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Oncomine™ Precision Assay GX

USER GUIDE

For use with the Genexus™ Integrated Sequencer

Catalog Number A46291

Publication Number MAN0018508

Revision K

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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

Revision history: MAN0018508 K (English)

Revision	Date	Description
K	1 October 2025	• Updated the detection of hotspot mutations feature description to include cfTNA assay workflow information. See "Product description" on page 8. • Updated required materials to include Genexus™ Primer Pool Tube Holder. See "Genexus™ Primer Pool Tube Holder" on page 14. • Updated sequencing instructions to include the use of the Genexus™ Primer Pool Tube Holder. See "Load the sequencer and start a run" on page 113 and "Clear the instrument deck and perform a UV Clean" on page 122. • Updated troubleshooting for Genexus™ Primer Pool Tube Holder. See "Genexus™ Integrated Sequencer—general and QC troubleshooting" on page 164. • Added information for cleaning the Genexus™ Primer Pool Tube Holder. See "Clean the Genexus™ Primer Pool Tube Holder" on page 181.
J	7 May 2024	• Added AcroMetrix™ Multi-Analyte ctDNA Plasma Controls. See "Recommended sample controls" on page 21. • Added DNA*Zap*™ PCR DNA Degradation Solutions, RNase*Zap*™ RNase Decontamination Solution, and RNase *AWAY*™ Decontamination Reagent. See "General laboratory supplies and reagents" on page 15. • Added guidelines for FFPE samples. See "Guidelines for FFPE samples" on page 24. • Added guidelines for RNA. See "Guidelines for RNA" on page 24. • Added guidelines for panel and reagent use and handling. See "Guidelines for panel and reagent use and handling" on page 24. • Added updated guidelines for preventing contamination. See "Guidelines for preventing contamination" on page 25. • Added reagent strip and panel tube fill volume guide. See "Genexus™ reagent strip and panel tube fill volume guide" on page 174. • Updated chip capacity per sequencing run. See "Chip capacity by sample type" on page 98. • Updated chip capacity by sample type. See "Load the sample plate—Sample to Result run" on page 104. • Added troubleshooting information to Genexus™ Integrated Sequencer section. See "Sample QC passes, but CF-1 Mean AQ20 Read Length QC fails" on page 167. • Updated example representative metrics for sequencing runs. See "Example metrics for a successful sequencing run" on page 179.
H.0	20 October 2023	• Updated "Review data and results" chapter (page 126). • Correction to the chip capacity information provided (page 98). • Added information about resequencing.
G.0	7 July 2023	Updates for Genexus™ Software 6.8.

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

Contents

1 Product information

Product description

The Oncomine™ Precision Assay GX (Cat. No. A46291) enables simultaneous detection of biomarkers across 50 genes, including key targets within EGFR, BRAF, KRAS, ALK, ROS1, NTRK, RET, HER2, and others, from solid tissue and liquid biopsy samples. The Oncomine™ Precision Assay GX uses a synchronous approach for detecting prevalent fusion isoforms and potential novel fusions. The following types of gene fusions can be detected using FusionSync™ Detection Technology.

- Targeted designs for known fusion isoforms, including prevalent isoforms such as EML4-ALK, ETV6-NTRK3, and KIF5B-RET.
- Novel combinations of any driver and partner genes included in the panel.
- Novel fusion isoforms of key driver genes using the exon tiling imbalance method.

Each kit provides the targeted pan-cancer panel and library reagents sufficient to perform up to 32 sequencing reactions on the Genexus™ Integrated Sequencer, using the GX5™ Chip (see “Contents and storage” on page 10). Additional Genexus™ Integrated Sequencer reagents and supplies must be ordered separately (see “Required materials not supplied for use with the Genexus™ Integrated Sequencer” on page 12).

This user guide provides the following instructions.

- How to use the Oncomine™ Precision Assay GX to perform a **Sample to Result** next-generation sequencing (NGS) run on the Ion Torrent™ Genexus™ Purification System for nucleic acid isolation, followed by sequencing on the Genexus™ Integrated Sequencer.
- How to use the Oncomine™ Precision Assay GX to perform a **Nucleic Acid to Result** next-generation sequencing (NGS) run on the Genexus™ Integrated Sequencer.
- How to perform data analysis of the sequencing run.

IMPORTANT! When using the Oncomine™ Precision Assay GX, genomic coverage of the assay does not ensure that all variants present in those genomic regions will be detected by the assay.

The Genexus™ Purification System automates the extraction and quantification of nucleic acids from various tissue types for use on the Genexus™ Integrated Sequencer. With a single touch point and 10 minutes of hands-on time, the Genexus™ Purification System can extract both DNA and RNA sequentially from FFPE lysates (Ion Torrent™ Genexus™ FFPE DNA and RNA Purification Kit) and

from cell-free plasma from whole blood samples (Ion Torrent™ Genexus™ Cell-Free Total Nucleic Acid Purification Kit). When connected to the Genexus™ Integrated Sequencer, the Ion Torrent™ Genexus™ Software streamlines the NGS workflow by integrating the sample-to-result workflow within a single software system.

The Oncomine™ Precision Assay GX includes the following features.

- Compatibility with both FFPE tissue and plasma sample types
- Compatibility with as little as 20 ng nucleic acid input per pool from cfTNA samples
- Detection of hotspot mutations (substitutions, insertions, and deletions), copy number variations (CNVs), and gene fusions across 50 cancer driver genes in the FFPE assay and cfTNA assay workflows.
- FFPE assay workflow can also report non-hotspot mutations detected in the sample, when using FFPE assay definition file (ADF) w3.4.2. Non-hotspot mutation detection is not enabled for earlier FFPE assay ADF versions or any cfTNA assay ADF versions. For more information see “Oncomine™ Precision Assay GX” on page 171.
- Sensitive and specific low-level variant detection using Ion AmpliSeq™ HD chemistry

Contents and storage

The Oncomine™ Precision Assay GX (Cat. No. A46291) includes the OPA Pool 1 panel, Genexus™ Strip 1, and Genexus™ Strip 2-HD sufficient for up to 32 reactions. The OPA Pool 1 panel is provided in 8 pairs of tubes, where each tube pair contains one tube with forward (FWD) primers and one tube with reverse (REV) primers. If needed, the Genexus™ Library Strips 1 and 2-HD combo kit (Cat. No. A40255) can be ordered separately.

Contents	Carrier color	Amount	Part No.	Storage
OPA Pool 1				
FWD primers (position 1, amber tube)	Magenta	8 × 2 tubes[1], 50 µL/tube	A44350	–30°C to –10°C
REV primers (position 2, natural tube)				
Genexus™ Library Strips 1 and 2-HD (Cat. No. A40255)				
Genexus™ Strip 1[2]	Light red	8 strips	A46812	2°C to 8°C
Genexus™ Strip 2-HD[2]	Violet	8 strips	A46814	–30°C to –10°C

[1] 4 reactions/tube pair
[2] 4 reactions/strip

The maximum number of samples that can be analyzed per kit depends on the assay workflow performed.

Workflow	# of samples
Simultaneous DNA- and RNA-based variant analysis	up to 16 FFPE tissue samples (one DNA reaction and one RNA reaction per sample)
DNA-only or RNA-only variant analysis	up to 32 FFPE tissue samples
Cell-free TNA variant analysis (combined DNA and RNA)	up to 24 plasma samples

Genexus™ Controls

Contact support for information about controls for the Oncomine™ Precision Assay GX.

Workflow overview

Oncomine™ Precision Assay GX workflow

Enter samples (page 34)

Enter samples in the Genexus™ Software to assign sample names and provide other information such as sample collection date, gender, type, and disease category. An assay is assigned to each sample.

Plan a run (page 35 or page 38)

Runs that are planned in Genexus™ Software contain the settings that are used in library preparation, templating, sequencing, and analysis, including sample information and plate location, assays, and barcodes.

Prepare samples

- Genexus™ FFPE DNA and RNA Purification—See Chapter 5, "Genexus™ FFPE DNA and RNA Purification protocol".
- Genexus™ Cell-Free Total Nucleic Acid Purification—See Chapter 6, "Genexus™ Cell-Free Total Nucleic Acid Purification protocol".

Note: You can also prepare samples manually. Proceed to the next step.

Dilute the samples and load the sample plate (page 98)

Note: This step is not required for Sample to Result runs.

Quantify and dilute the nucleic acid samples, then load samples and controls (optional) onto the sample plate.

Load the sequencer and start a run (page 109)

Follow the step-by-step instructions on the sequencer touch screen to load the sample plate and consumables in the Genexus™ Integrated Sequencer.

Review results (page 126)

Review data and results in Genexus™ Software.

2 Reagents, supplies, and required materials

This chapter lists the reagents, supplies, and materials needed to operate the Genexus™ Purification System and Genexus™ Integrated Sequencer, and provides consumables ordering and storage information. Reagents and supplies can be ordered as kits and starter packs, but most consumables can also be ordered individually as your needs require.

Note: Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.

Genexus™ Integrated Sequencer

Components	Cat. No.
Genexus™ Integrated Sequencer	A45727

Required materials not supplied for use with the Genexus™ Integrated Sequencer

Genexus™ Integrated Sequencer reagents and supplies can be ordered as kits and starter packs. In addition, most consumables can also be ordered individually. This section provides information about the various ordering options.

Note: Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.

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. Catalog numbers that appear as links open the web pages for those products.

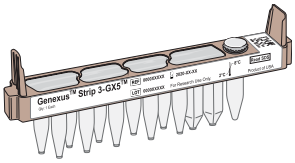
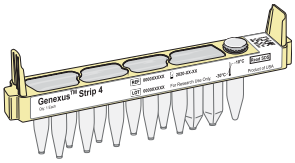
Genexus™ Barcodes 1–32 HD

Ion Torrent™ Genexus™ Barcodes 1–32 HD are supplied in a plate containing 32 dual barcodes.

Item	Label color	Cat. No.	Quantity	Storage
Genexus™ Barcodes 1–32 HD	Purple	A40261	1 plate	15°C to 30°C

Genexus™ Templating Strips 3-GX5™ and 4

Ion Torrent™ Genexus™ Templating Strips 3-GX5™ and 4 (Cat. No. A40263) are ordered as kits with 8 pairs of strips per kit.

Component	Carrier color	Part No.	Quantity per kit	Storage
Genexus™ Strip 3-GX5™ 	Brown	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4 	Yellow	A46816	8 strips	–30°C to –10°C

Genexus™ Pipette Tips

Ion Torrent™ Genexus™ Pipette Tips (Cat. No. A40266) are ordered in packs of 12 racks each. The number of pipette tip racks that are required for your experiment depends on the number of reactions and the sample type. For the Oncomine™ Precision Assay GX, 1 rack is sufficient for up to 6 reactions for FFPE samples or 2 reactions for cfTNA samples.

Item	Cat. No.	Quantity	Storage
Genexus™ Pipette Tips	A40266	12 racks	15°C to 30°C

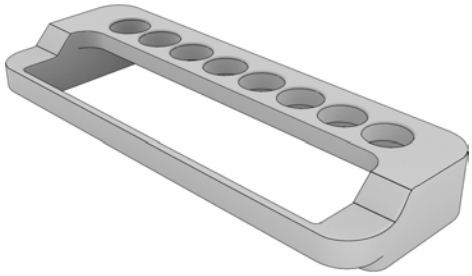
Genexus™ Conical Bottles

Genexus™ Conical Bottles (Cat. No. A40275) are installed in the sequencing reagents bay and serve as reservoirs for nucleotide reagent dilutions. For information on when and how to replace the bottles, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

Component	Quantity	Storage
Genexus™ Conical Bottles	5 bottles	15°C to 30°C

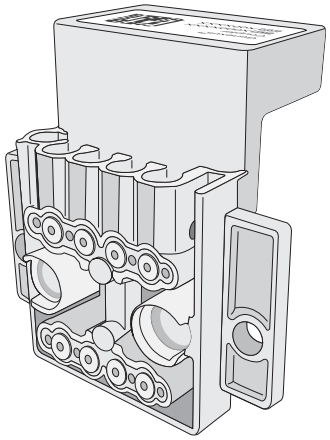
Genexus™ Primer Pool Tube Holder

The Genexus™ Primer Pool Tube Holder (Cat. No. A40004840) is used to help prevent the lifting of Genexus™ Primer Pool Tubes during a sequencing run.

Item	Cat. No.	Quantity
Genexus™ Primer Pool Tube Holder 	A40004840	1

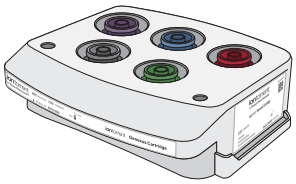
GX5™ Chip and Genexus™ Coupler

The GX5™ Chip and Genexus™ Coupler (Cat. No. A40269) are ordered as a set that contains two chips and two couplers. For the Oncomine™ Precision Assay GX, 1 chip is sufficient for sequencing up to 6 cfTNA samples, 16 FFPE DNA+RNA samples, or 32 FFPE DNA-only or RNA-only samples.

Component	Part No.	Quantity	Storage
GX5™ Chip 	100081364	2 chips	15°C to 30°C
Genexus™ Coupler 	100081252	2 couplers	

Genexus™ Sequencing Kit

The Ion Torrent™ Genexus™ Sequencing Kit (Cat. No. A40271) provides reagents and solutions sufficient to sequence up to 2 full chips.

Component	Part No.	Quantity	Storage
Genexus™ Cartridge 	A40272	2 cartridges	–30°C to –10°C
Genexus™ Bottle 2	A40273	4 bottles	15°C to 30°C
Genexus™ Bottles 1 and 3	A40274	2 bottles each (4 bottles total)	

General laboratory supplies and reagents

Item	Source
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	4483352, 4483354
Thermo Scientific™ Adhesive PCR Plate Foils	AB0626
Microcentrifuge[1]	**MLS**
2-, 20-, 200-, and 1,000-µL pipettes and appropriate filtered tips	**MLS**
Nuclease-free microcentrifuge tubes, 1.5-mL or 1.7-mL	**MLS**
Vortex mixer with a rubber platform	**MLS**
Gloves, powder-free nitrile	**MLS**
Ice buckets and ice	—
Nuclease-free water, molecular biology grade	**MLS**
Isopropyl alcohol, 70% solution	**MLS**
Wipes, disposable lint-free	**MLS**
DNA*Zap*™ PCR DNA Degradation Solutions	AM9890
RNase*Zap*™ RNase Decontamination Solution	AM9780

(continued)

Item	Source
RNase *AWAY*™ Surface Decontaminant	7005-11
(Optional) Uninterruptible Power Supply (UPS)[2]	**MLS**

[1] Must fit standard 0.2- and 1.5-mL microcentrifuge tubes and generate 15,000 × *g*. To convert the RPMs of your centrifuge to RCF in units of gravity, see **tools.thermofisher.com/content/sfs/brochures/TR0040-Centrifuge-speed.pdf**.

[2] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use an uninterruptible power supply that is compatible with 2500 W output or higher.

Genexus™ Purification System

The Genexus™ Purification System (Cat. No. A48148) includes the following components.

Components	Part. No.
Genexus™ Purification System	A47646
Genexus™ Purification System Install Kit	A48549[1]

[1] Not available for separate purchase.

Reagents and supplies for use with the Genexus™ Purification System

Genexus™ Purification System reagents and supplies can be ordered as kits and starter packs, but most consumables can also be ordered individually as your needs require.

Note: Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.

Ion Torrent™ Genexus™ FFPE DNA and RNA Purification Kit

The Ion Torrent™ Genexus™ FFPE DNA and RNA Purification Kit (Cat. No. A45539) includes the following subkits sufficient for 48 sequential DNA and RNA isolations from FFPE curls or slides.

IMPORTANT! Store all kit components in the upright orientation.

Component	Part No.	Storage
Genexus™ FFPE DNA and RNA Purification	A45532	15°C to 30°C
Genexus™ Nucleic Acid Quantitation	A45538	2°C to 8°C
Genexus™ Purification Supplies 2	A45574	15°C to 30°C

Genexus™ FFPE DNA and RNA Purification kit

The Genexus™ FFPE DNA and RNA Purification kit (Part No. A45532) includes sufficient reagents and consumables for 48 sequential DNA and RNA isolations.

IMPORTANT! Store all kit components in the upright orientation.

Component	Quantity	Storage
FFPE DNA and RNA Purification Plate 1	4 plates	15°C to 30°C
FFPE DNA and RNA Purification Plate 2	4 plates	
12-Well Tip Comb	4 each	
DNase (yellow cap)	115 µL	
DNase Buffer (blue cap)	4 × 1.2 mL	
Proteinase K (red cap)	1.2 mL	
FFPE Protease Buffer	15 mL	

Genexus™ Nucleic Acid Quantitation kit

The Genexus™ Nucleic Acid Quantitation kit (Part No. A45538) includes sufficient consumables for 48 DNA and 48 RNA quantitations.

IMPORTANT! Store all kit components in the upright orientation.

Component	Quantity	Storage
Quantitation Plate[1]	4 plates	2°C to 8°C
Quantitation Tube[2]	4 each	15°C to 30°C

[1] Store the Quantitation Plate in the dark to prevent photobleaching of the preloaded reagents.
[2] Can be stored at 15°C to 30°C upon receipt.

Genexus™ Purification Supplies 2 kit

The Genexus™ Purification Supplies 2 kit (Part No. A45574) includes sufficient consumables for 48 isolations.

Component	Quantity	Storage
48-Well Nucleic Acid Archive Plate	4 plates	15°C to 30°C
48-Well Nucleic Acid Archive Plate Seal	4 each	
Purification Tip Cartridge	8 each	

Ion Torrent™ Genexus™ Cell-Free Total Nucleic Acid Purification Kit

The Ion Torrent™ Genexus™ Cell-Free Total Nucleic Acid Purification Kit (Cat. No. A45542) includes the following subkits sufficient for 24 cell-free total nucleic acid (cfTNA) isolations from whole blood or cell-free plasma samples.

IMPORTANT! Store all kit components in the upright orientation.

Component	Part No.	Storage
Genexus™ Cell-Free Total Nucleic Acid Purification	A45535	15°C to 30°C
Genexus™ Nucleic Acid Quantitation	A45538	2°C to 8°C
Genexus™ Purification Supplies 1	A45529	15°C to 30°C

Genexus™ Cell-Free Total Nucleic Acid Purification kit

The Genexus™ Cell-Free Total Nucleic Acid Purification kit (Part No. A45535) includes sufficient reagents and consumables for 24 cell-free total nucleic acid (cfTNA) isolations.

IMPORTANT! Store all kit components in the upright orientation.

Component	Quantity	Storage
Cell-Free Total Nucleic Acid Purification Plate 1	4 plates	15°C to 30°C
Cell-Free Total Nucleic Acid Purification Plate 2	4 plates	
Cell-Free Total Nucleic Acid Purification Plate 3	4 plates	
6-Well Tip Comb	4 each	
12-Well Tip Comb	4 each	
Proteinase K (red cap)	1.2 mL	
cfTNA Lysis/Binding Solution	4 × 85 mL	

Genexus™ Nucleic Acid Quantitation kit

The Genexus™ Nucleic Acid Quantitation kit (Part No. A45538) includes sufficient consumables for 48 DNA and 48 RNA quantitations.

IMPORTANT! Store all kit components in the upright orientation.

Component	Quantity	Storage
Quantitation Plate[1]	4 plates	2°C to 8°C
Quantitation Tube[2]	4 each	15°C to 30°C

[1] Store the Quantitation Plate in the dark to prevent photobleaching of the preloaded reagents.
[2] Can be stored at 15°C to 30°C upon receipt.

Genexus™ Purification Supplies 1 kit

The Genexus™ Purification Supplies 1 kit (Part No. A45529) includes sufficient consumables for 48 isolations.

Component	Quantity	Storage
48-Well Nucleic Acid Archive Plate	4 plates	15°C to 30°C
48-Well Nucleic Acid Archive Plate Seal	4 each	
Purification Tip Cartridge	4 each	

Recommended materials not supplied for use with the Genexus™ Purification System

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

Item	Source
Equipment	
Bench top microcentrifuge	• Cole-Parmer EW-17414-06 • Eppendorf 022620304
1,000 µL Multichannel Pipette	**MLS**

(continued)

Item	Source
For use with Genexus™ FFPE DNA and RNA Purification Kit	
Sorvall™ ST 8 Small Benchtop Centrifuge (or equivalent)[1], with Thermo Scientific™ M10 Microplate Swinging Bucket Rotor (or equivalent)[2], and Sealed Bucket; Capacity: 4 Standard or 2 Midi-Deepwell plates (Set of 2) (or equivalent)	75007200 75005706 75005721
Economy Lab Incubator (2, 60°C and 90°C)	S50441A **fisherscientific.com**
Heating block (2, 60°C and 90°C)	**MLS**
Precision™ General Purpose Water Bath (or equivalent)	**MLS**
Equipment and consumables for AutoLys M FFPE sample extraction[3]	
AutoLys M Tubes and Caps kit	A38738
AutoLys M Tube Rack	A37955
AutoLys M Tube Locking Lid	A37954
AutoLys M TubeLifter or AutoLys M Tube Pliers	A37956 A38261
Tubes, plates, and other consumables	
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	4483354, 4483352
Adhesive PCR Plate Foils	AB0626
RNA*later*™ Stabilization Solution	AM7020
RNase*Zap*™ RNase Decontamination Solution	AM9780
CitriSolv™ Clearing Agent	22-143-975
Xylene	**MLS**
Ethanol, 100%	**MLS**

[1] Centrifuge must achieve an RCF of 2000 × *g*, have a swinging bucket rotor and accommodate deepwell plates.
[2] Swinging bucket rotor must carry deepwell plates in the landscape orientation (see page 48).
[3] For use with the Genexus™ FFPE DNA and RNA Purification Kit.

Recommended materials for nucleic acid isolation, quantification, and quality control

Item	Source
Manual nucleic acid isolation	
MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit	A36716
MagMAX™ FFPE DNA/RNA Ultra Kit	A31881
RNase*Zap*™ RNase Decontamination Solution	AM9780, AM9782, AM9784
Nucleic acid quantification	
Qubit™ 4 Fluorometer[1] and one or more of the following kits: • Qubit™ dsDNA HS Assay Kit *(DNA samples)* • Qubit™ RNA BR Assay Kit *(RNA samples)*	Q33238 Q32851, Q32854 Q10210, Q10211
Nucleic acid quality control	
Agilent™ 2100 Bioanalyzer™ Instrument and one or more of the following kits: • Agilent™ High Sensitivity DNA Kit *(cfTNA/FFPE DNA samples)* • Agilent™ RNA 6000 Nano Kit *(FFPE RNA samples)*	G2939BA 5067-4626 5067-1511 (Agilent)
(FFPE DNA samples only) Agilent™ 4200 TapeStation™ System and one or more of each of the following kits: • Agilent™ D5000 Reagents • Agilent™ D5000 ScreenTape	G2991AA 5067-5589 5067-5588 (Agilent)

[1] Qubit™ 2.0 Fluorometer and later are supported.

Recommended sample controls

Item	Source
FFPE DNA	
Horizon™ Structural Multiplex FFPE Reference Standard	HD789 (Horizon)
EGFR Gene-Specific Multiplex Reference Standard (FFPE) 5% AF	HD300 (Horizon)
KRAS Gene-Specific Multiplex Reference Standard (FFPE)	HD301 (Horizon)
Seraseq™ Lung & Brain CNV Mix, + 3 copies	0710-0414 (SeraCare)
Seraseq™ Breast CNV Mix, + 6 copies	0710-0412 (SeraCare)

(continued)

Item	Source
FFPE RNA	
Seraseq™ FFPE Tumor Fusion RNA v4 Reference Material	0710-0496 (SeraCare)
Seraseq™ FFPE NTRK Fusion RNA Reference Material	0710-1031 (SeraCare)
cfTNA	
Horizon™ Multiplex I cfDNA Reference Standard Set	HD780 (Horizon)
Thermo Scientific™ AcroMetrix™ Multi-Analyte ctDNA Plasma Control A	957563
Thermo Scientific™ AcroMetrix™ Multi-Analyte ctDNA Plasma Control B	957564
Thermo Scientific™ AcroMetrix™ Multi-Analyte ctDNA Plasma Control C	957565
Thermo Scientific™ AcroMetrix™ Multi-Analyte ctDNA Plasma Control D	957566
Thermo Scientific™ AcroMetrix™ Multi-Analyte ctDNA Plasma Control E	957567

3 Before you begin

Procedural requirements

IMPORTANT!

- Do **NOT** combine the contents of OPA Pool 1 tubes. Forward and reverse primers must remain separate, until they are combined by the Genexus™ Integrated Sequencer as part of the on-instrument library preparation workflow.
- Do **NOT** store the OPA Pool 1, Genexus™ Strip 1, and Genexus™ Strip 2-HD on the Genexus™ Integrated Sequencer for more than 24 hours before starting an instrument run.

- Use only the reagents and supplies that have been validated for the Oncomine™ Precision Assay GX. For a list of validated reagents and supplies, see Chapter 2, “Reagents, supplies, and required materials”.
- Use good laboratory practices to minimize cross-contamination of products. Keep all tubes sealed until immediately before loading onto the Genexus™ Integrated Sequencer.
- Use controls to identify or rule out reagent contamination. For a list of validated controls, see “Recommended sample controls” on page 21.
- Do not freeze-thaw the OPA Pool 1. Thaw only the number of panel tubes that are required for a given experiment. Discard unused panel tubes after they are thawed. Store panels at –30°C to –10°C.

Note: One OPA Pool 1 tube pair (one tube with forward primers and one tube with reverse primers) is sufficient for preparing up to 4 libraries.

Guidelines for FFPE samples

- For core needle biopsies and fine needle aspirates, macrodissection is not recommended due to the limiting tissue section surface areas.
- Necrotic samples: Up to 75% necrotic tissue in the region of interest does not appear to interfere with the assay. However, we recommend that you macrodissect highly necrotic areas or select alternate samples if possible.
- Nucleic acid integrity is important for sample performance. Factors such as age of the block, fixation process used, and sample source can impact the quality of the extracted nucleic acid.
- Nucleic acid yield can be impacted by overall tissue area. If an initial extraction leads to insufficient concentrations for DNA and RNA, repeat the extractions with more material whenever possible.
- We recommend that you extract sufficient nucleic acid from samples to enable repeat sequencing runs, if possible. This recommendation also applies to cfTNA from plasma samples.

Guidelines for RNA

- Wear clean gloves and a clean lab coat.
- Change gloves whenever they may be contaminated.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Clean lab benches and equipment (including gloves, tube racks, pipettes, centrifuges, and vortexers) with an RNase decontamination solution before and after use.
- Work in a designated RNase-free pre-PCR area.
- Keep RNA on ice or in a –30°C to –10°C chilled benchtop cold box during use.
- Do not vortex RNA. Flick 4 times to mix, then pulse centrifuge to collect.

Guidelines for panel and reagent use and handling

- Keep OPA Pool 1 panel tubes capped until immediately before loading in the Genexus™ Integrated Sequencer.
- Thaw positive controls on ice or at 4°C for up to 2 hours. After the positive controls are completely thawed, vortex on a platform vortexer, then centrifuge to collect tube contents. Return to ice or 4°C before loading into the sample plate.

  IMPORTANT! Ensure that contents of the control tubes are completely thawed before adding to the sample plate.

- Equilibrate Genexus™ Strip 1, Genexus™ Strip 2-HD, and Genexus™ Strip 3-GX5™ at room temperature for 30 minutes before loading in the sequencer.
- Thaw Genexus™ Strip 4 on ice or at 4°C for 30 minutes.

  IMPORTANT! Ensure that strip contents are completely thawed before loading in the sequencer.

- Vortex thawed library and templating strips on a platform vortexer to dissolve precipitate and dislodge air bubbles. After vortexing, centrifuge the strips to collect tube contents using the Genexus™ Strip Centrifuge Adapter to hold strips during centrifugation.

  IMPORTANT! Inspect the bottom of tube 3 of the Genexus™ Strip 2-HD for white precipitate before loading it in the sequencer.

  Note: For information about using the Genexus™ Strip Centrifuge Adapter, see "Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter" on page 112.

- Verify consistency of reagent volumes across strips and panel tubes by visually inspecting them two at a time after centrifugation. For instructions, see "Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter" on page 112. Alternatively, use the strip volume guide images provided page 174 to check appropriate fill volumes of strip tubes.
- Keep thawed panel, Genexus™ Strip 4, and the sample plate on ice or at 4°C until ready to use or load in the sequencer.
- Do not freeze-thaw the OPA Pool 1 panel tubes. Thaw only the number of panel tubes that are needed for an instrument run. Discard unused panel tubes after they are thawed. Store panel tubes at –30°C to –10°C.

  Note: One OPA Pool 1 panel tube pair (one tube with forward primers and one tube with reverse primers) is sufficient for preparing up to 4 libraries.

- Do not combine the contents of OPA Pool 1 panel tubes. Forward and reverse primers must remain separate, until they are combined by the sequencer as part of the on-instrument library preparation workflow.
- Do not store OPA Pool 1 panel tubes or reagent strips on the sequencer for more than 24 hours before starting an instrument run.

Guidelines for preventing contamination

We recommend following these guidelines to prevent cross-contamination of samples and controls between and within sequencing runs.

Personal protective equipment

- Wear a laboratory coat that is reserved for sequencing work and is laundered frequently. If possible, change to a fresh laboratory coat before setting up a run, or use new sleeve covers.
- Wear fresh gloves to load the instruments, including during the loading of the sequencing chip and coupler. Do not remove gloves to install the sequencing chip.
- Change gloves between dispensing positive controls and no-template control (NTC) in sample plate wells.

Equipment and instrument cleaning

- If you are preparing samples and sample plates in a PCR cabinet or laminar flow hood (recommended), illuminate the hood with UV light for at least 15 minutes before use.
- Before a run, decontaminate equipment or bench surfaces where samples or sample plates are handled. Use DNA*Zap*™ and either 1) the RNase*Zap*™ RNase Decontamination Solution or 2) the RNase *AWAY*™ Surface Decontaminant reagent. Follow manufacturer instructions for the proper storage and use of each decontamination reagent. Alternatively, a 10% solution of commercial bleach can be used for decontaminating working areas and equipment. Follow with wiping of surfaces with wipes moistened with 70% isopropanol or 70% ethanol.
- Before and after a run, sequentially wipe the deck surface of the Genexus™ Purification System and Genexus™ Integrated Sequencer with lint-free wipes moistened with the two DNA*Zap*™ decontamination solutions followed by wipes moistened with either 1) the RNase*Zap*™ RNase Decontamination Solution or 2) the RNase *AWAY*™ Surface Decontaminant reagent. Follow with wiping of deck surfaces with wipes moistened with 70% isopropanol or 70% ethanol, or with prepared alcohol wipes. The robotic pipettor arm of each instrument can also be cleaned in this manner.

  IMPORTANT! Do not spray decontamination solution or alcohol solution directly onto deck surfaces or into deck openings. Instead, use a lint-free wipe moistened with solution to clean surfaces. Do not use bleach to decontaminate instrument surfaces as it can cause corrosion of the metal surfaces.

- After a sequencing run has completed, ensure that you cover the PCR amplification plate with a foil seal before removing it from the instrument to prevent library contamination.

Workflow tips

- After dispensing a positive control or sample (if applicable) in a sample plate well, do not pass the end of the used tip over wells intended for other samples or NTC. This practice minimizes the chance of depositing microdroplets in adjacent wells.
- If you dilute samples on the sample plate and vortex the plate after sealing, apply the foil seal tightly between wells using an adhesive film applicator before vortexing to prevent contamination between wells. To prevent spillage, use a PCR plate holder while sealing.
- Before disposal, close or cap used sample and positive control tubes to avoid creation of aerosols.
- Avoid touching the foil seals of the reagent strips, barcode plate, and sample plate.
- When loading the sequencer deck for a run, install the sample plate last.
- After a run, immediately seal the PCR amplification plate with a foil seal before removing the plate from the PCR amplification station. Sealing the plate before removal helps prevent contamination of libraries if libraries are recovered for reuse.

Requirements for nucleic acid isolation, quantification, quality control, and input amount

- For automated nucleic acid isolation using the Genexus™ Purification System, see Chapter 6, "Genexus™ Cell-Free Total Nucleic Acid Purification protocol" or Chapter 5, "Genexus™ FFPE DNA and RNA Purification protocol".
- For verified kits to use for manual isolation, quantification, and quality control of nucleic acid samples, see "Recommended materials for nucleic acid isolation, quantification, and quality control" on page 21.
- Use the Qubit™ Fluorometer with the Qubit™ dsDNA HS Assay Kit for quantifying DNA samples.
- Use the Qubit™ Fluorometer with the Qubit™ RNA BR Assay Kit for quantifying RNA samples.
- Use the Agilent™ 2100 Bioanalyzer™ with the Agilent™ High Sensitivity DNA Kit for quality control of cfTNA samples.
- Use the Agilent™ 2100 Bioanalyzer™ with the Agilent™ High Sensitivity DNA Kit or the Agilent™ 4200 TapeStation™ System with the Agilent™ D5000 Reagents and Agilent™ D5000 ScreenTape for quality control of FFPE DNA samples.
- Use the Agilent™ 2100 Bioanalyzer™ with the Agilent™ RNA 6000 Nano Kit for quality control of FFPE RNA samples.
- Quantification methods such as densitometry (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA or RNA. Use of these methods can lead to gross overestimation of the concentration of sample nucleic acid, under-seeding of the target amplification reaction, and low library yields.
- Ensure that the input nucleic acid concentration is in the verified range.

Table 1 Sample concentration requirements

Sample type	Optimal nucleic acid concentration	Verified nucleic acid concentration range
FFPE DNA	0.67 ng/µL	0.33–1 ng/µL
FFPE RNA		
cfTNA	1.33 ng/µL	0.33–2.67 ng/µL

IMPORTANT! For samples with concentrations less than the recommended minimum of 0.33 ng/µL, there is an increased incidence of false-positive SNV or Indel calls.

- The Genexus™ Integrated Sequencer requires 20 µL total volume loaded into the sample input plate (15 µL of sample with 5-µL overage due to dead volume).

- For optimal assay performance, load 13.4 ng of nucleic acid input for each solid tumor sample and 26.6 ng of nucleic acid input for each liquid biopsy sample onto the instrument deck to account for instrument dead volume (Table 2). At optimal nucleic acid concentrations, the instrument uses 10 ng of nucleic acid for solid tumor samples and 20 ng of input for liquid biopsy samples for each sequencing reaction.

Table 2 Sample input requirements

Sample type	On-deck input requirement[1]	Assay input
Solid tumor (FFPE DNA or RNA)	13.4 ng *(Optimal)* 6.6 ng–20 ng *(Verified range)*	10 ng *(Optimal)* 5–15 ng *(Verified range)*
Liquid biopsy (cfTNA)	26.6 ng *(Optimal)* 6.6 ng–53.4 ng *(Verified range)*	20 ng *(Optimal)* 5–40 ng *(Verified range)*

[1] Assay input plus overage.

Before each use of the kit

Thaw assay reagents as indicated:

- Thaw OPA Pool 1 at room temperature for at least 30 minutes and keep on ice until immediately before loading onto the Genexus™ Integrated Sequencer.
- Equilibrate Genexus™ Strip 1, Genexus™ Strip 2-HD, and Genexus™ Strip 3-GX5™ at room temperature for at least 30 minutes before loading onto the Genexus™ Integrated Sequencer.
- Thaw Genexus™ Strip 4 on ice or at 4°C for at least 30 minutes and keep on ice until immediately before loading onto the Genexus™ Integrated Sequencer.

IMPORTANT! Ensure that the strip contents are completely thawed before installing in the sequencer.

When thawed, gently tap tubes and strips on the benchtop to remove any bubbles and collect the contents at the bottom of each tube. For information about preparing all reagents and consumables for loading onto the instrument, see “Before you begin” on page 109.

Note: If tapping fails to dislodge a bubble, you can dislodge large bubbles using the technique that is described in “Before you begin” on page 109.

Guidelines for Genexus™ Integrated Sequencer operation

- Follow guidance that is provided by Genexus™ Software when you plan a run to determine which consumables must be loaded and which consumables can be reused from a previous run.
- Follow guidance that is provided by the software when you plan a run to determine how many samples can be run with a given assay or assays in an instrument run. The number of samples that can be included in a sequencing run depends on multiple factors.

Limiting factor	Description
The number of available barcodes in the barcode plate	The maximum number of available barcodes per run is 48. **IMPORTANT!** When libraries are prepared on the Genexus™ Integrated Sequencer, each target amplification reaction for a sample requires a unique barcode.
Maximum number of target amplification reactions per run	One library strip pair has the reagents necessary for 6 target amplification reactions, or 6 barcodes. With a maximum of 8 library strip pairs loaded, a maximum of 48 samples can be run using an assay with one primer pool.
The number of primer pools per assay	Given the limits of 48 target amplification reactions, and 48 available barcodes, the number of samples in a run multiplied by the total number of primer pools in the assays that are used in a run cannot exceed 48. For one single-pool assay, a maximum of 48 samples can be run on a single chip. If you are using 2 assays with two primer pools each, you can sequence a maximum of 12 samples in a run. Similarly, for one assay with 4 primer pools, you can sequence a maximum of 12 samples in a run, if the minimum read count per sample allows it.
The number of unused lanes on an installed chip	A maximum of 4 lanes are available on a single GX5™ Chip. A maximum of 4 lanes are available on a single GX5™ Chip or GX7™ Chip.
The minimum read count per sample for an assay	The minimum read count per sample parameter is set during assay creation.

- Two assays cannot share a chip lane, so a maximum of 4 assays can be run per chip.
- The assays that are used in a single run must use the same chemistry (Ion AmpliSeq™ or Ion AmpliSeq™ HD), and have compatible cycling parameters to allow amplification in the instrument thermal cycler. The thermal cycler has two independently controlled heating zones. After you select an assay, Genexus™ Software restricts the list of available assays to use in the run to those that are compatible with the selected assay or assays.
- One library strip pair is needed for each primer tube position 1–8 that is filled in a run.
- One template strip pair is needed for each chip lane that is used in a run.
- Consumables are configured to support sample batch sizes in multiples of six samples. The most efficient use of consumables occurs when samples are run in multiples of six.
- If a chip installed in a sequencer has unused lanes, do not remove it unless you are sure that you want to replace it with a new chip. After a partially used chip has been removed from the sequencer, it cannot be reinserted and reused. The sequencer cannot track lane usage after chip removal.

- You can remove a chip in one of the following situations.
  - After all the lanes of a chip are used in a run, the chip shuttles to the install position and you are asked to remove the used chip.
  - When you select a run plan that requires more lanes than are available on the installed chip, you are asked to remove the partially used chip, and the sequencer performs a post-chip clean. In addition, you need to remove consumables from the lower sequencing reagents bay, even if only a single lane of the chip was used.
- The Genexus™ Integrated Sequencer can track used and unused barcodes in barcode plates enabling you to swap plates between runs if needed, and reload a partially used barcode plate for a run if a sufficient number of barcodes is available on the plate.

  IMPORTANT! Do not write or mark on the foil seal of the barcode plate. The instrument vision system will be unable to distinguish used wells from unused wells, which can result in a run failure.
- Before loading pipette tip boxes in the sequencer before a run, ensure that you remove the lids from the tip boxes.
- After loading in the sequencer, reusable consumables, such as barcode plate, chips, and sequencing reagents bay components, must be used within 14 days for optimal results.
- An assay that is selected in a Library to Result run cannot include library batches that share a library with the same barcode. However, two different assays in a run can include a barcode in common, because assays are run in separate lanes of a chip.
- If desired, manually dilute samples to the required concentration to avoid loss of sample due to the required volume overages during automated dilution.

Precautions

Avoid nucleic acid contamination

IMPORTANT! A primary source of contamination is spurious nucleic acid fragments from previous sample processing steps. Do not introduce amplified DNA into the work area where the instrument is located.

Avoid chip damage

IMPORTANT! To avoid possible damage to the chip due to electrostatic discharge, ground yourself before picking up a chip or placing a chip on a surface such as a lab bench. For example, touch the metal trim on the chip compartment before inserting or removing a chip from the chip clamp.

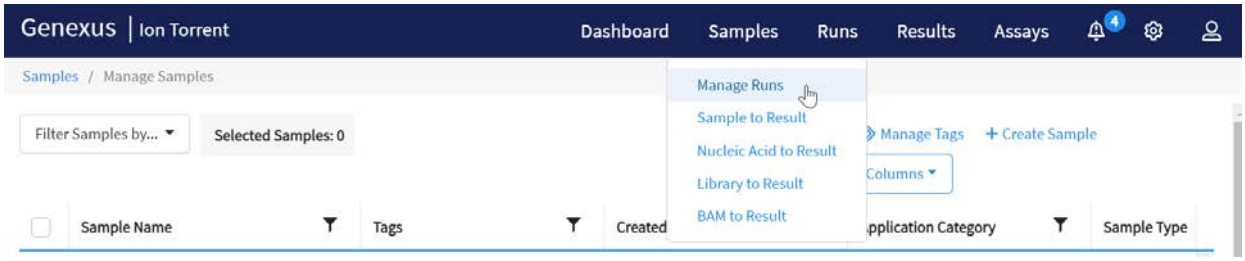
Avoid strong electromagnetic radiation

WARNING! Do not use the instrument in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources), as these sources can interfere with proper operation.

Protection by equipment

WARNING! The protection that is provided by the equipment can be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner that is not specified by the manufacturer (Thermo Fisher Scientific).

4 Plan and manage runs



Runs plans that are created in Genexus™ Software contain the settings that are used in sample purification, library preparation, templating, sequencing, and analysis, including sample information and plate location, assays, and barcodes. Run plans are used to track samples, consumables, and chips throughout purification, library preparation, templating, sequencing, and data analysis.

For more information about run planning, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the software help system.

Types of Oncomine™ Precision Assay GX runs

The type of run that you plan depends on your instrument configuration, assay, and sample type.

Genexus™ Software guides you step-by-step through the process to set up a run. The software prompts you to select required information and consumables, then provides a printed run setup guide to help you load consumables on the Genexus™ Integrated Sequencer and the Genexus™ Purification System, if applicable.

Table 3 Types of runs for use with the Oncomine™ Precision Assay GX

Run type	Description
Sample to Result	An integrated run for sequential and automated nucleic acid purification, quantification, and sequencing. This run type requires the Genexus™ Purification System to be connected to the sequencer.
Nucleic Acid to Result	A sequencing run that starts with purified nucleic acid samples of known concentration as input. Purified nucleic acids can be isolated and quantified using the Genexus™ Purification System in stand-alone configuration. Alternatively, you can use other manual purification and quantification kits to isolate purified nucleic acid samples. For a list of recommended kits, see “Recommended materials for nucleic acid isolation, quantification, and quality control” on page 21.

For more information about planning other types of runs, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the software help system.

System-installed assays for use with the Oncomine™ Precision Assay GX

An assay is a reusable experimental design that contains the settings and parameters for sample purification, library preparation, templating, controlling the sequencing run, analyzing, and reporting the results. Assays also define the panels, kits, and chips that are used in a run, and specify the reference files and threshold values for quality control and variant detection. The software files that include the assay settings and parameters are packaged in a ZIP file called an assay definition file (ADF).

An assay can be used to plan many runs and plays an important role in enabling rapid throughput across the purification and sequencing instruments. Assays help reduce the chance of errors, because information is stored and then applied to runs instead of entered manually for each run.

The following system-installed assays are available in the Genexus™ Software 6.8 or later for use with the Oncomine™ Precision Assay GX. Each system-installed assay is configured with settings that are optimized for a specific sample type.

Note: We recommend updating your Genexus™ Software to the latest available versions of the system-installed assays. Previous versions of the assay do not have all the functionality available in Genexus™ Software 6.8. For more information, contact your field service representative. For information about

how to perform software and assay package updates, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Assay name	Sample type
Oncomine™ Precision - GX5 - w3.4.2 - Liquid Biopsy cfTNA	cfTNA
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA	DNA
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - Fusions	RNA
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions	DNA and RNA

The Genexus-Oncomine-Precision-GX5-w3.4.2-Combined-SolidTumor-DNAandFusions_SW_6.8 assay definition file (ADF) contains 3 assays: DNA and Fusion, DNA, Fusion.

You can use the system-installed assays in your run plan without change. To modify any assay settings, you can copy the system-installed assay that best represents your sequencing experiment and sample type, then edit assay settings if needed. To modify a system-installed assay with custom settings, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

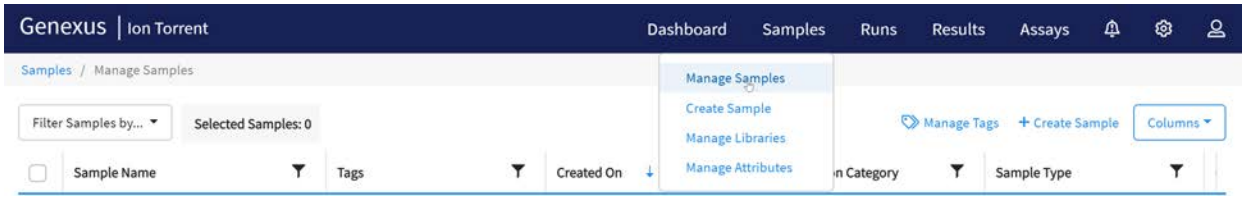
The Oncomine™ Precision - GX5 - w3.4.2 - Liquid Biopsy cfTNA assay includes resequencing by default. Resequencing is repeat sequencing of the same DNA template a second time using a different dNTP flow order. For more information about resequencing and sequencing flow order, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Enter samples in the Genexus™ Software

In Genexus™ Software, the data and attributes that characterize a purified nucleic acid—or a specimen that requires nucleic acid purification and quantitation—to be sequenced are called samples.

Before you plan a run in the Genexus™ Software, you must first enter sample information in the software to assign sample names and provide other information.

For more information about creating and managing samples, see the Genexus™ Software help system.



1. Sign in to Genexus™ Software.
2. In the menu bar, click **Samples** ▸ **Create Sample**, select an **Application Category**, complete the required fields and any optional fields, then click **Save**.
   The new sample is listed in the **Manage Samples** screen and is available to use in a run plan.

- To create a Sample to Result run, proceed to “Plan a Sample to Result run” on page 35.
- To create a Nucleic Acid to Result run, proceed to “Plan a Nucleic Acid to Result run” on page 38.

Plan a Sample to Result run

You can plan runs for sequencing that start from various sample types. In Genexus™ Software, a run that includes purification of nucleic acids from samples is called a **Sample to Result** run.

Planning a **Sample to Result** run is organized into steps: **Setup**, **Assays**, **Samples**, **Purification**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / Sample to Result** screen.

This procedure applies only if a Genexus™ Purification System is used with the Genexus™ Integrated Sequencer.

Ensure that the following prerequisites are complete before you plan a **Sample to Result** run.

- Integrate a Genexus™ Purification System with the system.
- Enter sample information into Genexus™ Software. For more information, see the "Enter samples in the Genexus™ Software" on page 34.
- The Genexus™ Cell-Free Total Nucleic Acid Purification assay has quantitation disabled by default to preserve the sample. Quantitation requires 5 µL of the final eluted sample. To include quantitation in cell-free TNA purification runs, create and install a custom assay. For more information about creating a custom assay, see the Genexus™ Software help system or contact your support representative.

1. In the menu bar, click **Runs** ▸ **Sample to Result**.
   You can also click + **Sample to Result** in the **Runs / Manage Runs** screen.

2. In the **Setup** step, enter or make the following selections.
   a. In the **Plan** section, enter a unique name.
      The name is limited to 50 characters and no spaces are allowed.

   b. *(Optional)* In the **Reporting (Optional)** section, enable **Generate Report** to generate a variant report that uses the default report template.
      To create a report template, click **Assays** ▸ **Manage Presets**, then in the **Report Templates** tab, click + **Add New**.

   c. Click **Next**.
      If a chip is installed in the sequencer, the **Chip View** graphic in the lower left corner indicates the lanes that are available for sequencing.

3. In the **Assays** step, select the **Research Application** of each assay that you want to use in the run.
   Use the **(Filter)** tools in table column headings or the **Filter Assays By** list to find assays of interest.

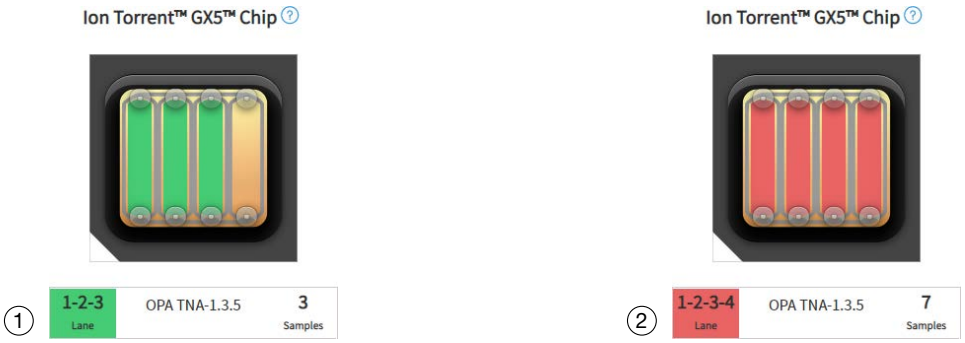
   IMPORTANT! Ensure that you select the assay that corresponds with the sample type that you use in the run. If you select the wrong assay when you plan a run, the instrument uses incorrect settings during the run, resulting in invalid sequencing results. Available assays are listed in the **Assays / Manage Assays** screen.

   Only assays that are configured for **Sample to Result** runs are listed.

After selecting an assay, the list is filtered to show compatible assays that can be selected and run at the same time.

4. Click **Next**.

5. In the **Samples** step, select the checkbox next to each sample from the list that you want to run with the assay, then in the **Selected Assays** pane, click **Assign**.
   The **Chip View** updates to show the lanes to be used in the run. Lane usage is calculated based on the number of samples (including a no template control or control sample, if selected) and minimum reads per sample entered at assay setup. Green denotes a chip lane to be used in the run containing assigned samples within lane capacity. If the `minimum reads per sample` × `number of samples` exceeds the chip or lane well capacity, a dialog box appears after you click **Assign** asking you to confirm that you want to continue. After confirmation, the **Chip View** updates and shows the lane color as red instead of green. The run is allowed if the lane capacity is exceeded, but you may not achieve the required reads per sample to pass QC metrics.



① ②

Green lane color ① denotes lane usage and sample assignment within lane capacity. Red lane color ② denotes sample assignment that exceeds lane capacity.

6. Select the **Include NTC** checkbox to include a no template control for the assay.

7. *(Optional)* Select the **Control Sample** checkbox to designate a sample as a positive control for the assay.

8. If you selected more than one assay, repeat step 5 through step 7 for each assay added.

9. Click **Next**.

10. If needed, edit samples in one of the following ways, then click **Next**.
    - Click **View & Remove**, make your selections, then click **Update**.
    - Click **Remove All**, make your selections, then click **Assign**.

11. In the **Purification** step, review and edit purification selections, then click **Next**.

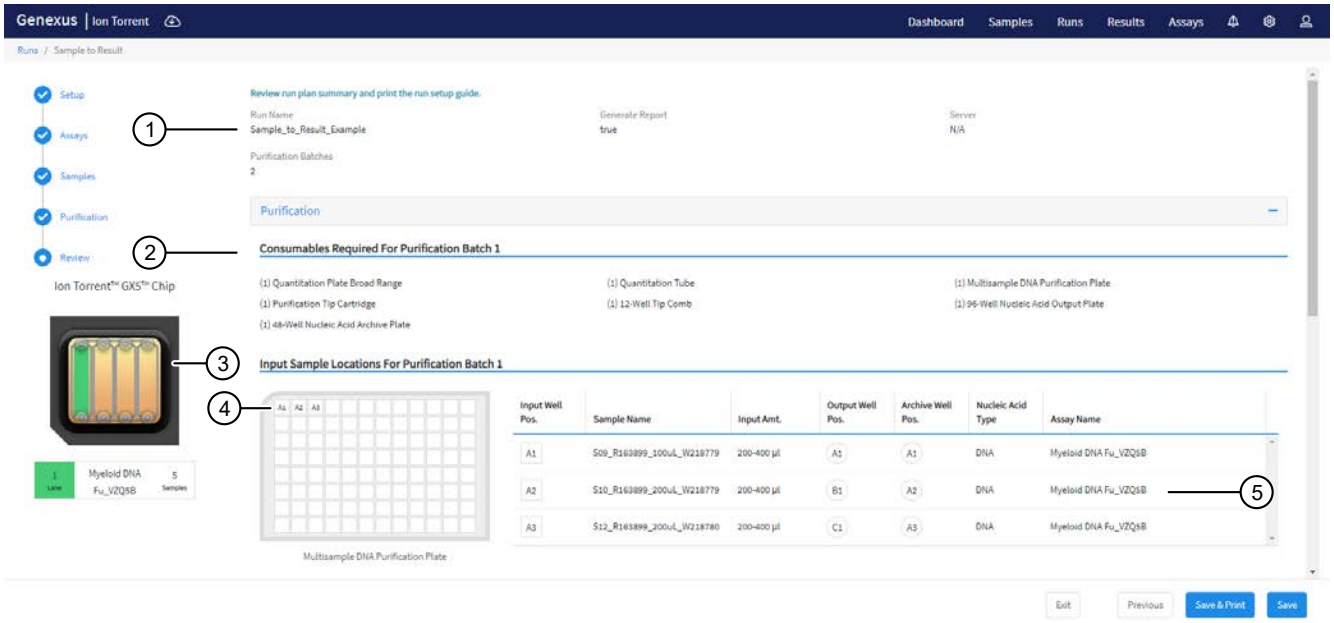
Option	Description
Protocol Selection	Select a protocol from the dropdown list for each purification kit.
Elution Vol. (µL)	Modify the elution volume within the allowable range, if needed. Quantitation requires up to 5 µL of the eluted sample. If retesting is needed, up to 10 µL of the eluted sample is used. If the expected sample yield is limiting, manual sample quantitation may be preferred to preserve the sample. Alternatively, you can increase the sample elution volume in the run plan before starting the purification.
Review?	• Select the checkbox in the row of a purification kit to review the sample concentrations after purification, before sequencing. This lets you review whether a sample concentration is out of range for automated dilution or below the minimum concentration needed threshold. You can then decide on a per sample-basis whether to sequence the samples. • Deselect the **Review?** checkbox in the row of a purification kit to exclude out of range samples from sequencing. This option does not require you to review samples during the run. By default, the **Review?** function is enabled for FFPE assays and disabled for cfTNA assays.

12. In the **Review** step, review the run plan summary.

    If desired, click **Save & Print** to print the run setup guide. Click **Save** to save the run without printing.

    The run plan summary lists the following details:

    - The consumables that are needed for this run and the consumables that are installed and ready to use
    - How much sample volume to load
    - Where to load samples and primer pool tubes
    - Details about the assay



① Run information
② List of consumables needed for the run
③ Chip view showing the lanes to be used in the run
④ Positions in the sample plate to load the samples
⑤ Table that lists the sample plate position, sample type, volume to load, concentration, dilution factor, and assay for each sample

Tip: Click **Sequencing** to expand the sequencing section of the run plan summary to view the sequencing plans and required consumables.

Note: If you are using an assay with Ion AmpliSeq™ HD library chemistry, the primer pool positions show that HD primer pools occupy both rows.

If the **Sample to Result** run requires more than one purification batch (for example a Oncomine™ Myeloid v2 - GX5 - DNA and Fusions run) you can select which purification batch to run first. Or, if there are multiple purification instruments integrated with one sequencer, select the instrument on which to purify each batch.

After saving, the run appears in the **Manage Runs** screen in the run list with the name you specified. Until a run plan is selected for use on the sequencer it can be edited. For information about how to edit run plans, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

After you select the run and load the purification instrument, the run is started on the instrument screen.

Plan a Nucleic Acid to Result run

You can plan a run for sequencing that starts with nucleic acid samples. In Genexus™ Software 6.6 and later, a run that starts with nucleic acid samples is called a **Nucleic Acid to Result** run.

Planning a **Nucleic Acid to Result** run is organized into steps: **Setup**, **Assays**, **Samples**, **Sample Plate**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / Nucleic Acid to Result** screen.

Ensure that the following prerequisites are complete before you plan a **Nucleic Acid to Result** run.

- Identify the system-installed or custom assay to use in the run. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
- Enter sample information into Genexus™ Software. For more information, see "Enter samples in the Genexus™ Software" on page 34.
- Quantify the sample concentration if you do not dilute sample concentrations manually to the target concentration of the assay. For more information, see the *Genexus™ Integrated Sequencer User Guide* (MAN0017910).

1. In the menu bar, click **Runs** ▸ **Nucleic Acid to Result**.

   Note: Alternatively, you can click + **Nucleic Acid to Result** in the **Runs / Manage Runs** screen.

2. In the **Setup** step, enter or make the following selections.
   a. In the **Plan** section, enter a unique name.

      The name is limited to 50 characters and no spaces are allowed.

   b. *(Optional)* In the **Reporting (Optional)** section, ensure that **Generate Report** is enabled to generate a **Variant Report** using the default report template.

   c. *(Optional)* In the **Reporting (Optional)** section, enable **Upload BAM files to Server** to upload BAM files to another server.

Option	Description
Upload BAM files to another Genexus™ Software server.	In the dropdown lists that appear, select the target Genexus™ Software server and version. To configure a Genexus™ Software account that you can use for data uploads, click **Set up Account**. For more information, see the Genexus™ Software help system.
Automatically upload data for further analysis with Ion Reporter™ Software.	In the dropdown lists that appear, select the Ion Reporter™ Software account and Ion Reporter™ Software version. To configure an Ion Reporter™ Server account that you can use for data uploads, click **Set up Account**. For more information, see the Genexus™ Software help system.

   d. Click **Next**.

3. In the **Assays** step, select one or more assays that you want to include in the run.
   a. Use the ▼ **(Filter)** tools in table column headings to find assays of interest, if desired.

   b. In the **Application Type** column for the assay of interest, select one or more application types, such as **DNA and Fusions** or **DNA**, to include each selected application type for the assay in the run plan.

      After selecting an assay and the research application for the assay, the list is filtered to show compatible assays that can be selected and run at the same time.

   c. If more assays are included in the run, repeat substep 3b for each extra assay.

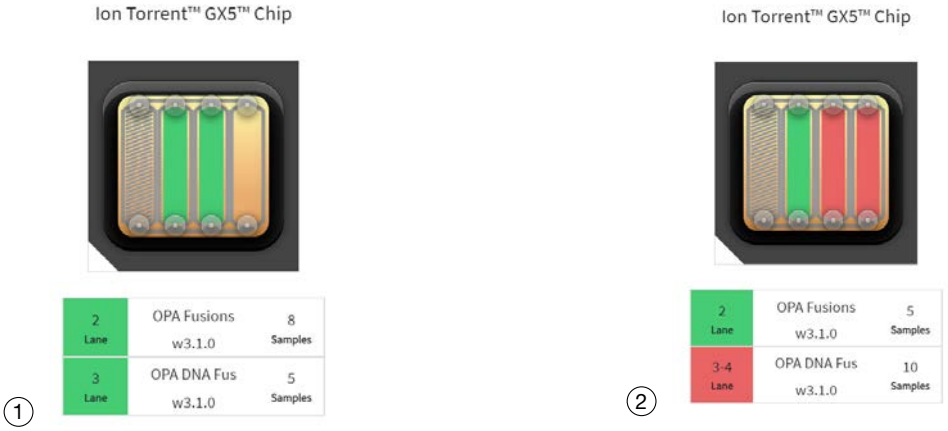
d. Click **Next**.

4. In the **Samples** step, select the samples that you want to run with each application type of each assay.

   a. Select the checkbox next to each sample that you want to assign to the application type of an assay, then in the **Selected Assays** pane, for the assay and application type that you want to use for the selected samples, click **Assign**.

   The **Chip View** updates to show the lanes used in the run. Lane usage is calculated based on the number of samples (including a no template control, if selected) and the minimum read counts per sample for the assay. For information about the GX5™ Chip lane usage for the Oncomine™ Precision Assay GX, see “Lane usage by sample type” on page 176. Green denotes a chip lane in the run containing assigned samples within lane capacity.

   If the *minimum reads per sample* × *number of samples* exceeds the chip or lane well capacity, a dialog box appears after you click **Assign** asking you to confirm that you want to continue. After confirmation, the **Chip View** updates and shows the lane color as red instead of green. The run is allowed if the lane capacity is exceeded, but you may not achieve the required reads per sample to pass QC metrics. If needed, remove extra samples from the run as described in substep 4c.



Green lane color ① indicates lane usage and sample assignment within lane capacity. In this example, lane 1 was used in a previous run and is not available.

Red lane color ② indicates sample assignment that exceeds lane capacity for lanes 3 & 4.

   b. If you selected more than one application type or assay, repeat substep 4a for each application type for each assay in the run plan.

   c. If needed, edit samples in one of the following ways.

   - Click **View & Remove**, make the selections, then click **Update**.
   - Click **Remove All**, to remove all sample assignments for all assays.

   d. If desired, for each application type of each assay in the run plan, select **NTC** to include a no template control.

   The **Chip View** updates to show the lanes used in the run for the included no template controls.

   e. Click **Next**.

5. In the **Sample Plate** step, review position assignments in the sample plate. Drag-and-drop samples and no template controls to edit the location of samples and controls, if applicable.
   a. If desired, enter the extraction kit barcode for one or more samples or controls. For a single sample, in the row of the sample of interest, in the **Kit Barcodes** column, enter the extraction kit barcode or control kit barcode, if applicable. For multiple samples or controls, select the samples and controls, then click **Assign Kit Barcodes**. In the **Assign Kit Barcodes** dialog box, enter the extraction kit barcode for the samples, and if applicable, enter the barcode for the no template control.

   b. Modify the concentration of samples, if needed. For a single sample, in the row of the sample of interest, in the **Conc. (ng/µl)** column, edit the concentration.

      To modify the concentration of multiple samples, select the samples of interest, then click **Edit Concentration**. In the **Bulk Edit** dialog box, enter the concentration for all selected samples, then click **Submit**. The concentration for each selected sample is updated to the new value.

      If a sample concentration is within the designated range, the instrument does not dilute the sample during the run. Sample concentrations must be within 0.11–1,000 ng/µL and cannot be higher than 1,024X of the target concentration. If a sample concentration is ≤1,024X of the target concentration, the sequencer dilutes the sample to the target concentration during the run. If a sample concentration is >1,024X of the target concentration, manually dilute the sample to the target concentration before loading on the sample plate. For more information about sample dilution, see “Dilute the samples (if needed) and load the sample plate” on page 101.

      Note: The sample volume that is required for library preparation is not adjustable. The volume depends on the number of primer pools in the assay, sample type, and library chemistry. For specific sample volumes to load onto the sample plate, see “Dilute the samples (if needed) and load the sample plate” on page 101.

   c. Ensure that sample plate information is correct, then click **Next**.

6. In the **Review** step, review the run plan summary, then click **Save & Print** to print the run setup guide, if desired. Click **Save** to save the run without printing.
   After saving, the run appears in the **Manage Runs** screen in the run list with the name you specified.

After you select the run and load the sequencer, the run is started on the sequencer screen.

When the sequencing run is complete, you can recover the leftover volume from each library that was prepared in the run. For more information to find, recover, and purify the leftover library preparations, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

5 Genexus™ FFPE DNA and RNA Purification protocol

Genexus™ FFPE DNA and RNA Purification workflow

5 min

Plan a purification run (page 44)

IMPORTANT! If performing a **Sample to Result** run, plan the run in the Genexus™ Software. See "Plan a Sample to Result run" on page 35.

To purify samples for use in **Nucleic Acid to Result** runs or other nonsequencing applications, run the Genexus™ Purification System in standalone configuration. Add a new purification run plan or copy-edit an existing purification run plan that best represents your experiment. Purification run plans contain instrument settings that are used in sample purification.

3 hr

Prepare samples from FFPE curls (page 50)

OR

Prepare samples from FFPE slides (page 52)

Samples are deparaffinized and digested with protease in preparation for isolation of DNA.

5 min

Load the Genexus™ Purification System (page 55)

The purification run plan is selected, and the run is initiated. The instrument performs a UV cleaning, then reagents and consumables are loaded onto the instrument.

2 hr

Start the run (page 65)

After the sample plate, reagents, and consumables are loaded, the instrument door is closed, and the run is started.

Depending on the number of samples purified, sample quantification adds up to 2.5 hours to the run time.

5 min

Unload the purified nucleic acids (page 66)

If performing a **Sample to Result** run, remove the 96-Well Nucleic Acid Output Plate and proceed to sequence the purified sample. Remove and seal the 48-Well Nucleic Acid Archive Plate, then store as directed.

If performing a **Nucleic Acid to Result** run or purifying nucleic acids in standalone configuration for use in other downstream applications, remove and seal the 48-Well Nucleic Acid Archive Plate, then store as directed or proceed to sequence the purified sample.

Used reagents and consumables are removed from the instrument and the instrument performs a UV cleaning.

Plan a purification run (standalone configuration)

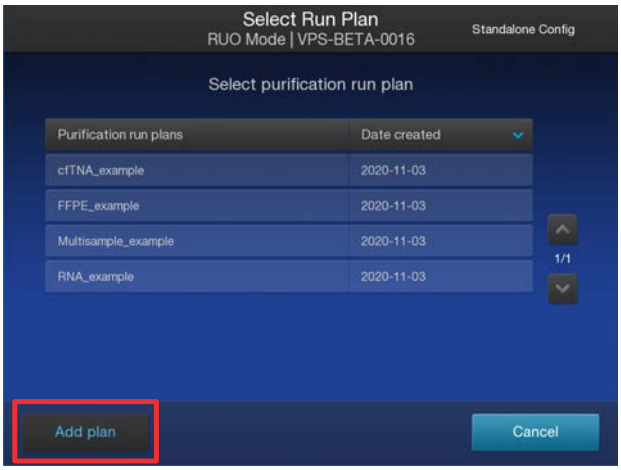
Note: If running the Genexus™ Purification System in integrated configuration, see "Plan a Sample to Result run" on page 35 to plan a **Sample to Result** run in the Genexus™ Software.

In standalone configuration, plan a purification-only run through the instrument touchscreen. After purification is complete, all purified samples are transferred to an archive plate for storage.

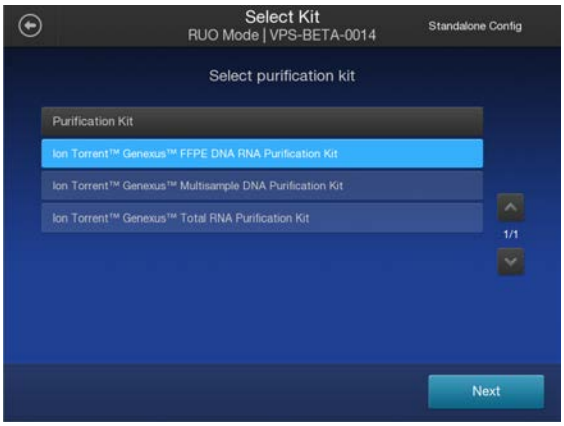
Add a purification run plan (standalone configuration)

Plan the run before you prepare samples and load the samples into the FFPE DNA and RNA Purification Plate 1. However, experienced users can save time if you plan the purification run during the protease digestion step of sample preparation.

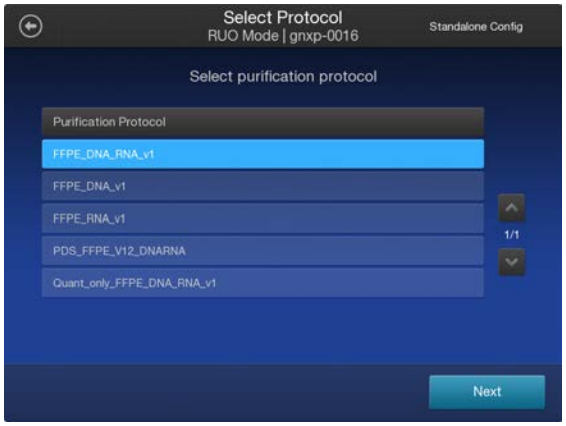
1. Enter your username and password to sign in to the instrument.
2. Tap **Run**, then tap **Add plan**.



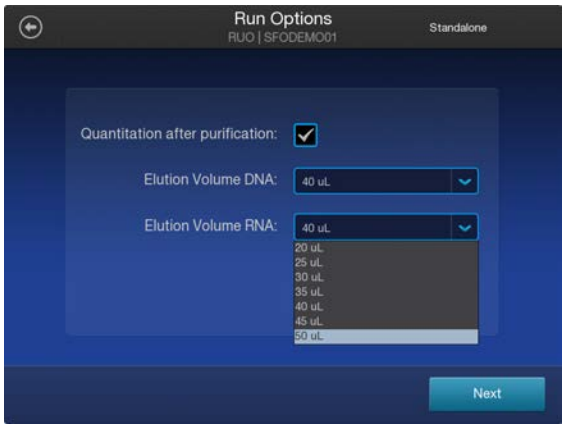
3. Tap in the entry box, enter a unique name for the run plan, then tap **Done** ▸ **Next**.
4. Select the **Ion Torrent™ Genexus™ FFPE DNA and RNA Purification Kit**, then tap **Next**.



5. Select the appropriate purification protocol and software version in use, then tap **Next**.
   - If sequentially purifying DNA and RNA, select **FFPE DNA RNA**.
   - If purifying DNA only, select **FFPE DNA**.
   - If purifying RNA only, select **FFPE RNA**.



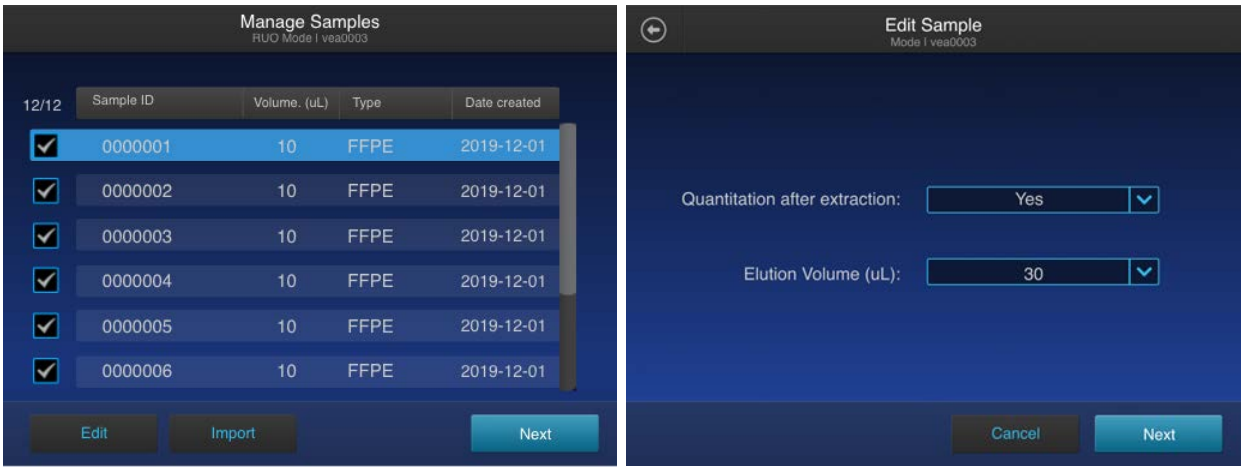
6. Enable or disable **Quantitation after Purification**.
   The Quantitation Plate is required even if **Quantitation after Purification** is disabled.
   Disabling **Quantitation after Purification** may reduce the purification run time by up to 2.5 hours.



7. Accept the default elution volume. If needed, select the desired elution volume from the dropdown list, then tap **Next**.

8. *(Optional)* Change the number of samples and the sample details.
   a. In the **Manage Samples** screen, deselect extra samples (for example, if you run only 10 samples, deselect samples 11 and 12).
   b. Tap on a sample ID to select the sample.
   c. Tap **Edit**, enter a new **Sample ID** and any **Notes**, then tap **Save**.
   d. Repeat substep 8b and substep 8c for each additional sample.

e. Click **Next**.



9. *(Optional)* Import sample information.

   Importing sample information overwrites the existing **Sample ID** and **Notes** information for each sample selected.

   In standalone configuration, prepare a CSV sample import file and save it to a USB drive to import sample information. See page 175.

   a. In the **Manage Samples** screen, select the samples to import the sample information, then tap **Import**.

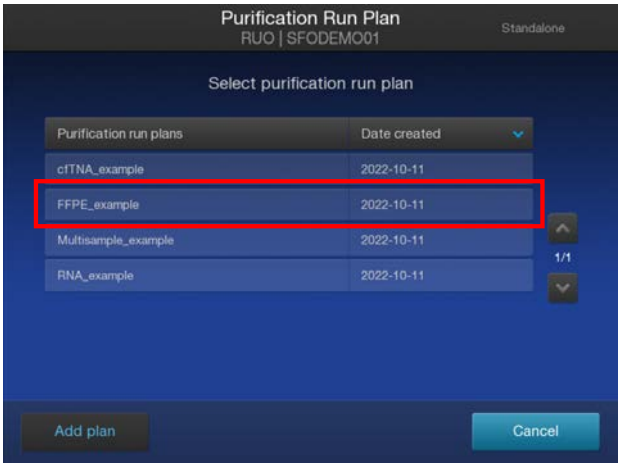
   b. In the **Sample Import** screen, tap **Import** to proceed.

   c. Insert the USB drive that contains the sample import CSV file into the USB port on the front of the purification instrument. In the **Sample Import** screen, select the USB drive, then navigate to and select the sample import file.

   d. *(Optional)* Tap **Details** to view the CSV file that lists the sample names to be imported.

   e. Tap **Import**, then in the **Import Successful** screen, tap **OK**.
   The imported sample information is shown in the **Manage Samples** screen. If needed, edit imported sample information as described in step 8.

10. Review the **Purification Run Plan Details**. Tap **Edit** to change any of your selections, otherwise tap **Next**.



The new purification run plan appears in the list of available **Purification Run Plans**.

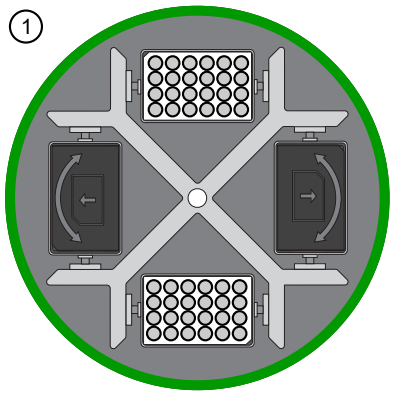
To delete an existing run plan, see the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

Prepare samples

Procedural guidelines

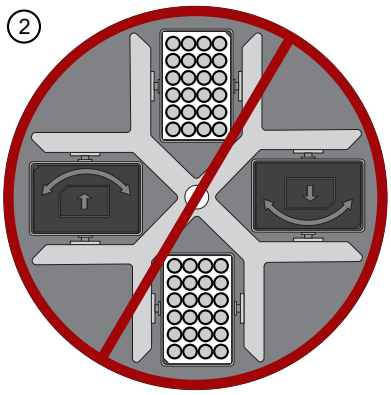
IMPORTANT! Store all kit components containing liquid in the upright orientation.

- Perform all steps at room temperature (15–30°C) unless otherwise noted.
- Thawing or storing on ice can be substituted with thawing or storing at 4°C (2–8°C refrigerator or prechilled benchtop cold block).
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Wear clean gloves and a clean laboratory coat.
- Change gloves whenever you suspect that they are contaminated.
- Open and close all sample tubes carefully. Avoid splashing or generating aerosols of the samples.
- When working with RNA:
  - Use a positive-displacement pipettor and RNase-free pipette tips.
  - Clean laboratory benches and equipment periodically with an RNase decontamination solution, such as RNase*Zap*™ RNase Decontamination Solution (Cat. No. AM9780).
  - Store RNA at –90°C to –70°C.
- Volumes for reagent mixes are given per sample. We recommend that you prepare master mixes for larger sample numbers. To calculate volumes for master mixes, see the per-well volume and add 5–10% overage.
- Incubation at 60°C can be extended 1 hour (2 hr total time) to increase DNA yields followed by the 90°C incubation for 1 hour.
- We recommend using a plate centrifuge that holds the AutoLys M Tube Rack in "landscape" orientation.



① Landscape orientation—recommended

Note: Place AutoLys M Tube Rack in the centrifuge with the arrow on the cover pointing outward as shown.



② Portrait orientation—not recommended

- The plate chiller on the Genexus™ Purification System shuts off 60 minutes after run completion. Remove the 96-Well Nucleic Acid Output Plate and 48-Well Nucleic Acid Archive Plate with purified nucleic acids from the instrument within 1 hour of run completion. Proceed immediately to sequencing or properly store the nucleic acids until use.
- The Quantitation Plate requires equilibration to room temperature for at least 30 minutes before use.

Before each use of the kit

- We recommend the use of incubators when using AutoLys M Tubes.
- Preheat incubators to 60°C and 90°C.
- Prepare Protease Digestion and DNase Digestion solutions immediately before use.
- Centrifuge purification plates for 30 seconds at 1,000 x *g* to collect the contents.

Materials required

- Genexus™ FFPE DNA and RNA Purification (Part. No. A45532)
  - FFPE DNA and RNA Purification Plate 1
  - Proteinase K (red cap)
  - FFPE Protease Buffer
- Genexus™ Purification Supplies 2 (Part. No. A45574)
  - AutoLys M Tubes and Caps
- AutoLys M TubeLifter or Pliers
- AutoLys M Tube Rack
- Plate centrifuge
- Incubators (see “Recommended materials for nucleic acid isolation, quantification, and quality control” on page 21 for a list of recommended incubators)

Recommended input amount

Sample type	Input range	Recommended input amount
FFPE tumor resections	1 x 5 µm curl/slide to 4 x 10 µm curls/slides	1 x 10 µm curl/slide
FFPE fine needle aspirate (FNA) FFPE core needle biopsy (CNB)	1 x 5 µm curl/slide to 6 x 10 µm curls/slides	4 x 10 µm curls/slides

Prepare 1X Protease Digestion Master Mix

Prepare the 1X Protease Digestion Master Mix immediately before use.

1. Invert the FFPE Protease Buffer and Proteinase K tubes supplied in the kit 5X each, then briefly centrifuge.

2. In a 5–10-mL tube, prepare a 1X Protease Digestion Master Mix as indicated, where n is the number of samples.

Component	Volume per reaction
FFPE Protease Buffer	(n + 1) × 225 µL
Proteinase K (red cap)	(n + 1) × 10 µL
Total volume	**(n + 1) × 235 µL**

3. Vortex for ~5 seconds to mix, then briefly centrifuge to collect the contents.

Prepare FFPE curl samples with AutoLys M Tubes

Use AutoLys M Tubes to prepare FFPE samples. Alternatively, CitriSolv™ Clearing Agent, xylene, or an equivalent method for removal of paraffin from the FFPE samples can be used to prepare samples. See the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

Digest with Protease in AutoLys M Tubes

Note: To minimize the amount of time between protease digestion and starting the purification run on the instrument, prepare the reagents and consumables that are required by the instrument during the 90°C incubation (step 6).

1. Label an AutoLys M Tube for each FFPE research sample.

2. Transfer each FFPE section curl from its storage tube to a separate labeled AutoLys M Tube.
   a. Invert a labeled AutoLys M Tube over an uncapped storage tube containing the curl, then carefully invert the 2 tubes together so that the storage tube is on top.
   b. Tap or flick the storage tube so that the curl falls into the AutoLys M Tube.

   If the curl adheres to the storage tube, gently grasp an edge of the curl with forceps, then transfer it to the AutoLys M Tube. Clean the forceps between samples by rinsing with nuclease-free water, followed by ethanol or wiping with ethanol wipes. Do not reuse the forceps without cleaning. Alternatively, use sterile disposable forceps.

3. Cap the AutoLys M Tubes tubes, place the tubes in an AutoLys M Tube Rack, then centrifuge at 2,000 × *g* for 1 minute to collapse the curl before the addition of buffer.

4. Pipet 235 µL 1X Protease Digestion Master Mix into each labeled tube.

   Ensure that the curls are submerged in the Protease Digestion Master Mix. If tissue adheres to the side of the tube, use a sterile pipette tip to push the tissue into the solution.

5. Securely recap each tube to limit evaporation, then incubate the capped tubes at 60°C for ≥60 minutes in an AutoLys M Tube Rack.

   Incubation at 60°C can be extended to 2 hours to increase DNA yields. We recommend that you incubate low yielding samples such as core needle biopsy and fine needle aspirate samples for 2 hours to maximize yield.

6. Securely retighten each tube cap to limit evaporation, then incubate at 90°C for 60 minutes.
   - If using a single incubator, keep the sample in the incubator while the temperature increases. Start timing when the temperature reaches 90°C.
   - Set up the FFPE DNA and RNA Purification Plate 1 during the incubation.
   - Prepare the reagents and consumables that are required by the instrument during the incubation. See “Prepare the consumables” on page 55.
   - Equilibrate the Quantitation Plate to room temperature during the incubation.

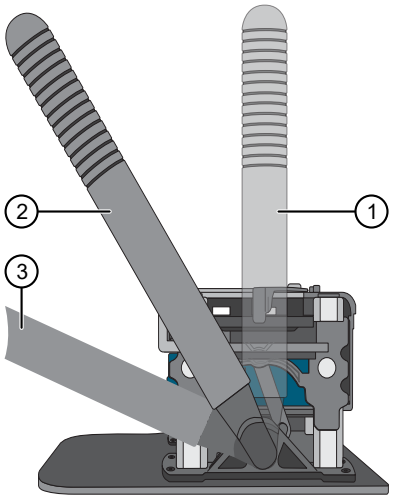
7. Allow samples to cool to room temperature for at least 10 minutes before proceeding to lift the tubes.

8. Lift the tubes. The following steps describe use of the AutoLys M TubeLifter to process up to 24 samples simultaneously. Alternatively, AutoLys M Tube Pliers can be used to process tubes individually.
   a. Ensure that the AutoLys M TubeLifter lever is in Position A (straight up) and the slider is in Position 1.

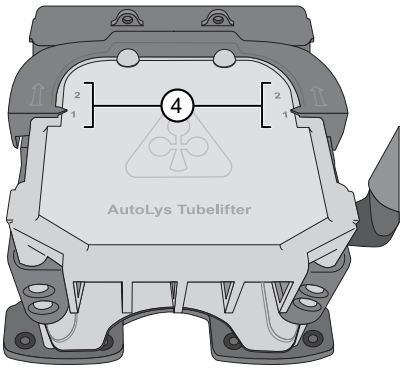
   b. Slide the 24-well AutoLys M Tube Rack containing the lysed samples into the AutoLys M TubeLifter.

   c. Press the lever down from Position A to Position B, then remove the rack from the lifter.

   For more information about use of the AutoLys M TubeLifter see the *AutoLys M TubeLifter User Guide* (Pub. No. MAN0017676).



① AutoLys M TubeLifter lever Position A
② AutoLys M TubeLifter lever Position B
③ AutoLys M TubeLifter lever Position C



④ AutoLys M TubeLifter slider positions 1 & 2

9. Slide the AutoLys M Tube Locking Lid onto the rack, then centrifuge the samples at 2,000 × *g* for 10 minutes.

   IMPORTANT! Ensure that the embossed arrow of the AutoLys M Tube Locking Lid points away from the center of rotation (landscape orientation) when placed in the centrifuge. See the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

10. Using the AutoLys M TubeLifter or AutoLys M Tube Pliers, separate the filter from the outer tube.
    a. Adjust the position of the AutoLys M TubeLifter slider to Position 2.
    b. Remove the AutoLys M Tube Locking Lid, then slide the rack into the AutoLys M TubeLifter.
    c. Press the lever down from Position B to Position C.

    Keep the samples on ice or at 4°C.

Proceed to "Load the instrument and start the run" on page 55.

STOPPING POINT If needed, transfer samples to a separate labelled 1.5-mL Eppendorf™ LoBind™ tube and store at 4°C for up to 4 days or frozen at –30°C to –10°C for up to 7 days.

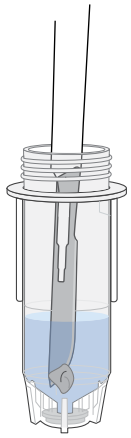
Prepare FFPE slide samples with AutoLys M Tubes

Use AutoLys M Tubes for the preparation of FFPE samples. Alternatively, CitriSolv™ Clearing Agent, xylene, or an equivalent method for removal of paraffin from the FFPE samples can be used to prepare samples. See the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

Collect the tissue

1. Label an AutoLys M Tube for each FFPE tissue sample.
   Label each tube (cap and side) with its Sample ID using a marker that is resistant to ethanol.
2. Pipet 235 µL 1X Protease Digestion Master Mix into each labeled tube.
3. Pipet 2–4 µL of 1X Protease Digestion Master Mix from the labeled tube evenly across the fixed tissue section on the slide to prewet the tissue section.
   Larger sections may need an additional 2–4 µL of 1X Protease Digestion Master Mix.
4. Use a sterile disposable scalpel with a flat blade to scrape the tissue in a single direction, then collect the tissue into a cohesive mass on the tip of the scalpel blade.

5. Carefully insert the scalpel blade with the tissue mass into the 1X Protease Digestion Master Mix in the AutoLys M Tube. Rinse the tissue from the blade into the buffer, then ensure that the entire mass is in solution.



6. Remove and inspect the blade to ensure that no tissue remains on it.

7. Inspect the slide to ensure that all the tissue is removed (the slide should be translucent). Discard the scalpel in a waste container for sharp objects.

8. Cap each tube securely, then gently flick the tube to mix and to immerse the tissue.

   If the tissue adheres to the side of the tube, use a sterile pipette tip to push the tissue into the solution.

Digest with protease

To minimize the amount of time between protease digestion and starting the purification run on the instrument, prepare the reagents and consumables that are needed by the instrument during the 90°C incubation (step 2).

1. Incubate at 60°C for ≥60 minutes in an AutoLys M Tube Rack.

   Incubation at 60°C can be extended to 2 hours to increase DNA yields. We recommend that you incubate low yielding samples such as core needle biopsy and fine needle aspirate samples for 2 hours to maximize yield.

   Note: Ensure that the incubator loaded with sample tubes has sufficient space to allow the rapid transfer of heat within the incubator—do not overcrowd with tubes.

2. Securely retighten each tube cap to limit evaporation, then incubate at 90°C for 60 minutes.
   - If using a single incubator, keep the sample in the incubator while the temperature increases. Start timing when the temperature reaches 90°C.
   - Set up the FFPE DNA and RNA Purification Plate 1 during the incubation.
   - Prepare the reagents and consumables that are needed by the instrument during the incubation. See “Prepare the consumables” on page 55.
   - Equilibrate the Quantitation Plate to room temperature during the incubation.

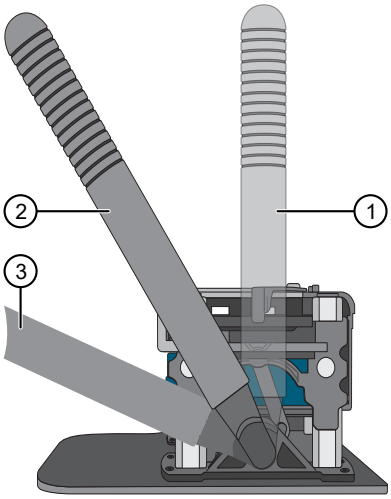
3. Allow samples to cool to room temperature for at least 10 minutes before proceeding to lift the tubes.

4. Lift the tubes. The following steps describe use of the AutoLys M TubeLifter to process up to 24 samples simultaneously. Alternatively, AutoLys M Tube Pliers can be used to process tubes individually.
   a. Ensure that the AutoLys M TubeLifter lever is in Position A (straight up) and the slider is in Position 1.

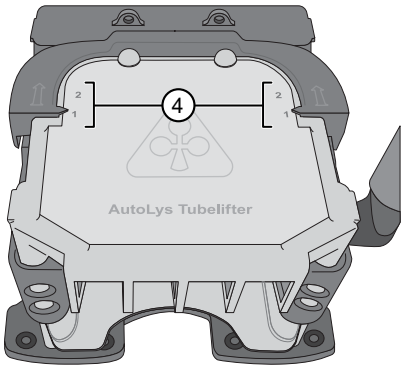
b. Slide the 24-well AutoLys M Tube Rack that contains the lysed samples into the AutoLys M TubeLifter.

c. Press the lever down from Position A to Position B, then remove the rack from the lifter.

For more information about use of the AutoLys M TubeLifter, see the *AutoLys M TubeLifter User Guide* (Pub. No. MAN0017676).



① AutoLys M TubeLifter lever Position A
② AutoLys M TubeLifter lever Position B
③ AutoLys M TubeLifter lever Position C



④ AutoLys M TubeLifter slider positions 1 & 2

5. Slide the AutoLys M Tube Locking Lid onto the rack, then centrifuge the samples at 2,000 × *g* for 10 minutes.

IMPORTANT!

- Ensure that the embossed arrow of the AutoLys M Tube Locking Lid points away from the center of rotation (landscape orientation) when placed in the centrifuge (see the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475)).
- Ensure that AutoLys M Tube caps are securely held by the AutoLys M Tube Locking Lid before you start the centrifugation.

6. Using the AutoLys M TubeLifter or AutoLys M Tube Pliers, separate the filter from the outer tube.
   a. Adjust the position of the AutoLys M TubeLifter slider to Position 2.
   b. Remove the AutoLys M Tube Locking Lid, then slide the rack into the AutoLys M TubeLifter.
   c. Press the lever down from Position B to Position C.

Keep the samples on ice or at 4°C.

Proceed to “Load the instrument and start the run” on page 55.

STOPPING POINT If needed, transfer samples to a separate labelled 1.5-mL Eppendorf™ LoBind™ tube and store at 4°C for up to 4 days or frozen at –30°C to –10°C for up to 7 days.

Load the instrument and start the run

This section describes how to perform the following procedures.

- Set up the instrument for use by loading all of the required reagents and consumables.
- Start a run.

Note: Do NOT load any consumables onto the instrument until after the instrument has performed the prerun UV cleaning.

Materials required

- Genexus™ FFPE DNA and RNA Purification (Part. No. A45532)
  - FFPE DNA and RNA Purification Plate 1
  - FFPE DNA and RNA Purification Plate 2
  - DNase (yellow cap)
  - DNase Buffer (blue cap)
  - 12-Well Tip Comb
- Genexus™ Nucleic Acid Quantitation (Part. No. A45538)
  - Quantitation Plate
  - Quantitation Tube
- Genexus™ Purification Supplies 2 (Part. No. A45574)
  - 2 Purification Tip Cartridges
  - 48-Well Nucleic Acid Archive Plate
  - 48-Well Nucleic Acid Archive Plate Seal
- 96-Well Nucleic Acid Output Plate
- P200 pipette and filtered tips

Prepare the consumables

Note: Consumables can be prepared during the protease digestion 90°C incubation step to save time.

Remove all cartridges and consumables from their packaging, then place them on the bench at room temperature. Prepare the following cartridges and consumables.

- Genexus™ Purification Supplies 2
  - 2 Purification Tip Cartridges
  - 48-Well Nucleic Acid Archive Plate
  - 48-Well Nucleic Acid Archive Plate Seal
- 12-Well Tip Comb

Equilibrate the Quantitation Plate

IMPORTANT! Allow at least 30 minutes for the Quantitation Plate to equilibrate to room temperature.

The Quantitation Plate is required even if your run plan does not include sample quantitation.

The Quantitation Plate can be equilibrated to room temperature during the protease digestion to save time.

Centrifuge the Quantitation Plate at 1,000 × *g* for 30 seconds to collect the contents.

Add 1X DNase Digestion Master Mix to the FFPE DNA and RNA Purification Plate 2

Note: Addition of DNase is not required if purifying only DNA (purification protocol **FFPE DNA** is selected for the run). Proceed directly to "Add samples to FFPE DNA and RNA Purification Plate 1" on page 57.

The FFPE DNA and RNA Purification Plate 2 contains magnetic beads in row H.

1. Vortex the DNase Buffer and DNase supplied in the kit for ~5 seconds each, then briefly centrifuge to collect the contents.

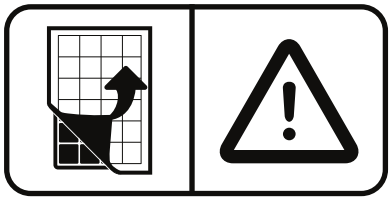
2. In a 1.5-mL low-retention microcentrifuge tube, prepare a 1X DNase Digestion Master Mix as indicated, where `n` is the number of samples.

Component	Volume per reaction
DNase Buffer (blue cap)	(`n` + 1) × 99 µL
DNase (yellow cap)	(`n` + 1) × 1.0 µL
Total volume	**(`n` + 1) × 100 µL**

3. Vortex for ~5 seconds to mix, then briefly centrifuge to collect the contents.

4. Centrifuge the FFPE DNA and RNA Purification Plate 2 at 1,000 × *g* for 30 seconds to collect the contents.

5. Carefully remove the plate seal without disturbing the contents.



6. Pipet 100 µL 1X DNase Digestion Master Mix into each well that is used in Row A of the FFPE DNA and RNA Purification Plate 2.

Add samples to FFPE DNA and RNA Purification Plate 1

The FFPE DNA and RNA Purification Plate 1 contains magnetic beads in row B.

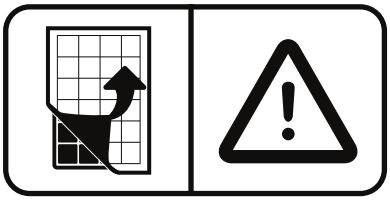
Note: You can add the samples to the FFPE DNA and RNA Purification Plate 1 and load the plate as the final steps of loading the instrument. This can be done to help ensure that other components are successfully loaded and accepted by the instrument before adding samples of possibly limited supply to the purification plate.

1. Centrifuge the plate at 1,000 x *g* for 30 seconds to collect the contents.

   IMPORTANT! Do not create bubbles when preparing the plate.

2. Inspect the plate to ensure that the contents of all rows are at the bottom of the wells.

3. Carefully remove the plate seal without disturbing the contents.



4. Transfer 200 µL of each sample to an individual well in row A of the prefilled FFPE DNA and RNA Purification Plate 1.

   Add samples to consecutive wells starting with sample 1 in well A1, through sample 12 in well A12 as defined in the run plan. Do not skip wells.

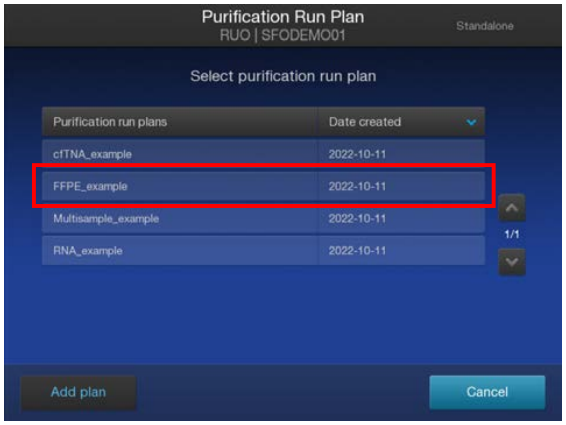
   A precipitate can form, but this does not interfere with the DNA binding. Proceed directly to the next step.

   Reagent consumables cannot be reused.

   IMPORTANT! Load the plate in the instrument (see page 60) within 15 minutes of adding samples to the plate.

Start the purification run

1. In the instrument touchscreen, tap **Run**, then tap to select the run plan that you created for this run.



2. Ensure that the correct run plan is selected, then tap **Next**.

3. *(Optional)* Import sample information.

   The import overwrites the existing **Sample ID** and **Notes** information for each sample. That is, if the run plan has 6 samples, the sample import file must include information for at least 6 samples.

   To import sample information, prepare a CSV sample import file and save it to a USB drive. See .

   a. In the **Sample Assignment** screen, tap **Manage Samples**.

   b. In the **Manage Samples** screen, tap **Import**.

   c. In the **Sample Import** screen, tap **Import** to proceed.

   d. Insert the USB drive that contains the sample import CSV file into the USB port on the front of the purification instrument. In the **Sample Import** screen, select the USB drive, then navigate to and select the sample import file.

   e. *(Optional)* Tap **Details** to view the CSV file that lists the sample names to be imported.

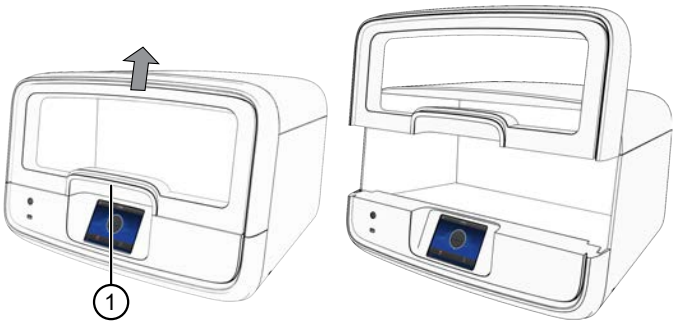
   f. Tap **Import**, then in the **Import Successful** screen, tap **OK**.

      The imported sample information is shown in the **Manage Samples** screen. If needed, select a sample, then tap **Edit** to modify the **Sample ID** or **Notes**.

4. Tap **Next**.

   The instrument performs a 2-minute UV cleaning, then unlocks the door.

5. Lift the instrument door to the stop.



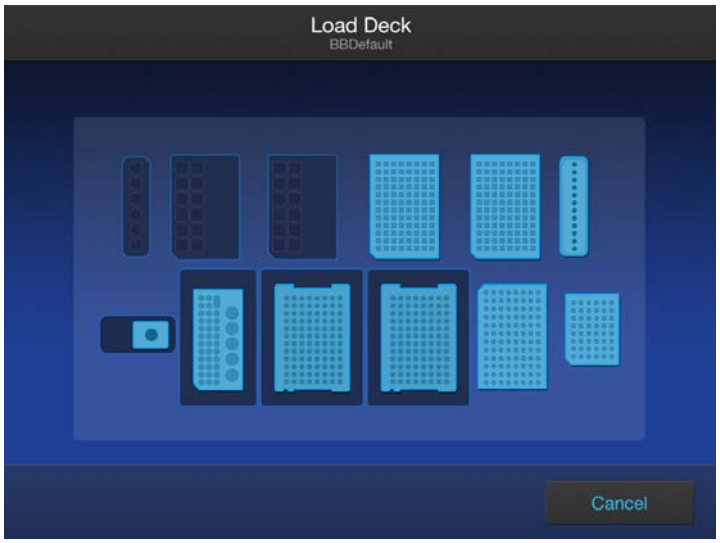
① Hold here, then lift.

Load the Genexus™ Purification System

IMPORTANT!

- Do NOT load any consumables onto the instrument until after the instrument has performed the prerun UV cleaning.
- Ensure that all components are clean and dry before loading them onto the instrument.
- Ensure that the reagent and quantitation station compartments are free of condensate before loading components. If needed, use a lint-free wipe to dry the compartment.

Follow the on-screen prompts to load the Genexus™ Purification System.

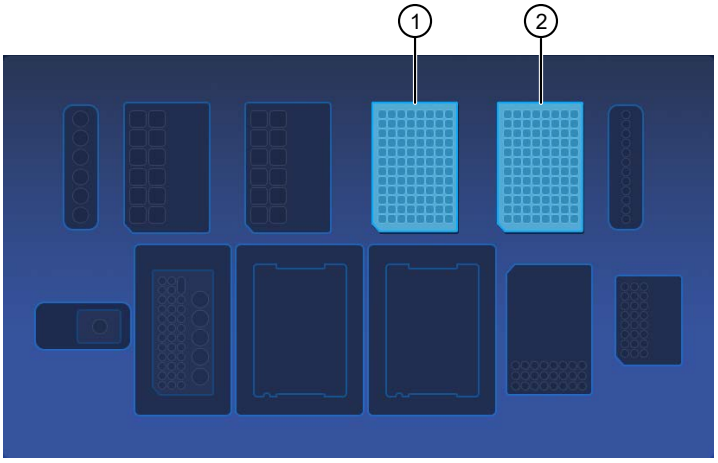


Screen display for an instrument fully loaded for an FFPE sample purification run.

Load FFPE DNA and RNA Purification Plate 1 & 2

1. Load the FFPE DNA and RNA Purification Plate 1 (DNA plate) prepared in step 4 of "Add samples to FFPE DNA and RNA Purification Plate 1" on page 57.

2. Load the FFPE DNA and RNA Purification Plate 2 (RNA plate) prepared in step 6 of "Add 1X DNase Digestion Master Mix to the FFPE DNA and RNA Purification Plate 2" on page 56.

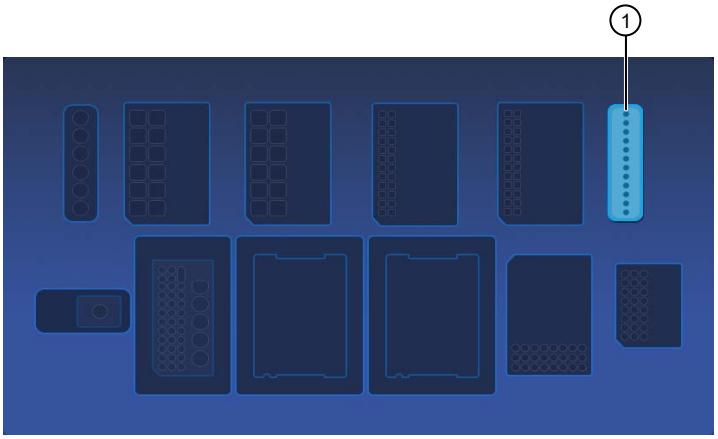


① FFPE DNA and RNA Purification Plate 1 position
② FFPE DNA and RNA Purification Plate 2 position

Load the 12-Well Tip Comb, Purification Tip Cartridges, 96-Well Nucleic Acid Output Plate, and 48-Well Nucleic Acid Archive Plate

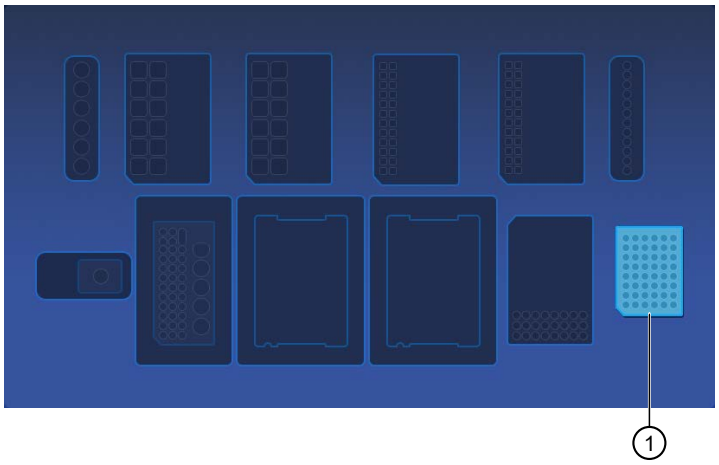
1. Unwrap, then load a new 12-Well Tip Comb.

   Ensure that the 12-Well Tip Comb is straight and that the tabs are not bent or broken. If needed, gently bend the tip comb in the opposite direction to the curvature to straighten the tip comb before installing it.



① 12-Well Tip Comb position

2. Unwrap, then load a new 48-Well Nucleic Acid Archive Plate.



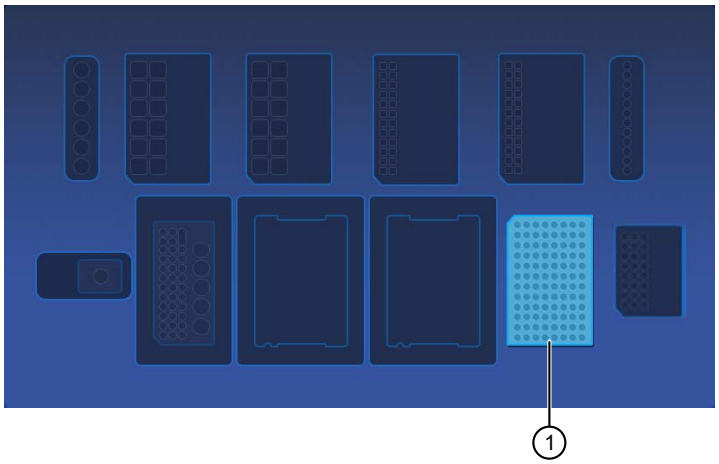
① 48-Well Nucleic Acid Archive Plate position

Note: Ensure that the 48-Well Nucleic Acid Archive Plate is seated properly. If the plate is curved or bent, gently bend the plate in the direction opposite to the curvature to straighten the plate before loading.

3. *(Integrated configuration)* Load a new 96-Well Nucleic Acid Output Plate into the output plate position.

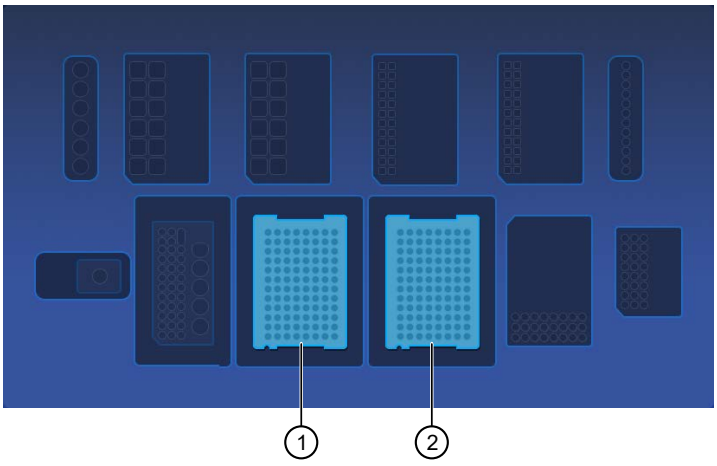
   The 96-Well Nucleic Acid Output Plate is not required when performing the purification in standalone configuration. The samples are in the 48-Well Nucleic Acid Archive Plate on completion of the purification run.

   After a **Sample to Result** purification run, the 96-Well Nucleic Acid Output Plate becomes the sample plate to be loaded in the Genexus™ Integrated Sequencer.



① 96-Well Nucleic Acid Output Plate position

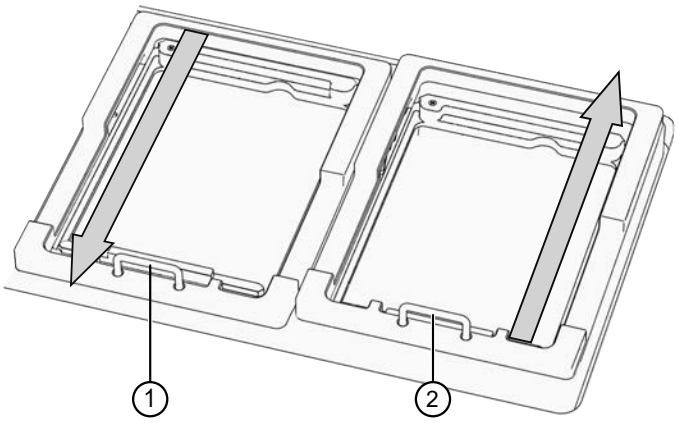
4. Unwrap two new Purification Tip Cartridges, remove the cover to expose the pipette tips, then load the cartridges in positions 1 and 2.



① Purification Tip Cartridges position 1
② Purification Tip Cartridges position 2

   a. Pull the locking mechanism handle forward (callout 1), then place the tip box in the open position.

   b. Push the locking mechanism handle back (callout 2) to lock the tip box in place.



① Unlocked (forward)
② Locked (back)

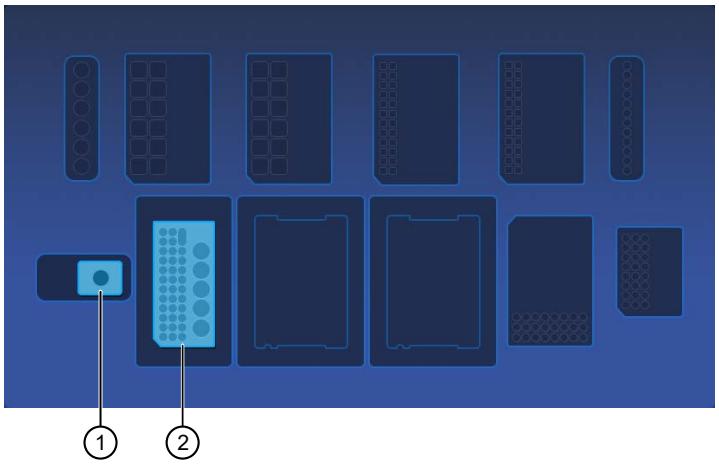
Load the quantitation reagents and consumables

IMPORTANT!

- Protect the Quantitation Plate from direct bright light to prevent photobleaching of the preloaded reagents.
- The Quantitation Plate is required even if your run plan does not include sample quantitation.
- The Quantitation Tube is not required if your run plan does not include sample quantitation.
- Ensure the Quantitation Plate and the Quantitation Tube have equilibrated to room temperature.

1. Centrifuge the Quantitation Plate at 1,000 × *g* for 30 seconds to collect the contents.

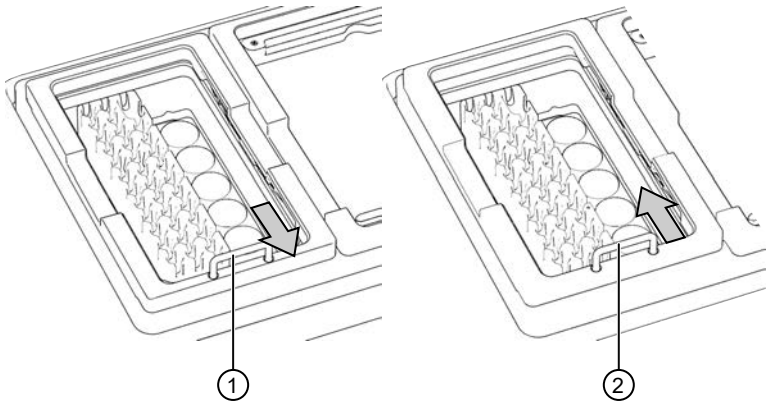
2. Load the Quantitation Plate in position 2.



① Quantitation Tube position
② Quantitation Plate position

   a. Pull the locking mechanism handle forward, then place the Quantitation Plate in the open position.

   b. Push the locking mechanism handle back to lock the plate in place.

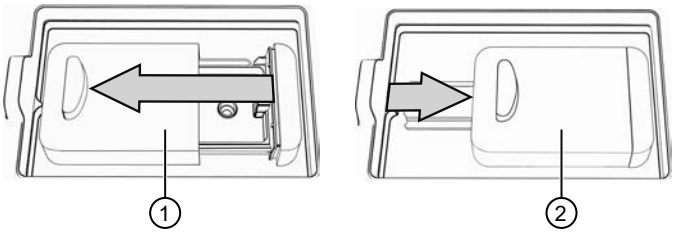


① Unlocked (forward)

② Locked (back)

3. *(If needed)* Slide and hold the quantitation module cover to the left, then insert the Quantitation Tube. **Touching only the top of the tube, press down firmly** to properly seat the tube, then allow the module cover to close.

WARNING! Do not force the module cover closed. Forcing the module cover closed can damage the instrument.



① Open ② Closed

Confirm that consumables are installed correctly

IMPORTANT! To support correct and safe instrument operation, confirm that all consumables are installed correctly on the deck before you start a run. The instrument vision system confirms that required reagents are in place, no reagents are expired, and foil seals are removed. The vision system does not verify all aspects of the consumable setup before beginning each run.

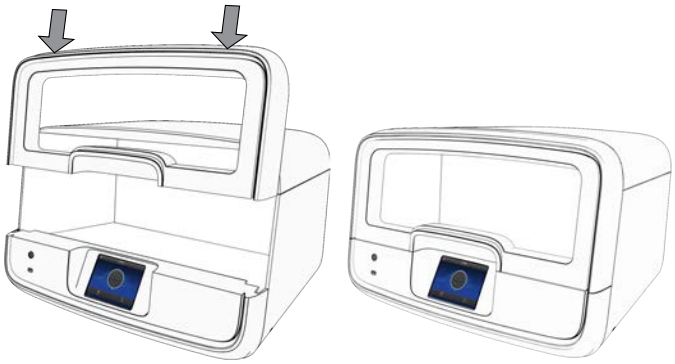
1. Confirm the following.
   - Foil seals are removed from the purification plates. Do not remove foil seal from the Quantitation Plate.
   - Each component is at the correct location and in the correct orientation. Press down on all plates and cartridges to ensure that they are firmly seated in place.
   - The Tip Combs are in place.
   - The Quantitation Plate is in the correct location, is in the correct orientation, and is locked in place.
   - *(If needed)* The Quantitation Tube is firmly seated in the quantitation module.
   - Each Purification Tip Cartridge is in the correct location, in the correct orientation, and locked in place.

   If the vision system detects an error, the location indicator does not turn gray in the touchscreen.

2. If needed, tap **Help**, then accept each warning message appropriately to proceed.

Start the run

1. When all reagents and consumables are loaded in the purification instrument, tap **Next**.
2. Close the instrument door by pressing down on both top corners. Ensure that the door is locked after closing it.



   The instrument vision system confirms that all reagents are in place and are not expired.

3. Tap **Start**.

   The time remaining until the purification is complete is displayed, and the interior lighting turns green.

   - If you need to stop the run for any reason, tap **Cancel**, then tap **Yes** to confirm the cancellation. A canceled run cannot be resumed. You must restart a run from the beginning.
   - The interior lighting turns off during quantitation, then turns blue when the run is complete.
   - If the instrument encounters a problem during the run, it aborts the run and displays the error on the instrument touchscreen. The interior lighting turns red.

When the run is complete, the interior light turns blue, and the touchscreen displays **Run Complete**. Quantitation results are available immediately. See “View and export quantitation results” on page 68.

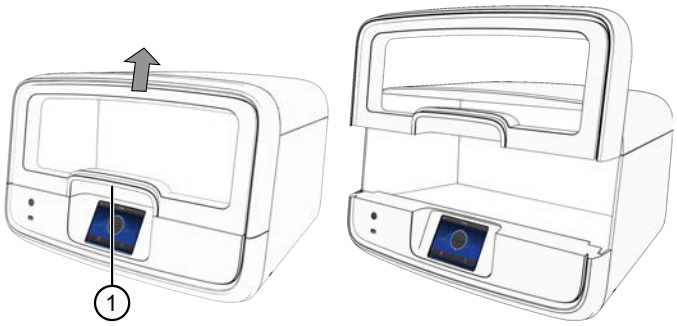
Unload purified DNA and RNA

IMPORTANT! Do not allow purified nucleic acids to sit on the instrument. Store as directed or use in sequencing reactions within 60 minutes.

1. In the touchscreen, tap **Unload**.

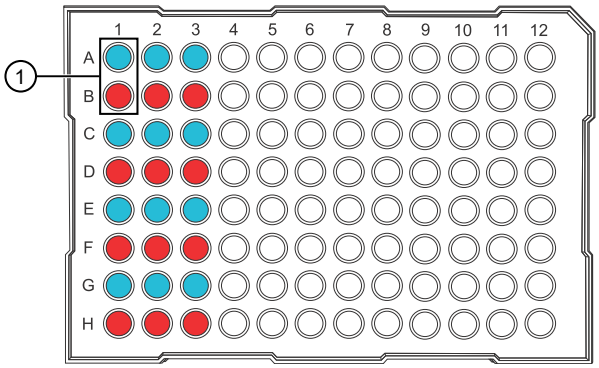
The door unlocks.

2. Lift the instrument door to access the instrument deck.



① Hold here, then lift.

3. ***(Sample to Result** run)* Remove the 96-Well Nucleic Acid Output Plate that contains the purified sample DNA and RNA that is ready for the addition of positive or nontemplate sample sequencing controls. For more information, see . Store on ice or at 4°C. If quantitation was performed, the sample concentration information is visible in the Genexus™ Software. Alternatively, determine sample concentrations manually, if needed.



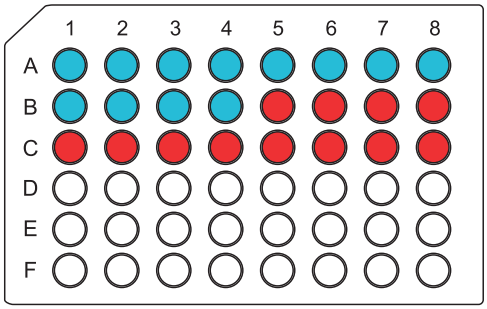
① Paired purified DNA-A1 and RNA-B1 from sample 1.
C1 + D1 = sample 2,
E1 + F1 = sample 3,
and so on.

DNA

RNA

STOPPING POINT If not sequencing immediately, seal the plate with an Adhesive PCR Plate Foil (Cat. No. AB0626), then store the plate at –20°C for up to 1 week. For long term storage (>1 week), transfer the samples to labeled low-retention tubes, then store the DNA samples at –30°C to –10°C and the RNA samples at –90°C to –70°C for up to 36 months.

4. Remove the 48-Well Nucleic Acid Archive Plate that contains the purified sample DNA and RNA.



DNA is in wells A1–B4. DNA samples always start in well A1.

RNA is in wells B5–C8. RNA samples always start in well B5 regardless of how many samples are purified.

Note: *(Purification only configuration)* If using the purified DNA or RNA immediately, transfer the sample to a sample input plate for sequencing. To determine the sample concentrations, see "View and export quantitation results" on page 68. For more information, see the relevant assay user guide for **Nucleic Acid to Result** run guidance.

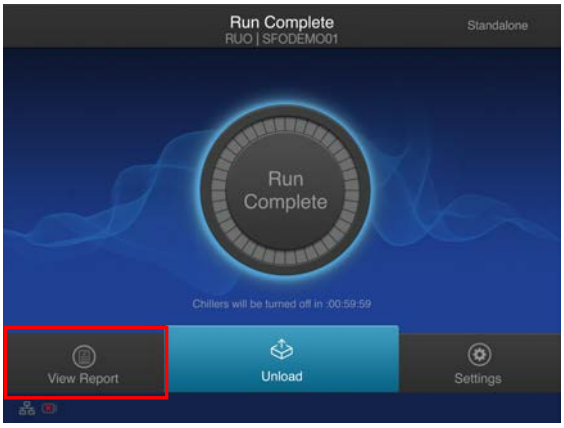
5. For short-term storage, seal the plate with a 48-Well Nucleic Acid Archive Plate Seal. Store the plate at –20°C for up to 3 months. For long-term storage (>3 months), transfer samples to labeled low-retention tubes, then store the DNA samples at –30°C to –10°C and the RNA samples at –90°C to –70°C for up to 36 months.

   If the archive plate is thawed during short-term storage, transfer the DNA and RNA into labeled low-retention tubes. Do not reseal the archive plate with the used plate seal.

View and export quantitation results

Purification runs that include sample quantitation produce sample concentration results (ng/µL) that can be accessed after the run is complete. In integrated configuration, view the **Run Report** that is available in the Genexus™ Software to see the sample concentrations. In standalone configuration, sample concentration results can be accessed from the **Run Complete** screen or the **Home** screen as described here.

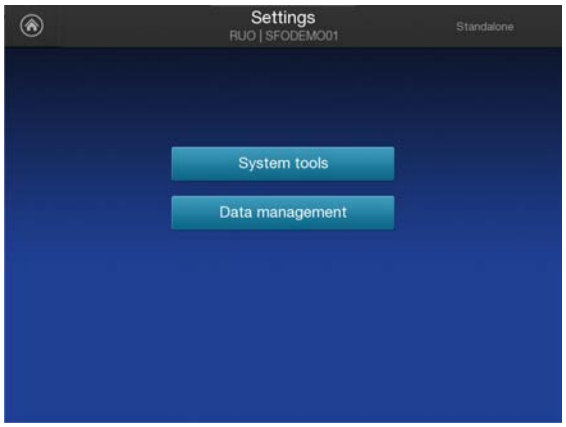
1. In the **Run Complete** screen, tap **View report**.



   The **Saved Experiment Reports** screen opens. See step 4.

2. At any time after unloading and UV cleaning the instrument, sample concentration results can be accessed through the **Home** screen. Tap ⚙ (**Settings**).

3. In the **Settings** screen, tap **Data Management**.



4. In the **Saved Experiment Reports** screen, tap ⌄ or ⌃ to page through the list. Locate the **Experiment Name** of interest, tap in the row to select the experiment, then tap **View Report**.

5. In the **Run Report** screen, tap **Quant Results** to view the sample concentration results. Results displayed in the **Concentration** column are in ng/µL.

6. Insert a USB drive into the USB port on the front of the instrument, then tap **Export CSV**. Navigate to the file destination, then tap **Save**.

Dispose of used consumables and UV clean the instrument

Unload purified nucleic acid samples before disposal of used consumables.

IMPORTANT! Do not allow purified nucleic acids to sit on the instrument. Store as directed or use in sequencing reactions within 60 minutes.

---

1. Remove and discard the deep-well sample input plates.
   a. Remove the FFPE DNA and RNA Purification Plate 1 from the instrument.
   b. Dispose of the liquid waste by tipping the deep-well plate on one corner and pouring the waste into an appropriate liquid biohazardous waste container.

WARNING! Liquid waste contains guanidine thiocyanate, dispose of properly.

   c. Dispose of the deep-well plate in an appropriate waste container.
   d. Repeat substep 1a through substep 1c to discard the FFPE DNA and RNA Purification Plate 2.
2. Unlock, then remove and dispose of the Purification Tip Cartridges in an appropriate waste container.

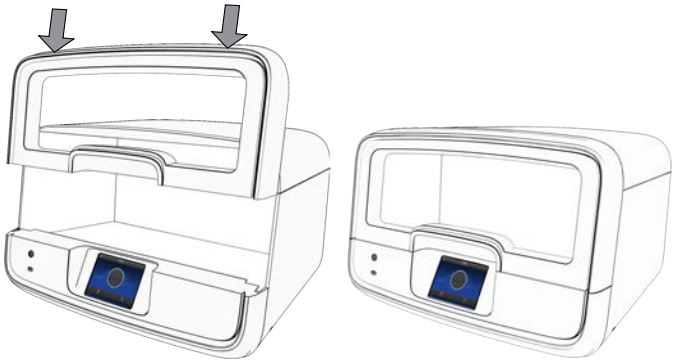
3. Unlock, then remove and dispose of the Quantitation Plate.
   a. Dispose of the liquid waste by tipping the deep-well plate on one corner and pouring the waste into an appropriate liquid biohazardous waste container.

WARNING! No data are currently available that address the mutagenicity or toxicity of the Qubit™ RNA BR Reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ RNA BR Reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

   b. Dispose of the deep-well plate in an appropriate waste container.

4. Open the quantitation module cover, remove and discard the Quantitation Tube, then allow the module cover to close.

5. Close and lock the instrument door by pressing down on both top corners, then tap **Start UV Clean**.



The time remaining in the UV cleaning is displayed. When complete, the instrument is ready to start a new purification run.

6 Genexus™ Cell-Free Total Nucleic Acid Purification protocol

Genexus™ Cell-Free Total Nucleic Acid Purification workflow

5 min

Plan a purification run (page 74)

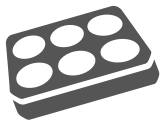
IMPORTANT! If performing a **Sample to Result** run, plan the run in the Genexus™ Software. See "Plan a Sample to Result run" on page 35.

To purify samples for use in **Nucleic Acid to Result** runs or other nonsequencing applications, run the Genexus™ Purification System in standalone configuration. Add a new purification run plan or copy-edit an existing purification run plan that best represents your experiment. Purification run plans contain instrument settings that are used in sample purification.

30 min

Equilibrate the Quantitation Plate to room temperature (page 77)

The Quantitation Plate requires equilibration to room temperature for at least 30 minutes before use. To save time, experienced users can take the Quantitation Plate out of 4°C storage before creating a run plan and preparing samples.



45 min

Prepare cell-free plasma from whole blood samples (page 78)

Whole blood samples are centrifuged to separate plasma from other components.

2 min

Load the Genexus™ Purification System (page 79)

The purification run plan is selected, and the run is initiated. The instrument performs a UV cleaning, then samples, reagents, and consumables are loaded onto the instrument.

2 hr

Start the run (page 91)

After the sample plate, reagents, and consumables are loaded, the instrument door is closed, and the run is started.

Depending on the number of samples purified, quantification adds ~45 minutes to the run time.

Genexus™ Cell-Free Total Nucleic Acid Purification workflow

5 min

Unload the purified nucleic acids (page 92)

In integrated configuration, remove the 96-Well Nucleic Acid Output Plate and proceed to sequence the purified sample.

In standalone configuration, remove and seal the 48-Well Nucleic Acid Archive Plate, then store as directed, or proceed to sequence the purified sample.

Used reagents and consumables are removed from the instrument and the instrument performs a UV cleaning.

Plan a purification run (standalone configuration)

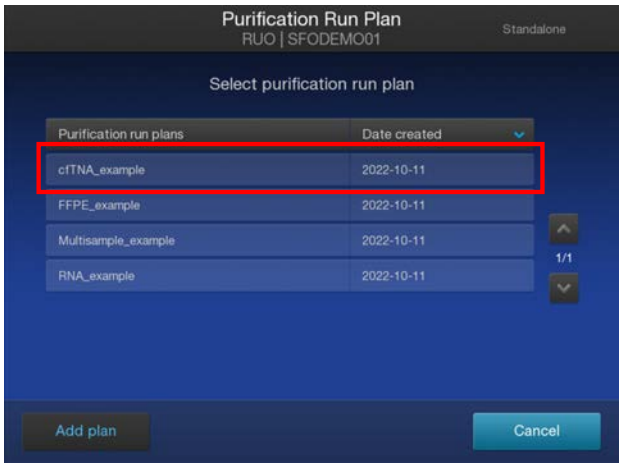
Note: If running the Genexus™ Purification System in integrated configuration, see "Plan a Sample to Result run" on page 35 to plan a **Sample to Result** run in the Genexus™ Software.

In standalone configuration, plan a purification-only run through the instrument touchscreen. After purification is complete, all purified samples are transferred to an archive plate for storage.

Plan a Genexus™ Cell-Free Total Nucleic Acid Purification run

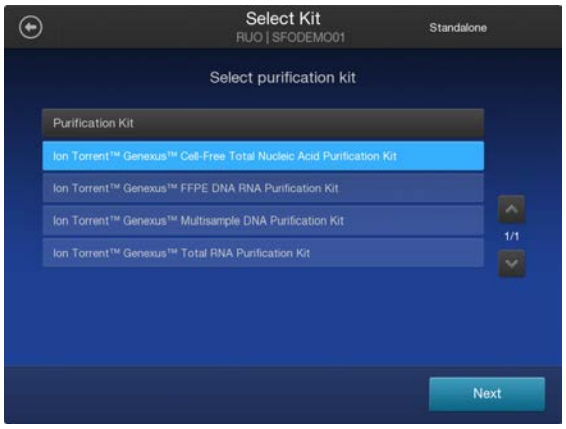
Create a run plan before you prepare samples and load the samples into the Cell-Free Total Nucleic Acid Purification Plate 1. However, experienced users can save time by creating the run plan during the centrifugation steps of sample preparation.

1. Enter your username and password to sign in to the instrument.
2. Tap **Run**, then tap **Add plan**.

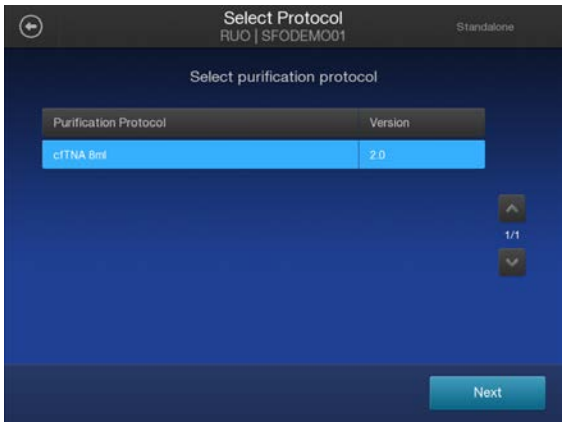


3. Tap in the entry box, enter a unique name for the run plan, then tap **Done** ▸ **Next**.

4. Select the Ion Torrent™ Genexus™ Cell-Free Total Nucleic Acid Purification Kit, then tap **Next**.



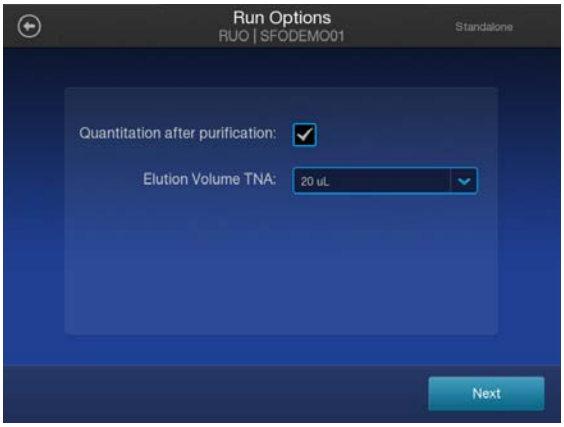
5. Select the appropriate purification protocol, then tap **Next**.



6. Enable or disable **Quantitation after Purification**.

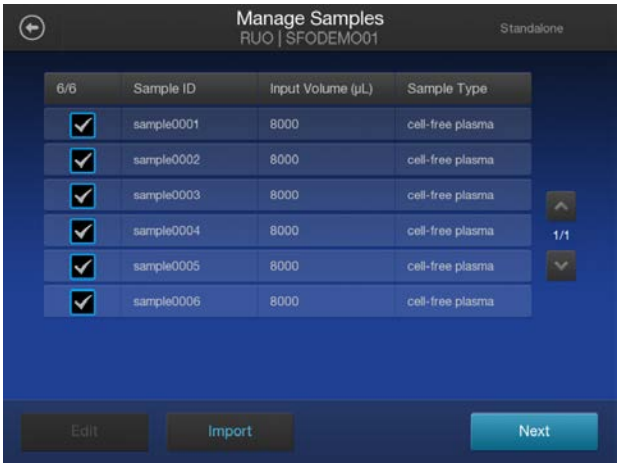
Note:

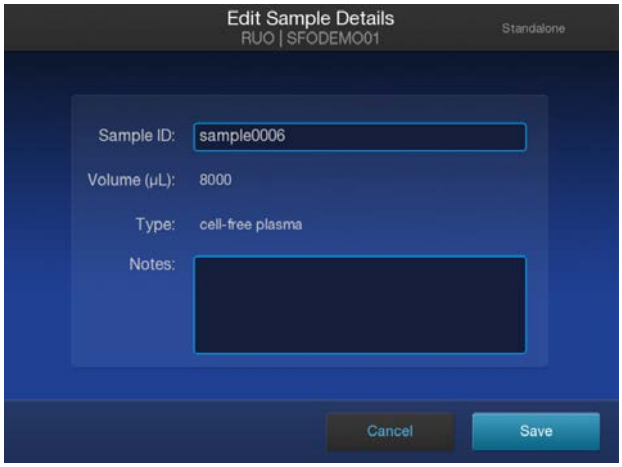
- The Quantitation Plate is required even if **Quantitation after Purification** is disabled.
- Disabling **Quantitation after Purification** can reduce the purification run time by up to 45 minutes.
- Quantitation requires up to 5 µL of the eluted sample. If the expected sample yield is limiting, manual sample quantitation using less volume may be preferred to preserve sample.



7. Select the desired elution volume from the dropdown list, then tap **Next**.

8. *(Optional)* Change the number of samples and the sample details.
   a. In the **Manage Samples** screen, deselect extra samples (for example, if you run only 4 samples, you must deselect samples 5 and 6). Do not deselect samples from in the middle of the range (for example, if you run only 4 samples, do not deselect sample 2, 3, or 4.)
   b. In the **Manage Samples** screen, tap on a sample ID to select the sample.
   c. Tap **Edit**, enter a new **Sample ID** and any **Notes**, then tap **Save**.
   d. Repeat substep 8b and substep 8c for each additional sample.
   e. Tap **Next**.





9. *(Optional)* Import sample information.

   The import overwrites the existing **Sample ID** and **Notes** information for each sample selected.

   In standalone configuration, prepare a CSV sample import file and save it to a USB drive to import sample information. See page 175.

   a. In the **Manage Samples** screen, select the samples to import sample information, then tap **Import**.

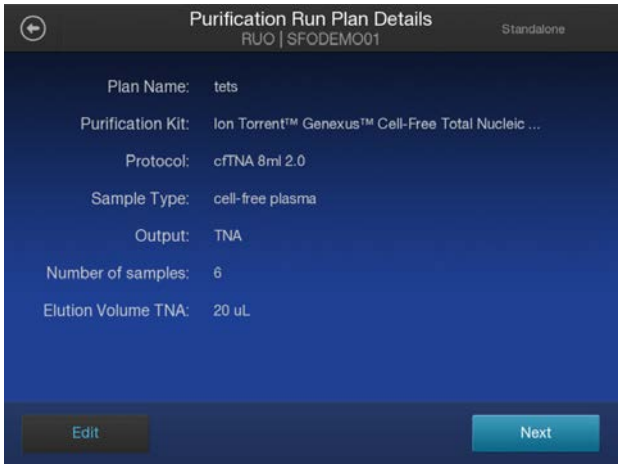
b. In the **Sample Import** screen, tap **Import** to proceed.

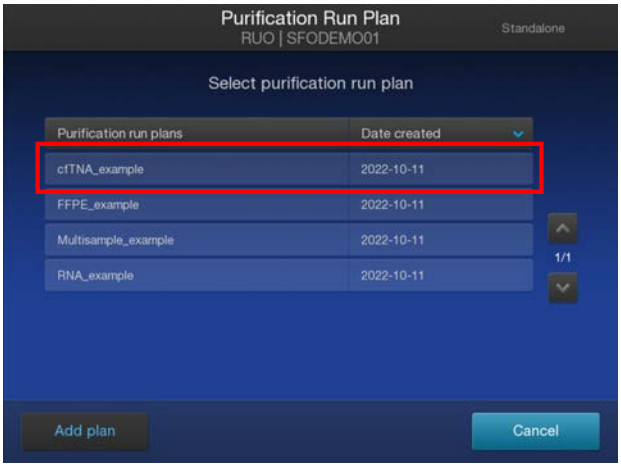
c. Insert the USB drive containing the sample import CSV file into the USB port on the front of the purification instrument. In the **Sample Import** screen, select the USB drive, then navigate to and select the sample import file.

d. *(Optional)* Tap **Details** to view the CSV file that lists the sample names to be imported.

e. Tap **Import**, then in the **Import Successful** screen, tap **OK**.
The imported sample information is shown in the **Manage Samples** screen. If needed, edit imported sample information as described in step 8.

10. Review the **Purification Run Plan Details**. Tap **Edit** to change selections. Otherwise tap **Next**.





The new purification run plan appears in the list of available **Purification run plans**.

Prepare the Quantitation Plate

Prepare the following cartridges and consumables:

- Genexus™ Nucleic Acid Quantitation (Part No. A45538)
  - Quantitation Plate
  - Quantitation Tube

Equilibrate the Quantitation Plate

IMPORTANT!

- Protect the Quantitation Plate from direct bright light to prevent photobleaching of the preloaded reagents.
- Allow at least 30 minutes for the Quantitation Plate to equilibrate to room temperature.
- The Quantitation Plate is required even if your run plan does not include sample quantitation.

1. Centrifuge the Quantitation Plate at 1,000 × *g* for 30 seconds to collect the contents.
2. Place the plate and Quantitation Tube on the bench next to the purification instrument.

Prepare cell-free plasma from whole blood samples

Procedural guidelines

IMPORTANT! Store all kit components that contain liquid in the upright orientation.

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Thawing or storing on ice can be substituted with thawing or storing at 4°C (2–8°C refrigerator or prechilled benchtop cold block).
- Blood specimens must be collected in K_2EDTA blood collection tubes.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- When working with whole blood, observe these guidelines.
  - Wear clean gloves and a clean laboratory coat.
  - Change gloves whenever you suspect that they are contaminated.
  - Open and close all sample tubes carefully. Avoid splashing or spraying samples.
  - Use a positive-displacement pipettor and RNase-free pipette tips.
  - Clean laboratory benches and equipment periodically with 10% bleach solution and rinse with 70% isopropanol.
  - When freezing whole blood or bone marrow aspirate samples for later use. We recommend that you freeze samples in aliquots up to 400 µL at –90°C to –70°C such that the entire sample is used for one purification.
- When working with cell-free RNA, observe these guidelines.
  - Wear clean gloves and a clean laboratory coat.
  - Change gloves whenever you suspect that they are contaminated.
  - Open and close all sample tubes carefully. Avoid splashing or spraying samples.
  - Use a positive-displacement pipettor and RNase-free pipette tips.
  - Clean laboratory benches and equipment periodically with an RNase decontamination solution, such as RNase*Zap*™ RNase Decontamination Solution (Cat. No. AM9780) or RNase *AWAY*™ Decontamination Reagent (Cat. No. 7005-11).
- The plate chiller shuts off 60 minutes after run completion. Remove the 96-Well Nucleic Acid Output Plate (integrated configuration) or 48-Well Nucleic Acid Archive Plate (standalone configuration) with purified nucleic acids from the instrument within 1 hour of run completion. Proceed immediately to sequencing, or properly store the nucleic acids until use.

Before each use of the kit

- If needed, thaw plasma samples—stored at –90°C to –70°C—at room temperature, then store on ice or at 4°C until use.
- Keep fresh plasma samples on ice or at 4°C until use.
- Centrifuge purification plates at 1,000 × *g* for 30 seconds to collect the contents.

Isolate cell-free plasma from whole blood

1. Centrifuge the blood sample at 2,000 × *g* for 10 minutes at 4°C.

   Up to 8 mL of plasma can be used as sample input. One 10 mL tube of whole blood yields approximately 4 mL plasma.

   Centrifuge the sample within 2 hours of blood draw. Ensure that the sample is centrifuged no later than 6 hours after blood draw.

   If a refrigerated centrifuge is not available, chill the sample on ice or in a prechilled 4°C cold block for 10 minutes, centrifuge at 2,000 × *g* for 10 minutes at room temperature, then immediately place the centrifuged sample on ice or 4°C cold block.

2. Transfer the plasma to a new 15-mL or 50-mL conical centrifuge tube. Volume should be 3–5 mL.

   IMPORTANT! Do not disturb the buffy coat layer when transferring the plasma layer.

   A 1X centrifuged plasma sample can be stored overnight at 4°C, or long term at –90°C to –70°C.

3. Centrifuge the plasma sample at 6,000 × *g* for 30 minutes at 4°C.

   Alternatively, centrifuge the plasma sample at 16,000 × *g* for 10 minutes at 4°C.

4. Transfer the supernatant to a fresh tube, note the volume of cell-free plasma, then keep on ice or at 4°C until use.

   Store cell-free plasma samples at –90°C to –70°C for long term storage.

Load the instrument and start the run

This section describes how to perform the following procedures.

- Set up the instrument for use by loading all required reagents and consumables.
- Start a run.

Note: Do NOT load any consumables onto the instrument until after the instrument has performed the prerun UV cleaning.

Materials required

- Genexus™ Cell-Free Total Nucleic Acid Purification (Part No. A45535)
  - Cell-Free Total Nucleic Acid Purification Plate 1
  - Cell-Free Total Nucleic Acid Purification Plate 2
  - Cell-Free Total Nucleic Acid Purification Plate 3
  - 6-Well Tip Comb
  - 12-Well Tip Comb
  - Proteinase K (red cap)
  - cfTNA Lysis/Binding Solution
- Genexus™ Nucleic Acid Quantitation (Part No. A45538)
  - Quantitation Plate
  - Quantitation Tube
- Genexus™ Purification Supplies 1 (Part No. A45529)
  - Purification Tip Cartridge
  - 48-Well Nucleic Acid Archive Plate (not required for integrated configuration)
  - 48-Well Nucleic Acid Archive Plate Seal (not required for integrated configuration)
- MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (96-Well Nucleic Acid Output Plate, integrated configuration only)
- 200- and 1000-µL pipettors, and filtered tips
- 1000-µL multichannel pipettor and reagent reservoir (recommended)

Prepare the consumables

Remove all cartridges and consumables from their packaging, then place them on the bench at room temperature.

Prepare the following cartridges and consumables:

- Genexus™ Purification Supplies 1
  - Purification Tip Cartridge
  - 48-Well Nucleic Acid Archive Plate
  - 48-Well Nucleic Acid Archive Plate Seal
- 12-Well Tip Comb
- 6-Well Tip Comb
- MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode (96-well output plate)

Add cfTNA Lysis/Binding Solution to Cell-Free Total Nucleic Acid Purification Plate 1 & 3

To prepare the Cell-Free Total Nucleic Acid Purification Plate 1 and the Cell-Free Total Nucleic Acid Purification Plate 3 for use, add cfTNA Lysis/Binding Solution to the plates.

1. Remove the plate seal from Cell-Free Total Nucleic Acid Purification Plate 1.

2. Add 2.5 mL of cfTNA Lysis/Binding Solution to each well in the plate, A1 through D6.

   a. Attach six 1,000 µL pipette tips on a 1,000 µL multichannel pipettor, then set the volume on the pipettor to 833 µL.

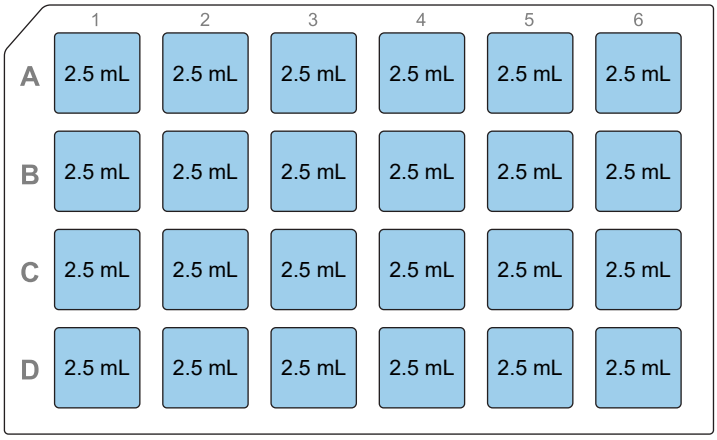
   If the tip spacing of the multichannel pipettor is not adjustable, attach tips into alternate channels to fit into plate 1 wells.

   b. Transfer ~70 mL of the cfTNA Lysis/Binding Solution to a 50–100-mL reagent reservoir.

   Use one bottle per run to avoid cross-contamination between runs.

   c. Use the multichannel pipettor to dispense 833 µL cfTNA Lysis/Binding Solution to each well A1–A6, then B1–B6, and so on. Well A1 is located at the notched corner of the plate.

   d. Repeat substep 2c two more times to load a total volume of 2.5 mL into each of the 24 wells.

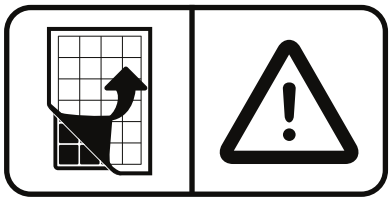


cfTNA Lysis/Binding Solution loaded in 24 wells of plate 1

   e. Visually inspect the plate to ensure that the wells are uniformly filled to 2.5 mL, then set the plate aside until you are ready to add samples. See “Add samples to Cell-Free Total Nucleic Acid Purification Plate 1” on page 89.

- If you are purifying fewer than 6 samples, add cfTNA Lysis/Binding Solution to wells in each row of the plate columns for the number of samples that you have, but start with wells A1, B1, and so on.
- If a multichannel pipettor is not available, use a single-channel pipettor or repeat pipettor to add cfTNA Lysis/Binding Solution to the plates. We do not recommend the use of serological pipettes.
- To prevent contaminants from falling into wells after adding the solution, cover the plate loosely with the original plate seal, or cover with a clean object such as a 96-well plate cover.

3. Carefully remove the plate seal from Cell-Free Total Nucleic Acid Purification Plate 3 without disturbing the contents of the plate.



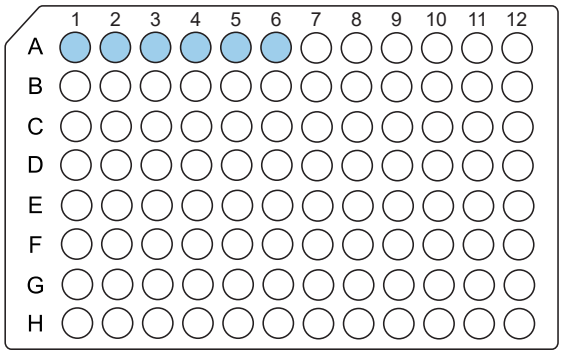
4. Add 440 µL of cfTNA Lysis/Binding Solution to wells A1 through A6.

   a. Attach six 1,000 µL pipette tips on a 1,000 µL multichannel pipettor, then set the volume on the pipettor to 440 µL.

   b. If needed, transfer additional cfTNA Lysis/Binding Solution from the bottle into the reagent reservoir. Use the remaining solution from substep 2b.

      Use one bottle per run to avoid cross-contamination between runs.

   c. Use the multichannel pipettor to dispense 440 µL cfTNA Lysis/Binding Solution to each well A1–A6.



cfTNA Lysis/Binding Solution loaded in wells A1–A6 of plate 3 (440 µL/well)

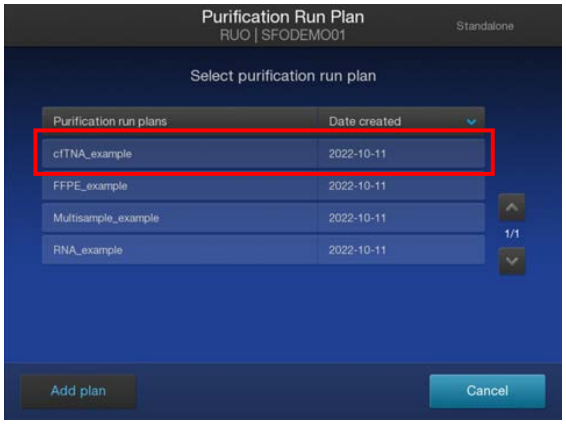
- If you are purifying fewer than 6 samples, add cfTNA Lysis/Binding Solution to row A wells of the plate columns for the number of samples to purify, but start with well A1.
- If a multichannel pipettor is not available, use a single-channel pipettor or repeat pipettor to add cfTNA Lysis/Binding Solution to the plates. We do not recommend the use of serological pipettes.

5. Discard excess cfTNA Lysis/Binding Solution into an appropriate liquid biohazardous waste container.

WARNING! Liquid waste contains guanidine thiocyanate. Dispose of waste appropriately.

Start the purification run

1. In the instrument touchscreen, tap **Run**, then tap to select the run plan that you created for this run.



2. Ensure that the run plan selected is correct, then tap **Next**.

3. *(Optional)* Import sample information.

   The import overwrites the existing **Sample ID** and **Notes** information for each sample. That is, if the run plan has 6 samples, the sample import file must include information for at least 6 samples.

   To import sample information, prepare a CSV sample import file and save it to a USB drive. See .

   a. In the **Sample Assignment** screen, tap **Manage Samples**.

   b. In the **Manage Samples** screen, tap **Import**.

   c. In the **Sample Import** screen, tap **Import** to proceed.

   d. Insert the USB drive that contains the sample import CSV file into the USB port on the front of the purification instrument. In the **Sample Import** screen, select the USB drive, then navigate to and select the sample import file.

   e. *(Optional)* Tap **Details** to view the CSV file listing the sample names that are to be imported.

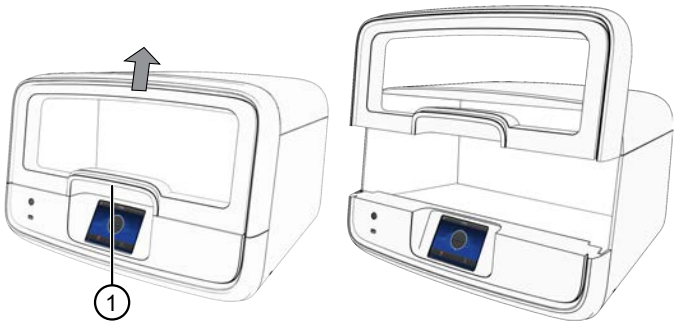
   f. Tap **Import**, then in the **Import Successful** screen, tap **OK**.

      The imported sample information is shown in the **Manage Samples** screen. If needed, select a sample then tap **Edit** to modify the **Sample ID** or **Notes**.

4. Tap **Next**.

   The instrument performs a 2-minute UV cleaning, then unlocks the door.

5. Lift the instrument door to the stop.



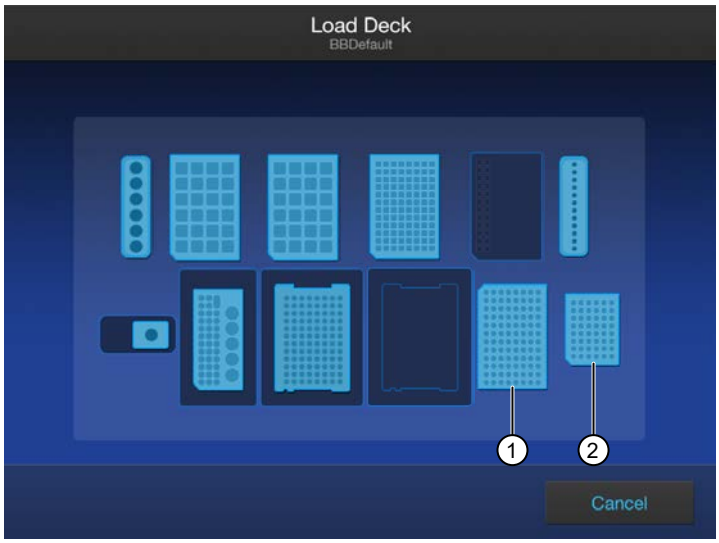
① Hold here, then lift.

Load the Genexus™ Purification System

IMPORTANT!

- Do NOT load any consumables onto the instrument until after the instrument has performed the prerun UV cleaning.
- Ensure that all components are clean and dry before loading them onto the instrument.
- Ensure that the reagent and quantitation station compartments are free of condensate before loading components. If needed, use a lint-free wipe to dry the compartment.

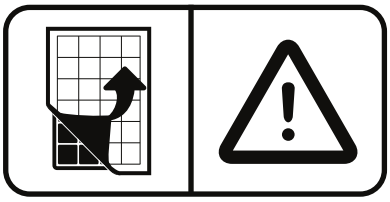
Follow the on-screen prompts to load the Genexus™ Purification System.



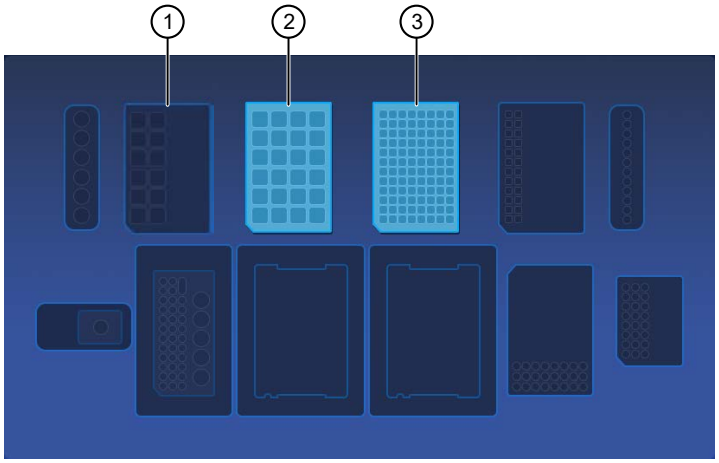
① 96-Well Nucleic Acid Output Plate, not needed when performing the purification in standalone configuration.
② 48-Well Nucleic Acid Archive Plate, not needed when performing the purification in integrated configuration.

Load Cell-Free Total Nucleic Acid Purification Plate 2 & 3

1. Carefully remove the plate seal from Cell-Free Total Nucleic Acid Purification Plate 2 without disturbing the contents of the plate.



2. Load the 24 deep-well Cell-Free Total Nucleic Acid Purification Plate 2 in position 2.



① Cell-Free Total Nucleic Acid Purification Plate 1 position (do not load yet)
② Cell-Free Total Nucleic Acid Purification Plate 2 position
③ Cell-Free Total Nucleic Acid Purification Plate 3 position

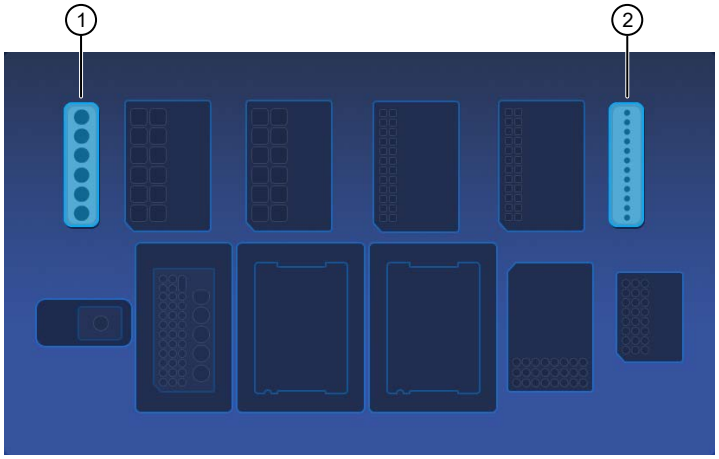
3. Load the 96 deep-well Cell-Free Total Nucleic Acid Purification Plate 3 in position 3.

Note: Ensure that the plate has 440 µL cfTNA Lysis/Binding Solution manually loaded in wells A1–D6.

Load the tip combs, Purification Tip Cartridge, 96-Well Nucleic Acid Output Plate, and 48-Well Nucleic Acid Archive Plate

1. Unwrap, then load a new 6-Well Tip Comb and 12-Well Tip Comb.

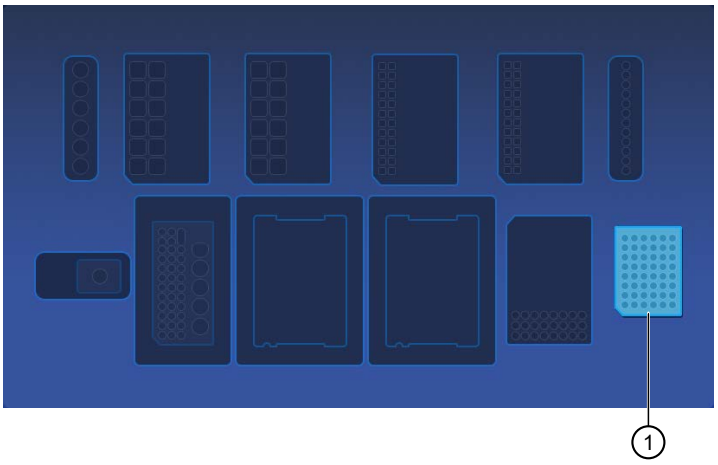
   Ensure that each tip comb is straight and that the tabs are not bent or broken. If needed, gently bend the tip comb in the opposite direction to the curvature to straighten the tip comb before installing it.



① 6-Well Tip Comb position
② 12-Well Tip Comb position

2. *(Standalone configuration only)* Unwrap, then load a new 48-Well Nucleic Acid Archive Plate.

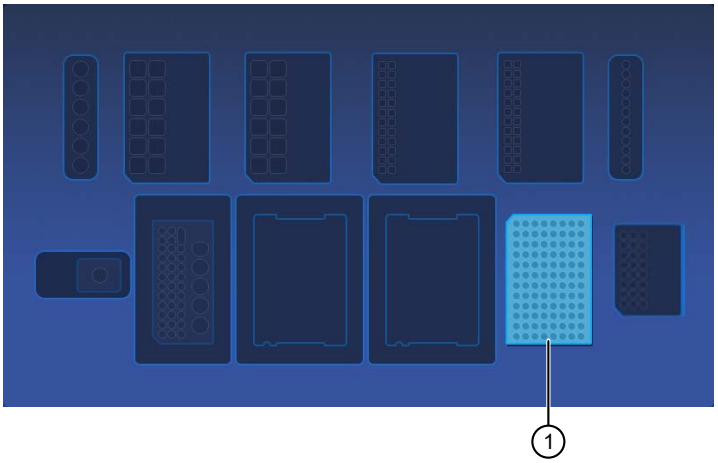
   The 96-Well Nucleic Acid Output Plate is not required when performing the purification in standalone configuration.



① 48-Well Nucleic Acid Archive Plate position

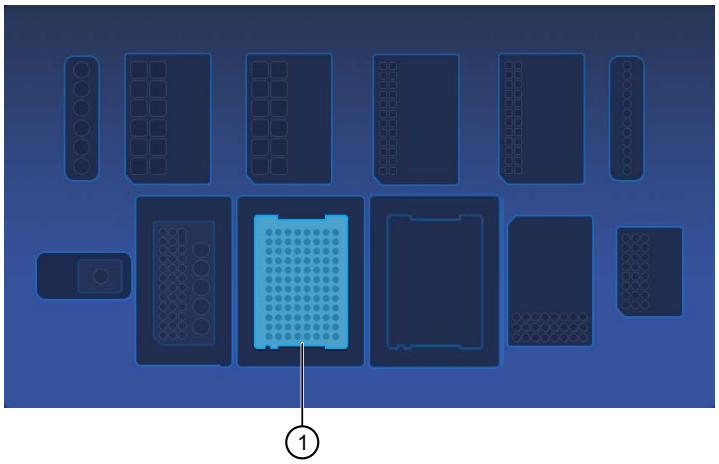
3. *(Integrated configuration only)* Load a new 96-Well Nucleic Acid Output Plate into the output plate position.

   After a purification run, the 96-Well Nucleic Acid Output Plate becomes the sample plate to be loaded in the Genexus™ Integrated Sequencer. The 48-Well Nucleic Acid Archive Plate is not required when performing the purification in integrated configuration.



① 96-Well Nucleic Acid Output Plate position

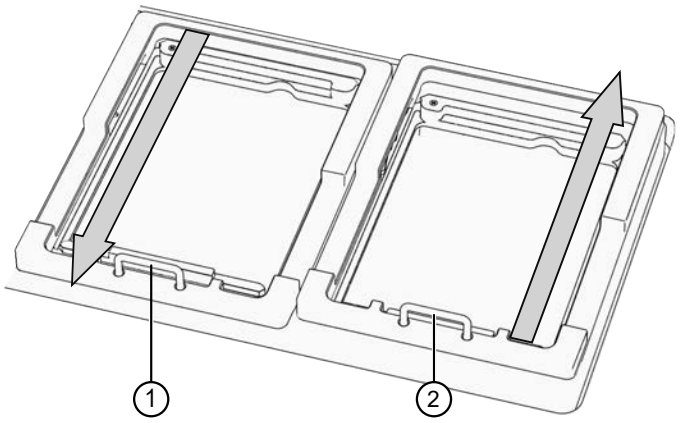
4. Unwrap a Purification Tip Cartridge, remove the cover to expose the pipette tips, then load it in position 1.



① Purification Tip Cartridge position 1

   a. Pull the locking mechanism handle forward (callout 1, below), then place the tip box in the open position.

   b. Push the locking mechanism handle back (callout 2) to lock the tip box in place.



① Unlocked (forward) ② Locked (back)

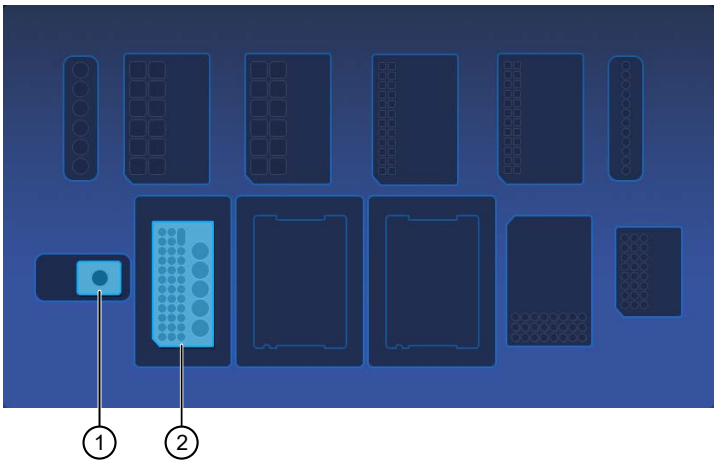
Load the quantitation reagents and consumables

IMPORTANT!

- Protect the Quantitation Plate from direct bright light to prevent photobleaching of the preloaded reagents.
- The Quantitation Plate is required even if your run plan does not include sample quantitation.
- The Quantitation Tube is not required if your run plan does not include sample quantitation.
- Ensure the Quantitation Plate and the Quantitation Tube have equilibrated to room temperature.

1. Centrifuge the Quantitation Plate at 1,000 × *g* for 30 seconds to collect the contents.

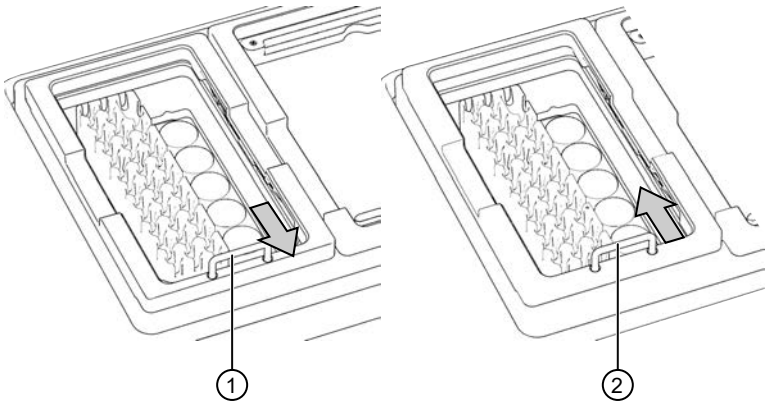
2. Load the Quantitation Plate in position 2.



① Quantitation Tube position
② Quantitation Plate position

   a. Pull the locking mechanism handle forward, then place the Quantitation Plate in the open position.

   b. Push the locking mechanism handle back to lock the plate in place.

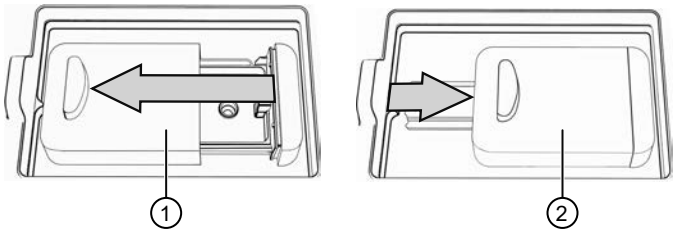


① Unlocked (forward)

② Locked (back)

3. *(If needed)* Slide and hold the quantitation module cover to the left, then insert the Quantitation Tube. **Touching only the top of the tube, press down firmly** to properly seat the tube, then allow the module cover to close.

WARNING! Do not force the module cover closed. Forcing the module cover closed can damage the instrument.



① Open ② Closed

Add samples to Cell-Free Total Nucleic Acid Purification Plate 1

IMPORTANT!

- Before adding samples, ensure that cfTNA Lysis/Binding Solution is added to the Cell-Free Total Nucleic Acid Purification Plate 1.
- Prepare and load all reagents and consumables, except the Cell-Free Total Nucleic Acid Purification Plate 1, on the instrument, before adding Proteinase K to the samples to avoid overdigestion.
- Do not create bubbles when preparing the plate.
- During the wash steps, the Wash Solution may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.

1. Inspect the plate to ensure that the contents of all rows are at the bottom of the tubes. If needed, centrifuge the plate at 1,000 × *g* for 30 seconds to collect the contents.

   Alternatively, gently flick or tap the plate on the bench to force the reagents to the bottoms of the tubes.

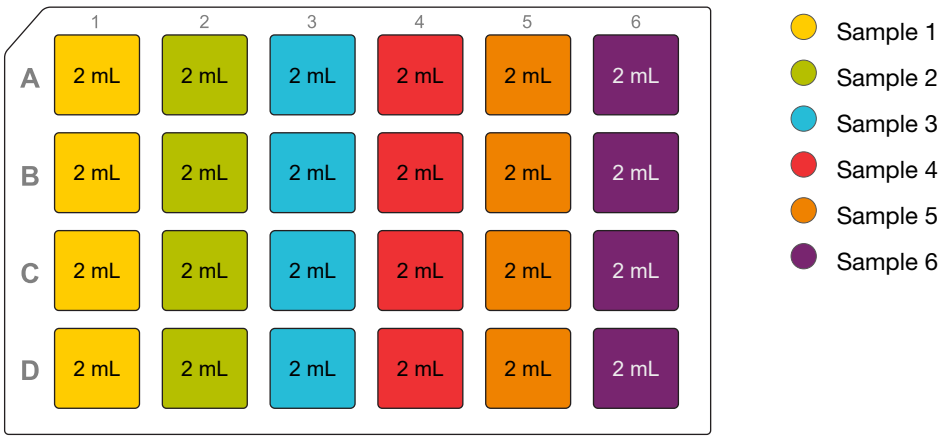
2. If the plasma sample volume is less than 8 mL, add 1X PBS to a final volume of 8 mL.

   Plasma sample volumes less than 8 mL can result in suboptimal performance.

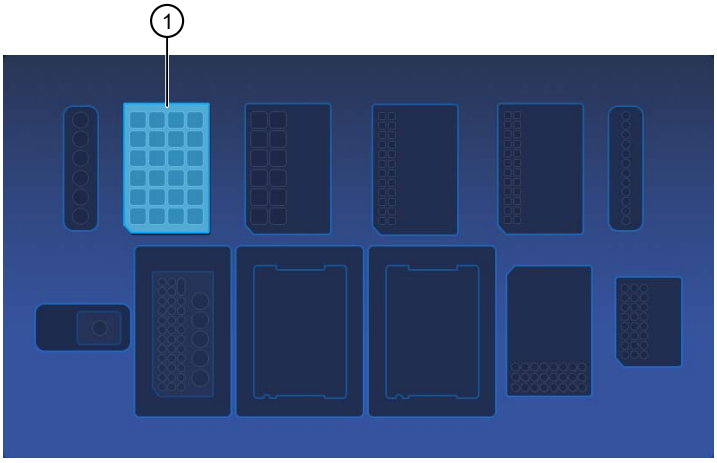
3. Add 50 µL Proteinase K to each sample, then mix by inverting the tube 5–10 times.

4. Transfer each sample into the 4 wells of a single column (2 mL per well) in the prefilled 24 deep-well Cell-Free Total Nucleic Acid Purification Plate 1.

   Add samples to consecutive columns beginning with sample 1 in column 1, through sample 6 in column 6. Do not skip wells.



5. Load the Cell-Free Total Nucleic Acid Purification Plate 1 with the samples in position 1.



① Cell-Free Total Nucleic Acid Purification Plate 1 position

Confirm that consumables are installed correctly

IMPORTANT! To support correct and safe instrument operation, confirm that all consumables are installed correctly on the deck before you start a run. The instrument vision system confirms that required reagents are in place, no reagents are expired, and foil seals are removed. The vision system does not verify all aspects of the consumable setup before beginning each run.

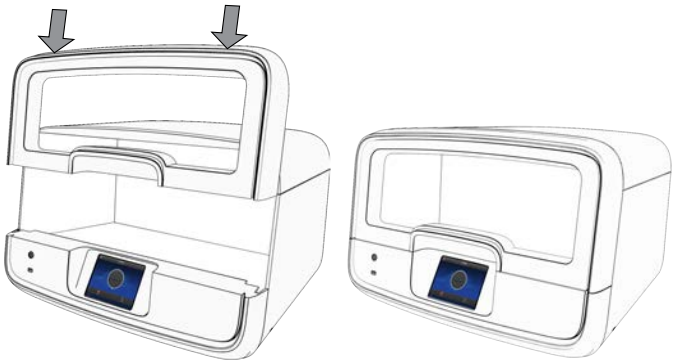
1. Confirm the following.
   - Foil seals are removed from the purification plates. Do not remove foil seal from the Quantitation Plate.
   - Each component is at the correct location and in the correct orientation. Press down on all plates and cartridges to ensure that they are firmly seated in place.
   - The Tip Combs are in place.
   - The Quantitation Plate is in the correct location, is in the correct orientation, and is locked in place.
   - *(If needed)* The Quantitation Tube is firmly seated in the quantitation module.
   - Each Purification Tip Cartridge is in the correct location, in the correct orientation, and locked in place.

   If the vision system detects an error, the location indicator does not turn gray in the touchscreen.

2. If needed, tap **Help**, then accept each warning message appropriately to proceed.

Start the run

1. When all reagents and consumables are loaded in the purification instrument, tap **Next**.
2. Close the instrument door by pressing down on both top corners. Ensure that the door is locked after closing it.



   The instrument vision system confirms that all reagents are in place and are not expired.

3. Tap **Start**.

   The time remaining until the purification is complete is displayed and the interior lighting turns green.

   - (Integrated configuration) Quantitation is turned off by default to preserve sample.
   - If you have to stop the run for any reason, tap **Cancel**, then tap **Yes** to confirm the cancellation. A canceled run cannot be resumed. You must restart a run from the beginning.
   - The interior lighting turns off during quantitation, then turns blue when the run is complete.
   - If the instrument encounters a problem during the run, it aborts the run and displays the error on the instrument touchscreen. The interior lighting turns red.

When the run is complete, the interior light turns blue, and the touchscreen displays **Run Complete**. Quantitation results are available immediately. See “View and export quantitation results” on page 94.

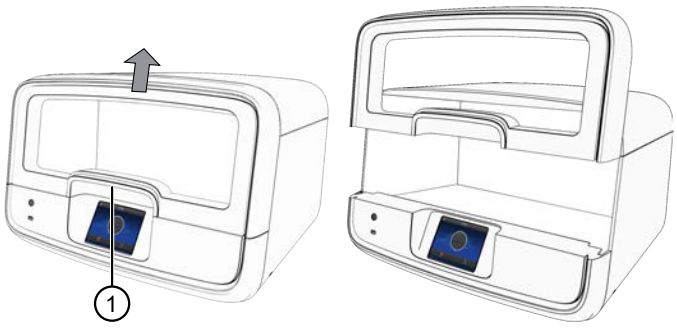
Unload purified cell-free TNA

IMPORTANT! Do not allow purified nucleic acids to sit on the instrument. Store as directed or use in sequencing reactions within 60 minutes.

1. In the touchscreen, tap **Unload**.

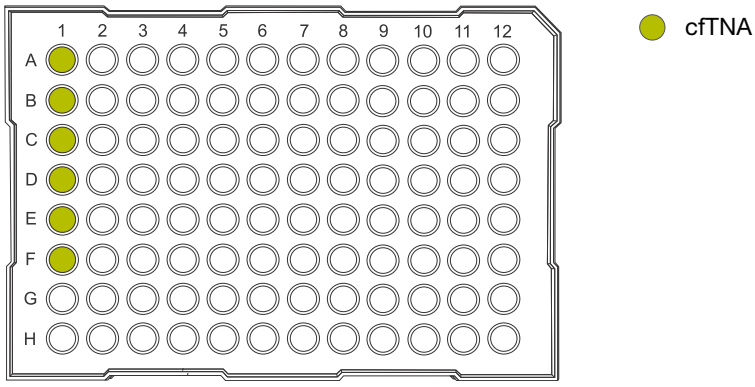
The door unlocks.

2. Lift the instrument door to access the instrument deck.



① Hold here, then lift.

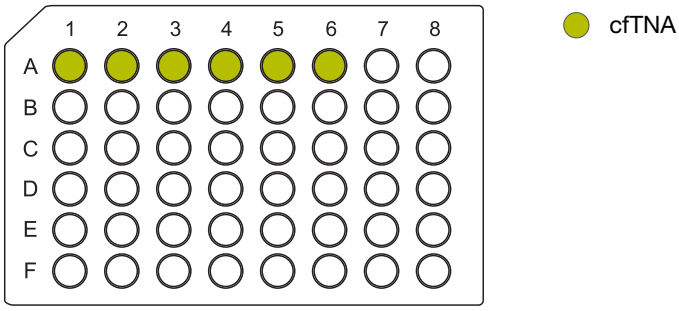
3. *(Integrated configuration)* Remove the 96-Well Nucleic Acid Output Plate that contains the purified sample cfTNA that is ready for the addition of positive or nontemplate sample sequencing controls. For more information, see "Transfer DNA and RNA samples to the sample input plate" on page 105. Store on ice or at 4°C. Quantitation is turned off by default to preserve the sample. Determine sample concentrations manually, if needed.



The 48-Well Nucleic Acid Archive Plate is not used.

STOPPING POINT If not sequencing immediately, seal the plate with an Adhesive PCR Plate Foil (Cat. No. AB0626), then store the plate at –20°C for up to 1 week. For long term storage (>1 week), transfer the samples to labeled low-retention tubes, then store at –90°C to –70°C for up to 12 months.

4. *(Standalone configuration)* Remove the 48-Well Nucleic Acid Archive Plate, containing the purified sample cell-free TNA in row A.



If using the purified cfTNA immediately, transfer the sample to a sample input plate for sequencing. To determine the sample concentrations, see "View and export quantitation results" on page 94 if quantitation was performed. Alternatively, determine sample concentrations manually, if needed. For more information, see the relevant assay user guide for **Nucleic Acid to Result** guidance.

5. For short term storage, seal the plate with a 48-Well Nucleic Acid Archive Plate Seal. Store the plate at –20°C for up to 3 months. For long-term storage (>3 months), transfer samples to labeled low-retention tubes, then store cfTNA samples at –90°C to –70°C for up to 12 months.

   If the archive plate is thawed during short-term storage, transfer the cfTNA into labeled low-retention tubes. Do not reseal the archive plate with the used plate seal.

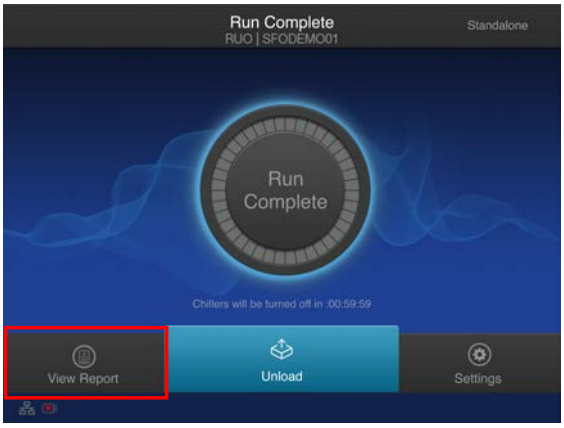
6. For long-term storage, transfer samples to labeled low-retention tubes, then store cfTNA samples at –90°C to –70°C for up to 6 months.

View and export quantitation results

Purification runs that include sample quantitation produce sample concentration results (ng/µL) that can be accessed after the run is complete. In standalone configuration, sample concentration results can be accessed from the **Run Complete** screen or the **Home** screen as described here.

Note: (Integrated configuration) Quantitation is turned off by default to preserve the sample.

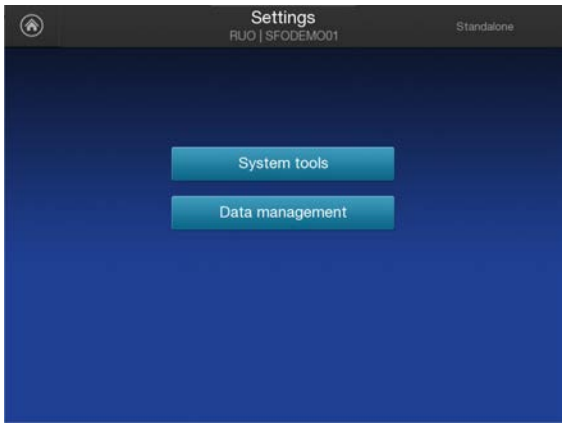
1. In the **Run Complete** screen, tap **View report**.



   The **Saved Experiment Reports** screen opens. See step 4.

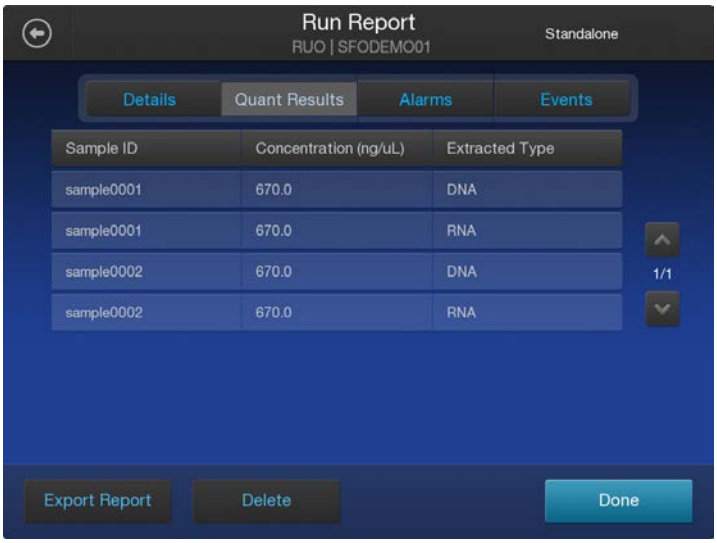
2. At any time after unloading and UV cleaning the instrument, sample concentration results can be accessed through the **Home** screen. Tap ⚙ (**Settings**).

3. In the **Settings** screen, tap **Data Management**.



4. In the **Saved Experiment Reports** screen, tap ⌄ or ⌃ to page through the list. Locate the **Experiment Name** of interest, tap in the row to select the experiment, then tap **View Report**.

5. In the **Run Report** screen, tap **Quant Results** to view the sample concentration results. Results displayed in the **Concentration** column are in ng/µL.



6. Insert a USB drive into the USB port on the front of the instrument, then tap **Export CSV**. Navigate to the file destination, then tap **Save**.

Dispose of used consumables and UV clean the instrument

Unload purified nucleic acid samples before disposal of used consumables.

IMPORTANT! Do not allow purified nucleic acids to sit on the instrument. Store as directed or use in sequencing reactions within 60 minutes.

---

1. Remove and discard the deep-well sample input plates.
   a. Remove the Cell-Free Total Nucleic Acid Purification Plate 1 from the instrument.
   b. Dispose of the liquid waste by tipping the deep-well plate on one corner and pouring the waste into an appropriate liquid biohazardous waste container.

WARNING! Liquid waste contains guanidine thiocyanate, dispose of properly.

   c. Dispose of the deep-well plate in an appropriate waste container.
   d. Repeat substep 1a through substep 1c to discard both the Cell-Free Total Nucleic Acid Purification Plate 2 and the Cell-Free Total Nucleic Acid Purification Plate 3.
2. Unlock, then remove and dispose of the Purification Tip Cartridges in an appropriate waste container.

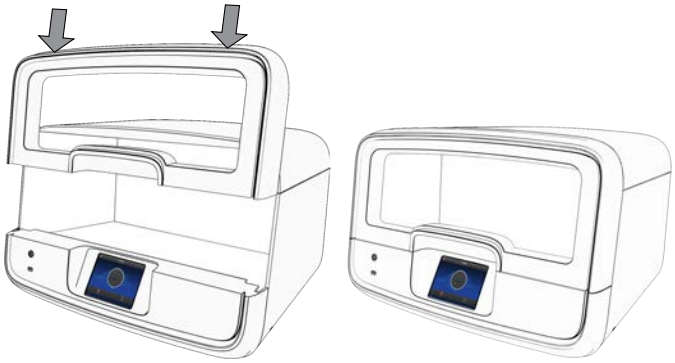
3. Unlock, then remove and dispose of the Quantitation Plate.
   a. Dispose of the liquid waste by tipping the deep-well plate on one corner and pouring the waste into an appropriate liquid biohazardous waste container.

WARNING! No data are currently available that address the mutagenicity or toxicity of the Qubit™ RNA BR Reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ RNA BR Reagent with the same safety precautions as all other potential mutagens and dispose of the dye in accordance with local regulations.

   b. Dispose of the deep-well plate in an appropriate waste container.

4. *(If quantitation was performed)* Open the quantitation module cover, remove and discard the Quantitation Tube, then allow the module cover to close.

5. Close and lock the instrument door by pressing down on both top corners, then tap **Start UV Clean**.



The time remaining in the UV cleaning is displayed. When complete, the instrument is ready to start a new purification run.

7 Prepare the sample input plate

After you have planned a run in Genexus™ Software, use the run setup guide provided by the software to load samples in the sample plate.

Chip capacity by sample type

Use the following guidance to determine how many samples you can load onto the GX5™ Chip. The capacity of the GX5™ Chip varies depending on the sample type. Chip capacity information applies to assays that use system-installed settings only.

IMPORTANT! Changes to system-installed assay settings have not been verified. We recommend using system-installed assay settings. Consult your local Field Service Engineer before changing default assay settings.

Table 4 GX5™ Chip max capacity by sample type – Nucleic Acid to Result Run

Assay	Capacity for 1 lane	Capacity for 2 lanes	Capacity for 3 lanes	Capacity for 4 lanes
No controls				
Oncomine™ Precision - GX5 - w3.4.2 - Liquid Biopsy cfTNA	1	3	4	6
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA	8	16	24	32
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - Fusions	8	16	24	32
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions	4	8	12	16
With NTC only				
Oncomine™ Precision - GX5 - w3.4.2 - Liquid Biopsy cfTNA	1	3	4	6

Table 4 GX5 Chip max capacity by sample type – Nucleic Acid to Result Run *(continued)*

Assay	Capacity for 1 lane	Capacity for 2 lanes	Capacity for 3 lanes	Capacity for 4 lanes
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA	8	16	24	31
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - Fusions	8	16	24	31
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions	4	8	12	15
With NTC and PC[1]				
Oncomine™ Precision - GX5 - w3.4.2 - Liquid Biopsy cfTNA	N/A	2	3	5
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA	7	15	23	30
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - Fusions	7	15	23	30
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions	3	7	11	14

[1] When running a Nucleic Acid to Report run, the PC cannot be added automatically. One sample must be treated as the positive control.

Dilute the samples and load the sample plate—Nucleic Acid to Result run

When performing a Nucleic Acid to Result run on the sequencer, you must quantify and dilute the samples, then load the sample input plate. If sample purification and quantification was performed using the Genexus™ Purification System in standalone configuration further sample quantification is not needed and sample concentrations are available in the instrument software. Alternatively, nucleic acid samples can be manually purified and quantified using a recommended kit, see “Recommended materials for nucleic acid isolation, quantification, and quality control” on page 21.

Note: The number of samples that can be purified in a single purification run on the Genexus™ Purification System can exceed the number of samples that can be accommodated in a single GX5™ Chip lane in a sequencing run.

Quantify nucleic acid samples

Isolate nucleic acid samples using one of the validated procedures and kits that are listed in "Recommended materials for nucleic acid isolation, quantification, and quality control" on page 21.

IMPORTANT! The correct input amount of nucleic acid is critical for assay success. Use a Qubit™ Fluorometer to accurately measure sample concentration before you proceed to "Dilute the samples (if needed) and load the sample plate" on page 101.

Analyze 1 µL of nucleic acid sample using a Qubit™ Fluorometer with one of the following assay kits. Follow the procedure in the corresponding user guide to prepare samples and standards, then calculate sample concentration.

Sample type	Kit	User Guide
DNA	Qubit™ dsDNA HS Assay Kit	*Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. MAN0002326)
RNA	Qubit™ RNA BR Assay Kit	*Qubit™ RNA BR Assay Kits User Guide* (Pub. No. MAN0001987)

Evaluate nucleic acid sample quality

In some cases, the sample concentration that is required for quality control analysis can be different from the sample concentration that is required for the sequencing reaction.

Note: Typically, cfTNA samples include enough gDNA to complete a successful sequencing run. If you have a limited amount of sample, you can omit the quality control analysis and proceed directly to "Dilute the samples (if needed) and load the sample plate" on page 101.

1. *(If needed)* Dilute a small portion of the sample to the recommended concentration range for quality control analysis.

2. Analyze 1 µL of each nucleic acid sample on the Agilent™ 2100 Bioanalyzer™ Instrument. For FFPE DNA samples, you can also use the Agilent™ 4200 TapeStation™ System. Follow the quality control procedure in the corresponding user guide.

Instrument	Kit	Sample type	Recommended concentration range
Agilent™ 2100 Bioanalyzer™ Instrument	Agilent™ High Sensitivity DNA Kit	FFPE DNA	5–500 pg/µL
		cfTNA	
	Agilent™ RNA 6000 Nano Kit	FFPE RNA	5–500 ng/µL
Agilent™ 4200 TapeStation™ System	Agilent™ D5000 Reagents and Agilent™ D5000 ScreenTape	FFPE DNA	0.1–50 ng/µL

When evaluated on the Agilent™ Bioanalyzer™ Instrument, FFPE DNA and FFPE RNA samples typically exhibit a broad smear that covers a size range of thousands of base pairs. The average size typically falls between 100 base pairs to ~3,000 base pairs, depending on the age and condition of the original sample. For best performance, use samples with an average size of ≥200 base pairs.

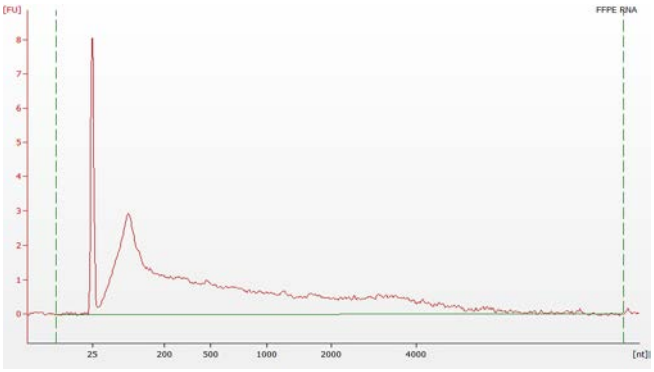


Figure 1 Example Bioanalyzer™ trace for an FFPE RNA sample

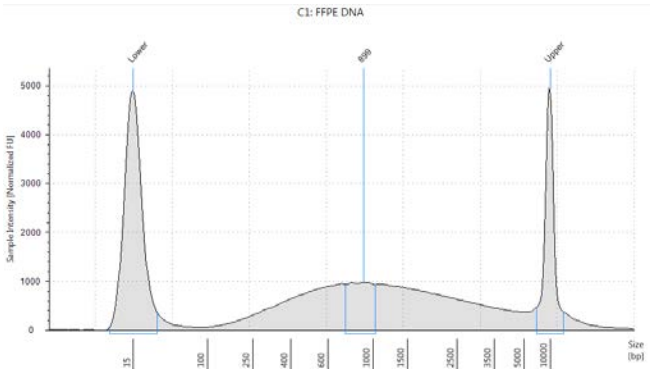


Figure 2 Example TapeStation™ trace for an FFPE DNA sample

When evaluated on the Agilent™ Bioanalyzer™ Instrument, cfTNA samples typically exhibit a distinct peak of ~170 base pairs, which indicates the cfDNA fraction of an extracted sample. Depending on the collection method, storage conditions, or transportation conditions, another high molecular weight peak of >1,000 base pairs can be visible on the Bioanalyzer™ trace (Figure 3).

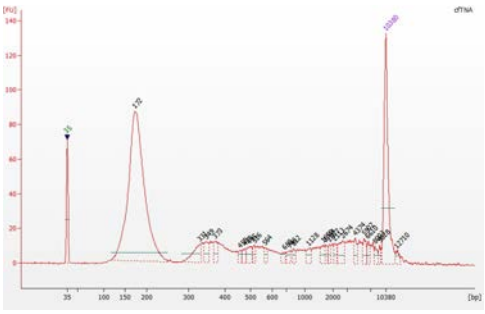


Figure 3 Example Bioanalyzer™ trace for a cfTNA sample

Note: For instructions about reviewing the size distribution for a sample on the Agilent™ 2100 Bioanalyzer™ System, see the *2100 Expert Software User's Guide* (Pub. No. SD-UF0000050), which can be downloaded from **agilent.com**.

Dilute the samples (if needed) and load the sample plate

Before you proceed, use a Qubit™ Fluorometer to accurately measure sample concentration. For more information, see “Quantify nucleic acid samples” on page 100. To run sample controls, select an appropriate control for your sample type as recommended in “Genexus™ Controls” on page 10.

Nucleic acid samples with concentrations up to 1,024X of the target concentration for an assay (displayed as default values in the **Sample Plate** step screen in run planning) are in range for automated dilution and require no manual dilution. Enter the concentrations during sample run planning at the **Sample Plate** step (see “Plan a Nucleic Acid to Result run” on page 38).

Note: You can manually dilute samples to the required concentration to avoid loss of sample due to the required volume overages during automated dilution.

1. For samples with concentrations that are out of range for automated dilution, manually dilute the samples to the required concentration with nuclease-free water as indicated in the run setup guide (see "Plan a Nucleic Acid to Result run" on page 38).

Table 5 Sample concentration requirements

Sample type	Optimal nucleic acid concentration	Verified nucleic acid concentration range
FFPE DNA	0.67 ng/µL	0.33–1 ng/µL
FFPE RNA		
cfTNA	1.33 ng/µL	0.33–2.67 ng/µL

Table 6 Sample input requirements

Sample type	On-deck input requirement[1]	Assay input
Solid tumor (FFPE DNA or RNA)	13.4 ng *(Optimal)* 6.6 ng–20 ng *(Verified range)*	10 ng *(Optimal)* 5–15 ng *(Verified range)*
Liquid biopsy (cfTNA)	26.6 ng *(Optimal)* 6.6 ng–53.4 ng *(Verified range)*	20 ng *(Optimal)* 5–40 ng *(Verified range)*

[1] Assay input plus overage.

2. Add diluted nucleic acid samples to the sample plate at the volume and well positions that are specified in the run setup guide.

Table 7 Sample volume per well

Sample type	Volume
DNA	20 µL
RNA	20 µL

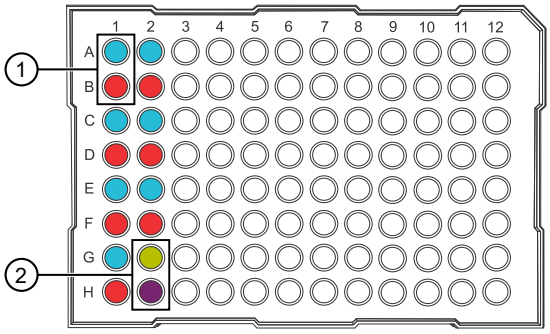


Figure 4 Example DNA and Fusions Nucleic Acid to Result sample input plate layout

Note: Sample position in the input plate can be rearranged when creating the Nucleic Acid to Result run plan to ease pipetting.

① Paired purified DNA-A1 and RNA-B1 from sample 1. C1 + D1 = sample 2, E1 + F1 = sample 3 etc.
② Paired DNA and RNA negative controls
DNA
No template control (DNA)
RNA
No template control (RNA)

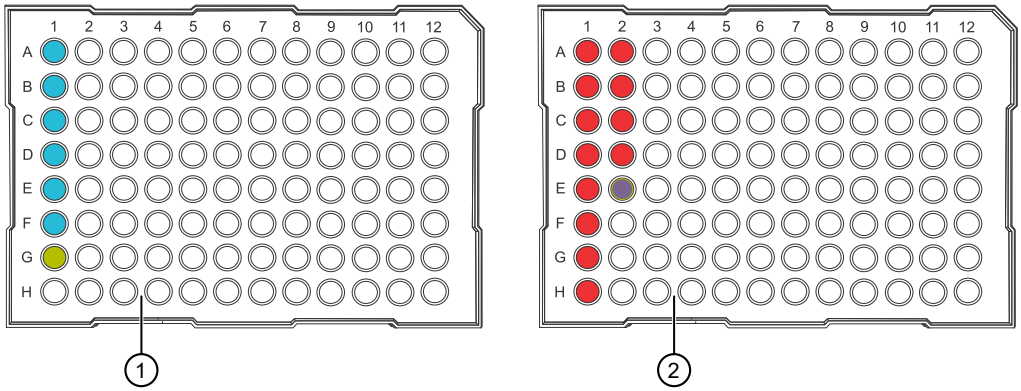


Figure 5 Example DNA-only or Fusions (RNA-only) Nucleic Acid to Result sample input plate layouts

Note: The examples provided are for sequencing in a single chip lane.

① DNA-only sample input plate layout
DNA
No template control (DNA)
② Fusions (RNA-only) sample input plate layout
RNA
No template control (RNA)

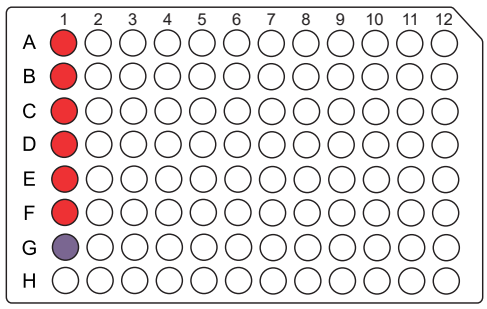


Figure 6 Example cfTNA Nucleic Acid to Result sample input plate layout

cfTNA
No template control (NTC)

3. Add controls to the sample input plate at the volume and well positions that are specified in the run setup guide.

4. Seal the plate with a sheet of Thermo Scientific™ Adhesive PCR Plate Foil.

   IMPORTANT! Do NOT use other brands of aluminum sealing tape. The use of other plate seals can affect performance.

5. Place the plate on ice until ready to load in the sequencer.

Load the sample plate—Sample to Result run

When performing a Sample to Result sequencing run, the 96-Well Nucleic Acid Output Plate from the purification instrument can be loaded directly on to the sequencer as the sample input plate for DNA-only or Fusions (RNA-only) workflows. For the DNA and Fusions sequencing run workflow, purified and quantified DNA and RNA samples, and no template controls must be transferred to a new 96-well input plate as specified in the run setup guide.

Note: No template controls included in the run plan are added to the 96-well output plate by the Genexus™ Purification System.

If positive controls were included in the DNA-only, Fusions (RNA-only), or DNA and Fusions run plan, you must manually add positive controls to the sample input plate, before loading the plate in the sequencer. For more information, contact support.

Note: The maximum number of samples that can be run on the Genexus™ Purification System is 12.

Table 8 GX5™ Chip max capacity by sample type – Sample to Result Integrated Run

Assay	Capacity for 1 lane	Capacity for 2 lanes	Capacity for 3 lanes	Capacity for 4 lanes
No controls				
Oncomine™ Precision - GX5 - w3.4.2 - Liquid Biopsy cfTNA	1	3	4	6
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA	8	12	N/A	N/A
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - Fusions	8	12	N/A	N/A
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions	4	8	12	N/A
With NTC only[1]				
Oncomine™ Precision - GX5 - w3.4.2 - Liquid Biopsy cfTNA	1	3	4	5

Table 8 GX5 Chip max capacity by sample type – Sample to Result Integrated Run *(continued)*

Assay	Capacity for 1 lane	Capacity for 2 lanes	Capacity for 3 lanes	Capacity for 4 lanes
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA	8	12	N/A	N/A
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - Fusions	8	12	N/A	N/A
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions	4	8	12	N/A
With NTC and PC[2]				
Oncomine™ Precision - GX5 - w3.4.2 - Liquid Biopsy cfTNA	N/A	2	3	5
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA	7	12	N/A	N/A
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - Fusions	7	12	N/A	N/A
Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions	3	7	11	12

[1] NTC must be added to the output plate after the run.
[2] NTC and PC must be added to the output plate after the run.

Transfer DNA and RNA samples to the sample input plate

To perform DNA and Fusions sequencing runs the DNA and RNA samples must be transferred from their respective purification output plates to a new 96-well sample plate for loading onto the sequencer. If any controls were included in the run plan, you must manually add positive controls and no template controls to the sample input plate, before loading the plate in the sequencer. For more information about the appropriate controls see “Genexus™ Controls” on page 10.

For **Sample to Result** runs nucleic acid sample concentrations are transferred automatically from the integrated Genexus™ Purification System to the Genexus™ Software. Samples up to 1,024X of the recommended target concentration for the assay (displayed as default values in the **Sample Plate** step screen in run planning) are diluted to the target concentration on the Genexus™ Integrated Sequencer during the run.

Note: You can manually dilute samples to the needed concentration to avoid loss of sample due to the required volume overages during automated dilution.

- DNA and Fusions sequencing runs
  a. Add diluted DNA and RNA samples to the sample plate at the volume and well positions that are specified in the run setup guide.

Table 9 Sample volume per well

Sample type	Verified nucleic acid concentration range	Volume
DNA	0.33–1 ng/µL	20 µL
RNA	0.33–1 ng/µL	20 µL

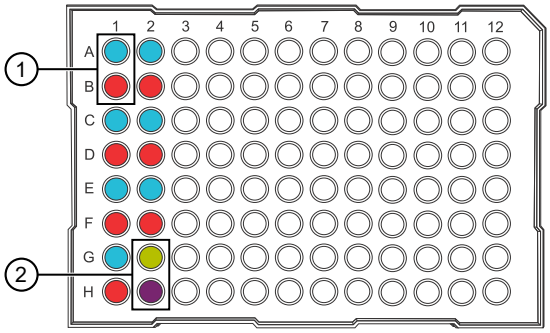


Figure 7 Example DNA and Fusions Sample to Result sample input plate layout without NTC

Note: Sample position in the input plate cannot be rearranged when creating the Sample to Result run plan.

(1) Paired purified DNA-A1 and RNA-B1 from sample 1. C1 + D1 = sample 2, E1 + F1 = sample 3 etc.
(2) Paired DNA and RNA positive controls

DNA
RNA
DNA positive control
RNA positive control

  b. Add controls to the sample input plate at the volume and well positions that are specified in the run setup guide.

  c. Seal the plate with a sheet of Thermo Scientific™ Adhesive PCR Plate Foil.

IMPORTANT! Do NOT use other brands of aluminum sealing tape.

  d. Place the plate on ice until ready to load in the sequencer.

- DNA-only or Fusions (RNA-only) sequencing runs

The 96-Well Nucleic Acid Output Plate generated by the Genexus™ Purification System can be used directly as the sample input plate for sequencing. Because the number of samples that can be accommodated in a single GX5™ Chip lane (DNA-only = 8, RNA-only = 32) does not correlate with the number of samples that can be purified in a single purification batch (DNA or RNA = 12) it can be preferable to transfer samples to new 96-well sample input plates (for example, multibatch purification of RNA samples).

  a. *(If needed)* Transfer samples to a new 96-well sample input plate.

b. Add controls to the sample input plate at the volume and well positions that are specified in the run setup guide.

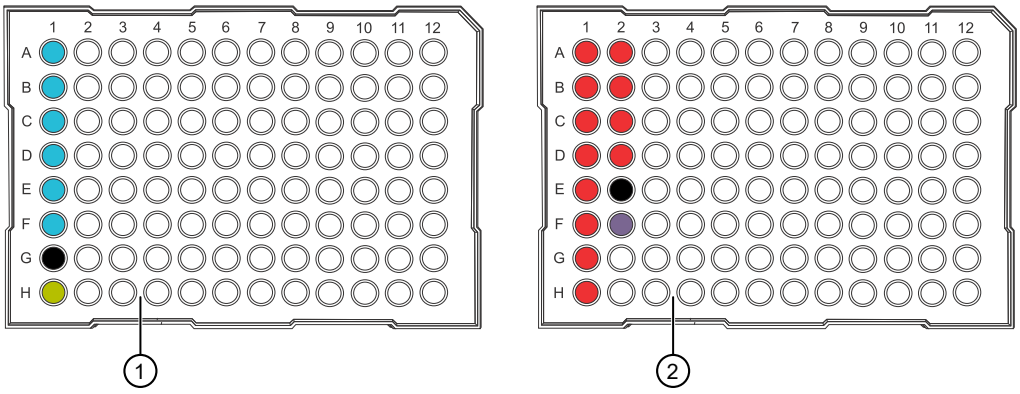


Figure 8 Example DNA-only or Fusions (RNA-only) Sample to Result sample input plate layouts

Note: The examples provided are for sequencing in a single chip lane.

① DNA-only sample input plate layout
- DNA
- No template control (DNA)
- DNA positive control

② Fusions (RNA-only) sample input plate layout
- RNA
- No template control (RNA)
- RNA positive control

- cfTNA sequencing runs

a. Add diluted DNA and RNA samples to the sample plate at the volume and well positions that are specified in the run setup guide.

Table 10 Sample volume per well

Sample type	Verified nucleic acid concentration range	Volume
cfTNA	0.33–1 ng/µL	20 µL

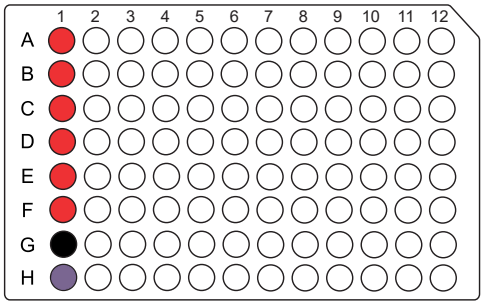


Figure 9 Example cfTNA Sample to Result sample input plate layout

Note: Sample position in the input plate cannot be rearranged when creating the Sample to Result run plan.

- cfTNA
- No template control (NTC)
- Positive control

b. Add controls to the sample input plate at the volume and well positions that are specified in the run setup guide.

c. Seal the plate with a sheet of Thermo Scientific™ Adhesive PCR Plate Foil.

IMPORTANT! Do NOT use other brands of aluminum sealing tape.

d. Place the plate on ice until ready to load in the sequencer.

8 Load the sequencer and start a run

After you have planned a run in Genexus™ Software, use the run setup guide provided by the software to determine which consumables to load in the sequencer. Follow the step-by-step instructions in the sequencer touchscreen during run setup. The vision system of the sequencer tracks the addition of consumables in real-time and alerts you if a component is loaded in the wrong position, or if the wrong quantity is loaded.

Before you begin

1. Remove the library and templating strips from their boxes in the refrigerator or freezer, and ready them for loading in the sequencer.
   a. Equilibrate Genexus™ Strip 1, Genexus™ Strip 2-HD, and Genexus™ Strip 3-GX5™ to room temperature for 30 minutes.

   b. Thaw Genexus™ Strip 4 on ice for 30 minutes. If you are delayed in loading, keep the strips on ice before proceeding.

   Note: These thawing conditions were optimized with strips lying flat on ice. If you are thawing many strips side-by-side, or if strips are submerged in ice, thaw time can be longer than 30 minutes.

   IMPORTANT! Ensure that the strip contents are completely thawed before proceeding.

2. Visually check tube 3 of the Genexus™ Strip 2-HD for precipitation. If needed, flick the tube to dissolve the precipitate.

   Note: If you have a Genexus™ Strip Centrifuge Adapter, you can vortex the strip on a platform vortexer to dissolve precipitate, then briefly centrifuge the strips using the adapter to collect the contents at the bottom of the tubes. For more information about obtaining and using the adapter, see “Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter” on page 112.

3. Remove primer pool tubes in tube carriers that are needed for the run from the freezer, then thaw for at least 30 minutes on ice or at 4°C. After thawing, gently tap the primer pool tube or tubes on a bench surface to ensure that contents are collected at the bottom of the tubes. Keep the tubes and carriers on ice or at 4°C until you load them in the sequencer.

4. If you are installing a new Genexus™ Cartridge, thaw the cartridge at room temperature for 30 minutes before installing in the sequencer.

5. Genexus™ Strip 1 and Genexus™ Strip 3-GX5™ contain magnetic beads in one or two positions, yellow or brown in color, that sometimes get trapped in the upper "keyhole" of the tube. Dislodge these beads from the keyhole before installing the strip in the sequencer. Use the following procedure for each strip.

Note: If you have a Genexus™ Strip Centrifuge Adapter, vortex the strips on a platform vortexer to dislodge air bubbles and magnetic beads or to dissolve precipitate, then briefly centrifuge the strips using the adapter to collect the contents at the bottom of the tubes. For more information, see “Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter” on page 112.

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a. Invert the strip 3–4 times to dislodge beads that are trapped in the keyholes.

b. To remove any remaining beads and liquid from the keyholes, grasp the strip at one end with the strip seal facing up, then swing the strip with a rapid, downward centrifugal arm motion, ending with a sharp wrist-flick.

c. Grasp the strip at the other end, then repeat the centrifugal motion.

d. Check tube positions for significant amounts of beads that remain trapped in keyholes (see Figure 10), then repeat the centrifugal motion, if needed. It is acceptable if a few beads remain in the keyhole or on the tube wall, but most should be either in suspension or in a pellet at the bottom of the tube.

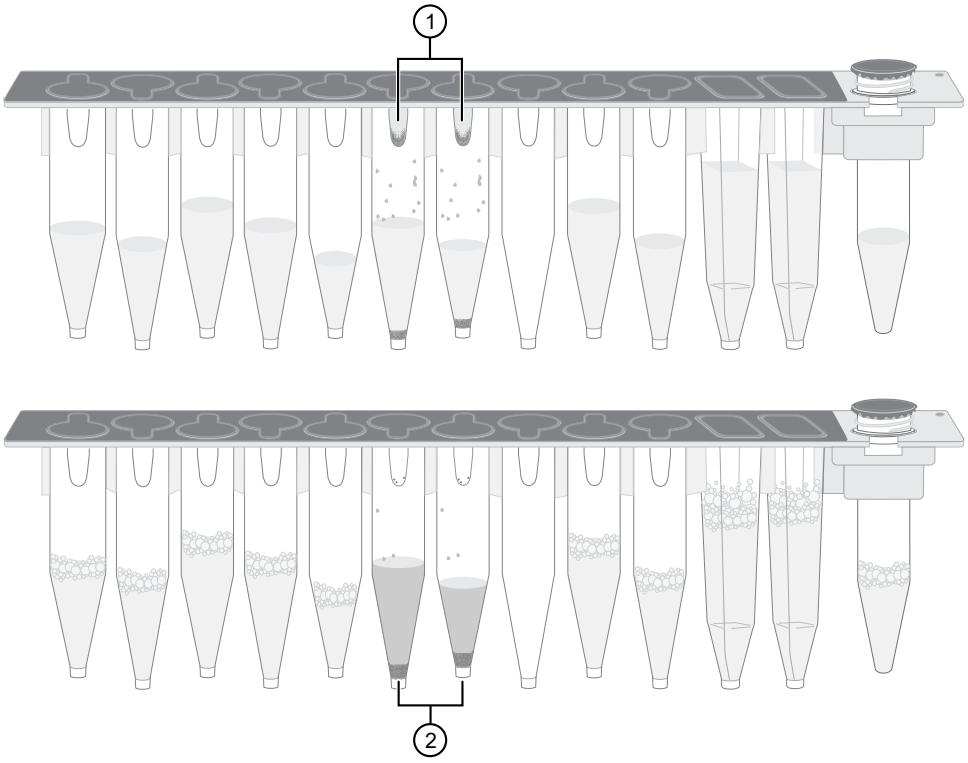


Figure 10 Example Genexus™ Strip 3-GX5™ before (upper) and after (lower) inversion. The carrier has been removed to show tube contents more easily.

① Magnetic beads trapped in keyholes ② Magnetic beads dislodged from keyholes

Note:

- It is not necessary to resuspend the magnetic beads completely—it is only necessary to dislodge most of the beads that may be trapped in the keyhole. The instrument resuspends the beads during the run when needed.
- Fine bubbles can form above the liquid in some tubes after inversion. These bubbles do not affect the run.

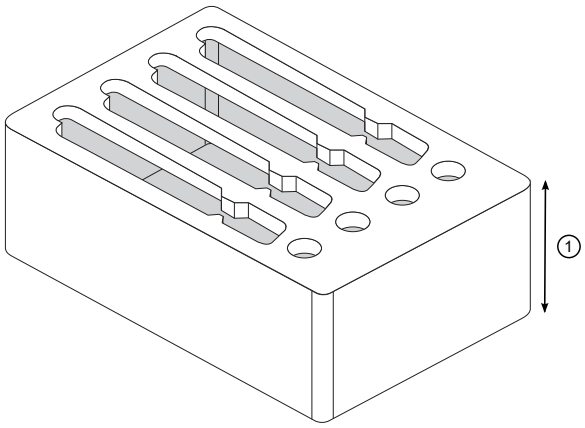
6. Inspect all strips for large bubbles lodged under the surface of the liquid or at the bottom of each tube or well. Gently tap the strips on a benchtop to dislodge any bubbles without splashing the contents onto the upper tube walls. If tapping fails to dislodge a bubble, use the technique that is described in substep 5b until large bubbles are dislodged.

Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter

You can use the Genexus™ Strip Centrifuge Adapter as a holder for centrifuging library and templating strips to collect contents after vortexing the strips. Vortexing and centrifuging strips is recommended to decrease errors in the workflow due to air bubbles in strip wells or beads trapped near the foil seal. Users can request this part from a Thermo Fisher Scientific Field Service Engineer.

Note: The Genexus™ Strip Centrifuge Adapter can also be used to centrifuge Genexus™ Primer Pool Tubes.

Use of the adapter requires a centrifuge with buckets that support the height of the adapter at 44 mm so that buckets swing freely in the centrifuge rotor when loaded with strips. For more information, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).



Genexus™ Strip Centrifuge Adapter

① 44 mm height

To use the Genexus™ Strip Centrifuge Adapter, follow these steps.

1. After thawing, vortex each strip at maximum speed for 5–10 seconds while rocking the strip from side to side.
2. Load the strips in the adapters in a balanced orientation, then place each adapter loaded with strips in the centrifuge. The centrifuge buckets must support the height of the adapters loaded with strips.
3. Centrifuge the strips at 300 × *g* for 15 seconds.
4. Remove strips from the adapters, then inspect the strips to ensure that contents have been collected and air bubbles are not present.
5. If brown magnetic beads are still visible in the tube keyhole near the foil seal, invert the strip to resuspend the contents, then repeat step 3.

   Note: It is not necessary to dislodge all the beads trapped in a keyhole—dislodging most beads is sufficient.

6. Repeat step 1 through step 4, if needed, for the remaining library and templating strips to be loaded in the sequencer.

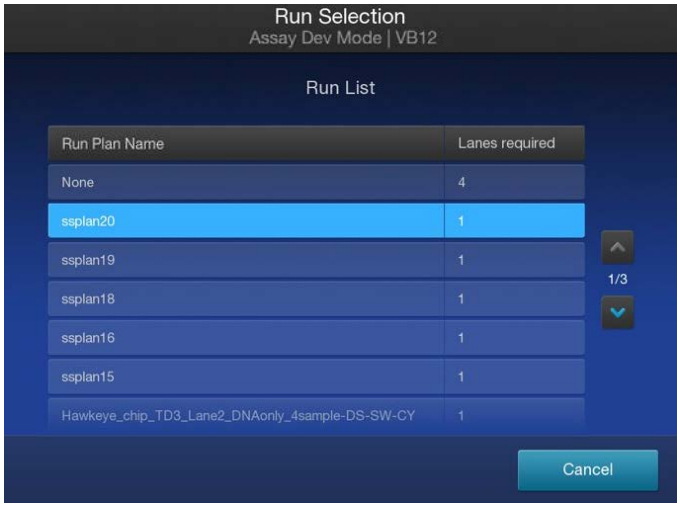
7. After centrifugation, return Genexus™ Strip 4 to ice or 4°C until you are ready to load the strips in the sequencer.

Load the sequencer and start a run

IMPORTANT! The GX5™ Chip is needed for the Oncomine™ Precision Assay GX. Ensure that the correct chip is loaded in step 7.

1. Tap **Run** on the sequencer home screen to start the loading procedure.

2. In the **Run Selection** screen, select the run that you want to use from the list.

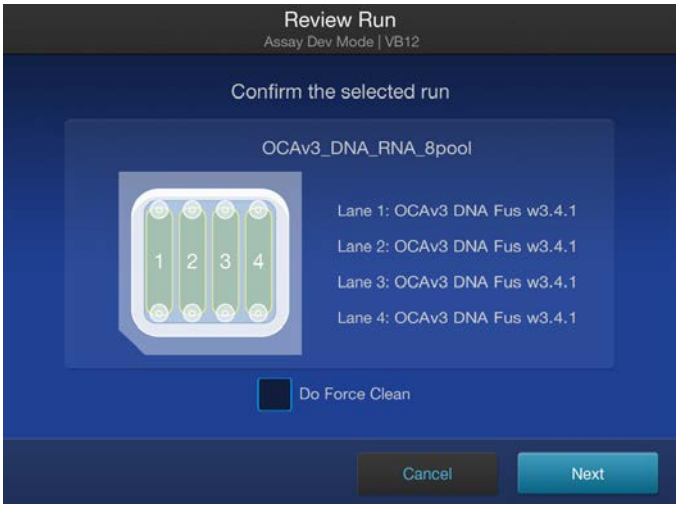


If you select a run that requires more lanes than are available on a currently installed chip, a dialog box appears giving you the option to install a new chip, or cancel. If you proceed with a new chip, a post-chip clean is performed, then the sequencer prompts you to perform the following steps.

- **Clear Deck**
- **UV Clean**
- **Load Deck**
- **Clear Sequencing Reagents**
- **Load Sequencing Reagents**

Note: A post-chip clean takes about 90 minutes to complete.

3. In the **Review Run** screen, confirm the run selections, then tap **Next**.



The deck door opens automatically.

Note:

- If the instrument vision system detects consumables loaded on the deck, the sequencer prompts you to remove the consumables, then starts a UV Clean.
- If all 4 lanes of the installed chip were previously used, the used chip shuttles to the install position. You are prompted to remove it before the UV Clean starts.
- Select the **Do Force Clean** checkbox if there will be 1–3 unused lanes on the installed chip after the run, but you want to start the next run on a new chip after the current run. A force clean automatically cleans the instrument after the run, eliminating the need for an operator to manually perform the **Clean instrument** procedure between the completion of the current run and the next run. Selecting **Do Force Clean** makes all lanes of the chip and installed sequencing reagents unusable after the run

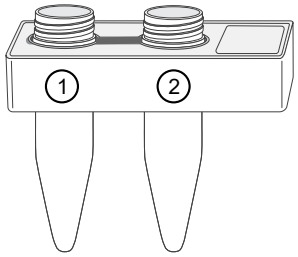
4. In the **Load Deck** screen, the sequencer instructs you step by step to load each required consumable in a highlighted position on the deck. The sequencer detects the loading of each consumable in real time and advances to the next component automatically.

IMPORTANT!

- Ensure that you remove the primer pool tube cap or caps before installing the tube carrier on the deck.
- Ensure that you load the correct type of barcode plate, library Strip 2, and templating Strip 3 for the type of run you are setting up. The sequencer displays a warning if you have installed consumables that are incompatible with the run you have selected, for example, a Genexus™ Barcodes AS plate or Genexus™ Strip 2-AS in an HD run.
- Ensure that you remove the lids from all the boxes of Genexus™ Pipette Tips before you load the boxes in the sequencer.
- After removing the tip box lid, visually inspect tip boxes to ensure that all tips are seated level in the tip rack before and after placing on the instrument deck. If a bent or damaged tip is suspected, replace the tip box with a new tip box. Reracking of tips from partially used boxes is not supported and can cause some tips to seat at an angle, which can affect run performance.
- Ensure that all tip boxes sit level on the deck by visually inspecting all boxes across the tip station. Reseat the boxes before starting the run, if needed.
- Do not use third-party pipette tips or rerack third-party tips into used Genexus™ Pipette Tips boxes. Use of third-party tips can result in sequencing run failure.
- Load instrument plates, including the PCR plate, barcode plate, enrichment plate, and sample plate, into position by pressing down firmly and evenly on all sides and corners of each plate.
- Check any errors carefully before proceeding. Tap **Help** to view the errors.

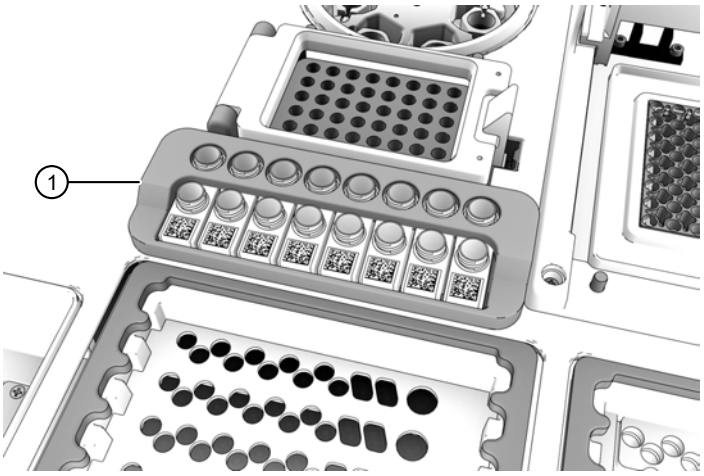
Note:

- A primer pool tube carrier can only be installed with the position 1 tube in the back row of the Primer Pool Tube Station. Follow the guidance in the run setup guide for loading the primer pool tube carrier or carriers in the correct position and order in the station.
- If the sequencer cannot read the correct loading of an unexpired consumable, tap **Help** in the lower left corner of the screen to override the block. After using this override, the name of the consumable will not appear in the run summary consumables list.



① Position 1
② Position 2

5. For Nucleic Acid to Report and Sample to Report sequencing runs using panel primer tubes, place the Genexus™ Primer Pool Tube Holder (Cat. No. A40004840) on top of the Genexus™ Primer Pool Tubes, lining up the holes in the tube holder to the tops of the primer tubes.



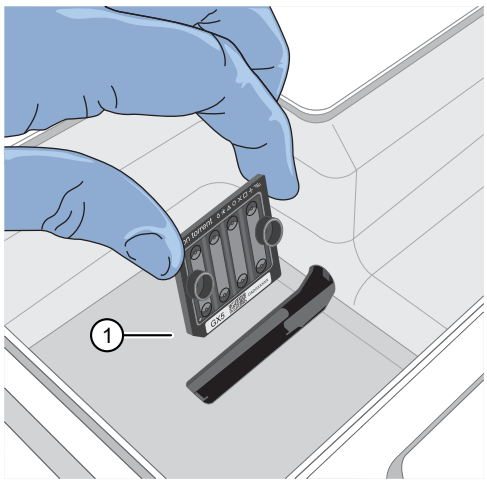
① Genexus™ Primer Pool Tube Holder

6. Ensure that all the primer pool tube carrier barcodes are visible and are accurately shown as loaded in the instrument software.

IMPORTANT! The instrument cannot detect if the Genexus™ Primer Pool Tube Holder is improperly placed. Ensure that the tube holder is correctly positioned before proceeding.

When used with a single primer pool tube, the tube holder can move after placement. This movement has not demonstrated an effect on run performance in our testing.

7. If prompted, insert a new GX5™ Chip and Genexus™ Coupler. Insert the chip into the chip install slot with the chip notch oriented down and toward the front of the instrument.



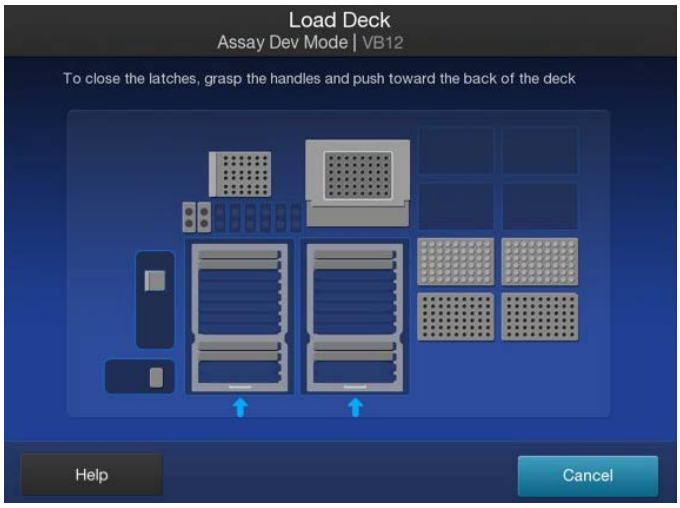
① Notched corner of chip

Note: A chip shuttle under the deck moves the installed chip to loading and sequencing positions during the run.

IMPORTANT! Insert the Genexus™ Coupler so that it is level to properly align with the GX5™ Chip. A coupler that is installed at an angle or is not level will not align properly to the chip and can result in a failed run.

8. When the deck consumables have been loaded, lock the library and templating strips in place by sliding the latches toward the rear of the deck.

Note: We recommend that you load the sample plate last.



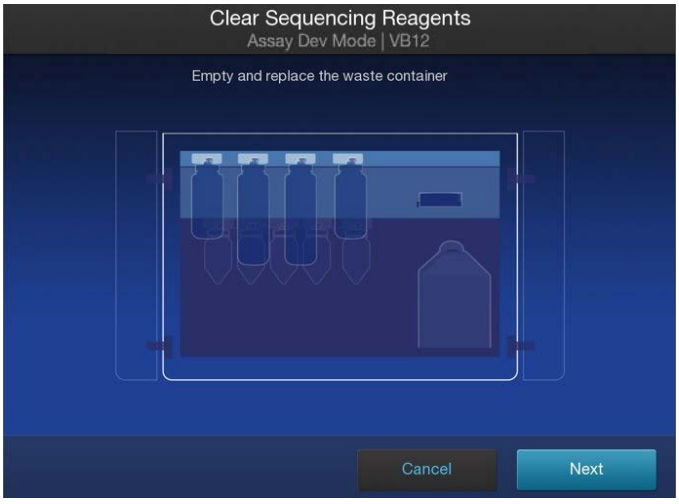
If a chip is detected and the strip latches are closed, the **Close Deck Door** screen appears.

9. Close the deck door, then tap **Next**.

- If you installed a new chip in the sequencer, the sequencer prompts you to open the sequencing reagents bay doors to empty the waste and remove used sequencing reagents bay consumables. Proceed to step 10.
- If you are using a chip that was previously installed and has sufficient lane capacity for the run, the sequencer starts the run.

IMPORTANT! The cartridge and bottles in the sequencing reagents bay must be replaced every time that a new chip is installed, regardless of how many lanes were used in the previous chip.

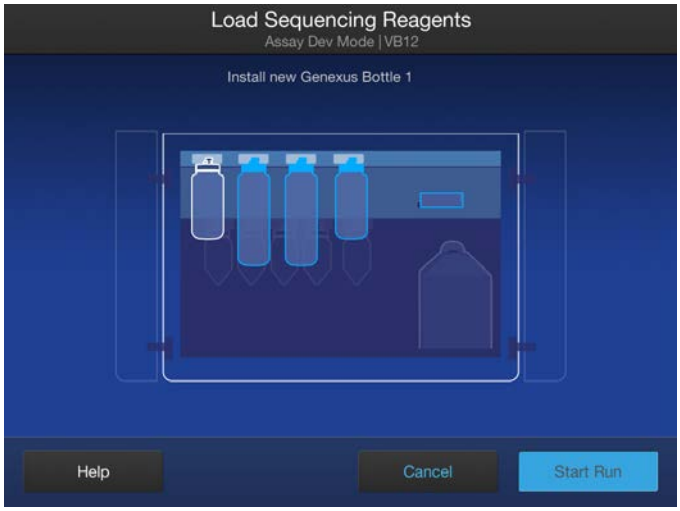
10. Follow on-screen instructions to empty the waste in the Waste carboy, remove waste pipette tips, remove the used Genexus™ Bottle 1, Genexus™ Bottle 2, Genexus™ Bottle 3, and Genexus™ Cartridge, then tap **Next**.



IMPORTANT!

- Ensure that you empty and replace the Waste carboy and the waste pipette tip bin.
- After replacing the emptied Waste carboy, ensure that you reinsert the waste tube into the carboy.
- Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of consumables and liquid waste.

11. Install a new Genexus™ Bottle 1, Genexus™ Bottle 2 (two required), Genexus™ Bottle 3, and Genexus™ Cartridge.

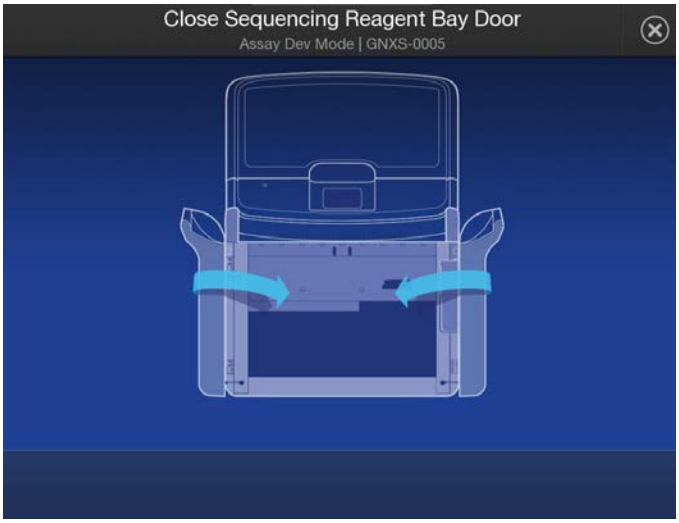


IMPORTANT!

- Before installing, *gently* invert each Genexus™ Bottle 2 five times to mix—avoid vigorous mixing. Inspect the plastic nozzles for any pinches or deformations. To avoid pinching or folding of the plastic nozzle, install the bottles straight-on, not at an angle.
- The installed reagents can be used for up to 14 days on the sequencer with full performance. After 14 days, you may observe reduced performance.

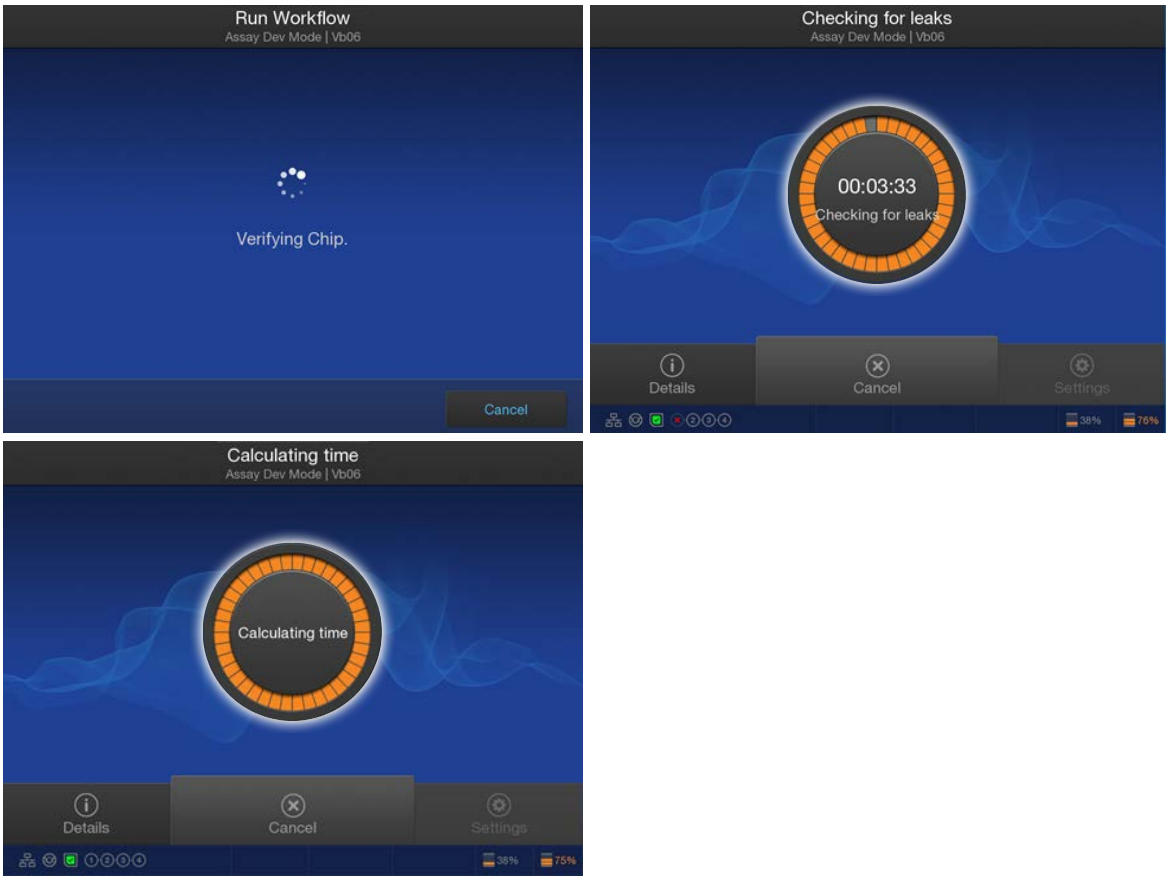
After reagents are installed, the **Close Sequencing Reagent Bay Door** screen appears.

12. Close the sequencing reagents bay doors.



After the doors are closed, the sequencer starts the run.

At the beginning of the run, the instrument chip coupler check verifies the chip, checks for leaks, then calculates run time.



A sequencing run encompasses the following stages.

1. Starting
2. Initializing
3. Library Prep
4. Templating
5. Pre-sequencing
6. Sequencing
7. Cleaning

At each stage, the instrument shows the time remaining on the touchscreen.

Note: The time remaining shown on the screen does not include run analysis time.

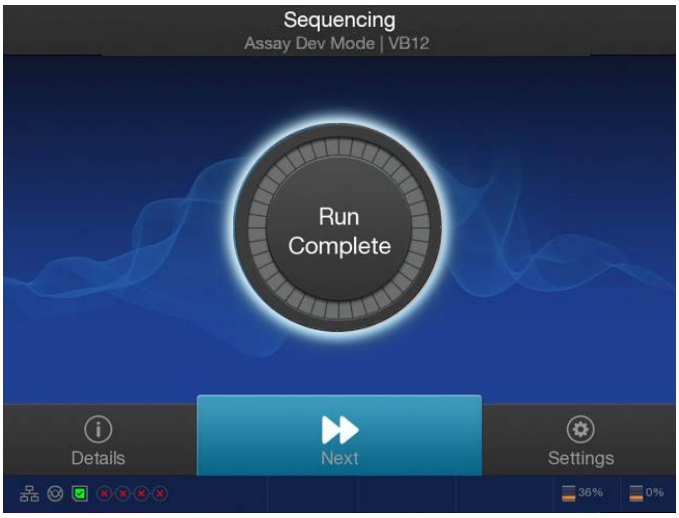
When the run finishes, the sequencer displays the **Run Complete** screen.

Note: If all the lanes of a chip are used in a run, the used chip remains engaged in the sequencer until a new run is started. The chip then shuttles to the install position, and you are prompted to remove the chip before loading the deck with new consumables.

Clear the instrument deck and perform a UV Clean

After a run completes, remove used consumables from the deck and perform a **UV Clean** to ready the instrument for the next run.

1. In the **Run Complete** screen, tap **Next** to start removal of used consumables.



The deck door opens.

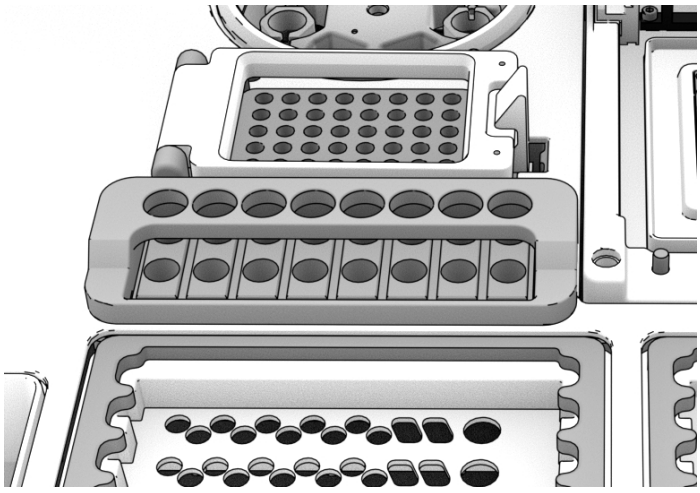
2. In the **Clear Deck** screen, the sequencer provides step-by-step instructions by highlighting the components to be removed. Unlock the library and templating strips by sliding the latches toward the front of the deck, then remove the used strips. Remove the remaining deck components specified by the sequencer.

3. If panel primer tubes were used, remove the Genexus™ Primer Pool Tube Holder and discard the primer pool carriers from the deck.

   Note: If the Genexus™ Primer Pool Tube Holder shows reagent droplet accumulation from normal operation, it should be cleaned before proceeding. For more information, "Clean the Genexus™ Primer Pool Tube Holder" on page 181.

4. Place the Genexus™ Primer Pool Tube Holder back on top of the Primer Pool Tube Station on the deck and store it there until next use.

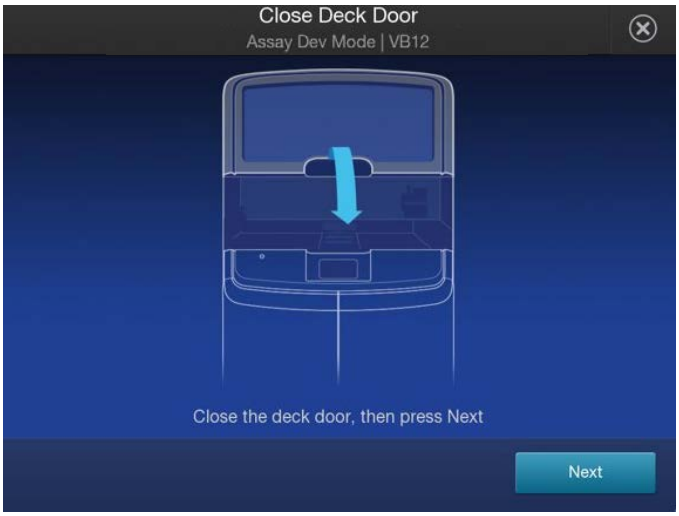
   IMPORTANT! Leave the Genexus™ Primer Pool Tube Holder in this location for the **UV Clean** step and to help reduce contamination.



5. Inspect the Genexus™ Filter in the liquid waste disposal port and verify that no standing liquid is present. If standing liquid is present, manually remove the liquid with a pipette, then pull out the filter. Test the filter with water to determine if a clog is present.

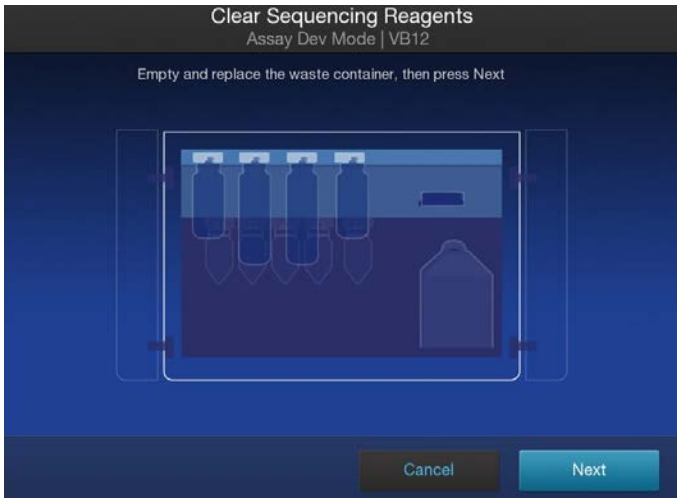
   - If the Genexus™ Filter is clogged, replace it with a new filter. For more information, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).
   - If the Genexus™ Filter does not appear to be clogged, a line clog downstream of the filter is implicated. Contact Technical Support and report a possible deck liquid waste line clog.

6. When finished, close the deck door, then tap **Next**.



A two-minute **UV Clean** starts.

7. After UV cleaning, if all the chip lanes were used, the sequencing reagents bay doors unlock. Open the doors, remove used components from the bay and empty the Waste carboy, then tap **Next**.



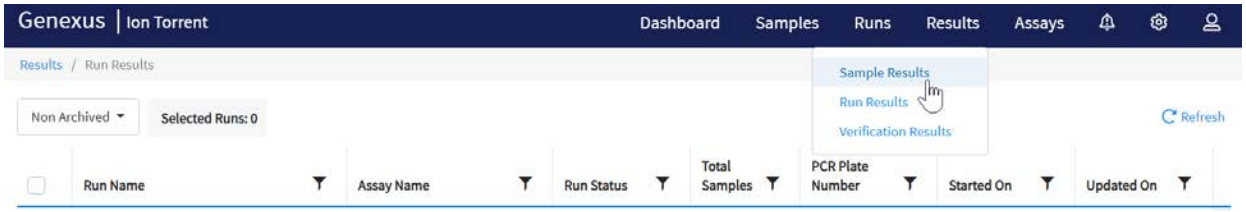
IMPORTANT! Do **not** discard or remove the conical bottles, unless alerted by the sequencer to replace the bottles after a conical bottle flow rate test. For more information, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

IMPORTANT! Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Genexus™ Integrated Sequencer consumables and liquid waste.

CAUTION! The Genexus™ Bottle 1 (small waste bottle) contains small amounts of formamide. Dispose of this waste appropriately.

8. After removal of used components, close the sequencing reagents bay doors, then tap **Next**. The sequencer returns to the home screen.

9 Review data and results



Use the **Results** menu to review run results and data analysis and perform data management tasks. You can view results sorted by sample or by run.

Oncomine™ Precision Assay GX

The Oncomine™ Precision Assay GX cfTNA assay workflow is a hotspot-only assay. In addition, the Oncomine™ Precision Assay GX FFPE assay workflow reports non-hotspot de novo (non-targeted) variants detected in the sample. These de novo variants are usually found in unknown or unidentified genes.

Hotspots are amino acid positions that exhibit recurrent somatic mutations. Based on empirical evidence, the hotspots targeted by the assay are derived from genomic representations of observed alleles for select hotspot codons as well as less-frequently observed variants and are expected to provide selective advantage to tumor cells.

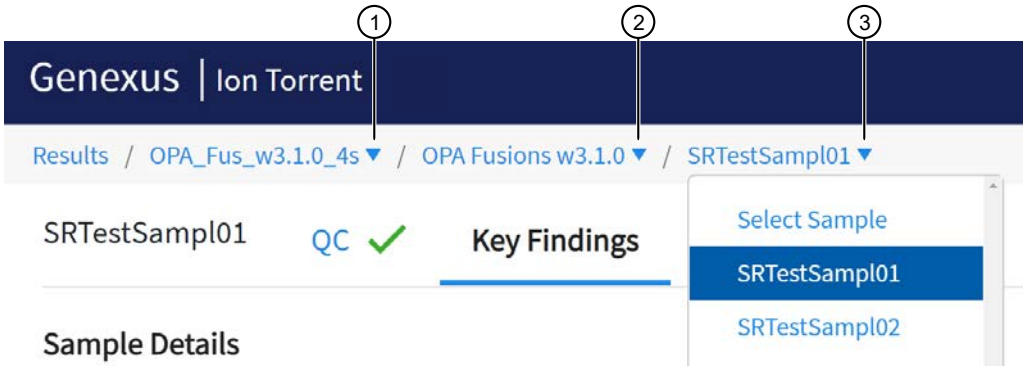
Hotspots, or variant alleles of interest, for the assay are defined in a browser extensible data (BED) file. Hotspot BED files instruct the variant calling pipeline to call out the defined variant alleles in output files, including evidence for the presence of a variant and the filtering thresholds that can disqualify a variant candidate. Variant calls and the filtering metrics for each hotspot allele are reported in the output variant call format (VCF) file, including data for "ABSENT" or "NO CALL" variants.

Results navigation bar overview

After you select a run result or a sample result, a results navigation bar appears and allows you to easily toggle between different results screens for each run. You can quickly switch between run-level results, assay-level results, and sample-level results for the run.

Assay-level results include assay metrics, such as final read data. Assay metrics are also shown in the **Run Report** tab. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Samples that are run with the same assay in the same run share the same **Run Report**. All other results are specific to the sample and are shown in the sample results.



Callout	Dropdown menu	Description
1	**Run Name**	The run name is listed. Multiple runs are listed only if the run has been reanalyzed. For more information, see “Reanalysis” on page 178.
2	**Select Assay**	You can click the assay name of interest to view assay metrics for the run. You can toggle between different assays used in the run. If only one assay is associated with the run, only one assay name is listed. Some results from a plugin that is included in the run, such as the customer support archive (CSA) plugin, apply to all of the samples for an assay in a run. In this case, the results from assay-level plugin are shown in the **Plugins** tab when you select an assay but do not select any samples for the run. For more information about plugins, see “Review plugin results in Genexus™ Software” on page 150. You can choose **Select Assay** from the dropdown list to remove the assay selection. Removing the assay selection opens the **Run Summary** tab for the run selected in the **Run Results** dropdown list.
3	**Select Sample**	You can click a sample name to view the sequencing results for the sample. You can click a different sample name to view other sample results for other samples in the run for the assay that is selected in **Select Assay**. You can choose **Select Sample** from the dropdown list to remove the sample selection. Removing the sample selection opens the **Run Report** tab for the assay selected in the **Select Assay** dropdown list. For **Sample to Result** runs that include a positive control, results for the positive control are shown when you select **Control Sample**. For **Sample to Result** runs that include a non-template control (NTC), results for the NTC are shown only in the **QC** tab under **Sample QC**. The NTC sample is not listed in the **Select Sample** dropdown list.

View sample results

1. Click **Results** ▸ **Sample Results** to view sequencing results, including DNA variant and fusion calls, for a particular sample.
2. In the **Sample Name** column, click a sample name.
3. In the **Results** screen, click the tabs to view the different types of sample-specific results and data.

Tab	Description
QC	The quality metrics for the sample sequenced in the run. For more information, see "View the QC results" on page 129.
Key Findings	An overview of the results for the sample, including **Sample Details**, **Key Metrics**, **Key Variants** and **Coverage Graphs**. For more information, see "View key findings" on page 129.
Variants	Detailed variant results for **SNVs/Indels**, **Fusions**, and **CNVs** For more information, see "View SNV/INDEL results" on page 137, "View Fusion results" on page 141, and "View CNV results" on page 146.
Plugins	Results generated from the plugins associated with the assay used to analyze the sequenced sample. For more information, see "Review plugin results in Genexus™ Software" on page 150.
Reports	You can download and generate summaries of run results. There are two types of reports: • Run Reports – include assay metrics and the record of reagents that were used in a run. For more information, see "Download a run report" on page 159. • Variant Reports – include the variant results for each sample in a sequencing run, reagents used, and QC evaluation metrics. For more information, see "Download a variant report" on page 160.

View the QC results

You can view the quality control (QC) metrics for each sample that was sequenced in a run in Genexus™ Software.

To view QC metrics, in the **Results / Sample Results** screen, click a sample name in the **Sample Name** column. In the **Results** screen, click the **QC** tab.

For details about QC metrics, see the Genexus™ Software help system.

The QC status for each metric is indicated beneath each QC test (**Run QC**, **Templating Control QC–CF–1**, **Sample QC–DNA**, **Sample QC–RNA** and if applicable, **Purification QC**, **NTC QC–DNA**, and **NTC QC–RNA**).

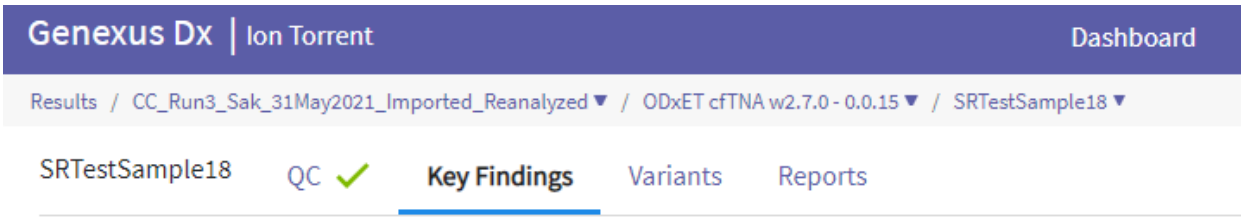
View key findings

You can view **Sample Details**, a summary of the metrics for the run, biomarker results and the **Variants** matrix for genes that were assayed in the run in Genexus™ Software.

You can view the **Key Findings** screen for a sample starting from either sample results or run results.

In the menu bar:

- Click **Results** ▸ **Sample Results**, then click a sample name.
- Click **Results** ▸ **Run Results** to open the **Results / Run Results** screen, then then click a run name in the **Run Name** column, click a run name to open the **Run Summary** screen, You can then select an assay from the **Select Assay** dropdown list and a sample name from the **Select Sample** dropdown list.



You can toggle between different assays used in a run with the **Assays** dropdown list at the top of the screen.

Review the key findings. For more information, see “View key findings” on page 129.

Key Findings

The **Key Findings** table shows details about the sample, a summary of key metrics for the run, and coverage plots for genes assayed in the run.

Note: The **Key Findings** table is by default the first view that is first shown for sample results.

Table 11 Sample Details

Section	Description
Sample Name	A unique identifier representing the sample.
Collection Date	The date that the sample was collected.
Gender	The biological sex of the sample: **Female**, **Male**, or **Unknown**.
Sample Type	A term that describes the sample, for example, FFPE, DNA, DNA & RNA.
Application Category	The application type of the sample.
Cancer Type	The type of cancer that is represented by the sample.
Cancer Stage	The stage of the cancer from which the sample was collected.
%Cellularity	The percentage of tumor cellularity in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is entered when a sample is created. The attribute is applicable to FFPE samples only. The attribute is shown but not required.

Table 12 Key Metrics

Section	Description
Target Coverage[1]	
Target base coverage at *N*x	The percentage of reference genome bases covered by at least *N* reads.
Amplicon Summary	
Average Base Coverage Depth	The average number of reads of all targeted reference bases. This is the total number of base reads on target divided by the number of targeted bases, and therefore includes any bases that had no coverage.
Uniformity Of Base Coverage	The percentage of bases in all targeted regions (or whole genome) with a depth of coverage ≥20% of the mean read coverage. Cumulative coverage is linearly interpolated between the nearest integer base read depths.
Percent Reads On Target	The percentage of filtered reads that are mapped to any targeted region relative to all reads mapped to the reference. A read is considered on target if at least one aligned base overlaps at least one target region. If no target regions (file) was specified, this value is the percentage of reads passing uniquely mapped and/or non-duplicate filters, or 100% if no filters were specified.
Uniformity of Amplicon Coverage	Uniformity of molecular coverage for all amplicons. This metric is generated from the MolecularCoverageAnalysis plugin.

[1] Metrics are shown only for analyses that run the **coverageAnalysis** plugin.

View the Key Variants matrix

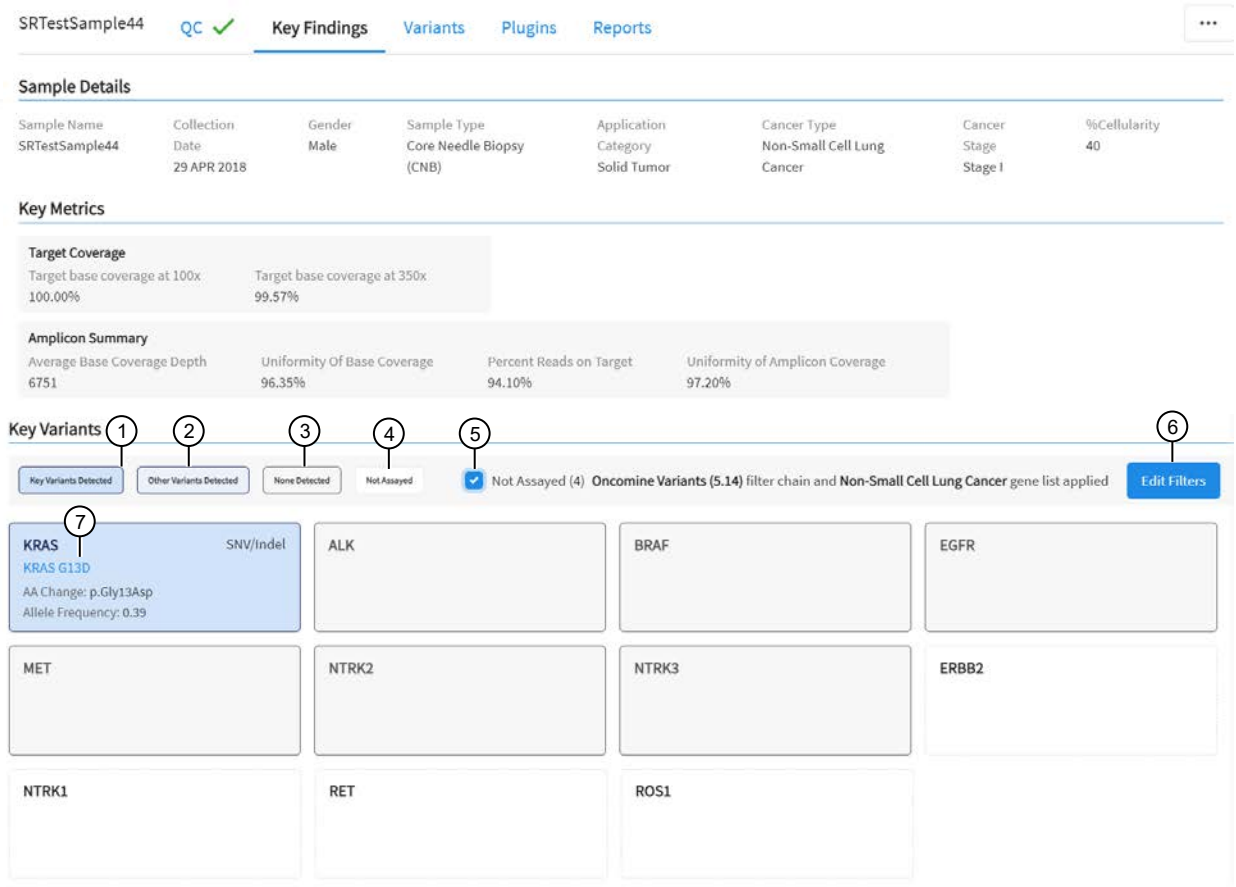
The **Key Variants** matrix provides a color-coded visual representation of the variant results. When the application category of the sample is **Cancer** or **Cancer (Germline)** the genes that are shown in the **Key Variants** matrix are determined by the filter chain and the gene list applied to the results. You can change the filters that are applied to expand or restrict the genes that are shown.

Each tile in the **Key Variants** matrix represents one gene and indicates whether a variant is detected or not. Note that a single gene is represented with multiple tiles when there are multiple mutations detected in that gene.

You can view a color-coded visual summary of variant results in Genexus™ Software.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name.
   The **Results** screen opens to the **Key Findings** tab, which shows the **Sample Details**, **Key Metrics**, the **Key Variants**, and the **Coverage Graphs**.
3. Refine the genes that are shown in the **Key Variants** matrix.

Action	Procedure
Hide genes that were not included in an assay but are in a gene list.	Select or deselect the **Not Assayed** checkbox.
Refine the genes that are shown in the **Key Variants** matrix.	a. Click **Edit Filters**. b. To refine or expand the genes, change one or both of the filters. • Select a different filter chain or no filter chain. • Select a different gene list or no gene list. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409). c. Click **Save**.



Example **Key Variants** matrix

① Color coded legend to the variant tile matrix.

Key Variants Detected —A gene is present in the gene list, variants are annotated by the Oncomine™ Variant Annotator plugin, and the variants are **Key Variants**. Genes that are called are listed in the **Variants** tab with **Present** in the **Call** column. **Key Variants** are listed in the **Variants** tab, with a value of **Yes** in the **Key Variant** column. For more information about gene lists, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Other Variants Detected —A gene is present in the gene list and variants are annotated by the Oncomine™ Variant Annotator plugin, but the variants are not **Key Variants**. Genes that are called are listed in the **Variants** tab with **Present** in the **Call** column. Variants that are not **Key Variants** are listed in the **Variants** tab, with a value of **No** in the **Key Variant** column.

None Detected —A gene is present in the gene list but no variants are annotated by the Oncomine™ Variant Annotator plugin.

Not assayed —A gene is present in the gene list, but is not included in the panel used in the assay.

② Checkbox to hide or show genes that are not assayed.

③ **Edit filters**—Allows you to select an available filter chain and gene list. Changes of the filter chain or gene list change the genes that appear in the **Key Variants** matrix.

④ **Variant Name** —The name of the variant. The **Variant Name** is a hyperlink to the pileup for the variant in the **Variants** tab.

View the amplicon coverage

You can view DNA exon amplicon coverage in Genexus™ Software to help you determine whether the sequencing reads across a gene are uniform and sufficient. The default genes that are shown are determined by the gene list that is designated as default for the cancer type of the sample. You can also view amplicon coverage graphs for more genes. These amplicon coverage graphs provide a high-level overview of coverage. More detailed coverage information is also available in the software.

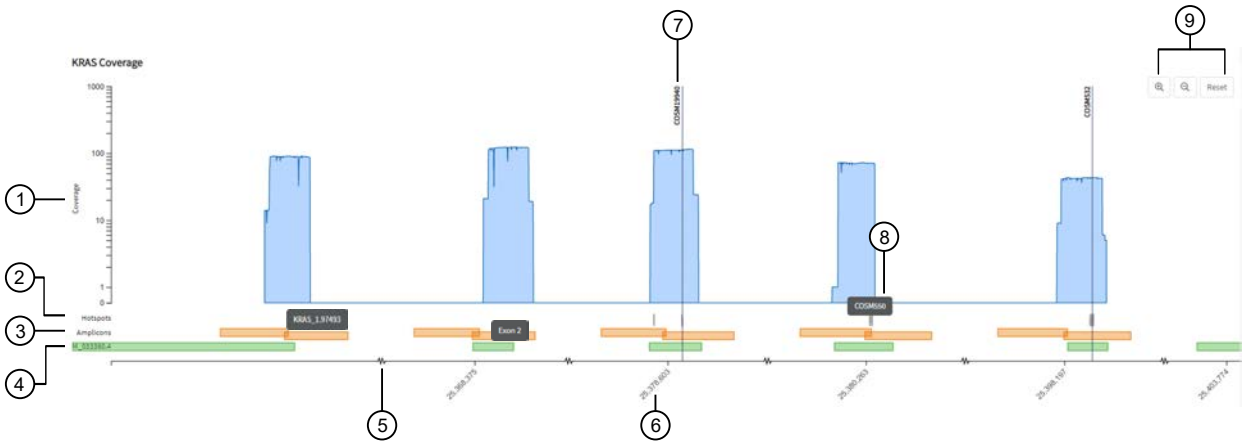
1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Key Findings** tab in the **Results** screen.
3. Scroll to the amplicon **Coverage Graphs**.
4. Review the coverage graphs.
5. To show coverage graphs for more genes, scroll to the bottom of the screen. Click **Show Coverage for Gene**, then select the gene of interest.
6. You can adjust the amplicon coverage graph with the pointer and the buttons in the upper right corner of each graph. The coverage graphs change to show coverage for the selected gene.

Action	Procedure
Zoom in on a region of interest.	Click one or multiple times.
Zoom out for an expanded view.	Click one or multiple times.
Revert to the default view.	• Click one or multiple times. • Click **Reset**.
Move the image left or right in the screen.	After you zoom in, click-drag at any position in the image.

Note: Amplicons that extend beyond the gene start or end coordinates are not displayed in the amplicon coverage graph. Coverage graphs are affected for 6 genes due to this issue: DDX41, ZRSR2, CALR, CSF3R, ANKRD26, and CEBPA. Sequencing results and variant calls are not affected.

Example amplicon coverage graph

Shown here is an example of an amplicon coverage graph for the *KRAS* gene.



1. Base coverage is shown on the y-axis on a logarithmic scale.
2. The location of the known hotspots are denoted with gray bars.
3. The location and span of the amplicons are represented with orange bars. You can hover the mouse over an orange bar to view the amplicon name.
4. The location and span of the transcript track is denoted with green bars. You can hover the mouse over the green bar to view the exon number.
5. The line along the x-axis is broken to indicate that the graph is discontinuous to show exons.
6. The genome coordinate position is shown on the x-axis.
7. Called variants are indicated with a vertical line above the blue coverage plot. The variants noted in the graph depend on the filter chain applied to the results in the **Key Variants** matrix. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
8. You can place the pointer over the hotspots that are represented with gray lines to view the hotspot name.
9. You can adjust the amplicon coverage graph. For more information, see “View the amplicon coverage” on page 134.

View variant results

You can view detailed variant results in the **Variants** table. In the default view, all variants are listed. You can also limit the types of variants that are shown and toggle between different variant results: SNVs/Indels, Fusions, and CNVs.

Table 13 Variant Tables

Variant type	Description
SNVs/Indels	• **SNVs**: missense and nonsense single nucleotide variants. Multi-nucleotide variants are also included. • **Indels**: insertion and deletion variants.
Fusions	Translocations of genetic material.
CNVs	Copy number variations (CNVs) are variations of the number of copies of a given gene.

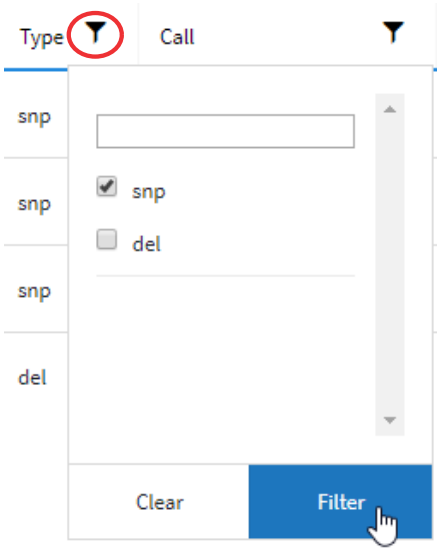
Search and filter variant results

You can search and filter to narrow the list of results that are shown in the variant tables. You can apply filters to columns of information that appear in the screen. The filters, available at the top of each column, narrow the list of information in any columns to which filters are applied.

For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name in the **Sample Name** column.
3. In the **Results** screen, click the **Variants** tab.
4. Select the variant class to display the results: **SNVs/Indels**, **Fusions**, or **CNVs**.
5. In the table of variants, in the column heading of interest, click **(Filter)**.
   - In the search field, enter at least 3 characters, then click **Filter**.
   - Select the checkbox in the row of each filter that you want to apply, then click **Filter**.

   The options that are available depend on the column and variant class. For example, you can filter data in the **Type** column to show one specific variant type.
6. Click **Clear Filters** to remove all filters and view the full list of run results.



The column or columns to which you applied a filter change to reflect the filter and selected options.

System-installed filter chains

The following Genexus™ Software system-installed filter chains are available for use with the Oncomine™ Precision Assay GX results.

Note: System-installed filter chains are locked and cannot be changed.

System-installed filter chain	Description
Oncomine™ Extended 6.8	Default filter chain that is optimized for reporting detected variants in FFPE samples with the Oncomine™ Precision Assay GX. This filter chain includes all Oncomine™-annotated variants and variants that can be relevant to cancer due to their inclusion in one or more of the following classes. • Non-targeted fusions. • CNV variants with **FILTER** value of **GAIN** or **LOSS**. • Likely somatic mutations based on **dbSNP**, **5000Exomes**, **ExAC**, and **UCSC Common SNPs** annotation source databases. The minor allele frequencies range lies between 0.0 and 1.0E-6. Mutations must also be non-synonymous and occur in exonic or splice-site regions. • Variants with **ClinVar** annotations of pathogenic or likely pathogenic.
No Filter	Select this filter chain to remove a previously applied filter chain and view all called variants.

View SNV/INDEL results

The **SNVs/Indels** table lists the calls and other information for the SNV and INDEL variants that are analyzed in each sample in a run.

To view the **SNVs/Indels** table for a sample, click **Results** ▸ **Sample Results** in the menu bar, then in the **Sample name** column, click the name of the sample of interest. Click the **Variants** tab, then click **SNVs/Indels**. To export the data in tabular format, click **Export** in the upper right corner of the screen.

Molecular Tag and Hybrid-based variant calling

The Oncomine™ Precision Assay GX analysis of sequencing data from cfTNA samples uses Molecular Tags, or Unique Molecular Indices (UMIs), to remove systematic and homopolymer errors when calling SNV and Indel variants. Alternative allele variant calls are supported by families/molecules, resulting in a very low limit of detection.

The Oncomine™ Precision Assay GX analysis of sequencing data from DNA samples uses UMIs to remove homopolymer errors when calling SNV and Indel variants. Alternative allele variant calls are supported by reads. Because both tags and reads are used in the variant calling algorithm, this approach is referred to as the "Hybrid" method.

SNVs/Indels table

The data displayed in the **SNVs/Indels** table depends on the assay that was used in the run.

You can filter the results list in the table using filtering tools and filter chains. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Column	Description
User Classifications	User-defined classification to select from the list. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
Variant ID	The name of the hotspot as defined in the Browser Extensible Data (BED) file. Click the link to view more annotation information. For more information, see . Click the link to view more annotation information.
Variant Name	The name of the variant.
Key Variant	Indicates whether the variant is a key variant. Possible values are **Yes** or **No**. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Locus	The chromosome and position of the detected variant.
Oncomine Variant Class	The type of SNV or INDEL at the locus based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • **Gain-of-function**—The altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • **Loss-of-function**—The altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Gene	The gene name.
AA Change	Identification of the amino acid change using Human Genome Variation Society (HGVS) nomenclature.
Ref	The reference base or bases at that locus.
Alt	The alternate base or bases at that locus.

(continued)

Column	Description
Type	The type of variant that is detected. • **snp** (single nucleotide polymorphism) • **mnp** (multi-nucleotide polymorphism) • **ins** (insertion) • **del** (deletion) • **complex** • **FLT3-ITD**
Call	Indicates the presence or absence of an SNV/Indel variant. When the default filter chain is applied, only the variant calls that are designated with **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants** (VCF) file (see "Results files" on page 153). • **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)**—Indicates a high confidence call that passes all filter thresholds at a given variant position. – When the default filter chain is applied, **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** indicates the presence of the ALT (alternative) allele. – When the **No Filter** option is applied or when viewing the **Variants** (VCF) file, **Present** does not imply the presence of the ALT (alternative) allele. To infer the presence of the ALT allele, see the **Alt** column. • **NO CALL**—Although some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call. • **ABSENT**—Indicates that the variant is unlikely to be present in the sample.
Call Details	The reason why a variant is reported as **NO CALL**.
Phred QUAL Score	The relative probability of either the "reference" hypothesis interval [0,cutoff], or the "variant" hypothesis interval [cutoff,1], Phred-scaled (-10*log10). A higher score means more evidence for the variant call.
Raw Read Depth	Total read coverage across amplicon containing SNV/Indel hotspot locations. Count of chip-level reads aligned at this locus that participate in variant calling.
Effective Read Depth	The number of reads covering the position.
Alt Allele Read Counts	The number of reads containing the alternate allele.
Coverage	The number of total reads at a locus, wild-type + alt allele.
Nuc Change	The position and identity of the nucleic acid change.
Allele Frequency	The number of variant read counts divided by the total number of read counts for the sample.
Allele Frequency (%)	The allele frequency, represented as a percentage.
Allele Ratio	The relative frequency of each allele.

(continued)

Column	Description
Mut/WT	The ratio of mutant allele to wild-type allele.
Zygosity	Describes whether the variant is homozygous (0) or heterozygous (1).
Filtered Read Coverage	Coverage at the position considering only filtered reads.
Allele read Count	The number of reads detected for the allele. The Allele Read Count filter sets the minimum count of the genotype alleles.
Raw Alt Allele Read Counts	The number of unfiltered reads containing the alternate allele.
PPA	Possible Polyploidy Alleles (PPA). A value of **Yes** indicates variants that are PPA alleles. A value of **No** indicates variants that are not PPA alleles.
Homopolymer Length	The number of consecutive identical nucleotides adjacent to a variant.
P-Value	The probability value for the detection of variant calls.
Mol Depth	The reports number of interrogated DNA molecules containing target. This metric defines the limit of detection at a hotspot position in a particular run and sample. For reference calls, molecular depth provides a measurable metric that serves as confirmation of variant absence among a large number of interrogated molecules. For instance, if molecular depth is ≥1,500, you can have high confidence that no variant is present at ≥0.2% variant allele frequency. If molecular depth is ≥2,500, you can have high confidence that no variant is present at ≥0.1% variant allele frequency.
WT Mol Counts	The number of detected molecules containing the wildtype allele.
Alt Allele Mol Counts	The number of detected molecules containing the alternate allele.
Mol Freq %	Molecular frequency percentage. The percentage of alternate allele reads over total reads at the locus.
% LOD	the limit of detection (LOD) of a variant allele expressed as a percentage of the WT allele. LOD is the lowest possible variant frequency in the sample that can be detected by the system with a true positive rate greater than 98% for FFPE samples or 95% for cfTNA samples. LOD is dependent on the molecular read depth at the locus. %LOD is reported when there are no variant calls for the gene.

View Fusion results

You can view calls and other information for the fusions that are analyzed for each sample in a run.

1. To view the **Fusions** table for a sample, click **Results** ▸ **Sample Results** in the menu bar, then in the **Sample name** column, click the name of the sample of interest.
2. Click the **Variants** tab, then click **Fusions**.
3. To export the data in tabular format, click **Export** in the upper right corner of the screen.

Fusions table

The data displayed in the **Fusions** table depends on the assay that was used in the run.

You can filter the results list in the table using filtering tools and filter chains. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
Variant ID	The name of the fusion target as defined in the BED file. Click the link to view more annotation information. For more information, see .
Variant Name	The name of the variant. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
Key Variant	Indicates whether the variant is a key variant. Possible values are **Yes** or **No**. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Locus	The chromosome positions in the reference genome that define the fusion junction.
Oncomine Variant Class	Oncomine variant class annotation that indicates fusion type based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • **Gain-of-function**—the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • **Loss-of-function**—the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.

(continued)

Column	Description
Genes (Exon)	The name of fusion target and representative acceptor and donor exons.
Read Counts	The frequency that the fusion was detected in the sample.
Type	Assay type, (for example, Fusion, RNA exon variant (exon skipping), RNAExon Tile, Proc Control).
Call	Indicates the presence or absence of a fusion or RNA exon variant. When the default filter chain is applied, only the fusion/RNA exon variant calls that are designated with **PRESENT** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants** (VCF) file (see "Results files" on page 153). • **PRESENT**—indicates a high confidence call that passes all filter thresholds at a given variant position. • **ABSENT**—indicates the absence of a fusion due to a variant call that falls below thresholds. • **NO CALL**—although some evidence for the presence of a fusion exists, the call does not pass one or more filters that are required for a high confidence fusion call.
Call Details	The reason for reporting a fusion as **NO CALL** or **ABSENT**.
Read Counts Per Million	The number of fusion read counts detected per million total reads.
Oncomine Driver Gene	The gene believed to be associated with increased oncogenic properties. The gene is inappropriately activated by the fusion.
Gene Isoform	The name of the fusion target as defined in the BED file.
Mol Cov. Mutant	The median molecular coverage across a fusion amplicon.

(continued)

Column	Description
Imbalance Score[1]	Each fusion gene exhibits a characteristic **Imbalance Score** threshold. Scores that exceed the threshold value indicate a high likelihood of the presence of the fusion in the sample. Imbalance score calculation starts with the normalization of reads in the exon-tiling amplicons of the gene, followed by correction with a baseline that represents expression values in normal samples. Reads for observed imbalance scores come from samples. Baseline scores come from normal, fusion-negative samples and are stored in the exon tile assay baseline file for the assay. Imbalance score = Observed imbalance (in samples) / Expected imbalance (in the baseline) where Observed imbalance = 3' of breakpoint (in samples) / 5' of breakpoint (in samples) • 3' of breakpoint is the sum of the normalized reads of amplicons 3' of a predicted breakpoint for the gene in the sample. • 5' of breakpoint is the total normalized reads of the amplicons for the gene in the sample. Expected imbalance = 3' of breakpoint (in the baseline) / 5' of breakpoint (in the baseline) • 3' of breakpoint is the sum of normalized baseline values of amplicons 3' of the breakpoint for the gene. • 5' of breakpoint is the total normalized baseline values of the amplicons for the gene.
Imbalance P-Value[1]	The statistical significance of measure of imbalance relative to a control gene.
Predicted Break-point Range[1]	The exonic range for predicted fusion break point in exon tiling assays.
Ratio To Wild Type[1]	The molecular ratio for exon skipping assays relative to wild type control amplicons.
Norm Count Within Gene[1]	Exon skipping assay coverage normalized to molecular coverage of wild type (WT) MET control amplicons.

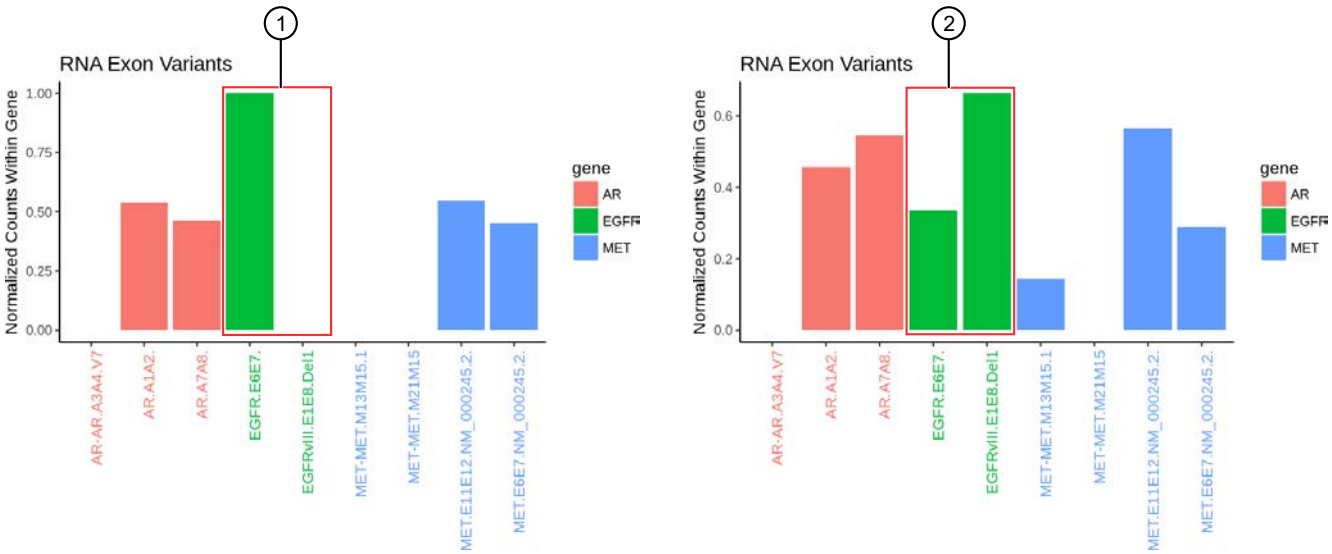
[1] Column appears only in analyses that use the exon tiling fusion detection method.

View RNA Exon Variants

The **RNA Exon Variant** data view displays a bar graph summary of intragenic exon rearrangements or fusions. The displayed RNA exon variants are defined in the BED file that is associated with an assay. The **RNA Exon Variant** data view is available for all RNA and fusion assays.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample name** column, click the name of the sample of interest.
3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
4. In the top right corner of the screen, click **Visualization** ▸ **RNA Exon Variant**, then review the **RNA Exon Variants** plot.

Representative RNA Exon Variant plots



The X-axis represents specific exon variants, where each variant is labeled with a gene ID followed by a sequence of adjacent exons. The Y-axis measures the read counts for each variant, normalized to the wild type.

① Example result where only the wild type EFGR (EFGR.E6E7) was detected.

② Example result where RNA exon 2–7 deletion occurred in the EFGR gene. The deletion of exons 2–7 resulted in an increase of normalized read counts for the EFGR variant that contains the intragenic fusion of exon 1 and exon 8 (EFGR.E1E8.Del1) and a decrease of normalized read counts for the wild type EFGR (EFGR.E6E7).

To return to the table view of fusions, click ✕ **(Remove)** next to the **Visualization** dropdown menu.

View RNA Exon Tile Fusion Imbalance

The **RNA Exon Tile Fusion Imbalance** data view provides a visual representation of the RNA fusion imbalance analyses.

Driver genes, for example ALK, RET, NTRK1, have amplicons that span exon-exon junctions to probe the expression difference between the 3' and the 5' regions of the gene.

An imbalance score is calculated for each driver gene in a sample to quantify the magnitude of the expression imbalance change between the two parts of the gene. For example, the 3':5' ratio in ALK; and the 5':3' ratio in FGFR2.

Note:

- The exon tiling method for fusion detection and the **RNA Exon Tile Fusion Imbalance** data view are specific to the Oncomine™ Precision Assay GX and Oncomine™ Comprehensive Assay Plus GX assays.
- The exon tiling method for fusion detection and the **RNA Exon Tile Fusion Imbalance** data view are not enabled in the Oncomine™ Precision Assay GX cfTNA assay.

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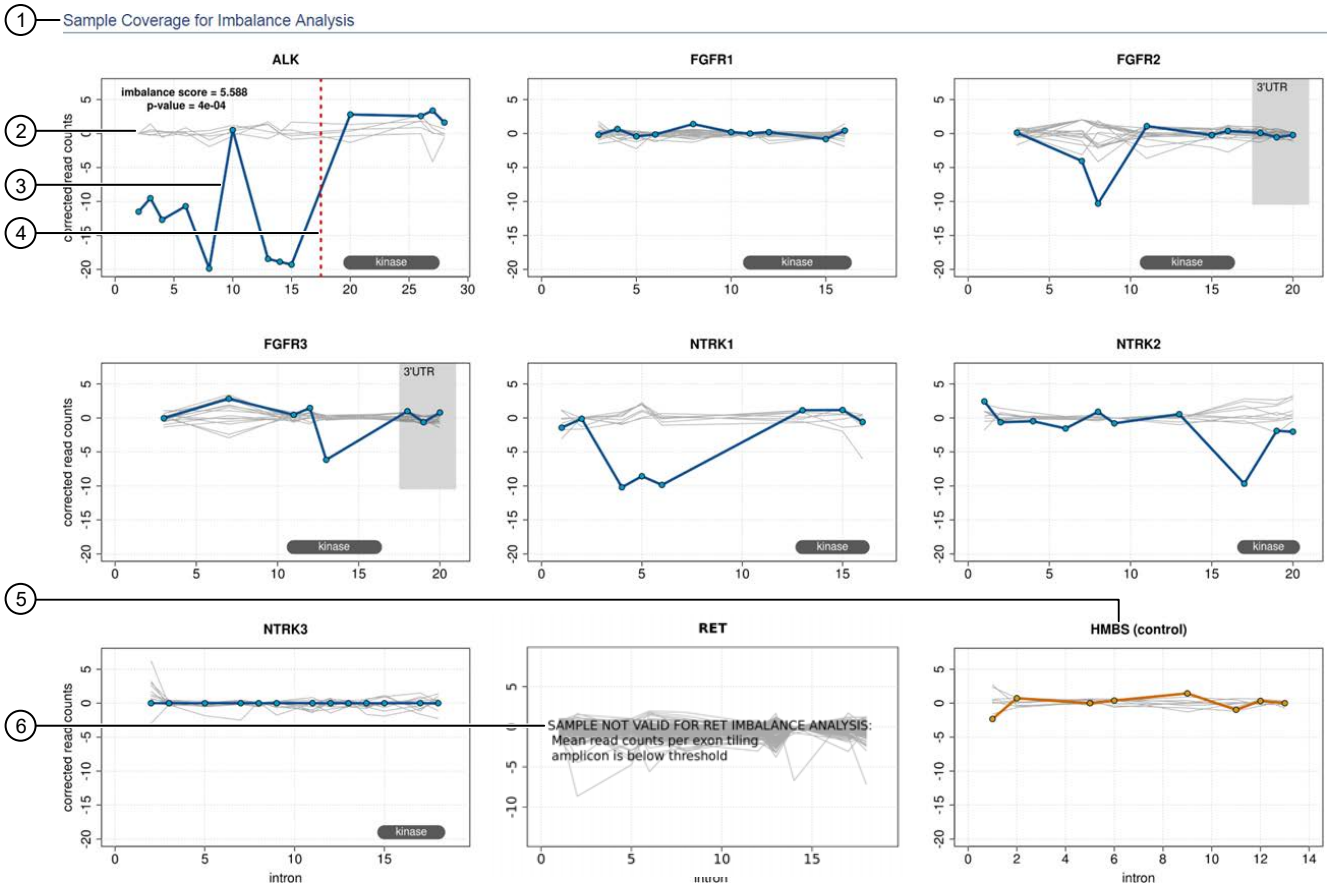
1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample name** column, click the name of the sample of interest.

3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

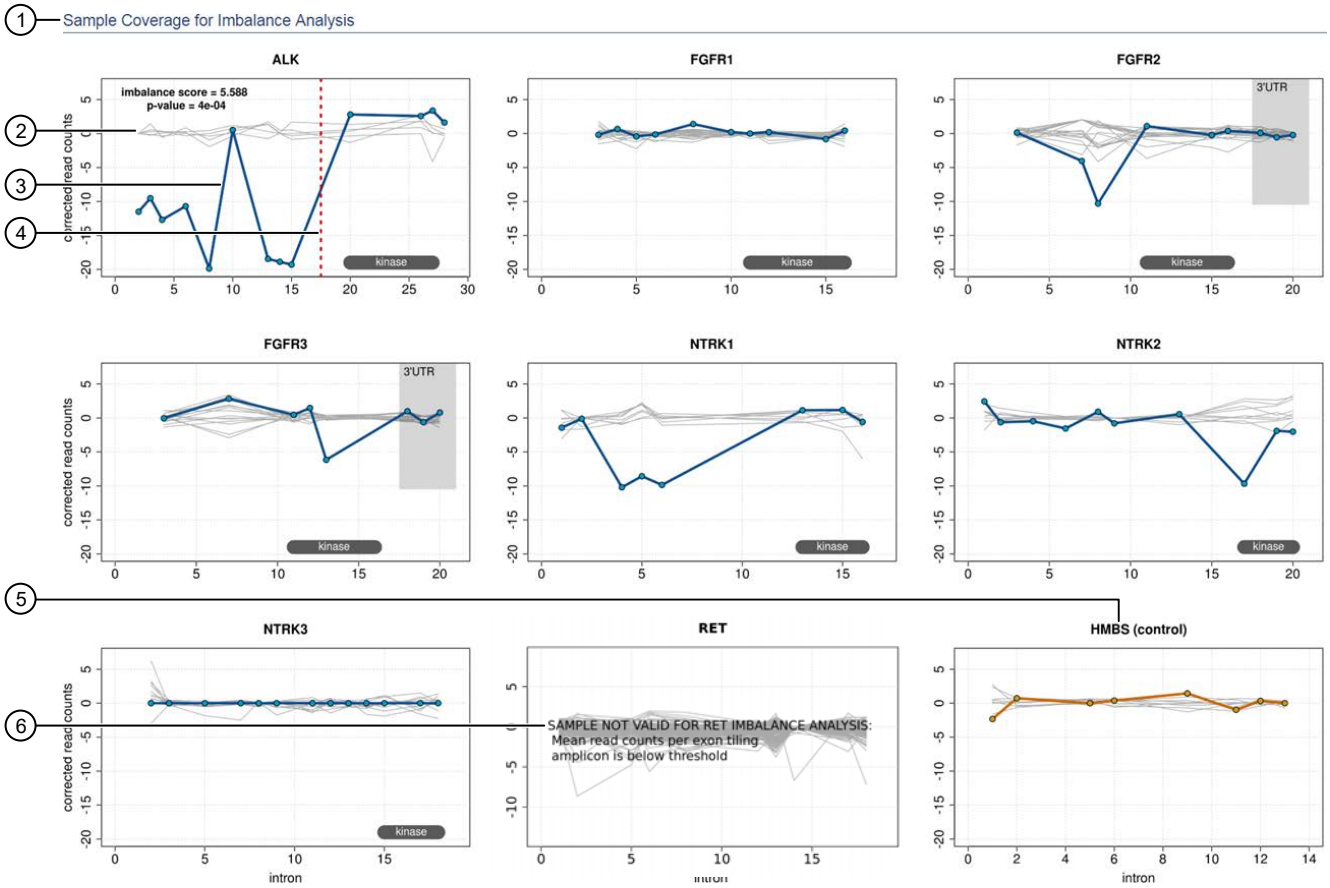
4. In the top right corner of the screen, click **Visualization** ▸ **RNA Exon Tile Fusion Imbalance**, then review the **RNA Exon Tile Fusion Imbalance** plots.

Representative RNA Exon Tile Fusion Imbalance plots



① The **Fusion Imbalance Call: Imbalance Score & P-value** plot shows the imbalance scores and p-values for all the genes in the selected sample. The dashed gray lines mark the threshold for an imbalance call, which is applied to all genes across all samples. Points that fall within the blue shaded area of the plot represent imbalance-score positive genes (✚). All other points that are outside of the blue shaded area represent imbalance-score negative genes (◯). Control genes are marked with ◆.

② The **Normalized Read Depth by Gene** plot shows the mean read counts of each gene that is captured on the chip for the selected sample. For each gene, the read counts are normalized to the number of amplicons.



① The **Sample Coverage for Imbalance Analysis** plots show the expression profile for each exon-exon tiling amplicon for each gene. The Y-axis represents the corrected molecular counts. The X-axis represents individual exon-exon junctions, which are listed from 5′ to 3′. The **imbalance score** and **p-value** are listed in the panel of each gene that was called positive for fusion.

② Baseline (a cluster of gray lines), generated from a fusion-negative sample.

③ Test sample corrected read coverage (blue line), normalized to the baseline. Each point on the line represents a unique exon-exon junction that was covered by the assay and normalized to the baseline.

④ Predicted range for the fusion break point for a fusion-positive gene (dashed red line).

⑤ Sample coverage profile for the control gene (orange line).

⑥ If the collected data are insufficient to determine an imbalance score, the **SAMPLE NOT VALID FOR <gene> IMBALANCE ANALYSIS** message appears in the panel for that gene.

To return to the table view of fusions, click ✕ **(Remove)** next to the **Visualization** dropdown menu.

View CNV results

The **CNVs** table lists the calls and other information for the copy number variants (CNVs) analyzed in each sample in a run.

To view the **CNVs** table for a sample, click **Results** ▸ **Sample Results** in the menu bar, then in the **Sample name** column, click the name of the sample of interest. In the **Variants** tab, click **CNVs** to display the data. To export the data in tabular format, click **Export** in the upper right corner of the screen.

CNVs table

The data displayed in the table depend on the assay that was used in the run. Columns not displayed by default can be added or removed from the table through the **Columns** dropdown list. You can filter the results list in the table using filtering tools and filter chains.

IMPORTANT! *(FFPE samples only)* If the **%Cellularity** value for a sample is set to <100, then the magnitude of copy number gain or loss can be decreased.

For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Column	Description
User Classifications	A user-defined classification selected from the list.
Variant ID	The identifier of the CNV variant. Click the link to view more annotation information.
Variant Name	The standardized name of the variant.
Key Variant	Indicates whether the variant is a key variant. Possible values are **Yes** or **No**. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run.
Locus	The starting position of the first amplicon covering the CNV gene.
Oncomine Variant Class	Annotation that indicates whether CNV is an amplification or deletion. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations. • **Gain-of-function**—The altered gene product has a new molecular function or pattern of gene expression, compared to the wild-type gene. • Loss-of-**function**—The altered gene product lacks the molecular function of the wild-type gene. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run.
Gene	The gene name.
Copy Number	The copy number of a CNV gene locus per genome.
Relative Fold Difference	Ratio of the gene copy number to the copy number of the chromosome arm on which the gene is situated.

(continued)

Column	Description
Call	Indicates the presence or absence of a CNV. When the default filter chain is applied, only the CNV-positive calls that are designated with **PRESENT** (GAIN) and **PRESENT** (LOSS) are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **All Variants** (VCF) file (see "Results files" on page 153). • **PRESENT (GAIN)**—A CNV-positive call that indicates gene amplification; a high confidence variant call that passes all filter thresholds. • **PRESENT (LOSS)**—A CNV-positive call that indicates gene deletion; a high confidence variant call that passes all filter thresholds. • **PRESENT** —Indicates a high confidence call that passes all filter thresholds. • **ABSENT**—The absence of a variant; result is below the detection threshold for a CNV call. • **NO CALL**—Although some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call.
P-Value	The statistical significance of the CNV ratio measurement.
Call Details	The reason for reporting a CNV as NO CALL.
Subtype	The CNV subtype. • **BigDel**—Deletion of at least one exon. • **BigDup**—Duplication of at least one exon. • **GeneCNV**—Whole BRCA1/BRCA2 gene deletion or duplication. • **NOCALL**—Read count differs from baseline by non-integer amount; evidence for a BigDel or BigDup call is weak. • **REF**—Read count matches reference baseline. • **ARM**—Aneuploidy of a chromosome arm.
CNV Confidence *(Optional)*	The CNV confidence interval associated with the call. The 5% lower confidence bound value is the ploidy estimate, where there is a 5% chance that the true ploidy is below that value. The 95% upper confidence bound is the ploidy estimate, where it is 95% certain that the true ploidy is below that value.
Valid CNV Amplicons *(Optional)*	The number of amplicons spanning the CNV call.
Type *(Optional)*	The type of variant that is detected. • **CNV**—Copy number variant • **LOH**—Loss of heterozygosity

Compare sample results

In Genexus™ Software, you can compare variant results across samples and create customized comparison plots. To compare samples from different assay runs, the research application (such as DNA or DNA and Fusions) must be comparable. That is, results from a research application must include the type of results from the other research application. For example, results generated from DNA-only assay runs cannot be compared to results generated from Fusion-only assays. Comparisons that can be made are:

- DNA-only vs DNA-only
- DNA-only vs DNA and Fusions
- DNA and Fusions vs Fusions-only
- Fusions-only vs Fusions-only

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Select the checkbox in the row of each sample that you want to compare, then click **Compare**.

   Note: **Compare** is not enabled until at least two samples are selected.

   The **Results / Compare** screen opens and displays a list of variants and variant information in the **Compare Samples** table. The **Oncomine™ Extended** filter chain is the default filter chain that is applied to the data that are displayed in the tables. Only variants with a Variant ID are shown.
3. In the **Compare Samples** table, select the checkbox in the row of one or more variants of interest to view the variants plot (see callout 1 in Figure 11).
4. Customize the variant view if needed.

Option	Description
Customize table columns	Click **Table Columns**, then select the columns that you want to view in the table, or deselect the columns that you want to hide (see callout 2 in Figure 11).
Customize plot axes	Click **Chart Options** (see callout 3 in Figure 11). In the **Preferences** dialog box, select the desired X-axis, then select the desired Y-axis for each variant type.
Change the default filter chain	From the **Filter Chain** dropdown list, select a filter that you want to apply to the variants that appear in the **Compare Samples** table (see callout 4 in Figure 11). For a description of system-installed filters, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

5. Click **Export Variants** to download the information in the **Compare Samples** variant table for the selected variants.

   See callout 5 in Figure 11.

   The information is downloaded in XLSX format.
6. Click **Download Chart** to download the variants plot in PNG format (see callout 6 in Figure 11).

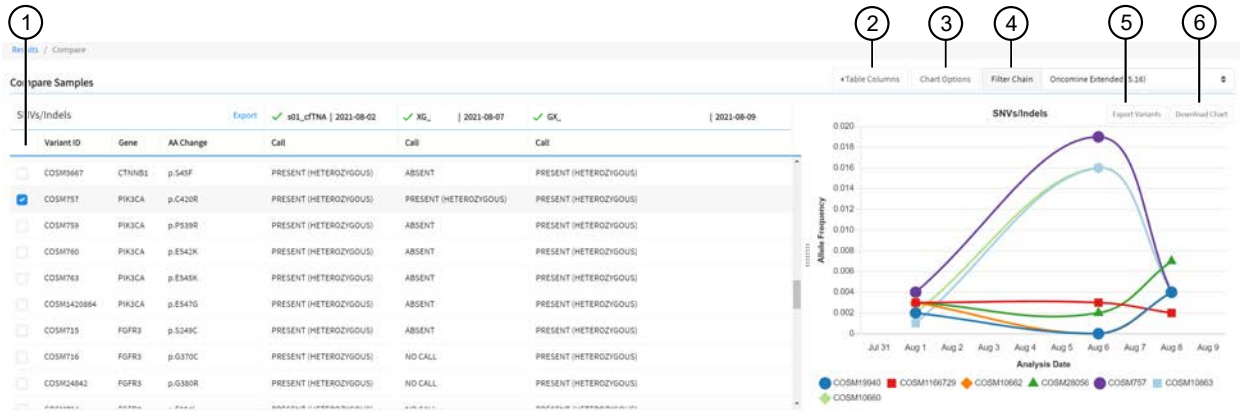


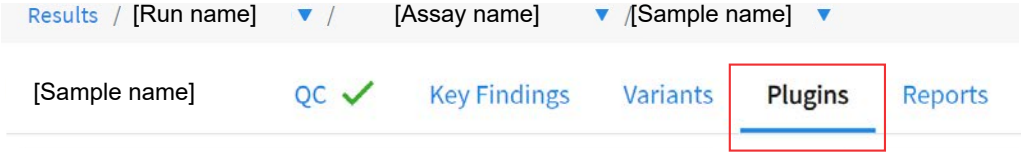
Figure 11 Compare variants

1. Select one or more variants to compare in the variants plot.
2. Customize columns that appear in the **Compare Samples** table.
3. Customize the X-axis and Y-axis that appear in the variants plot.
4. Select a filter chain to filter the results that appear in the **Compare Samples** table.
5. Download in XLSX format, the information in the **Compare Samples** variant table for the selected variants.
6. Download a PNG file of the plot.

Review plugin results in Genexus™ Software

In Genexus™ Software, you can review plugin results in the **Results** screen for a sample.

Each plugin that was selected for an assay during assay creation is listed in the **Plugins** tab.



Review coverageAnalysis plugin results

The coverageAnalysis plugin generates a **Coverage Analysis Report**. This report includes read statistics and several charts. The statistics and charts that are presented depend on the library type for the analysis.

The report summary lists the barcode, the sample, the number of mapped reads, the percentage of on target reads, mean base coverage depth, and base coverage uniformity.

You can download coverageAnalysis plugin output files from the **Results** screen for a sample. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample of interest.

3. Click the **Plugins** tab.
   A summary table of the coverage analysis, by barcode, is included in the **coverageAnalysis** summary pane.

4. *(Optional)* From the **Executed At** dropdown list, select an alternate timestamp, if available, to view additional reports.

5. *(Optional)* Click **View Log** to view the coverageAnalysis log.

6. *(Optional)* Click **Delete** to delete the coverageAnalysis plugin output for the selected timestamp.

   IMPORTANT! If you click **Delete**, the report is deleted without the appearance of the confirmation window. Ensure that you intend to delete the report before clicking **Delete**.

7. Click ••• **(More)** ▸ **Download Files** to download coverageAnalysis plugin results files.

   Note: Sometimes the file name can be too long to open in applications such as Microsoft™ Excel™. To resolve this problem, right-click the file and click **Save As** to rename the downloaded file.

8. In the **coverageAnalysis** summary pane, in the **Barcode Name** column, click the link in the row of the barcode of interest.
   The detailed **Coverage Analysis Report** for the barcode opens in a separate window.

Review molecularCoverageAnalysis plugin results

The molecularCoverageAnalysis plugin generates a Molecular Coverage Analysis Report. This report includes molecule statistics and several charts. The report summary lists the barcode, the sample, the median molecular coverage, the molecular uniformity, the median reads per functional molecule, and the median percentage of functional reads. Additional details regarding molecular coverage are also provided on a per-barcode basis, along with amplicon information that is included in the BED file.

You can download molecularCoverageAnalysis plugin output files from the **Results** screen for a sample. For more information, see “Results files” on page 153.

For more information on how to review and interpret read statistics and output files, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Note: For Oncomine™ Precision Assay GX, the molecularCoverageAnalysis output is applicable to liquid biopsy assays only.

1. In the menu bar, click **Results** ▸ **Sample Results**.

2. In the **Sample Results** screen, in the **Sample Name** column, click the sample of interest.

3. Click the **Plugins** tab.
   A summary table of the coverage analysis, by barcode, is included in the **molecularCoverage Analysis** summary pane.

4. *(Optional)* From the **Executed At** dropdown list, select an alternate timestamp, if available, to view additional reports.

5. *(Optional)* Click **View Log** to view the molecularCoverageAnalysis log.

6. *(Optional)* Click **Delete** to delete the molecularCoverageAnalysis plugin output for the selected timestamp.

   IMPORTANT! If you click **Delete**, the report is deleted without the appearance of confirmation dialog window. Ensure that you intend to delete the report before clicking **Delete**.

7. Click **(More)** ▸ **Download Files** to download coverageAnalysis plugin results files.

   Note: Sometimes the file name can be too long to open in applications such as Microsoft™ Excel™. To resolve this problem, right-click the file and click **Save As** to rename the downloaded file.

8. In the **molecularCoverageAnalysis** summary pane, in the **Barcode Name** column, click the link in the row of the barcode of interest.
   The detailed **Molecular Coverage Analysis Report** for the barcode opens in a separate window.

Download results files for a sample result

You can download results files for a sample result in Genexus™ Software.

1. In the menu bar, click **Results** ▸ **Sample Results**, or **Results** ▸ **Run Results**, then do one of the following procedures.

Option	Selection
Download files for a sample	
Download results files for a sample from the list of sample results.	In the **Results / Sample Results** screen, place the pointer over the row of a sample of interest, then click **Download Files**.
Download results files for a selected sample from the **Results** screen.	1. In the **Results / Sample Results** screen, in the **Sample Name** column, click the sample name of interest. 2. Click ··· **(More Options)** ▸ **Download Files**.
Download files for a run or assay	
Download results files for a run from the list of run results.	In the **Results / Run Results** screen, place the pointer over the row of a run of interest, then click **Download Files**.
Download results files for a selected run or assay from the **Results** screen.	1. In the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest. 2. Click the run or assay of interest from the results navigation bar. 3. Click ··· **(More Options)** ▸ **Download Files**.

2. In the **Download Files** dialog box, select the files to download, then click **Download**.
   For information about the files, see “Results files” on page 153.

The selected results files are downloaded in one ZIP folder.

Results files

The following files can be downloaded from the **View Results** screen for each sample. The files that are available for download vary depending on the assay used. Results files include the sequencing data, results from the analyses, such as variant files, and audit and log files. For a list and descriptions of plugin output files, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Option	File name	Description
Variants		
Filtered Variants (.vcf)[1]	`<Filter_name>_filtered.vcf`	Summary of filtered variant results in variant call format (VCF).
All Variants (.vcf)[1]	`Oncomine_<LibPrepID>_ <analysisID>.vcf`	Summary of variant results in variant call format (VCF).
Snvindel (.tsv)	`Snvindel.tsv`	File that lists SNV/INDEL variant results in tab-separated value format (TSV).
Fusion (.tsv)	`Fusion.tsv`	File that lists fusion results in tab-separated value format (TSV).
CNV (.tsv)	`Cnv.tsv`	File that lists copy number variant results in tab-separated value format (TSV).
Sequencing Results		
DNA Unmapped Bam File (.bam)	`<barcode> _rawlib.basecaller.bam`	Unmapped DNA barcode BAM file; output after mapping reads to reference.
DNA mapped bam file (.bam)	`merged.bam`	Mapped BAM file of combined barcode reads.
DNA Mapped Bam Index File (.bai)	`merged.bam.bai`	Mapped BAM Index file.
DNA Basecaller FASTAQ File (.fastq)	`<barcode>_rawlib. basecaller.fastq`	FASTQ file of the DNA barcodes used.
DNA Processed Bam File	`merged.bam.ptrim.bam`	Mapped BAM file of combined barcode reads.
DNA Processed Bam Index	`merged.bam.ptrim.bam.bai`	Mapped BAM index file.
RNA Unmapped Bam File (.bam)	`<barcode>_rawlib. basecaller.bam`	Unmapped RNA BAM file; output of base calling, contains unmapped reads.
RNA Mapped Bam File (.bam)	`<barcode>_rawlib. basecaller_alignments.bam`	Mapped BAM file of combined barcode reads.
RNA Mapped Bam Index File (.bai)	`<barcode>_rawlib. basecaller_alignments.bam.bai`	Mapped BAM index file.

(continued)

Option	File name	Description
RNA Basecaller FASTAQ File (.fastq)	`<barcode>_rawlib.basecaller.fastq`	FASTQ file generated from the unmapped BAM file of the RNA barcodes used.
Test Fragment Basecaller FASTAQ File (.fastq)	`rawtf.basecaller.fastq`	FASTQ file for the test fragment.
Audit and Log		
Analysis Log File	`analysis.log`	Analysis log file.
Run Summary[2]	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.
Run Audit	`PlannedRun-AuditTrail.pdf`	Contains all audit records pertaining to the run.
Reports		
Report	`<language>_<sampleName>_<mode>_<templateName>_<assayName>_<date>.pdf`	A PDF report that contains sample-specific results. For more information, see “Variant report” on page 159.
Sample Summary[3]	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.
Troubleshooting Files		
Log Files[4]	`analysis.log`	Analysis log file.
	`summary.<timestamp>.log`	Start and end time for each time an assay module is executed for the analysis.
	`various.err` `various.out`	Analysis pipeline logs used by field service engineers for troubleshooting.
	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.

(continued)

Option	File name	Description
Log Files[4]	`PlannedRun-AuditTrail.pdf`	Contains the audit trail of the run plan in PDF format.
Other[4]	`analysis.ini` `analysisSamples.json`	Analysis configuration files, including secondary and tertiary INI files.
VCF Files	`analysis.vcf`	Summary of variant results in variant call format (VCF).

[1] You can view the extracted files individually, or upload a VCF file to a software application that accepts VCF files, such as Ion Torrent™Oncomine™ Reporter software.

[2] Files are available only for assays from Genexus™ Software version 6.2 and earlier.

[3] Files are available for both custom and system installed assays in Genexus™ Software version 6.6.

[4] Separate folders are generated for each sample. If included in the run, separate folders are also generated for an NTC and positive control.

Review run results

Use the **Results / Run Results** screen, runs that are pending, in progress, or completed are listed. Runs with a status of failed, aborted, or stalled are also listed.

You can search the list of results when you click **(Filter)** in a column of interest, then entering a full or partial run or assay name, or other applicable filter term.

The following run information appears in the **Results / Run Results** screen.

Column	Description
Run Name	The unique name of the run given when it was created in the software. Click a run name to open the **Run Summary**. Runs that are reanalyzed are listed with **(Reanalysis)** after the run name.
Assay Name	The name of the assay selected for the run. You can view the **Assay Name** and corresponding **Assay Full Name** for all assays in the **Assays ▸ Manage Assays** screen.
Run Status	The status of the run. For example, **Analysis In Progress**, **Executing Plugin**, **Analysis Completed**, **Terminated**, **Archival: In Progress**, **Purification In Progress**, or **Purification Completed**).
Total Samples	The total number of samples in a run.
PCR Plate Number	A unique identifier for the 96-well plate used for library preparation and templating. For more information, see “Assign the PCR Plate” on page 157.
Started On	The date and time when the run was started.
Updated On	The date and time when the last action was completed on the run.

Users with appropriate permissions can perform the following actions in the **Results / Run Results** screen.

Option	Description
Actions	Available action links for a run are shown when you place the pointer over the row of a run. The actions that are available depend on the type of run. • **BAM Uploader**—Upload run information to another Genexus™ Software server or to Ion Reporter™ Software for further analysis. **BAM Uploader** is not available for BAM run results or for archived runs in which BAM files have been removed. • **Audit**—View the audit trail for the run. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting. • **Assign PCR Plate**—Enter a unique identifier for the 96-well plate used for library preparation and templating. This action is available only in **Sample to Result** and **Nucleic Acid to Result** runs after the run completes. • **Download Files**—Select the **Variants**, **Reports**, **Sequencing Results**, **Audit and Log**, and **Troubleshooting Files** to download.

View the run summary

The run summary provides an overview of the run. The information that is displayed includes the name of the assay used in the run, sample locations, information about the reagents used in the run, primer tube positions, and instrument information. Metrics from sample purification are also provided, if applicable.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Name** column, click the run name of interest.
   The **Run Summary** tab opens.
3. Review the run summary.

Action	Procedure
View the assay metrics.	In the **Assays** section, click the assay name of interest.
View the sample locations in an image of a 96-well sample plate.	In the **Sample Locations** section, click **PCR Plate View**.
Reanalyze a run with a new assay.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **Reanalyze**.
Run plugins on the sequencing data after a sequencing run is complete.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **Run Plugin**.
Download customer support archive (CSA) log files for the run to help with troubleshooting.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **CSA**.
Upload results to another Genexus™ Software server or to Ion Reporter™ Software for further analysis.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **BAM Uploader**.
View the history of variant classifications.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **Variant Audit**.
View the run report.	Click the **Run Report** tab.

Assign the PCR Plate

In **Sample to Result** and **Nucleic Acid to Result** workflows, Genexus™ Software allows you to track and associate a run with the PCR plate used in the run. The PCR plate is the 96-well plate that is used for library preparation and templating. You can assign a unique identifier, a **PCR Plate Number**, to runs that have a status of **Library Preparation Completed**, **Sequencing Completed**, or **Run Completed**. The PCR plate number that you enter is shown in the **Run Results** screen and if needed, can help you track libraries and troubleshoot. Later, if you sequence the remaining libraries in a different sequencing run and assign the PCR plate number to the run, you can easily search for and find all run results associated with the libraries in the PCR plate.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, place the pointer over the row of a run of interest, then click **Assign PCR Plate number**.
3. In the **Assign PCR Plate Number** dialog box, confirm, edit, or enter the **PCR Plate Number**.

   The PCR plate number must be between 1 and 10 characters. Only alphanumeric characters (numbers 0 to 9 and letters A to Z), period (.), underscore (_), or hyphen (-) are allowed. Spaces are not allowed.
4. Click **Submit** to associate the PCR plate with the run.

View assay metrics and the run report

The run report provides detailed information about a run, such as the **Total Bases** and **Final Reads**. For runs with multiple assays, metrics are provided for each assay in the run. The run report contains the assay metrics for all assays in the run.

For runs with only one assay, the run report and assay metrics show the same information for assay metrics. In addition to the metrics for the assay, the run report shows information about the run and the instruments used in the run.

1. To view the **Run Report**, in the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest. In the **Run Summary** screen, click the **Run Report** tab.

   The **Run Report** is assay-specific and cannot be viewed within the sample results screens. To view the **Run Report**, ensure that **Select Sample** is selected in the **Select Sample** dropdown menu.
2. Click **Results** ▸ **Run Results** to view sequencing results for a particular sample.
3. In the **Run Name** column, click the run name of interest.

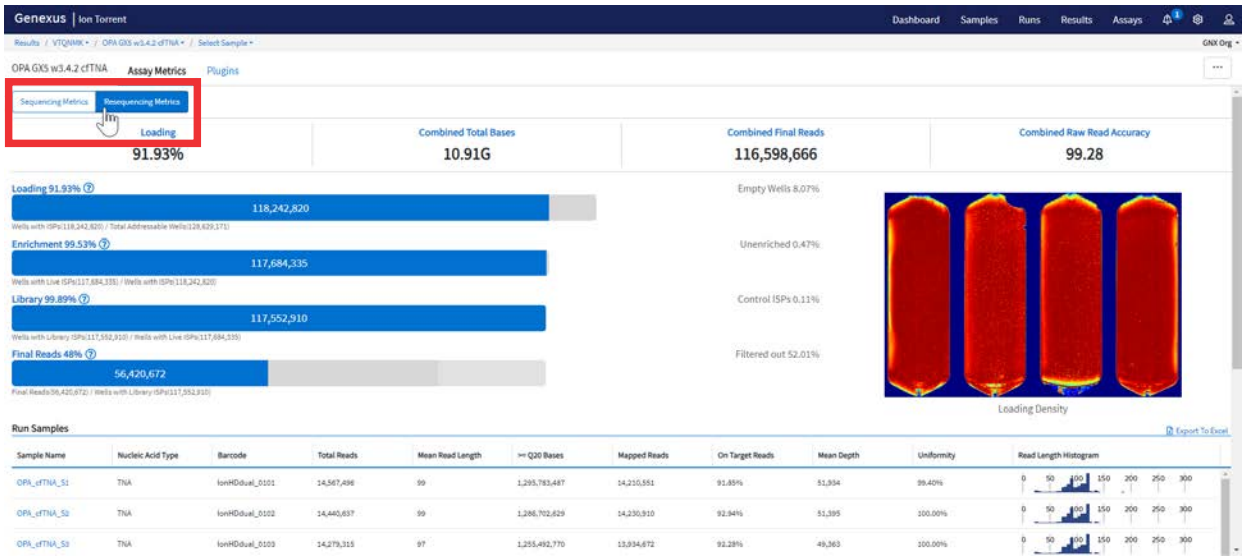
Action	Procedure
View the run report	Click the **Run Report** tab.
View the assay metrics for a single assay	In the **Select Assay** dropdown list at the top of the screen, select the assay of interest.

For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Assay metrics and the run report

The run report provides detailed information, such as chip metrics for the run, well, and Ion Sphere™ Particles (ISPs) statistics. For runs with multiple assays, metrics are provided for each assay in the run. Sequencing metrics are shown at the top of the screen, followed by sample-specific metrics in the **Run Samples** table. Read data for individual samples for the assay are listed in the **Run Samples** table.

Barcode-specific metrics for barcodes that are included in the run are listed in the **Barcodes With Reads Reported** table, which follows the **Run Samples** table. The CSA file for the run contains information for barcodes that are not assigned to samples in the run. Information in the CSA file can help you troubleshoot results, if needed. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).



For descriptions of each assay metric, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

View resequencing metrics

For assays that include resequencing, you can view resequencing metrics when a run is complete.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, in the **Run Name** column, click the run name of interest.
3. In **Select Assay**, select the assay name of interest.
   The assay metrics for the sequencing run are shown.
4. Click **Resequencing Metrics**.
   The resequencing metrics for the assay are shown.

Download a run report

You can download a run report summary in PDF format. The run report includes assay metrics and the record of reagents that were used in a run. For information about the contents of the run report, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409). If you entered extraction kit barcodes for samples when you prepared library batches or when you planned the run, the extraction kit barcodes are listed in the run report.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample name of interest.
3. Click the **Reports** tab.
   Multiple panes including a **Run Report** pane, a **Variant Report** pane, and any panes for customized reports that have been generated are shown.
4. In the **Run Report** pane, click **Download Report** to download a run report summary in PDF format.

Variant report

A variant report is a PDF report of the results for each sample in a sequencing run. You can use a system-installed report template. For system installed report templates, the assay used in the run determines the data that are included in the report.

Alternatively, you can customize the layout and contents of a variant report.

To automatically generate a variant report for each sample during data analysis of a run, enable **Generate Report** in the **Setup** step when you plan the run (for more information, see Chapter 4, "Plan and manage runs"). Alternatively, if you did not enable this feature, you can generate a variant report for each sample after a run is complete. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

When a variant report has been generated for a sample, it is available for download in two places:

- A link is available in the **Results / Sample Results** screen when you place the pointer over the row for that sample. Click the link to download the PDF.
- A **Download Report** button is available in the **Variant Report** pane in the **Reports** tab.

Variant reports can be electronically or manually signed by users. Electronically signed reports have **(Sign off)** after the sample name in the **Sample Results** screen. The electronic signature is included in the footer of the report. If included, electronic signatures also appear in the Electronic Signature section of the report. A sample name followed by **(Locked)** indicates that the variant report is locked. For more information, see "Sign off on the run results" on page 160.

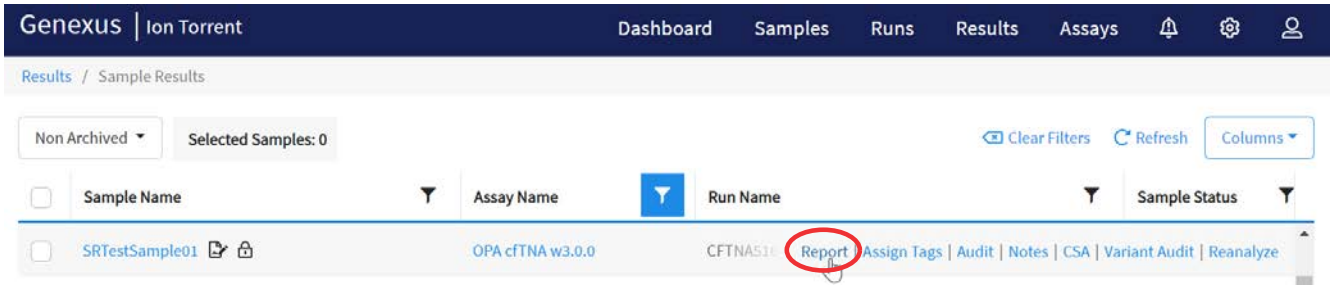
For more information about variant reports and report templates, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Download a variant report

You can download a variant report for a sample result of interest from the **Results / Sample Results** screen.

Note: The variant report is also available for download as part of the results files in the **Results** screen for a specific sample. For more information, see "Results files" on page 153.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, place the pointer over the row of the sample of interest, then click **Report**.



A ZIP file that contains the PDF report downloads automatically.

3. Extract the downloaded files, then open the PDF file in an appropriate viewer.

Sign off on the run results

Multiple users can sign variant reports. In the **Results / Sample Results** screen, a sample name followed by indicates that the sample results are signed off by at least one user. A sample name followed by indicates that the variant report is locked. After a variant report is locked, the report is no longer available to be signed by any other user. The signature information appears in the variant report PDF file. For more information, see "Variant report" on page 159.

Multilanguage support for PDF report generation is provided.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

Permission to sign reports must be enabled in order to sign reports. An administrator-level user manages electronic signature permissions for user accounts. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample result of interest.
3. In the upper right corner of the screen, click ··· **(More Options)** ▸ **Sign Off**.
4. In **Password**, enter the password.

5. In the **Electronic Signature** dropdown list, select the meaning of the signature.

   Administrator-level users configure the meaning of electronic signatures. One type of signature is designated to lock a variant report when the report is signed.

6. In the **Report Template** dropdown list, select the report template that you want to use.

   The option to select the report template is available only before the variant report has been signed by any user.

7. In **Sign Off Comments**, enter a comment.

8. In **Report Name**, change the name of the report if needed.

   The option to change the report name is available only before the variant report has been signed by any user.

9. In **Select Your Language**, change the language of the report if needed.

   The option to change the language is available only before the variant report has been signed by any user.

10. In **Footer Field**, enter any text that you want to appear in the footer of the PDF report pages.

    If you entered footer information in the **Footer Field** when you created a report template, the same footer information appears in the **Electronic Signature** dialog box. You can enter new footer information to override the report template.

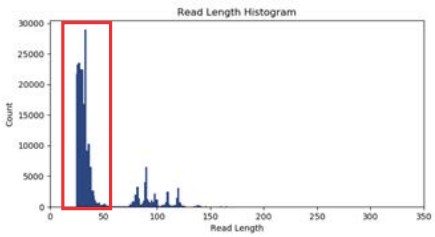
11. Click **Sign Off** to confirm the electronic signature.

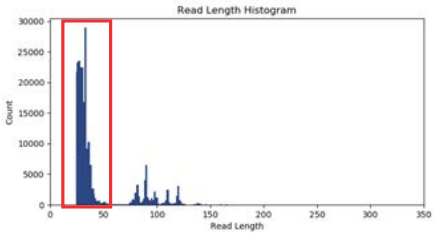
The report is signed. If the signature is designated to lock the report, the variant report is signed and locked.

For information to download the signed report, see “Download a variant report” on page 160.

A Troubleshooting

Oncomine™ Precision Assay GX

Observation	Possible cause	Recommended action
The number of assigned GX5™ Chip lanes is 2X more than expected when planning a run for FFPE samples using the Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions assay.	In the **Samples** step of run plan creation, FFPE DNA and FFPE RNA samples are assigned to an assay separately.	When entering samples for use with the Oncomine™ Precision - GX5 - w3.4.2 - Combined - Solid Tumor - DNA and Fusions assay, in the **Add New Sample** dialog box, select **DNA & RNA** from **Sample Type** dropdown list (see "Enter samples in the Genexus™ Software" on page 34). The minimum reads assigned to each FFPE sample that includes DNA and RNA is 3,000,000. Each sample is assigned as a DNA and RNA set on the plate layout when planning the run. When the run completes, the results display both DNA and Fusions results for the sample.
High byproduct peak is observed in the **Read Length Histogram** for an FFPE or catnap sample.  In the **Results** > **Run Results** > **View Results** screen for the sample, click **QC** to review the **Read Length Histogram**.	The quality of the FFPE sample is poor due to crosslinking, which cannot be measured by the Agilent™ Bioanalyzer™ Instrument or TapeStation. Chemical fixation of tissue can cause excessive crosslinking of samples, resulting in poor DNA or cDNA amplification.	Repeat the sequencing run using a good quality FFPE sample. Use a sample control in your sequencing run to determine if the quality of your sample is poor (see "Recommended sample controls" on page 21).

Observation	Possible cause	Recommended action
High byproduct peak is observed in the **Read Length Histogram** for an FFPE or catnap sample.  In the **Results** > **Run Results** > **View Results** screen for the sample, click **QC** to review the **Read Length Histogram**. *(continued)*	Sample input is low due to the use of incorrect sample concentration, dilution, or volume.	Ensure sample concentration and dilutions as described in "Quantify nucleic acid samples" on page 100 and "Dilute the samples (if needed) and load the sample plate" on page 101, then repeat the sequencing run using the correct sample concentration and volume.
Significantly lower number of reads observed for the last one or two samples. **Note:** The number of reads for each sample that was used in a run can be viewed in the **Run Metrics** screen of the run results, in the **Total Reads** column of the **Run Samples** table.	When setting up the run, reagents were not collected at the bottom of the strips. This can cause insufficient reagent volume for the last few samples.	Before you load the reagents into the sequencer, prepare the strips as described in "Before you begin" on page 109, then repeat the sequencing run.
cfTNA sample – CNV QC failed. **Details:** The **MAPD** QC metric did not fall within the range that was specified by the assay.	The extraction of cfTNA was unsuccessful.	Use the recommenced kits for cfTNA extraction and carefully follow the manufacturer's protocol. For more information, see "Recommended materials for nucleic acid isolation, quantification, and quality control" on page 21.
		Ensure that the extracted cfTNA samples pass quality control before starting the sequencing run. For more information, see "Evaluate nucleic acid sample quality" on page 100.
	DNA is degraded.	Follow the instructions from the blood collection tube manufacturer for sample collection and storage.
		Use the RNase*Zap*™ RNase Decontamination Solution to decontaminate your workspace before sample processing.
		Use a sample control in your sequencing run to determine whether the quality of your sample is poor (see "Recommended sample controls" on page 21).

Observation	Possible cause	Recommended action
Fusion QC failed. **Details:** The **RNA Expression Ctrls Detected** QC metric did not fall within the range that was specified by the assay.	The RNA yield from cfTNA or FFPE sample was low.	Use the recommended kits for sample preparation. For more information, see "Recommended materials for nucleic acid isolation, quantification, and quality control" on page 21.
	RNA was degraded in cfTNA or FFPE samples.	Follow the instructions from the blood collection tube manufacturer for sample collection and storage.
		Store cfTNA samples at –80°C to prevent RNA degradation.
		Use a sample control in your sequencing run to determine whether the quality of your sample is poor (see "Recommended sample controls" on page 21).

Genexus™ Integrated Sequencer—general and QC troubleshooting

Observation	Possible cause	Recommended action
A consumable is not recognized by the sequencer after loading on the deck	The consumable (for example, a strip, pipette tip box) is correctly placed but is not completely inserted into its position, causing it to be misaligned with its expected position.	Ensure that the consumable is pressed completely into place. Apply firm pressure on the item so that it fits snugly into its deck position.
	The barcode of the consumable is not readable by the instrument.	Tap **Help** in the lower left corner of the **Load Instrument** screen and follow on-screen instructions to override the block manually. Note that the name of the consumable does not appear in the list of consumables in the run summary.
		If the behavior continues in subsequent runs, contact Technical Support.
	Consumable version does not match the Genexus™ Software version. For example, a consumable compatible with Genexus™ Software 6.6 is installed in a sequencer updated for Genexus™ Software 6.8.	Ensure that you are using consumables compatible with the software installed on the sequencer.

Observation	Possible cause	Recommended action
A consumable is not recognized by the sequencer after loading on the deck *(continued)*	The expiration date of the consumable has passed.	Ensure that you load consumables that have not exceeded their expiration dates.
	The instrument camera is not functioning properly.	If no other cause can be found for the issue, there may be a problem with the camera. Contact Technical Support.
Run Status = **Failed** **Details:** In the Genexus™ Software **Run Result** screen, the **Run Status** for a completed run is listed as "**Failed**". In the **Sample Results** screen, the **Sample Status** is listed as "**BaseCallingActor FAILED**".	Chip calibration failed due to a chip problem, or an instrument problem.	Repeat the run with a new chip. If the problem persists, contact Technical Support.
A lane that has been used is not crossed out in the sequencer screen **Details:** After completion of a run, the lane used in the run was not crossed out, so that the next run could reuse the lane.	A chip problem caused a datacollect failure to read efuse.	In the sequencer screen, tap **Settings** ▸ **Clean instrument** to perform a clean instrument. For details, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910). After cleaning, start a new run. **Note:** The **Clean instrument** procedure renders remaining sequencing reagents and unused lanes on the installed chip unusable in a sequencing run after the cleaning.
The number of sample reads is low, CF-1 metrics pass QC, but read ratio of inline controls is low **Details:** If CF-1 reads per lane, accuracy, and mean AQ20 read length are good, and read ratio of inline controls (endogenous vs. spike-in) is low (<< 3), a problem with sample input is indicated. For more information, see “Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls” on page 168.	Nucleic acid input may have been insufficient, and/or the nucleic acid was degraded.	For a sample run, requantify nucleic acid samples and/or perform sample QC to ensure that the expected nucleic acid input and size was loaded.
		If needed, reisolate and purify nucleic acid samples.
The number of sample reads is low, but CF-1 metrics pass QC, and read ratio of inline controls is normal **Details:** If CF-1 metrics passed QC, and read ratio of inline controls is normal (~ 3), a problem in library preparation unrelated to sample input or quality may be indicated. For more information, see “Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls” on page 168.	One or more of the Genexus™ Strip 1 strips used in the run had magnetic beads trapped in the tube 5 keyhole.	Repeat the run with strips that you have verified have no trapped beads. For more information, see “Before you begin” on page 109.
	An incorrect assay was selected for the run, or library amplification parameters were not optimal.	Ensure that you have selected the correct assay and reviewed assay parameters.

Observation	Possible cause	Recommended action
The number of sample reads is low, but CF-1 metrics pass QC, and read ratio of inline controls is normal **Details:** If CF-1 metrics passed QC, and read ratio of inline controls is normal (~ 3), a problem in library preparation unrelated to sample input or quality may be indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 168. *(continued)*	Library strips were inadequately equilibrated to room temperature (Genexus™ Strip 1), or incompletely thawed (Genexus™ Strip 2-AS or Genexus™ Strip 2-HD) before loading in the sequencer.	Ensure that Genexus™ Strip 1 strips are fully equilibrated to room temperature, and Genexus™ Strip 2-AS strips are completely thawed before loading in the sequencer.
The number of sample reads is low, and CF-1 metrics fail QC **Details:** If CF-1 metrics failed QC, a problem in templating or sequencing is indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 168.	One or more of the Genexus™ Strip 3-GX5™ strips used in the run may have had an excessive amount of magnetic beads trapped in the tube 6 or 7 keyhole.	Repeat the run with strips that you have verified have no trapped beads. For more information, see "Before you begin" on page 109.
	Template strips were inadequately equilibrated to room temperature (Genexus™ Strip 3-GX5™), or incompletely thawed (Genexus™ Strip 4) before loading in the sequencer.	Ensure that Genexus™ Strip 3-GX5™ strips are fully equilibrated to room temperature, and Genexus™ Strip 4 strips are completely thawed before loading in the sequencer.
	The sequencing chip or coupler was faulty or leaky.	Repeat the run with new chip and coupler. If low performance continues, contact Technical Support.
	The run was started >14 days after the last initialization was performed, or on an expired initialization.	Perform a Clean instrument procedure (**Settings ▸ Clean instrument**), install new a chip, and new sequencing reagent bottles and cartridge in the sequencing reagents bay, then repeat the run. **Note:** Reagents are stable on the sequencer for 14 days, after which you may experience reduced performance. For further information, see *Appendix A: Troubleshooting* in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

Observation	Possible cause	Recommended action
Panel primer tubes lifted from the Genexus™ Primer Pool Tube station	The tubes became loose.	Use the Genexus™ Primer Pool Tube Holder to secure the tubes in place. For information about using the Genexus™ Primer Pool Tube Holder, see "Load the sequencer and start a run" on page 113.
Sample QC passes, but CF-1 Mean AQ20 Read Length QC fails	Reads were filtered due to low quality ISPs caused by too much library input in the templating reaction.	Recover the unused pooled sample library from the run, then plan and perform a Library to Result run with diluted library. 1. To recover the pooled sample libraries from well E4 of the PCR amplification plate after the run, follow the procedure described in the *Recover the pooled sample libraries* section in the *Genexus™ Integrated Sequencer User Guide* (MAN0017910). 2. Add the recovered pooled sample libraries to well A1 of a new sample plate and dilute. • For a 4-lane run, add 49 µL of the pooled libraries to well A1, then add 91 µL of nuclease-free water or Low TE to bring the total volume to 140 µL. • For a 2-lane run, add 28 µL of the pooled sample libraries to well A1, then add 52 µL of nuclease-free water or Low TE to bring the total volume to 80 µL. 3. Pipet the contents of well A1 up and down 4–5 times to mix. 4. Plan and perform a Library to Result run with the diluted library. For more information, see the *Genexus™ Integrated Sequencer User Guide* (MAN0017910).

Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls

You can use quality control results to troubleshoot Genexus™ Integrated Sequencer runs to help identify the cause of performance problems. If you select the **Include Inline Controls** checkboxes for DNA and RNA in the **Reagent** step when you create an assay (see), you include the inline control analysis in the post-run results analysis. Inclusion of a set of six control amplicons (covering a range of amplicon length) and spike-in nucleic acid into sample library preparation reactions helps determine whether poor performance is due to insufficient sample input and/or poor sample quality, or is unrelated to sample input and quality. With 10 ng sample input, the read ratio of endogenous sample reads to spike-in control reads is expected to be ~3. Using more than 10 ng sample input results in a proportionally higher read ratio. For example, if you load 20 ng of sample, the read ratio should be ~6.

The CF-1 templating control serves as a check on templating and sequencing performance that is independent of library preparation.

Use the following table as a guide to help identify the source of performance problems. For recommended actions, see the troubleshooting topics under "Genexus™ Integrated Sequencer—general and QC troubleshooting" on page 164.

QC category	Run diagnostic			
	Successful run	Sample input and/or quality problem	Library preparation problem unrelated to sample	Templating or Sequencing problem
Sample QC (endogenous sample reads)	Passed	Failed	Failed	Failed
Read ratio for inline controls (endogenous to spike-in reads)	Normal Read ratio ~3	Low Read ratio <<3	Normal or variable	—
Templating Control QC - CF-1	Passed	Passed	Passed	Failed

Genexus™ Software

Observation	Possible cause	Recommended action
Cannot sign in to the Genexus™ Software	You have either forgotten your password or are signed out due to several failed login attempts.	Contact the Genexus™ Software system administrator.
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique sample name.	Every sample name in the software must be unique. Ensure that the spreadsheet does not contain any duplicate sample names, then repeat the import. Note that the system check is not case-sensitive, so a sample name of ABC1 conflicts with abc1.
	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors. Map a sample attribute if needed.
Cannot upload my panel or hotspots	Issues with BED file format or files do not end in ***.bed***.	Ensure your file is in the correct BED format and has a ***.bed*** extension.
Variants tab is missing hotspot entries **Details:** The remaining entries are present.	Hotspot BED file contains entries that are incorrectly formatted.	Check that BED file entry is correctly formatted. See the following examples: SNP entry: chr1 2337276 2337277 SVA_322 0 + REF=C;OBS=T;ANCHOR=G AMPL Deletion entry: chr1 201341175 201341180 SVA_497 0 + REF=AGAAG;OBS=;ANCHOR=C AMPL Insertion entry: chr1 236978992 236978992 SVA_621 0 + REF=;OBS=TCTG;ANCHOR=T AMPL
		Ensure that the REF values match the actual reference coordinate of hg19.

Observation	Possible cause	Recommended action
No information for my loci of interest in the results	The wrong hotspot or BED file is associated with the assay.	1. Check the hotspot and BED files associated with the assay. If either is incorrect, create a new assay. 2. Plan a new run for the sample or sample library with the correct assay. 3. Repeat sequencing of the sample or sample library.
The results of the run do not appear in the **Results / Run Results** screen	The instrument disk space is full.	Clear disk space on the sequencer. For more information, see "*Manually delete run data*" in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).
Cannot download run result files	The run failed.	Create an assay with the correct configuration for the samples, then reanalyze the samples.

B

Supplemental information

Oncomine™ Precision Assay GX

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Copy number deletion	**Loss-of-Function**	**Deletion**	• SVTYPE = "CNV" • FILTER = "LOSS" • Occurs in a designated copy-loss gene
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Expression imbalance	**Gain-of-Function**	**ExpressionImbalance**	• SVTYPE = "RNAExonTiles" • FILTER = "PASS" • Record meets Targeted Isoforms Detected Requirement
Loss-of-function truncating non-hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and coding syntax occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and coding syntax occur in predefined intronic hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

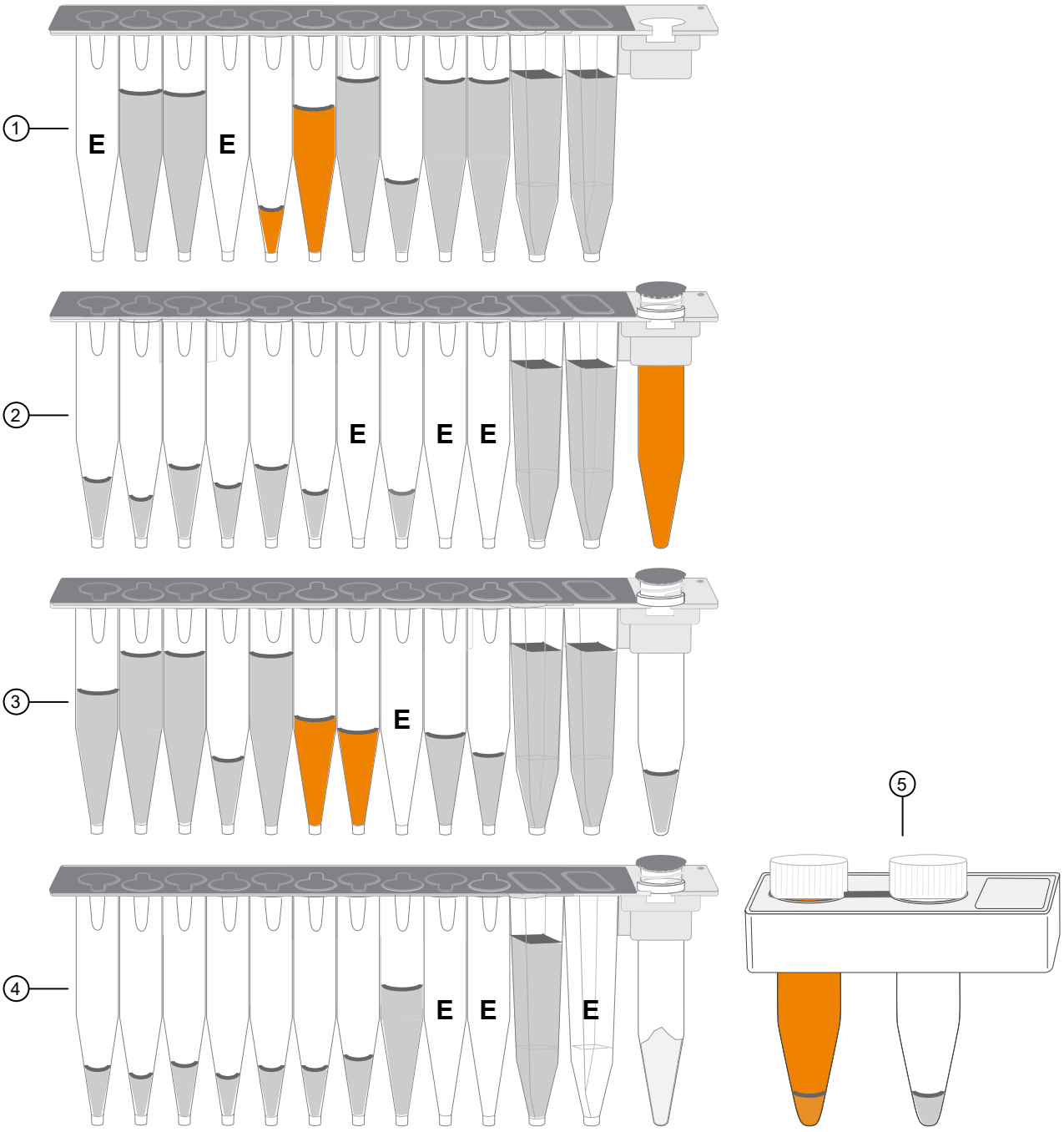
(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
EGFR exon 19 deletion	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR
EGFR exon 20 insertion	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list
ERBB2 exon 20 insertion	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2
KIT exon 11 deletion	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 550–580 of KIT • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11
KIT exon 11 insertion	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 550–580 of KIT
MET exon 14 skipping	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET Exon 14 Skipping confirmed list
PDGFRA exon 18 deletion	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 841–847 of PDGFRA

For more information, see the Genexus™ Software help system or contact support.

Genexus™ reagent strip and panel tube fill volume guide

You can use the figures below to check for appropriate fill volume of library and template strip tubes, and Oncomine™ Precision Assay GX primer pool tubes. Print this page at 100% and keep as a reference. If a fill volume appears inaccurate, contact Technical Support to report the problem. "E" indicates an empty tube.



① Genexus™ Strip 1
② Genexus™ Strip 2-HD
③ Genexus™ Strip 3-GX5™
④ Genexus™ Strip 4
⑤ Assay primer pool tubes

Create a template for importing samples in standalone configuration purification run plans

To use the **Import** function to import sample information from a USB drive into a standalone configuration run plan for the Genexus™ Purification System, add the sample information to a CSV file with a specific format. Follow these steps to create an import template file with a plain text editor, or with Microsoft™ Excel™ software.

1. Use the format in the follow examples to set up two columns in a CSV file for **Sample Name** and **Notes**. Populate the sample rows with sample name and notes.

```
1   Sample Name,Notes
2   CSV Sample 1,CSV notes for sample 1
3   CSV Sample 2,CSV notes for sample 2
4   CSV Sample 3,CSV notes for sample 3
5   CSV Sample 4,CSV notes for sample 4
6   CSV Sample 5,CSV notes for sample 5
7   CSV Sample 6,CSV notes for sample 6
8   CSV Sample 7,CSV notes for sample 7
9   CSV Sample 8,CSV notes for sample 8
10  CSV Sample 9,CSV notes for sample 9
11  CSV Sample 10,CSV notes for sample 10
12  CSV Sample 11,CSV notes for sample 11
13  CSV Sample 12,CSV notes for sample 12
```

	A	B
1	Sample Name	Notes
2	CSV Sample 1	CSV notes for sample 1
3	CSV Sample 2	CSV notes for sample 2
4	CSV Sample 3	CSV notes for sample 3
5	CSV Sample 4	CSV notes for sample 4
6	CSV Sample 5	CSV notes for sample 5
7	CSV Sample 6	CSV notes for sample 6
8	CSV Sample 7	CSV notes for sample 7
9	CSV Sample 8	CSV notes for sample 8
10	CSV Sample 9	CSV notes for sample 9
11	CSV Sample 10	CSV notes for sample 10
12	CSV Sample 11	CSV notes for sample 11
13	CSV Sample 12	CSV notes for sample 12

① Example file if in a plain text editor.

② Example file in Microsoft™ Excel™ software.

The **Sample Name** field populates the **Sample ID** field in the instrument screen.

2. Save the file using a CSV file format to a USB drive.

IMPORTANT!

- Do not add extra spaces in the headings, or add other columns in either file type.
- If you transfer files between macOS™ and Windows™ computers, extra characters that can cause errors can be added to files. Check for the presence of hidden characters, then delete if found.
- An alert is shown if fewer samples are in the CSV file than are selected for the run, or if two samples have the same name in the CSV file. No samples are imported until the error is corrected.

Lane usage by sample type

The capacity of the GX5™ Chip varies depending on the sample type. Table 14 provides GX5™ Chip lane usage information for run configurations with smaller sample batch sizes. For other run configurations, including larger batch sizes, use the following lane usage and sample plexy recommendations.

- For plasma cfTNA samples, the assay is configured to accommodate 1.5 samples per GX5™ Chip lane (or 6 samples on a single GX5™ Chip).
- For FFPE DNA+RNA samples, the assay is configured to accommodate 4 samples per GX5™ Chip lane.
- For FFPE DNA-only or RNA-only samples, the assay is configured to accommodate 8 samples per GX5™ Chip lane.

Table 14 Lane usage by sample type for small sample batch sizes

Sample type	Number of available lanes	Capacity
cfTNA	4	6 samples
FFPE DNA+RNA	1	4 samples
	2	8 samples
FFPE DNA-only or RNA-only	1	8 samples

Fusion detection methods

Note: For fusion variants detected by expression imbalance alone (RNAExonTiles), we recommend verifying the result using an orthogonal method.

The Oncomine™ Precision Assay GX uses the targeted panel design in combination with software algorithms to detect known and novel gene fusion isoforms. The assay employs the following methods to detect fusions.

Targeted method

In the targeted fusion detection method, panel primers are designed to target specific exon-exon junctions of fusions where the driver gene, the partner gene, and the breakpoint between the driver and the partner gene are known. The sequencing reads are mapped to a reference file that contains only the known gene fusions.

Non-targeted method

In the non-targeted fusion detection method, the panel primers are used to detect fusions between novel combinations of known driver and partner genes. The sequencing reads are mapped to a broader reference, such as the whole exome. Mapping the reads to a broader reference allows for the detection of multiple configurations of driver and partner genes as well as detection of novel breakpoints between the known partner and driver genes.

Exon tiling method

The exon tiling method is a partner agnostic fusion detection method that enables the discovery of novel fusion isoforms and breakpoints. In this method, the primers are designed to target a subset of exon-exon junctions of several driver genes (see Figure 12). Each driver gene in the test sample is analyzed individually. After the sequencing reads undergo normalization and baseline correction, the software measures the intragenic 3′ to 5′ expression ratio for each gene and compares the ratio to the baseline (normal sample). Genes that do not undergo a fusion event are expected to have a 3′ to 5′ expression ratio similar to the baseline (see Figure 13). Genes that undergo a fusion event typically have a 3′ to 5′ expression ratio greater than the baseline (see Figure 14). The imbalance score measures the magnitude of change in 3′ to 5′ expression ratio relative to the baseline. For each driver gene in which fusion was detected, the software also predicts the most likely position of fusion breakpoint. This allows for discovery of novel fusion breakpoints.

Imbalance score = Observed imbalance (test sample) ÷ Expected imbalance (normal sample)

For example, if the observed (test sample) 3′ to 5′ expression ratio is 3, although the expected 3′ to 5′ imbalance for a wild type transcript is 1.5, the imbalance score is 2. Typically, an imbalance score of ≥1.75–2 is indicative of a gene fusion event.

The significance of the expression imbalance is measured by the imbalance p-value. The p-value measures the significance of the imbalance at the predicted breakpoint compared to the negative control gene in the sample. Both, the p-value and the imbalance score are used to determine the occurrence of a fusion event.

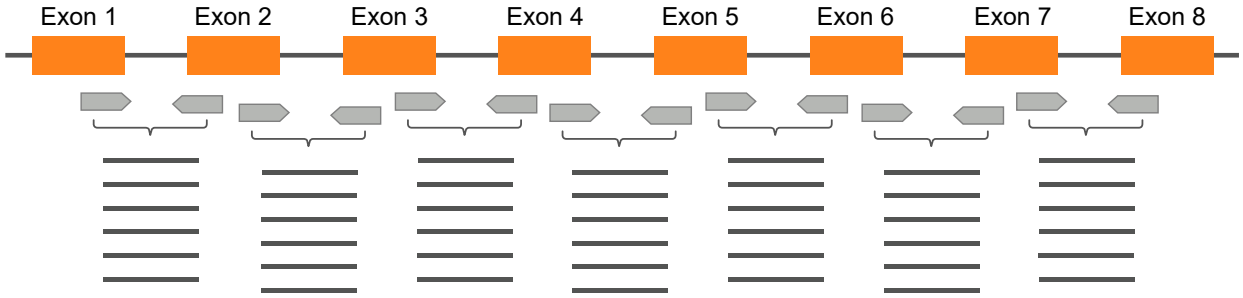


Figure 12 Representative primer design for an exon tiling assay

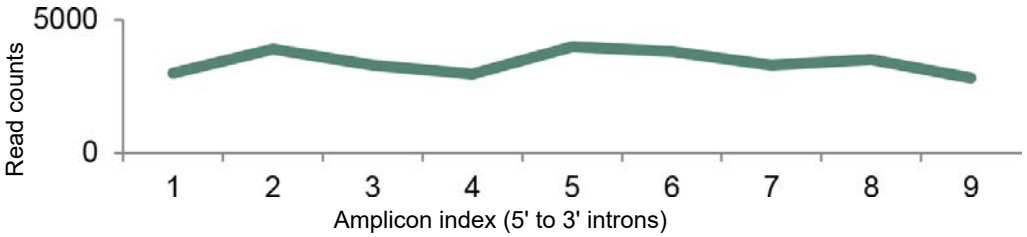


Figure 13 Example coverage profile for a sample with no fusion present In this example, no fusion is present in the sample. The wild type transcript has uniform coverage of 3′ and 5′ introns.

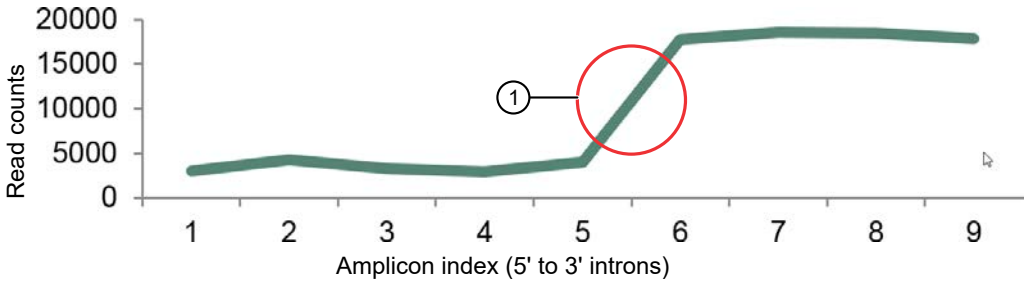


Figure 14 Example coverage profile for a sample with fusion present In this example, a mixture of wild type and fusion transcript is present in the sample. The presence of the fusion transcript accounts for the elevated expression of the 3′ gene region.

① Predicted fusion breakpoint

No-template controls

You can select to include a no-template control (NTC) when you plan a run in Genexus™ Software. NTCs do not contain nucleic acid but are processed in a run alongside test samples. NTCs are used to monitor contamination or primer-dimer formations that could produce false-positive results. Data that is generated from the NTC is displayed in the sample QC results. For more information, see “View the QC results” on page 129.

Reanalysis

If a run fails to meet one or more QC parameters defined by the assay, you can adjust the assay parameters and reanalyze a run or a sample from the run.

Reanalysis of runs can start from the alignment, basecalling, or signal processing steps. A sample can be reanalyzed starting only from the alignment step.

For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Example metrics for a successful sequencing run

The following tables summarize example metric values that have been observed from successful sequencing runs.

IMPORTANT! The values provided in these tables are examples only. The default QC metric values are embedded in the system-installed assay. If needed, you can set values based on your own internal data and the quality of samples that you typically test in the lab.

Table 15 Example metrics for FFPE DNA+RNA isolated from tumor FFPE samples

Metric	Value
Run QC	
Key Signal	59–82
Percent Loading	75–95%
Raw Read Accuracy	98–99%
Templating QC – CF-1 Control	
Average Reads Per Lane	14,000–57,000
Base Call Accuracy	93–99%
Mean AQ20 Read Length (bp)	80–130
Sample QC – DNA	
MAPD	0.17–0.50
Mapped Reads	350,000–2,300,000
Mean AQ20 Read Length (bp)	54–92
Mean Read Length (bp)	56–99
Uniformity Of Base Coverage	76–99%
Sample QC – RNA	
Mapped Reads	12,000–310,000
Mean Read Length (bp)	36–102
RNA Expression Ctrls Detected	3–7

Table 16 Example metrics for cfTNA isolated from normal plasma

Metric	Value
Run QC	
Key Signal	57-73
Percent Loading	89–98%
Raw Read Accuracy	98–99%
Templating QC – CF-1 Control	
Average Reads Per Lane	39,000–63,000
Base Call Accuracy	98–99%
Mean AQ20 Read Length (bp)	94–116
Sample QC – DNA	
MAPD	0.15–0.41
Mapped Reads	5,700,000–15,000,000
Mean AQ20 Read Length (bp)	77–93
Mean Read Length (bp)	82–101
Median Molecular Cov	325–2,315
Median Read Cov	10,000–56,000
Uniformity Of Base Coverage	94–99%
Sample QC – RNA	
Mapped Reads	150,000–670,000
Mean Read Length (bp)	82–102
RNA Expression Ctrls Detected	6–7

Clean the Genexus™ Primer Pool Tube Holder

Use this procedure to clean the Genexus™ Primer Pool Tube Holder.

Note: Dispose of all waste in appropriate liquid or solid waste containers.

1. Inspect the Genexus™ Primer Pool Tube Holder for signs of reagent accumulation.
2. Using lint-free wipes, wipe the Genexus™ Primer Pool Tube Holder with 70% isopropanol.
3. Place the Genexus™ Primer Pool Tube Holder back on top of the Primer Pool Tube Station on the deck and allow to air-dry.
4. When the Genexus™ Primer Pool Tube Holder is dry, proceed with the **UV Clean**.

Generate a variant report

When generating a customized report, you can update any report template selections. For more information about results files, see “Results files” on page 153.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Results / Sample Results** screen, click the sample of interest in the **Sample Name** column.
3. Click the **Reports** tab.
4. In the **Variant Report** pane, click **Generate Report**.
5. In the **Generate Report** dialog box, enter a name for the report, an optional description, and select the report template and language of the report.
   a. If the report template includes an image from the results, select the images to include in the report.
   b. If the custom text that was designated as **Editable on Report Generation** when the report template was created, enter text if needed.

   If you have previously generated a variant report that has not been locked and then you select the same report template that was used to generate the variant report, the new report and any new selections you make override the previous variant report.
6. Click **Generate**.
   A ZIP file that contains all the selected reports and other files is downloaded.

Reports that have been generated are available for download in the **Reports** tab and in the **Sample Results** screen.

C

Safety

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

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

Biological hazard safety

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

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

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

Chemical safety

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

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

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

D Documentation and support

Related documentation

Document	Publication number
Genexus™ Integrated Sequencer User Guide	MAN0017910
Genexus™ Integrated Sequencer Quick Reference	MAN0017912
Genexus™ Purification System User Guide	MAN0018475
Genexus™ Primer Pool Tube Holder Product Information Sheet	MAN1001399
Genexus™ Software 6.8 User Guide	MAN0026409
Genexus™ Software help system	Available in the software
Ion Reporter™ Software 5.16 User Guide	MAN0019148
Ion Reporter™ Software 5.16 Help	Available in the software
Oncomine™ Reporter User Guide	MAN0018068
Qubit™ dsDNA HS Assay Kits User Guide	MAN0002326
Qubit™ RNA BR Assay Kits User Guide	MAN0001987
Agilent™ 2100 Bioanalyzer™ System: 2100 Expert Software User's Guide	SD-UF0000050 (available at **agilent.com**)

Note: For additional documentation, see “Customer and technical support”.

Customer and technical support

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

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

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

Limited product warranty

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