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Oncomine™ BRCA Assay GX

USER GUIDE

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Revision C.0

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: MAN0018514 C.0 (English)

Revision	Date	Description
C.0	12 January 2024	Updates for Genexus™ Software 6.8.1 • Updated ADF file version to w4.1.1 • Instructions were revised for planning a sample to result run (“Plan a Sample to Result run” on page 33). • Instructions were revised for planning a nucleic acid to result run (“Plan a Nucleic Acid to Result run” on page 35). • Instructions for assigning the PCR plate were removed from Chapter 8, “Review data and results”. • Updated information in the QC results table (“QC results” on page 123). • Instructions were revised for export (“View CNV results” on page 134). • Added Variant Name to the SNVs/Indels table (“SNVs/Indels table” on page 132). • Instructions were revised for visualizing exon and whole gene CNVs (“Visualize exon and whole gene CNVs” on page 137). • Instructions were revised for searching on and filtering results in variant tables (“Search and filter variant results” on page 139). • Updated information for Variant name and Subtype in the CNVs table (“CNVs table” on page 134). • Updated information in the results file table (“Results files” on page 142). • Updated this topic to bring it into alignment with the other Genexus™ assay user guides (“Reanalysis” on page 157) • Removed Thermo Fisher Connect topics to bring the Supplemental information appendix into alignment with the other Genexus™ assay user guides. • Added information about output files generated by sampleID plugin (“Output files generated by the sampleID plugin” on page 165).
B.0	19 April 2023	Update ADF file version to w3.7.0.
A.0	13 January 2022	New document for the launch of the Oncomine™ BRCA Assay GX.

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

Contents

1 Product information

Product description

The Oncomine™ BRCA Assay GX (Cat. No. A47912) is a comprehensive targeted next generation sequencing (NGS) assay designed for sensitive detection of all coding regions of the human BRCA1 and BRCA2 genes.

Each kit includes 2 pools of Ion AmpliSeq™ oligonucleotide primers (DNA panel) and Genexus™ library reagents sufficient to sequence up to 32 samples on the Genexus™ Integrated Sequencer, using a GX5™ Chip (see “Oncomine™ BRCA Assay GX” on page 9). One end-to-end sequencing run uses 1 lane on a GX5™ Chip and accommodates up to 16 samples. The provided reagents are sufficient for 2 end-to-end runs. 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 20).

When used with the Oncomine™ BRCA Assay GX, the Genexus™ Integrated Sequencer performs library preparation, sequencing, analysis, and reporting in an automated sample-to-result workflow (when using the Genexus™ Purification System). Depending on the workflow, results can be obtained in as little as a single day. Multiplex primer design and sample barcoding leverage Ion AmpliSeq™ technology to generate results from multiple samples in a single run. Sequencing results are automatically analyzed in the Genexus™ Software using an optimized assay-specific analysis workflow.

This user guide provides the following instructions.

- How to use the Oncomine™ BRCA Assay GX to perform a **Sample to Result** next-generation sequencing (NGS) run on the Genexus™ Purification Instrument for nucleic acid isolation, followed by sequencing on the Genexus™ Integrated Sequencer.
- How to use the Oncomine™ BRCA 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.

About BRCA genes

BRCA1 and BRCA2 genes are tumor suppressor genes that code for proteins that are vital components of the homologous recombination pathway of DNA damage repair. BRCA mutations that result in the deficiency of either gene have been shown to result in inefficient activation of DNA damage repair and are linked to hereditary predisposition to cancer. Errors in the coding sequence of the BRCA genes have been detected in both inherited DNA found in all cells and in mutated DNA found in localized tumor cells. The Oncomine™ BRCA Assay GX is a complete kit that supports the amplification of the entire exonic region of both BRCA genes.

Oncomine™ BRCA Assay GX

The Oncomine™ BRCA Assay GX (Cat. No. A47912) includes a 2-pool DNA panel, Genexus™ Strip 1, and Genexus™ Strip 2-AS. The contents of each kit are sufficient for up to 32 × 2-pool reactions. The panel is provided in 2 packs of 8 tubes (4 tubes of BRCA DNA Pool 1 and 4 tubes of BRCA DNA Pool 2 per pack). Each primer pool in the panel is provided in pairs of tubes, where each tube pair contains one tube with primers in position 1 and one empty uncapped tube in position 2. Two 8-strip packs of the Genexus™ Strip 1 and Genexus™ Strip 2-AS (16 total of each strip) are provided with each kit. If needed, the Genexus™ Library Strips 1 and 2-AS combo kit (Cat. No. A40252) can be ordered separately.

Table 1 Oncomine™ BRCA Assay GX (Cat. No. A47912)

Contents	Carrier color	Number of reactions	Amount	Part No.	Storage
Oncomine™ BRCA Assay GX pools					
BRCA DNA Pool 1 (position 1)	Magenta	2 × 16 (4 reactions/tube)	2 × 4 tubes, 140 µL/tube	A44353	–30°C to –10°C
BRCA DNA Pool 2 (position 1)	Pale green	2 × 16 (4 reactions/tube)	2 × 4 tubes, 140 µL/tube		
Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252)					
Genexus™ Strip 1	Light red	2 × 32 (4 single-pool reactions/strip)	2 × 8 strips	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	2 × 32 (4 single-pool reactions/strip)	2 × 8 strips	A46813	–30°C to –10°C

Sample to Result workflow

Using the Oncomine™ BRCA Assay GX, the **Sample to Result** workflow starts from unprocessed samples and isolates nucleic acid on the Genexus™ Purification Instrument. The resultant 96-well output plate can be loaded directly into the Genexus™ Integrated Sequencer for sequencing.

Note: If using the Genexus™ Purification Instrument in standalone configuration, proceed to the appropriate purification chapter and follow the guidance for on-instrument run planning.

30 min

Select a system-installed assay (page 31)

System-installed assays are available in the Genexus™ Software for use with the Genexus™ Purification Instrument. Each system-installed assay is configured with settings that are optimized for a specific sample type. Create a new assay by copying an existing system-installed assay and modifying the parameters.

Enter samples (page 32)

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

Create a Sample to Result run plan (page 33)

In the Genexus™ Software, create a Sample to Result run plan to integrate both nucleic acid purification and sequencing. The run plan defines the samples, assays, purification kit, sequencing reagents, and the number of sequencing chip lanes that will be required.

up to 3 hr

Prepare FFPE samples (page 38)

AND/OR

Prepare whole blood samples (page 67)

Sample processing prior to adding to the appropriate purification plate and loading onto the instrument for purification of DNA.

5 min

Load the Genexus™ Purification Instrument

The Sample to Result run plan is selected and the run initiated. The instrument performs a prerun UV clean, then samples, reagents, and consumables are loaded on to the instrument.

1–2 hr

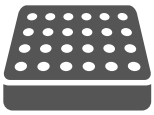
Start the run

After the sample plate and all reagents and consumables have been loaded the instrument door is closed and the run started.

5 min

Unload the purified nucleic acids

Remove the 96-Well Nucleic Acid Output Plate and proceed immediately to sequencing of the purified sample. Remove and seal the 48-Well Nucleic Acid Archive Plate, then store as directed for later use. Used reagents and consumables are removed from the Genexus™ Purification Instrument and a UV clean is performed.



up to 24 hr

Sequence the purified nucleic acids on the Genexus™ Integrated Sequencer (page 93)

Load the output plate with the purified nucleic acids, and all reagents and consumables required for the run, onto the integrated sequencer. Select the **Sample to Result** run plan, then press start.

Nucleic Acid to Result workflow

Using the Oncomine™ BRCA Assay GX, the **Nucleic Acid to Result** workflow starts from purified and quantified nucleic acid samples. Samples are loaded into a 96-well sample input plate and then loaded into the Genexus™ Integrated Sequencer for library preparation, templating, and sequencing.

Note: If using the Genexus™ Purification Instrument in standalone configuration, proceed to the appropriate purification chapter and follow the guidance for on-instrument run planning.

up to 3 hr

Prepare purified DNA

The Genexus™ Purification Instrument is not required for Nucleic Acid to Result sequencing runs. Any of the recommended nucleic acid purification kits can be used to purify DNA for use in Nucleic Acid to Result runs.

If sample quantitation was not performed as part of the purification run, use a Qubit™ Fluorometer to accurately measure sample concentration.

For more information see the appropriate purification protocol chapter.

Enter samples (page 32)

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

Create a Nucleic Acid to Result run plan (page 35)

In the Genexus™ Software, create a Nucleic Acid to Result run plan to create a sequencing run that starts with nucleic acid samples. The run plan defines the samples, assays, sequencing reagents, and the number of sequencing chip lanes that will be required.

up to 24 hr

Sequence the purified DNA on the Genexus™ Integrated Sequencer (page 93)

Transfer extracted nucleic acid, from the archive plate if a Genexus™ Purification Instrument was used, to a 96-well plate which serves as the sample input plate for the Genexus™ Integrated Sequencer. Load the input plate with the purified nucleic acids, and all reagents and consumables required for the run, onto the integrated sequencer. Select the Nucleic Acid to Result run plan, then press start.

Purification-only workflow (standalone configuration)

The following workflow outlines using the Genexus™ Purification Instrument in standalone configuration to prepare purified DNA for use in **Nucleic Acid to Result** runs or other non-sequencing applications.

Note: When using the Genexus™ Purification Instrument in standalone configuration, proceed to the appropriate purification chapter and follow the guidance for on-instrument run planning.

5 min

Create a purification run plan

Create a new purification run plan or copy/edit an existing purification run plan that best represents your experiment, then edit the settings as needed. Purification run plans contain instrument settings that are used in sample purification.

up to 3 hr

Prepare samples

Depending on the sample type, samples may require preparation before loading into the appropriate purification plate and starting the run. For more information see the appropriate purification protocol chapter.

5 min

Load the Genexus™ Purification Instrument

Using the instrument touchscreen, the purification run plan is selected and the run initiated. The instrument performs a prerun UV clean, then samples and consumables are loaded on to the instrument.

1–2 hr

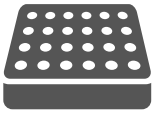
Start the run

After the sample plate and all reagents and consumables have been loaded the instrument door is closed and the run started.

5 min

Unload the purified nucleic acids

Remove and seal the 48-Well Nucleic Acid Archive Plate, then store as directed for later use. Used reagents and consumables are removed from the Genexus™ Purification Instrument and a UV clean is performed.



up to 24 hr

Sequence the purified nucleic acids on the Genexus™ Integrated Sequencer (page 93)

Transfer purified and quantified DNA samples from the archive plate to a 96-well plate which serves as the sample input plate for the Genexus™ Integrated Sequencer. Load the sample input plate with the purified nucleic acids, and all reagents and consumables required for the run, onto the integrated sequencer. Select the **Nucleic Acid to Result** run plan, then press start.

2 Reagents, supplies, and required materials

This chapter lists the reagents, supplies, and materials needed to operate the Genexus™ Purification Instrument 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.

Recommended materials for use with the Oncomine™ BRCA Assay GX

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.

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

Note: These materials are only recommended for Nucleic Acid to Result runs when not using the Genexus™ Purification System.

Item	Source
Nucleic acid isolation	
MagMAX™ DNA Multi-Sample Ultra 2.0 Kit *(Blood samples)*	A36570
PureLink™ Genomic DNA Mini Kit *(Blood samples)*	K1820-01
MagMAX™ FFPE DNA/RNA Ultra Kit *(FFPE samples)*	A31881
RecoverAll™ Multi-Sample RNA/DNA Workflow *(FFPE samples)*	A26069, A26135
Nucleic acid quantification	
Qubit™ 4 Fluorometer[1] and one or more of the following kits: • Qubit™ dsDNA BR Assay Kit *(Blood samples)* • Qubit™ dsDNA HS Assay Kit *(FFPE samples)*	Q33238 Q32850, Q32853 Q32851, Q32854
Nucleic acid quality control	
Agilent™ 2100 Bioanalyzer™ Instrument and one or more of the following kits: • Agilent™ High Sensitivity DNA Kit *(DNA samples)*	G2939BA 5067-4626 (Agilent)

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

Recommended sample controls

Item	Source
Seraseq™ Inherited Cancer DNA Mix v1	0730-0003 (SeraCare)
BRCA Germline I (gDNA)	HD793 (Horizon)
BRCA Somatic Multiplex I (FFPE)	HD810 (Horizon)

Genexus™ Controls

The Ion Torrent™ Genexus™ Controls kit (Cat. No. A40267) provides sufficient Genexus™ Control Library-AS to perform four **Library to Result** runs. The kit also provides sufficient Genexus™ Control Panel-AS and Genexus™ DNA Control to perform eight **Nucleic Acid to Result** runs.

Note: The Genexus™ Control Library-AS is barcoded with IonCode™ 0101.

Component	Quantity	Storage
Genexus™ Control Library-AS	1 tube	–30°C to –10°C
Genexus™ Control Panel-AS	8 carriers (white)	
Genexus™ DNA Control	2 tubes	

Reagents and supplies for use with the Genexus™ Purification Instrument

Genexus™ Purification Instrument 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™ Purification System

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

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

[1] Not available for separate purchase.

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

Ion Torrent™ Genexus™ Multisample DNA Purification Kit

The Ion Torrent™ Genexus™ Multisample DNA Purification Kit (Cat. No. A45540) includes the following subkits sufficient for 48 DNA isolations from whole blood, bone marrow, peripheral blood leukocytes, cultured cells, and fresh or frozen tissue samples.

IMPORTANT! Store all kit components in the upright orientation.

Component	Part No.	Storage
Genexus™ Multisample DNA Purification	A45533	15°C to 30°C
Genexus™ Nucleic Acid Quantitation, Broad Range	A45537	2°C to 8°C
Genexus™ Purification Supplies 1	A45529	15°C to 30°C

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

(continued)

Item	Source
For use with Genexus™ FFPE DNA and RNA Purification Kit	
Sorvall™ ST 8 Small Benchtop Centrifuge, with Thermo Scientific™ M10 Microplate Swinging Bucket Rotor, 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[1]	
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
For use with Genexus™ Multisample DNA Purification kit	
Fisherbrand™ Bead Mill 24 Homogenizer (or equivalent)	15-340-163
Polytron homogenizer	Pro Scientific, PRO250
Tubes, plates, and other consumables	
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	4483354, 4483352
Adhesive PCR Plate Foils	AB0626
CitriSolv™ Clearing Agent	22-143-975
Xylene	**MLS**
Ethanol, 100%	**MLS**

[1] For use with the Genexus™ FFPE DNA and RNA Purification Kit.

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. The following tables provide information about the various ordering options.

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

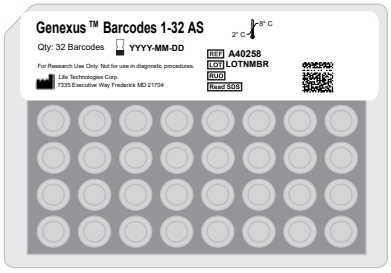
Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Genexus™ Integrated Sequencer

Components	Cat. No.
Genexus™ Integrated Sequencer	A45727

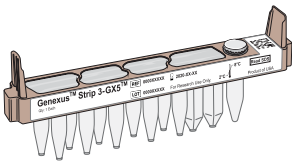
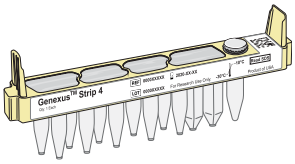
Genexus™ Barcodes AS

Ion Torrent™ Genexus™ Barcodes AS are supplied in plates containing 32 dual barcodes per plate. The barcodes can be ordered as a set of three plates (Cat. No. A40257), or ordered individually.

Item	Label color	Cat. No.	Quantity	Storage
Genexus™ Barcodes 1–96 AS	Blue	A40257	3 plates	15°C to 30°C
Genexus™ Barcodes 1–32 AS 	Blue	A40258	1 plate	
Genexus™ Barcodes 33–64 AS	Blue	A40259	1 plate	
Genexus™ Barcodes 65–96 AS	Blue	A40260	1 plate	

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™ BRCA Assay GX, 4 racks are sufficient for sequencing up to 16 samples on a single GX5™ Chip lane.

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

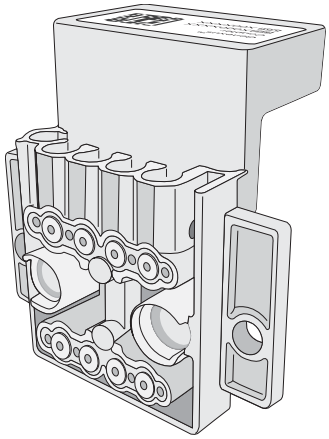
Genexus™ Conical Bottles

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 *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

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

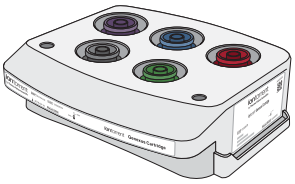
GX5™ Chip and Genexus™ Coupler

The GX5™ Chip and Genexus™ Coupler (Cat. No. A40269) are ordered as a set that contains two chips and two couplers. For the Oncomine™ BRCA Assay GX, 1 chip is sufficient for sequencing up to 64 samples (16 samples per lane).

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**
(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.

3 Before you begin

Guidelines for assay handling and preparation

- Use only the reagents and supplies that have been verified for the Oncomine™ BRCA Assay GX. For a list of verified reagents and supplies, see "Required materials not supplied for use with the Genexus™ Integrated Sequencer" on page 20 and "Recommended materials for use with the Oncomine™ BRCA Assay GX" on page 16.
- Use good laboratory practices to minimize cross-contamination of products. Keep all tubes sealed until immediately before loading onto the Genexus™ Integrated Sequencer.
- We recommed using controls to identify or rule out reagent contamination. For a list of verified controls, see "Genexus™ Controls" on page 17.
- Minimize freeze-thaw cycles of the BRCA DNA Pool 1 and BRCA DNA Pool 2 tubes. Thaw only the number of panel tubes that are required for a given experiment and keep the thawed panels at 4°C until ready to use. Store unused panels at –30°C to –10°C.

  Note: One set of panels (BRCA DNA Pool 1 and BRCA DNA Pool 2 tubes) is sufficient for processing up to 16 samples.

- Do **NOT** store the BRCA DNA Pool 1 and BRCA DNA Pool 2, Genexus™ Strip 1, and Genexus™ Strip 2-AS on the Genexus™ Integrated 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 lab coat that is reserved for sequencing work and is laundered frequently. If possible, change to a fresh lab 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.
- If you are using positive controls and a no-template control (NTC) in the run, change gloves between dispensing these controls, and if applicable, between dispensing samples and controls in sample plate wells.

Equipment and instrument cleaning

- If you are preparing samples and sample plates in a hood (recommended), illuminate the hood with UV light for 15 minutes before use.
- Before use, wipe working surfaces of the hood or bench where samples or sample plates are handled, and other equipment such as vortexers, microcentrifuges, and pipettors with lint-free wipes moistened with DNA*Zap*™ decontamination solutions. Wipe with solution 1 first, then follow with solution 2. Alternatively, a 10% solution of commercial bleach can be used. Follow with wiping of bench and equipment surfaces with wipes moistened with 70% isopropanol or 70% ethanol.
- Before and after a run, sequentially wipe instrument deck surfaces with lint-free wipes moistened with the two DNA*Zap*™ decontamination solutions. Follow with wiping of deck surfaces with wipes moistened with 70% isopropanol or 70% ethanol. The robotic pipettor arm 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 clean instrument surfaces.

- We recommend that you centrifuge the sample plate after sealing. Ensure that the centrifuge has been wiped down and cleaned before centrifuging the plate.

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 carefully between wells with an applicator before vortexing to ensure that the seal is complete and contamination between wells does not occur.
- If possible, have a pipettor and tips reserved for dispensing only NTC.
- 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, 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.

Guidelines for panel and reagent use and handling

- Use only the reagents and supplies that have been recommended in "Recommended materials for use with the Oncomine™ BRCA Assay GX" on page 16 and "Recommended materials for nucleic acid isolation, quantification, and quality control" on page 16.
- Keep panel tubes capped until immediately before loading in the Genexus™ Integrated Sequencer.
- If using, thaw positive controls on ice for 30 minutes. After the positive controls are completely thawed, vortex the tubes, then centrifuge to collect tube contents. Return to ice before loading into sample plate.

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

- Equilibrate or thaw the following reagent strips at room temperature for 30 minutes before loading in the sequencer.
  - Genexus™ Strip 1
  - Genexus™ Strip 2-AS (or Genexus™ Strip 2-HD)
  - Genexus™ Strip 3-GX5™ (or Genexus™ Strip 3-GX7™)
- Thaw Genexus™ Strip 4 on ice for 30 minutes before loading in the sequencer.

  IMPORTANT! Ensure that the contents of strips that are stored frozen are completely thawed before loading in the sequencer.

- Thawed library and templating strips can be vortexed on a platform vortexer to dissolve precipitate or dislodge air bubbles. If you vortex, you must centrifuge the strips to collect tube contents using the Genexus™ Strip Centrifuge Adapter to hold strips during centrifugation.

  For information about obtaining and using the Genexus™ Strip Centrifuge Adapter, see "Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter" on page 96.
- Keep the sample plate, thawed panel, Genexus™ Strip 2-AS (or Genexus™ Strip 2-HD), and Genexus™ Strip 4 on ice or at 4°C until ready to load in the sequencer.
- Do not freeze-thaw the panel. Thaw only the number of panel tubes that are required for an instrument run. Discard unused panel tubes after they are thawed. Store panel tubes at –30°C to –10°C.
- If you are using an assay that uses Ion AmpliSeq™ HD chemistry, do not combine the contents of 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 primer pool tubes or reagent strips on the sequencer for more than 24 hours before starting an instrument run.

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 32. **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 4 target amplification reactions, or 4 barcodes. With a maximum of 8 library strip pairs loaded, a maximum of 16 samples can be run using the Oncomine™ BRCA Assay GX, which contains 2 primer pools.
The number of primer pools per assay	Given the limits of 32 target amplification reactions, and 32 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 32.
The number of unused lanes on an installed chip	A maximum of 4 lanes are available on a single GX5™ Chip.
The minimum read count per sample for an assay	The minimum read count per sample parameter is set during assay creation.

- 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 four samples. The most efficient use of consumables occurs when samples are run in multiples of four.
- Each GX5™ Chip can accommodate up to 16 samples per lane, or 15 samples and 1 no-template control.
- 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 must clear 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 in Genexus™ Software 6.2.0 and later, 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 are available on the plate.
- 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.

Before each use of the kit

Thaw assay reagents as indicated:

- Thaw BRCA DNA Pool 1 and BRCA DNA Pool 2 on ice or at 4°C for at least 30 minutes and keep on ice until immediately before loading onto the Genexus™ Integrated Sequencer.
- Equilibrate Genexus™ Strip 1 and Genexus™ Strip 3-GX5™ at room temperature for at least 30 minutes before loading onto the Genexus™ Integrated Sequencer.
- Thaw Genexus™ Strip 2-AS and 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 Chapter 3, “Before you begin”.

Note: If tapping fails to dislodge a bubble, you can dislodge large bubbles using the technique that is described in Chapter 3, “Before you begin”.

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.

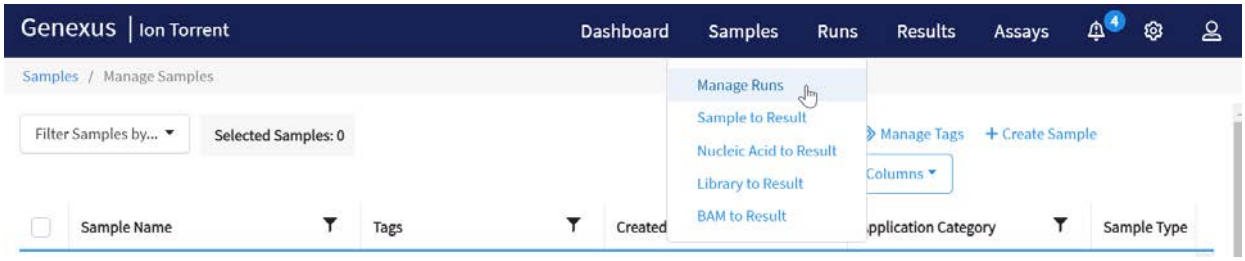
Avoid strong electromagnetic radiation

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™ BRCA 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 Instrument, if applicable.

Table 2 Types of runs for use with the Oncomine™ BRCA 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 Instrument 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 Instrument 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 16.

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™ BRCA Assay GX

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

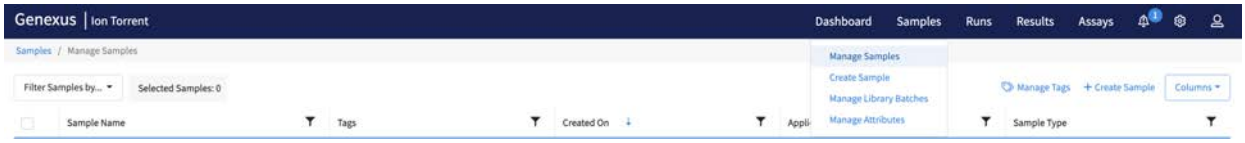
Assay name	Sample type
Oncomine™ BRCA - GX5 Germline - DNA - w4.1.1 (Whole blood DNA only)	DNA
Oncomine™ BRCA - GX5 Somatic - DNA - w.4.1.1 (FFPE DNA only)	

Note: Make sure to update your software to the latest available versions of the system-installed assays. For more information, contact your field service representative. For information on how to perform software and assay package updates, see the software help system.

You can use the system-installed assays in your run plan without change. To modify any assay settings, copy the system-installed assay that best represents your sequencing experiment and sample type, then edit assay settings as needed.

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

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.

From the **Samples** menu, you can add samples in multiple ways. For more information on creating and managing samples, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

About integrated run planning

The Genexus™ Purification Instrument can be used in either a standalone configuration or an integrated configuration to purify nucleic acids from up to 12 samples in a single run. The standalone configuration yields an archive plate with purified and quantified nucleic acid samples that can be used in multiple downstream applications. The integrated configuration yields a 96-well output plate, in addition to the archive plate, that can be used directly in a Genexus™ Integrated Sequencer **Sample to Result** run.

Note: If using the Genexus™ Purification Instrument in standalone configuration, proceed to the appropriate purification chapter and follow the guidance for on instrument run planning.

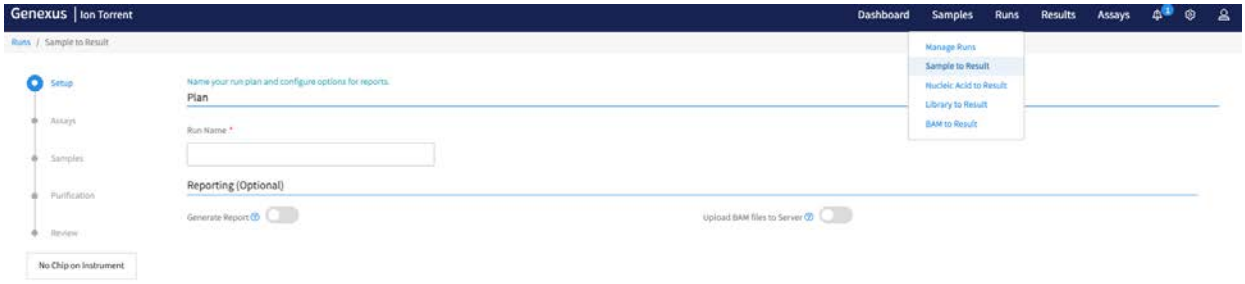
When using the Genexus™ Purification Instrument in integrated configuration, plan a **Sample to Result** ("Plan a Sample to Result run" on page 33) or **Nucleic Acid to Result** ("Plan a Nucleic Acid to Result run" on page 35) sequencing run in the Genexus™ Software. A **Sample to Result** run integrates automated nucleic acid purification and sequencing.

- **Sample to Result**—start from unprocessed samples and isolate nucleic acid on the Genexus™ Purification Instrument, then load the purified and quantified nucleic acid samples directly into the Genexus™ Integrated Sequencer for library preparation, templating, and sequencing.
- **Nucleic Acid to Result**—pipet purified and quantified nucleic acid samples into a 96-well sample input plate and load into the Genexus™ Integrated Sequencer for library preparation, templating, and sequencing.

  Note: The Genexus™ Purification Instrument is not required for **Nucleic Acid to Result** sequencing runs.

When you plan a **Sample to Result** run, nucleic acid isolation is performed in a separate batch for each sample type (for example, FFPE samples and whole blood). The Genexus™ Purification Instrument can run one nucleic acid isolation batch at a time. You can run different nucleic acid isolation batches simultaneously on multiple purification instruments or sequentially on a single instrument.

Plan a Sample to Result run



This procedure applies only if a Genexus™ Purification Instrument 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 Instrument with the system.
- Enter sample information into Genexus™ Software. For more information, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

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

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

   Note: 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 run name.

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

   b. In the **Reporting (Optional)** section, enable **Generate Report** to generate a variant report that uses the default report template.

   c. Click **Next**.

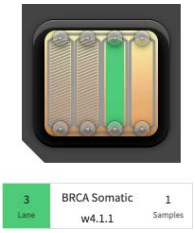
3. In the **Assays** step, select the Oncomine™ BRCA - GX5 Germline - DNA - w.4.1.1, the Oncomine™ BRCA - GX5 Somatic - DNA - w.4.1.1 assay, or both to include in the run.

   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.
   a. In the **Research Application** column for the assay of interest, select research application **DNA** to include the assay in the run plan.

   b. If more than one assay is included in the run, repeat substep 3a for each additional assay.

   c. Click **Next**.

4. In the **Samples** step, select the samples that you want to run with each assay.
   a. Select the checkbox next to each sample that you want to assign to an assay, then in the **Selected Assays** pane, for the assay 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. Green denotes a chip lane in the run containing assigned samples within lane capacity. The maximum number of samples per lane is 16 and cannot be exceeded because the maximum number of barcodes per plate is 32.



Green lane color denotes lane usage and sample assignment within lane capacity.

   b. If desired, select **NTC** to include a no template control and select **Control Sample** to include a positive control.
   The **Chip View** updates to show the lanes used in the run for the included controls.

Note: The total number of samples that can be run per lane is 16, including any NTC and/or Control Sample added to the run plan.

---

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

   d. If needed, edit samples using one of the following options.
      - Click **View & Remove**, make the selections, then click **Update**.
      - Click **Remove All**, to remove all sample assignments for all assays.

   e. Click **Next**.

5. In the **Purification** step, review and edit selections.
   a. Review and edit purification selections.

Option	Description
Protocol Selection	Select a protocol from the dropdown list.
Elution Vol. (µL)	Modify the elution volume within the allowable range, if needed.
Review?	• If selected, the system waits for manual concentration review if the sample concentration is out of range. • If deselected, samples with concentration that is out of range are automatically excluded from sequencing. **Note:** Regardless of the setting, this selection overrides the **Review?** setting of the assay.

b. Review and edit the information for control samples, if applicable.

Option	Description
Conc. (ng/µL)	Review and edit the concentration of control samples, if needed.
Kit Barcodes	Enter the extraction kit barcode for each control sample, if needed.

c. 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.

   The run plan appears in the **Manage Runs** screen with the name that you specified.

   Note: A run can include multiple purification batches. For more information, see the software help system, or the the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

   The run plan summary lists the following details:

   - the consumables that are required for purification
   - how much volume to load
   - where to load samples
   - the consumables that are required for sequencing
   - details about the assay or assays.

   Tip: Click **Sequencing** to expand the sequencing section of the run plan summary.

   IMPORTANT! The consumables required for sequencing can change. After purification, review the run plan in the **Runs / Manage Runs** screen for updated sequencing consumable information. For more information, see the software help system, or the the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Proceed to the appropriate purification kit chapter to prepare and purify samples.

Plan a Nucleic Acid to Result run

You can plan a run for sequencing that starts with nucleic acid samples. In Genexus™ Software 6.8 and later, a run that starts with nucleic acid samples is called a **Nucleic Acid to Result** run.

When performing a **Nucleic Acid to Result** run, start from purified nucleic acid samples that are loaded in the Genexus™ Integrated Sequencer for library preparation, templating, and sequencing

IMPORTANT! The Genexus™ Purification Instrument is not required for **Nucleic Acid to Result** sequencing runs.

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. Click **Next**.

3. In the **Assays** step, select the Oncomine™ BRCA - GX5 Germline - DNA - w.4.1.1, the Oncomine™ BRCA - GX5 Somatic - DNA - w.4.1.1 assay, or both to include in the run.
   a. In the **Research Application** column for the assay of interest, select the research application **DNA** to include each 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.

   b. If more than one assay is included in the run, repeat substep 3a for each extra assay.

   c. Click **Next**.

4. In the **Samples** step, select the samples that you want to run with each assay.
   a. Select the checkbox next to each sample that you want to sequence, then in the **Selected Assays** pane, for the assay that you want to use for the selected samples, click **Assign**.

   b. If you selected more than one assay, repeat substep 4a 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. 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.

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.

Before sequencing, the DNA samples must be transfered to a 96-well sample input plate.

- If starting from FFPE samples, see "Dilute the samples and load the sample input plate—Nucleic Acid to Result run" on page 64.
- If starting from blood, see "Dilute the samples and load the sample input plate—Nucleic Acid to Result run" on page 91.

Lane usage by sample type

The capacity of the GX5™ Chip varies depending on the sample type. Chip capacity information provided in Table 3 applies to assays that use system-installed settings only. For Oncomine™ BRCA Assay GX, each run uses one lane on the GX5™ Chip, which accommodates 16 samples or 15 samples with NTC. If needed, you can modify the number of samples that can be loaded onto a chip lane by copying a system-installed assay and adjusting the **Minimum Read Count Per Sample** parameter. For more information, see the software help system, or *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409), and "Guidelines for Oncomine™ BRCA Assay GX settings" on page 154.

IMPORTANT! Modifications to system-installed assay settings have not been validated. We recommend using system-installed assay settings. Consult your local Field Service Engineer before modifying default assay settings.

Table 3 Lane usage by sample type (system-installed Oncomine™ BRCA Assay GX settings only)

Sample type[1]	Number of available lanes[2]	Capacity
FFPE DNA or Blood DNA	1	16 samples or 15 samples with NTC

[1] Only one sample type per lane.
[2] A maximum of 1 lane can be used in a single run.

5 Genexus™ FFPE DNA and RNA Purification Kit protocol

Note: The procedures described in this chapter only apply to purification of DNA from FFPE samples on the Genexus™ Purification Instrument using the Ion Torrent™ Genexus™ FFPE DNA and RNA Purification Kit. For information on purification of DNA from other sample types using the Genexus™ Purification Instrument, see Chapter 6, “Genexus™ Multisample DNA Purification Kit protocol”.

Genexus™ FFPE DNA Purification workflow

5 min

Plan a purification run (page 40)

IMPORTANT! If performing a **Sample to Result** sequencing run, plan the run in the Genexus™ Software. For more information see "Plan a Sample to Result run" on page 33.

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

3 hr

Prepare samples from FFPE curls (page 45)

OR

Prepare samples from FFPE slides (page 47)

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

5 min

Load the Genexus™ Purification Instrument (page 49)

The purification run plan is selected and the run initiated. The instrument performs a prerun UV clean, then reagents and consumables are loaded on to the instrument.

2 hr

Start the run (page 59)

After the sample plate and all reagents and consumables have been loaded the instrument door is closed and the run started.

Note: Sample quantification adds ~2 hours to the run time.

Genexus™ FFPE DNA Purification workflow

Unload the purified nucleic acids (page 60)

5 min

If performing a Sample to Result run, remove the 96-Well Nucleic Acid Output Plate, add any required control samples and proceed to sequencing of the purified samples. 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 sequencing of the purified sample.

Used reagents and consumables are removed from the instrument and a UV clean is performed.

Note: 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 purification run plan and preparing samples.

Plan a purification run (standalone configuration)

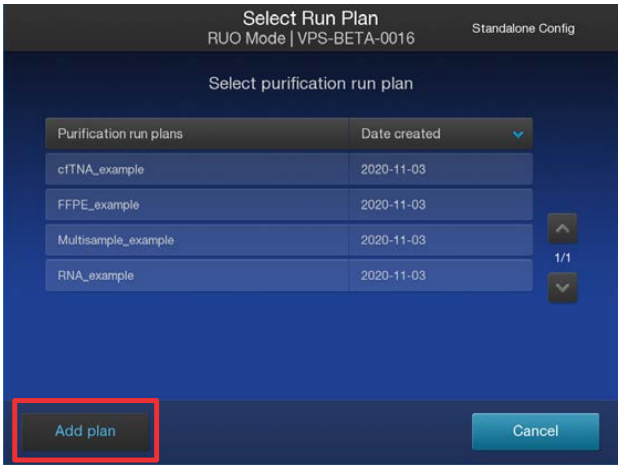
Note: If running the Genexus™ Purification System in integrated configuration, see "Plan a Sample to Result run" on page 33 to plan a **Sample to Result** run in the Genexus™ Software.

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.

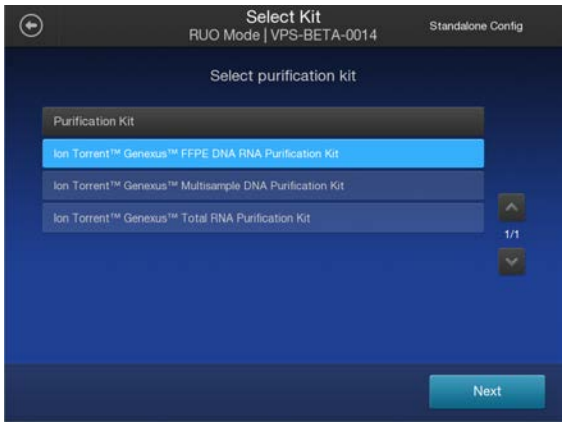
Add a purification run plan (standalone configuration)

Note: We recommend that you plan the run before preparing your samples and loading into the FFPE DNA and RNA Purification Plate 1. However, experienced users can save time by creating the purification run plan 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**.



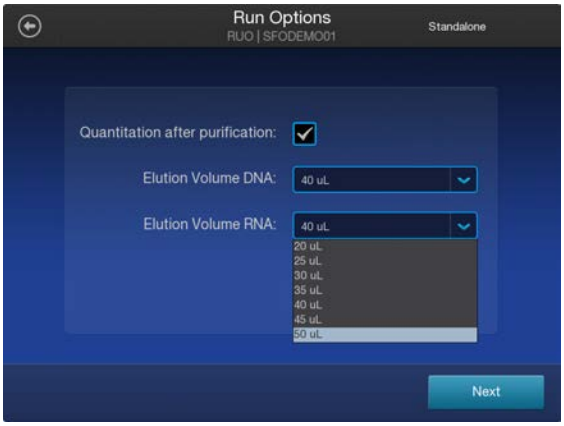
3. Tap in the entry box, enter a unique name for the run plan, then tap **Done** ▸ **Next**.
4. Select the Ion Torrent™ Genexus™ FFPE DNA and RNA Purification Kit, then tap **Next**.



5. Select the **FFPE_DNA_v1** purification protocol, then tap **Next**.

Note: The **FFPE_DNA_RNA_v1** and **FFPE_RNA_v1** purification protocols are not currently supported with the Oncomine™ BRCA Assay GX.

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



Note:

- 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.
- Quantitation requires up to 5 µL of the eluted sample. If the expected sample yield is limiting, manual sample quantitation may be preferred to preserve sample.

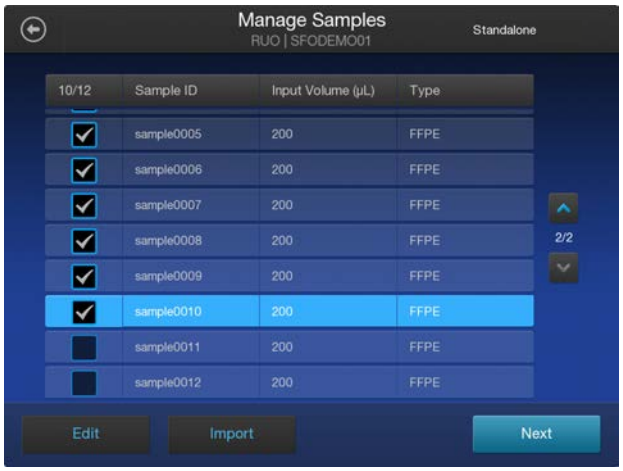
7. Accept the default elution volume. If needed, select the desired elution volume from the dropdown list, then tap **Next**.

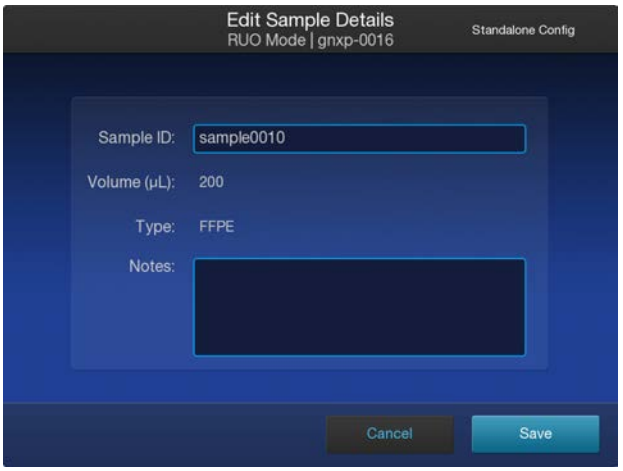
8. *(Optional)* Change the number of samples and the sample details.
   a. In the **Manage Samples** screen, deselect extra samples (for example, if you are only running ten samples, deselect samples 11 and 12).
   b. Tap on a sample ID to select the sample.

c. Tap **Edit**, enter a new **Sample ID** and any **Notes**, then tap **Save**.

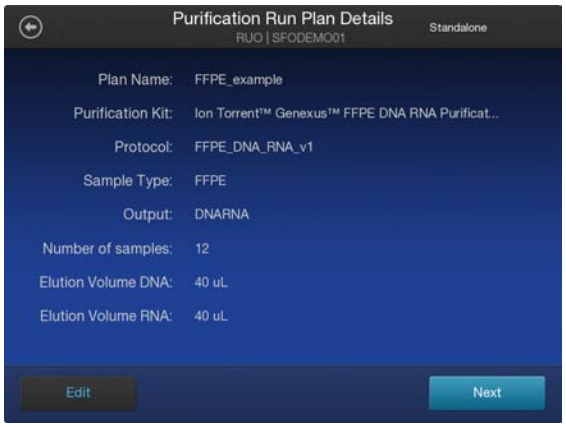
d. Repeat substep 8b and substep 8c for each additional sample.

e. Click **Next**.





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



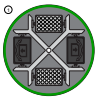
The new purification run plan appears in the list of available **Purification Run Plans**.

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

Prepare samples

Procedural guidelines

- Perform all steps at room temperature (20°C–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Wear clean gloves and a clean lab 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.
- 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, refer to 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 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

- AutoLys M TubeLifter or Pliers
- AutoLys M Tubes and Caps
- AutoLys M Tube Rack
- Plate centrifuge
- Incubators (see "Recommended materials for nucleic acid isolation, quantification, and quality control" on page 16 for a list of recommended incubators)

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 1.5-mL low-retention microcentrifuge tube, prepare a 1X Protease Digestion Master Mix as indicated, where `n` is the number of tissue 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

Note: We recommend the use of AutoLys M Tubes for the preparation of FFPE samples. Alternatively, CitriSolv™ Clearing Agent, xylene or equivalent solution can be used for removal of paraffin from the FFPE samples. For more information, 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 we recommend that you 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 tissue sample.
2. Add each FFPE section curl to a separate labeled tube.
3. Place AutoLys M tubes in an AutoLys M Tube Rack, then centrifuge at 2000 x *g* for 1 minute to collapse the curl prior to the addition of buffer.
4. Pipet 235 µL 1X Protease Digestion Master Mix into each labeled tube.

   Note: Ensure the samples are submerged in the Protease Digestion Master Mix.

5. Cap each tube securely to limit evaporation, then incubate at 60°C for ≥60 minutes in an AutoLys M Tube Rack.

Note: Incubation at 60°C can be extended to 2 hours to increase DNA yields.

6. Incubate at 90°C for 60 minutes.

Note:

- If using a single incubator, keep 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 50.
- Equilibrate the Quantitation Plate to room temperature during the incubation.

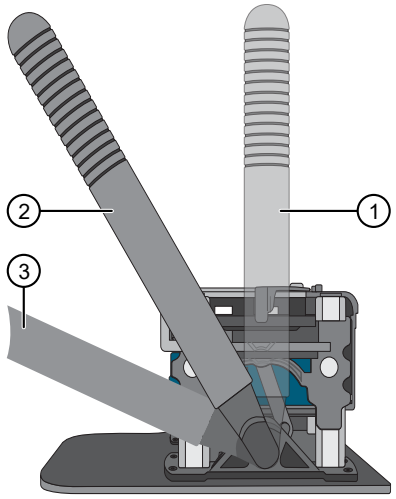
7. Allow samples to cool to room temperature for 3–5 minutes before proceeding to lift the tubes.

8. Lift the tubes. The following steps describe use of the AutoLys M TubeLifter to process up to 24 samples simultaneously. For more information on use of the AutoLys M TubeLifter see the *AutoLys M TubeLifter User Guide* (Pub. No. MAN0017676). Alternatively, AutoLys M Tube Pliers can be used to process tubes individually.

   a. Ensure 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.



① Position A
② Position B
③ Position C

9. Slide the AutoLys M Tube Locking Lid onto the rack, then centrifuge the samples at 2000 × *g* for 10 minutes.

Note: Ensure 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 page 44).

10. Separate 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.

Proceed to "Add samples to FFPE DNA and RNA Purification Plate 1" on page 51.

STOPPING POINT If needed, samples can be stored over night at –20°C.

Prepare FFPE slide samples with AutoLys M Tubes

Note: We recommend the use of AutoLys M Tubes for the preparation of FFPE samples. Alternatively, CitriSolv™ Clearing Agent, xylene or equivalent solution can be used for removal of paraffin from the FFPE samples. For more information, see *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 xylene and ethanol.

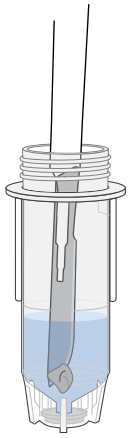
2. Pipet 235 µL 1X Protease Digestion Master Mix into each labeled tube.

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 pre-wet the tissue section.

   Larger sections may need an additional 2–4 µL of 1X Protease Digestion Master Mix.

4. Use a sterile disposable scalpel or clean razor 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. Gently flick the tube to mix and to immerse the tissue.

   If the tissue adheres to the sides of the tube, use a pipette tip to push the tissue into the solution or centrifuge briefly to immerse the tissue in 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.

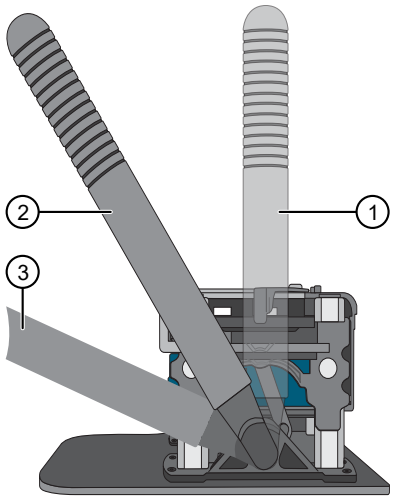
   Incubation at 60°C can be extended to 2 hours to increase DNA yields.

2. 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 50.
   - Equilibrate the Quantitation Plate to room temperature during the incubation.

3. Allow samples to cool to room temperature for 5 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 Position A
② Position B
③ Position C

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 “Procedural guidelines” on page 44).

6. 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 "Add samples to FFPE DNA and RNA Purification Plate 1" on page 51.

STOPPING POINT If needed, samples can be stored overnight at 4°C or frozen at –30°C to –10°C.

Load the Genexus™ Purification 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 Genexus™ Purification Instrument 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 51.

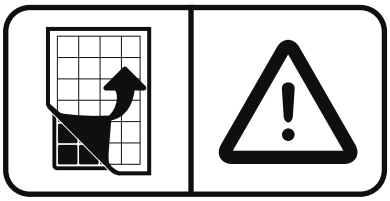
The FFPE DNA and RNA Purification Plate 2 contains magnetic beads in row H.

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 tissue samples.

Component	Volume per reaction
DNase Buffer	(n + 1) × 99 µL
DNase	(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 x *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

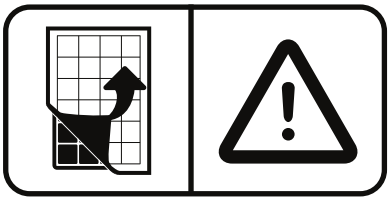
The FFPE DNA and RNA Purification Plate 1 contains magnetic beads in row B.

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.

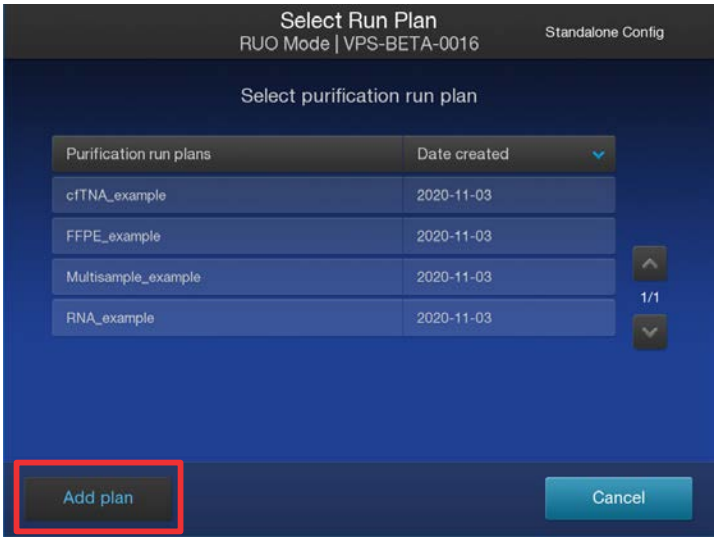
   A precipitate can form, but this does not interfere with the DNA binding. Proceed directly to the next step.

   Reagent consumables cannot be reused.

   You can add the samples to the FFPE DNA and RNA Purification Plate 1 and load the plate in the Genexus™ Purification Instrument 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.

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, standalone configuration)* 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 “Create a template for importing samples in standalone configuration purification run plans” on page 156.

   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.

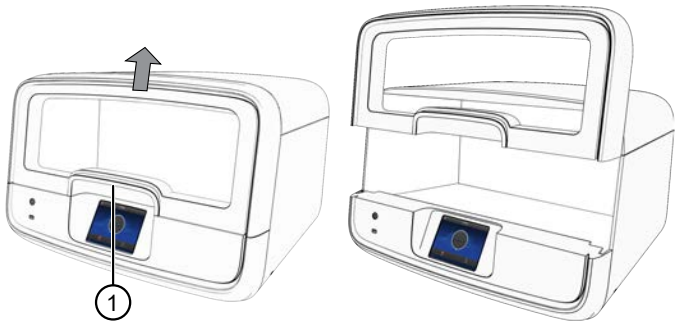
   f. Tap **Import**, then in the **Import Successful** screen, tap **OK**.

   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.



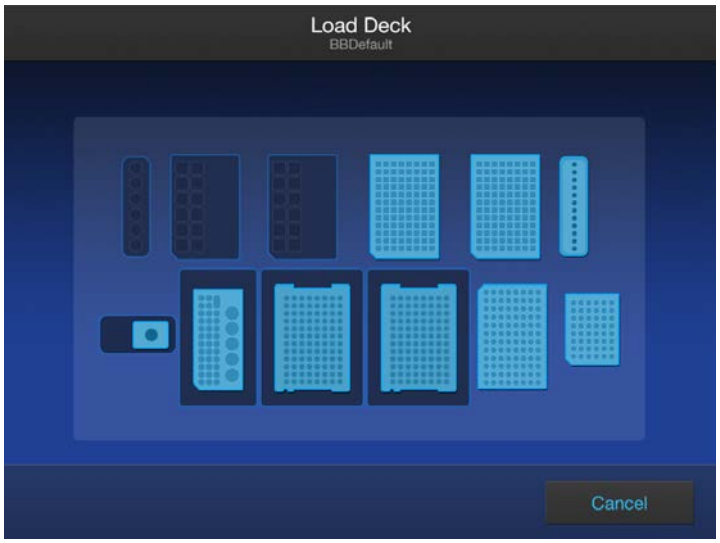
① Hold here, then lift.

Load the Genexus™ Purification Instrument

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.

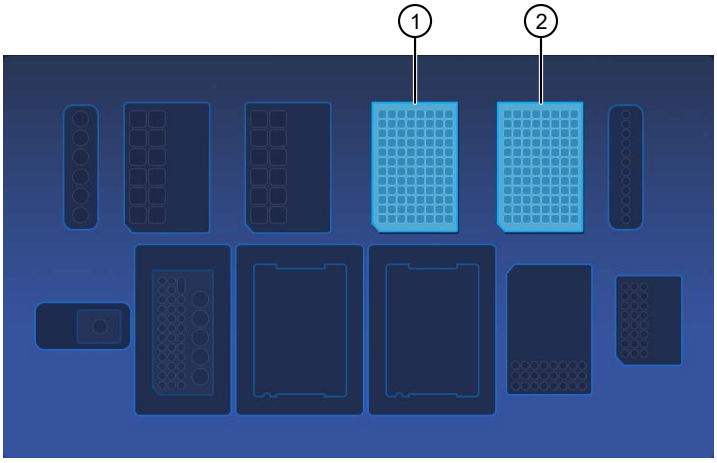
Follow the on-screen prompts to load the instrument.



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 51.

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 50.

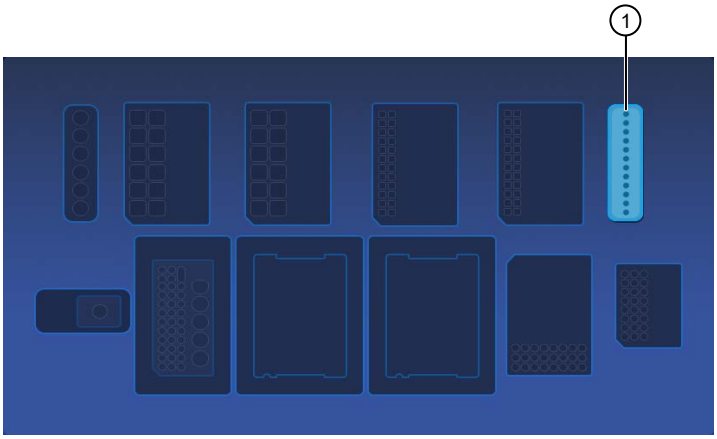


① FFPE DNA and RNA Purification Plate 1 position
② FFPE DNA and RNA Purification Plate 2 position

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

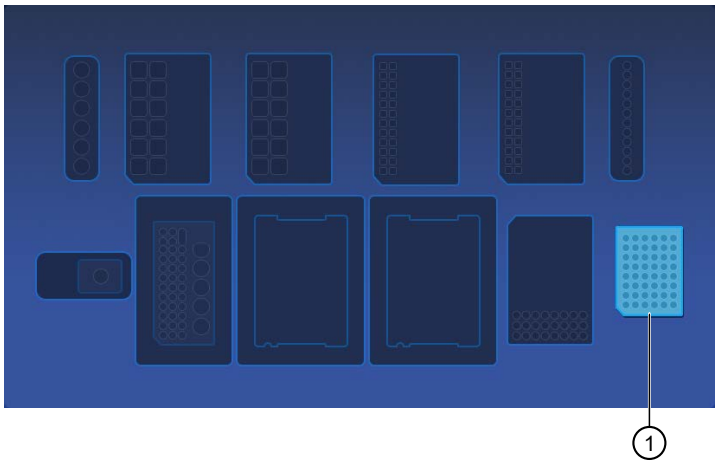
1. Unwrap, then load a new 12-Well Tip Comb.

   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.

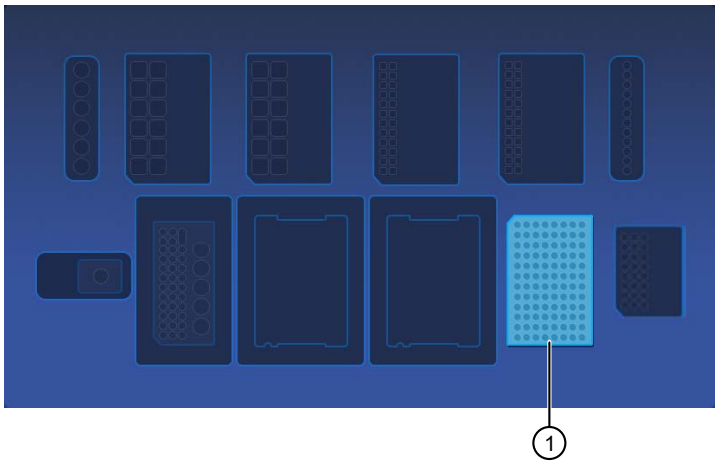


① 48-Well Nucleic Acid Archive Plate position

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

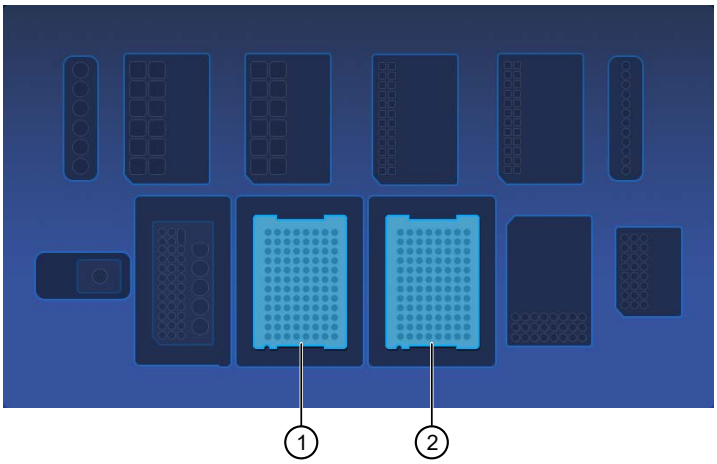
   The 96-Well Nucleic Acid Output Plate is not required when performing the purification in standalone configuration. The samples are in the 48-Well Nucleic Acid Archive Plate on completion of the purification run.

   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

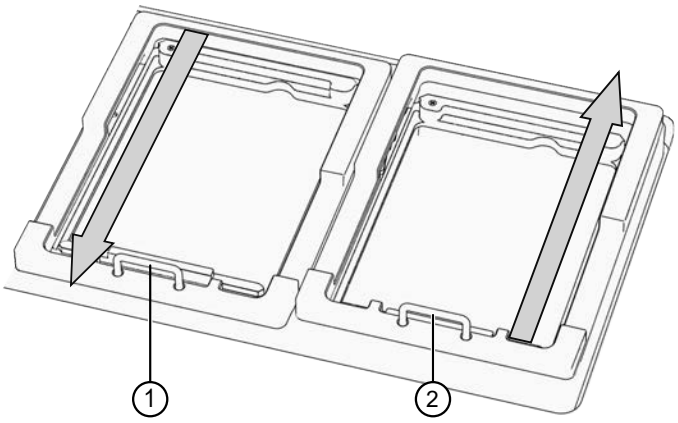
4. Unwrap two new Purification Tip Cartridges, remove the cover to expose the pipette tips, then load the cartridges in positions 1 and 2.



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)

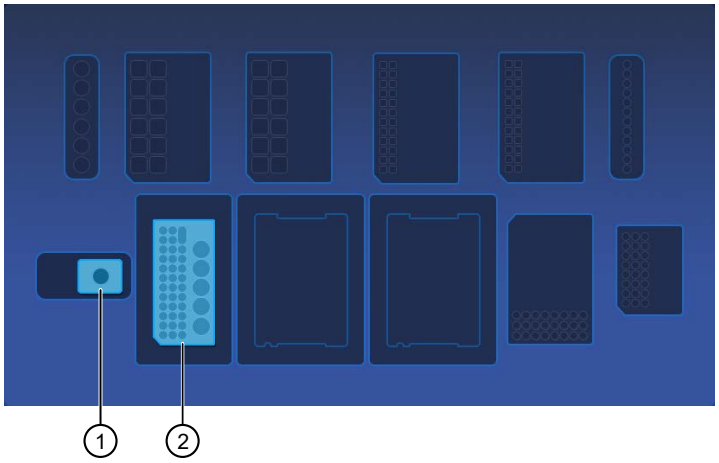
② Locked (back)

Load the quantitation reagents and consumables

Note:

- Protect the Quantitation Plate from 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.

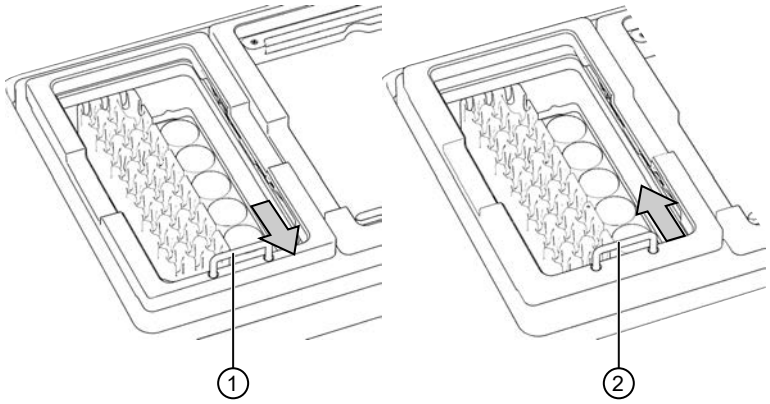
1. Centrifuge the Quantitation Plate at 1,000 × *g* for 30 seconds to collect the contents.
2. Load the Quantitation Plate in position 2.



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.

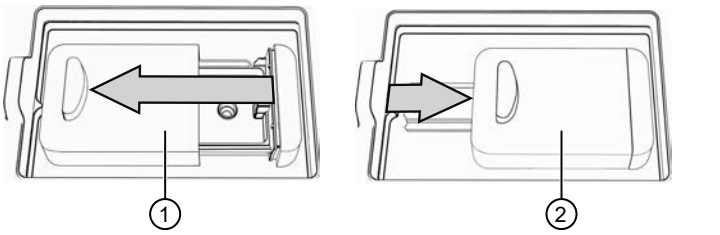


① Unlocked (forward)

② Locked (back)

3. *(If needed)* Slide and hold the quantitation module cover to the left, then insert the Quantitation Tube. **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 ensure 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.

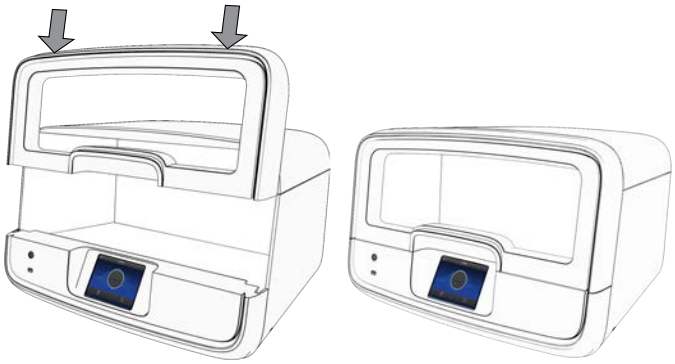
1. Confirm the following.
   - Foil seals are removed from the purification plates. Do not remove foil seal from the Quantitation Plate.
   - Each component is at the correct location and in the correct orientation. Press down on all plates and cartridges to ensure that they are firmly seated in place.
   - 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 Genexus™ 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.

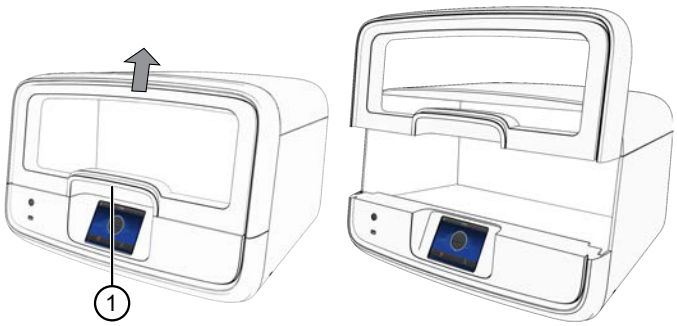
When the run is complete, the interior light turns blue, and the touchscreen displays **Run Complete**. Quantitation results are available immediately. See “View and export quantitation results” on page 62.

Unload purified samples

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

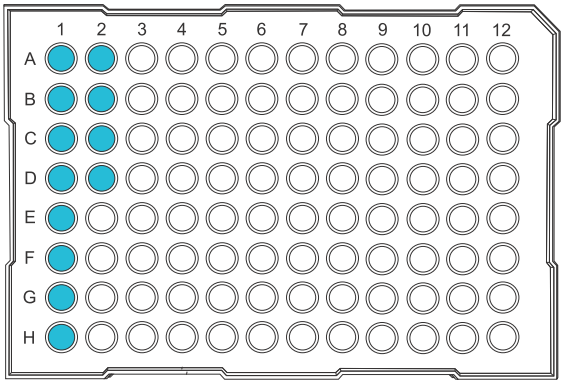
1. In the touchscreen, tap **Unload**. The door unlocks.

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



① Hold here, then lift.

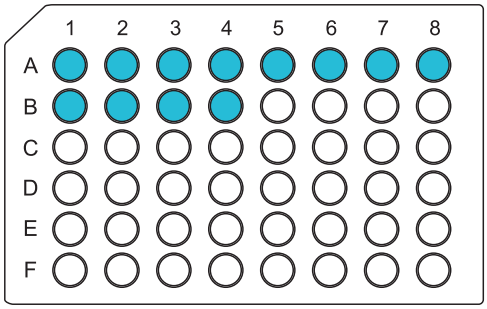
3. *(Integrated configuration)* Remove the 96-Well Nucleic Acid Output Plate, containing the purified sample DNA, ready for the addition of positive or non template sample sequencing controls (see "Add controls to the sample plate—Sample to Result run" on page 64). Store on ice.
If quantitation was performed, the sample concentration information is visible in the Genexus™ Software. Alternatively, use a Qubit™ Fluorometer to accurately measure sample concentration manually if needed. For more information, see "Quantify nucleic acid samples" on page 157.



DNA

Note: If not sequencing immediately, seal the plate with an Adhesive PCR Plate Foil (Thermo Fisher Scientific Cat. No. AB0626), then store purified samples 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.

4. Remove the 48-Well Nucleic Acid Archive Plate, containing the purified sample DNA.



DNA is in wells A1–B4.

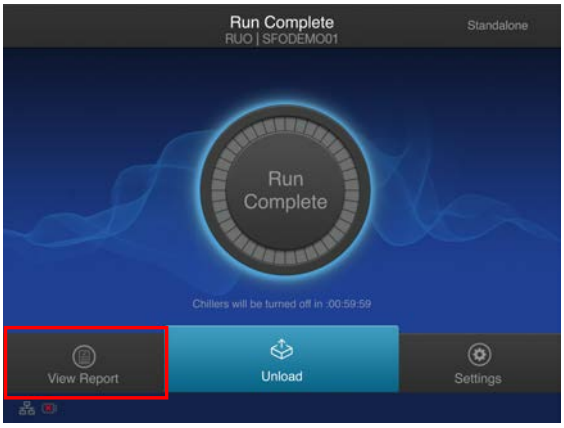
Note: *(Standalone configuration)* If using the purified DNA immediately, transfer the sample DNA to a sample input plate for sequencing. For more information, see "Dilute the samples and load the sample input plate—Nucleic Acid to Result run" on page 64. To determine the sample concentrations, see "View and export quantitation results" on page 62.

5. Seal the plate with a 48-Well Nucleic Acid Archive Plate Seal.
Store purified samples 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.

View and export quantitation results

Purification runs that include sample quantitation produce sample concentration results 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.

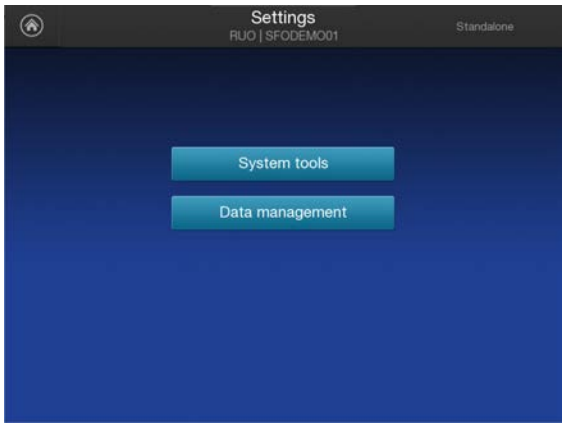
1. In the **Run Complete** screen, tap **View report**.



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

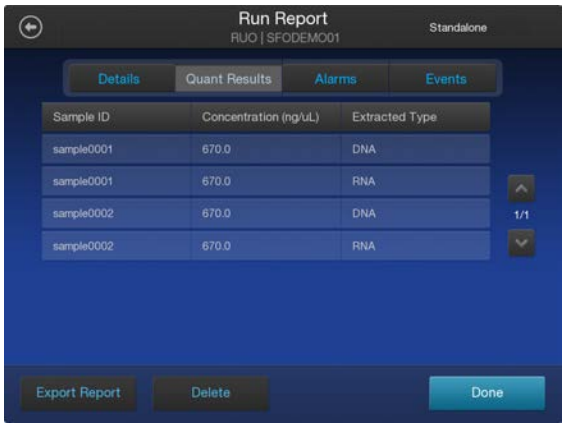
2. At any time after unloading and UV cleaning the instrument, sample concentration results can be accessed through the **Home** screen. Tap ⚙ (**Settings**).

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.



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

Add controls to the sample plate—Sample to Result run

Sample plates created by the Genexus™ Purification Instrument for Sample to Result runs have the correct volume of samples loaded in the positions specified in the run setup guide. If any controls were included in the run plan, you need to manually add positive controls and no template controls to the sample plate, before loading the plate in the sequencer. For more information on the appropriate controls see “Recommended sample controls” on page 16.

1. Add controls to the sample plate with the volume and in the well positions that are specified in the run setup guide.
2. Seal the plate with an Adhesive PCR Plate Foil (Thermo Fisher Scientific Cat. No. AB0626).

   Note: The use of other plate seals can affect performance.
3. Keep the plate on ice or at 4°C until you are ready to load it in the sequencer.

Proceed to Chapter 7, “Load the sequencer and start a run”.

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

Note: The Genexus™ Purification Instrument is not required for Nucleic Acid to Result sequencing runs. Any of the recommended nucleic acid purification kits can be used to purify DNA for use in Nucleic Acid to Result runs. For more information, see “Recommended materials for nucleic acid isolation, quantification, and quality control” on page 16.

If sample quantitation was not performed as part of the purification run, use a Qubit™ Fluorometer to accurately measure sample concentration (see page 157). If you want to run no template controls or positive sample controls, select an appropriate control for your sample type as recommended in “Recommended sample controls” on page 16.

1. Use the run setup guide to dilute samples and sample controls, if used, to the required concentration using nuclease-free water.

   IMPORTANT! Make sure to dilute samples to the concentration that was specified in substep 5b in “Plan a Nucleic Acid to Result run” on page 35.

   Table 4 Recommended sample concentration for Oncomine™ BRCA Assay GX

Sample type	Recommended nucleic acid concentration
DNA from FFPE	0.8 ng/µL

2. Add 25 µL of diluted samples or sample control to the wells in the sample input plate as specified in the run setup guide.

3. Seal the plate with a sheet of Thermo Scientific™ Adhesive PCR Plate Foil (Cat. No. AB0626).

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

4. Keep the sample input plate on ice until ready to load in the sequencer.

Proceed to Chapter 7, “Load the sequencer and start a run”.

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.

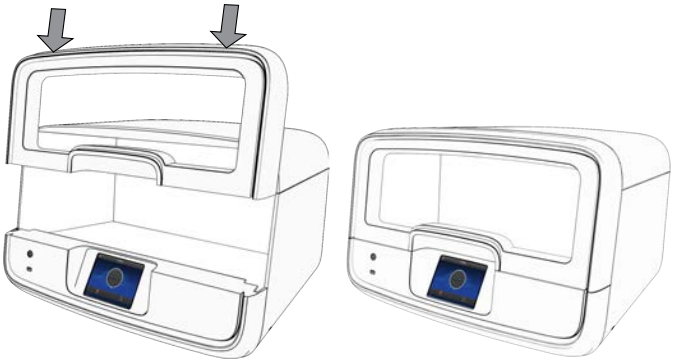
WARNING! Liquid waste contains guanidine thiocyanate, dispose of properly.

   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™ Multisample DNA Purification Kit protocol

Note: The procedures described in this chapter apply to purification of DNA on the Genexus™ Purification Instrument using the Ion Torrent™ Genexus™ Multisample DNA Purification Kit for the following sample types.

- Whole blood
- Fresh frozen tissue
- Cell lines

For information on purification of DNA from FFPE samples using the Genexus™ Purification Instrument, see Chapter 5, “Genexus™ FFPE DNA and RNA Purification Kit protocol”.

Genexus™ Multisample DNA Purification workflow

5 min

Plan a purification run (page 70)

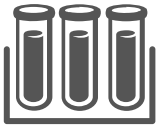
IMPORTANT! If performing a **Sample to Result** sequencing run, plan the run in the Genexus™ Software. For more information see “Plan a Sample to Result run” on page 33.

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

5–30 min

Prepare samples (page 74)

Samples are processed based on the sample type prior to adding to the Multisample DNA Purification Plate and loading on to the instrument for purification of DNA.



2 min

Load the Genexus™ Purification Instrument (page 79)

The purification run plan is selected and the run initiated. The instrument performs a pre-run UV clean, then consumables are loaded on to the instrument. DNA Enhancer solution, sample, and Proteinase K are added to the Multisample DNA Purification Plate, then immediately loaded on to the instrument for purification of DNA.

1 hr

Start the run (page 85)

After the sample plate and all reagents and consumables have been loaded the instrument door is closed and the run started.

Note: Depending on the number of samples, quantification adds ~1 hour to the run time.

Genexus™ Multisample DNA Purification workflow

5 min

Unload the purified nucleic acids (page 86)

If performing a Sample to Result run, remove the 96-Well Nucleic Acid Output Plate, add any required control samples and proceed to sequencing of the purified samples. 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 sequencing of the purified sample.

Used reagents and consumables are removed from the instrument and a UV clean is performed.

Note: 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 purification run plan and preparing samples.

Plan a purification run (standalone configuration)

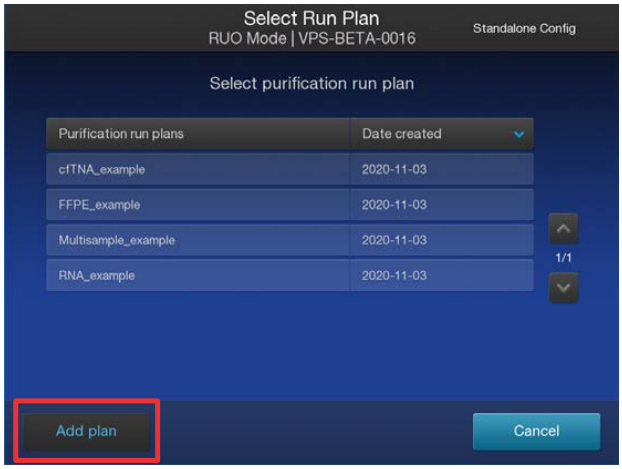
Note: If running the Genexus™ Purification System in integrated configuration, see "Plan a Sample to Result run" on page 33 to plan a **Sample to Result** run in the Genexus™ Software.

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.

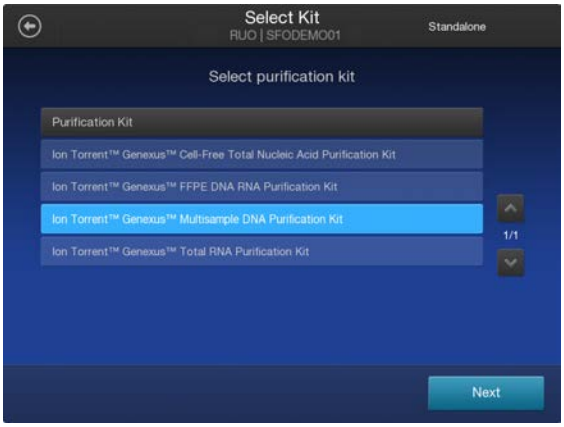
Add a purification run plan (standalone configuration)

Note: We recommend that you create the run plan before preparing your samples and loading into the Multisample DNA Purification Plate.

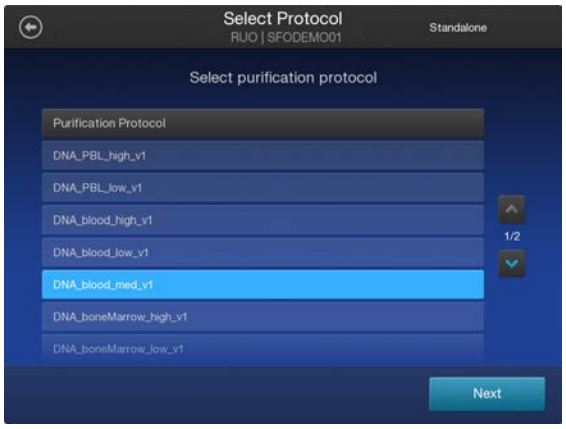
1. Enter your username and password to sign in to the instrument.
2. Tap **Run**, select **Purification Only**, then tap **Add plan**.



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



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



Sample type	Input volume	Select
Whole blood	50–100 µL	Blood 50–100 µL
	100–200 µL[1]	Blood 100–200 µL
	200–400 µL	Blood 200–400 µL

[1] Default value in ADF

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

Note:

- 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 1 hour.
- Quantitation requires up to 10 µL of the eluted sample. If the expected sample yield is limiting, manual sample quantitation may be preferred to preserve sample.

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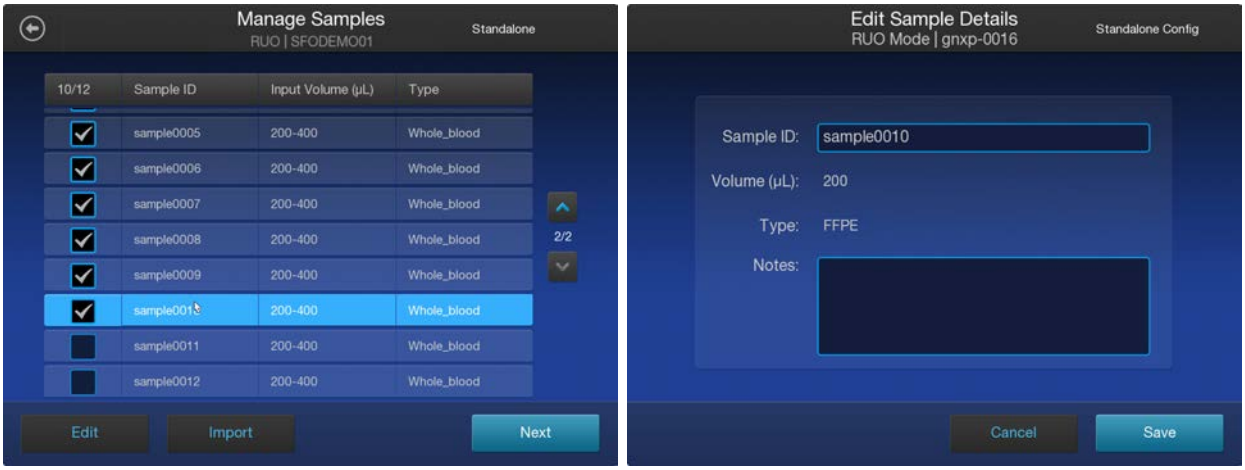
7. Accept the default elution volume. If needed, select the desired elution volume from the dropdown list, then tap **Next**.

8. *(Optional)* Change the number of samples and the sample details.
   a. In the **Manage Samples** screen, deselect extra samples (for example, if you are only running 11 samples, deselect sample 12).
   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. Click **Next**.



9. *(Optional)* Import sample information.

Note:

- Importing sample information overwrites the existing **Sample ID** and **Notes** information for each sample selected.
- In standalone configuration, you must prepare a CSV sample import file and save it to a USB drive to import sample information. For more information, see page 156.

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a. In the **Manage Samples** screen, select the samples for which sample information is being imported, then tap **Import**.

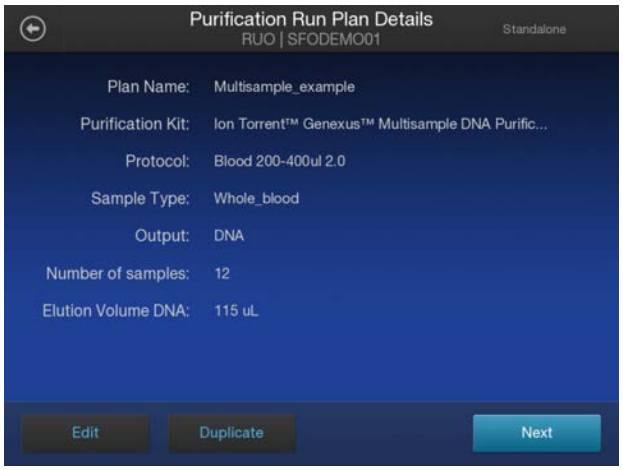
b. The **Sample Import** screen opens, tap **Import** to proceed.

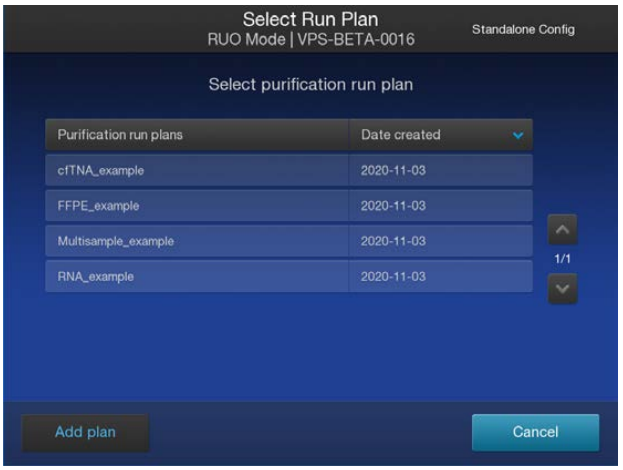
c. Insert the USB drive containing the sample import CSV file into the USB port on the front of the purification instrument. In the **Sample Import** screen, select the USB drive, then navigate to and select the sample import file.

d. *(Optional)* Tap **Details** to view the CSV file listing 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 **Run Plan** will now appear in the list of available **Purification Run Plans**.

Prepare the Quantitation Plate and consumables

Cartridges and consumables needed:

- Genexus™ Multisample DNA Purification (Part. No. A45533)
  - Multisample DNA Purification Plate
  - 12-Well Tip Comb
- Genexus™ Nucleic Acid Quantitation, Broad Range (Part. No. A45537)
  - Quantitation Plate Broad Range
  - Quantitation Tube
- Genexus™ Purification Supplies 1 (Part. No. A45529)
  - Purification Tip Cartridge
  - 48-Well Nucleic Acid Archive Plate
  - 48-Well Nucleic Acid Archive Plate Seal
- P200 pipet and filtered tips
- 96-Well Nucleic Acid Output Plate

Equilibrate the Quantitation Plate

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

IMPORTANT!

- Protect the Quantitation Plate from light to prevent photobleaching of the preloaded reagents.
- Allow at least 30 minutes for the Quantitation Plate to equilibrate to room temperature.
- Experienced users can save time by isolating cell-free plasma from whole blood while the Quantitation Plate equilibrates.

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 Genexus™ Purification Instrument.

Prepare the consumables

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

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

Prepare samples

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- When working with whole blood:
  - Wear clean gloves and a clean lab 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 lab benches and equipment periodically with 10% bleach solution and rinse with 70% ethanol.
- 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

- Keep samples on ice or at 4°C until use.
- Centrifuge purification plates for 30 seconds at 1,000 × *g* to collect the contents.

Materials required

Genexus™ Multisample DNA Purification (Part No. A45535)

- Multisample DNA Purification Plate
- Enhancer solution
- Proteinase K

Fisherbrand™ Bead Mill 24 Homogenizer

Whole Blood Samples

1. Determine the volume (≥50–400 µL) of each whole blood sample.
2. To each sample, add DNA Homogenization Buffer to a total volume of 400 µL.
   For example, to 200 µL whole blood add 200 µL DNA Homogenization Buffer. To 400 µL whole blood do not add DNA Homogenization Buffer.

   IMPORTANT! For frozen samples, add DNA Homogenization Buffer directly to the frozen sample to help in thawing.
3. Vortex, or pipet up and down at least 10 times, to thoroughly mix.

Proceed directly to "Start the purification run" on page 52. Samples are loaded in step 4 of "Add samples to Multisample DNA Purification Plate" on page 83.

Prepare tissue samples

Fresh or frozen tissue DNA yields can vary based on the tissue type. Use the amounts suggested in Table 5. For more information on expected DNA yield by tissue type, see "Example tissue yields" on page 77. Adjust input amount based on your results.

Use a bead mill homogenizer when processing small amounts of tissue and use a rotor-stator tissue homogenizer when processing larger amounts of tissue. Alternate methods of cell disruption can also be used. Do not exceed 5 mg of homogenized tissue for high yielding tissues or 10 mg of homogenized tissue for low yielding tissues as sample input to the Multisample DNA Purification Plate.

Table 5 Recommended input amount based on tissue type

Tissue type	Recommended tissue:DNA Homogenization Buffer ratio[1] (mg:µL)	Maximum Multisample DNA Purification Plate tissue input amount[2]
Low-yield tissue	1:40	10 mg
High-yield tissue	1:80	5 mg

[1] Do not exceed the indicated ratio when homogenizing the sample before loading on the purification plate.
[2] Do not exceed the indicated amount as input into the sample purification plate.

1. Cut the sample into appropriately sized pieces. For larger samples, we recommend cutting the material into long, thin strips for faster homogenization.

2. Weigh the tissue sample, then calculate and add the recommended volume of DNA Homogenization Buffer.
   For example, for 5.0 mg high-yield tissue add 400 µL DNA Homogenization Buffer or for 25 mg low-yield tissue add 1.0 mL DNA Homogenization Buffer.
   Maintain the recommended tissue-to-buffer ratio if using more or less tissue.

3. Homogenize the samples following manufacturer instructions for your homogenizer.
   When homogenizing large amounts of tissue, use a rotator-stator tissue homogenizer in 10 second pulses on ice or at 4°C.
   Visually inspect the samples. If homogenization is incomplete, repeat step 3.

4. Transfer the lysate to a new tube. Ensure that no beads are carried over if using a bead mill homogenizer.
   Keep homogenized samples on ice or at 4°C until use.
   Proceed to "Start the purification run" on page 52. Samples are loaded in step 4 of "Add samples to Multisample DNA Purification Plate" on page 83.

STOPPING POINT If not proceeding directly to sample loading, store homogenized samples at –90°C to –70°C.

Prepare cultured cell samples

Up to 4 × 10^6 cultured cells can be processed per sample.

1. Centrifuge cells in culture media at 100 × *g* for 5 minutes at 4°C, then carefully remove the supernatant without disturbing the cell pellet.
   Thaw previously frozen cell pellets on ice or at 4°C, then remove as much culture media as possible without disturbing the cell pellet.

2. *(Optional)* Wash the cell pellet.
   a. Resuspend the cell pellet in 1/2 volume of 1X PBS.
   b. Centrifuge cells at 100 × *g* for 5 minutes at 4°C, then carefully remove the supernatant without disturbing the cell pellet.

3. Add 400 µL of DNA Homogenization Buffer (provided) to each sample cell pellet, set a P1000 pipettor to 300 µL, then slowly pipet up and down 10–15 times.

   IMPORTANT! The sample can be viscous. Pipet up and down thoroughly to ensure complete mixing.

Proceed to "Start the purification run" on page 52. Samples are loaded in step 4 of "Add samples to Multisample DNA Purification Plate" on page 83.

Example tissue yields

Table 6 Recommended input amount based on tissue type

Tissue type	Recommended tissue:DNA Homogenization Buffer ratio[1] (mg:µL)	Maximum Multisample DNA Purification Plate tissue input amount[2]	Potential yield[3]
High yielding tissues			
High-yield tissue (25 mg)	1:80	5 mg	10–30 µg
Liver (25 mg)	1:80	5 mg	10–30 µg
Brain (25 mg)	1:80	5 mg	15–30 µg
Thymus (25 mg)	1:80	5 mg	15–30 µg
Kidney (25 mg)	1:80	5 mg	15–30 µg
Spleen (25 mg)	1:80	5 mg	15–75 µg
Mouse tail (1.2 cm tip)	1:80	5 mg	10–25 µg
Low yielding tissues			
Low-yield tissue (25 mg)	1:40	10 mg	5–10 µg
Lung (25 mg)	1:40	10 mg	5–10 µg
Heart (25 mg)	1:40	10 mg	5–10 µg
Breast (25 mg)	1:40	10 mg	5–10 µg

[1] Do not exceed the indicated ratio when homogenizing the sample before loading on the purification plate.
[2] Do not exceed the indicated amount as input into the sample purification plate.
[3] All yields are approximate and not indicative of purification performance.

Load the Genexus™ Purification Instrument and start the run

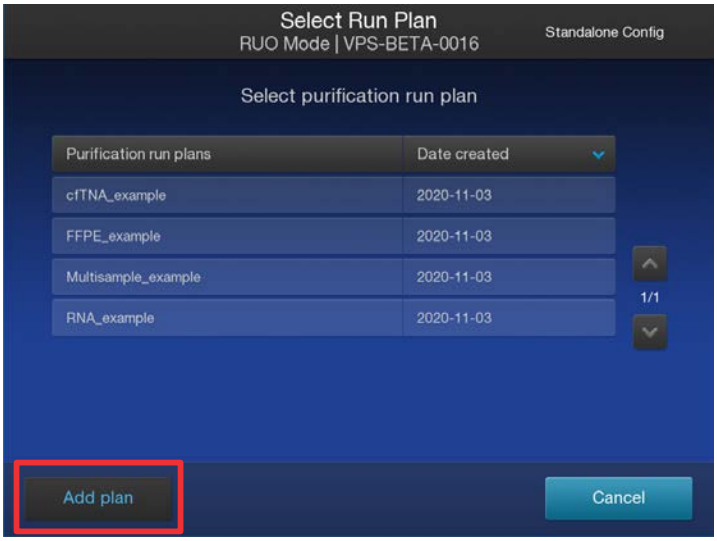
This section describes how to perform the following procedures.

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

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

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, standalone configuration)* 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 “Create a template for importing samples in standalone configuration purification run plans” on page 156.

   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.

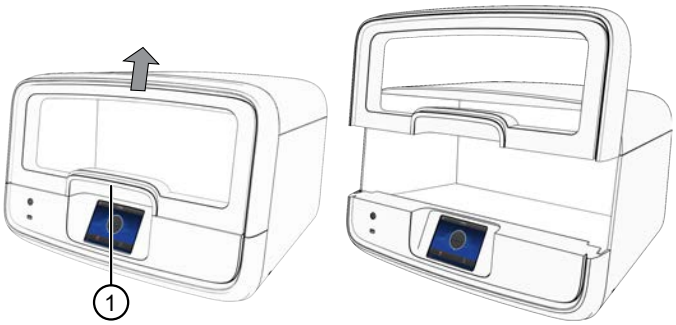
   f. Tap **Import**, then in the **Import Successful** screen, tap **OK**.

   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.



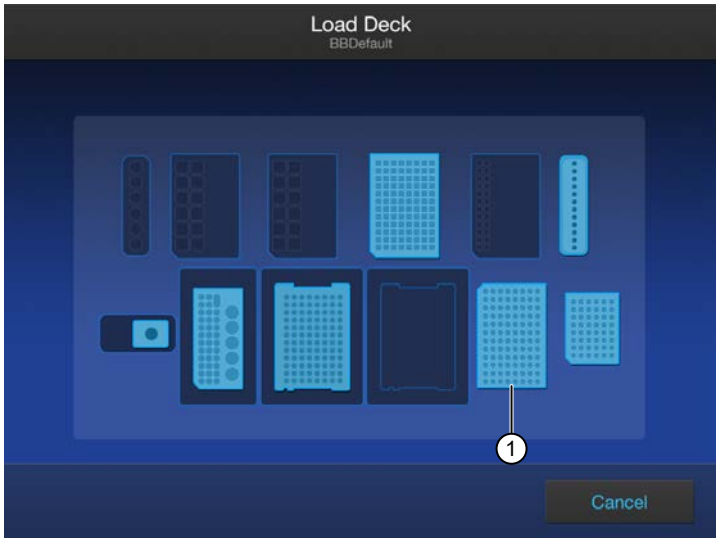
① Hold here, then lift.

Load the Genexus™ Purification Instrument

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 instrument.

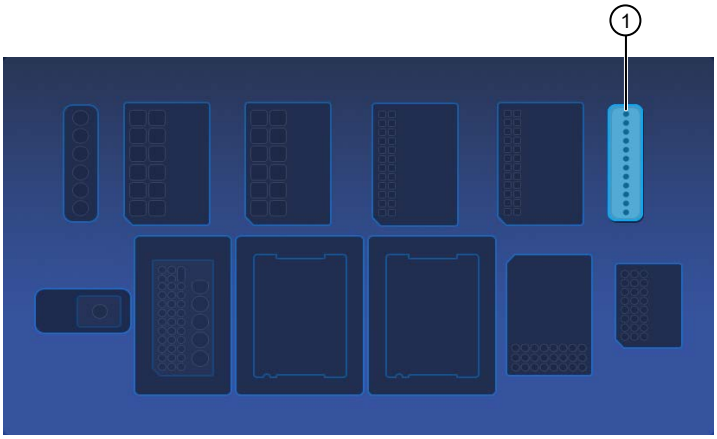


① 96-Well Nucleic Acid Output Plate, only needed when performing the purification in integrated configuration.

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

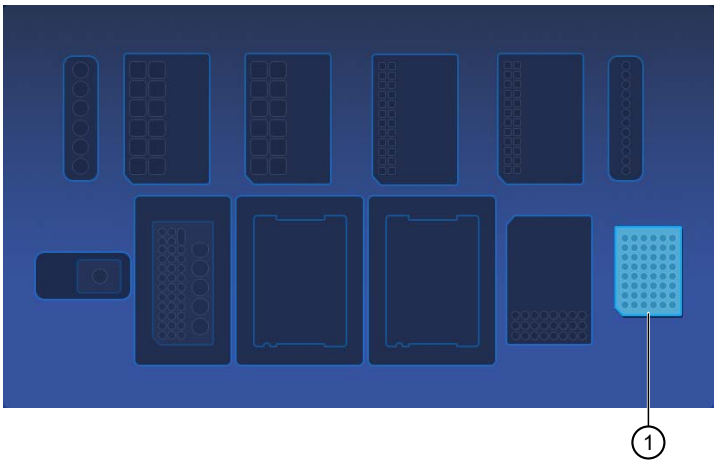
1. Unwrap, then load a new 12-Well Tip Comb.

   Ensure that the 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.

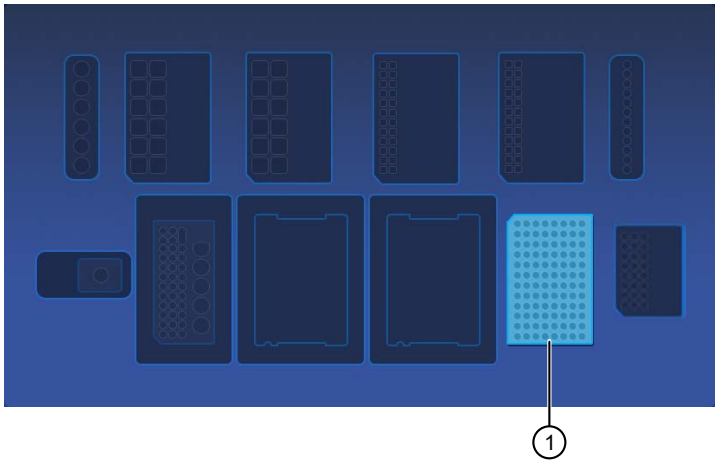


① 48-Well Nucleic Acid Archive Plate position

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

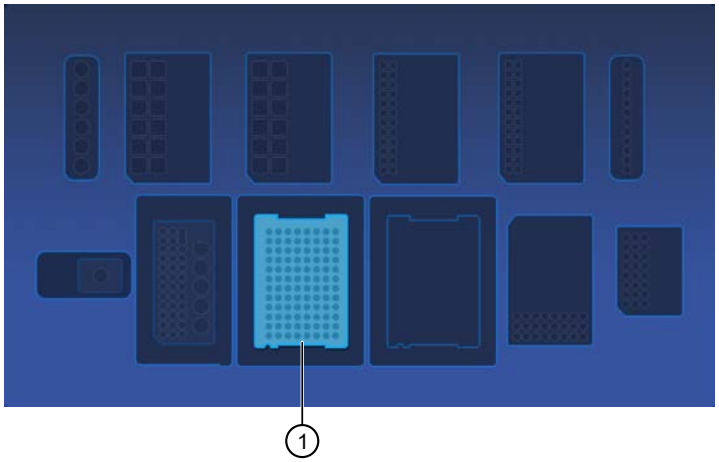
   The 96-Well Nucleic Acid Output Plate is not required when performing the purification in standalone configuration.

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



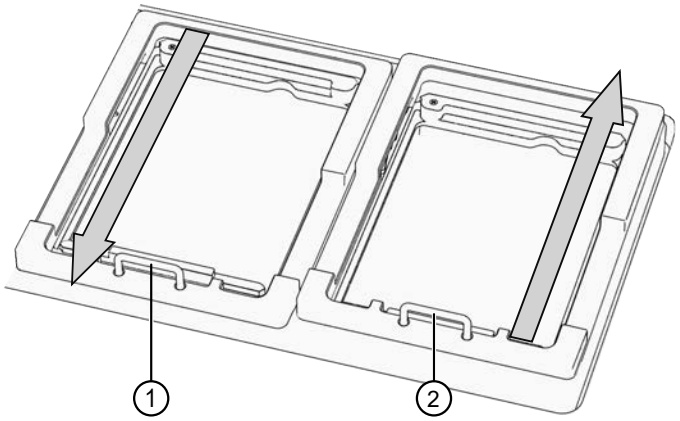
① 96-Well Nucleic Acid Output Plate position

4. Unwrap 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, then place the tip box in the open position.



① Unlocked (forward) ② Locked (back)

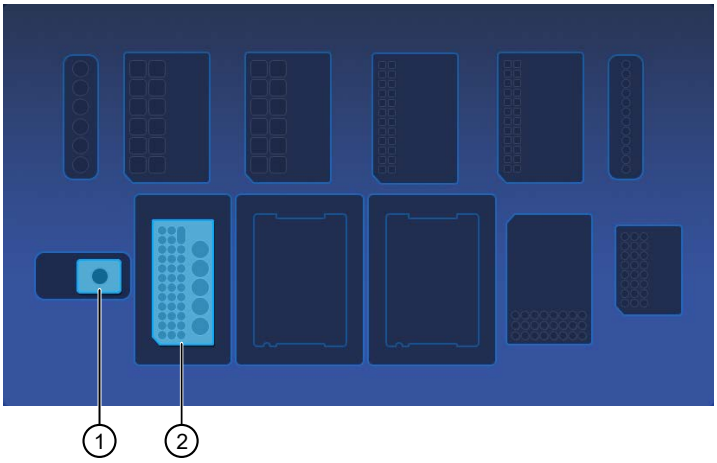
b. Push the locking mechanism handle back to lock the tip box in place.

Load the quantitation reagents and consumables

Note:

- Protect the Quantitation Plate from 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.

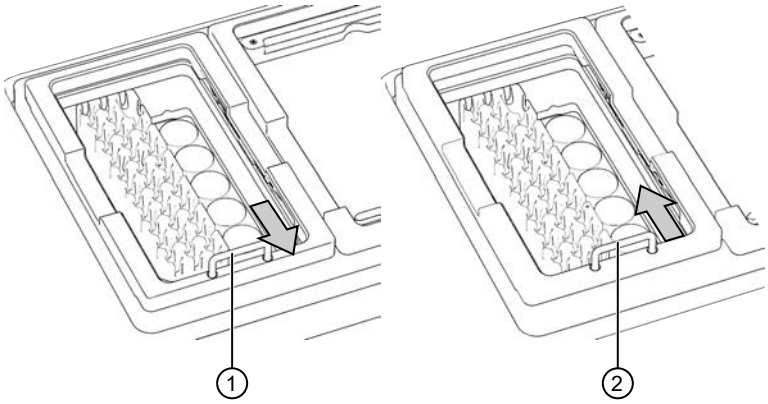
1. Centrifuge the Quantitation Plate at 1,000 × *g* for 30 seconds to collect the contents.
2. Load the Quantitation Plate in position 2.



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.

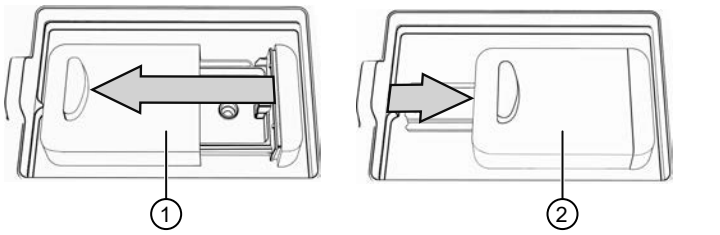


① Unlocked (forward)

② Locked (back)

3. *(If needed)* Slide and hold the quantitation module cover to the left, then insert the Quantitation Tube. **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 Multisample DNA Purification Plate

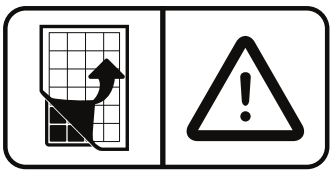
The Multisample DNA Purification Plate contains magnetic beads in row D.

1. Briefly centrifuge the sealed Multisample DNA Purification Plate at 1000 x *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.

IMPORTANT! Do not create bubbles when preparing the plate.

2. Inspect the plate to ensure the contents of all rows are at the bottom of the wells. If needed repeat step 1.

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



4. Add the samples to the Multisample DNA Purification Plate as indicated in Table 7.

IMPORTANT!

- Do not pre-mix the DNA Enhancer solution and Proteinase K.
- Do not change the order of pipetting.
- Add samples to consecutive wells beginning with sample 1 in well A1, through sample 12 in well A12. Do not skip wells.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

a. Add X µL DNA Enhancer solution to each well in Row A of the Multisample DNA Purification Plate.

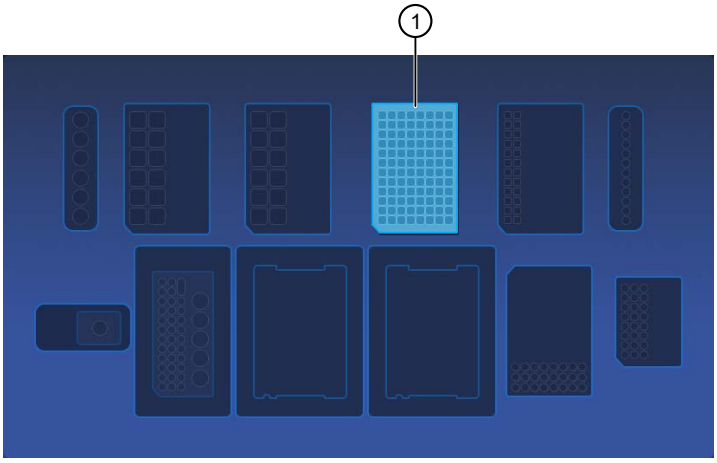
b. Add Y µL sample to each well in Row A.

c. Add Z µL Proteinase K solution to each well in Row A.

Table 7

DNA Enhancer solution (X µL)	Sample Volume (Y µL)	Proteinase K Volume (Z µL)
Sample type: whole blood		
5	50	5
10	100	10
20	200	20
30	300	30
40	400	40
Sample type: fresh frozen tissue		
—	400	—
Sample type: cell lines		
40	400	40

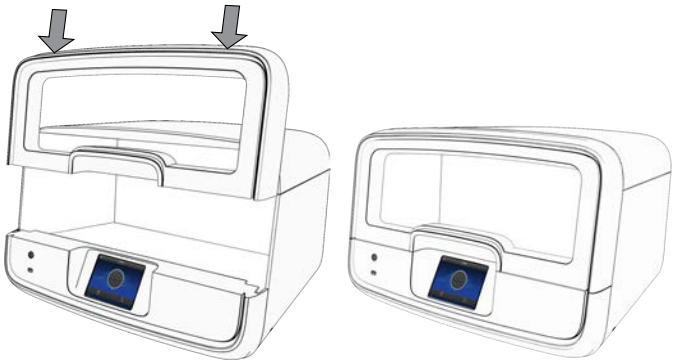
5. Immediately load the 96 deep-well Multisample DNA Purification Plate with the samples in position 1.



① Multisample DNA Purification Plate position

Start the run

1. When all reagents and consumables are loaded in the Genexus™ 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.
   - 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.

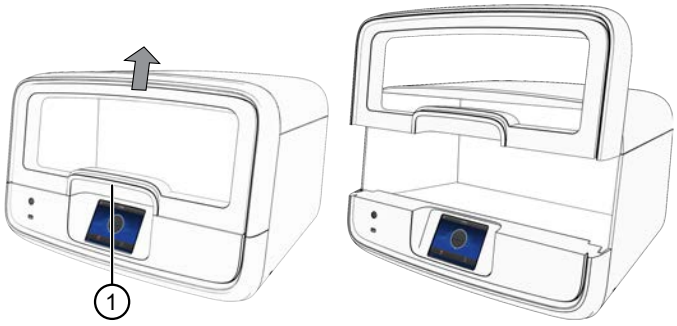
When the run is complete, the interior light turns blue, and the touchscreen displays **Run Complete**. Quantitation results are available immediately. See “View and export quantitation results” on page 88.

Unload purified samples

IMPORTANT! Do not allow purified samples 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.

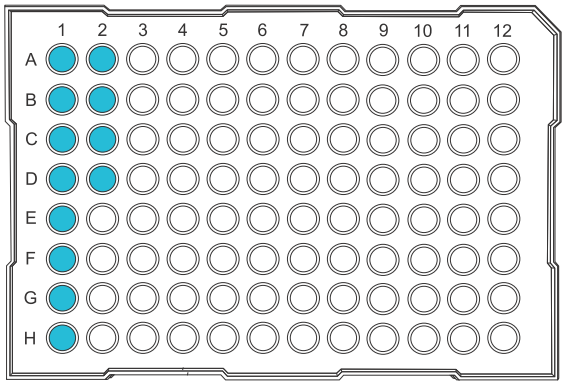
2. Lift the instrument door to access the instrument deck.



① Hold here, then lift.

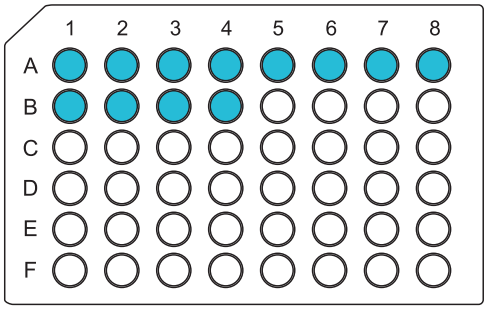
3. *(**Sample to Result** run)* Remove the 96-Well Nucleic Acid Output Plate that contains the purified sample DNA that is ready for the addition of positive or nontemplate sample sequencing controls. For more information, see “Add controls to the sample input plate—Sample to Result run” on page 90.. Store on ice or at 4°C. If quantitation was performed, the sample concentration information is visible in the Genexus™ Software. Alternatively, use a Qubit™ Fluorometer to accurately measure sample concentration manually, if needed.

   For more information, see “Quantify nucleic acid samples” on page 157.



STOPPING POINT If not sequencing immediately, for example while waiting for a second purification batch, seal the plate with an Adhesive PCR Plate Foil (Cat. No. AB0626), then store the plate at –20°C for up to 3 months. For long-term storage (>3 months), transfer the samples to labeled low-retention tubes, then store the samples at –30°C to –10°C for up to 36 months.

4. Remove the 48-Well Nucleic Acid Archive Plate that contains the purified sample DNA in rows A1–8 and B1–4.



Note: *(Standalone configuration)* If using the purified DNA immediately, transfer the sample DNA to a sample input plate for sequencing. For more information, see the relevant assay user guide for **Nucleic Acid to Result** run guidance. To determine the sample concentrations, see “View and export quantitation results” on page 88.

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 for up to 36 months.

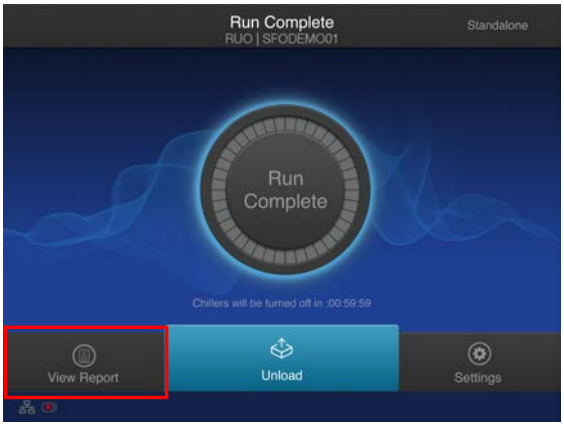
   If the archive plate is thawed during short-term storage, transfer the samples into labeled low-retention tubes. Do not reseal the archive plate with the used plate seal.

View and export quantitation results

Genexus™ Purification Instrument runs that include sample quantitation produce sample concentration results that can be accessed after the run is complete. When integrated with the Genexus™ Software, sample concentration information is automatically available in the software and used for **Sample to Result** runs.

In standalone configuration, results can be accessed from the **Run Complete** screen or the **Home** screen, then exported to a USB for transfer to the Genexus™ Software.

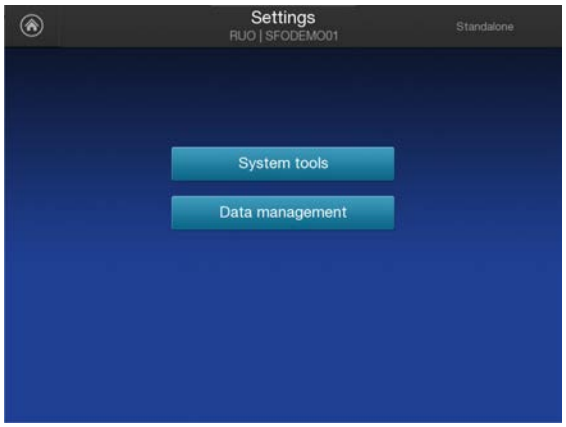
1. In the **Run Complete** screen, tap **View report**.



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

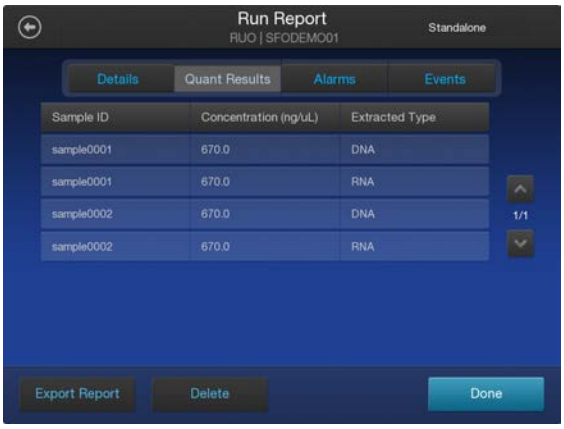
2. At any time after unloading and UV cleaning the instrument, sample concentration results can be accessed through the **Home** screen. Tap ⚙ (**Settings**).

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.



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

   Sample concentration information is automatically transferred to the Genexus™ Software and used for **Sample to Result** runs.

Add controls to the sample input plate—Sample to Result run

The 96-Well Nucleic Acid Output Plate created by the Genexus™ Purification Instrument for Sample to Result runs have the correct volume of samples loaded in the positions specified in the run setup guide for use as the sample input plate on the Genexus™ Integrated Sequencer. If any controls were included in the run plan, you need to manually add positive controls and no template controls to the sample input plate, before loading the plate in the sequencer. For more information on the appropriate controls see “Recommended sample controls” on page 16.

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

2. Seal the plate with a sheet of Thermo Scientific™ Adhesive PCR Plate Foil (Cat. No. AB0626).

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

3. Keep the sample input plate on ice until ready to load in the sequencer.

Proceed to Chapter 7, “Load the sequencer and start a run”.

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

Note: The Genexus™ Purification Instrument is not required for Nucleic Acid to Result sequencing runs. Any of the recommended nucleic acid purification kits can be used to purify DNA for use in Nucleic Acid to Result runs. For more information, see "Recommended materials for nucleic acid isolation, quantification, and quality control" on page 16.

If sample quantitation was not performed as part of the purification run, use a Qubit™ Fluorometer to accurately measure sample concentration. If you want to run no template controls or positive sample controls, select an appropriate control for your sample type as recommended in "Recommended sample controls" on page 16.

1. Use the run setup guide to dilute samples and sample controls, if used, to the required concentration using nuclease-free water.

   IMPORTANT! Make sure to dilute samples to the concentration that was specified in substep 5b in "Plan a Nucleic Acid to Result run" on page 35.

   Table 8 Recommended sample concentration for Oncomine™ BRCA Assay GX

Sample type	Recommended nucleic acid concentration
DNA from blood	0.8 ng/µL

2. Add 25 µL of diluted samples or sample control to the wells in the sample input plate as specified in the run setup guide.

3. Seal the plate with a sheet of Thermo Scientific™ Adhesive PCR Plate Foil (Cat. No. AB0626).

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

4. Keep the sample input plate on ice until ready to load in the sequencer.

Proceed to Chapter 7, "Load the sequencer and start a run".

Dispose of used consumables and UV clean the instrument

Unload purified DNA samples before disposal of used consumables.

IMPORTANT! Do not allow purified DNA 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 Multisample DNA Purification Plate 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.

2. Unlock, then remove and dispose of the Purification Tip Cartridge 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.

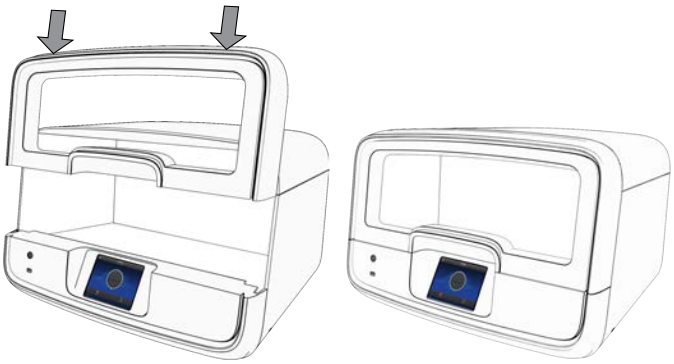
WARNING! No data are currently available that address the mutagenicity or toxicity of the Qubit™ RNA BR Reagent (Component A). This reagent is known to bind nucleic acid and is provided as a solution in DMSO. Treat the Qubit™ RNA BR Reagent with the same safety precautions as all other potential mutagens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

IMPORTANT! Do not allow the module cover to spring shut.

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 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 load samples in the sample plate, and 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 an incorrect position, or if an incorrect quantity is loaded.

Before you begin

Before setting up a sequencing run, review general procedural guidelines for handling panels, reagents, and samples to minimize the chance of contamination. For more information, see "Guidelines for panel and reagent use and handling" on page 26 and "Guidelines for preventing contamination" on page 25.

1. Remove the library and templating strips from their packaging in the refrigerator or freezer, and prepare them for loading in the sequencer.
   - Equilibrate or thaw the following reagent strips at room temperature for 30 minutes.
     - Genexus™ Strip 1
     - Genexus™ Strip 2-AS. If you are delayed in loading, keep the thawed strips on ice or at 4°C until you load them in the sequencer.
     - Genexus™ Strip 3-GX5™ (or Genexus™ Strip 3-GX7™)
   - Thaw Genexus™ Strip 4 by laying the strips on ice for 30 minutes, or incubating at 4°C for 30 minutes. If you are delayed in loading, keep the thawed strips on ice or at 4°C until you load them in the sequencer.

   IMPORTANT! Ensure that contents of strips that are stored frozen are completely thawed before installing in the sequencer.

2. Remove primer pool tubes in tube carriers that are needed for the run from the freezer, then thaw at room temperature for 30 minutes. 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.

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

4. Genexus™ Strip 1, Genexus™ Strip 3-GX5™, and Genexus™ Strip 3-GX7™ 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 96.

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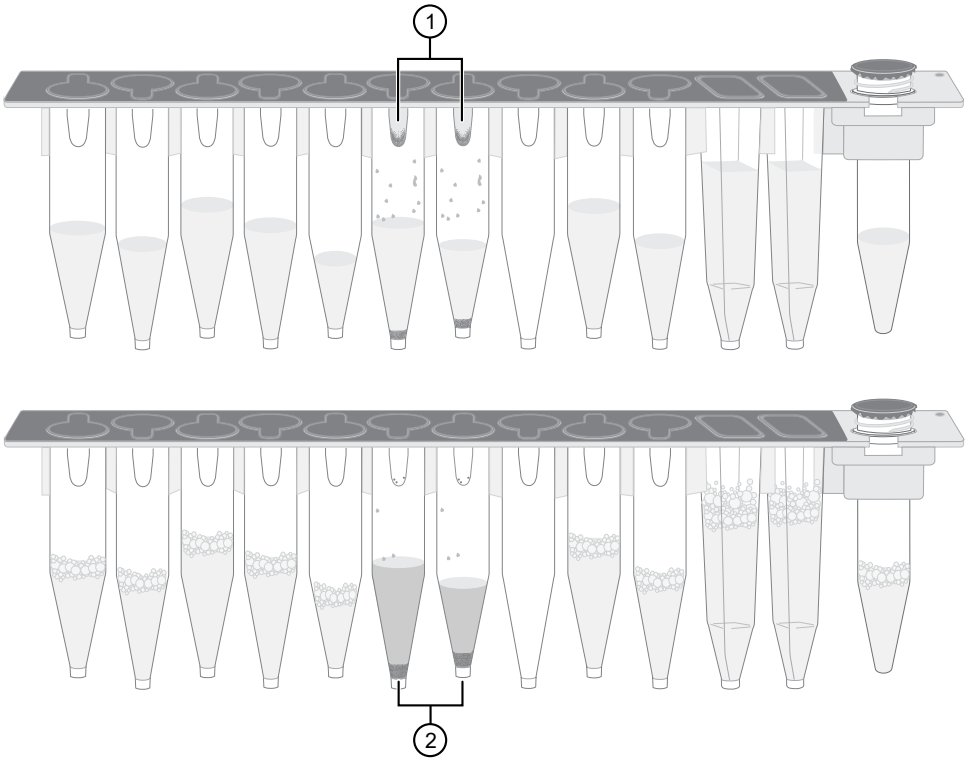
a. Invert the strip 3–4 times to dislodge beads that are trapped in the keyholes.

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 are still trapped in keyholes (see the following figure), 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.



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 can 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.

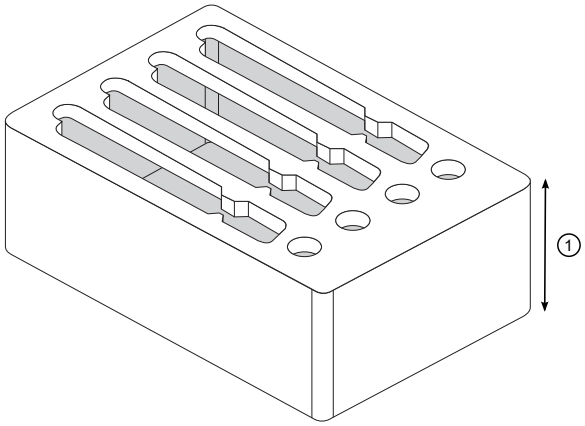
5. Inspect all strips for large bubbles lodged under the surface of the liquid or at the bottom of each tube or well. Gently tap the strips on a benchtop to dislodge any bubbles without splashing the contents onto the upper tube walls. If tapping fails to dislodge a bubble, use the technique that is described in substep 4b 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 “Recommended materials for use with the Oncomine™ BRCA Assay GX” on page 16.



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.

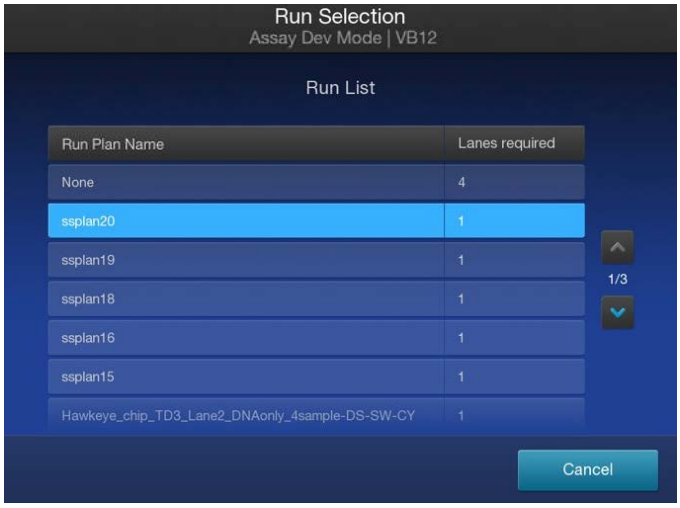
6. Repeat step 1 through step 4, if needed, for the remaining library and templating strips to be loaded in the sequencer.

7. After centrifugation, keep each strip on ice or 4°C until you are ready to load the strips in the sequencer.

Load the sequencer and start a run

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

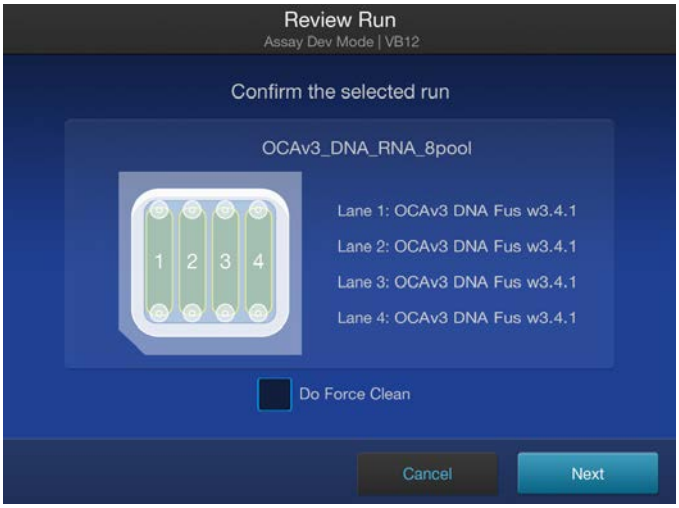
2. In the **Run Selection** screen, select the run that you want to use from the list.



Note: 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**

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.
- Select the **Do Force Clean** checkbox if there will be an unused lane or lanes on the installed chip after the run, but you want to start your 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 perform the cleaning procedure manually between the completion of the current run and the next run. Selecting **Do Force Clean** renders all lanes of the installed chip unusable after the run.

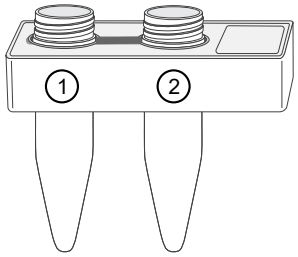
4. In the **Load Deck** screen, the sequencer instructs you step by step to load each required consumable in a highlighted position on the deck. The sequencer detects the loading of each consumable in real time and advances to the next component automatically.

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 and library strip 2 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. The sequencer vision system cannot distinguish tip boxes with lids from boxes without lids.
- 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. Re-racking 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 re-rack 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.

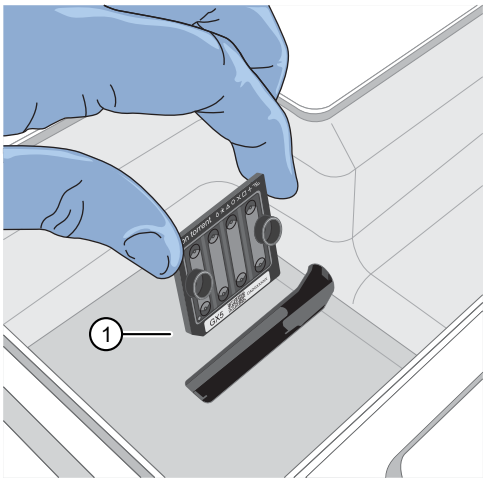
Note:

- 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. 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.

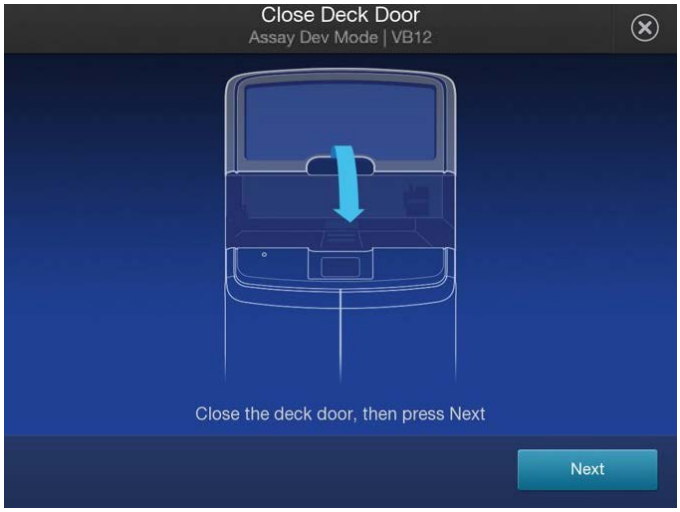
IMPORTANT! Insert the Genexus™ Coupler so that it is level to properly align with the GX5™ Chip. A coupler that is installed at an angle or is not level will not align properly to the chip and can result in a failed run.

6. 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.

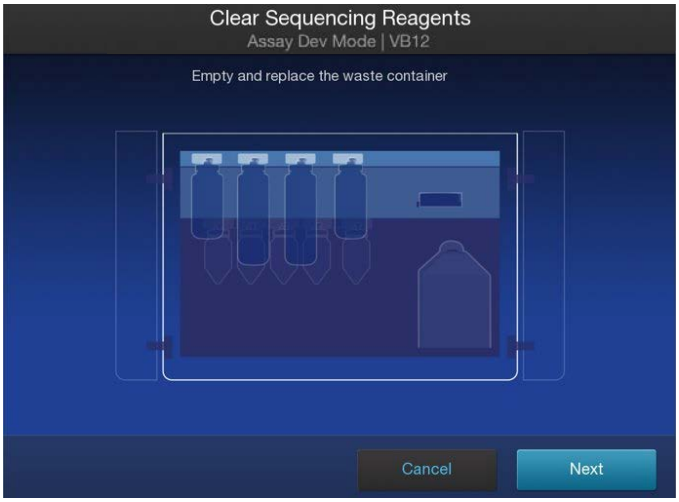
7. Close the deck door, then tap **Next**.



- 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 8.
- If you are using a chip that was previously installed and has sufficient lane capacity for the run, the sequencer prompts you to start 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.

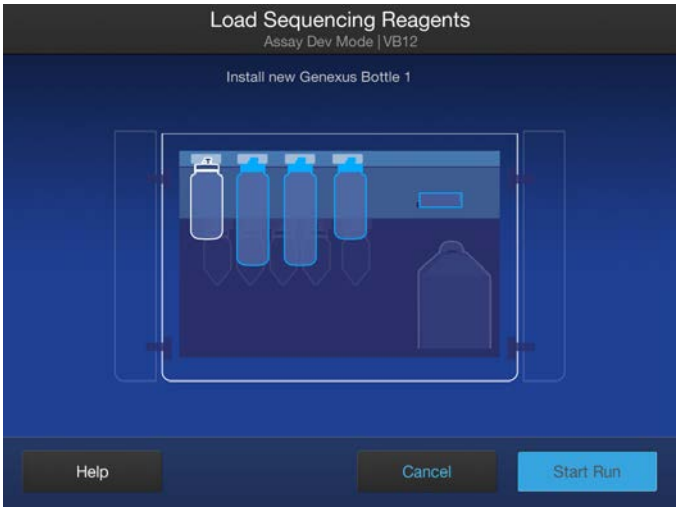
8. Follow on-screen instructions to empty the waste in the Waste carboy, remove waste pipette tips, remove the used Genexus™ Bottle 1, Genexus™ Bottle 2, Genexus™ Bottle 3, and Genexus™ Cartridge, then tap **Next**.



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.

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

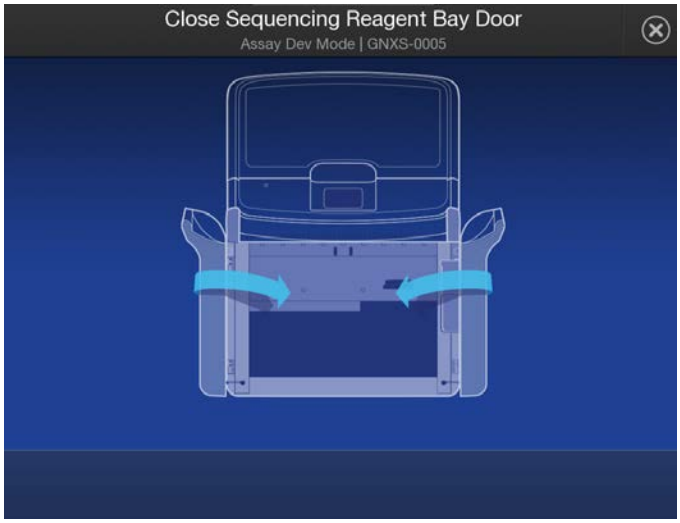


IMPORTANT!

- 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 have been installed, the **Close Sequencing Reagent Bay Door** screen appears.

10. Close the sequencing reagents bay doors.



After the doors are closed, the sequencer automatically 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, the chip shuttles to the install position. You are asked to remove the chip and coupler, and clear the sequencing reagents.

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.

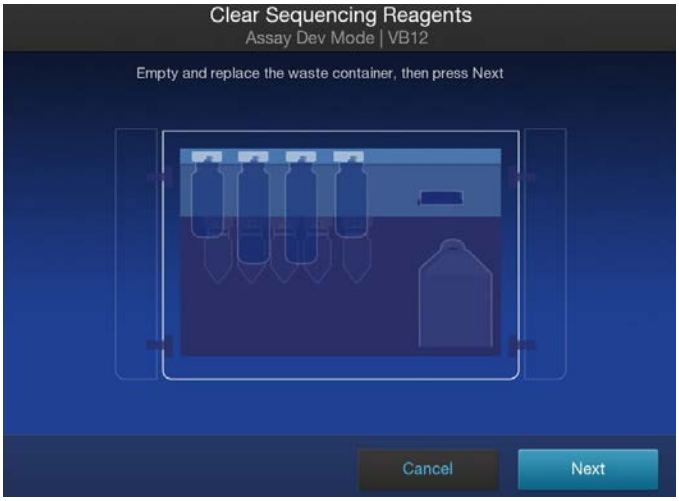
3. Inspect the Genexus™ Filter in the liquid waste disposal port and verify that no standing liquid is present. If standing liquid is present, manually remove the liquid with a pipette, then pull out the filter. Test the filter with water to determine if a clog is present.

   - If the Genexus™ Filter is clogged, replace it with a new filter. For more information, see "*Replace the Genexus™ Filter*" in 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.

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

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

5. 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 *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.

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

8 Review data and results

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

Selection	Description
Click **Results** ▸ **Sample Results**	Select this option to review completed sample results and reports.
Click **Results** ▸ **Run Results**	Select this option to review completed run results and reports by assay.
Click **Results** ▸ **Verification Results**	Select this option to review data from completed verification runs that were performed during sequencer installation or performance qualification.

Review run results

In the **Results / Run Results** screen, runs that are pending, in progress, or complete are listed. Runs with a status of failed, aborted, or stalled are also listed.

You can filter the list of run results by clicking **(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. For more information, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
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 Plugins**, **Analysis Completed**, **Terminated**, **Archival: In Progress**, **Purification In Progress**, or **Purification Completed**). For purification runs that have a status of **Purification In Progress**, **Purification Complete**, or for failed or aborted purification runs, you can place the pointer over the shaded number, in the **Status** column to view the status of each purification batch for the run. The shaded number, such as 1 or 2, represents a purification batch for the run. For example, a status can be **1 Completed**, **2 Started**, and **3 Planned**, which indicates that the first batch is complete, the second batch has started, and the third purification batch has not yet started, but is planned. For more information about purification batches, see “About integrated run planning” on page 32.
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 *Genexus™ Software Help*.
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.

You can also 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. • **Delete**—Delete the run. • **BAM Uploader**—Upload run information to another Genexus™ Integrated Sequencer 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. For more information, see the software help system. • **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.
Actions	These actions are available only for Sample to Result runs. • **View Plan**—View detailed run plan information. • **Review**—Review samples that do not have a concentration within a specified threshold after purification, but before library preparation. For more information, see “Review samples for Sample to Result runs” on page 114. • **Abort**—Enables you to abort a run after purification, but before sequencing. This action is available when the run status is **Purification Review Required**, or when the run status is **Purification Completed** and some purification samples have been excluded from sequencing.
Actions	Available action links for a run are shown when you select the checkbox to the left of the **Run Name**. • **Clear**—Deselect the selected run or runs. • **Retain Data**—Click to keep run data regardless of disk cleanup settings. To undo, select **Release Data**. • **Archive Data**—Click to archive run data. Select run data options (**Sequencing Output**, **Intermediate Files**) in the **Archive Confirmation** dialog box. **Analysis Results** are selected by default and are not optional. • **Delete**—Delete the run data of the selected run or runs.

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™ Integrated Sequencer 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**. The information is available only for results that include variant classifications.
View the run report.	Click the **Run Report** tab.

The run summary

The run summary displays run information, assays used in the run, sample locations, metrics from sample purification, information about the reagents used in the run, primer tube positions, and instrument information.

Depending on the type of the run and the selections made in the run plan, the run information can include the following items.

Table 9 Run information

Item	Description
Run Name	The name of the run.
Started On	The date and time when the run was started.
Completed On	The date and time when the run was completed.
Run Status	The status of the run.
Report Template	If the option to generate a report was enabled in the run plan, the report template used is listed.
Chip Type	The semiconductor sequencing chip used in the run, such as the Ion Torrent™ GX5™ Chip.
Library Chemistry	The type of library chemistry used in the run, such as Ion AmpliSeq™ HD.
Server	The Ion Reporter™ Software account or the Genexus™ Software account and the respective software version that was selected for uploading BAM files when the run is complete.
Run Type	The type of run. For example, Nucleic Acid to Result.

Table 10 Sequencing instrument information

Item	Description
Instrument Name	The name of the sequencer.
Instrument Serial Number	The serial number of the sequencer.
Status	The status of the sequencing portion of the run.
Operator	The name of the person who was signed in the Genexus™ Integrated Sequencer when the sequencing run was performed.
Start Date	The date and time when the run was started on the sequencer.
Completion Date	The date and time when the run was completed on the sequencer.
Sequencing Flows	The number of flows performed by the sequencer.

Table 11 Purification instrument information

Item	Description
Purif. Instrument	The name of the purification instrument.
Instrument Serial Number	The serial number of the purification instrument.
Status	The status of the purification portion of the run for the batch.
Operator	The name of the person who was signed in the Genexus™ Purification Instrument when purification was performed.
Start Date	The date and time when the batch was started on the purification instrument.
Completion Date	The date and time when the batch was completed on the purification instrument.
Purif. Batch	The nucleic acid isolation batch.

Table 12 Assays table

Item	Description
Assay Full Name	The name of the assay used in the run. Click the assay name to display the details of the assay.
Assay Name	An abbreviated name of the assay or assays used in the run.
Analysis Version	The version of the assay used for analysis.
Research Application	The research application for the assay, such as **DNA** or **DNA and Fusions**.
Lane	The chip lane or lanes used in the sequencing run for the assay.
Total Samples	The total number of samples sequenced for the assay. A single sample can correlate with multiple wells on the plate for some assays when multiple nucleic acid types are contained within the sample.
Updated Annotations	The information that is shown depends on the settings and the type of the assay. • The name and version of the annotation source that is applied to the sample result is shown when an assay is configured to use the latest annotations but the annotation that is applied to the results is not the latest annotation source. • No information is shown in the column (the value is blank) when the latest annotation set version is applied to the result, regardless of whether the assay is configured to use the latest annotation source. • No information is shown in the column (the value is blank) when an assay is configured to not use the latest annotation source, and instead uses a fixed version of an annotation source. • **N/A** is shown when an assay does not use an annotation set.

Table 13 Purification Samples table

Item	Description
Batch ID	When the run includes more than one purification batch, the **Batch ID** is listed. The **Batch ID**, such as **1** or **2**, indicates the purification group for each sample.
Sample Name	The unique identifier created when the sample was entered into the software.
Sample Type	A term that describes the sample, for example, **FFPE** or **Blood (Plasma)**.
Nucleic Acid Type	**DNA**, **RNA**, or **DNA+RNA**.
Conc. (ng/µl)	The concentration of the sample measured by the purification instrument. In some instances, such as when purification is in progress or when the sample is a no-template control, the values is listed as N/A. If the concentration is not within the QC concentration range specified by the assay, a **Quantity Not Sufficient** (QNS) alert is displayed.
QC Conc. Range (ng/µl)	The QC concentration range for the assay.
Batch Status	The run status of a purification batch. The status can be **N/A**, **Planned**, **Review**, **Started**, **Aborted**, or **Completed**.
Archive Position	The sample positions in the archive plate. Sample positions can be in rows A to D and in columns 1 to 8.
Library Prep	An indicator of whether the sample is selected for sequencing. • ✔(Checkmark) – indicates that the sample is selected for sequencing. • ⊘– (No icon) indicates that the sample is not selected for sequencing. • **N/A** – indicates that purification has not started or is in progress.

Table 14 Sample Locations table

Item	Description
PCR Plate View	Click to view the sample locations in an image of a 96-well sample plate.
Well Pos.	The well position indicates the location of the sample on the plate.
Library Batch ID	The unique identifier created for the library batch. This information is available only for Library to Result runs.
Sample Name	The unique identifier created when the sample was entered into the software.
Nucleic Acid Type	The sample nucleic acid type, such as DNA, RNA, or TNA.
Vol. (µl)	The volume of the sample.
Conc. (ng/µl)	The concentration of the sample.
Dilution Factor	The dilution factor of the sample.

Table 14 Sample Locations table *(continued)*

Item	Description
Kit Barcodes	The barcode for the kit used for nucleic acid extraction, if applicable.
Barcode	The name of the barcode adapter or adapters that are associated with the sample, for example, **IonHDdual_0101**.
Assay Name	The assay that was used to sequence the sample for the indicated well position.
Library Position	The plate location of libraries. For completed runs that were planned using samples, the **Library Position** indicates the location of unused libraries that can be sequenced by planning and initiating a library run.

Table 15 Reagents table

Item	Description
Consumable	Consumables used in the run, such as the **PanelKit** and **LibraryKit**, are listed.
Barcode	The consumable barcode, if applicable.
Part #	The consumable part number, if applicable.
Expiration Date	The consumable expiration date, if applicable.
Lot #	The consumable lot number, if applicable.

The **Primer Tube Positions** table shows an image that indicates the locations of the primer pool tubes.

Review samples for Sample to Result runs

A **Sample to Result** run integrates nucleic acid purification and sequencing. When you create a **Sample to Result** run plan, you can include an option that allows samples to be reviewed before library preparation starts. Samples that do not meet a specified concentration threshold after purification have a run status of **Purification Review Required**.

For more information about **Sample to Result** runs, see “About integrated run planning” on page 32.

You can also apply this option when you create an assay. For more information, see the software help system.

1. In the menu bar, click **Results** ▸ **Run Results**.

   Note: Sample to Result runs that require review after purification have a **Run Status** of **Purification Review Required**.

2. In the **Run Results** screen, place the pointer over the row of the run plan of interest, then click **Review**.
   The **Purification Samples** screen opens.

3. Review the concentration for each sample, then select the checkbox in the row for each sample you want to sequence.

4. Click **Submit**.

The samples are ready for sequencing. For more information, see the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

Variant report

The variant report is a PDF report of the results for each sample in a sequencing run. You can use a system-installed report template to generate a variant report, or you can customize the layout and contents of a variant report.

To make a variant report available for each sample result upon completion of a sequencing run, enable **Generate Report** in the **Setup** step when you plan the run. For more information, see Chapter 4, "Plan and manage runs"). To generate a variant report after a run is complete, see "Generate a variant report" on page 115.

When a variant report has been generated for a sample result, it is available for download in three places:

- In the **Results / Sample Results** screen when you place the pointer over the row for that sample, then click the **Report** link to download the PDF.
- In the variant report pane for the sample results in the **Reports** tab when you click ⋮ **(More) ▸ Download Report**.
- In the **Results** screen for the sample when you click ··· **(More Options) ▸ Download Files**.

Variant reports can be electronically or manually signed by users. Electronic signatures are shown in the Electronically Signed By section of the report, if the section is included in the report template.

Generate a variant report

You can generate a new variant report for sample results after a run is complete. A 🔒 **(Lock)** in the variant report indicates that the electronic signature option for the report is locked. After a variant report is locked, the report cannot be electronically signed by any other user.

You can generate multiple reports for a sample result, if each report is named uniquely, and is generated in a different language, or uses a different report template.

When generating a customized report, you can update any report template selections. You can use this procedure to generate multiple reports for a sample, if a unique report name is entered for each report. For example, you may want to generate reports for different languages, or reports that use different templates.

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. Select the **Reports** tab, then click **+ Generate Variant Report**.

4. In the **Generate Report** dialog box, change the name of the report that is generated by the software, if needed, then select the report template and language of the report.
   a. If the report template includes the option to include custom images from the results, click **Upload Image**, then select the images to include in the report, and enter a title for the image, and if needed enter a description and footnote for the image.

   b. If the option to make custom text **Editable on Report Generation** was selected when the report template was created, enter a title in the **Custom Text** section and if needed, a description.

   The **Report Template** list includes the report templates that are associated with the assay that was used in the run. For information about creating report templates, see the software help system, or the *Genexus™ Software 6.8 User Guide*.

   If you select the same report template that was used to generate a variant report, and have not locked that report, the new selections you make override the previous variant report.

5. Click **Generate**.
   A draft version of the report is added to the **Reports** tab.

A pane for the new report is added next to the Run Report pane in the **Reports** tab. Reports that have been generated are available for download in the **Reports** tab, in the **Sample Results** screen, and in the ZIP package that contains results files.

Download a variant report

You can download a variant report for a sample result of interest from the **Results / Sample Results** screen, from the pane that shows the report, or as part of a download of results files.

1. In the menu bar, click **Results** ▸ **Sample Results**.

2. Download the report with one of the following options.

Option	Procedure
Download a ZIP file that contains all variant reports for a sample result.	In the **Sample Results** screen, click the sample name of interest in the **Sample Name** column, then click ··· **(More Options)** ▸ **Download Files**.
Download the variant report in PDF format from the **Reports** tab for the sample results.	Click the **Reports** tab then, in the pane of the report of interest, click ⋮ **(More)** ▸ **Download Report**.
Download the variant report as part of the results files for a specific sample in the **Results** screen.	

   A ZIP file that contains the PDF report is downloaded to the computer if you download reports from the **Sample Results** screen or as part of the results files. A report in PDF format downloads if you use the **Reports** tab for the download.

3. 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.

4. Extract the downloaded files, then open the PDF file in an appropriate viewer.

View sequencing results

For every run, you can view assay-specific results and sample-specific results. Assay-specific results include assay metrics, such as final read data, and assay-level plugin information, such as execution of the customer support archive. For more information, see “Assay metrics and the run report” on page 118 and “Review plugin results in Genexus™ Software” on page 140.

The following sample-specific result information is available.

1. Click **Results** ▸ **Sample Results** to view sequencing results 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 “QC results” on page 123.
Key Findings	An overview of the results for the sample, including **Sample Details**, **Key Metrics**, **Genes**, and **Coverage** graphs. For more information, see the software help system.
Variants	Detailed variant results for **SNVs/Indels** and **CNVs**. For more information, see “View SNV/INDEL results” on page 131 and “View CNV results” on page 134.
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 140.
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 122. • Variant reports – include the variant results for each sample in a sequencing run, reagents used, and QC evaluation metrics. For more information, see “Variant report” on page 115.

Assay metrics and the run report

The run report provides detailed information, such as various chip metrics for the run, and 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 software help system.

Table 16 Run Report

Metric	Description
Loading	The number and percentage of total addressable wells on the chip that contain an ISP.
Enrichment	The number and percentage of wells ISPs that contain live ISPs.
Library	The number and percentage of wells with live ISPs that contain Library ISPs.
Combined Final Reads	For runs that include resequencing, the combined final reads is the sum of library reads from both sequencing and resequencing.
Final Reads	Library reads passing all filters that are recorded in the output BAM files. This value can be different from the total number of reads due to technicalities associated with read trimming beyond a minimal requirement.
Total Bases	The number of filtered and trimmed base pairs that are reported in the output BAM file.
Combined Total Bases	For runs that include resequencing, the combined total bases is the sum from sequencing and resequencing.
Raw Read Accuracy	The raw read accuracy across each individual base position in a read calculated as, (1–[total errors in the sequenced reads]/[total bases sequenced]) × 100. Raw read accuracy is measured at each base across the length of the read and is based on 1x sequencing coverage; raw read accuracy is *not* based on consensus accuracy across multiple reads for the same base position.
Wells with ISPs	The number of wells that contain an ISP.
Unenriched	The number and percentage of wells with ISPs that do not contain live ISPs.
Total Addressable Wells	Wells on the chip that can be physically reached by a library.
Empty Wells	The percentage of total addressable wells on the chip that do not contain an ISP.
Wells with Live ISPs	Loaded wells with ISPs with a signal of sufficient strength and composition to be associated with the library or control fragment key.
Wells with Library ISPs	Loaded wells with live ISPs with a key signal that is identical to the library key signal.

Table 16 Run Report *(continued)*

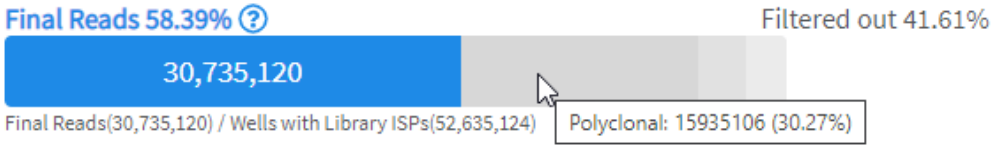
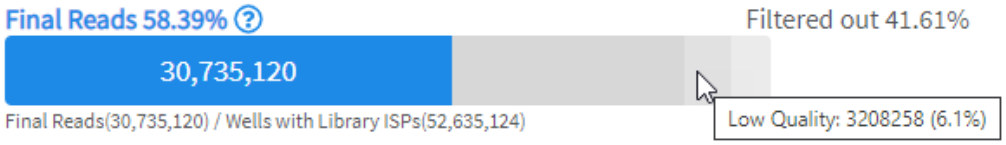
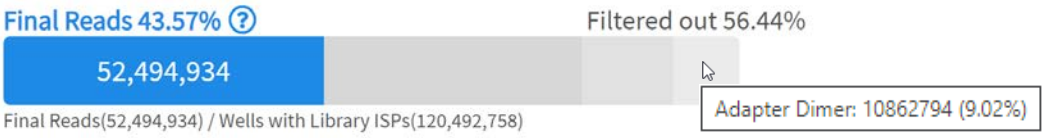
Metric	Description
Control ISPs	Loaded wells with live ISPs with a key signal that is identical to the control fragment key signal.
Filtered out	The total percentage of filtered reads, or the sum of the percentages of polyclonal, low quality, and adapter dimer reads.
Polyclonal	Wells with a live ISP that carries clones from two or more templates. To view polyclonal metrics, mouse over the first low quality portion (gray) of the **Final Reads** bar plot. 
Low Quality	Loaded wells with a low or unrecognizable signal. To view polyclonal metrics, mouse over the second low quality portion (gray) of the **Final Reads** bar plot. 
Adapter Dimer	Loaded wells with a library template of an insert size less than 8 bases. To view adapter dimer metrics, mouse over the lightest gray portion of the **Final Reads** bar plot.  **Note:** In assays using Ion AmpliSeq™ HD library chemistry, adapter dimer reads represent a small proportion of total reads and can be seen by hovering the pointer over the right end of the **Final Reads** bar.

Table 16 Run Report *(continued)*

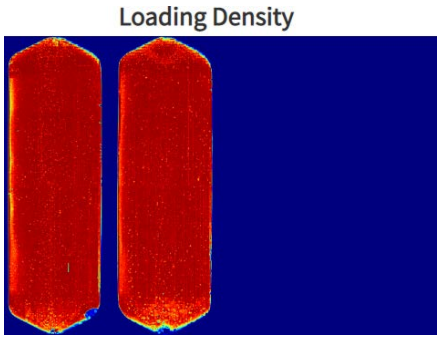
Metric	Description
Loading Density	A visual representation of chip loading. Red color indicates areas of higher density of loading. Blue color indicates areas of lower density of loading. The following example shows a sequencing experiment where two lanes on the chip are uniformly loaded with ISPs. 

Table 17 Run Samples

The **Run Samples** table lists read data for each individual sample in the assay.

Column	Description
Sample Name	The unique identifier created when the sample was entered in the software.
Nucleic Acid Type	The sample nucleic acid type, such as DNA, RNA, or TNA.
Barcode	The unique identifiers of the dual barcode pair assigned to the DNA and/or RNA library for a sample.
Total Reads	The total number of filtered and trimmed reads with the listed dual barcodes assigned to the sample.
Mean Read Length	The average length, in base pairs, of usable library reads for each sample.
≥Q20 Bases	The total number of called bases that have ≥99% accuracy (or less than 1% error rate) aligned to the reference for the sample.
Mapped Reads	The number of reads that are mapped to the reference file.
On Target Reads	The percentage of sequencing reads mapped to any target region of the reference.
Mean Depth	The average number of reads of all targeted reference bases.
Uniformity	The percentage of bases in all targeted regions (or whole genome) with a depth of coverage ≥20% of the mean read coverage.
Read Length Histogram	A histogram that presents all filtered and trimmed library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). 

Table 18 Barcodes with Reads Reported

The **Barcodes with Reads Reported** table lists barcode-specific metrics

Column	Description
Barcode	The unique identifiers of the dual barcode pair assigned to the DNA and/or RNA library for a sample.
Total Reads	The total number of filtered and trimmed reads with the listed dual barcodes assigned to the sample. The reads are independent of length reported in the output BAM file.
Mean Read Length	The average length, in base pairs, of usable library reads for each sample.
≥Q20 Bases	The total number of called bases that have ≥99% accuracy (or less than 1% error rate) aligned to the reference for the sample.

Download information about run samples

Information about each sample in a run, such as the barcode for the sample and the total reads, are shown in the run samples table. You can download the information shown in the run samples table.

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. Click the **Run Report** tab, then scroll to the **Run Samples** table.
4. In the upper right corner above the **Run Samples** table, click **Export to Excel**.

An XLSX file that contains the information shown in the run samples table is downloaded.

Read length histograms are not included in the file.

Download reads reported by barcodes

Metrics for the barcodes that are included in a run are listed in the barcodes with reads reported table. You can download the information shown in the barcodes with reads reported table.

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. Click the **Run Report** tab, then scroll to the **Barcodes with Reads Reported** table.
4. In the upper right corner above the **Barcodes with Reads Reported** table, click **Export to Excel**.

An XLSX file that contains the information shown in the barcodes with reads reported table is downloaded.

Read length histograms are not included in the file.

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 assay metrics are shown. For more information, see “Assay metrics and the run report” on page 118.

Run report

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 “Assay metrics and the run report” on page 118. If you entered extraction kit barcodes for samples or control samples when you prepared library batches or when you planned the run, the extraction kit barcodes are listed in the run report.

View a run report

You can view or download a run report in Genexus™ Software.

For information to download a run report, see “Download a run report” on page 122.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, click the sample name of interest in the **Sample Name** column.
3. Click the **Reports** tab.
   A pane for the run report and each variant report that has been generated is shown. The name of the report is listed at the top of the pane.
4. In the **Run Report** pane, click **View Report**.

The run report opens in a new browser tab.

Download a run report

You can download a run report summary in PDF format.

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.

QC results

The quality control (QC) metrics for each sample that was sequenced in a run are displayed in Genexus™ Software.

If a sample fails a single test metric, the sample fails that QC test. A sample must meet all QC parameter thresholds of a particular QC test in order to pass. The QC status is divided into the following categories.

- ✔ (Passed) indicates the sample passed all QC metrics.
- ✘ (Failed) indicates the sample failed a QC metric.
- — (Not Calculated) indicates a sample did not undergo QC analysis.

The data displayed in the screen depend on the assay that was used in the run.

Metric[1]	Description
Purification QC	**Quality control information for nucleic acid extraction that is performed on a Genexus™ Purification Instrument.**
Sample Concentration RNA	The concentration of extracted RNA after purification.
Sample Concentration DNA	The concentration of extracted DNA after purification.
Sample Concentration TNA	The concentration of extracted TNA after purification.
Run QC	**General run quality control information.**
Final Reads	Library reads passing all filters that are recorded in the output BAM files. This value can be different from the total number of reads due to technicalities associated with read trimming beyond a minimal requirement.
Key Signal	The average signal after software processing for library ISPs that identically match the library key (TCAG).
Key Signal – Resequencing	The average signal after software processing for library ISPs that identically match the library key (TCAG) for the resequencing run. This metric is only shown for assays that include resequencing.
Percent Loading	The number of wells with ISPs divided by the number of the total addressable wells in a run.
Percent Loading – Resequencing	The number of wells with ISPs divided by the number of the total addressable wells in the resequencing run. This metric is shown only for assays that include resequencing.
Raw Read Accuracy	The average raw accuracy across each individual base position in a read, where raw read accuracy is calculated as 100 * (1 - (sum(per base error)/sum(per base depth))).

(continued)

Metric[1]	Description
Templating QC—CF-1 Control	**Sequencing quality metrics of the control fragment. These metrics indicate templating success.**
Average Reads Per Lane	The number of CF-1 reads divided by the number of chip lanes used in the run.
Base Call Accuracy	The probability that a given base is called correctly. 1 – (total number of errors for all positions in CF-1) / (total number of CF-1 base reads).
Mean AQ20 Read Length (bp)	Average length, in base pairs, at which the accuracy rate is ≥99% for CF-1 reads.
Sample QC—DNA	**Sequencing quality metrics of the sample DNA library.**
MAPD	MAPD (Median of the Absolute values of all Pairwise Differences) is a quality metric that estimates coverage variability between adjacent amplicons in CNV analyses. A MAPD value of ≤0.5 indicates an acceptable level of coverage variability. High MAPD value typically translates to a lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. If the MAPD QC threshold is not met, CNVs are not called. The MAPD metric does not affect SNVs/INDEL calls.
Mapped Reads	The total valid mapped reads.
Mean AQ20 Read Length (bp)	The average length, in base pairs, at which the accuracy rate is ≥99% for all aligned reads of a library.
Mean Read Cov	The number of average reads per amplicon.
Molecular Uniformity	Uniformity of molecular coverage for all amplicons.
Mean Read Length (bp)	The average length, in base pairs, of final library reads for the sample.
Uniformity of Amplicon Coverage	The percentage of amplicons that had at least 20 percent of the average number of reads per amplicon. Cumulative coverage is linearly interpolated between nearest integer read depth counts.
Median Mol Cov	The median number of functional molecule reads per amplicon calculated over all amplicons in the assay. This metric is applicable to Ion AmpliSeq™ HD library chemistry only.
Uniformity of Base Coverage	The percentage of reads with a depth of coverage ≥20% of the mean read coverage at each position. This metric is applicable to Ion AmpliSeq™ HD library chemistry only.
Read Length Histogram	The histogram presents all filtered and trimmed DNA library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences.

(continued)

Metric[1]	Description
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of genomic sample reads to control reads for each inline control amplicon. **Note:** The **Read Ratio** for each inline control amplicon is expected to be approximately 3 with 10 ng DNA input for both Ion AmpliSeq™ and Ion AmpliSeq™ HD chemistries.
NTC QC – DNA	**DNA Sequencing quality metrics of the no-template control.**
Average Base Coverage Depth	The average number of DNA reads of all targeted reference bases.
Mean Read Length (bp)	The average length, in base pairs, of final DNA library reads for the no template control.
Read Length Histogram	The histogram presents all filtered and trimmed reads for the no template control that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis).
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of no template control sample reads to control reads for each DNA inline control amplicon.

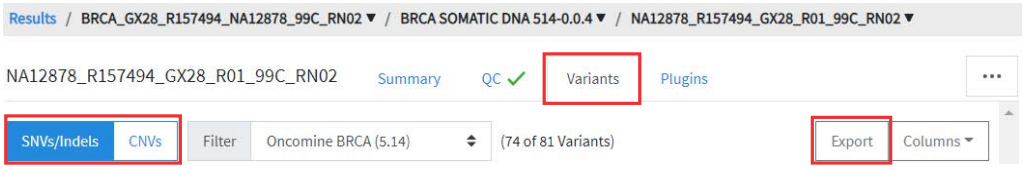
[1] For **BAM to Result** runs, only the **Sample QC—DNA** metrics are shown.

View key findings

You can view the **Key Findings** 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**, then click a run name to open the **Results / Run Results** screen. In the **Run Name** column, click a run name to open the **Results** screen, then select a sample from the **Select Sample** dropdown list.



Key Findings

The **Key Findings** table shows details about the sample, a summary of key metrics for the run, key variants detected in the sample, 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.

The information that is displayed depends on the assay that was used in the run.

Table 19 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 or DNA.
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[1]	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.

[1] Metric is shown only for some assays.

Table 20 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.

Table 20 Key Metrics *(continued)*

Section	Description
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 will be the percentage of reads passing uniquely mapped and/or non-duplicate filters, or 100% if no filters were specified.

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

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 **Solid Tumor** 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. For more information, see “View the Key Variants matrix” on page 127. For more information about gene lists, see the software help system.

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.

View the Key Variants matrix

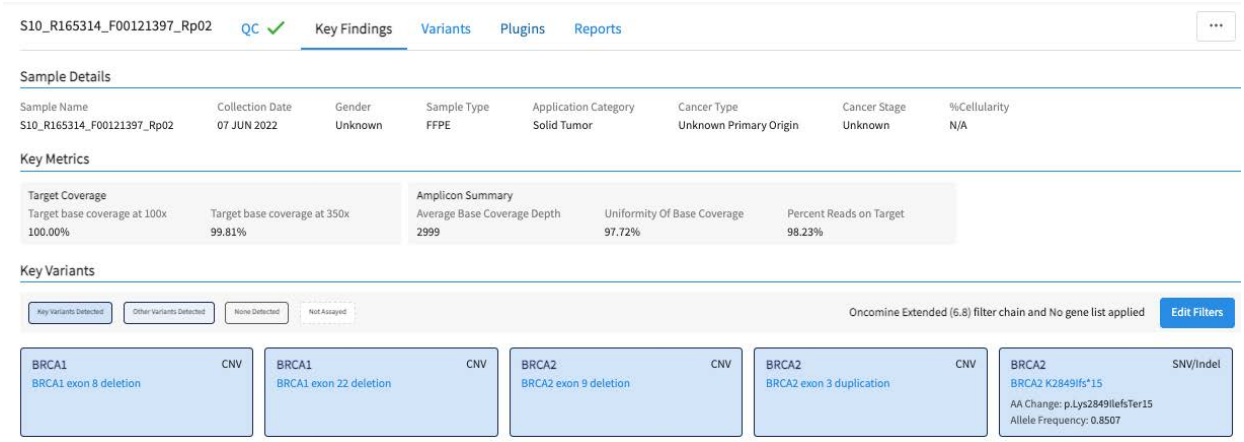
The **Key Variants** matrix provides a color-coded visual representation of the variant results. When the application category of the sample is **Cancer** or **Cancer (Germline)** the genes that are shown in the **Key Variants** matrix are determined by the filter chain and the gene list applied to the 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 “Search and filter variant results” on page 139. 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 called 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 software help system.

Other Variants Detected —A gene is present in the gene list and variants are called 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 called by the Oncomine™ Variant Annotator plugin.

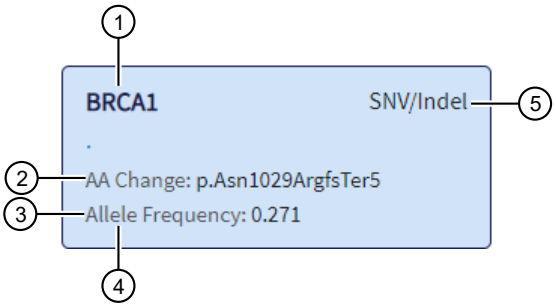
Not assayed —A gene is present in the gene list, but is not included in the panel used in the assay.

② **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.

③ **Variant Name** —The name of the variant. The **Variant Name** is a hyperlink to the pileup for the variant in the **Variants** tab.

Gene tiles in the Key Variants matrix

Each blue tile in the **Key Variants** matrix represents a variant and summarizes information for the variant.



① The gene name.

② The **Variant Name** – link to the variant details in the **Variants** tab.

③ The amino acid change using Human Genome Variation Society (HGVS) nomenclature.

④ The number of variant read counts divided by the total number of read counts for the sample.

⑤ The variant type.

View the amplicon coverage

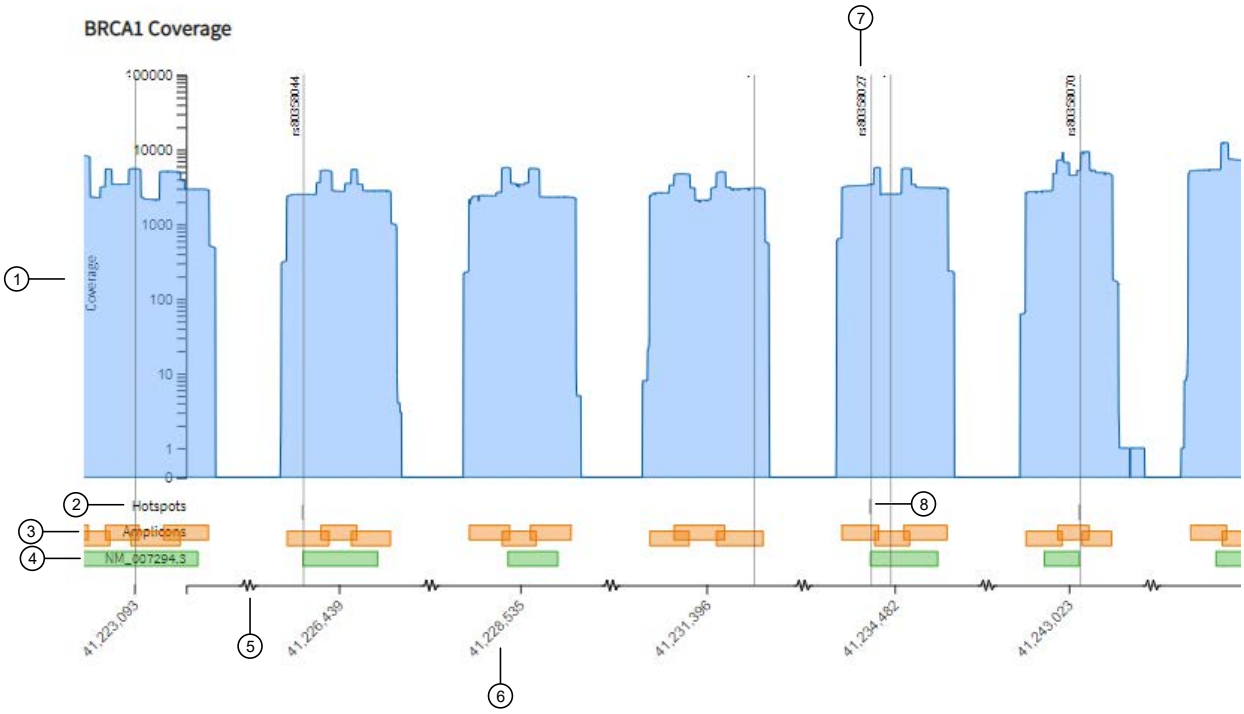
You can view DNA exon amplicon coverage in Genexus™ Software to help you determine whether the sequencing reads across a gene are uniform and sufficient. The default genes that are shown are determined by the gene list that is designated as default for the cancer type of the sample. You can also view 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.

Example amplicon coverage graph

Here is an example of an amplicon coverage graph for the *BRCA* 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 Key Variants matrix” on page 127.

⑧ You can place the pointer over the hotspots that are represented with gray lines to view the hotspot name.

You can zoom into or out of, or reset, the graph. For more information, see “View the amplicon coverage” on page 130.

View SNV/INDEL results

You can view the **SNVs/Indels** results for a sample. 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.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Results** screen.
3. Click the **Variants** tab.

4. Click **SNVs/Indels** to view the **SNVs/Indels** table.

   For more information about the data displayed in the table, see “SNVs/Indels table” on page 132.

5. *(Optional)* Click **Export** in the upper right corner of the screen to export the results data in XLS or TSV format, if needed.

   The information that is included in the file is based on the gene list, the filters that are applied to each column, and filter chain that is applied

   - The file in XLS format contains the information that is shown in the SNV/Indels variant table.
   - The file in in tab-separated value format (TSV) format contains the information that is shown in the SNV/Indels variant table, and information for table columns that are available but not currently shown in the SNV/Indels table. The file also includes information that is listed in the Annotations table.

SNVs/Indels table

The data displayed in the table depend on the assay that was used in the run. Results in the table can be filtered using the filtering tools. For more information, see “Search and filter variant results” on page 139.

Column	Description
User Classifications	User-defined classification to selected from the list. For more information, see *Genexus™ Software Help*.
Variant ID	The name of the hotspot as defined in the Browser Extensible Data (BED) file. Click the link to view more annotation information.
Variant Name	The name of the variant.
Key Variant	Indicates whether or not 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. • LongDel — A 40-bp deletion in BRCA1 called by the end-point detection method. 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: • 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
Gene	The gene name. Click the link to open the **View Annotation Sources** window to view additional information. For more information, see *Genexus™ Software Help*.
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 (A, G, C, T).
Type	The type of variant that is detected. • snp (single nucleotide polymorphism) • mnp (multi-nucleotide polymorphism) • ins (insertion) • del (deletion) • complex
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 142). • **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, refer to the **Alt** column. • **NO CALL**—While 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 the presence of a variant that differs from the reference allele at a given position, however, this nucleotide is not an ALT allele that is targeted by this assay.
Call Details	The reason why a variant is reported as **No Call**.
Effective Read Depth	The number of reads covering the position.
Alt Allele Read Count	The number of reads containing the alternate allele.
Nuc Change	The position and identity of the nucleic acid change.
Allele Fraction	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.

View CNV results

You can view the **CNVs** results for a sample. The **CNVs** table lists the calls and other information for the copy number variants (CNVs) analyzed in each sample in a run.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Results** screen.
3. Click the **Variants** tab.
4. Click **CNVs** to view the **CNVs** table.

   For more information about the data displayed in the table, see “CNVs table” on page 134.
5. *(Optional)* Click **Export** in the upper right corner of the screen to export the results data in XLS or TSV format, if needed.

   The information that is included in the file is based on the gene list, the filters that are applied to each column, and filter chain that is applied

   - The file in XLS format contains the information that is shown in the SNV/Indels variant table.
   - The file in tab-separated value (TSV) format contains the information that is shown in the SNV/Indels variant table, and information for table columns that are available but not currently shown in the SNV/Indels table. The file also includes information that is listed in the Annotations table.

CNVs table

The data displayed in the table depend on the assay that was used in the run. Results in the table can be filtered using the filtering tools. For more information, see “Search and filter variant results” on page 139.

IMPORTANT! *(FFPE samples only)* If **% 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 software help system.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see the software help system.
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.
Variant Name	The name of the variant.
Locus	The starting position of the first amplicon covering the CNV gene.

(continued)

Column	Description
Oncomine Variant Class	Annotates gene-level and exon-level CNV deletions and duplications. 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 the following type of Oncomine™ annotations. • 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, which provides a link to the **View Annotation Sources** dialog box with additional information about the **HotSpot ID**. For more information, see the software help system.
Copy Number	The copy number of a CNV gene locus per genome. This column is available when a positive call is made.
Type	Indicates variant type, such as CNV or NA (non-CNV).
Subtype	Indicates variant subtype. • REF – Read count matches reference baseline. • NO CALL – Read count differs from baseline by non-integer amount; evidence for a BigDel or BigDup call is weak. • BigDel – deletion of at least one exon, or multiple exons. • BigDup – Duplication of at least one exon, or multiple exons. • GeneCNV – whole BRCA1/BRCA2 gene deletion or duplication. **Note:** You can use the visualizations in the software to confirm deletions and duplications. Use the post-corrected visualization to confirm exon deletions and duplications. Whole gene deletion or duplication results can be confirmed only in the precorrected view. That is, if a BRCA1 deletion or duplication (**BRCA1DEL** or **BRCA1DUP**) or a BRCA2 deletion or duplication (**BRCA2DEL**or **BRCA2DUP**) for a GeneCNV subtype is shown in the **Call Details** of the CNV variants table, review the visualization to verify the direction of the GeneCNV subtype. Click **Pre-corrected** and Compare the SampleID (sid) amplicons to the BRCA1 or BRCA2 genes.

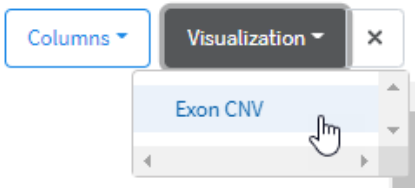
(continued)

Column	Description
Call Details	Indicates exon number or exon region for exon-level CNV calls or GeneCNV subtype (BRCA1Del and BRCA2Del) and the following calls: • BRCA1Del • BRCA2Del • BRCA1Dup • BRCA2Dup • BRCA1NC • BRCA2NC (for NOCALL)
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** 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 142). • **PRESENT** – indicates a high confidence call that passes all filter thresholds. • **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. • **ABSENT** – the absence of a variant; result is below detection threshold for a CNV call. • **NO CALL**– while 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.
No Call Reason	The reason for reporting a CNV as No Call.
CNV Confidence	For BRCA Exon CNV and GeneCNV calls, the **CNV Confidence** is a Phred-scale score corresponding to the quality of the CNV call. The 95% upper confidence bound is the ploidy estimate, where it is 95% certain that the true ploidy is below that value.
Variant ID	The name of the hotspot as defined in the BED file.
CNV Ratio	The ratio of measured CNV gene locus coverage relative to coverage of non-CNV loci.
Med Read Cov Gene	The median read coverage of targeted CNV gene.
Med Read Cov Ref	The median read coverage of non-CNV reference loci.
Valid CNV Amplicons	The valid number of amplicons spanning the CNV call that pass internal QC. This number can vary in each run, depending on the quality of the data.

Visualize exon and whole gene CNVs

In Genexus™ Software, you can visualize read counts of BRCA1 and BRCA2 exons that are normalized to the Oncomine™ BRCA DNA baseline.

1. In the menu bar, click **Results** ▸ **Sample Results**, then in the **Sample name** column, click the name of the sample of interest.
2. In the **Variants** tab, click **CNVs** to open the CNV results screen.
3. Click **Visualization** ▸ **Exon CNV** to visualize exon read counts.



A plot opens with **Normalized Read Counts** in log2 scale for each exon CNV that is detected.

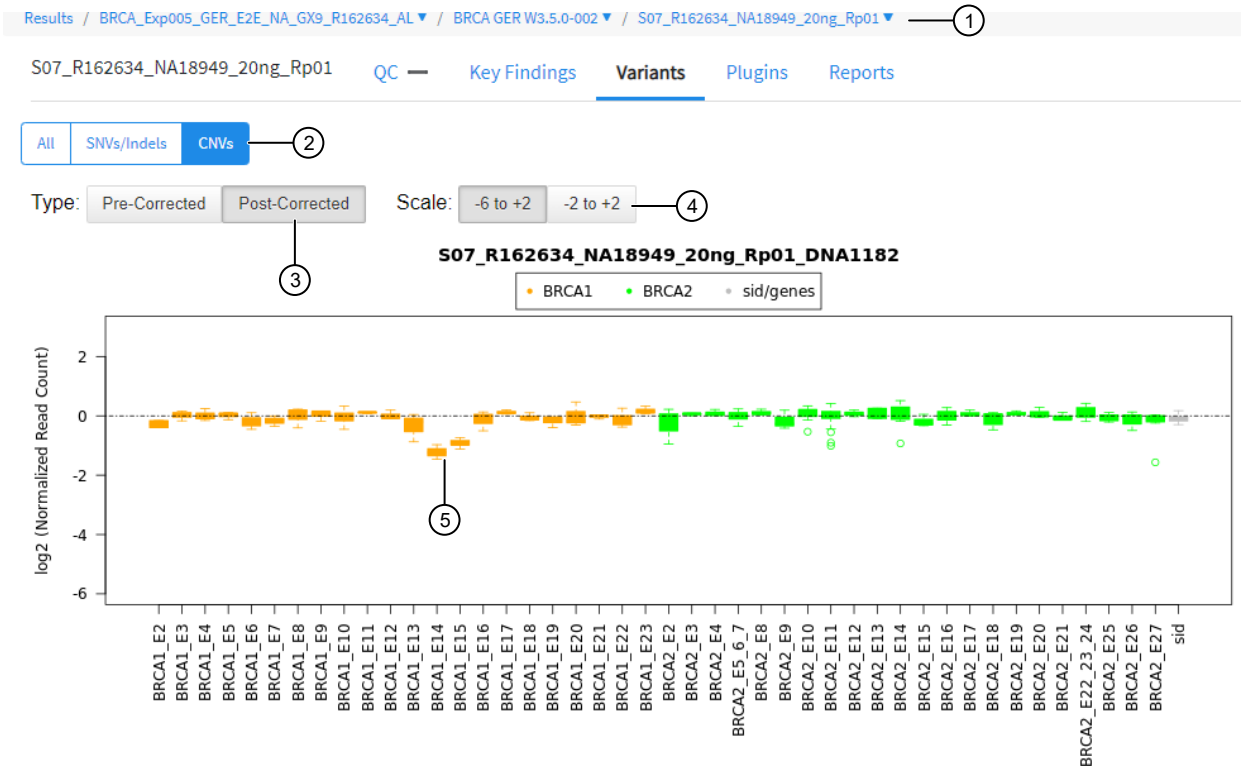


Figure 1 Exon-level deletion detected with Oncomine™ BRCA Assay GX.

① Menu bar that shows the selected results, the assay, and the sample name.
② **CNVs** selection shows the CNV results screen.
③ Toggle selection for **Pre-corrected** and **Post-Corrected** plot views.

- Pre-corrected plot shows amplicon coverage data normalized to total reads in a sample.
- Post-corrected plot shows amplicon coverage after applying GC-content, amplicon length, and principal components analysis corrections to the log2 ratios.

④ **Scale** values of **–6 to +2** or **–2 to +2**, to zoom out or zoom in on the plot.
⑤ This example shows an exon-level deletion. Exons 14 and 15 are deleted in this example.

You can use the visualizations in the software to confirm deletions and duplications.

- Use the post-corrected visualization to confirm exon deletions and duplications. For example, if a BRCA1 deletion (BRCA1DEL) or a BRCA2 deletion (BRCA2DEL) for a GeneCNV subtype is shown in the Call Details of the CNV results table, review the visualization to verify the deletion of the gene. Click Pre-corrected and Compare the SampleID (sid) amplicons to the BRCA1 genes.
- Use the pre-corrected view to confirm whole gene deletion or duplication results. If a BRCA1 deletion (**BRCA1DEL**) for a GeneCNV subtype is shown in the **Call Details** of the CNV variants table, review the visualization to verify the deletion of the gene. Click **Pre-corrected** and Compare the SampleID (sid) amplicons to the BRCA1 genes.

Note: Whole gene deletions and duplications can be confirmed only in the pre-corrected view.

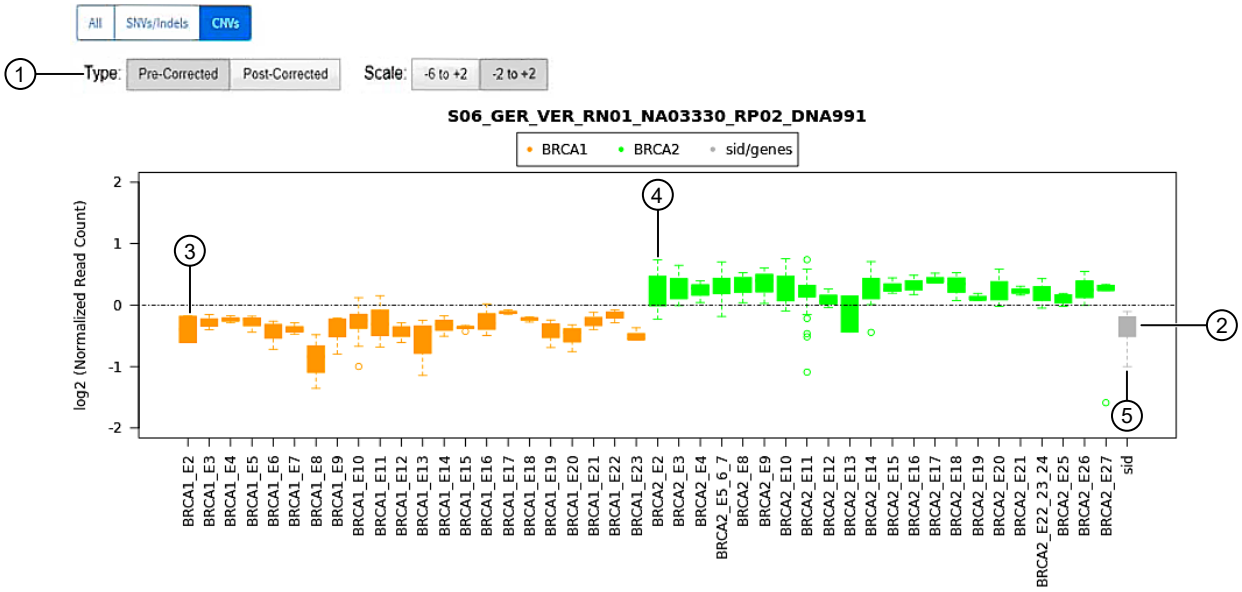


Figure 2 Verification of duplications with the Oncomine™ BRCA Assay GX.

① **Pre-corrected** selection.
② The location of the SampleID (sid) amplicons show information about BRCA1 and BRCA2 genes.
③ BRCA1 genes
④ BRCA2 genes
⑤ SampleID (sid) amplicons

In this example, a BRCA2 duplication can be verified because the sid amplicons are aligned with the BRCA1 genes in the visualization. Therefore the BRCA1 gene is normal, the sid amplicons are normal and the BRCA2 gene is a duplication.

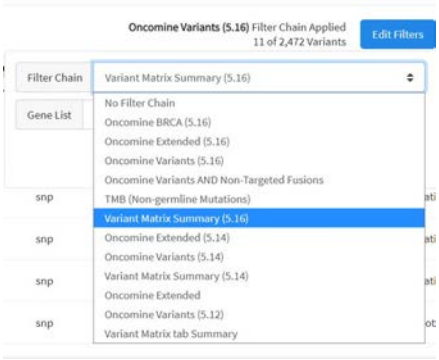
Search and filter variant results

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 6.8 User Guide* (Pub. No. MAN0026409) or the software help system.

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™ BRCA Assay GX results.

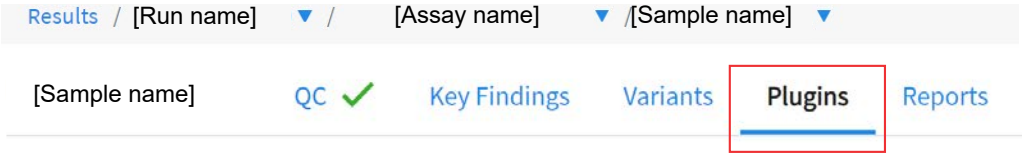
Note: System-installed filter chains are locked and cannot be changed.

System-installed filter chain	Description
Oncomine™ Variants (6.8)	This filter chain is recommended for use with the Oncomine™ BRCA - GX5 Germline - DNA) Assay. The filter chain reports only Oncomine™-annotated variants.
Oncomine Extended (6.8)	This filter chain is recommended for use with the Oncomine™ BRCA - GX5 Somatic - DNA) Assay. The filter chain reports all Oncomine™-annotated variants and variants that can be relevant to cancer due to their inclusion in one or more of the following classes: • 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 nonsynonymous and occur in exonic or splice-site regions. • Variants with ClinVar annotations of pathogenic or likely pathogenic.
Oncomine™ BRCA (6.8)	Filter chain to filter out non-BRCA1 and non-BRCA2 genes.
No Filter	Select this filter chain to remove a previously applied filter chain and view all called variants.

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. Microsoft™ Excel™-compatible reports are also generated, including differential expression tables. Additional details regarding read

coverage are also provided on a per-barcode basis, along with a list of gene annotations for each sequenced region.

You can download coverageAnalysis plugin output files from the **Results** screen for a sample. For more information, see "Results files" on page 142 and "Output files generated by the coverageAnalysis plugin" on page 161.

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 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 **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 sampleID plugin results in Genexus™ Software

Use the sampleID plugin to track samples and identify misassignment or possible mix up between samples and barcodes in a sequencing run. The sampleID plugin produces a unique identification code (**SampleID**) for each barcode.

The sampleID plugin is preconfigured and does not require input. For more information about the sampleID plugin, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

After the sequencing run completes, review the plugin results.

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 **sampleID** results is shown.
4. Click a barcode name to open the **Sample ID Report**.
5. To return to Genexus™ Software, click back in the browser.

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. For lists and descriptions of plugin output files, see "Output files generated by the coverageAnalysis plugin" on page 161 and "Output files generated by the sampleID plugin" on page 165.

To download the files, click **Results** ▸ **Sample Results**, then click a sample name in the **Sample Name** column. In the **Results** screen, click ··· **(More Options)** ▸ **Download Files**. In the **Download Files** dialog box, select the files to download, then click **Download**. The selected results files are downloaded in one ZIP folder.

Selection	File name	Description
Variants		
Filtered Variants (.vcf)	`<Sample_name>_<LibPrepID>_filtered.vcf`	Summary of filtered variant results in variant call format (VCF). The variants that are included in the VCF file depend on the filter chain that is applied to the sample results. For more information, see *Genexus™ Software Help*.
All Variants (.vcf)	`<Sample_name>_<LibPrepID>_<analysisID>.vcf`	Summary of variant results in variant call format (VCF).
All Variants (.tsv)	`<Sample_name>_<LibPrepID>_<analysisID>.tsv`	The file in TSV format contains the information that is shown in the variants table in tab-separated value format (TSV). The information that is included in the file is based on the gene list and filter chain that are applied. In addition to the information shown in the variants table, the file includes information for the columns that are available, but are not selected. The file also includes information that is listed in the Annotations table.
Variant Summary (.tsv)	`Summary.tsv`	File that lists SNV/INDEL and copy number results in tab-separated value format (TSV).
Snvs/Indels (.tsv)	`Snvindel.tsv`	File that lists SNV/INDEL variant results in tab-separated value format (TSV).
CNV (.tsv)	`Cnv.tsv`	File that lists copy number variant results in tab-separated value format (TSV).

(continued)

Selection	File name	Description
Pre-Corrected / -6 to +2 (.png)	`boxplot_before_renorm.png`	Visualization of pre-corrected BRCA plot. The portable network graphic (PNG) image shows amplicon coverage data normalized to total reads in a sample with scale values of -6 to +2.
Pre-Corrected / -2 to +2 (.png)	`boxplot_before_renorm_alt.png`	Visualization of pre-corrected BRCA plot. The portable network graphic (PNG) image shows amplicon coverage data normalized to total reads in a sample with scale values of -2 to +2.
Post-Corrected / -6 to +2 (.png)	`boxplot_after_vcib.png`	Visualization of post-corrected BRCA plot. The portable network graphic (PNG) image shows amplicon coverage data normalized to total reads in a sample with scale values of -6 to +2.
Post-Corrected / -2 to +2 (.png)	`boxplot_after_vcib_alt.png`	Visualization of post-corrected BRCA plot. The portable network graphic (PNG) image shows amplicon coverage data normalized to total reads in a sample with scale values of -2 to +2.
Reports		
Lab Report(s)	`<language>_<samplename>_AD <reporttemplatename>_<date>.pdf`	A PDF report that contains sample-specific results. For more information, see “Variant report” on page 115.
Sample Summary[1]	`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.
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.

(continued)

Selection	File name	Description
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.
Test Fragment Basecaller FASTAQ File (.fastq)	`rawtf.basecaller.fastq`	FASTQ file for the test fragment.
Coverage Analysis		
DNA Unmapped Bam File (.bam)	`<barcode>_rawlib.basecaller.bam`	Unmapped DNA barcode BAM file; output after mapping reads to reference.
DNA Coverage Statistics	`<DNA Barcode><ExpName>.stats.cov.txt`	A summary of the statistics presented in the tables at the top of the plugin report. The first line is the title. Each subsequent line is either blank or a particular statistic title followed by a colon (:) and its value.
DNA Chromosome base coverage summary	`<DNA Barcode><ExpName>.chr.cov.xls`	Base reads per chromosome summary data that is used to create the default view of the Reference Coverage Chart.
DNA Base depth of coverage	`<DNA Barcode><ExpName>.base.cov.xls`	Coverage summary data used to create the Depth of Coverage Chart.
DNA Amplicon coverage summary	`DNA Coverage Statistics <DNA Barcode><ExpName>.amplicon.cov.xls`	Coverage summary data used to create the Amplicon Coverage Chart.
DNA Coverage Analysis Summary (.pdf)	`DNA Coverage Statistics <DNA Barcode><ExpName>.summary.pdf`	A PDF file that contains the Coverage Analysis Report, including read statistics and charts that are generated by the coverageAnalysis plugin.
Audit and Log		
Analysis Log File	`analysis.log`	Analysis log file.
Run Audit	`PlannedRun-AuditTrail.pdf`	Contains all audit records pertaining to the run.
Troubleshooting Files		
Log Files[2]	`analysis.log`	Analysis log file.
	`summary.<timestamp>.log`	Start and end time for each time an assay module is executed for the analysis.

(continued)

Selection	File name	Description
Log Files[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.
	`PlannedRun-AuditTrail.pdf`	Contains the audit trail of the run plan in PDF format.
	`various.err` `various.out`	Analysis pipeline logs used by field service engineers for troubleshooting.
Other	`analysis.ini` `analysisSamples.json`	Analysis configuration files, including secondary and tertiary INI files.

VCF Files	`analysis.vcf`
	`SmallVariants.vcf`
	`SmallVariants.filtered.vcf`
		Summary of variant results in variant call format (VCF).
		Summary of all results for small variant in variant call format (VCF).
		Summary of filtered results for small variant in variant call format (VCF).

[1] Files are available for both custom and system installed assays in Genexus™ Software version 6.8.

[2] Separate folders are generated for each sample. If included in the run, separate folders are also generated for an NTC and positive control.

Sign off variant reports

Manager, administrator, and report-level users can electronically sign a variant report for sample results in Genexus™ Software. A sample result can have multiple variant reports only if each report uses a different report template, and a different language. However, only one of the reports can include electronic signatures.

IMPORTANT! If you use the same template and language to regenerate an existing report, the existing report will be overwritten.

Multiple signatures can be added to the variant report that is used for sign-offs. Account permissions for electronic signatures are associated with a signature type. A lock-level signature type locks the report. If your signature permissions are associated with a lock-level signature type and you sign the report, you will be the last person to sign the report before it is locked.

The filter chain that is applied to the results must be locked before you can electronically sign a variant report. After users begin to electronic sign-offs, changes cannot be made to the filter chain, gene list, or classification, and plugins cannot be run for the sample results.

The signature information appears in the variant report PDF file or a user-created report, if selected. For more information, see “Variant report” on page 115. For information about how to create a report template, see the software help system, or the *Genexus™ Software 6.8 User Guide*.

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.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, click the sample result of interest in the **Sample Name** column.
3. In the **Reports** tab, in a variant report pane that is labeled as **Draft**, click **Sign Off**.
   The **Sign Off Report** dialog box opens and shows the name of the report, the template used for the report, and the following information, which is based on the profile of the user who signs the report.
   - **Electronic Signature**
   - **User Name**
   - **Language**
4. In **Password**, enter the password.
5. In **Sign Off Comments**, enter a comment.
6. 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, or if text was entered during a previous sign off, the same footer information is shown in the **Sign Off Report** dialog box. You can edit the text in the **Footer Field** to change the information that will be shown in the footer of the PDF report.
7. Select an image to an include with the electronic signature, and enter a description and footnote for the image, if needed.
   Only one image can be added when a report is signed off. If an image was previously added, the option to add an image is not available.
8. Click **Sign Off** to confirm the electronic signature.
   The pane for the signed variant report is updated to include the signature information for the variant report. If the electronic signature that is associated with a lock-level signature type is used, a **(Lock)** is shown in the variant report pane and the report is locked.

The report is signed. The **Electronic Signatures** section of the report pane is updated to include your username and the signature type for your account. You can hover over the signature type to show the list of signature types that are completed for the report. If multiple electronic signatures are added to the report, an electronic signature type for each signer is shown in the **Electronic Signatures** column of the sample results table. For more information, see the software help system, or the *Genexus™ Software 6.8 User Guide*.

If a signature type used is designated to lock the report, the variant report is signed and locked, and no other variant reports for the sample result can be signed or generated. If you must change the report, reanalyze the sample and generate a report for the reanalyzed sample.

You can send notifications for report sign-offs to other users. For more information, see the software help system, or the *Genexus™ Software 6.8 User Guide*.

A Troubleshooting

Oncomine™ BRCA Assay GX

Observation	Possible cause	Recommended action
Sample QC failed **Details:** ✗ (Failed) appears in the **QC Status** column in the **Results / Sample Results** screen and the **QC** tab in the **Results** screen for a sample.	The concentration of a nucleic acid sample was overestimated, resulting in under-seeding of the target amplification reaction and low library yield.	Quantify nucleic acid samples using one of the recommended quantification methods listed in "Recommended materials for nucleic acid isolation, quantification, and quality control" on page 16. Do **NOT** use densitometry methods (such as NanoDrop™ spectrophotometer) for quantifying nucleic acid samples.

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.

Observation	Possible cause	Recommended action
A consumable is not recognized by the sequencer after loading on the deck *(continued)*	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.
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 "*Perform a Clean instrument procedure*" in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910). After cleaning, start a new run.
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 152.	Nucleic acid input may have been insufficient, and/or the nucleic acid was degraded.	For a sample run, re-quantify nucleic acid samples and/or perform sample QC to ensure that the expected nucleic acid input and size was loaded.
		If needed, re-isolate and purify nucleic acid samples.

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 152.	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 Chapter 3, "Before you begin".
	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.
	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 152.	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 Chapter 3, "Before you begin".
	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 defective or leaky.	Repeat the run with new chip and coupler. If low performance continues, contact Technical Support.

Observation	Possible cause	Recommended action
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 152. *(continued)*	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**). For more information, see "*Perform a Clean instrument procedure*" in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910). After the Clean instrument procedure, 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 decreased performance. For more information, see *Appendix A: Troubleshooting* in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. 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, 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 148.

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.

Observation	Possible cause	Recommended action
Batch sample import fails *(continued)*	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.
The assay I created does not appear in the menu when I plan a run	Forgot to lock your assay.	Go to **Assay tab ▸ Manage Assays** and make sure that the assay is locked.
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.
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

Guidelines for Oncomine™ BRCA Assay GX settings

In the Genexus™ Software, the system-installed assays are configured with settings that are optimized for each sample type (see "System-installed assays for use with the Oncomine™ BRCA Assay GX" on page 31). If needed, you can copy a system-installed assay, then modify settings as needed. For more information see, the software help system, or *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Representative metrics for a successful sequencing run

The following tables summarize metric values that are expected from successful sequencing runs.

IMPORTANT! The values provided are examples from good quality samples that you can use as guidelines to set QC threshold values. Set values based on your own internal data to represent the quality of samples that you typically test in the lab.

Table 21 Example metrics for the Oncomine™ BRCA - GX5 Germline - DNA using DNA isolated from blood tissue samples

QC metric	Value
Run QC	
Key Signal	60–87
Percent Loading	75–89%
Raw Read Accuracy	98–99%
Templating QC – CF-1 Control	
Average Reads Per Lane	12,000–36,000
Base Call Accuracy	97–99%

Table 21 Example metrics for the Oncomine BRCA - GX5 Germline - DNA using DNA isolated from blood tissue samples *(continued)*

QC metric	Value
Mean AQ20 Read Length (bp)	93–115
Sample QC – DNA	
MAPD	0.1–0.3
Mapped Reads	600,000–1,100,000
Mean AQ20 Read Length (bp)	86–98
Mean Read Length (bp)	108–138
Uniformity Of Base Coverage	98–100%
NTC QC – DNA	
Average Base Coverage Depth	1–46
MeanRead Length (bp)	40–53

Table 22 Example metrics for the Oncomine™ BRCA - GX5 Somatic - DNA using DNA isolated from tumor FFPE tissue samples

QC metric	Value
Run QC	
Key Signal	60–87
Percent Loading	75–89%
Raw Read Accuracy	98–99%
Templating QC – CF-1 Control	
Average Reads Per Lane	12,000–36,000
Base Call Accuracy	97–99%
Mean AQ20 Read Length (bp)	93–115
Sample QC – DNA	
MAPD	0.1–0.4
Mapped Reads	300,000–1,400,000
Mean AQ20 Read Length (bp)	80–95
Mean Read Length (bp)	92–126
Uniformity Of Base Coverage	85–100%

Table 22 Example metrics for the Oncomine BRCA - GX5 Somatic - DNA using DNA isolated from tumor FFPE tissue samples *(continued)*

QC metric	Value
NTC QC – DNA	
Average Base Coverage Depth	4–37
MeanRead Length (bp)	49–67

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 Instrument, 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
```

① Example file if in a plain text editor.

	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

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 extra 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.

Quantify nucleic acid samples

Isolate nucleic acid samples using one of the verified procedures and kits that are listed in “Recommended materials for nucleic acid isolation, quantification, and quality control” on page 16.

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 transferring into a sample input plate..

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)
	Qubit™ dsDNA BR Assay Kit	*Qubit™ dsDNA BR Kits User Guide* (Pub. No. MAN0002325)

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 6.8 Help* or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

coverageAnalysis plugin in Genexus™ Software

Use the coverageAnalysis plugin to view statistics and graphs that describe the level of sequence coverage produced for targeted genomic regions. The results for a run analyzed with the plugin vary based on the library type that you select when you configure the plugin. You can export some

charts as graphics, such as the Amplicon Coverage Chart and the Reference Coverage Chart. The coverageAnalysis plugin runs by default with the Oncomine™ BRCA Assay GX.

Reads statistics

The library type determines which statistics are presented. The following tables describe the statistics that are generated by the coverageAnalysis plugin. The statistics that are displayed in your report depend on the type of library that is used in your sequencing experiment. Definitions are in tooltips. Almost every statistic, plot, link, and functional widget in the report provides tooltips with definitions. Place the pointer over a heading or description in the report to view the tooltip.

Table 23 General statistics

Statistic	Description
Number of mapped reads	The total number of reads mapped to the reference genome.
Percent reads on target	The percentage of filtered reads mapped to any targeted region relative to all reads mapped to the reference. If no target regions file is specified, this value will be the percentage of reads passing uniquely mapped and/or nonduplicate filters, or 100% if no filters were specified. A read is considered on target if at least one aligned base overlaps at least one target region. A read that overlaps a targeted region but where only flanking sequence is aligned, for example, due to poor matching of 5' bases of the read, is not counted.

Amplicon read coverage statistics

The following statistics describe the reads that are assigned to specific amplicons. Each sequence read is assigned to exactly one of the amplicons specified by the targets file. If a read spans multiple amplicon targets, the target region that the reads covers most is assigned. In the event of a tie, the target that is the closest to the 3' end is assigned.

Statistic	Description
Number of amplicons	The number of amplicons that is specified in the target regions file.
Percent assigned amplicon reads	The percentage of reads that were assigned to individual amplicons relative to all reads mapped to the reference. A read is assigned to a particular (inner) amplicon region if any aligned bases overlap that region. If a read might be associated with multiple amplicons, it is assigned to the amplicon region that has the greatest overlap of aligned sequence.
Average reads per amplicon	The average number of reads assigned to amplicons.
Uniformity of amplicon coverage	The percentage of amplicons that had at least 20% of the average number of reads per amplicon. Cumulative coverage is linearly interpolated between nearest integer read depth counts.
Amplicons with at least *N* reads	The percentage of all amplicons that had at least *N* reads.

(continued)

Statistic	Description
Amplicons with no strand bias	The percentage of all amplicons that did not show a bias towards forward or reverse strand read alignments. An individual amplicon has read bias if it has ≥10 reads and the percentage of forward or reverse reads to total reads is greater than 70%. Amplicons with <10 reads are considered to have no strand bias.
Amplicons reading end-to-end	The percentage of all amplicons that were considered to have a sufficient proportion of assigned reads (70%) that covered the whole amplicon target from 'end-to-end'. To allow for error, the effective ends of the amplicon region for read alignment are within 2 bases of the actual ends of the region.
Amplicon base composition bias	A number that represents the proportion of amplicons showing low representation (<0.2x mean reads) in the lower and/or upper quartiles of amplicons ordered by increasing G/C base pair content of their insert sequences. The value is relative to that in the center 50th percentile of amplicons and weighted by the standard deviation of representation over all amplicons. An RMS (root mean square) value is used so that a bias greater in either upper or lower quartiles produces a larger value than a mean bias seen more equally in both outer quartiles. The value is 0 if the uniformity of amplicon coverage metric is 100%, however, the value is not necessarily high at lower amplicon uniformity.

Target base coverage statistics

The following statistics describe the targeted base reads of the reference. A base covered by multiple target regions is counted only once per sequencing read.

Statistic	Description
Bases in target regions	The total number of bases in all specified target regions of the reference.
Percent base reads on target	The percent of all bases covered by reads aligned to the reference that covered bases in target regions. Clipped bases, deletions, and insertions (relative to the reference) are not included in this percentage. If no specific target regions were specified, the whole genome is the targeted regions.
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) that is covered by at least 20% of the average base coverage depth reads. Cumulative coverage is linearly interpolated between nearest integer base read depths.
Target base coverage at *N*x	The percentage of target bases covered by at least *N* reads.

(continued)

Statistic	Description
Target bases with no strand bias	The percentage of all target bases that did not show a bias toward forward or reverse strand read alignments. An individual target base is considered to have read bias if it has ≥10 reads and the percentage of forward or reverse reads to total reads is greater than 70%. Target bases with <10 reads are considered to have no strand bias.
Percent end-to-end reads	The percentage of on-target reads that fully cover their assigned amplicon (insert) from 'end-to-end'. To allow for error, the effective ends of the amplicon region for read alignment are within 2 bases of the actual ends of the region.

Example charts generated by the coverageAnalysis plugin

The charts that are generated by the coverageAnalysis plugin include **Plot**, **Overlay**, or **Display** menus that allow you to customize the data that is displayed in each chart.

Click **(Search)** (in the top right corner of a chart) to open the chart **Viewing Options** panel, where you can further customize a chart. Click **(Help)** to open a description of the chart.

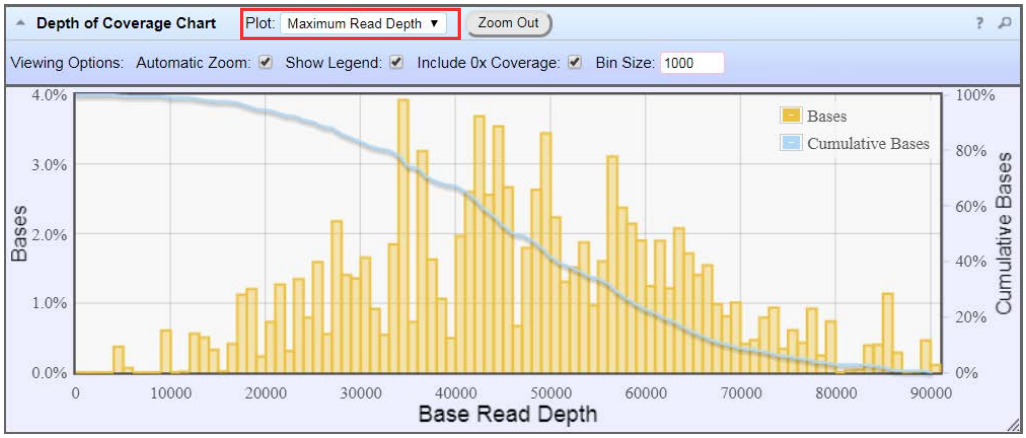


Figure 3 Representative Depth of Coverage Chart

The Depth of Coverage Chart shows the distribution of targeted base coverage. The X-axis represents the base read depth. The left Y-axis represents the number of reads at a given base read depth or a range (bin) of base read depths, as a percentage of the total number of base reads. The right Y-axis represents the cumulative count of the number of reads at a given read depth or greater, as a percentage of the total number of reads. The individual orange bars represent the percentage of reads in the specific range of base read depths. The blue curve measures the cumulative reads at a given base read depth or greater. If your analysis includes a region of interest file, this chart reflects only target regions (reads that fall within a region of interest). Use the **Plot** dropdown list to switch between **Maximum Read Depth**, **99.9% of All Reads**, and **Normalized Coverage** plots.

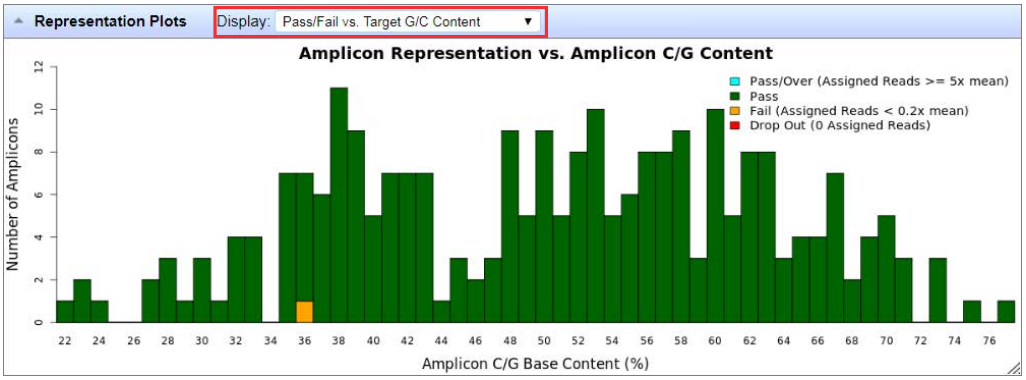


Figure 4 Representation Plots

Use the **Display** dropdown list to switch between **Pass/Fail vs. Target G/C Content**, **Pass/Fail vs. Target Length**, **Representation vs. Target G/C Content**, **Amplicon Coverage Chart**, **Mean Target Reads vs Pool**, **Reference Coverage Chart**, and **Representation vs. Target Length** plots. This figure shows an example **Pass/Fail vs. Target G/C Content** plot.

Output files generated by the coverageAnalysis plugin

You can download coverageAnalysis plugin results files from the **Results** screen for a sample. For more information, see "Results files" on page 142.

Note: Sometimes the downloaded file name can be too long to open a file in applications such as Microsoft™ Excel™. To resolve this problem, right-click the file, click **Save As**, then rename the downloaded file with a shorter name.

The following tables describe the files that can be generated by the coverageAnalysis plugin. The list of files depends on the type of assay template that was selected during assay creation.

Table 24 File download selections in the Genexus™ Software

Selection	File Name
DNA Coverage Statistics	`<DNA Barcode><ExpName>.stats.cov.txt`
DNA Chromosome base coverage summary	`<DNA Barcode><ExpName>.chr.cov.xls`
DNA Base depth of coverage	`<DNA Barcode><ExpName>.base.cov.xls`
DNA Amplicon coverage summary	`<DNA Barcode><ExpName>.amplicon.cov.xls`
DNA Coverage Analysis Summary (.pdf)	`<DNA Barcode><ExpName>.summary.pdf`
RNA Coverage Statistics	`<RNA Barcode><ExpName>.stats.cov.txt`
RNA Amplicon coverage summary	`<RNA Barcode><ExpName>.amplicon.cov.xls`
RNA Coverage Analysis Summary (.pdf)	`<RNA Barcode><ExpName>.summary.pdf`
TNA Coverage Statistics	`<TNA Barcode><ExpName>.stats.cov.txt`
TNA Chromosome base coverage summary	`<TNA Barcode><ExpName>.chr.cov.xls`
TNA Base depth of coverage	`<TNA Barcode><ExpName>.base.cov.xls`

Table 24 File download selections in the Genexus Software *(continued)*

Selection	File Name
TNA Amplicon coverage summary	`<TNA Barcode><ExpName>.amplicon.cov.xls`
TNA Coverage Analysis Summary (.pdf)	`<TNA Barcode><ExpName>.summary.pdf`

Table 25 File contents

File	Description
Coverage Statistics	A summary of the statistics presented in the tables at the top of the plugin report. The first line is the title. Each subsequent line is either blank or a particular statistic title followed by a colon (:) and its value.
Chromosome base coverage summary	Base reads per chromosome summary data that is used to create the default view of the Reference Coverage Chart. This file contains the following fields. • `chrom`: the name of the chromosome or contig of the reference. • `start`: the coordinate of the first base in this chromosome. This is always 1. • `end`: the coordinate of the last base of this chromosome. Also its length in bases. • `fwd_basereads`: the total number of forward strand base reads for the chromosome. • `rev_basereads`: the total number reverse strand base reads for the chromosome. • `fwd_trg_basereads` (if present): the total number of forward strand base reads that mapped over at least one target region. • `rev_trg_basereads` (if present): the total number of reverse strand base reads that mapped over at least one target region. • `total_reads`: the total number of sequencing reads that are mapped to individual contigs.
Base depth of coverage	Coverage summary data used to create the Depth of Coverage Chart. This file contains the following fields. • `read_depth`: the depth at which a (targeted) reference base has been read. • `base_cov`: the number of times any base was read (covered) at this depth. **Note:** Lines (read depths) for which `base_cov` is 0 are omitted to avoid excessively large files being produced in specific situations. • `base_cum_cov`: the cumulative number of reads (coverage) at this read depth or greater. • `norm_read_depth`: the normalized read depth (depth divided by average base read depth). • `pc_base_cum_cov`: same as base_cum_cov but represented as a percentage of the total base reads.

Table 25 File contents *(continued)*

File	Description
Amplicon coverage summary	Coverage summary data used to create the Amplicon Coverage Chart. This file contains the following fields: • `contig_id`: the name of the chromosome or contig of the reference for this amplicon. • `contig_srt`: the start location of the amplicon target region. **Note:** This coordinate is 1-based, unlike the corresponding 0-based coordinate in the original targets BED file. • `contig_end`: the last base coordinate of this amplicon target region. **Note:** The length of the amplicon target is given as tlen = (contig_end - contig_srt + 1). • `region_id`: the ID for this amplicon as given as the fourth column of the targets BED file. • `gene_id` or `attributes`: the gene symbol or attributes field as provided in the targets BED file. • `gc_count`: the number of G and C bases in the target region. The %GC that is uses this count divided by the amplicon (insert) length. • `overlaps`: the number of times this target was overlapped by any read by at least one base. **Note:** Individual reads might overlap multiple amplicons where the amplicon regions themselves overlap. • `fwd_e2e`: the number of assigned forward strand reads that read from one end of the amplicon region to the other end. • `rev_e2e`: the number of assigned reverse strand reads that read from one end of the amplicon region to the other end. • `total_reads`: the total number of reads assigned to this amplicon. This value is the sum of fwd_reads and rev_reads and is the field that rows of this file are ordered by (then by contig id, srt, and end). • `fwd_reads`: the number of forward strand reads that are assigned to this amplicon. • `rev_reads`: the number of reverse strand reads that are assigned to this amplicon. • `covNx`: the number of bases of the amplicon target that had at least N reads. There are 3 such columns for the specified coverage tiers, which by default are `cov100x`, `cov350x`, and `cov500x`.
Coverage Analysis Summary (.pdf)	A PDF file that contains the Coverage Analysis Report, including read statistics and charts that are generated by the coverageAnalysis plugin.

sampleID plugin

The sampleID plugin produces an identification code (**SampleID**) for each barcode in a sample.

The sampleID plugin runs by default with the Oncomine™ BRCA Assay GX, which is a human SNP genotyping panel, to help ensure that the accuracy of samples increase confidence in sample data management. The sample ID identification codes produced are composed of the identified human

sample gender and IUPAC base letters for eight high-frequency noncoding SNPs. Ion AmpliSeq™ Sample ID Panel contains nine primer pairs.

For the samples to work with this plugin, the Ion AmpliSeq™ library must have been prepared with Ion AmpliSeq™ sample tracking amplicons. SampleID amplicons are part of the panel.

The sampleID plugin is preconfigured and does not require input.

Sample ID Report

Statistic	Description
Number of mapped reads	The total number of reads mapped to the reference genome.
Number of reads in sample ID regions	The total number of reads mapped to Sample ID regions.
Percent reads in sample ID regions	The percent of all reads aligned to the reference that covered bases in Sample ID regions.
Total base reads in sample ID regions	The total base reads aligned to the reference that covered bases in Sample ID regions.
Percent base reads in sample ID regions	The percent of all bases covered by reads aligned to the reference that covered bases in Sample ID regions.
Male sample ID region reads	Number of reads mapped to the specific male (Y) identification target(s).
Female sample ID region reads	Number of reads mapped to the specific female (X) identification target(s).
Sample ID Regions and Sample ID SNPs	
Bases in target regions	The total number of bases in all specified target regions of the reference.
Average base coverage depth	The average number of DNA reads of all targeted reference bases.
Uniformity of coverage	The percentage of target bases covered by at least 0.2x of the average base coverage depth.
Coverage at 1x	The percentage of target bases covered by at least 1 read.
Coverage at 20x	The percentage of target bases covered by at least 20 reads.
Coverage at 100x	The percentage of target bases covered by at least 100 reads.

Output files generated by the sampleID plugin

You can download sampleID plugin results files from the **Results** screen for a sample. For more information, see “Results files” on page 142.

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.

Table 26 describes the files that can be generated by the sampleID plugin. The list of files depends on the application type that was selected during assay creation.

Table 26 File download selections in the Genexus™ Software

Selection	File Name
Tracking Loci Regions (SNPs) File	`tracking_loci.bed`
Aligned Tracking Reads Index (BAI) File	`<DNA Barcode><ExpName>.bam.bai`
Hard-copy A4 PDF Image Of This Report Page	`<DNA Barcode><ExpName>.sampleID.pdf`
Tracking Target Regions File	`tracking_regions.bed`
Aligned Tracking Reads (BAM) File	`<DNA Barcode><ExpName>.bam`
All Variant Calls As a Table File	`allele_counts.xls`

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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
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

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.

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™ Software 6.8 User Guide	MAN0026409
Genexus™ Software 6.8 Help	MAN0026408 (available in the software)
Qubit™ dsDNA HS Assay Kits User Guide	MAN0002326
Qubit™ dsDNA BR Kits User Guide	MAN0002325

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/or its affiliate(s) 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 any questions, please contact Life Technologies at **www.thermofisher.com/support**.