitan™ reagent trays for array processing cannot be loaded until the array plate has finished hybridization, and they should not be prepared more than 1.5 hours before hybridization will finish. The GeneTitan™ reagent trays cannot be prepared ahead of time and stored.

Table 11 Day 4 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using overnight precipitation.

Activity	Plate	Approximate times	
		Start time[1]	End time
Denaturation and hybridization.	B	8:45 a.m.	9:30 a.m. on day 5[2]
Centrifuge and dry.	C	9:30 a.m.	10:50 a.m.
Resuspension and hybridization preparation.	C	10:50 a.m.	11:15 a.m.
Sample QC.	C	11:15 a.m.	11:35 a.m.
Sample quantification (OD)[3]	C	11:35 a.m.	11:40 a.m.
Fragmentation Gel QC.	C	11:35 a.m.	12:00 p.m.
Prepare and load GeneTitan™ reagent tray.	A	3:30 p.m.	5:00 p.m.
Denaturation and hybridization.	C	4:15 p.m.	5:00 p.m. on day 5[2]

[1] Approximate start time indicates start of thawing of reagents.
[2] 45 minutes setup, 23.5–24 hours hybridization.
[3] Sample quantification runs concurrently with Fragmentation gel QC run. Load the Gel QC Plate first, then read the OD QC Plate.

Day 4 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using overnight precipitation



User activities

- Warm array plate to room temperature
- Prepare reagents for resuspension and hyb preparation
- Prepare reagents for GeneTitan reagent preparation
- Load array plate into GTMC, begin Wash-Scan
- Centrifugation and drying pellets
- Resuspension and hybridization preparation
- Sample QC
- Sample quantification—OD
- Fragmentation gel QC
- GeneTitan™ reagent tray preparation and loading
- Denaturation and hybridization

Background activities

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

Day 5 activities

- GeneTitan™ reagents preparation and loading for plates B and C.

Reagent and plate handling

- Start preparing the reagents at least 30 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.

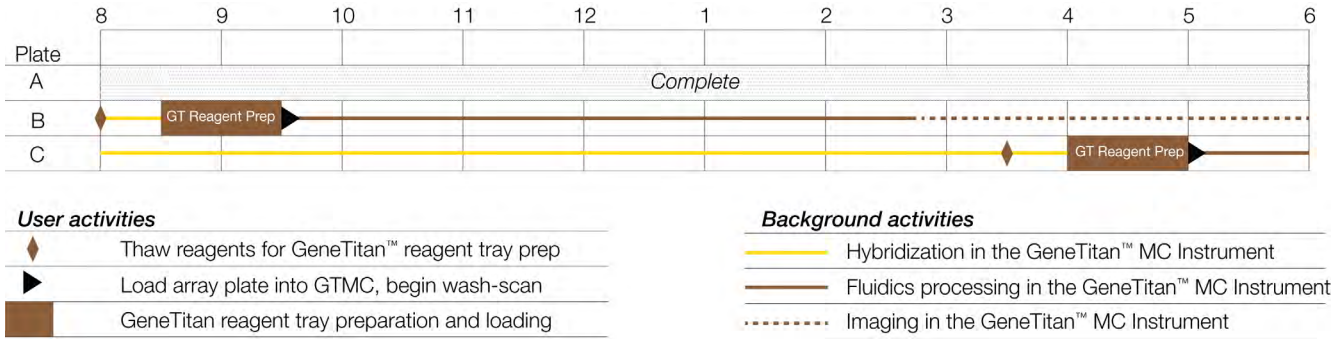
IMPORTANT! The GeneTitan™ reagent trays for array processing cannot be loaded into the GeneTitan™ MC Instrument until the array plate has finished hybridization. Do not prepare the reagent trays more than 1.5 hours before hybridization finishes. The GeneTitan™ reagent trays cannot be prepared ahead of time and stored.

Table 12 Day 5 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using overnight precipitation.

Activity	Plate	Approximate times	
		Start time[1]	End time
Prepare and load GeneTitan™ reagent tray.	B	8:00 a.m.	9:30 a.m.
Prepare and load GeneTitan™ reagent tray.	C	3:30 p.m.	5:00 p.m.

[1] Approximate start time indicates start of thawing of reagents.

Day 5 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using overnight precipitation



7 Three-plate workflow for CytoScan™ HT-CMA Array Plates using a 3-hour DNA precipitation step

The CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using a 3-hour DNA precipitation step allows faster assay turnaround time. Using this workflow, plate 1 CEL files are available in 72 hours, and CEL files for all 3 plates are available by day 5. One to 2 people can process 3 CytoScan™ HT-CMA 96-array format plates in 1 work week. This chapter describes the timing of the steps for each sample and array plate that are required to perform this workflow. Note that this workflow requires approximately 9–10-hour workdays.

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

For detailed instructions on the manual target preparation protocol and array plate processing, see the following chapters:

- Chapter 4, “Target preparation”
- Chapter 5, “Process array plates with the GeneTitan™ Multi-Channel Instrument”

Overview of the 3-plate workflow for manual target preparation using a 3-hour precipitation step

The figure and table below show the timing and duration of the hands-on processing necessary for performing the 3-plate workflow.

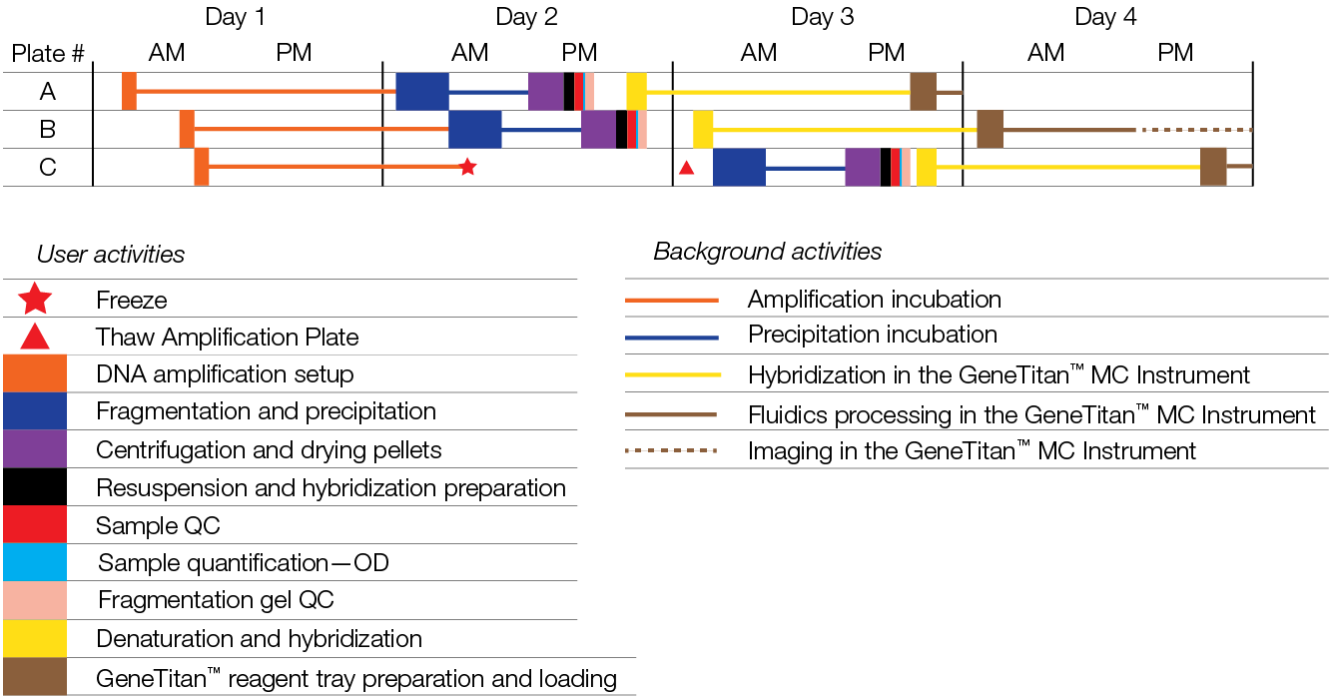


Figure 20 Full week target preparation and array processing activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using a 3-hour precipitation step.

The 3 plates are referred to as plates A, B, and C in the manual target preparation and in the GeneTitan™ MC Instrument array processing steps.

To process 3 plates during a 40-hour week, the steps must be performed in the order and with the timing that is described in this chapter. It is helpful to have 2 people working together on day 2 and day 3.

Table 13 Daily steps for manual target preparation workflow

Day	Activities	Plates
1	Amplify 3 plates of genomic DNA.	A, B, C
2	• Fragment and precipitate 2 plates amplified on day 1. • Freeze 1 plate of amplified DNA for fragmentation and precipitation on day 3. • Centrifuge, dry, resuspend, prepare hybridization, and QC 2 plates. • Denature and begin hybridization for 1 plate of hybridization-ready samples on the GeneTitan™ MC Instrument. • Freeze 1 hybridization-ready plate.	• A, B • C • A, B • A • B

Table 13 Daily steps for manual target preparation workflow *(continued)*

Day	Activities	Plates
3	• Fragment and precipitate 1 plate amplified on day 1. • Centrifuge, dry, resuspend, prepare hybridization, and QC 1 plate. • Denature and begin hybridization for 2 plates of hybridization-ready samples on the GeneTitan™ MC Instrument. • Prepare GeneTitan™ reagents and load GeneTitan™ reagent trays.	• C • C • B, C • A
4	Prepare GeneTitan™ reagents and load GeneTitan™ reagent trays.	B, C

The timing of these steps is critical because of constraints on both the target preparation, done on the lab bench, and the array processing, done using the GeneTitan™ MC Instrument. These constraints are described in more detail in the following sections:

- "Timing considerations for manual target preparation" on page 130
- "Timing considerations for processing arrays on the GeneTitan™ MC Instrument" on page 131

Timing considerations for manual target preparation

The GeneTitan™ reagent trays for array processing cannot be loaded into the GeneTitan™ MC Instrument until the array plate has finished hybridization. Do not prepare the reagent trays more than 1.5 hours before hybridization finishes. The GeneTitan™ reagent trays cannot be prepared ahead of time and stored.

Table 14 Time required for manual target preparation steps using 3-hour precipitation step.

Stage	Hands-on time	Total preparation time[1]	Incubation/hybridization/ processing
"Stage 1: Amplify the genomic DNA" on page 38	30 minutes	1.5 hours	23 ±1 hours
"Stage 2: Fragment and precipitate the DNA" on page 47	2 hours	2 hours	3-hour precipitation
"Stage 3A: Centrifuge the Precipitation Plate and dry the DNA pellets" on page 60	30 minutes	1 hour 20 minutes	N/A
"Stage 3B: Resuspend the pellets and prepare for hybridization" on page 62	25 minutes	25 minutes	N/A
"Stage 3C: Perform quantification and fragmentation QC checks" on page 66	45 minutes	45 minutes	N/A
"Stage 4: Denature and hybridize the sample plate" on page 72	25 minutes	45 minutes	23.5-hour to 24-hour hybridization
"Stage 5: Prepare GeneTitan™ reagents and trays" on page 78	1 hour	1.5 hours	Additional time for processing: 96 arrays: 12.5 hours

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

Timing considerations for processing arrays on the GeneTitan™ MC Instrument

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

The hybridization time for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow on the GeneTitan™ MC Instrument is 23.5–24 hours. This time range provides a 30-minute window during which the instrument control software prompts you to load the reagents that are required for washing and staining.

Table 15 Time required for array plate processing steps on the GeneTitan™ MC Instrument.

Step	Time required
Hybridization of 2 plates in 1 day • First plate loaded at 9:30 a.m. • Second plate loaded at 5:00 p.m.	23.5 hours per plate
Loading reagent trays	10 minutes
Fluidics	5 hours per plate
Scanning[1]	Up to 7.5 hours depending on array format

[1] For labs that run several array plate formats, scanning times can vary.

Oven temperatures for the 3-plate workflow

Multiple ovens are required for manual target preparation. If you are running the 3 plate/week workflow, 3 ovens are recommended. Table 16 lists the different temperatures that are required for each step. Though only 2 ovens are strictly required, we recommend maintaining separate 37°C ovens for the amplification and fragmentation stages. The additional oven helps to avoid confusion of plates and to minimize excess opening and closing of oven doors during incubation. Table 17 provides a list of suggested settings for 3 ovens when performing the 3 plate/week workflow.

Table 16 Oven temperatures needed for each step of the workflow.

Workflow step	Oven temperature
Amplification	37°C
Stopping amplification	65°C
Pre-fragmentation incubation	37°C
Fragmentation incubation	37°C
Drying	37°C
Hybridization[1]	48°C

[1] For preheating of the 96-well metal chamber for hybridization transfer.

Table 17 Suggested oven settings for the 3 plate/week manual target preparation workflow using the 3-hour precipitation step.

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

Thermal cycler requirements for the 3-plate workflow

One thermal cycler is required to conduct the 3-plate workflow. See the *CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide* (Pub. No. MAN0018215) for a list of verified thermal cyclers.

Thaw frozen plates of amplified DNA

1. Place the deep-well plate in a small water bath.
   For example, pour ultra-pure water into a small tray. Place the frozen plate on the water in the tray.
2. Leave the plate in the water bath for ~1 hour until all wells have thawed, especially the middle wells.
3. Centrifuge at 1,000 rpm for 30 seconds.
4. Avoid cross-contamination of wells during vortexing.
   a. Remove the seal, then blot the top of the plate with a laboratory tissue.
   b. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 seconds to mix. See “Guidelines for handling plates and tubes” on page 27.
6. Centrifuge at 1,000 rpm for 30 seconds.

Manual target preparation and array processing for the 3-plate workflow using a 3-hour DNA precipitation step

Day 1 activities

- On this day, you start whole-genome amplification of the 3 plates. Each plate must incubate 23 ±1 hours after amplification starts.
- All amplifications are set up on day 1 to allow for a 23 ±1-hour amplification incubation for each plate. This schedule helps to minimize movement between preamplification and post-amplification areas.

Reagent and plate handling

- Start thawing the amplification reagents, particularly the Amp Solution, 60 minutes before the start of each reaction.

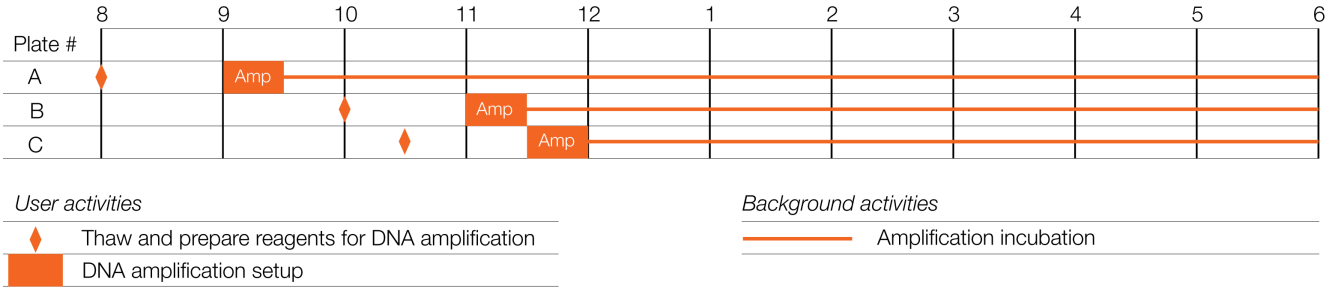
IMPORTANT! Amplification preparation must take place in an Amplification Staging Room or dedicated area with a biosafety hood, dedicated pipettes, tips, vortexer, and other equipment.

Table 18 Day 1 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using 3-hour precipitation.

Activity	Plate	Approximate times	
		Start time[1]	End time
DNA amplification	A	8:00 a.m.	9:30 a.m.
DNA amplification	B	10:00 a.m.	11:30 p.m.
DNA amplification	C	10:30 a.m.	12:00 p.m.

[1] Approximate start time indicates start of thawing of reagents.

Day 1 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using 3-hour precipitation



Day 2 activities

- It is recommended that 2 people work together for day 2 activities.
- Plates A and B are fragmented and precipitated on day 2. Precipitation is carried out at –20°C for 3 hours.
- Centrifuge, dry, resuspend, prepare hybridization, and QC plates A and B.
- Perform denaturation and hybridization for plate A.
- Transfer denatured hybridization samples to hybridization tray for plate A.
- Load the hybridization tray and array plate into the GeneTitan™ MC Instrument and start hybridization for plate A.
- Freeze plate C at –20°C at the end of 23-hour amplification incubation.
- Store plate B hybridization-ready samples at –20°C.

Reagent and plate handling

- Start thawing the fragmentation reagents 30 minutes before the start of the fragmentation step.
- Start preparing the resuspension reagents at least 60 minutes before the start of the resuspension step.
- Before opening the array plate pouch or removing the array plate, warm the array plate to room temperature for at least 25 minutes.
- Warm the hybridization-ready sample plate at room temperature for at least 5 minutes before the denature step.

CAUTION! Hybridization tray preparation must take place under a running fume hood.

IMPORTANT! Store plate C at –20°C immediately after the end of the 23-hour amplification reaction without performing the 65°C stop amplification reaction step.

Table 19 Day 2 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using 3-hour precipitation.

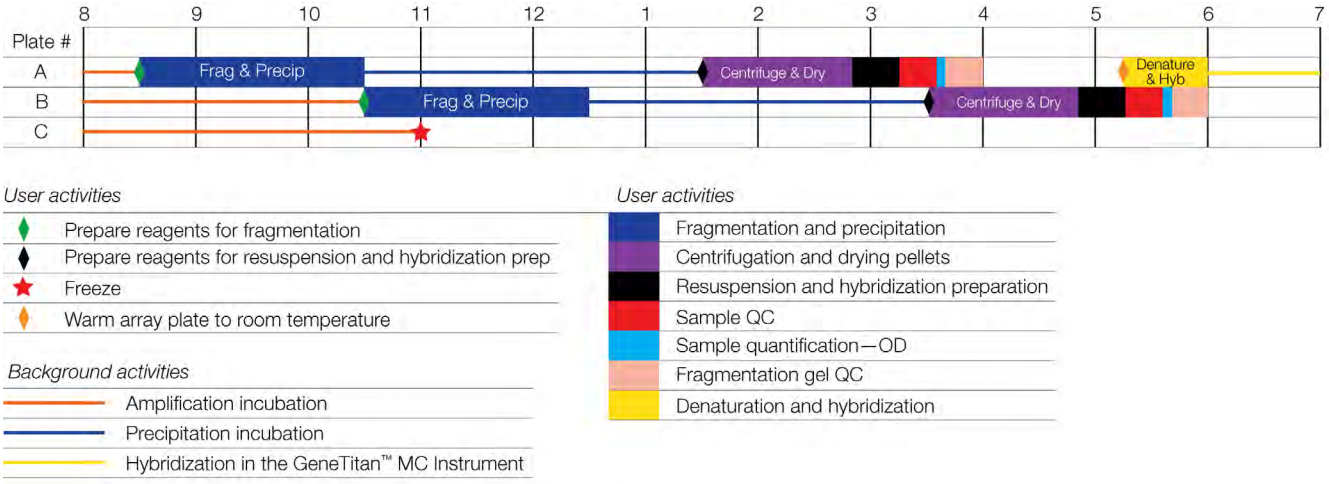
Activity	Plate	Approximate times	
		Start time[1]	End time
Fragment and precipitate.	A	8:30 a.m.	10:30 a.m.
Incubate Precipitation Plate at –20°C.	A	10:30 a.m.	1:30 p.m.
Fragment and precipitate.	B	10:30 a.m.	12:30 p.m.
Freeze at –20°C at end of 23-hour DNA amplification.	C	11:00 a.m.	—
Incubate Precipitation Plate at –20°C.	B	12:30 p.m.	3:30 p.m.
Centrifuge and dry.	A	1:30 p.m.	2:50 p.m.

Table 19 Day 2 activities for the CytoScan HT-CMA Assay 96-Array Format Manual Workflow using 3-hour precipitation. *(continued)*

Activity	Plate	Approximate times	
		Start time[1]	End time
Resuspension and hybridization preparation.	A	2:50 p.m.	3:15 p.m.
Sample QC.	A	3:15 p.m.	3:35 p.m.
Centrifuge and dry.	B	3:30 p.m.	4:50 p.m.
Sample quantification (OD).[2]	A	3:35 p.m.	3:40 p.m.
Fragmentation gel QC.	A	3:35 p.m.	4:00 p.m.
Resuspension and hybridization preparation.	B	4:50 p.m.	5:15 p.m.
Sample QC	B	5:15 p.m.	5:35 p.m.
Denaturation and hybridization.	A	5:15 p.m.	6:00 p.m. on day 3[3]
Sample quantification (OD).[2]	B	5:35 p.m.	5:40 p.m.
Fragmentation gel QC.	B	5:35 p.m.	6:00 p.m.
Store Hyb-Ready Plate at –20°C	B	5:40 p.m.	—

[1] Approximate start time indicates start of thawing of reagents.
[2] Sample quantification runs concurrently with fragmentation gel QC run. Load Gel QC Plate first, then read the OD QC Plate.
[3] 45 minutes setup, 23.5—24 hours hybridization.

Day 2 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using 3-hour precipitation



Day 3 activities

- It is recommended that 2 people work together for day 3 activities.
- Perform denaturation and hybridization for plates B and C.
- Transfer denatured hybridization samples to the hybridization trays for plates B and C.
- Load the hybridization trays and array plates into the GeneTitan™ MC Instrument and start hybridization for plates B and C.
- Thaw plate C. See "Thaw frozen plates of amplified DNA" on page 120.
- Fragment (including the 65°C stop amplification reaction step) and precipitate plate C. Precipitation is carried out at –20°C for 3 hours.
- Centrifuge, dry, resuspend, prepare hybridization, and QC plate C.
- Prepare and load the GeneTitan™ reagent trays for plate A.

CAUTION! Hybridization tray preparation must take place under a running fume hood.

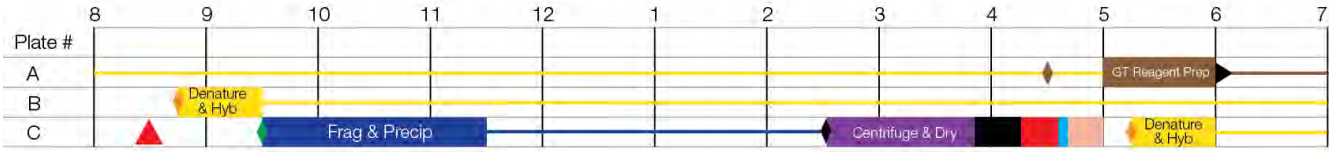
IMPORTANT! Amplified plates that are frozen must be thawed and thoroughly mixed. See "Thaw frozen plates of amplified DNA" on page 120.

Table 20 Day 3 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using 3-hour precipitation.

Activity	Plate	Approximate times	
		Start time[1]	End time
Denaturation and hybridization.	B	8:45 a.m.	9:30 a.m. on day 4[2]
Thaw DNA amplification plate C.	C	8:30 a.m.	9:30 a.m.
Fragment and precipitate.	C	9:30 a.m.	11:30 a.m.
Incubate Precipitation Plate at –20°C.	C	11:30 a.m.	2:30 p.m.
Centrifuge and dry	C	2:30 p.m.	3:50 p.m.
Resuspension and hybridization preparation.	C	3:50 p.m.	4:15 p.m.
Sample QC.	C	4:15 p.m.	4:35 p.m.
GeneTitan™ reagent tray preparation and loading.	A	4:30 p.m.	6:00 p.m.
Sample quantification (OD).[3]	C	4:35 p.m.	4:40 p.m.
Fragmentation gel QC.	C	4:35 p.m.	5:00 p.m.
Denaturation and hybridization.	C	5:15 p.m.	6:00 p.m. on day 4[2]

[1] Approximate start time indicates start of thawing of reagents.
[2] 45 minutes setup, 23.5–24 hours hybridization.
[3] Sample quantification runs concurrently with Fragmentation gel QC run. Load the Gel QC Plate first, then read the OD QC Plate.

Day 3 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using 3-hour precipitation



User activities

- Thaw Amplification Plate
- Warm array plate to room temperature
- Prepare reagents for fragmentation
- Prepare reagents for resuspension and hybridization prep
- Thaw reagents for GeneTitan reagent tray preparation
- Load array plate into GTMC, begin wash-scan
- Fragmentation and precipitation
- Centrifugation and drying pellets
- Resuspension and hybridization preparation
- Sample QC
- Sample quantification—OD
- Fragmentation gel QC
- Denaturation and hybridization
- GeneTitan™ reagent tray preparation and loading

Background activities

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

Day 4 activities

- GeneTitan™ reagents preparation and loading for plates B and C.

Reagent and plate handling

- Start preparing the reagents at least 30 minutes before the start of each GeneTitan™ reagent tray preparation and loading step.

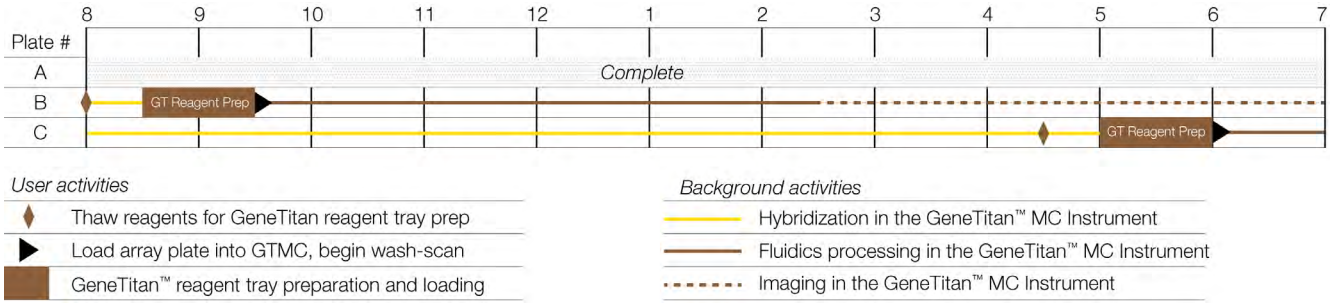
IMPORTANT! The GeneTitan™ reagent trays for array processing cannot be loaded into the GeneTitan™ MC Instrument until the array plate has finished hybridization. Do not prepare the reagent trays more than 1.5 hours before hybridization finishes. The GeneTitan™ reagent trays cannot be prepared ahead of time and stored.

Table 21 Day 4 activities for the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow using 3-hour precipitation.

Activity	Plate	Approximate times	
		Start time[1]	End time
GeneTitan™ reagent tray preparation and loading.	B	8:00 a.m.	9:30 a.m.
GeneTitan™ reagent tray preparation and loading.	C	4:30 p.m.	6:00 p.m.

[1] Approximate start time indicates start of thawing of reagents.

Day 4 activities for the CytoScan™ HT-CMA Assay 96-Array Format 3-plate Manual Workflow using 3-hour precipitation

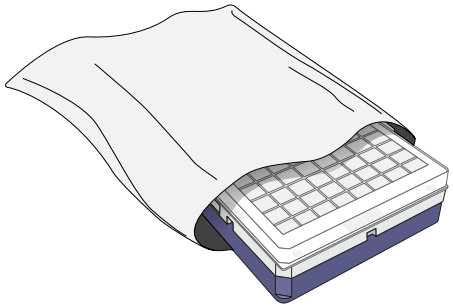
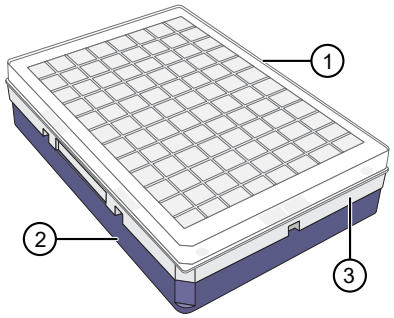


A

Recommended techniques for GeneTitan™ MC Instrument operation

This chapter describes the recommended techniques and procedures to follow when using the GeneTitan™ Multi-Channel (MC) Instrument for the fluidics processing and array scanning steps of the CytoScan™ HT-CMA Assay 96-Array Format Manual 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* (Pub. No. 08-0308) and the *GeneChip™ Command Console™ User Guide* (Pub. No. 702569).

Array plate packaging

Item	Image	Details
CytoScan™ HT-CMA 96-Array Plate	  ① Shipping cover (to be discarded) ② Array plate protective base ③ Array plate	The array plate package includes the following: • Clear plastic cover/shipping cover: The function of the clear 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 1 notched corner. The notched corner of plates, trays, covers, and bases must be in vertical alignment with each other and placed in position A1 per the Tray Alignment guide inside each GeneTitan™ MC Instrument drawer.

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

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

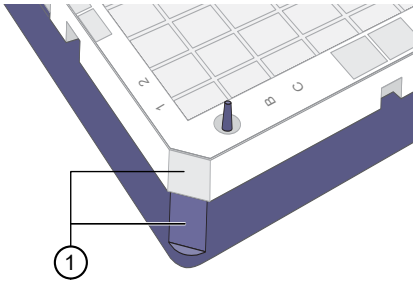


Figure 21 Notched corners aligned.

(1) Notched corner of array plate that is aligned with notched corner of blue base.

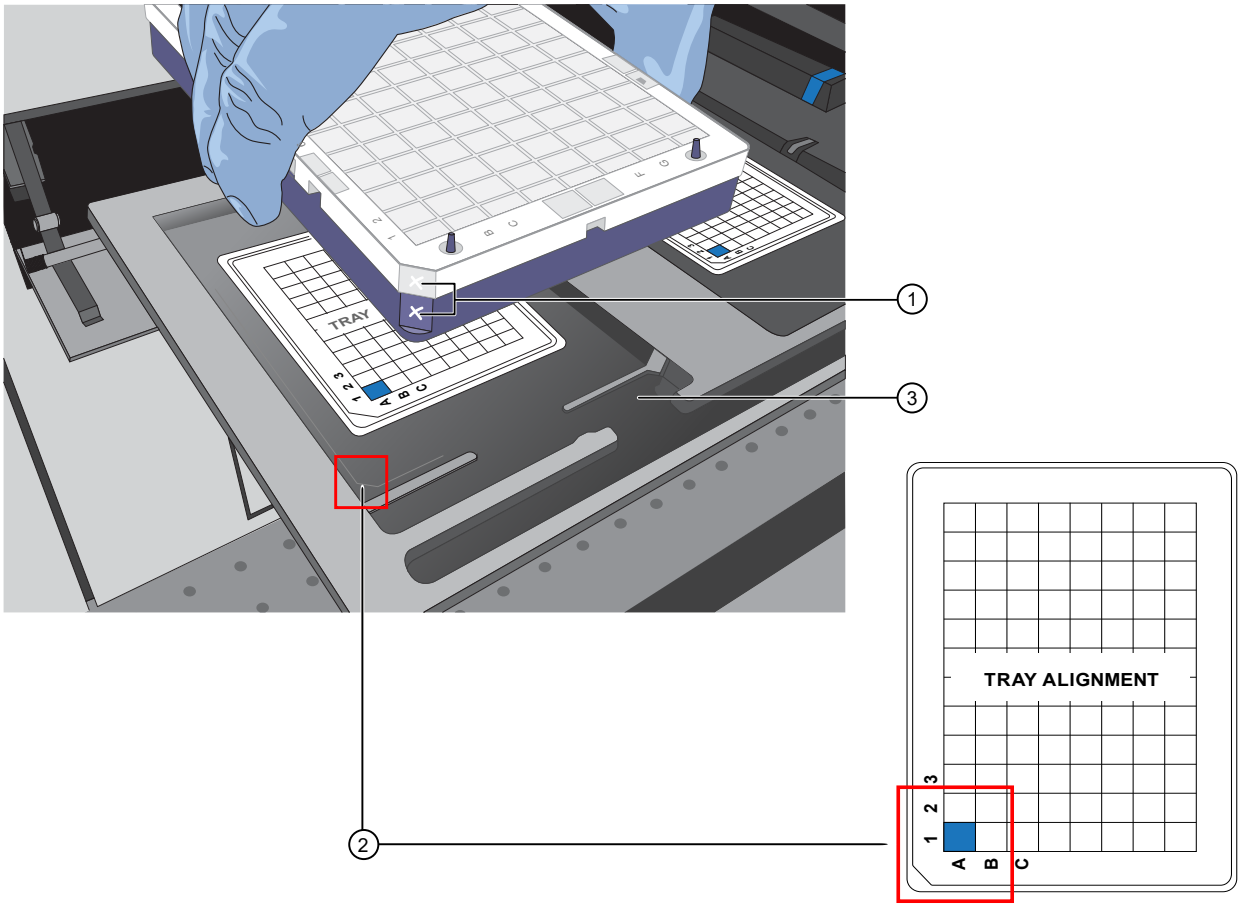


Figure 22 Notched corners marked and aligned with tray alignment guide.

(1) Notched corners of the array plate and blue base marked with a permanent marker to help ensure proper alignment and loading.

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

(3) Plates and trays must be seated in this groove.

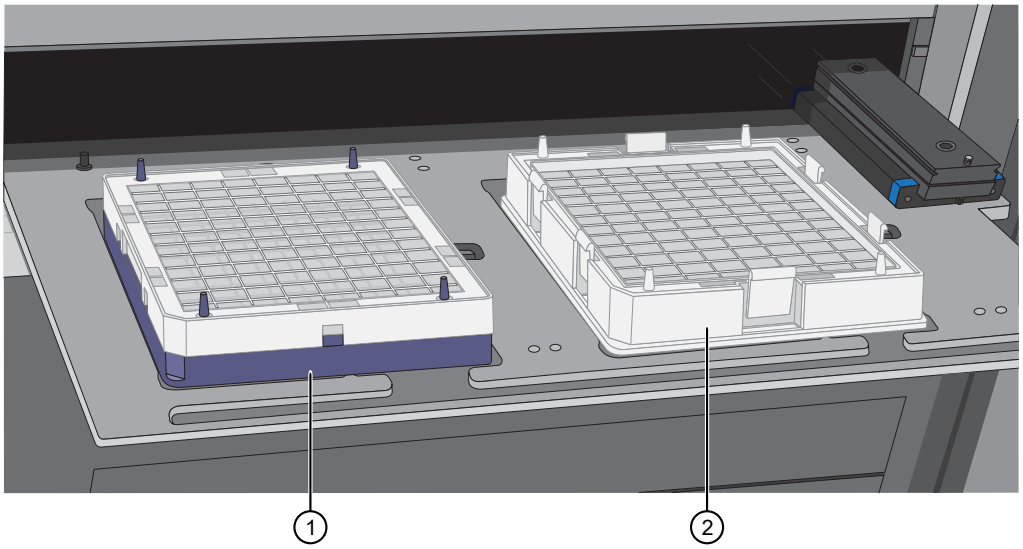


Figure 23 Array plate with protective blue base and the hybridization tray aligned properly loaded into drawer 6.

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

Scan tray

The scan tray must be loaded into the GeneTitan™ Instrument with the scan tray cover only.

Do not load the scan tray while still on the protective base.

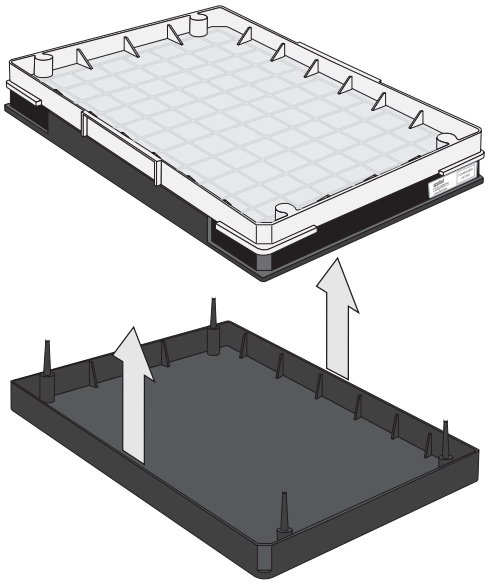


Figure 24 Remove the scan tray and cover from the base before loading in the GeneTitan™ Instrument.

Proper orientation of consumables

It is important that consumables be oriented properly when loaded into/onto the GeneTitan™ MC Instrument. The barcodes face into the instrument.

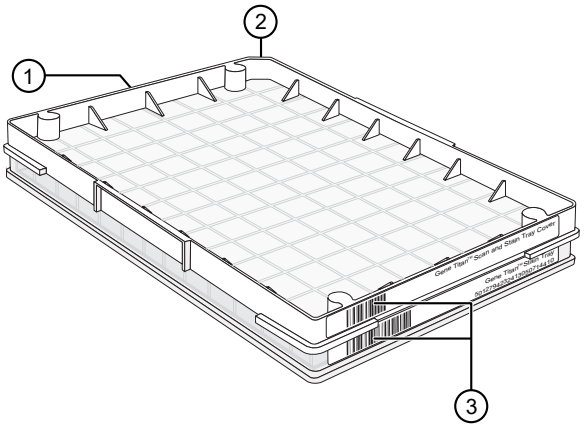


Figure 25 Example shows consumables that must be rotated and loaded on the drawer so that the barcodes face into the instrument.

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.

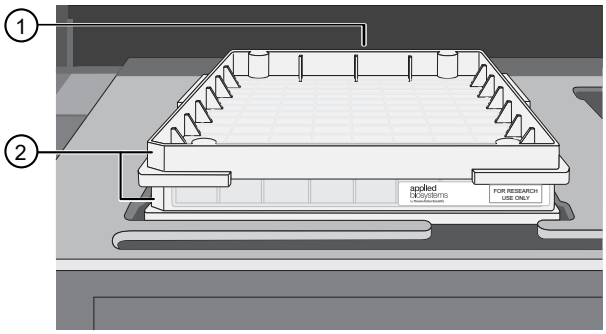


Figure 26 Example of properly loaded GeneTitan™ tray consumables. A GeneTitan™ stain tray and the stain tray cover are shown in this example.

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

Drawer tabs in the GeneTitan™ MC Instrument

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

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

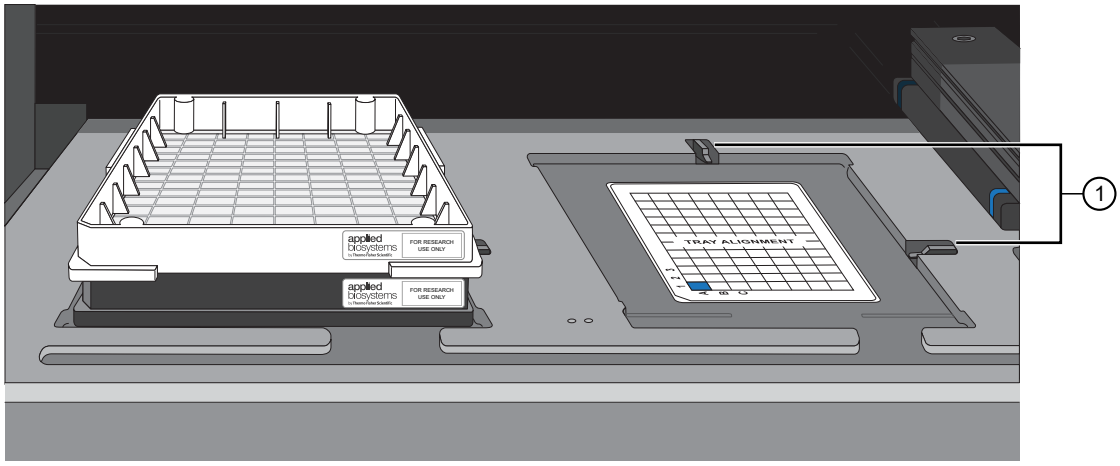


Figure 27 Location of drawer tabs, or fingers.

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

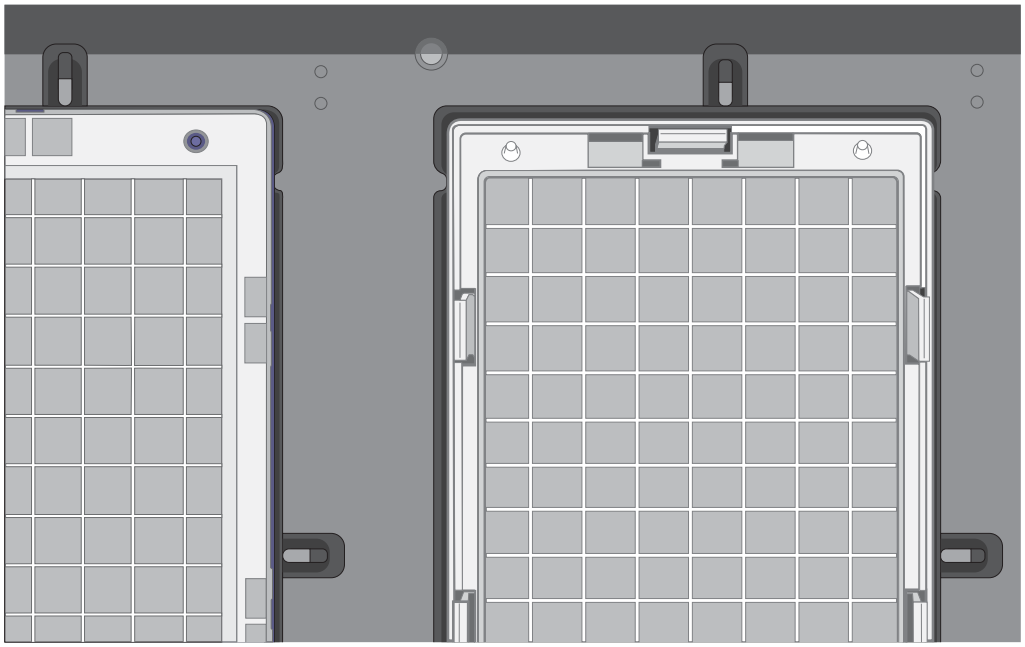


Figure 28 Trays are placed on the drawer when the tabs, or fingers, are retracted.

Stain trays and covers

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

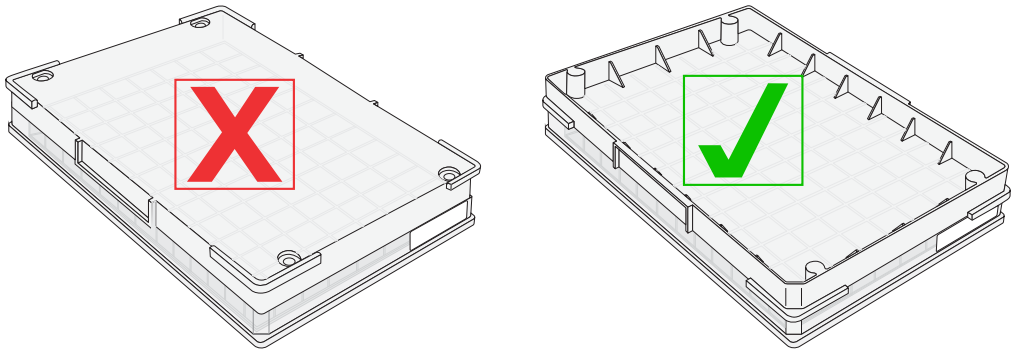


Figure 29 Placement of covers on trays.

Label GeneTitan™ hybridization and reagent trays

When preparing the hybridization and reagent trays to be loaded onto the GeneTitan™ MC Instrument, it is helpful to mark each tray in a way that identifies its contents.

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

Label the GeneTitan™ 96-layout Hybridization Tray

Label the GeneTitan™ 96-layout Hybridization Tray on the front part of the short side of the tray, next to the notch at the left, as shown in the following image. The proper section for labeling is nearest to the notched corner, corresponding to the A1 and B1 wells.

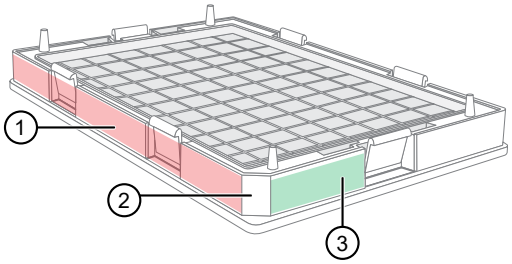


Figure 30 Correct area to label the GeneTitan™ 96-layout hybridization tray.

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

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

Label the GeneTitan™ reagent trays

You can label the GeneTitan™ reagent trays on the left side of the front of the tray as shown in the following image. The correct side is nearest to the notched corner, corresponding to the A1 through C1 wells.

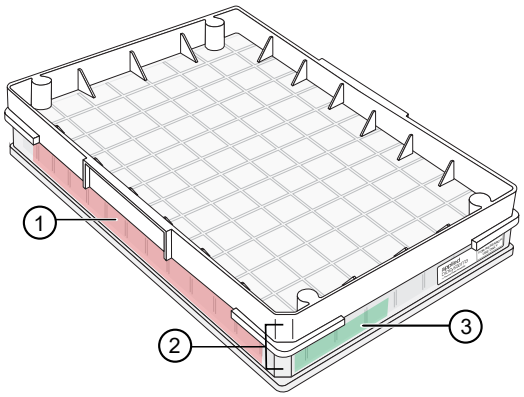
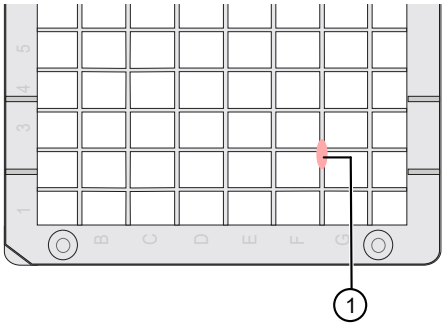


Figure 31 Correct area to label the GeneTitan™ reagent trays (96-layout stain tray with cover shown).

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

Guidelines for aliquoting reagents to GeneTitan™ trays

IMPORTANT! Droplets near or on the top of the well dividers can cause the cover to stick to the tray during GeneTitan™ MC Instrument processing. Remember to deionize the stain trays and the covers before aliquoting master mixes.



① Example of a droplet of liquid that has splashed onto the well divider of a stain tray during reagent aliquoting. Ensure that no droplets of liquid are on top of the well dividers. Blot with a laboratory tissue to remove.

- If the trays are not being used immediately, protect them from light by covering with foil or placing in a cabinet.
- After aliquoting the Ligation, Stain 1, Stain 2, and Stabilization Master Mix reagents to the trays, it is not necessary to spread the reagent to each corner of the well. The reagent spreads evenly when the array plate is inserted into the reagent tray during processing with the GeneTitan™ MC Instrument.

Deionization of GeneTitan™ trays and covers

We recommend the use of the GeneTitan™ ZeroStat AntiStatic Gun (Cat. No. 74-0014) to deionize GeneTitan™ MC Instrument stain trays and covers.

IMPORTANT! Except for the array plate, scan tray, and the hybridization tray, you must deionize all GeneTitan™ stain trays, stain tray covers, and the scan tray cover using an antistatic gun. Always deionize before you fill the trays with reagents and before you place the covers on the trays. Deionization removes the static electricity. Static electricity on the underside of the cover can cause the gripper to lift the tray along with the tray cover and can result in an aborted run.

Deionize the inner surface of each tray and cover:

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

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

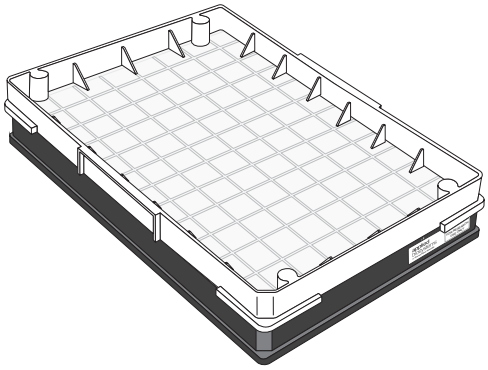


Figure 32 96-format scan tray and cover. Deionize only the cover.

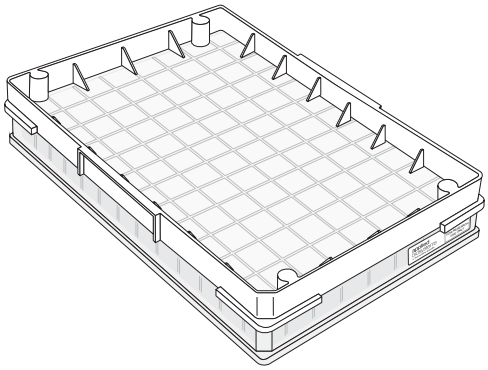


Figure 33 96-format stain tray and cover. Deionize the cover and the tray.

Deionize GeneTitan™ trays and covers

WARNING! The deionization steps damage the arrays on the plate. Before using the antistatic gun, ensure that the array plates remain in their protective pouch and placed away from the deionization area.

Place the scan tray and hybridization tray away from the area where you are performing deionization.

During this procedure, treat the plate or cover as if it were divided into 6 sections. See Figure 34.

1. Place a laboratory tissue on the benchtop.
2. Place the stain tray on a table top. Use the antistatic gun, then squeeze, then release the trigger slowly 3 times over the center of each section, squeezing for approximately 2 seconds, then releasing for approximately 2 seconds.

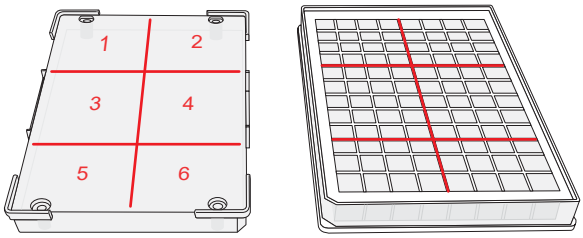
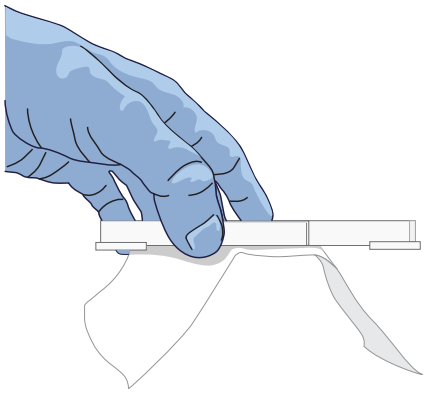


Figure 34 Deionization zones for GeneTitan™ 96-format trays and covers.
Ensure that a stream of ionized particles settles on all wells of the stain tray to dissipate the static electricity.

3. Place the stain tray cover on the tissue with the flat surface facing upward.
4. From ~13 mm away, aim the antistatic gun at the flat surface of the stain tray cover, then squeeze the trigger. As you squeeze the trigger, move the gun across the cover so that the stream of ionized particles settles on all areas of the cover to dissipate the static electricity.
   Squeeze and release the trigger slowly 3 times over each section, squeezing for approximately 2 seconds and releasing for approximately 2 seconds.
5. Place the treated cover or tray on the laboratory tissue, then lift it up.



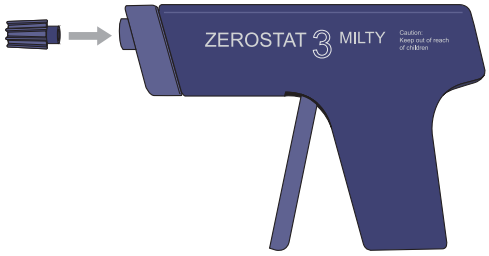
6. Do one of the following:
   - If the tissue does not cling to the plastic, proceed with the protocol.
   - If the tissue clings to the plastic, then repeat step 2 through step 5. If the tissue continues to cling to the plastic, test the device using the ion-indicator cap to determine if the unit is still releasing ions. Otherwise, consider replacing the unit.

Ion-indicator cap

The GeneTitan™ ZeroStat AntiStatic Gun includes an ion-indicator cap. The cap is a device that is used to test the release of ions when the antistatic gun is in use.

Test the antistatic gun for ion release

1. Insert the ion-indicator cap into the nose of the GeneTitan™ ZeroStat AntiStatic Gun.



IMPORTANT! Do not leave the ion-indicator cap on the antistatic gun when deionizing trays and covers.

2. Slowly squeeze the release trigger, then observe the discharge through the viewing window on the ion-indicator cap.

   A visible light is observed in the viewing window on the cap when charged ions are discharged.

3. If you cannot see a light through the viewing window, replace the antistatic gun as it is unusable.

   Each GeneTitan™ ZeroStat AntiStatic Gun produces approximately 50,000 trigger operations, which are sufficient for approximately 200-250 runs on the GeneTitan™ MC Instrument.

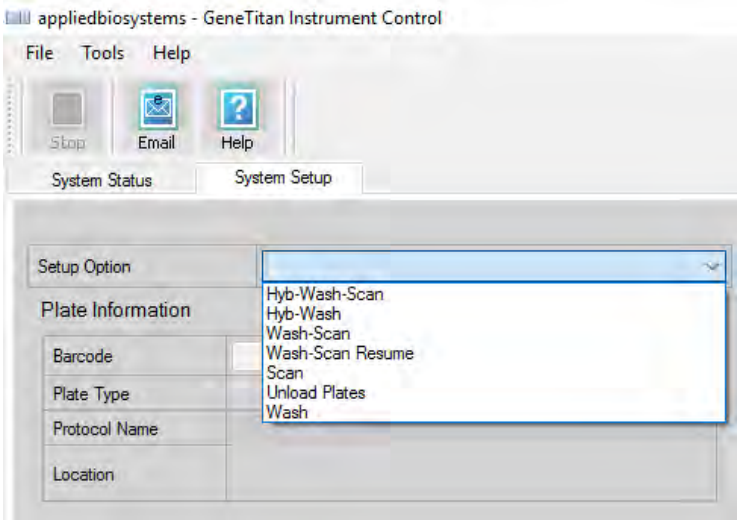
4. If you can see a light through the viewing window, then have determined that the gun is functional, remove the cap from the gun before deionizing a tray or cover.

Setup options for array plate processing

There are 3 steps performed by the GeneTitan™ MC Instrument for array plate processing:

- Hybridization
- Wash and Stain
- Imaging (Scan)

The GeneChip™ Command Console™ software provides options to perform all these steps, or only some of the steps. This section describes the **System Setup** options.



Hyb-Wash-Scan

The **Hyb-Wash-Scan** setup option enables you to hybridize, wash-ligate-stain-stabilize, and scan an array plate on the GeneTitan™ MC Instrument.

- **Hyb**: The array plate is moved to the hybridization oven inside the instrument. Each denatured sample in the hybridization tray is hybridized to an array on the array plate.
  - Time that is required for 96 samples = 23.5 hours
- **Wash**: Samples on arrays are ligated, washed, stained, and stabilized.
  - Time that is required for 96 samples = ~5 hours
- **Scan**: The array plate is moved to the imaging device in the GeneTitan™ MC Instrument and each array is scanned.
  - Time that is required for 96 samples = ~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.

Wash-Scan Resume

Use the **Wash-Scan Resume** option if fluidics processing has been interrupted (for example, a power failure at your facility). This allows you to resume an interrupted workflow at any point in the **Wash** stage.

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

The step at which the run was aborted is identified by:

- Viewing the **System Status** window if you are aborting the last plate through the fluidics system.
- Starting the **Resume** process.

Select **Wash/Scan Resume** from the **System Setup** tab, then follow the prompts to unload and reload all drawers.

The trays are loaded. It is up to you to determine whether to load fresh reagents or reuse the trays already in the GeneTitan™ Multi-Channel (MC) Instrument. Base your decision on the step where the problem occurred.

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

- Load new stain trays with fresh reagents.
- Load a new scan tray.

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

Scan

Use the scan option:

- To rescan an entire array plate or specific arrays on a plate that failed to scan for reasons such as bubbles or gridding failure.
- If you have hybridized and performed the fluidics processes on a different GeneTitan™ MC Instrument than the one currently being used for the scan, or at a different time.
- To queue a second plate for scanning. Using the scan option allows you to start a second scan workflow although another scan workflow is already running.

Unload Plates

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

Wash

The **Wash** workflow enables you to bypass the scan step, performing only wash and stain.

Note: When the **Wash** option is selected, allow 25–30 minutes to warm up the Wash B.

IMPORTANT! After the **Wash** workflow is complete, scan the array plate as soon as possible. Array plate data can be affected when the plate is not scanned immediately after washing.

Load an array plate and hybridization tray into the GeneTitan™ MC Instrument (for Hyb-Wash-Scan or Hyb-Wash)

1. When drawer 6 opens, load the array plate and hybridization tray in the following manner:
   a. Examine the wells of the hybridization tray for bubbles, then puncture any bubbles with a 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 140.

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

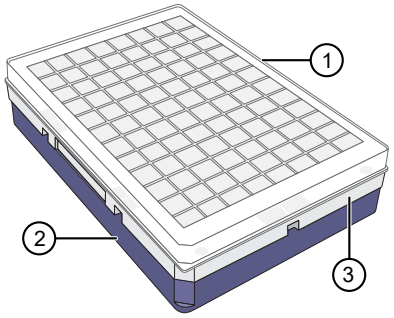


Figure 35 Array plate components, as shipped.

1. Clear shipping cover to be discarded.
2. Array plate protective base.
3. Array plate.

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

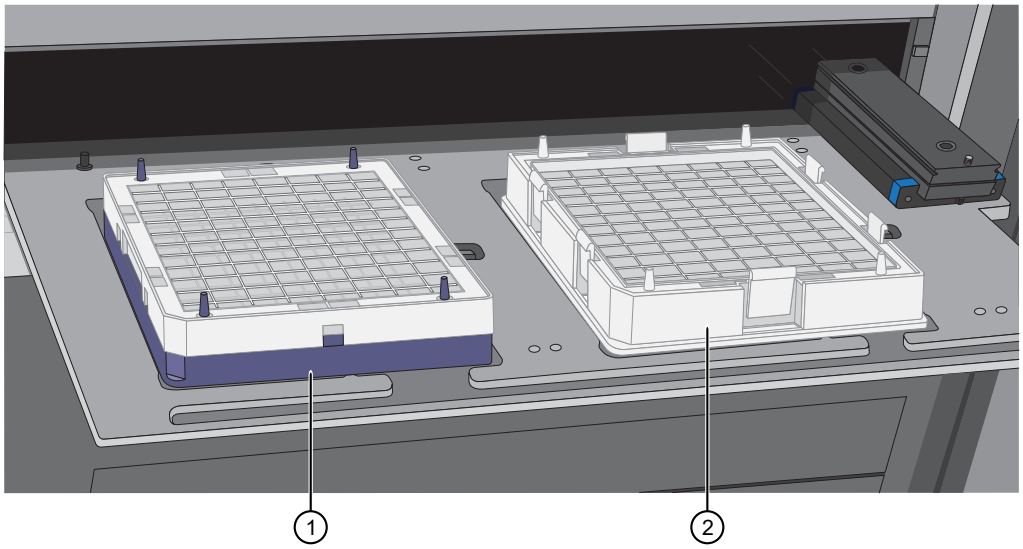


Figure 36 Array plate on protective blue base and the hybridization tray properly loaded into drawer 6.

① Array plate on protective base.
② Hybridization tray.

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

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

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

e. Press the 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 clamping the array plate onto the hybridization tray. A **Clamping in Progress** dialog appears.

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

5. Verify the plate clamping step to ensure that the array plate is securely fastened to the hybridization tray. Using your thumbs, press the array plate downward following the positions that are specified in Figure 37. *No clicking sound indicates proper clamping.*

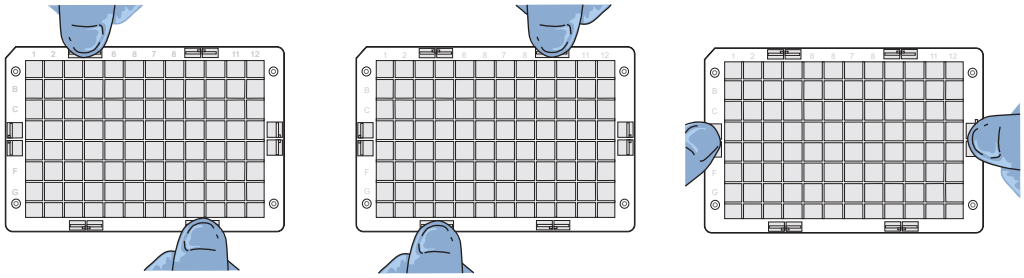


Figure 37 Clamping verification steps.

6. Inspect the array plate for bubbles.
   a. **Keeping the plate stack level**, inspect the bottom for bubbles under the arrays—*do not* tilt or invert the plates.
   b. If bubbles are present, gently tap the plate until the bubbles move out from under the arrays—*do not* unclamp the plate stack.

7. Return the plate stack to the drawer with the notched corner facing you, then press the blue confirmation button on the GeneTitan™ Instrument to proceed.

8. 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.

9. Continue to “Load a second array plate and hybridization tray” on page 106.

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 three minutes. The status window displays “AbortRequested”, 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 to remove the aborted plate or plates.
- 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.

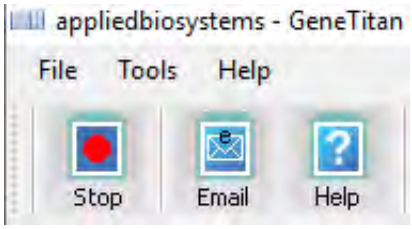
The operator can initiate an abort on one plate and the gripper can continue to process other plates in the instrument.

How to abort a process

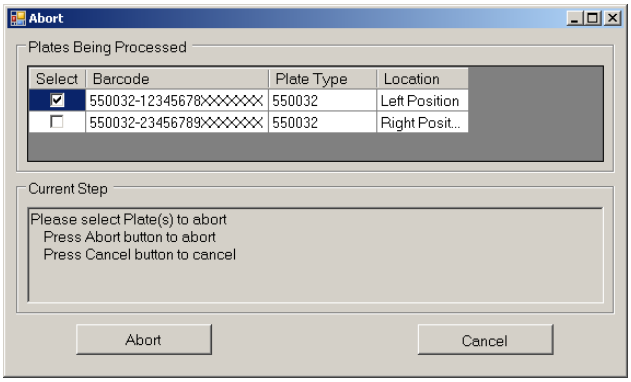
Use the following procedure to abort a process underway in the GeneTitan™ MC Instrument.

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**

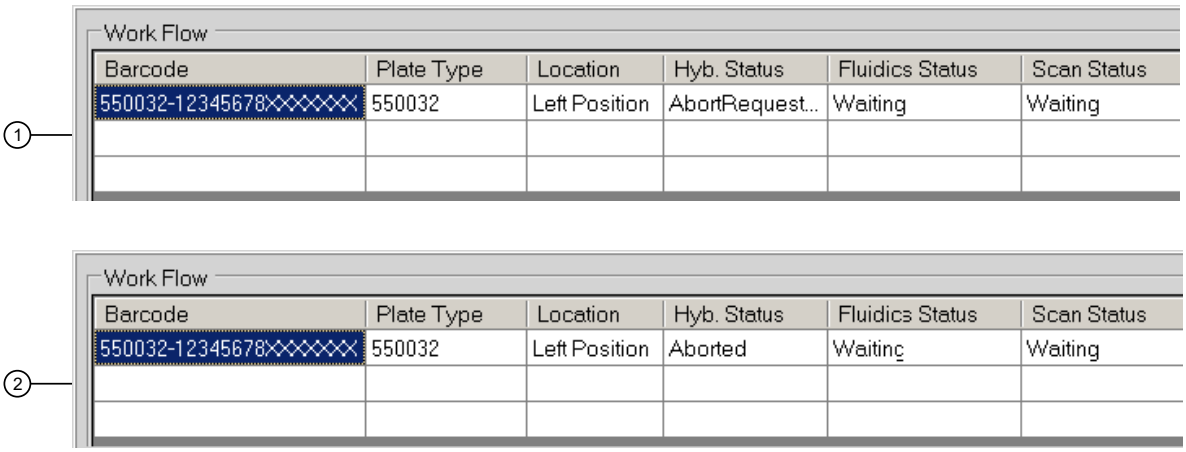


3. When a confirmation dialog opens, click **Yes**.

4. Wait until the status of the array plate in the **WorkFlow** pane in the **Instrument Control** display changes from “AbortRequested” 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 “AbortRequested” state until the gripper becomes available.



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.

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.

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* (Pub. No. 702569).

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* (Pub. No. 08-0308).

See the user guide for the Lambda LS and Smart controller system. Never manually switch the lamp and the controller on or off. The GeneTitan™ Instrument control software manages the lamp activity and switches the lamp on and off as required. It takes 10 minutes to warm up the lamp. In idle mode, the lamp remains on for 2 hours before it is automatically switched off if there are no more plates

being transferred from the fluidics to the imaging station. This automatic switching is by design and is intended behavior. Do not try to save the lamp life by powering off the switch on the lamp.

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

B Register samples in GeneChip™ Command Console™

GeneTitan™ Array Plate Registration file

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

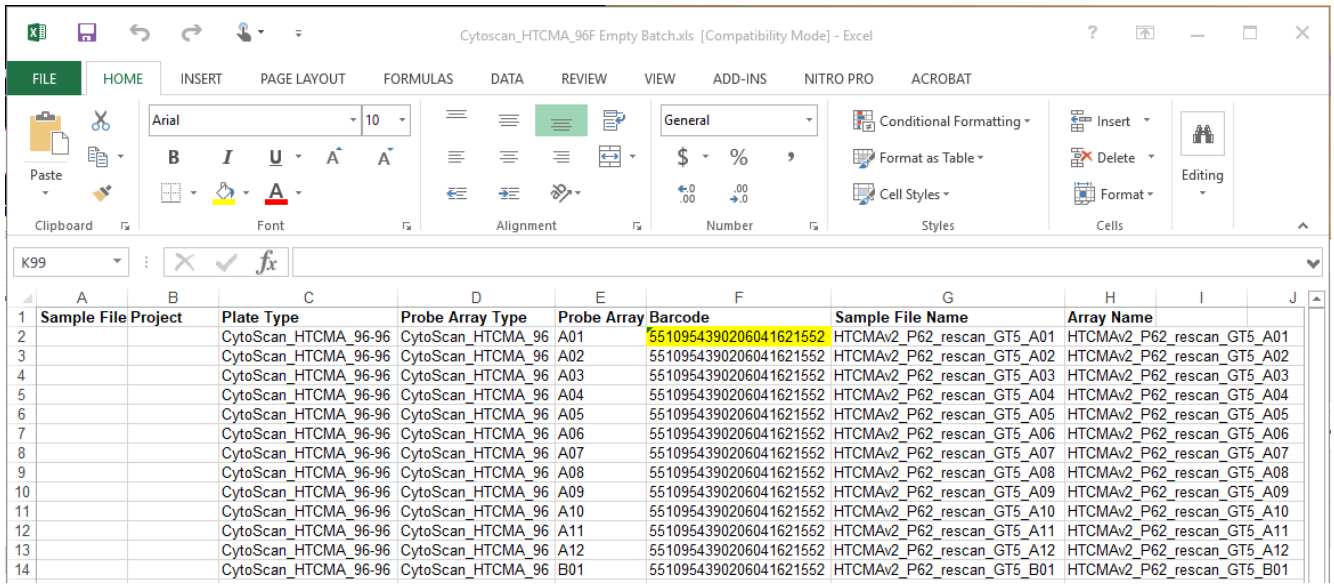
Note: The GeneTitan™ Array Plate Registration file uses the *.xls Microsoft™ Excel™ file extension. Do not use the*.xlsx file extension.

Create a GeneTitan™ Array Plate Registration file

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

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

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



	A	B	C	D	E	F	G	H	I	J
1	Sample File	Project	Plate Type	Probe Array Type	Probe Array	Barcode	Sample File Name	Array Name		
2			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A01	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A01	HTCMAv2_P62_rescan_GT5_A01		
3			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A02	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A02	HTCMAv2_P62_rescan_GT5_A02		
4			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A03	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A03	HTCMAv2_P62_rescan_GT5_A03		
5			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A04	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A04	HTCMAv2_P62_rescan_GT5_A04		
6			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A05	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A05	HTCMAv2_P62_rescan_GT5_A05		
7			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A06	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A06	HTCMAv2_P62_rescan_GT5_A06		
8			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A07	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A07	HTCMAv2_P62_rescan_GT5_A07		
9			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A08	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A08	HTCMAv2_P62_rescan_GT5_A08		
10			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A09	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A09	HTCMAv2_P62_rescan_GT5_A09		
11			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A10	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A10	HTCMAv2_P62_rescan_GT5_A10		
12			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A11	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A11	HTCMAv2_P62_rescan_GT5_A11		
13			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	A12	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_A12	HTCMAv2_P62_rescan_GT5_A12		
14			CytoScan_HTCMA_96-96	CytoScan_HTCMA_96	B01	551095439020604162155 2	HTCMAv2_P62_rescan_GT5_B01	HTCMAv2_P62_rescan_GT5_B01		

9. Enter a unique name for each sample in the **Sample File Name** column and any additional information, such as array plate barcode.

Note: The array plate's barcode can be scanned into the **Barcode** field. The barcode is stored in the sample file for each array.

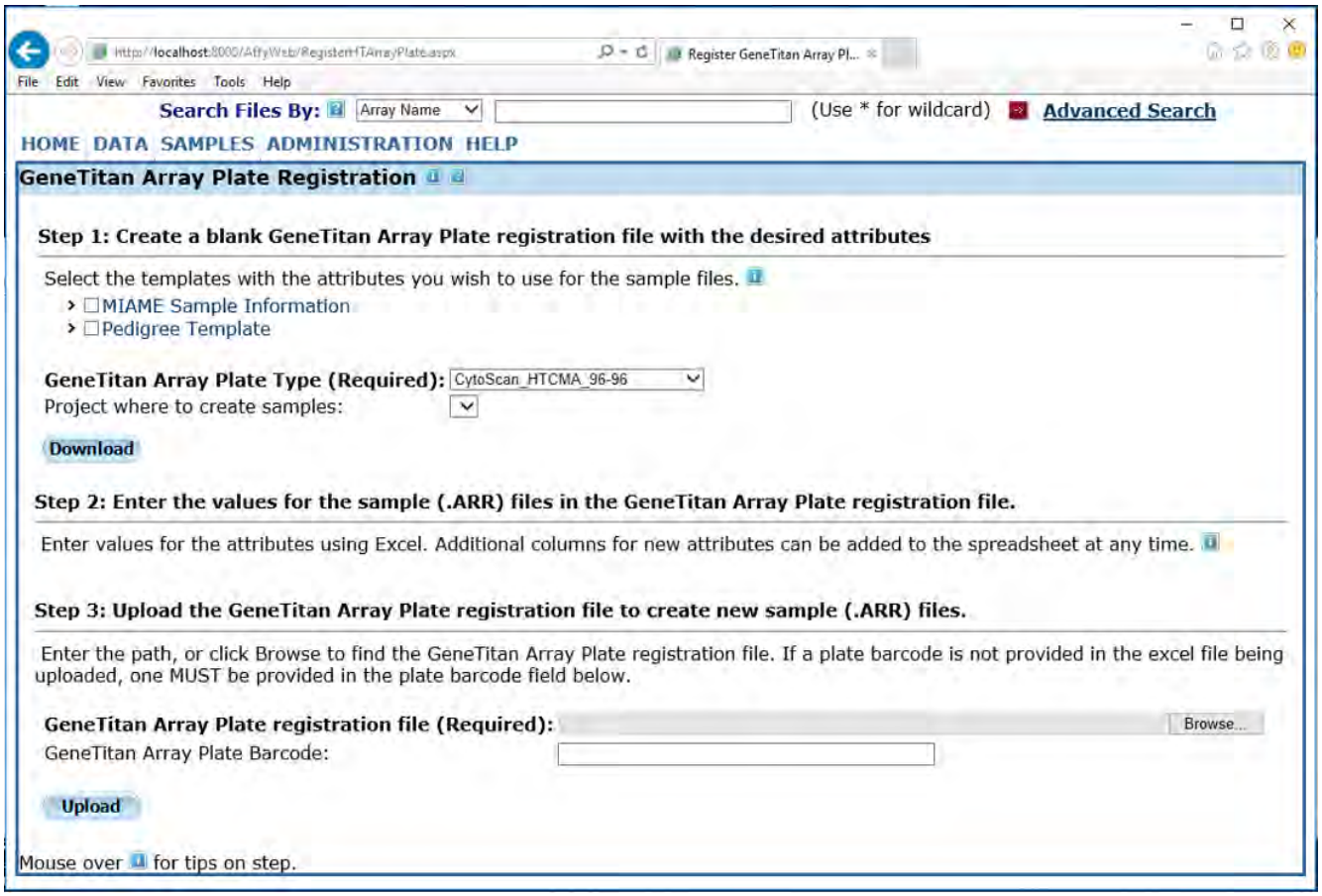
---

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

11. Follow these steps to save the file:
    a. Click **File** ▸ **Save As**.
    b. Enter a name for the array plate registration file.
    c. Click **Save**.

12. Follow these steps when you are ready to load the array plate onto the GeneTitan™ MC Instrument.
    a. Click **Browse**, navigate to the GeneTitan™ Array Plate Registration file, then click **Open**.
    b. Scan the array plate barcode, if it has not already been scanned, and save the registration file.

c. Click **Upload**, wait for the information to load, then click **Save** found at the bottom of the next window that is displayed.



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

Item	Source
Mother E-Base™ Device	EBM03
Daughter E-Base™ Device	EBD03
E-Gel™ *48* Agarose Gels, 4%	G800804
25 bp DNA Ladder, or a similar product prepared as instructed by the manufacturer	931343

(continued)

Item	Source
TrackIt™ Cyan/Orange Loading Buffer	10482028
Nuclease-free water	MLS

Consumables required

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

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

Prepare the gel diluent

Dilute the TrackIt™ Cyan/Orange Loading Buffer

A 100-fold dilution of the TrackIt™ Cyan/Orange Loading Buffer can be used in “Stage 3C: Perform quantification and fragmentation QC checks” on page 66.

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.

Dilute the 25 bp DNA ladder

Follow the product instructions for dilution method.

Run the fragmentation QC gel

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

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

1. Tightly seal the Gel QC Plate that is prepared during automated target preparation.
2. Vortex the center of the plate for 3 seconds. Centrifuge at 1,000 rpm for 30 seconds to get the droplets down.
3. Connect an E-Base™ device to an electrical outlet.
4. Push the **Power/Prg** button on each to ensure the gel base is in **EG** mode, not EP mode.
5. Take the gel out of the pouch, then remove the combs.
6. Place the E-Gel™ *48* Agarose Gel into the base unit.
7. Load 20 µL from each well of the Gel QC Plate onto the gel.
8. Load 25 bp DNA Ladder into the marker wells (M).
9. Load 20 µL nuclease-free water into any unused wells.
10. Run the gels for 22 minutes.
11. Capture a gel image.

Fragmentation QC gel images should look similar to the gel shown in Figure 38.

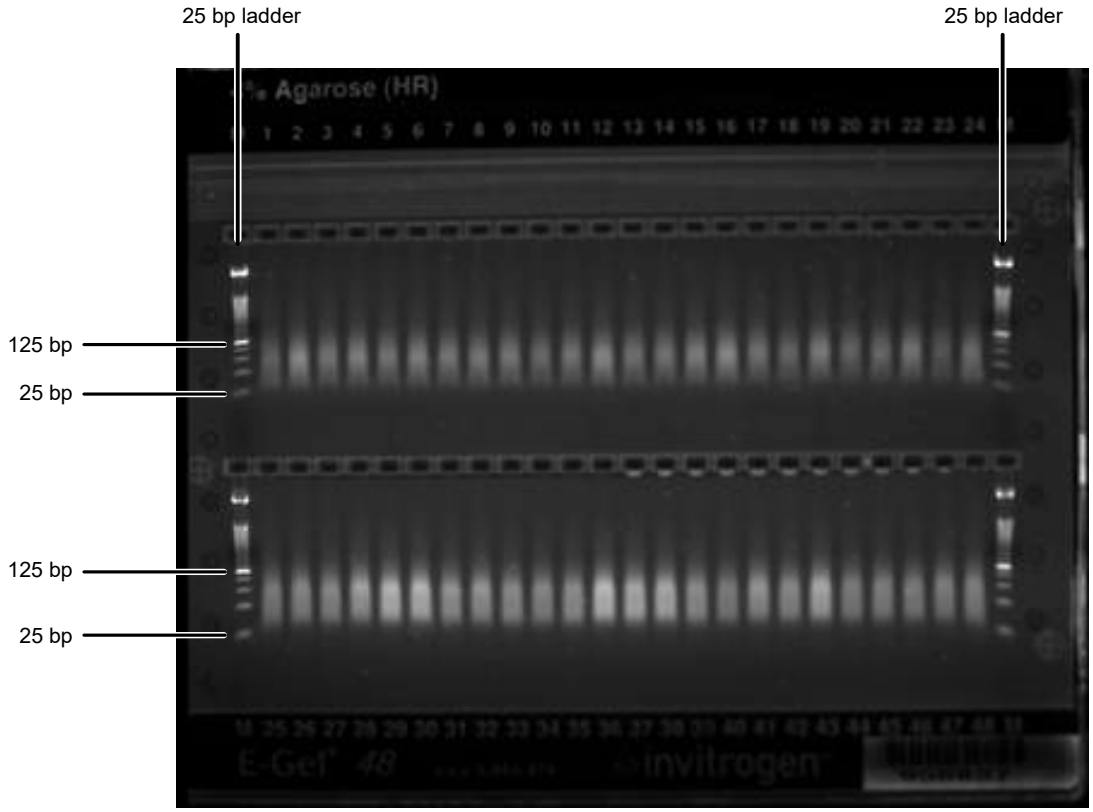


Figure 38 Fragments fall between 125 bp and 25 bp on a successful gel image.

D

Sample quantification after resuspension

Equipment required

Spectrophotometer

We recommend that you use one of the following spectrophotometers, or the equivalent.

Item	Source
Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer	51119600
SpectraMax® Plus 384 Microplate Reader	Molecular Devices® PLUS 384
DTX 880 Multimode Detector with genomic filter slide	Beckman Coulter™ Detector 987921 Filter slide A30184

Quantify the diluted samples

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

For OD quantification, readings must be taken at wavelengths of 260 nm, 280 nm, and 320 nm. See “Plate reader guidelines for sample quantification” on page 170.

OD yield evaluation guidelines

The measurement of the yield of DNA after resuspension of the pellets is an important QC checkpoint in the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow. If the median yield for the plate is <1,000 µg DNA per sample:

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

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

Plate reader guidelines for sample quantification

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

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

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

Where:

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

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

The optical path length depends on the type of plate and can depend on the spectrophotometer used. Check the recommendations for the path length for your instrument and plate type or for recommendations on how to measure this quantity. The SpectraMax® Plus 384 Microplate Reader can use an automated path length detection system. Consult the SpectraMax® Plus 384 Microplate Reader user guide for more information.

E Troubleshooting

GeneTitan™ 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.

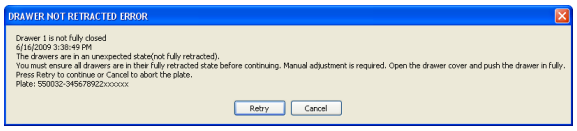
GeneTitan™ MC Instrument

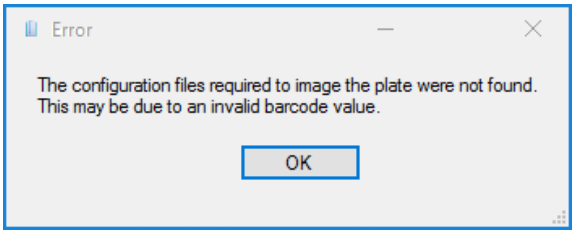
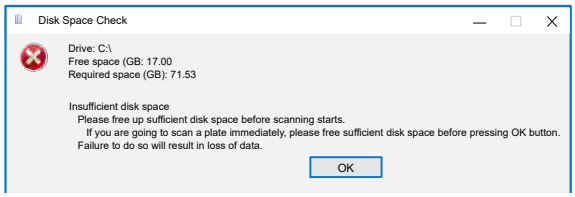
This section provides instructions on how to identify and solve simple problems with the GeneTitan™ MC Instrument. If a problem or error occurs that is not listed in this chapter, contact Thermo Fisher Scientific Technical Support for help.

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

Observation	Possible cause	Recommended action
Plate trapped in the GeneTitan™ MC Instrument	• Plate (or plate with lid) not properly loaded in drawer. • Notched edge of lid and plate not aligned. • Gripper failed to retrieve plate. • System requires adjustment.	1. Restart the GeneTitan™ MC Instrument by unplugging and reconnecting power cord. 2. Run the **Unload Plates** setup option. 3. If the plate remains trapped in the instrument, call Thermo Fisher Scientific support.
Computer frozen	• Too many processes running. • Attempting to transfer data while an array plate is being scanned (imaged).	Restart the computer and unload all of the plates. • Plates in the hybridization station: finish hybridization off line. • Plate in the scanner: rescan using **Scan Only** function. • Plate in fluidics: use **Wash/Scan Resume** to resume the fluidics process. **IMPORTANT!** Do not manually, or through the GCC transfer utility, move any data associated with the current plate that is being processed/scanned.

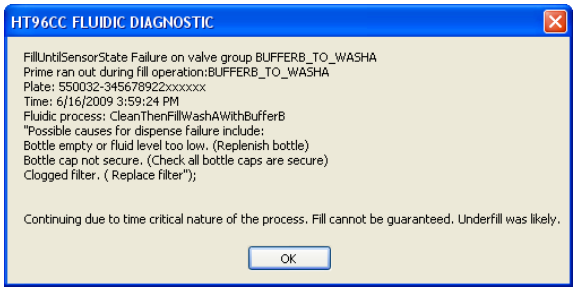
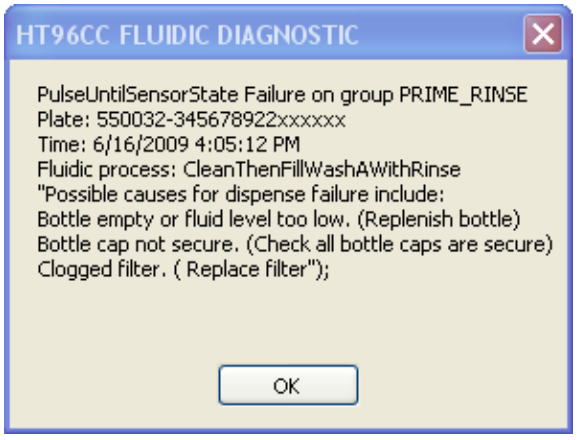
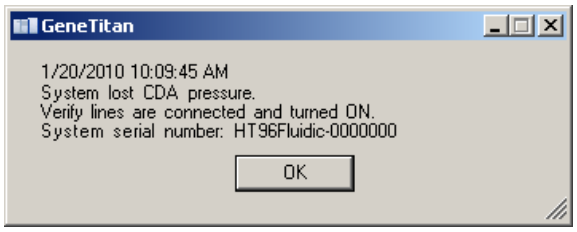
Observation	Possible cause	Recommended action
Hybridization aborted	• System-initiated abort: power loss. • User-initiated abort: – User error – Other	If the array plate and hybridization tray are still clamped, contact your local field service engineer with information on the workstation model.
		If the plate stack is moved to drawer 1: 1. Remove the plate stack and finish hybridization offline. 2. Return the hybridized array plate stack to the GeneTitan™ MC Instrument and finish processing using the **Wash/Scan** process.
Fluidics aborted	• System-initiated abort: power loss. • User-initiated abort: incorrect protocol selected.	Follow the recommendations and instructions under "Wash-Scan Resume" on page 152.

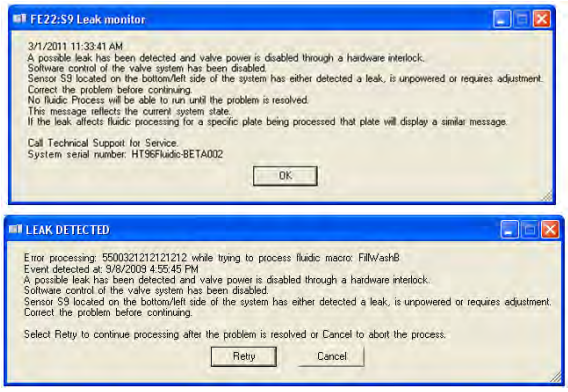
Observation	Possible cause	Recommended action
Homing recovery of gripped item 	Indicates that an item is in the gripper, and normal startup of the GeneTitan™ MC Instrument is not possible. The item must be removed from the instrument before you can start processing array plates.	Recommendation: click **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 Hyb Tray and Array Plate Drawer(n)L/R_Hta_DOWN where n is the drawer number and L or R to indicate the left or right side. The _Hta_ (second term) indicates that the item held. An example is drawer1R_HtaHyb_DOWN indicating it is an array plate with a hybridization tray or Drawer2L_ScanHta_Pk_DOWN indicating it is an array plate with a scan tray
Drawer not retracted error 	The drawer that is listed in the message is not fully closed.	Manually push the drawer back into the instrument until it is fully closed. There are 2 stop positions with audible clicks. Push until you hear the second click and the drawer is fully seated.

Observation	Possible cause	Recommended action
Array registration error message 	The protocol file for the array plate barcode could not be found.	Check that the array plate barcode has been entered correctly. Ensure that the library files required for the type of array plate you are using have been installed, and are installed in the correct directory. Restart the GeneTitan™ Instrument control software after library files have been installed.
Insufficient disk space notice  This message appears when you first initialize the software and instrument, or when you select arrays for imaging.	There is not sufficient memory on the computer hard drive to save the data from an array plate.	Free up sufficient disk space before starting imaging with the GeneTitan™ MC Instrument.

GeneTitan™ 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 Wash Buffer A and Water are above the 50% mark when setting up the system to process an array plate. We recommend that all 600 mL of the Wash Buffer B from the HT Target Prep Reagent Kit 96F be emptied into the GeneTitan™ Wash B bottle when setting up the system to process a plate. Using all 600 mL of the Wash Buffer B ensures that the bottle is filled to more than the requisite 35% of Wash B bottle volume.

Observation	Possible cause	Recommended action
Fluidics diagnostic dispense error  BUFFER*x* = Buffer bottle A, B, or Rinse. WASH*x* = Wash A or B reservoir in the fluidics station.  	Reagent bottle is empty or too low.	Replenish fluid level in the Rinse or Wash Bottle B to the 1-L mark. Do not overfill. **IMPORTANT!** Only replenish bottles when prompted by the UI. Replenishing during fluidic processing can cause system malfunction including overflowing inside the system and more problems. The only thing to do when a plate is running is to ensure that bottle caps are secure.
		Replenish fluid level in Wash Bottle A to 2 L.
	GeneTitan™ reagent bottle cap is loose.	Fasten the bottle cap.
	The GeneTitan™ reagent bottle filter is clogged.	Replace the filter. See “Bottle filter replacement” on page 181.
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* (Pub. No. 08-0305) for the portable compressor model that has been verified with the GeneTitan™ MC Instrument.
		Contact your local field application specialist and notify the engineer about the error message.

Observation	Possible cause	Recommended action
Leak detected Leak checks are performed at application startup and any time a fluidic process, such as priming, filling, draining, is performed. Leak detection is a hard-wired sensor that shuts off fluid flow without software control. Leaks are normally confined to the drip pan found inside the system. 	• System malfunction. • The GCC application being manually closed using Windows™ Task Manager during a fill operation resulting in an application exit without stopping flow.	Contact Thermo Fisher Scientific support. The system cannot be used for fluidic processing until the problem is resolved.
Filter change required error message The software displays warning messages for the filter in each reagent bottle when it detects a problem or shows a trend of increased fill times during fluid fill operations. When an error is detected, a message box is displayed along with the information on the specific operation (dispense-related check or fill-related check).	One or more reagent bottle filters are clogged or worn out.	Change all 3 reagent bottle filters, even if only 1 is reported as problematic. See “Bottle filter replacement” on page 181.

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 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 39 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.

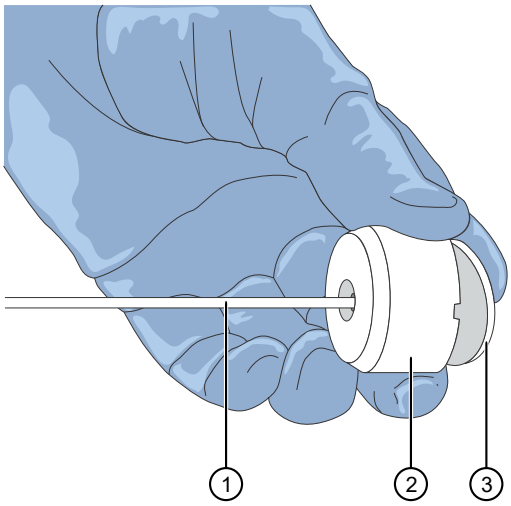


Figure 40 Components of the reagent buffer supply line and filter.

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 40).

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 184, and “Replace the xenon lamp” on page 185. After changing the lamp, you must manually reset the lamp life clock.

Remove the xenon lamp

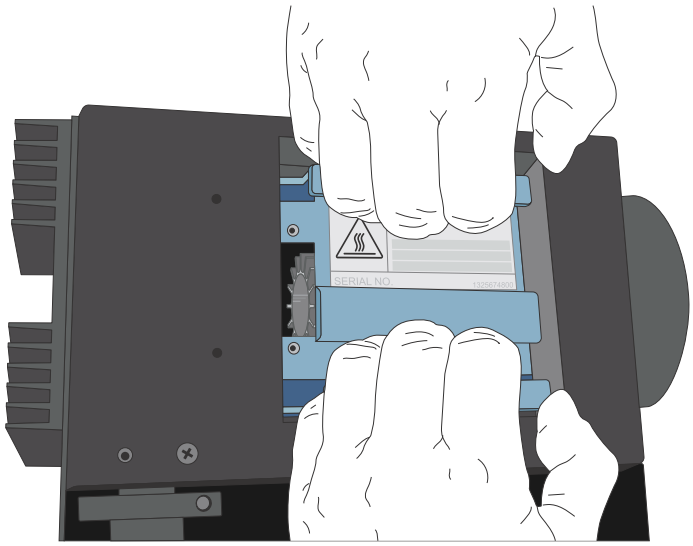
WARNING! Power off the lamp using the switch in the rear of the unit and then disconnect the power cord. Allow to cool before attempting to replace the lamp.

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.

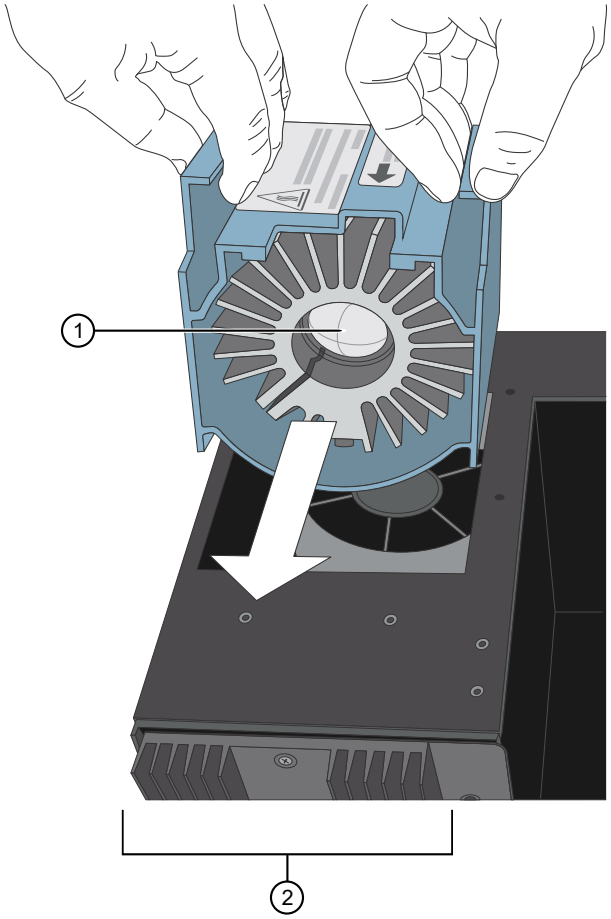


Replace the xenon lamp

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.



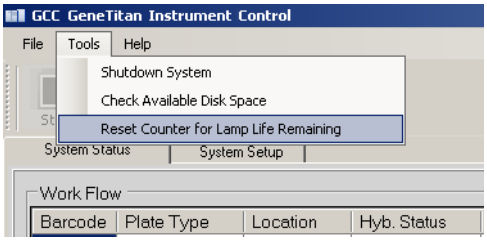
① Xenon bulb faces away from the fan and towards the heat sink.
② Heat sink on the Lambda LS unit.

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, see the “Documentation and Support” section in this document.

Chemical safety

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

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

AVERTISSEMENT ! **PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES.** Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

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

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

Biological hazard safety

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

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

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

Documentation and support

Related documentation

Document	Publication number	Description
CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Site Preparation Guide	MAN0018215	Provides guidance on reagents, instruments, and supplies required to run the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow.
CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow Quick Reference	MAN0018216	An abbreviated reference for the target preparation step of the CytoScan™ HT-CMA Assay 96-Array Format Manual Workflow. This quick reference document is for experienced users.
GeneTitan™ MC Protocol for Axiom™ Array Plate Processing Quick Reference	MAN0017718	An abbreviated reference for processing array plates with the GeneTitan™ Multi-Channel Instrument.
GeneTitan™ Multi-Channel Instrument User Guide	08-0308	The GeneTitan™ Multi-Channel Instrument automates array processing from target hybridization to data generation by combining a hybridization oven, fluidics processing, and state-of-the art imaging device into a single benchtop instrument. This document details the use, care, and maintenance for the GeneTitan™ Multi-Channel Instrument.
GeneTitan™ Multi-Channel Instrument Site Preparation Guide	08-0305	Provides guidance on creating and maintaining the proper environment required for the GeneTitan™ Multi-Channel Instrument.
Analysis and software		
Reproductive Health Research Analysis Software User Guide	703517	This user guide provides instructions on using the Reproductive Health Research Analysis Software (RHAS) to analyzes CEL files and generate result CHP files. Cytogenetic and variant analysis enables visualization and summarization of chromosomal aberrations and genotyping of specified variants, as well as SMN analyzing for up to 500 samples at a time. **Note:** This user guide includes the documentation for the Multi Sample Viewer Software.

(continued)

Document	Publication number	Description
Chromosome Analysis Suite User Guide	702943	This user guide provides instructions on using the Chromosome Analysis Suite Software (ChAS) for in-depth CN result exploration. The software enables cytogenetic analysis to view and summarize chromosomal aberrations, including copy number gain or loss, loss of heterozygosity segments, or variant data, across the genome.
GeneChip™ Command Console™ User Guide	702569	This user guide provides instructions on using GeneChip™ Command Console™ (GCC) used to control GeneChip™ instrument systems. GeneChip™ Command Console™ software provides an intuitive set of tools for instrument control and data management used in the processing of GeneChip™ arrays.

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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