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Oncomine™ Comprehensive Assay v3 GX

USER GUIDE

For use with the Genexus™ Integrated Sequencer

Catalog Numbers A46296, A46294, A46295

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Revision E

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: MAN0018512 E (English)

Revision	Date	Description
E	29 April 2025	• Updated system-installed assays. See "System-installed assays for use with the Oncomine™ Comprehensive Assay v3 GX" on page 30. – Oncomine™ Comprehensive v3 - GX5 - DNA – w6.0.2 – Oncomine™ Comprehensive v3 - GX5 - Fusions – w6.0.2 – Oncomine™ Comprehensive v3 - GX5 - DNA and Fusions – w6.0.2 • Added information for variant annotation. See "Requirements for Oncomine™ Comprehensive Assay v3 GX variant annotation" on page 123. • Updated information about class-based variants. See "Ion Torrent™ Class-Based Variants" on page 126. • Added information about the coverageAnalysis plugin. See "Review coverageAnalysis plugin results" on page 92. • Added an appendix with ADF release note information. See Appendix C, "Release Notes".
D	22 July 2024	• Corrected lane usage information for GX5™ Chip for DNA and RNA (3 samples). See "Chip capacity by sample type" on page 36. • Added information about class-based variants. See "Ion Torrent™ Class-Based Variants" on page 126. • Updated system-installed assays. See "System-installed assays for use with the Oncomine™ Comprehensive Assay v3 GX" on page 30. – Oncomine™ Comprehensive v3 - GX5 - DNA – w5.0.3 - EVAL – Oncomine™ Comprehensive v3 - GX5 - Fusions – w5.0.3 - EVAL – Oncomine™ Comprehensive v3 - GX5 - DNA and Fusions – w5.0.3 - EVAL
C.0	15 May 2023	• Support added for performing sample to result runs in Genexus™ Software. • Support added for purification of nucleic acids from FFPE using the Genexus™ Purification Instrument. • Added support for updated Genexus™ Software workflows – Oncomine™ Comprehensive v3 - GX5 - DNA – w5.0.2 – Oncomine™ Comprehensive v3 - GX5 - Fusions – w5.0.2 – Oncomine™ Comprehensive v3 - GX5 - DNA and Fusions – w5.0.2

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

Contents

1 Product information

Product description

The Ion Torrent™ Oncomine™ Comprehensive Assay v3 GX (Cat. No. A46296) is a targeted, next-generation sequencing (NGS) assay that enables the detection of single-nucleotide variants (SNVs), insertions and deletions (INDELs), copy number variations (CNVs), and gene fusions from 161 unique genes. The assay is optimized for use with formalin-fixed paraffin embedded (FFPE) samples.

The assay kit provides 4 pools of multiplex PCR primers (2 pools for the DNA assay and 2 pools for the RNA assay) and library reagents sufficient to perform up to sixteen 2-pool or eight 4-pool reactions on the Ion Torrent™ Genexus™ Integrated Sequencer System. The assay is optimized for use with the Ion Torrent™ Genexus™ GX5™ Chip. Additional Genexus™ Integrated Sequencer reagents and supplies must be purchased separately (see "Required materials not supplied for use with the Genexus™ Integrated Sequencer" on page 15).

This user guide provides the following instructions.

- How to use the Oncomine™ Comprehensive Assay v3 GX to perform a **Sample to Result** next-generation sequencing run on the Ion Torrent™ Genexus™ Purification Instrument for nucleic acid isolation, followed by sequencing on the Genexus™ Integrated Sequencer.
- How to use the Oncomine™ Comprehensive Assay v3 GX to perform a **Nucleic Acid to Result** next-generation sequencing run on the Genexus™ Integrated Sequencer.
- How to perform data analysis of the sequencing run.

The Oncomine™ Comprehensive Assay v3 GX includes the following features.

- Analysis of variants across 161 genes
- Automated **Sample to Result** workflow on the Genexus™ Integrated Sequencer in less than 1 day
- Detection of SNVs and INDELs, CNVs, and gene fusions
- Robust performance from as little as 10 ng of nucleic acid input per pool (20 ng of DNA and 20 ng of RNA) isolated from FFPE samples, including fine needle biopsies
- Molecular standards and controls
- Content driven by the Oncomine™ Knowledgebase
- Ion AmpliSeq™ chemistry

- System-installed assays preconfigured with settings that are optimized for specific application and sample type
- Optimized Oncomine™ informatics

The Genexus™ Purification System automates the extraction and quantification of nucleic acids from various tissue types for use on the Genexus™ Integrated Sequencer. With a single touch point and 10 minutes of hands-on time, the Genexus™ Purification System can extract both DNA and RNA sequentially from FFPE lysates (Ion Torrent™ Genexus™ FFPE DNA and RNA Purification Kit). When connected to the Genexus™ Integrated Sequencer, the Ion Torrent™ Genexus™ Software streamlines the NGS workflow by integrating the **Sample to Result** workflow within a single software system.

Contents and storage

The Oncomine™ Comprehensive Assay v3 GX (Cat. No. A46296) includes the 2-pool DNA panel, the 2-pool RNA panel, Genexus™ Strip 1, and Genexus™ Strip 2-AS. The Oncomine™ Comprehensive Assay v3 GX DNA only (Cat. No. A46294) includes the 2-pool DNA panel, Genexus™ Strip 1, and Genexus™ Strip 2-AS. The Oncomine™ Comprehensive Assay v3 GX RNA only (Cat. No. A46295) includes the 2-pool RNA panel, Genexus™ Strip 1, and Genexus™ Strip 2-AS.

Table 1 Oncomine™ Comprehensive Assay v3 GX

Contents	Carrier color	Number of reactions	Part No.	Storage
Oncomine™ Comprehensive Assay v3 GX pools[1]				
OCAv3 DNA Pool 1 (position 1, white cap)	Magenta	16 (4 reactions/tube x 4 tubes)	A40281	–30°C to –10°C
OCAv3 DNA Pool 2 (position 1, white cap)	Pale green	16 (4 reactions/tube x 4 tubes)		
OCAv3 RNA Pool 1 (position 1, white cap)	Pale orange	16 (4 reactions/tube x 4 tubes)	A44351	
OCAv3 RNA Pool 2 (position 1, white cap)	Blue	16 (4 reactions/tube x 4 tubes)		
Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252)[2,3]				
Genexus™ Strip 1	Light red	2 × 32 (4 single-pool reactions/strip)[4]	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	2 × 32 (4 single-pool reactions/strip)[4]	A46813	–30°C to –10°C

[1] Each primer pool in the panel is provided in 4 pairs of tubes, where each tube pair contains one tube with primers in position 1 and one empty uncapped tube in position 2.
[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.
[3] Can be ordered separately.
[4] Oncomine™ Comprehensive Assay v3 GX limits the reaction to 4 reactions per strip.

The maximum number of samples that can be analyzed per kit depends on the type of run that you want to perform.

Workflow	# of samples
Simultaneous DNA- and RNA-based variant analysis	16 FFPE tissue samples—One DNA reaction and one RNA reaction for each sample.
DNA-only or RNA-only variant analysis	32 FFPE tissue samples—16 DNA only and 16 RNA only samples

Prerequisites

Category	Prerequisites
Instrument	• *(Optional)* Access to the Genexus™ Purification Instrument • Access to the Genexus™ Integrated Sequencer • Stock of reagents and supplies that are compatible with the Oncomine™ Comprehensive Assay v3 GX and the Genexus™ Integrated Sequencer For ordering information, see “Required materials not supplied for use with the Genexus™ Integrated Sequencer” on page 15.
Software	• Genexus™ Software 6.8.2 or later
Functional knowledge and understanding	• Key steps in a next-generation sequencing (NGS) workflow • Main functions of the Genexus™ Integrated Sequencer • Main features of the Genexus™ Software 6.8.2 or later

Workflow overview

The following workflow outlines the key steps in performing the Oncomine™ Comprehensive Assay v3 GX run, from nucleic acid purification to sequencing and data analysis.

Plan a run in Genexus™ Software

Select an assay (page 30)

System-installed assays that are specifically configured for each sample type are available in the Genexus™ Software. You can use the system-installed assays in the run plan without change. To modify any assay settings, copy the system-installed assay that best represents the experiment, then edit the assay settings.

Enter samples (page 31)

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

Plan a run (page 29)

Run plans created in the Genexus™ Software contain all of the settings that are used in sample purification, library preparation, templating, sequencing, and analysis, including sample information and plate location, assays, and barcodes.

Prepare samples (page 38)

Note: If you are starting with purified nucleic acid samples, proceed to the next step.

Dilute the samples and load the sample plate (page 56)

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

Quantify and dilute the nucleic acid samples, then load the sample plate.

(Optional) Add controls to the sample plate.

Plan a run in Genexus™ Software

Load the sequencer and start a run (page 59)

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

Review data and results in the Genexus™ Software (page 67)

View variant calls and visualize results in Genexus™ Software.

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.

Guidelines for the usage of reagents and supplies, and sample capacity

Table 2 provides guidelines for the usage of reagents and supplies, and sample capacity for each sample-to-result Oncomine™ Comprehensive Assay v3 GX workflow. Table 2 provides guidelines for the amount of each kit required based on the number of samples and the type of workflow that you are planning to run.

Table 2 Guidelines for the amount of reagents, supplies required for each workflow type

Assay component	Workflow			
	DNA-only	RNA-only		DNA + RNA
	7 samples per run[1]	8 samples per run	16 samples per run	6 samples per run
Oncomine™ Comprehensive Assay v3 GX (Cat. No. A46296)	**½ kit**	**½ kit**	**1 kit**	**½ kit**
OCAv3 DNA Pool 1	2 tubes	—	—	2 tubes
OCAv3 DNA Pool 2	2 tubes	—	—	2 tubes
OCAv3 RNA Pool 1	—	2 tubes	4 tubes	2 tubes
OCAv3 RNA Pool 2	—	2 tubes	4 tubes	2 tubes
Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252)	**¼ kit**	**¼ kit**	**¼ kit**	**½ kit**
Genexus™ Strip 1 (Part No. A46812)	4 strips	4 strips	4 strips	8 strips
Genexus™ Strip 2-AS (Part No. A46813)	4 strips	4 strips	4 strips	8 strips
Genexus™ Templating Strips 3-GX5™ and 4 (Cat. No. A40263)	**½ kit**	**⅛ kit**	**¼ kit**	**½ kit**
Genexus™ Strip 3-GX5™ (Part No. A46815)	4 strips	1 strip	2 strips	4 strips
Genexus™ Strip 4 (Part No. A46816)	4 strips	1 strip	2 strips	4 strips
Genexus™ Barcodes AS (Cat. No. A40257, A40258, A40259, or A40260)	**14 barcodes[2]**	**16 barcodes**	**32 barcodes**	**24 barcodes**
GX5™ Chip and Genexus™ Coupler (Cat. No. A40269)	**½ set**	**⅛ set**	**¼ set**	**½ set**

Table 2 Guidelines for the amount of reagents, supplies required for each workflow type *(continued)*

Assay component	Workflow			
	DNA-only	RNA-only		DNA + RNA
	7 samples per run[1]	8 samples per run	16 samples per run	6 samples per run
GX5™ Chip (Part No. 100081364)	1 chip (4 lanes)	¼ chip (1 lane)	½ chip (2 lanes)	1 chip (4 lanes)
Genexus™ Sequencing Kit (Cat. No. A40271)	**½ kit**	**⅛ kit**	**¼ kit**	**½ kit**
Genexus™ Pipette Tips (Cat. No. A40266)	**⅓ pack**	**¼ pack**	**½ pack**	**½ pack**
Genexus™ Pipette Tips (Cat. No. A40266)	4 racks	3 racks	6 racks	6 racks

[1] In addition to 7 samples, you can also include a NTC.
[2] If you are running a NTC, the NTC uses 2 barcodes, for a total of 16 barcodes.

IMPORTANT! The information provided in Table 2 is for estimation purposes only. Only full-sized kits are available for purchase. For ordering information, see "Contents and storage" on page 8 and "Required materials not supplied for use with the Genexus™ Integrated Sequencer" on page 15.

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

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

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

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

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

Genexus™ Integrated Sequencer

Components	Cat. No.
Genexus™ Integrated Sequencer	A45727

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**

(continued)

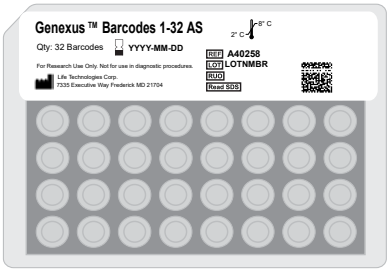
Item	Source
Vortex mixer with a rubber platform	MLS
Gloves, powder-free nitrile	MLS
Ice buckets and ice	—
Nuclease-free water, molecular biology grade	MLS
Isopropyl alcohol, 70% solution	MLS
Wipes, disposable lint-free	MLS
(Optional) Uninterruptible Power Supply (UPS)[2]	MLS

[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.

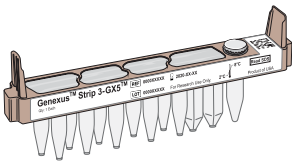
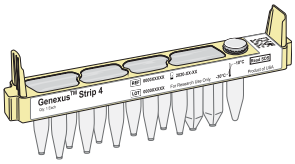
Genexus™ Barcodes AS

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	

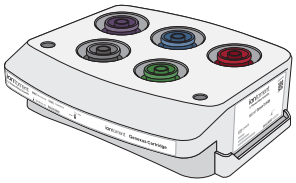
Genexus™ Templating Strips 3-GX5™ and 4

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™ 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)	

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™ Comprehensive Assay v3 GX, see “Guidelines for the usage of reagents and supplies, and sample capacity” on page 13 to determine the number of racks required for your experiment.

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

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.

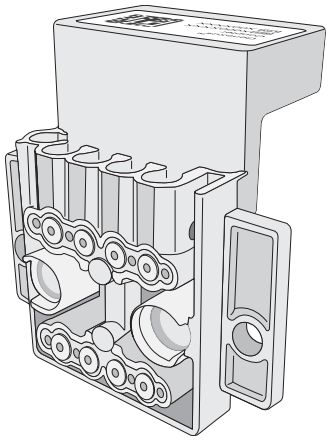
Note: The Genexus™ Control Library-AS is barcoded with IonCode™ 0101.

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	

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™ Comprehensive Assay v3 GX, see “Guidelines for the usage of reagents and supplies, and sample capacity” on page 13 to determine the number of chips required for your experiment.

Each chip can accommodate up to 6 DNA and RNA samples, or 6 samples and one no-template control.

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

Genexus™ Conical Bottles

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

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

Recommended materials for use with the Oncomine™ Comprehensive Assay v3 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 not supplied for use with the Genexus™ Purification System

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

Item	Source
Equipment	
Bench top microcentrifuge	• Cole-Parmer EW-17414-06 • Eppendorf 022620304
1,000 µL Multichannel Pipette	**MLS**
For use with Genexus™ FFPE DNA and RNA Purification Kit	
Sorvall™ ST 8 Small Benchtop Centrifuge (or equivalent)[1], with Thermo Scientific™ M10 Microplate Swinging Bucket Rotor (or equivalent)[2], and Sealed Bucket; Capacity: 4 Standard or 2 Midi-Deepwell plates (Set of 2) (or equivalent)	75007200 75005706 75005721
Economy Lab Incubator (2, 60°C and 90°C)	S50441A **fisherscientific.com**
Heating block (2, 60°C and 90°C)	**MLS**
Precision™ General Purpose Water Bath (or equivalent)	**MLS**
Equipment and consumables for AutoLys M FFPE sample extraction[3]	
AutoLys M Tubes and Caps kit	A38738
AutoLys M Tube Rack	A37955
AutoLys M Tube Locking Lid	A37954
AutoLys M TubeLifter or AutoLys M Tube Pliers	A37956 A38261
Tubes, plates, and other consumables	
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	4483354, 4483352

(continued)

Item	Source
Adhesive PCR Plate Foils	AB0626
RNA*later*™ Stabilization Solution	AM7020
RNase*Zap*™ RNase Decontamination Solution	AM9780
CitriSolv™ Clearing Agent	22-143-975
Xylene	**MLS**
Ethanol, 100%	**MLS**

[1] Centrifuge must achieve an RCF of 2000 × *g*, have a swinging bucket rotor and accommodate deepwell plates.
[2] Swinging bucket rotor must carry deepwell plates in the landscape orientation (see page 42).
[3] For use with the Genexus™ FFPE DNA and RNA Purification Kit.

Recommended sample controls

Item	Source
AcroMetrix™ Oncology Hotspot Control	969056
Seraseq™ Tri-Level Tumor Mutation DNA Mix v2 HC	0710-0097 (SeraCare)
Seraseq™ Lung & Brain CNV Mix, + 6 copies	0710-0415 (SeraCare)
Seraseq™ Breast CNV Mix, + 6 copies	0710-0412 (SeraCare)
Seraseq™ Fusion RNA Mix v3	0710-0431 (SeraCare)
Seraseq™ Fusion RNA Mix v4	0710-0497 (SeraCare)
Horizon™ ALK RET ROS RNA fusion	HD784 (Horizon)

Recommended materials for nucleic acid isolation and quantification

Item	Source
Nucleic acid isolation—One of the following kits:	
MagMAX™ FFPE DNA/RNA Ultra Kit	A31881
RecoverAll™ Multi-Sample RNA/DNA Workflow	A26069, A26135

(continued)

Item	Source
Nucleic acid quantification—One of the following kits:	
Qubit™ 4 Fluorometer[1] and one or more of the following kits: • Qubit™ dsDNA HS Assay Kit *(DNA samples)* • Qubit™ RNA HS Assay Kit *(RNA samples)*	Q33238 Q32854, Q32851 Q32852, Q32855
TaqMan™ RNase P Detection Reagents Kit	4316831
Nucleic acid quality control	
Agilent™ Bioanalyzer™ Instrument and one or more of the following kits: • Agilent™ High Sensitivity DNA Kit *(DNA samples)* • Agilent™ RNA 6000 Pico Kit *(RNA samples)*	G2939BA 5067-4626 50671513 (Agilent)

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

3 Before you begin

Procedural guidelines

- Use only the reagents and supplies that have been verified for the Oncomine™ Comprehensive Assay v3 GX. For a list of verified reagents and supplies, see "Required materials not supplied for use with the Genexus™ Integrated Sequencer" on page 15 and "Recommended materials for use with the Oncomine™ Comprehensive Assay v3 GX" on page 20.
- Use good laboratory practices to minimize cross-contamination of products. Keep all tubes sealed until immediately before loading onto the Genexus™ Integrated Sequencer.
- Minimize freeze-thaw cycles of the OCAv3 DNA Pool 1, OCAv3 DNA Pool 2, OCAv3 RNA Pool 1, and OCAv3 RNA 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 (OCAv3 DNA Pool 1, OCAv3 DNA Pool 2, OCAv3 RNA Pool 1, and OCAv3 RNA Pool 2) is sufficient for preparing up to 4 libraries.

- Do **NOT** store the OCAv3 DNA Pool 1, OCAv3 DNA Pool 2, OCAv3 RNA Pool 1, OCAv3 RNA 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 laboratory coat that is reserved for sequencing work and is laundered frequently. If possible, change to a fresh laboratory coat before setting up a run, or use new sleeve covers.
- Wear fresh gloves to load the instruments, including during the loading of the sequencing chip and coupler. Do not remove gloves to install the sequencing chip.
- Change gloves between dispensing positive controls and no-template control (NTC) in sample plate wells.

Equipment and instrument cleaning

- If you are preparing samples and sample plates in a hood (recommended), illuminate the hood with UV light for 15 minutes before use.
- Before preparing for a sequencing run, decontaminate surfaces of the hood or bench where samples or sample plates are handled, and other equipment such as vortexers, microcentrifuges, and pipettors. Use DNA*Zap*™ and either the RNase*Zap*™ RNase Decontamination Solution or the RNase *AWAY*™ Surface Decontaminant reagent. Follow manufacturer instructions for the proper storage and use of each decontamination reagent. Alternatively, a 10% solution of commercial bleach can be used. 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, followed by wipes moistened with either 1) the RNase*Zap*™ RNase Decontamination Solution or 2) the RNase *AWAY*™ Surface Decontaminant reagent. Follow with wiping of deck surfaces with wipes moistened with 70% isopropanol or 70% ethanol. 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, 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, reserve a pipettor and tips 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 not supplied for use with the Genexus™ Purification System" on page 20 and "Recommended materials for nucleic acid isolation and quantification" on page 21.
- 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 60.
- 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.

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

These are general guidelines for manual isolation and quantification of DNA and RNA for Nucleic Acid to Result runs. If you do not dilute sample concentrations manually to the target concentration of the assay, quantify sample nucleic acid concentrations ahead of time so concentrations are available to enter during run planning.

- For verified kits to use for isolation and quantification of nucleic acid samples, see "Recommended materials for nucleic acid isolation and quantification" on page 21.
- Quantification methods such as densitometry (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA or RNA. Use of these methods can lead to gross overestimation of the concentration of sample nucleic acid, under-seeding of the target amplification reaction, and low library yields.
- The recommended sample input concentration is 1.11 ng/µL for DNA samples and 0.95 ng/µL for RNA samples. At the recommended input amount and concentrations, the instrument requires 25 µL of input for DNA and RNA samples. For more information, see "Dilute the samples and load the sample plate" on page 57.

Before each use of the kit

Thaw assay reagents as indicated:

- Thaw OCAv3 DNA Pool 1, OCAv3 DNA Pool 2, OCAv3 RNA Pool 1, OCAv3 RNA 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 "Before you begin" on page 59.

Note: If tapping fails to dislodge a bubble, you can dislodge large bubbles using the technique that is described in "Before you begin" on page 59.

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.
- One template strip pair is needed for each chip lane that is used in a run.
- 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.
- 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.

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.

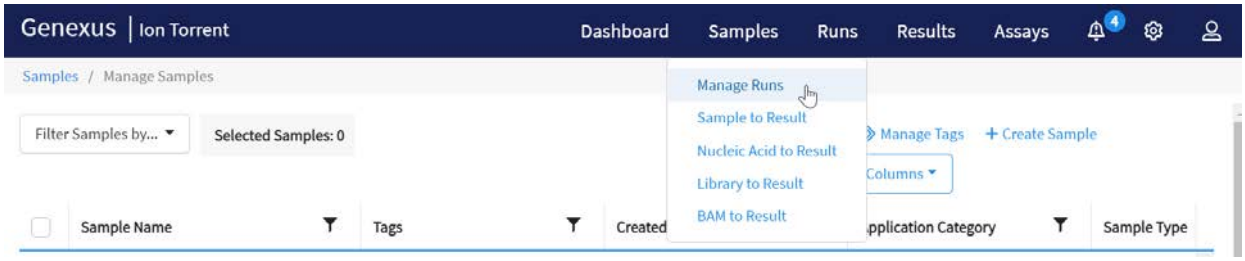
Avoid strong electromagnetic radiation

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™ Comprehensive Assay v3 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 3 Types of runs for use with the Oncomine™ Comprehensive Assay v3 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 and quantification” on page 21.

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

System-installed assays for use with the Oncomine™ Comprehensive Assay v3 GX

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

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

The following system-installed assays are available in the Genexus™ Software 6.8.2 or later for use with the Oncomine™ Comprehensive Assay v3 GX. Each system-installed assay is configured with settings that are optimized for a specific sample type.

Note: We recommend that you update software to the latest available versions of the system-installed assays. For more information, contact your field service representative. For information about how to perform software and assay package updates, see “Install software and assay definition file updates” on page 129.

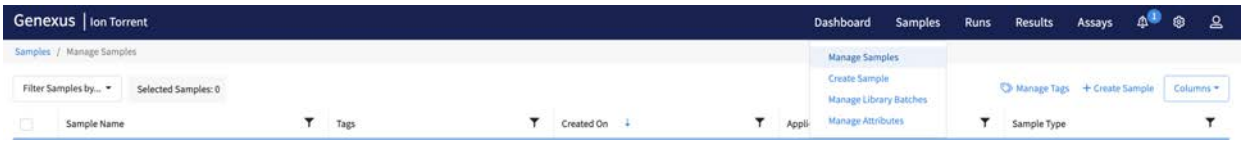
The Genexus-Oncomine-Comprehensive-GX5-w6.0.2-Combined-DNA-DNAandFusions_SW_6.8 assay definition file (ADF) contains 3 assays: DNA, Fusions, and DNA and Fusions.

Assay name	Sample type
Oncomine™ Comprehensive v3 - GX5 - DNA – w6.0.2	DNA
Oncomine™ Comprehensive v3 - GX5 - Fusions – w6.0.2	RNA
Oncomine™ Comprehensive v3 - GX5 - DNA and Fusions – w6.0.2	DNA and RNA

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

- If you are using a system-installed assay without changes, proceed directly to "Enter samples in the Genexus™ Software" on page 31.
- To modify a system-installed assay with custom settings, see the *Genexus™ Software Help* .

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).

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 *Genexus™ Software Help*.

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

2. In the **Setup** step, enter or make the following selections.
   a. In the **Plan** section, enter a unique name.
      The name is limited to 50 characters and no spaces are allowed.
   b. *(Optional)* In the **Reporting (Optional)** section, enable **Generate Report** to generate a variant report that uses the default report template.
   c. Click **Next**.

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

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

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

4. Click **Next**.

5. In the **Samples** step, select the checkbox next to each sample from the list that you want to run with the assay, then in the **Selected Assays** pane, click **Assign**.

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

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

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

9. Click **Next**.

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

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

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

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

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

    After saving, the run appears in the **Manage Runs** screen in the run list with the name you specified.

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

Plan a Nucleic Acid to Result run

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

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

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

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

      The **Chip View** updates to show the lanes used in the run. Lane usage is calculated based on the number of samples (including a no template control, if selected) and the minimum read counts per sample for the assay. For information about the GX5™ Chip lane usage for the Oncomine™ Comprehensive Assay v3 GX, see “Chip capacity by sample type” on page 36. Green denotes a chip lane in the run containing assigned samples within lane capacity.

      If the

      $$\textit{minimum reads per sample} \times \textit{number of samples}$$

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

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

Red lane color denotes sample assignment that exceeds lane capacity for lane 3.

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

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

d. To include a no template control, for each application type of each assay in the run plan, in the **Selected Assays** pane, select **NTC**.
   The **Chip View** updates to show the lanes used in the run for the included no template controls.

e. Click **Next**.

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

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

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

      If a sample concentration is within the designated range, the instrument does not dilute the sample during the run. Sample concentrations must be within 0.11–1,000 ng/µL and cannot be higher than 1,024X of the target concentration. If a sample concentration is ≤1,024X of the target concentration, the sequencer dilutes the sample to the target concentration during the run. If a sample concentration is >1,024X of the target concentration, manually dilute the sample to the target concentration before loading on the sample plate. For more information about sample dilution, see “Dilute the samples and load the sample plate” on page 57. If you want to modify the sample concentration range for an assay, see “Guidelines for Oncomine™ Comprehensive Assay v3 GX settings” on page 115 and the *Genexus™ Software Help*.

      Note: The sample volume that is required for library preparation is not adjustable. The volume depends on the number of primer pools in the assay, sample type, and library chemistry. For specific sample volumes to load onto the sample plate, see “Dilute the samples and load the sample plate” on page 57.

      ---

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

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

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

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

Chip capacity by sample type

Use the following guidance to determine how many samples you can load onto the GX5™ Chip. The capacity of the GX5™ Chip varies depending on the sample type. Chip capacity information provided in Table 4 applies to assays that use system-installed settings only.

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

Table 4 GX5™ Chip lane usage by sample type (system-installed Oncomine™ Comprehensive Assay v3 GX settings only)

Workflow	Number of samples	Number of lanes	Number of strips				Number of bar codes	DNA		RNA	
			Strip 1	Strip 2 AS	Strip 3	Strip 4		Tubes of Pool 1	Tubes of Pool 2	Tubes of Pool 1	Tubes of Pool 2
DNA only	1	1	2	2	1	1	2	1	1	—	—
	2	2	2	2	2	2	4	1	1	—	—
	3	2	2	2	2	2	6	1	1	—	—
	4	3	2	2	3	3	8	1	1	—	—
	5	3	4	4	3	3	10	2	2	—	—
	6	4	4	4	4	4	12	2	2	—	—
	7	4	4	4	4	4	14	2	2	—	—
DNA and RNA	1	1	4	4	1	1	4	1	1	1	1
	2	2	4	4	2	2	8	1	1	1	1
	3	2	4	4	2	2	12	1	1	1	1
	4	3	4	4	3	3	16	1	1	1	1
	5	4	8	8	4	4	20	2	2	2	2
	6	4	8	8	4	4	24	2	2	2	2
RNA only	1	1	2	2	1	1	2	—	—	1	1
	2	1	2	2	1	1	4	—	—	1	1
	3	1	2	2	1	1	6	—	—	1	1

Table 4 GX5 Chip lane usage by sample type (system-installed Oncomine Comprehensive Assay v3 GX settings only) *(continued)*

Workflow	Number of samples	Number of lanes	Number of strips				Number of bar codes	DNA		RNA	
			Strip 1	Strip 2 AS	Strip 3	Strip 4		Tubes of Pool 1	Tubes of Pool 2	Tubes of Pool 1	Tubes of Pool 2
RNA only	4	1	2	2	1	1	8	—	—	1	1
	5	1	4	4	1	1	10	—	—	2	2
	6	1	4	4	1	1	12	—	—	2	2
	7	1	4	4	1	1	14	—	—	2	2
	8	1	4	4	1	1	16	—	—	2	2
	9	2	6	6	1	1	18	—	—	3	3
	10	2	6	6	2	2	20	—	—	3	3
	11	2	6	6	2	2	22	—	—	3	3
	12	2	6	6	2	2	24	—	—	3	3
	13	2	8	8	2	2	26	—	—	4	4
	14	2	8	8	2	2	28	—	—	4	4
	15	2	8	8	2	2	30	—	—	4	4
	16	2	8	8	2	2	32	—	—	4	4

5 Genexus™ FFPE DNA and RNA Purification protocol

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *Genexus™ Purification System User Guide* (Pub. No. MAN0018475). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

The Genexus™ Purification System, with a single touch point and 10 minutes of hands-on time, automates the extraction and quantitation of both DNA and RNA sequentially from FFPE lysates for use on the Genexus™ Integrated Sequencer. When connected to the Genexus™ Integrated Sequencer, the Genexus™ Software streamlines the NGS workflow by integrating the **Sample to Result** workflow within a single software ecosystem. For more information on creating assays, adding or importing samples, and creating plans for **Sample to Result** runs, see the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475). This quick reference assumes familiarity with Genexus™ Software and the Genexus™ Purification Instrument, and is intended for more experienced users.

Plan a purification run (standalone configuration)

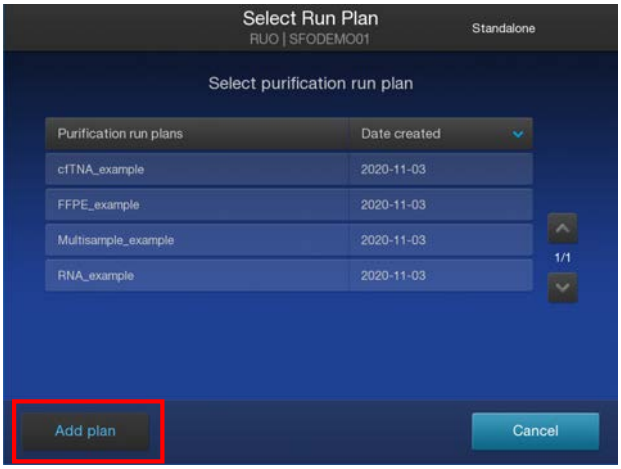
Note: If running the Genexus™ Purification System in integrated configuration, see "Plan a Sample to Result run" on page 31 to plan a **Sample to Result** run in the Genexus™ Software.

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.

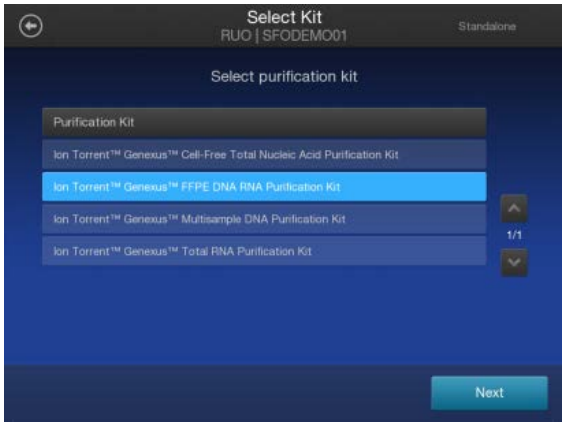
Add a purification run plan (standalone configuration)

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 planning the purification run during the protease digestion step of sample preparation.

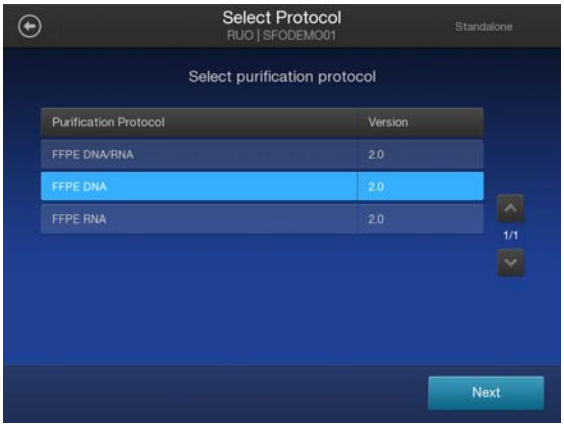
1. Enter your username and password to sign in to the instrument.
2. Tap **Run**, then tap **Add plan**.



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



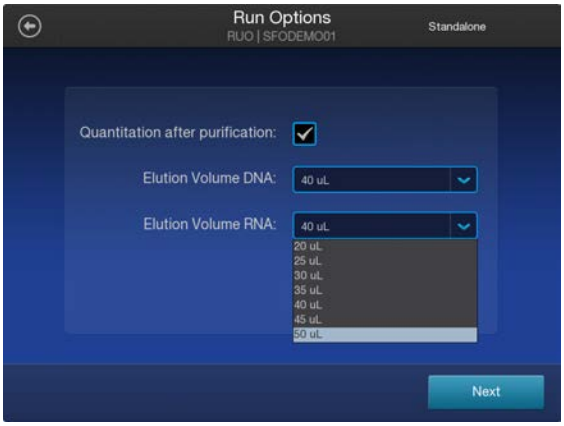
5. Select the appropriate purification protocol, then tap **Next**.
   - If sequentially purifying DNA and RNA, select **FFPE_DNA_RNA_v*X*** (v*X* is software version dependent).
   - If purifying DNA only, select **FFPE_DNA_v*X***.
   - If purifying RNA only, select **FFPE_RNA_v*X***.



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.



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

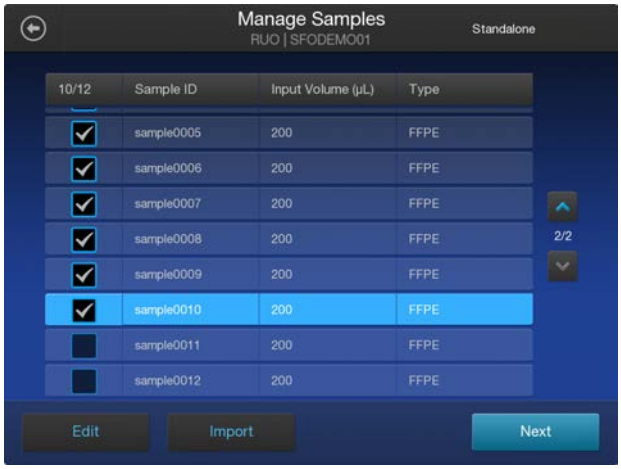
8. *(Optional)* Change the number of samples and the sample details.
   a. In the **Manage Samples** screen, deselect extra samples (for example, if you are only running ten samples, deselect samples 11 and 12).

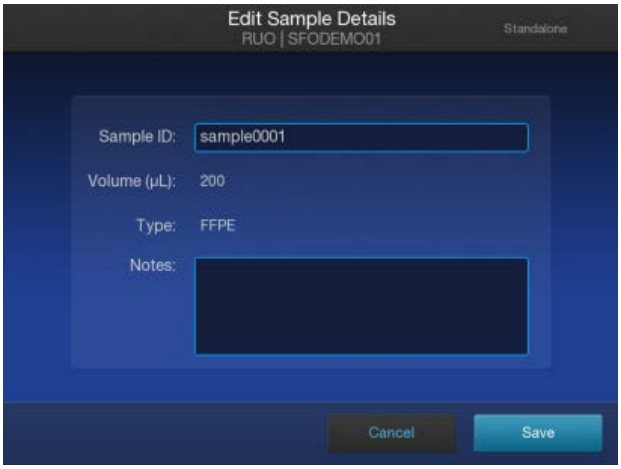
   b. Tap on a sample ID to select the sample.

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

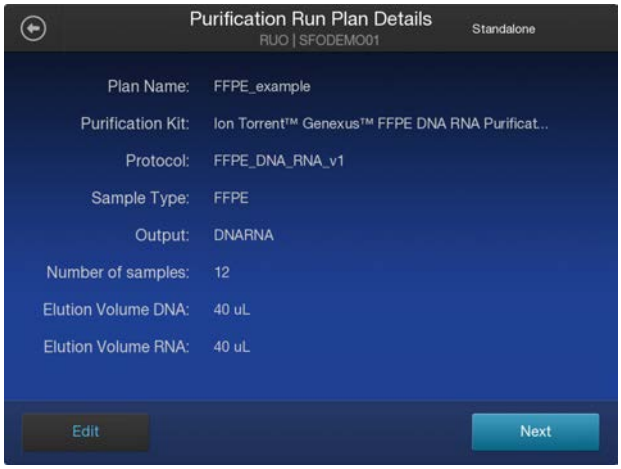
d. Repeat substep 8b and substep 8c for each additional sample.

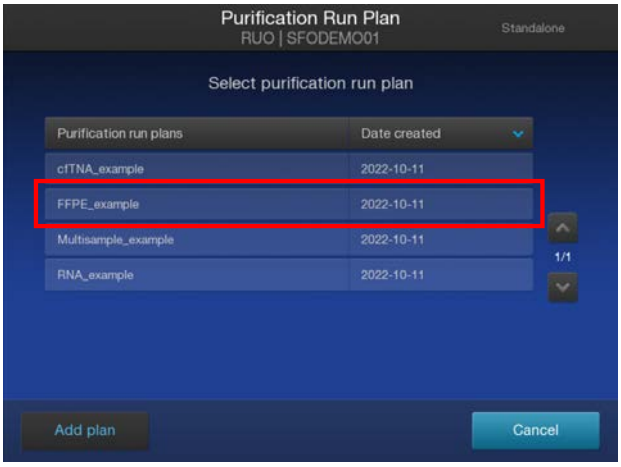
e. Click **Next**.





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**.

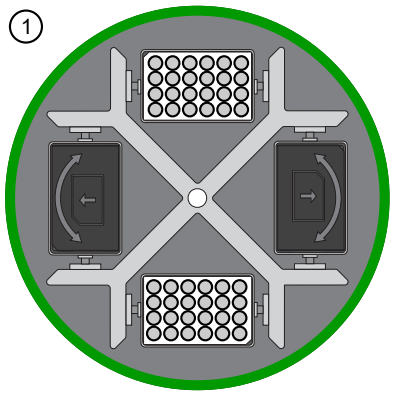
To delete an existing run plan, see the *Genexus™ Purification System User Guide*.

Prepare samples

Procedural guidelines

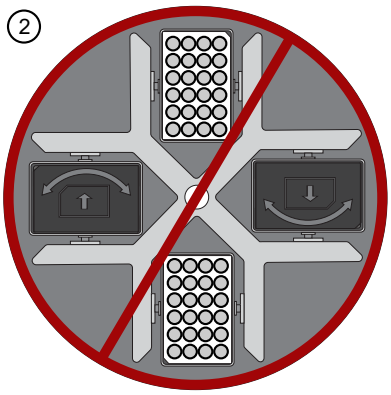
IMPORTANT! Store all kit components containing liquid in the upright orientation.

- Perform all steps at room temperature (20°C–25°C) unless otherwise noted.
- Thawing or storing on ice can be substituted with thawing or storing at 4°C (2–8°C refrigerator or prechilled benchtop cold block).
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Wear clean gloves and a clean laboratory coat.
- Change gloves whenever you suspect that they are contaminated.
- Open and close all sample tubes carefully. Avoid splashing or generating aerosols of the samples.
- When working with RNA:
  - Use a positive-displacement pipettor and RNase-free pipette tips.
  - Clean laboratory benches and equipment periodically with an RNase decontamination solution, such as RNase*Zap*™ RNase Decontamination Solution (Cat. No. AM9780).
  - Store RNA at –90°C to –70°C.
- Volumes for reagent mixes are given per sample. We recommend that you prepare master mixes for larger sample numbers. To calculate volumes for master mixes, see the per-well volume and add 5–10% overage.
- Incubation at 60°C can be extended 1 hour (2 hr total time) to increase DNA yields followed by the 90°C incubation for 1 hour.
- We recommend using a plate centrifuge that holds the AutoLys M Tube Rack in "landscape" orientation.



① Landscape orientation—recommended

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



② Portrait orientation—not recommended

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

Before each use of the kit

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

Materials required

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

Recommended input amount

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

Prepare 1X Protease Digestion Master Mix

1. 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**

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

Prepare FFPE curl samples with AutoLys M Tubes

Alternatively, CitriSolv™ Clearing Agent, xylene, or an equivalent method for removal of paraffin from the FFPE samples can be used to prepare samples. For more information, see appendix B in the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

Digest with Protease in AutoLys M Tubes

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

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

   b. Tap or flick the storage tube so that the curl falls into the AutoLys M Tube.

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

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

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

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

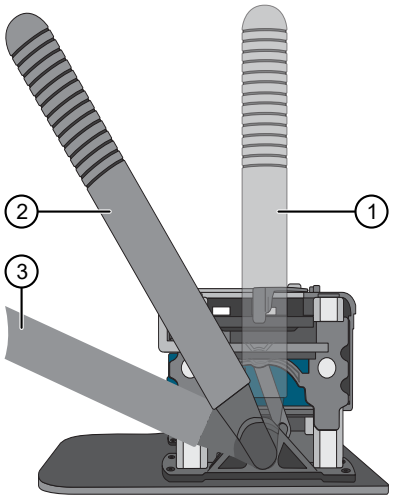
6. Incubate at 90°C for 60 minutes.

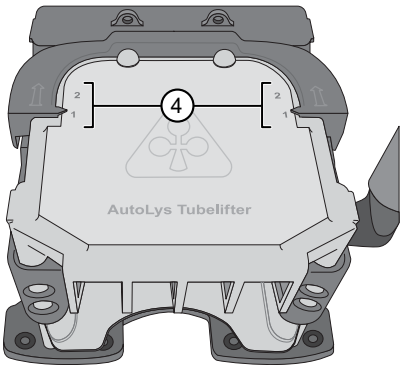
7. Allow samples to cool to room temperature for 5 minutes before proceeding to lift the tubes.

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

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

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





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

④ AutoLys M TubeLifter slider positions 1 & 2

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

IMPORTANT! Ensure that the embossed arrow of the AutoLys M Tube Locking Lid points away from the center of rotation.

10. 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 49.

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

Prepare FFPE slide samples with AutoLys M Tubes

Alternatively, CitriSolv™ Clearing Agent, xylene, or an equivalent method for removal of paraffin from the FFPE samples can be used to prepare samples. For more information, see appendix B in the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

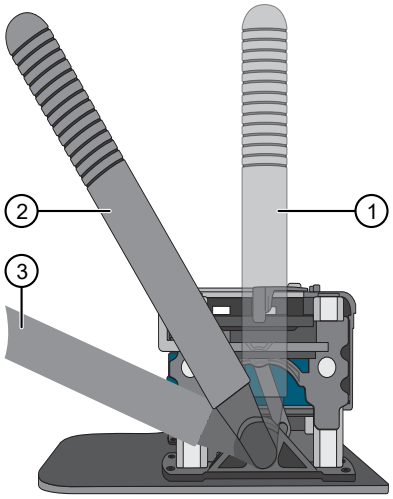
Collect the tissue

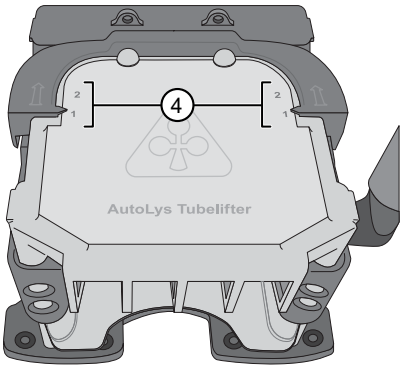
1. Label an AutoLys M Tube for each FFPE tissue sample.
2. Pipet 235 µL 1X Protease Digestion Master Mix into each labeled tube.
3. Use a sterile disposable scalpel with a flat blade to scrape the tissue in a single direction, then collect the tissue into a cohesive mass on the tip of the scalpel blade.
4. 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.
5. Remove and inspect the blade to ensure that no tissue remains on it.
6. 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.
7. Cap each tube securely, then gently flick the tube to mix and to immerse the tissue.

Digest with protease

1. Incubate at 60°C for ≥60 minutes in an AutoLys M Tube Rack.
2. Incubate at 90°C for 60 minutes.
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.





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

④ AutoLys M TubeLifter slider positions 1 & 2

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

IMPORTANT! Ensure that the embossed arrow of the AutoLys M Tube Locking Lid points away from the center of rotation.

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 49.

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

Load the Genexus™ Purification Instrument and start the 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

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

Equilibrate the Quantitation Plate

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

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 49.

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.

Start the purification run

1. In the instrument touchscreen, tap **Run**, then tap to select the run plan that you created for this run.
2. Ensure that the run plan selected is correct, then tap **Next**.

3. *(Optional)* Import sample information.

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

   To import sample information, prepare a CSV sample import file and save it to a USB drive. See the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

   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.

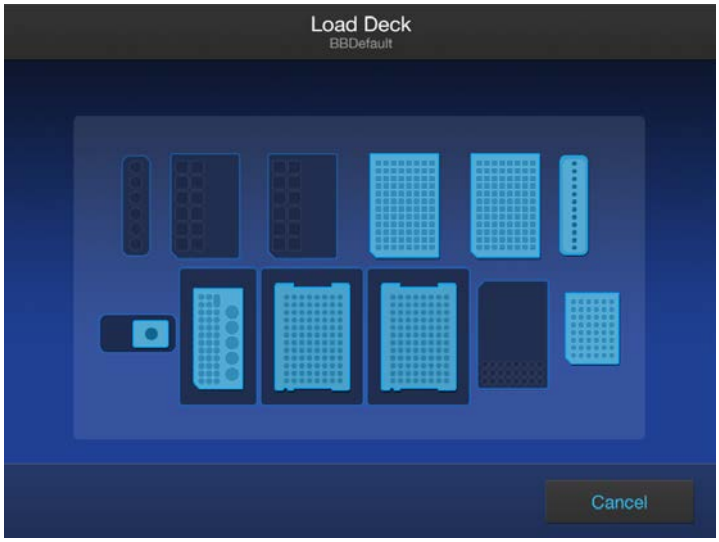
   f. Tap **Import**, then in the **Import Successful** screen, tap **OK**.
   The imported sample information is shown in the **Manage Samples** screen. If needed, select a sample, then tap **Edit** to modify the **Sample ID** or **Notes**.

4. Tap **Next**.
   The instrument performs a 2-minute UV cleaning, then unlocks the door.

5. Open the instrument door.

Load the Genexus™ Purification Instrument

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



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

Load FFPE DNA and RNA Purification Plate 1 & 2

1. Load the FFPE DNA and RNA Purification Plate 1 (DNA plate) prepared in step 4 of "Add samples to FFPE DNA and RNA Purification Plate 1" on page 49.
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 49.

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

   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.
4. Unwrap two new Purification Tip Cartridges, remove the cover to expose the pipette tips, then load the cartridges in both tip box positions.

Load the quantitation reagents and consumables

IMPORTANT!

- 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.
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.

Confirm that consumables are installed correctly

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.
3. Tap **Start**.

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

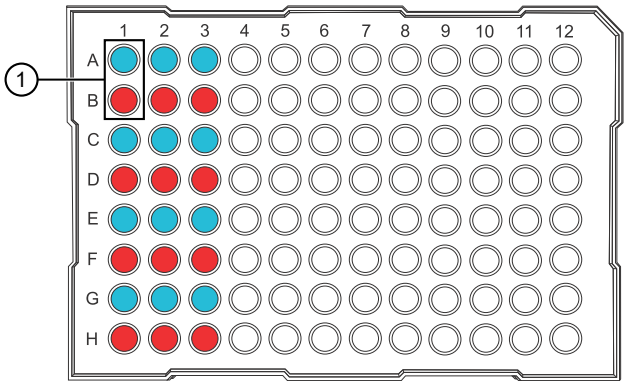
When the run is complete, the interior light turns blue, and the touchscreen displays **Run Complete**. Quantitation results are available immediately. See “View and export quantitation results” on page 54.

Unload purified DNA and RNA

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

1. In the touchscreen, tap **Unload**.
   The door unlocks.
2. Lift the instrument door to access the instrument deck.

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



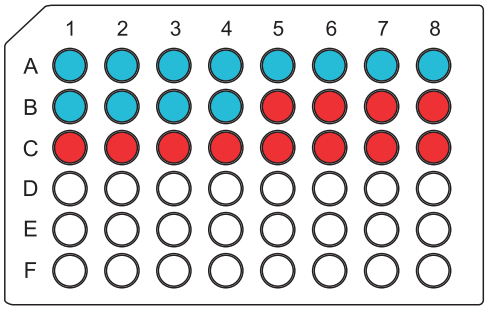
① Paired purified DNA-A1 and RNA-B1 from sample 1. C1 + D1 = sample 2, E1 + F1 = sample 3, etc. For a given purification run, reference the plate map in the run setup guide for the well location of each purified nucleic acid.

● DNA

● RNA

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

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



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

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

Note: *(Purification configuration only)* If using the purified DNA or RNA immediately, transfer the sample to a sample input plate for sequencing. To determine the sample concentrations, see "View and export quantitation results" on page 54.

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

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.

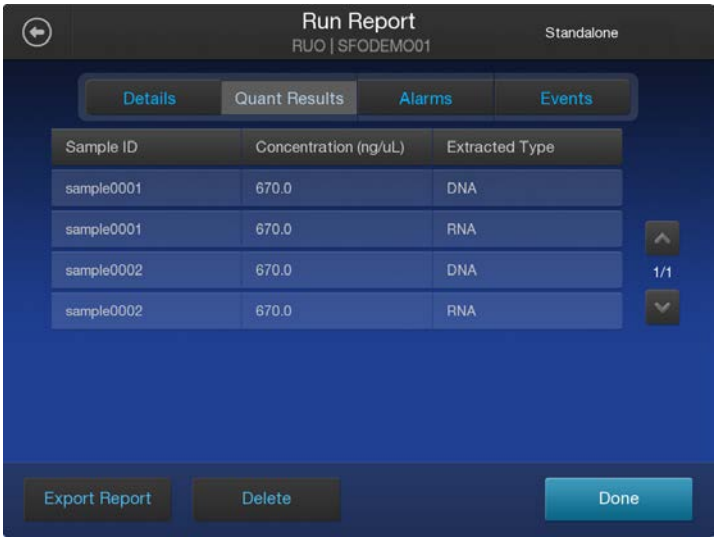
1. In the **Run Complete** screen, tap **View report**.
   The **Saved Experiment Reports** screen opens.

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 CSV**. Navigate to the file destination, then tap **Save**.

Dispose of used consumables and UV clean the instrument

Unload purified nucleic acid samples before disposal of used consumables.

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

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

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

   c. Dispose of the deep-well plate in an appropriate waste container.
   d. Repeat substep 1a through substep 1c to discard the FFPE DNA and RNA Purification Plate 2.
2. Unlock, then remove and dispose of the Purification Tip Cartridges in an appropriate waste container.
3. Unlock, then remove and dispose of the Quantitation Plate.
   a. Dispose of the liquid waste by tipping the deep-well plate on one corner and pouring the waste into an appropriate liquid biohazardous waste container.

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

   b. Dispose of the deep-well plate in an appropriate waste container.
4. Open the quantitation module cover, remove and discard the Quantitation Tube, then allow the module cover to close.
5. Close and lock the instrument door by pressing down on both top corners, then tap **Start UV Clean**.
   The time remaining in the UV cleaning is displayed. When complete, the instrument is ready to start a new purification run.

6 Prepare the sample input plate

Nucleic Acid to Result runs

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

For information about chip capacity, see “Chip capacity by sample type” on page 36.

Nucleic acid samples with concentrations up to 1,024X of the target concentration for an assay (displayed as default values in the **Sample Plate** step screen in run planning) are in range for automated dilution and require no manual dilution. Enter the concentrations during run planning at the **Sample Plate** step (see “Plan a Nucleic Acid to Result run” on page 33).

Quantify nucleic acid samples

Isolate nucleic acid samples using one of the verified procedures and kits listed in “Recommended materials for nucleic acid isolation and quantification” on page 21.

IMPORTANT! The correct input amount of nucleic acid is critical for assay success. Use a Qubit™ Fluorometer to accurately measure sample concentration before you proceed to “Dilute the samples and load the sample plate” on page 57.

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

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

Dilute the samples and load the sample plate

Before you proceed, use a Qubit™ Fluorometer to accurately measure sample concentration. For more information, see “Quantify nucleic acid samples” on page 56. If you want to run sample controls, select an appropriate control for your sample type as recommended in “Recommended sample controls” on page 21.

1. Use the run setup guide to dilute samples and sample controls, if used, to the required concentration using nuclease-free water (see “Plan a Nucleic Acid to Result run” on page 33).

   IMPORTANT! Make sure to dilute samples to the concentration that was specified in step 5 in “Plan a Nucleic Acid to Result run” on page 33.

   Table 5 Sample concentration for Oncomine™ Comprehensive Assay v3 GX

Sample type	Recommended nucleic acid concentration
FFPE DNA	1.1 ng/µL
FFPE RNA	0.95 ng/µL

2. Add 25 µL of diluted samples to the sample plate, to the wells that are specified in the run setup guide.

   Note: The Oncomine™ Comprehensive Assay v3 GX requires 20 µL of sample with 5-µL overage.

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

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

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

Sample to Result runs

If the DNA and RNA samples were not quantified on the Genexus™ Purification Instrument, you must quantify the samples. See “Quantify nucleic acid samples” on page 56.

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

For information about chip capacity, see “Chip capacity by sample type” on page 36.

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

If positive controls were included in the DNA-only, Fusions (RNA-only), or DNA and Fusions run plan, you must manually add positive controls to the sample input plate, before loading the plate in the sequencer. For more information, see “Nucleic Acid to Result runs” on page 56.

Transfer DNA and RNA samples to the sample input plate

To perform DNA and Fusions sequencing runs the DNA and RNA samples must be transferred from their respective purification output plates to a new 96-well sample plate for loading onto the sequencer. If any controls were included in the run plan, you must manually add positive controls and no template controls to the sample input plate, before loading the plate in the sequencer. For more information on the appropriate controls see “Recommended sample controls” on page 21.

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

7 Load the sequencer and start a run

After you have planned a run in Genexus™ Software (see Chapter 4, "Plan and manage runs"), 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, an incorrect quantity is loaded, an incorrect consumable is loaded, or if an expired reagent is loaded.

Instrument run times

Table 6 Total instrument run time required for each workflow type

DNA-only	RNA-only		DNA + RNA
7 samples per run	8 samples per run	16 samples per run	6 samples per run
~22.5 hrs	~18 hrs	~22.5 hrs	~30 hrs

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 25 and "Guidelines for preventing contamination" on page 24.

1. 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.

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

3. 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.

4. Vortex each strip at maximum speed for 5–10 seconds while rocking the strips from side to side.

   Note: Vortexing helps dislodge magnetic beads that can be trapped in keyholes of Genexus™ Strip 1 and Genexus™ Strip 3-GX5™, and to dissolve precipitate in Genexus™ Strip 2-HD, if present.

Note: Load the Genexus™ Integrated Sequencer within 2 hours of thawing reagents.

Proceed to “Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter”.

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

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

You can use the Genexus™ Strip Centrifuge Adapter as a holder for centrifuging library and templating strips to collect contents in the bottom of strip wells after vortexing the strips. Users can contact Technical Support or a Thermo Fisher Scientific Field Service Engineer to request the adapter (Part No. A53852). 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. 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

IMPORTANT! The GX5™ Chip is needed for the Oncomine™ Comprehensive Assay v3 GX. Ensure that the correct chip is loaded in step 5.

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

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

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

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

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

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

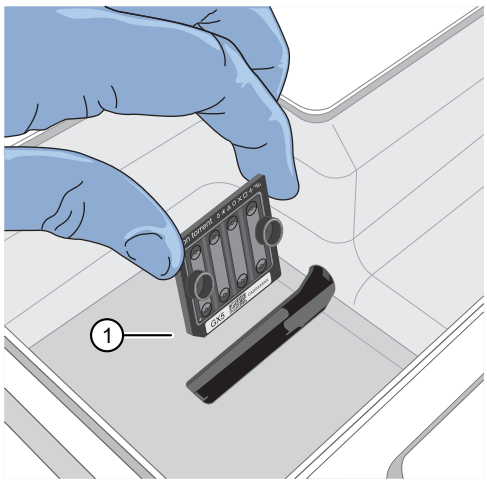
   The deck door opens automatically.

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

   IMPORTANT!

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

5. If prompted, insert a new 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

IMPORTANT! Insert the Genexus™ Coupler so that it is level to properly align with the 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.
   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 starts the run.

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

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

10. Close the sequencing reagents bay doors.

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

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

    A sequencing run encompasses the following stages.

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

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

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

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**.

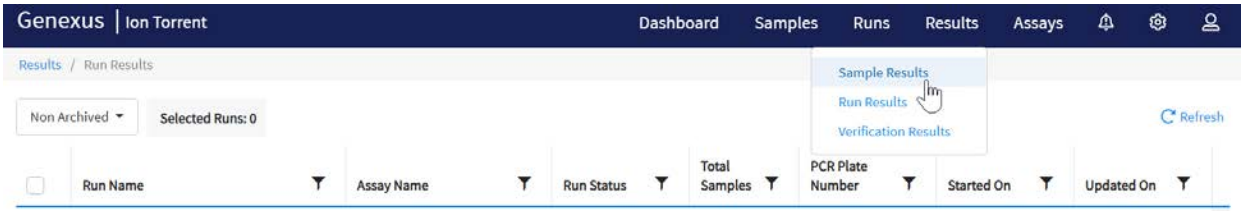
IMPORTANT! Do **not** discard or remove the conical bottles, unless alerted by the sequencer to replace the bottles after a conical bottle flow rate test. For more information, see the *Genexus™ Integrated Sequencer User Guide*.

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 run results and data analysis and perform data management tasks. You can view results sorted by sample or by run.

View data and results

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

The run report provides detailed information about a run, such as the **Total Bases** and **Final Reads**. The run report contains the assay metrics for all assays in the run.

Assay-level results include assay metrics, such as final read data. Assay metrics are also shown in the **Run Report** tab. For more information, see "View assay metrics and the run report" on page 116.

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

In the menu bar, select one of the following options.

Selection	Description
Click **Results** ▸ **Run Results**	Select this option to review completed run results and reports by assay. Proceed to "Review run results" on page 70.
Click **Results** ▸ **Sample Results**	Select this option to review completed sample results and reports. Proceed to "View sample results" on page 73.
Click **Results** ▸ **Verification Results**	Select this option to review data from completed verification runs that were performed during sequencer installation or performance qualification. We recommend selecting this option only when recommended by support.

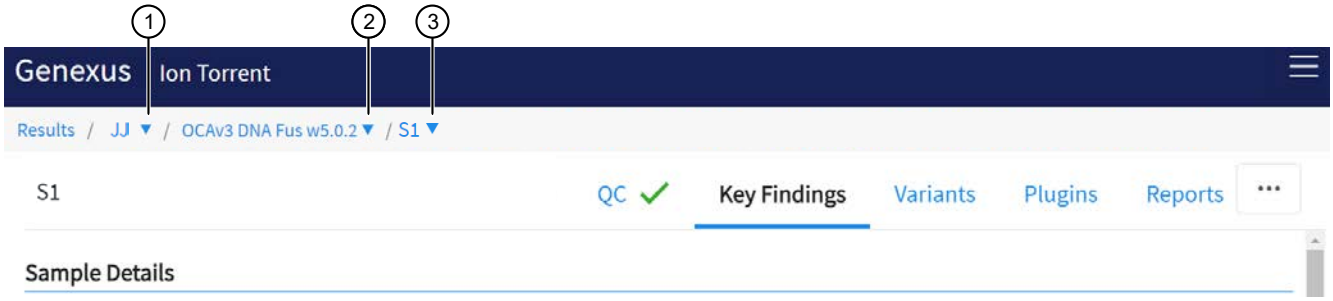
After you select a run result or a sample result, a results navigation bar appears and allows you to easily toggle between different results screens for each run. You can switch between run-level results, assay-level results, and sample-level results for the run.

Results navigation bar overview

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

Assay-level results include assay metrics, such as final read data. Assay metrics are also shown in the **Run Report** tab. For more information, see “View assay metrics and the run report” on page 116.

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



Callout	Dropdown menu	Description
1	**Run Name**	The run name is listed. Multiple runs are listed only if the run has been reanalyzed. For more information, see “Reanalysis” on page 129.
2	**Select Assay**	You can click the assay name of interest to view assay metrics for the run. You can toggle between different assays used in the run. If only one assay is associated with the run, only one assay name is listed. Some results from a plugin that is included in the run, such as the customer support archive (CSA) plugin, apply to all of the samples for an assay in a run. In this case, the results from assay-level plugin are shown in the **Plugins** tab when you select an assay but do not select any samples for the run. For more information about plugins, see “Review plugin results in Genexus™ Software” on page 92. You can choose **Select Assay** from the dropdown list to remove the assay selection. Removing the assay selection opens the **Run Summary** tab for the run selected in the **Run Results** dropdown list.
3	**Select Sample**	You can click a sample name to view the sequencing results for the sample. You can click a different sample name to view other sample results for other samples in the run for the assay that is selected in **Select Assay**. You can choose **Select Sample** from the dropdown list to remove the sample selection. Removing the sample selection opens the **Run Report** tab for the assay selected in the **Select Assay** dropdown list. For **Sample to Result** runs that include a positive control, results for the positive control are shown when you select **Control Sample**. For **Sample to Result** runs that include a non-template control (NTC), results for the NTC are shown only in the **QC** tab under **Sample QC**. The NTC sample is not listed in the **Select Sample** dropdown list.

Review run results

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

You can search the list of results when you click **(Filter)** in a column of interest, then entering a full or partial run or assay name, or other applicable filter term.

The following run information appears in the **Results / Run Results** screen.

Column	Description
Run Name	The unique name of the run given when it was created in the software. Click a run name to open the **Run Summary**. Runs that are reanalyzed are listed with **(Reanalysis)** after the run name.
Assay Name	The name of the assay selected for the run. You can view the **Assay Name** and corresponding **Assay Full Name** for all assays in the **Assays** ▸ **Manage Assays** screen.

(continued)

Column	Description
Run Status	The status of the run. For example, **Analysis In Progress**, **Executing Plugin**, **Analysis Completed**, **Terminated**, **Archival: In Progress**, **Purification In Progress**, or **Purification Completed**).
Total Samples	The total number of samples in a run.
PCR Plate Number	A unique identifier for the 96-well plate used for library preparation and templating. For more information, see the software help system.
Started On	The date and time when the run was started.
Updated On	The date and time when the last action was completed on the run.

Users with appropriate permissions can perform the following actions in the **Results / Run Results** screen.

Option	Description
Actions	Available action links for a run are shown when you place the pointer over the row of a run. The actions that are available depend on the type of run. • **BAM Uploader**—Upload run information to another Genexus™ Software server or to Ion Reporter™ Software for further analysis. **BAM Uploader** is not available for BAM run results or for archived runs in which BAM files have been removed. • **Audit**—View the audit trail for the run. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting. • **Assign PCR Plate**—Enter a unique identifier for the 96-well plate used for library preparation and templating. This action is available only in **Sample to Result** and **Nucleic Acid to Result** runs after the run completes. • **Download Files**—Select the **Variants**, **Reports**, **Sequencing Results**, **Audit and Log**, and **Troubleshooting Files** to download.
Actions[1]	Available action links for a run are shown when you place the pointer over the row of a run. • **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. • **Abort**—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.

[1] More actions that are available only for Sample to Result 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™ Software server or to Ion Reporter™ Software for further analysis.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **BAM Uploader**.
View the history of variant classifications.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **Variant Audit**.
View the run report.	Click the **Run Report** tab.

Assign the PCR Plate

In **Sample to Result** and **Nucleic Acid to Result** workflows, Genexus™ Software allows you to track and associate a run with the PCR plate used in the run. The PCR plate is the 96-well plate that is used for library preparation and templating. You can assign a unique identifier, a **PCR Plate Number**, to runs that have a status of **Library Preparation Completed**, **Sequencing Completed**, or **Run Completed**. The PCR plate number that you enter is shown in the **Run Results** screen and if needed, can help you track libraries and troubleshoot. Later, if you sequence the remaining libraries in a different sequencing run and assign the PCR plate number to the run, you can easily search for and find all run results associated with the libraries in the PCR plate.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, place the pointer over the row of a run of interest, then click **Assign PCR Plate number**.

3. In the **Assign PCR Plate Number** dialog box, confirm, edit, or enter the **PCR Plate Number**.

   The PCR plate number must be between 1 and 10 characters. Only alphanumeric characters (numbers 0 to 9 and letters A to Z), period (.), underscore (_), or hyphen (-) are allowed. Spaces are not allowed.

4. Click **Submit** to associate the PCR plate with the run.

View sample results

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

Tab	Description
QC	The quality metrics for the sample sequenced in the run. For more information, see “QC results” on page 74.
Key Findings	An overview of the results for the sample, including **Sample Details**, **Key Metrics**, **Key Variants** and **Coverage Graphs**. For more information, see “Key Findings” on page 76.
Variants	Detailed variant results for **SNVs/Indels**, **Fusions**, and **CNVs**. For more information, see “View SNV/INDEL results” on page 83, “View Fusion results” on page 86, and “View CNV results” on page 88.
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 92.
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 120. • Variant Reports – include the variant results for each sample in a sequencing run, reagents used, and QC evaluation metrics. For more information, see “Download a variant report” on page 103.

View the QC results

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

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

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

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

QC results

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.

Note: If a sample fails to meet one or more QC parameters, you can reanalyze the sample or the run after adjusting the parameter or parameters. For more information, see "Reanalysis" on page 129.

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

Metric	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.**
Key Signal	The average signal for library ISPs that identically match the library key (TCAG) after software processing.
Percent Loading	The number of wells with ISPs divided by the number of the total addressable wells in a run.
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))).
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.

(continued)

Metric	Description
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.
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 Length (bp)	The average length, in base pairs, of final library reads for the sample.
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).
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.
Sample QC – RNA	**Sequencing quality metrics of the sample RNA library.**
Mapped Reads	The total valid mapped reads. That is, the mapped reads that pass the thresholds and filters across all of the RNA targets in the reference file. This number is a subset of the mapped reads shown in the **Run Samples** table in the **Assay Metrics**. The minimum **Mapped Reads** are 20,000.
Mean Read Length (bp)	The average length, in base pairs, of the final library reads for the sample. The minimum **Mean Read** length is 60.
Read Length Histogram	The histogram 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).
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.

(continued)

Metric	Description
NTC QC – RNA	**RNA Sequencing quality metrics of the no template control.**
Mapped Reads	The total number of valid mapped reads for the NTC.
Mean Read Length (bp)	The average length, in base pairs, of final RNA 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 RNA inline control amplicon.

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, and coverage plots for genes assayed in the run.

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

Table 7 Sample Details

Section	Description
Sample Name	A unique identifier representing the sample.
Collection Date	The date that the sample was collected.
Gender	The biological sex of the sample: **Female**, **Male**, or **Unknown**.
Sample Type	A term that describes the sample, for example, FFPE, DNA, DNA & RNA.
Application Category	The application type of the sample.
Cancer Type	The type of cancer that is represented by the sample.

Table 7 Sample Details *(continued)*

Section	Description
Cancer Stage	The stage of the cancer from which the sample was collected.
%Cellularity	The percentage of tumor cellularity in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is entered when a sample is created. The attribute is applicable to FFPE samples only. The attribute is shown but not required.

Table 8 Key Metrics

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

[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 **Cancer**, 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 78.

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

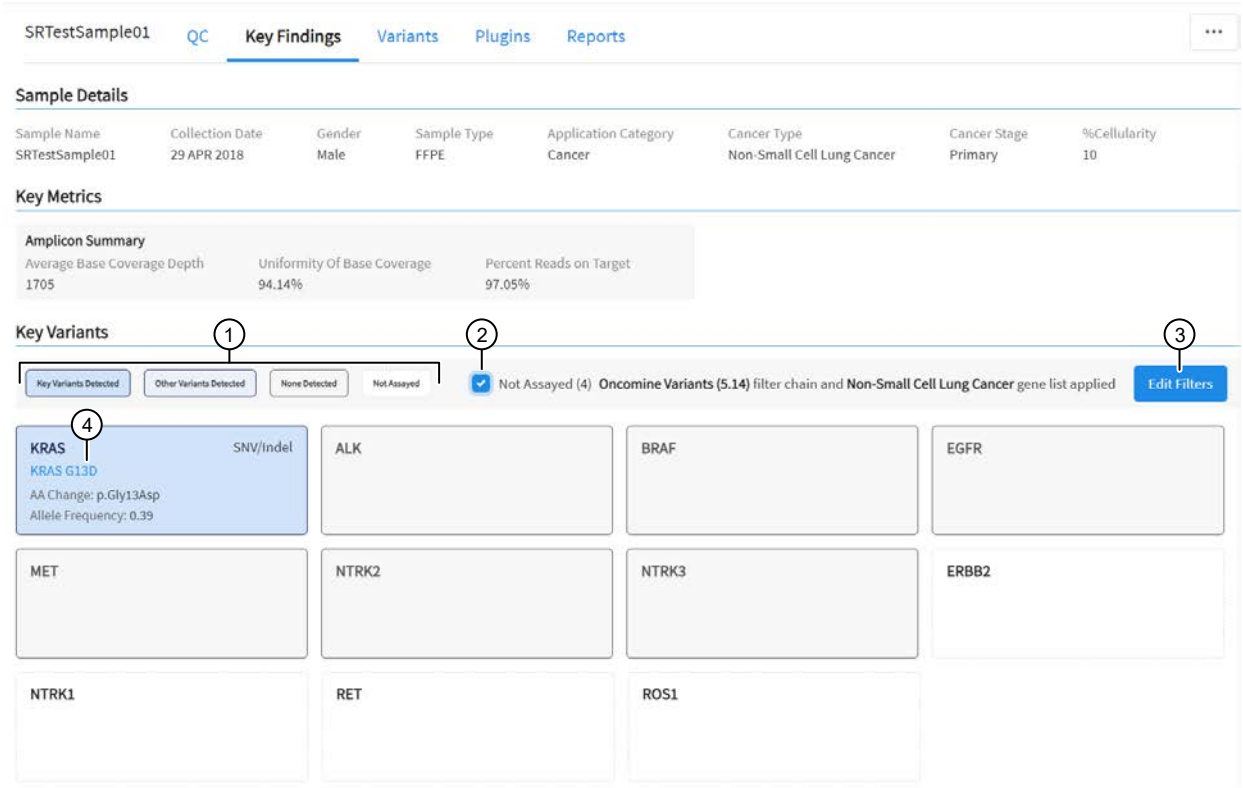
The **Key Variants** matrix provides a color-coded visual representation of the variant results. When the application category of the sample is **Cancer** or **Cancer (Germline)** the genes that are shown in the **Key Variants** matrix are determined by the filter chain and the gene list applied to the 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 82. c. Click **Save**.



Example **Key Variants** matrix

① Color coded legend to the variant tile matrix.

Key Variants Detected —A gene is present in the gene list, variants are annotated by the Oncomine™ Variant Annotator plugin, and the variants are **Key Variants**. Genes that are called are listed in the **Variants** tab with **Present** in the **Call** column. **Key Variants** are listed in the **Variants** tab, with a value of **Yes** in the **Key Variant** column. For more information about gene lists, see the *Genexus™ Software Help*.

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

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

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

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

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

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

View the amplicon coverage

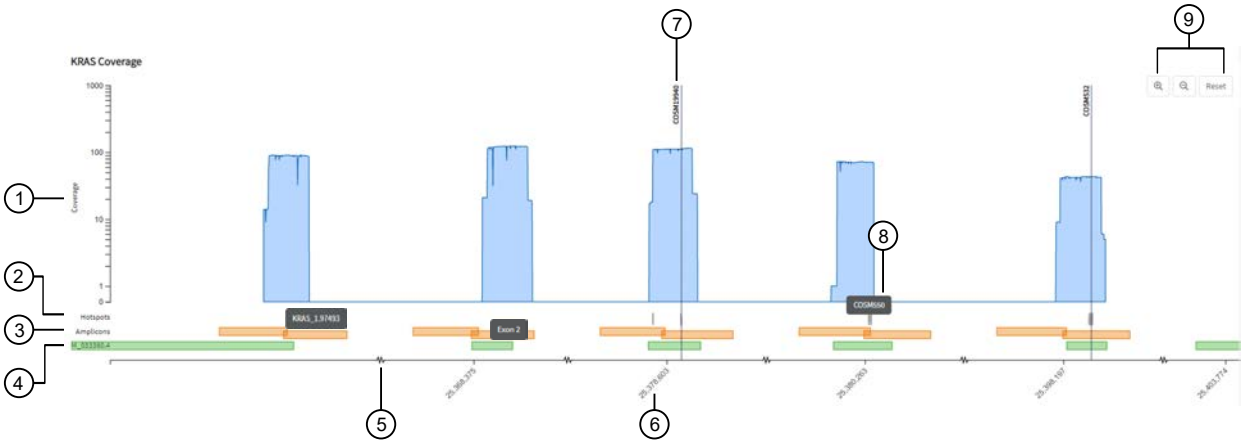
You can view DNA exon amplicon coverage in Genexus™ Software to help you determine whether the sequencing reads across a gene are uniform and sufficient. The default genes that are shown are determined by the gene list that is designated as default for the cancer type of the sample. You can also view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

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

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

Example amplicon coverage graph

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



1. Base coverage is shown on the y-axis on a logarithmic scale.
2. The location of the known hotspots are denoted with gray bars.
3. The location and span of the amplicons are represented with orange bars. You can hover the mouse over an orange bar to view the amplicon name.
4. The location and span of the transcript track is denoted with green bars. You can hover the mouse over the green bar to view the exon number.
5. The line along the x-axis is broken to indicate that the graph is discontinuous to show exons.
6. The genome coordinate position is shown on the x-axis.
7. Called variants are indicated with a vertical line above the blue coverage plot. The variants noted in the graph depend on the filter chain applied to the results in the **Key Variants** matrix. For more information, see the Genexus™ Software help system or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
8. You can place the pointer over the hotspots that are represented with gray lines to view the hotspot name.
9. You can adjust the amplicon coverage graph. For more information, see “View the amplicon coverage” on page 80.

View variant results

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

Table 9 Variant Tables

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

Search and filter variant results

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

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

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

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

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

System-installed filter chains

The following Genexus™ Software system-installed filter chains are available for use with the Oncomine™ Comprehensive Assay v3 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 includes all Oncomine™-annotated variants.
Oncomine™ Extended (6.8)	Default filter chain that includes all Oncomine™-annotated variants and variants that are 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 is 0.0 to 1.0E-6. Mutations must also be non-synonymous and occur in exonic or splice-site regions. • Variants with ClinVar annotations of pathogenic or likely pathogenic.
No Filter	Select this option to remove a previously applied filter chain and view all called variants.

View SNV/INDEL results

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

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

SNVs/Indels table

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

You can filter the results list in the table using filtering tools and filter chains. For more information, see “Search and filter variant results” on page 82.

Column	Description
User Classifications	User-defined classification to select from the list. For more information, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
Variant ID	The name of the hotspot as defined in the Browser Extensible Data (BED) file. Click the link to view more annotation information.
Variant Name	The name of the variant.

(continued)

Column	Description
Key Variant	Indicates whether the variant is a key variant. Possible values are **Yes** or **No**. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Locus	The chromosome and position of the detected variant.
Oncomine Variant Class	The type of SNV or INDEL at the locus based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • **Gain-of-function**—The altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • **Loss-of-function**—The altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Gene	The gene name.
AA Change	Identification of the amino acid change using Human Genome Variation Society (HGVS) nomenclature.
Ref	The reference base or bases at that locus.
Alt	The alternate base or bases at that locus.
Type	The type of variant that is detected. • **snp** (single nucleotide polymorphism) • **mnp** (multi-nucleotide polymorphism) • **ins** (insertion) • **del** (deletion) • **complex** • **FLT3-ITD**

(continued)

Column	Description
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 100). • **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)**—Indicates a high confidence call that passes all filter thresholds at a given variant position. – When the default filter chain is applied, **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** indicates the presence of the ALT (alternative) allele. – When the **No Filter** option is applied or when viewing the **Variants** (VCF) file, **Present** does not imply the presence of the ALT (alternative) allele. To infer the presence of the ALT allele, see the **Alt** column. • **NO CALL**—Although some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call. • **ABSENT**—Indicates that the variant is unlikely to be present in the sample.
Call Details	The reason why a variant is reported as **NO CALL**.
Phred QUAL Score	The relative probability of either the "reference" hypothesis interval [0,cutoff], or the "variant" hypothesis interval [cutoff,1], Phred-scaled (-10*log10). A higher score means more evidence for the variant call.
Raw Read Depth	Total read coverage across amplicon containing SNV/Indel hotspot locations. Count of chip-level reads aligned at this locus that participate in variant calling.
Effective Read Depth	The number of reads covering the position.
Alt Allele Read Counts	The number of reads containing the alternate allele.
Coverage	The number of total reads at a locus, wild-type + alt allele.
Nuc Change	The position and identity of the nucleic acid change.
Allele Frequency	The number of variant read counts divided by the total number of read counts for the sample.
Allele Frequency (%)	The allele frequency, represented as a percentage.
Allele Ratio	The relative frequency of each allele.
Mut/WT	The ratio of mutant allele to wild-type allele.
Zygosity	Describes whether the variant is homozygous (0) or heterozygous (1).
Filtered Read Coverage	Coverage at the position considering only filtered reads.
Allele read Count	The number of reads detected for the allele. The Allele Read Count filter sets the minimum count of the genotype alleles.

(continued)

Column	Description
Raw Alt Allele Read Counts	The number of unfiltered reads containing the alternate allele.
PPA	Possible Polyploidy Alleles (PPA). A value of **Yes** indicates variants that are PPA alleles. A value of **No** indicates variants that are not PPA alleles.
P-Value	The probability value for the detection of variant calls.

View Fusion results

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

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

Fusions 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 filtering tools. For more information, see “Search and filter variant results” on page 82.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).
Variant ID	The name of the fusion target as defined in the BED file.
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 positions in the reference genome that define the fusion junction.
Oncomine Variant Class	Oncomine variant class annotation that indicates fusion type based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run.

(continued)

Column	Description
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function—the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • Loss-of-function—the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Genes (Exon)	The name of fusion target and representative acceptor and donor exons.
Read Counts	The frequency that the fusion was detected in the sample.
Type	Assay type (for example, Fusion, RNA exon variant (exon skipping), Proc Control).
Call	Indicates the presence or absence of a fusion or RNA exon variant. When the default filter chain is applied, only the fusion/RNA exon variant calls that are designated with **PRESENT** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants** (VCF) file (see "Results files" on page 100). • **PRESENT** – indicates a high confidence call that passes all filter thresholds at a given variant position. • **ABSENT** – indicates the absence of a fusion due to a variant call that falls below thresholds. • **NO CALL** – although some evidence for the presence of a fusion exists, the call does not pass one or more filters that are required for a high confidence fusion call.
Call Details	The reason for reporting a fusion as **NO CALL** or **ABSENT**.
Read Counts Per Million	The number of fusion read counts detected per million total reads.
Oncomine Driver Gene	The gene believed to be associated with increased oncogenic properties. The gene is inappropriately activated by the fusion.
Gene Isoform	The name of the fusion target as defined in the BED file.

View RNA Exon Variants

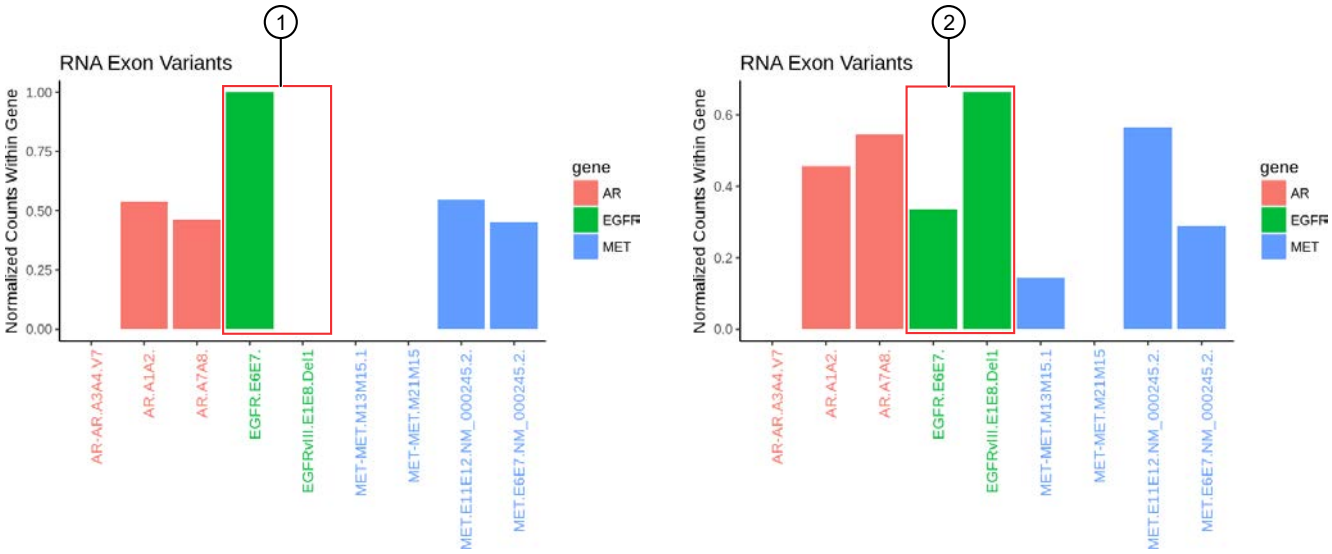
The **RNA Exon Variant** data view displays a bar graph summary of intragenic exon rearrangements or fusions. The displayed RNA exon variants are defined in the BED file that is associated with an assay. The **RNA Exon Variant** data view is available for all RNA and fusion assays.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample name** column, click the name of the sample of interest.

3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results. For more information, see “Fusions table” on page 86.

4. In the top right corner of the screen, click **Visualization** ▸ **RNA Exon Variant**, then review the **RNA Exon Variants** plot.

Representative RNA Exon Variant plots



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

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

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

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

View CNV results

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

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

CNVs table

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

You can filter the results list in the table using filtering tools and filter chains. For more information, see “Search and filter variant results” on page 82.

IMPORTANT! *(FFPE samples only)* If the **%Cellularity** value for a sample is set to <100, then the magnitude of copy number gain or loss can be decreased. For more information, see the online help system.

Column	Description
User Classifications	A user-defined classification selected from the list.
Variant ID	The identifier of the CNV variant. Click the link to view more annotation information.
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 starting position of the first amplicon covering the CNV gene.
Oncomine Variant Class	Annotation that indicates whether CNV is an amplification or deletion. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function—the altered gene product has a new molecular function or pattern of gene expression, compared to the wild-type gene • Loss-of-function—the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Gene	The gene name.
Copy Number	The copy number of a CNV gene locus per genome. This column is available when a positive call is made.

(continued)

Column	Description
Call	Indicates the presence or absence of a CNV. When the default filter chain is applied, only the CNV-positive calls that are designated with **PRESENT** 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 100). • **PRESENT** – indicates a high confidence call that passes all filter thresholds. • **ABSENT** – the absence of a variant; result is below the detection threshold for a CNV call. • **NO CALL**– although some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call.
P-Value	The statistical significance of the CNV ratio measurement.
Call Details	The reason for reporting a CNV as NO CALL.
CNV Confidence	The CNV confidence interval associated with the call. The 5% lower confidence bound value is the ploidy estimate, where there is a 5% chance that the true ploidy is below that value. The 95% upper confidence bound is the ploidy estimate, where it is 95% certain that the true ploidy is below that value.
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 number of amplicons spanning the CNV call.
Type	The type of variant that is detected. CNV– copy number variant

Compare sample results

In Genexus™ Software, you can compare variant results across samples and create customized comparison plots. To compare samples from different assays, the research application (such as DNA or DNA and Fusions) must be comparable. That is, results generated from DNA-only assays cannot be compared to results generated from RNA-only or fusion-only assays. However, if the results from a research application include the type of results from another research application, the comparison can be made. That is, you can compare variant results generated from a DNA and Fusion assay to the variant results generated from a DNA-only assay.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Select the checkbox in the row of each sample that you want to compare, then click **Compare**.

   Note: **Compare** is not enabled until at least two samples are selected.

   The **Results / Compare** screen opens and displays a list of variants and variant information in the **Compare Samples** table. The **Oncomine™ Extended (6.8)** filter chain is the default filter chain that is applied to the data that are displayed in the tables. Only variants with a Variant ID are shown.

3. In the **Compare Samples** table, select the checkbox in the row of one or more variants of interest to view the variants plot (see callout 1 in Figure 1).

4. Customize the variant view if needed.

Option	Description
Customize table columns	Click **Table Columns**, then select the columns that you want to view in the table, or deselect the columns that you want to hide (see callout 2 in Figure 1).
Customize plot axes	Click **Chart Options** (see callout 3 in Figure 1). In the **Preferences** dialog box, select the desired X-axis, then select the desired Y-axis for each variant type.
Change the default filter chain	From the **Filter Chain** dropdown list, select a filter that you want to apply to the variants that appear in the **Compare Samples** table (see callout 4 in Figure 1). For a description of system-installed filters, see “System-installed filter chains” on page 83.

5. Click **Export Variants** to download the information in the **Compare Samples** variant table for the selected variants.

   See callout 5 in Figure 1.

   The information is downloaded in XLSX format.

6. Click **Download Chart** to download the variants plot in PNG format (see callout 6 in Figure 1).

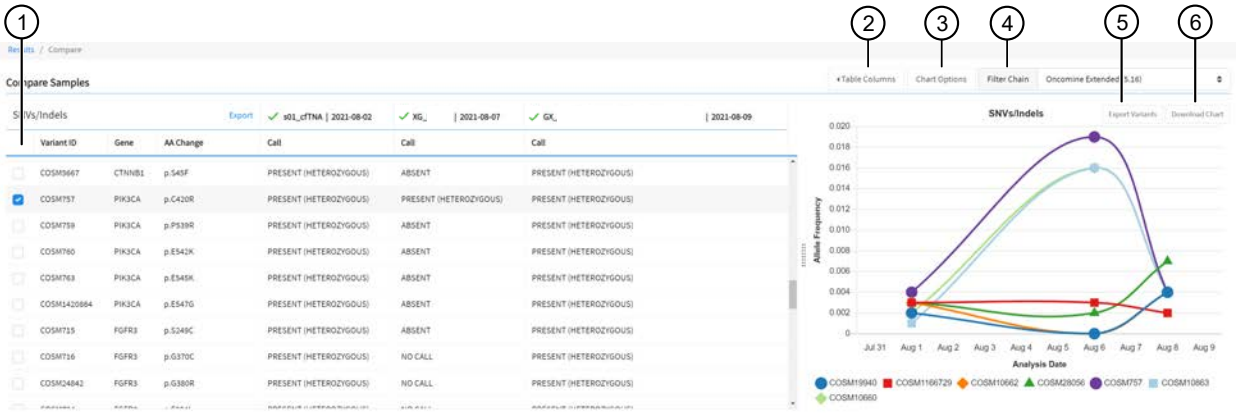


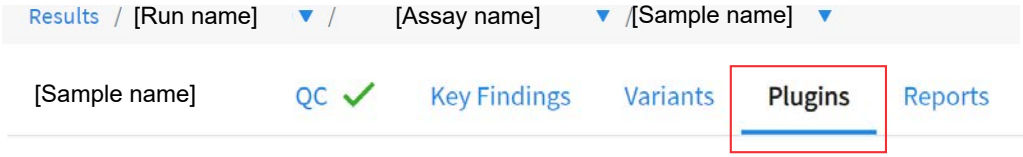
Figure 1 Compare variants

1. Select one or more variants to compare in the variants plot.
2. Customize columns that appear in the **Compare Samples** table.
3. Customize the X-axis and Y-axis that appear in the variants plot.
4. Select a filter chain to filter the results that appear in the **Compare Samples** table.
5. Download in XLSX format, the information in the **Compare Samples** variant table for the selected variants.
6. Download a PNG file of the plot.

Review plugin results in Genexus™ Software

In Genexus™ Software, you can review plugin results in the **Results** screen for a sample.

Each plugin that was selected for an assay during assay creation is listed in the **Plugins** tab.



Review coverageAnalysis plugin results

The coverageAnalysis plugin generates a **Coverage Analysis Report**. This report includes read statistics and several charts. The statistics and charts that are presented depend on the library type for the analysis.

The report summary lists the barcode, the sample, the number of mapped reads, the percentage of on target reads, mean base coverage depth, and base coverage uniformity.

You can download coverageAnalysis plugin output files from the **Results** screen for a sample. For more information, see the *Genexus™ Software Help*.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample of interest.
3. Click the **Plugins** tab.
   A summary table of the coverage analysis, by barcode, is included in the **coverageAnalysis** summary pane.
4. *(Optional)* From the **Executed At** dropdown list, select an alternate timestamp, if available, to view additional reports.
5. *(Optional)* Click **View Log** to view the coverageAnalysis log.
6. *(Optional)* Click **Delete** to delete the coverageAnalysis plugin output for the selected timestamp.

   IMPORTANT! If you click **Delete**, the report is deleted without the appearance of the confirmation window. Ensure that you intend to delete the report before clicking **Delete**.
7. Click ··· **(More)** ▸ **Download Files** to download coverageAnalysis plugin results files.

   Note: Sometimes the file name can be too long to open in applications such as Microsoft™ Excel™. To resolve this problem, right-click the file and click **Save As** to rename the downloaded file.
8. In the **coverageAnalysis** summary pane, in the **Barcode Name** column, click the link in the row of the barcode of interest.
   The detailed **Coverage Analysis Report** for the barcode opens in a separate window.

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 the report depend on the type of library that is used in the sequencing experiment. Definitions are in tooltips. Almost every statistic, plot, link, and functional widget in the report provides tooltips with definitions. Hover over a heading or description in the report to view the tooltip.

Table 10 General statistics

Statistic	Description
Number of mapped reads	The total number of reads mapped to the reference genome. **Note:** For Oncomine™ Comprehensive Assay v3 GX, do not use the number of mapped reads from coverageAnalysis plugin for RNA sample quality assessment. Use the QC results in the Genexus™ Software instead. For more information, see **Mapped Reads** in **Sample QC – RNA** in "QC results" on page 74.
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 is the percentage of reads passing uniquely mapped and/or non-duplicate 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.

Table 11 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. If there is 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.

Table 11 Amplicon read coverage statistics *(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 GC 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.

Table 12 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.

Table 12 Target base coverage statistics *(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.

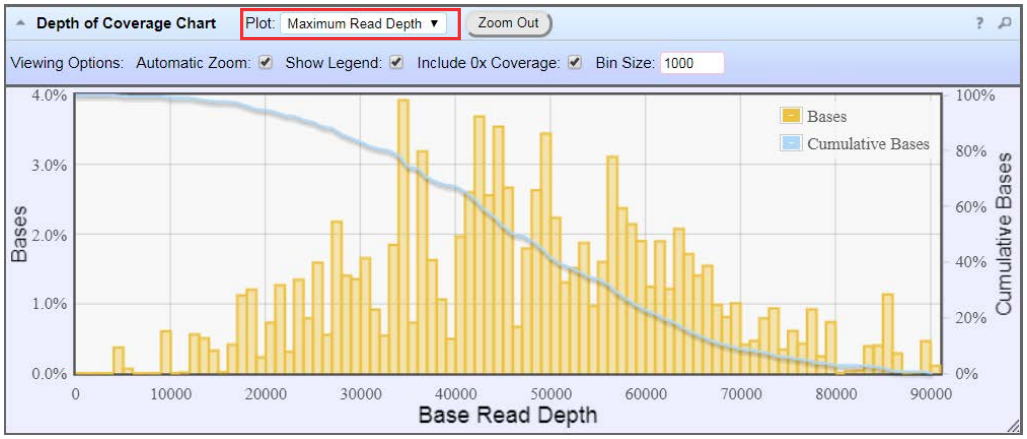


Figure 2 Representative Depth of Coverage Chart The Depth of Coverage Chart shows the distribution of targeted base coverage. The X-axis represents the base read depth. The left Y-axis represents the number of reads at a given base read depth or a range (bin) of base read depths, as a percentage of the 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.

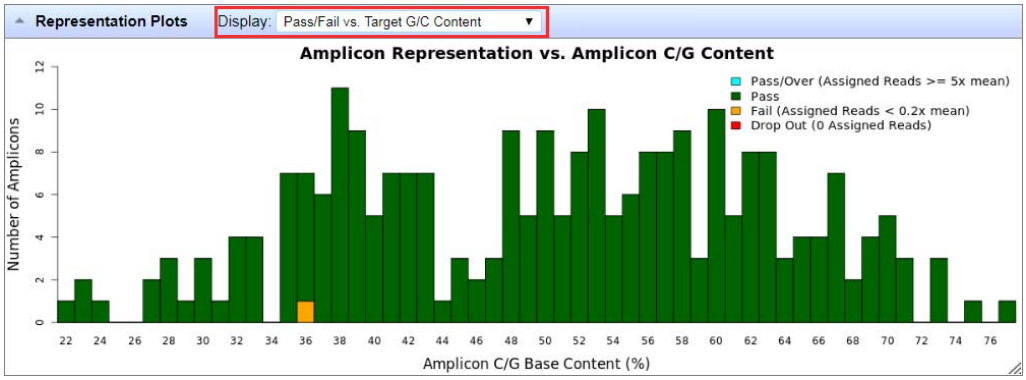


Figure 3 Representation Plots Use the **Display** dropdown list to switch between **Pass/Fail vs. Target G/C Content**, **Pass/Fail vs. Target Length**, **Representation vs. Target G/C Content**, **Amplicon Coverage Chart**, **Mean Target Reads vs Pool**, **Reference Coverage Chart**, and **Representation 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 100.

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.

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

Table 13 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`

Table 14 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.

Table 14 File contents *(continued)*

File	Description
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 14 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 `cov20x`, `cov100x`, and `cov350x`.
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.

Review sampleID plugin results in Genexus™ Software

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 toGenexus™ Software, click back in the browser.

Download results files for a sample result

You can download results files for a sample result in Genexus™ Software.

1. In the menu bar, click **Results** ▸ **Sample Results**, or **Results** ▸ **Run Results**, then do one of the following procedures.

Option	Selection
Download files for a sample	
Download results files for a sample from the list of sample results.	In the **Results / Sample Results** screen, place the pointer over the row of a sample of interest, then click **Download Files**.
Download results files for a selected sample from the **Results** screen.	1. In the **Results / Sample Results** screen, in the **Sample Name** column, click the sample name of interest. 2. Click ··· **(More Options)** ▸ **Download Files**.
Download files for a run or assay	
Download results files for a run from the list of run results.	In the **Results / Run Results** screen, place the pointer over the row of a run of interest, then click **Download Files**.
Download results files for a selected run or assay from the **Results** screen.	1. In the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest. 2. Click the run or assay of interest from the results navigation bar. 3. Click ··· **(More Options)** ▸ **Download Files**.

2. In the **Download Files** dialog box, select the files to download, then click **Download**.
   For information about the files, see “Results files” on page 100.

The selected results files are downloaded in one ZIP folder.

Results files

The following files can be downloaded from the **View Results** screen for each sample. The files that are available for download vary depending on the assay used. Results files include the sequencing data, results from the analyses, such as variant files, and audit and log files. For a list and descriptions of plugin output files, see the *Genexus™ Software Help*.

Option	File name	Description
Variants		
Filtered Variants (.vcf)[1]	`<Filter_name>_filtered.vcf`	Summary of filtered variant results in variant call format (VCF).
All Variants (.vcf)[1]	`Oncomine_<LibPrepID>_<analysisID>.vcf`	Summary of variant results in variant call format (VCF).
Snvindel (.tsv)	`Snvindel.tsv`	File that lists SNV/INDEL variant results in tab-separated value format (TSV).
Fusion (.tsv)	`Fusion.tsv`	File that lists fusion results in tab-separated value format (TSV).
CNV (.tsv)	`Cnv.tsv`	File that lists copy number variant results in tab-separated value format (TSV).
Sequencing Results		
DNA Unmapped Bam File (.bam)	`<barcode>_rawlib.basecaller.bam`	Unmapped DNA barcode BAM file; output after mapping reads to reference.
DNA mapped bam file (.bam)	`merged.bam`	Mapped BAM file of combined barcode reads.
DNA Mapped Bam Index File (.bai)	`merged.bam.bai`	Mapped BAM Index file.
DNA Basecaller FASTAQ File (.fastq)	`<barcode>_rawlib.basecaller.fastq`	FASTQ file of the DNA barcodes used.
DNA Processed Bam File	`merged.bam.ptrim.bam`	Mapped BAM file of combined barcode reads.
DNA Processed Bam Index	`merged.bam.ptrim.bam.bai`	Mapped BAM index file.
RNA Unmapped Bam File (.bam)	`<barcode>_rawlib.basecaller.bam`	Unmapped RNA BAM file; output of base calling, contains unmapped reads.
RNA Mapped Bam File (.bam)	`<barcode>_rawlib.basecaller_alignments.bam`	Mapped BAM file of combined barcode reads.
RNA Mapped Bam Index File (.bai)	`<barcode>_rawlib.basecaller_alignments.bam.bai`	Mapped BAM index file.

(continued)

Option	File name	Description
RNA Basecaller FASTAQ File (.fastq)	`<barcode>_rawlib.basecaller.fastq`	FASTQ file generated from the unmapped BAM file of the RNA barcodes used.
Test Fragment Basecaller FASTAQ File (.fastq)	`rawtf.basecaller.fastq`	FASTQ file for the test fragment.
Audit and Log		
Analysis Log File	`analysis.log`	Analysis log file.
Run Summary[2]	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.
Run Audit	`PlannedRun-AuditTrail.pdf`	Contains all audit records pertaining to the run.
Reports		
Report	`<language>_<sampleName>_<mode>_<templateName>_<assayName>_<date>.pdf`	A PDF report that contains sample-specific results. For more information, see “Variant report” on page 102.
Sample Summary[3]	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.
Troubleshooting Files		
Log Files[4]	`analysis.log`	Analysis log file.
	`summary.<timestamp>.log`	Start and end time for each time an assay module is executed for the analysis.
	`various.err` `various.out`	Analysis pipeline logs used by field service engineers for troubleshooting.
	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.

(continued)

Option	File name	Description
Log Files[4]	`PlannedRun-AuditTrail.pdf`	Contains the audit trail of the run plan in PDF format.
Other[4]	`analysis.ini` `analysisSamples.json`	Analysis configuration files, including secondary and tertiary INI files.
VCF Files	`analysis.vcf`	Summary of variant results in variant call format (VCF).

[1] You can view the extracted files individually, or upload a VCF file to a software application that accepts VCF files, such as Ion Torrent™Oncomine™ Reporter software.

[2] Files are available only for assays from Genexus™ Software version 6.2 and earlier.

[3] Files are available for both custom and system installed assays in Genexus™ Software version 6.6.

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

Variant report

A variant report is a PDF report of the results for each sample in a sequencing run. You can use a system-installed report template. For system installed report templates, the assay used in the run determines the data that are included in the report.

Alternatively, you can customize the layout and contents of a variant report.

To automatically generate a variant report for each sample during data analysis of a run, enable **Generate Report** in the **Setup** step when you plan the run (for more information, see Chapter 4, "Plan and manage runs"). Alternatively, if you did not enable this feature, you can generate a variant report for each sample after a run is complete. For more information, see the software help system.

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

- A link is available in the **Results / Sample Results** screen when you place the pointer over the row for that sample. Click the link to download the PDF.
- A **Download Report** button is available in the **Variant Report** pane in the **Reports** tab.

Variant reports can be electronically or manually signed by users. Electronically signed reports have **(Sign off)** after the sample name in the **Sample Results** screen. The electronic signature is included in the footer of the report. If included, electronic signatures also appear in the Electronic Signature section of the report. A sample name followed by **(Locked)** indicates that the variant report is locked. For more information, see "Sign off on the run results" on page 103.

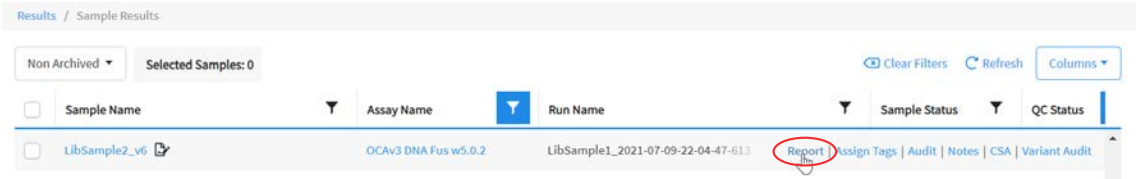
For more information about variant reports and report templates, see the software help system.

Download a variant report

You can download a variant report for a sample result of interest from the **Results / Sample Results** screen.

Note: The variant report is also available for download as part of the results files in the **Results** screen for a specific sample. For more information, see “Results files” on page 100.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, place the pointer over the row of the sample of interest, then click **Report**.



A ZIP file that contains the PDF report downloads automatically.

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

Sign off on the run results

Multiple users can sign variant reports. In the **Results / Sample Results** screen, a sample name followed by indicates that the sample results are signed off by at least one user. A sample name followed by indicates that the variant report is locked. After a variant report is locked, the report is no longer available to be signed by any other user. The signature information appears in the variant report PDF file. For more information, see “Variant report” on page 102.

Multilanguage support for PDF report generation is provided.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

Permission to sign reports must be enabled in order to sign reports. An administrator-level user manages electronic signature permissions for user accounts. For more information, see the software help system.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample result of interest.
3. In the upper right corner of the screen, click ··· **(More Options)** ▸ **Sign Off**.
4. In **Password**, enter the password.
5. In the **Electronic Signature** dropdown list, select the meaning of the signature.

6. In the **Report Template** dropdown list, select the report template that you want to use.

   The option to select the report template is available only before the variant report has been signed by any user.

7. In **Sign Off Comments**, enter a comment.

8. In **Report Name**, change the name of the report if needed.

   The option to change the report name is available only before the variant report has been signed by any user.

9. In **Select Your Language**, change the language of the report if needed.

   The option to change the language is available only before the variant report has been signed by any user.

10. In **Footer Field**, enter any text that you want to appear in the footer of the PDF report pages.

    If you entered footer information in the **Footer Field** when you created a report template, the same footer information appears in the **Electronic Signature** dialog box. You can enter new footer information to override the report template.

11. Click **Sign Off** to confirm the electronic signature.

The report is signed. If the signature is designated to lock the report, the variant report is signed and locked.

For information to download the signed report, see “Download a variant report” on page 103.

Example metrics for a successful Oncomine™ Comprehensive Assay v3 GX sequencing run

The following table summarizes typical QC metric values that are obtained from successful sequencing runs. The QC metric values in the table are recommendations and not absolute thresholds. Sequencing runs with values below those specified in the table may still be acceptable, but require more scrutiny. For help, contact support.

IMPORTANT! Actual QC threshold values can be viewed by clicking on the assay name in the **Assays** ▸ **Manage Assays** screen. To edit the QC threshold values, see “Edit the QC parameters of an assay” on page 121.

Table 15 Example QC metrics for successful sequencing runs

QC metric	Value
Run QC	
Key Signal	≥55
Percent Loading	≥75%
Raw Read Accuracy	≥98%

Table 15 Example QC metrics for successful sequencing runs *(continued)*

QC metric	Value
Templating QC – CF-1 Control	
Average Reads Per Lane	≥400
Sample QC – DNA	
Mapped Reads	≥3,000,000
Mean Read Length (bp)	≥75
Uniformity Of Amplicon Coverage	≥85%
Sample QC – RNA	
Mapped Reads	≥200,000
Mean Read Length (bp)	≥60

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Troubleshooting

Genexus™ Integrated Sequencer—general and QC troubleshooting

Observation	Possible cause	Recommended action
A consumable is not recognized by the sequencer after loading on the deck	The consumable (for example, a strip, pipette tip box) is correctly placed but is not completely inserted into its position, causing it to be misaligned with its expected position.	Ensure that the consumable is pressed completely into place. Apply firm pressure on the item so that it fits snugly into its deck position.
	The barcode of the consumable is not readable by the instrument.	Tap **Help** in the lower left corner of the **Load Instrument** screen and follow on-screen instructions to override the block manually. Note that the name of the consumable does not appear in the list of consumables in the run summary.
		If the behavior continues in subsequent runs, contact Technical Support.
	Consumable version does not match the Genexus™ Software version. For example, a consumable compatible with Genexus™ Software 6.6 is installed in a sequencer updated for Genexus™ Software 6.6.	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.

Observation	Possible cause	Recommended action
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. After cleaning, start a new run. **Note:** The **Clean instrument** procedure renders remaining sequencing reagents and unused lanes on the installed chip unusable in a sequencing run after the cleaning.
The number of sample reads is low, CF-1 metrics pass QC, but read ratio of inline controls is low **Details:** If CF-1 reads per lane, accuracy, and mean AQ20 read length are good, and read ratio of inline controls (endogenous vs. spike-in) is low (<< ~3), a problem with sample input is indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 109.	Nucleic acid input may have been insufficient, and/or the nucleic acid was degraded.	For a sample run, requantify nucleic acid samples and/or perform sample QC to ensure that the expected nucleic acid input and size was loaded.
		If needed, reisolate and purify nucleic acid samples.
The number of sample reads is low, but CF-1 metrics pass QC, and read ratio of inline controls is normal **Details:** If CF-1 metrics passed QC, and read ratio of inline controls is normal (~~3), a problem in library preparation unrelated to sample input or quality may be indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 109.	One or more of the Genexus™ Strip 1 strips used in the run had magnetic beads trapped in the tube 5 keyhole.	Repeat the run with strips that you have verified have no trapped beads. For more information, see "Before you begin" on page 59.
	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 109.	One or more of the Genexus™ Strip 3-GX5™ strips used in the run may have had an excessive amount of magnetic beads trapped in the tube 6 or 7 keyhole.	Repeat the run with strips that you have verified have no trapped beads. For more information, see "Before you begin" on page 59.

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 109. *(continued)*	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.
	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 the *Genexus™ Integrated Sequencer User Guide*. 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).
An Oncomine™ Comprehensive Assay v3 GX DNA and Fusion run fails the QC threshold for the MAPD parameter due to a decrease in mean read length (MRL) of DNA pool 2.	At high temperatures (within instrument specifications), the Genexus™ Integrated Sequencer PCR block is susceptible to temperature variation in the lower, middle area of the cycler block. This variation can affect assay performance.	A reduction from 99°C to 98°C for both the activation and denaturation steps in the library preparation target amplification PCR profile for Ion AmpliSeq™ Assays results in a more consistent library preparation performance, which in effect also produces more stable and predictable sequencing results. Contact your field service representative to obtain the ADF package (version w6.0.2 or later) that contains these temperature settings.

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 106.

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.

Observation	Possible cause	Recommended action
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique sample name.	Every sample name in the software must be unique. Ensure that the spreadsheet does not contain any duplicate sample names, then repeat the import. Note that the system check is not case-sensitive, so a sample name of ABC1 conflicts with abc1.
	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors. Map a sample attribute if needed.
Cannot upload my panel or hotspots	Issues with BED file format or files do not end in ***.bed***.	Ensure your file is in the correct BED format and has a ***.bed*** extension.
Variants tab is missing hotspot entries **Details:** The remaining entries are present.	Hotspot BED file contains entries that are incorrectly formatted.	Check that BED file entry is correctly formatted. See the following examples: SNP entry: chr1 2337276 2337277 SVA_322 0 + REF=C;OBS=T;ANCHOR=G AMPL Deletion entry: chr1 201341175 201341180 SVA_497 0 + REF=AGAAG;OBS=;ANCHOR=C AMPL Insertion entry: chr1 236978992 236978992 SVA_621 0 + REF=;OBS=TCTG;ANCHOR=T AMPL
		Ensure that the REF values match the actual reference coordinate of hg19.
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 *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

Observation	Possible cause	Recommended action
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

Working with samples

System-installed sample attributes

The following table lists and describes system-installed sample attributes. System-installed sample attributes cannot be edited.

Note: Custom sample attributes are not listed in this table.

Sample attribute	Description
Sample Name[1]	A unique identifier representing the sample. The sample name can contain only alphanumeric characters (0–9, Aa–Zz), periods (.), underscores (_), or hyphens (-), cannot contain spaces, and is limited to a maximum of 20 characters. **IMPORTANT!** To prevent erroneous sample selection during run planning, ensure that you assign a unique and distinguishable sample name for each sample. **Note:** • Samples that have been used in a run cannot be deleted. • To prevent duplication, the software checks all sample names and returns an error message if a non-unique sample name is detected.
Collection Date[1]	The date that the sample was collected. Click **Calendar** to select the date in the correct format.

(continued)

Sample attribute	Description
Gender[1]	The biological sex of the sample: **Female**, **Male**, or **Unknown**. **IMPORTANT!** **Male** or **Female** must be selected for correct measurement of AR CNV.
Sample Type[1]	A term that describes the sample, for example, FFPE, DNA, DNA & RNA. You can also select **Other**, then enter a custom sample type.
Nucleic Acid Type	The sample nucleic acid type, such as DNA, RNA, or TNA.
Application Category[1]	The sample application category, such as **Cancer (Germline)** or **Solid Tumor**. **Note:** If you select an oncology application category in this list, the **Cancer Stage**, **Cancer Type**, **% Cellularity**, and **% Necrosis** attributes listed below become available in the **Add New Sample** dialog box.
Cancer Type[1]	The type of cancer that is represented by the sample. Select the type of solid or hematologic cancer. If cancer type is unknown, select **Unknown Primary Origin**.
Cancer Stage[1]	The stage of the cancer from which the sample was collected. Select **Stage 0–IV**, or **Primary**, **Unknown**, or **Other**.
% Cellularity	The percentage of tumor cells over normal cells in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is applicable only to FFPE samples. **IMPORTANT!** • If this value is not set, **% Cellularity** is assumed to be 100% in calculations that use this attribute. • **% Cellularity** is a required attribute for CNV analyses. Do not leave the field blank. • *(FFPE samples only)* If **% Cellularity** value is set to <100, then the magnitude of copy number gain or loss can be decreased. For more information, see the *Genexus™ Software Help*.
% Necrosis	The percentage of cellular necrosis in the sample. This is a whole number between 1 and 100.
Notes	An open-entry field for more sample information.

[1] Required attribute

Import samples

You can enter sample information for multiple samples directly in Genexus™ Software. When you want to create more than a few samples, an easy and fast way to add multiple samples in the software is to create a file of information for a group of samples and import that file.

Sample data files can be used to capture, manage, and edit sample data. You can import sample data files in TSV, XLS, XLSX, or CSV file formats. For a list of the sample attributes that are included in the import file, see the *Genexus™ Software Help*. For ease of use, you can download a Microsoft™ Excel™ template file to create an import file.

1. In the menu bar, click **Samples** ▸ **Create Sample**.
2. In the **Create Sample** screen, click the **Multiple (.xls, .csv, .tsv)** tab.
3. In the **Application Category** dropdown list, select the application category for the samples.

   Tip: Use the search field to search for the application category of interest.

4. Set up a sample file using one of two options.

Option	Description
Download a template file, then edit it to create a new file.	For more information, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409). Upload the edited file using the **Browse** button. **Note:** When you select **Other** as the **Application Category**, you must enter text in the **Application Category** text box, then click anywhere in the screen in order for the template file to be available.
Upload the sample data from an existing file.	a. Click **Browse**. b. Navigate to the file, then click **Open**. The data contained in the file populates the table in the screen.

5. In the sample file, edit the sample table and data, if needed.

Option	Description
Remove an attribute column.	Click **Columns**, then deselect the column name.
Add an attribute column to the table.	Click **Columns**, then select the column name. The column names that are listed are **Application Category**-specific.
Edit sample data.	Click the sample row of interest, then edit the text field or dropdown list for the sample.
Add more samples to the table.	Click **Add Row**, then enter the sample data.
Remove a sample from the table.	Select the checkbox for the row, then click **Remove Row**.

6. Select the checkbox in the row for each sample to create. To select all samples, select the checkbox in the column heading row.

   IMPORTANT! The information for samples that are not selected is not retained by the software. Ensure that you select the checkbox for every sample you intend to create.

7. Click **Save**.

   You can place the pointer over ⓘ **(alert)** to view more information if needed.

The new samples are listed in the **Manage Samples** screen and are available to assign to a run plan.

Guidelines for Oncomine™ Comprehensive Assay v3 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™ Comprehensive Assay v3 GX” on page 30). To copy a system-installed assay, see the software help system. The following table provides guidelines on modifying key assay settings and recommendations for quality control metric threshold values.

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

Workflow step	Setting	Guidelines
Panel	**Minimum Read Count Per Sample**	This value determines the number of samples that are allowed on a chip. The Genexus™ Software uses this value to calculate how many lanes a run plan uses, then assigns samples accordingly. *Minimum Read Count Per Sample = (12,000,000 × number of lanes)÷(number of samples)* To increase or decrease the sequencing depth, adjust the **Minimum Read Count Per Sample** parameter. If higher sequencing depth is desired, decrease the number of samples per lane or increase the number of lanes used. For more information, see “Chip capacity by sample type” on page 36.
QC (Sample QC - DNA)	**MAPD**	0.5
QC (Sample QC - RNA)	**Mapped Reads**	200,000
	Mean Read Length (bp)	60

(continued)

Workflow step	Setting	Guidelines
Parameters	**Sample input Maximum (ng)**	By default, the target sample concentration for the Oncomine™ Comprehensive Assay v3 GX is set to a single value in the assay settings, where a sample input maximum is set to the same value as sample input minimum. You can modify the parameters to set a sample concentration range by adjusting the sample input maximum and minimum values if needed. The target concentration for a sample is the middle point of the range. When you modify the sample input maximum and minimum values, the **Sample Concentration Maximum (ng)** and **Sample Concentration Minimum (ng)** parameters are auto-adjusted to reflect the new maximum concentration values.
	Sample input Minimum (ng)	**Note:** The sample volume that is required for library preparation is not adjustable. The volume depends on the number of primer pools in the assay, sample type, and library chemistry. For specific sample volumes to load onto the sample plate, see “Dilute the samples and load the sample plate” on page 57. When you set a range for sample concentration in the assay settings, the instrument does not dilute the sample during the run as long as the concentration of a sample is within the designated range. If the sample concentration is outside of the range that is designated in the assay settings, the instrument dilutes the sample to the target concentration (the middle point of the range). For more information, see “Plan a Nucleic Acid to Result run” on page 33.

View assay metrics and the run report

The run report provides detailed information about a run, such as the **Total Bases** and **Final Reads**. The run report contains the assay metrics for all assays in the run.

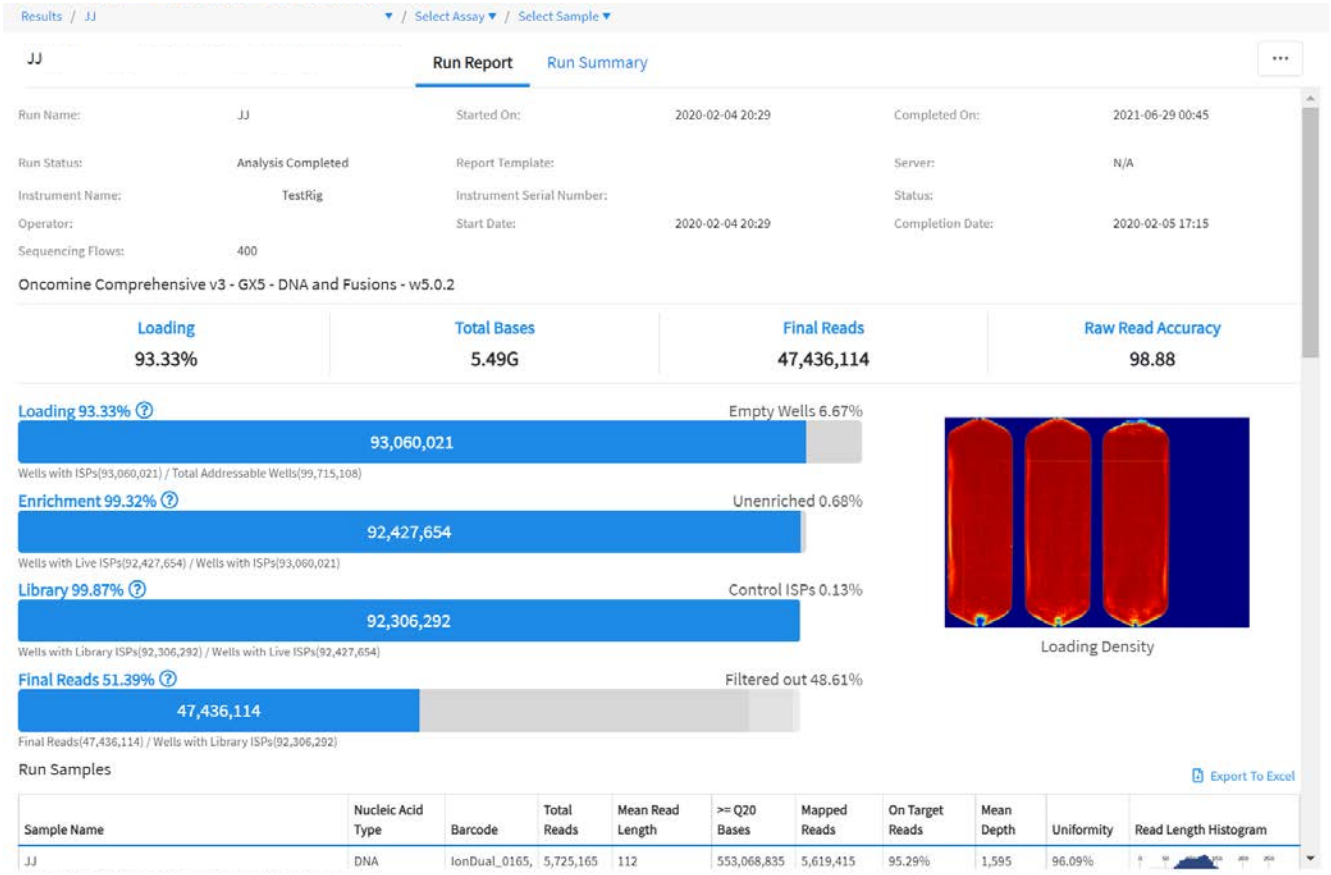
For a run with only one assay, the run report and assay metrics show the same information for assay metrics. In addition to the metrics for the assay, the run report shows information about the run and the instruments used in the run.

To view the **Run Report**, in the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest. In the **Run Summary**, click the **Run Report** tab.

The **Run Report** is assay-specific and cannot be viewed within the sample results screens. To view the **Run Report**, ensure that **Select Sample** is selected in the **Select Sample** dropdown menu.

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.



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 information about how to download the CSA file, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

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.
Final Reads	Library reads passing all filters that are recorded in the output BAM files.

Table 16 Run Report *(continued)*

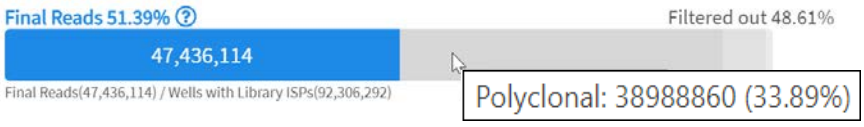
Metric	Description
Total Bases	The number of filtered and trimmed base pairs that are reported in the output BAM file.
Raw Read Accuracy	The raw read accuracy across each individual base position in a read calculated as, (1–[total errors in the sequenced reads]/[total bases sequenced]) × 100. Raw read 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.
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. 

Table 16 Run Report *(continued)*

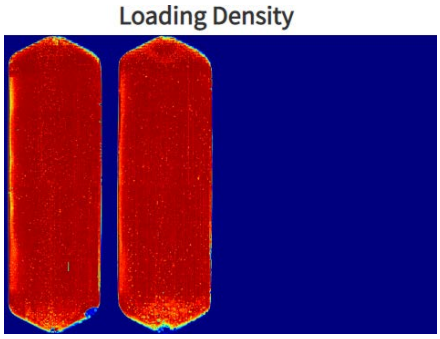
Metric	Description
Adapter Dimer	Loaded wells with a library template of an insert size less than 8 bases. To view adapter dimer metrics, mouse over the lightest gray portion of the **Final Reads** bar plot. 
Loading Density	A visual representation of chip loading. Red color indicates areas of higher 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 or RNA.
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.

Table 17 Run Samples *(continued)*

Column	Description
Uniformity	Uniformity of Base Coverage. The percentage of bases in all targeted regions (or whole genome) with a depth of coverage ≥20% of the mean base 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 dual barcode assigned to a single DNA or RNA library for a sample.
Total Reads	The total number of filtered and trimmed reads assigned to the specific dual barcode. 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 barcode.
≥Q20 Bases	The total number of called bases that have ≥99% accuracy (or less than 1% error rate) aligned to the reference for the barcode.

Download a run report

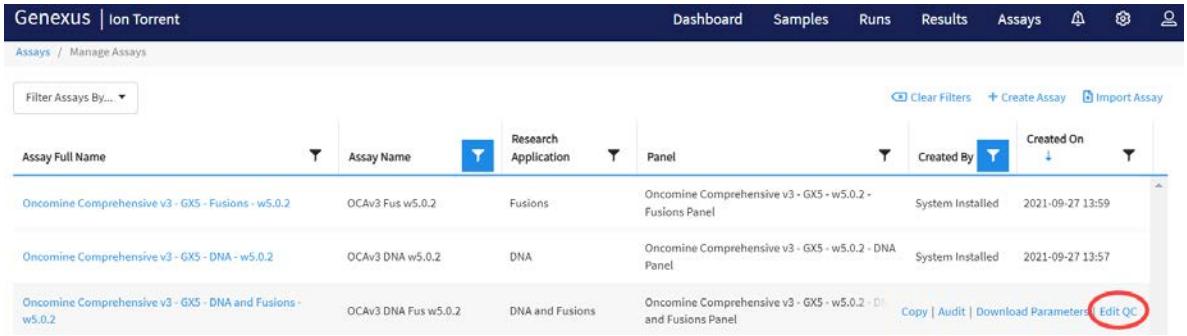
You can download a run report summary in PDF format. The run report includes assay metrics and the record of reagents that were used in a run. For information about the contents of the run report, see "View assay metrics and the run report" on page 116. If you entered extraction kit barcodes for samples when you prepared library batches or when you planned the run, the extraction kit barcodes are listed in the run report.

1. In the menu bar, click **Results ▸ Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample name of interest.
3. Click the **Reports** tab.
   Multiple panes including a **Run Report** pane, a **Variant Report** pane, and any panes for customized reports that have been generated are shown.
4. In the **Run Report** pane, click **Download Report** to download a run report summary in PDF format.

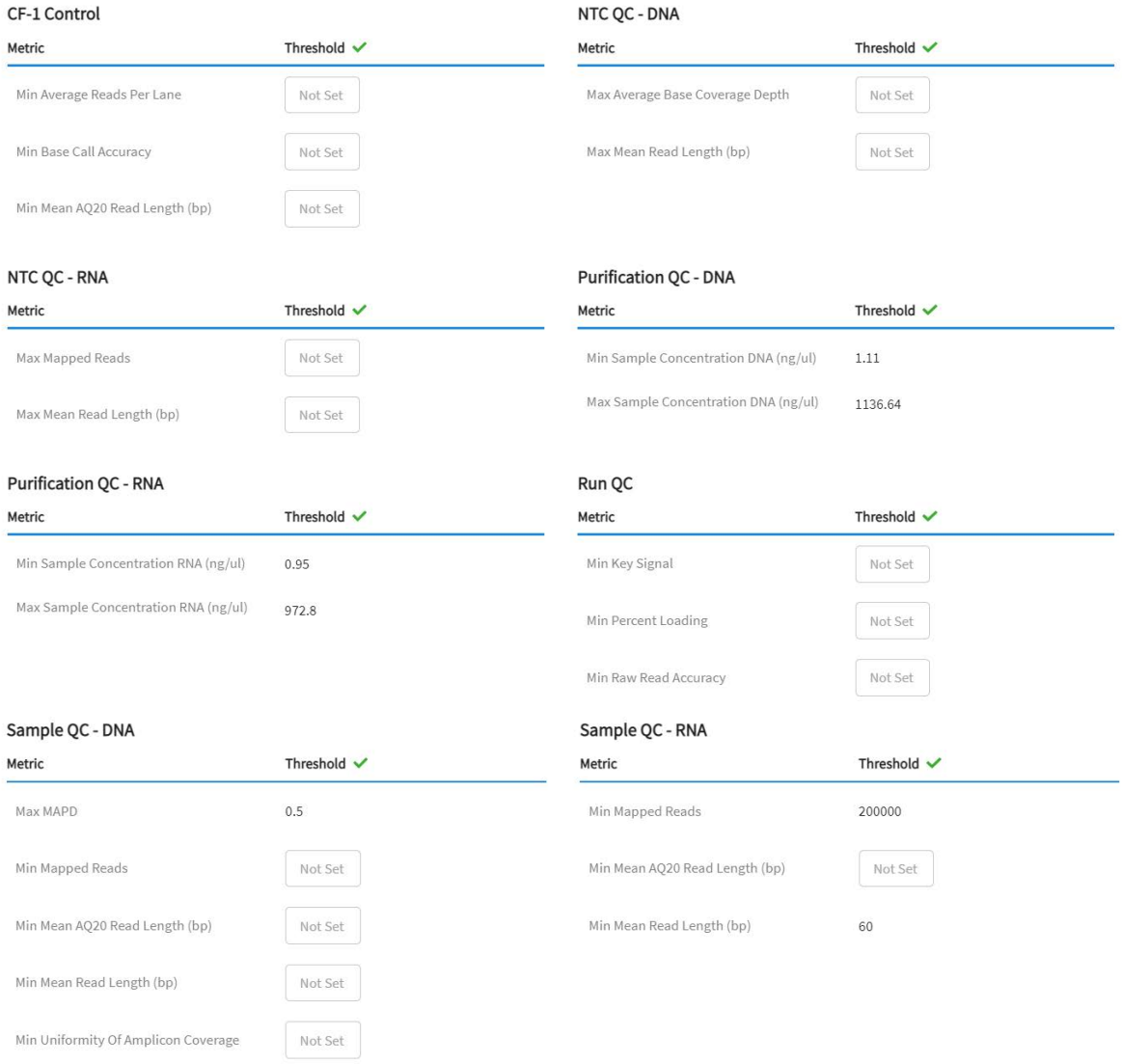
Edit the QC parameters of an assay

Manager- and administrator-level users can edit the QC parameters of any locked assay, including system-installed assays, if needed. When the QC parameters are changed, the edited QC parameters are applied to any new run that uses the assay. Edits to QC parameters do not affect runs that use the assay and are completed before edits are made.

1. In the menu bar, click **Assays** ▸ **Manage Assays**.
2. In the **Manage Assays** screen, hover the pointer over the row of the assay, then click **Edit QC**.



The **Edit QC** dialog window opens.



3. Edit the QC parameters as desired.

   To reset the QC parameters to the original values in a subsection, click **Reset** in the upper right corner of the subsection.

4. Click **Save**.

The new QC parameter values are saved and applied in any run that uses the assay after the changes are saved.

Requirements for Oncomine™ Comprehensive Assay v3 GX variant annotation

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	**Gain-of-Function**	**Amplification**	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Long deletion	**Loss-of-Function**	**LongDel**	• Positive mutation call • SVTYPE = "LongDel"
Gene fusion	**Gain-of-Function**	**Fusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
Non-targeted fusion	**Gain-of-Function**	**NonTargetedFusion**	• SVTYPE = "Fusion" • FILTER = "PASS" • Non-Targeted field is present • NT_FUSION_IN_FRAME is not FALSE • NT_FUSION_DRIVER_INVOLVED is not FALSE • NT_FUSION_SECONDARY is not TRUE
RNA exon variant	**Gain-of-Function**	**RNAExonVariant**	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function truncating de novo mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function gene
Missense hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Splice site hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and coding syntax occur in predefined intronic hotspot list
Promoter hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript, location, and coding syntax occur in predefined promoter hotspot list
Gain-of-function truncating hotspot mutation	**Gain-of-Function**	**Hotspot**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	**Loss-of-Function**	**Truncating**	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	**Gain-of-Function** **Loss-of-Function**	**Hotspot**	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
EGFR exon 19 deletion[1]	**Gain-of-Function**	**EGFRExon19Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744–761 of EGFR
EGFR exon 20 insertion[1]	**Gain-of-Function**	**EGFRExon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762–775 of EGFR or variant is in EGFR exon 20 insertion confirmed list

(continued)

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
ERBB2 exon 20 insertion[1]	**Gain-of-Function**	**ERBB2Exon20Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770–783 of ERBB2
KIT exon 11 deletion[1]	**Gain-of-Function**	**KITExon11Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 550–580 of KIT • Alternatively, mutation occurs in splice site flanking the 5' end of exon 11
KIT exon 11 insertion[1]	**Gain-of-Function**	**KITExon11Insertion**	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 550–580 of KIT
MET exon 14 skipping[1]	**Gain-of-Function**	**METExon14Skipping**	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET Exon 14 Skipping confirmed list
PDGFRA exon 18 deletion[1]	**Gain-of-Function**	**PDGFRAExon18Deletion**	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 841–847 of PDGFRA

[1] For more information, see "Ion Torrent™ Class-Based Variants" on page 126.

Ion Torrent™ Class-Based Variants

Ion Torrent™ Class-Based Variants are a set of logic-based rules that Genexus™ Software uses to computationally identify and annotate novel mutations in important genomic variant classes. The annotation logic is based on scientific literature evidence. Details about the logic follow.

EGFR exon 19 deletion

- **Background:** Epidermal growth factor receptor (EGFR) is a transmembrane receptor protein made up of 1210 amino acids and located on chromosome 7p11.2. EGFR has 31 exons— exons 2-4 and 8-12 encode the ligand binding domains, exons 5-7 and 13-16 encode the cysteine rich domain, exons 18–24 encode the tyrosine kinase domain (TKD), and exons 25-28 encode the autophosphorylation region [PMID: 17318210]. Mutations in EGFR often target the TKD which make up the ATP-binding pocket of the enzyme [PMID: 17318210]. Exon 19 deletions are indels that range between 15-18 bp in length and usually occur within codons 746-756 [PMID: 23768755, PMID: 22190593, PMID: 26933124, PMID: 24163741]. In non-small cell lung cancer (NSCLC), EGFR exon 19 deletions, located within codons 746-750, represent 45-50% of somatic mutations [PMID: 26933124, PMID: 24163741]. Exon 19 deletions result in constitutive activation of the receptor tyrosine kinase and hyperactivation of downstream signaling pathways [PMID: 29455648].
- **CBV Criteria:** The boundaries defined to capture EGFR exon 19 deletions as a class-based variant start at codon 744 and end at codon 761, which encompass common and rare indels [PMID: 30473385].

EGFR exon 20 insertion

- **Background:** Epidermal growth factor receptor (EGFR) is a transmembrane receptor protein made up of 1210 amino acids located on chromosome 7p11.2. EGFR has 31 exons— exons 2-4 and 8-12 encode the ligand binding domains, exons 5-7 and 13-16 encode the cysteine rich domain, exons 18–24 encode the tyrosine kinase domain (TKD), and exons 25-28 encode the autophosphorylation region [PMID: 17318210]. Mutations in EGFR often target the TKD which make up the ATP-binding pocket of the enzyme [PMID: 17318210]. In-frame insertions within exon 20 of EGFR are the third most common type of mutation found in NSCLC, representing 4-12% of all EGFR mutations in NSCLC [PMID: 31208370]. EGFR exon 20 encompasses codons 762 to 823. Exon 20 insertions commonly involve codons 762 to 774 wherein codons 762-766 make up the c-helix and codons 767-774 make up the activation loop [PMID: 31208370, PMID: 30854234]. EGFR exon 20 insertions do not alter the receptor binding affinity; however, it is suggested that the location of exon 20 insertions stabilizes its active confirmation [PMID: 30854234, PMID: 27843613].
- **CBV Criteria:** The boundaries defined to capture EGFR exon 20 insertions as a class-based variant start at codon 762 and end at codon 775. Additionally, known EGFR exon 20 insertions p.A763_Y764insFQEA and p.A763_Y764insLQEA, represented as c.2284–6_2284–5insTCCAGGAAGCCT and c.2284–9_2284–8insCCCTCCAGGAAG due to left alignment into the intronic region, are included in this rule.

ERBB2 exon 20 insertion

- **Background:** Erb-b2 receptor tyrosine kinase 2 (ERBB2) is a transmembrane glycoprotein located on chromosome 17q12 [PMID: 25276427]. ERBB2 (also known as HER2) has 27 exons— exons 2-4 and 9-12 encode the extracellular receptor L domains, exons 5-8 encode the furin like domain, exons 13-16 encode the growth factor receptor domain IV, exon 17 encodes the transmembrane domain, and exons 18–24 encode the tyrosine kinase domain (TKD) [PMID: 22761469, PMID: 29420467]. In lung cancer, the most recurrent ERBB2 activating mutations include in-frame exon 20 insertions [PMID: 30425522]. ERBB2 exon 20 involves codons 770 to 831 and majority of exon 20 insertions occur between codons 775 and 781 within the kinase domain [PMID: 29686424, PMID:22761469]. Insertions at the C-terminal end of ERBB2 exon 20 induce a change in conformation of the α-C helix leading to a constitutively active formation which affects the drug-binding pocket [PMID: 29686424].
- **CBV Criteria:** The boundaries defined to capture ERBB2 exon 20 insertions as a class-based variant start at codon 770 and end at codon 783.

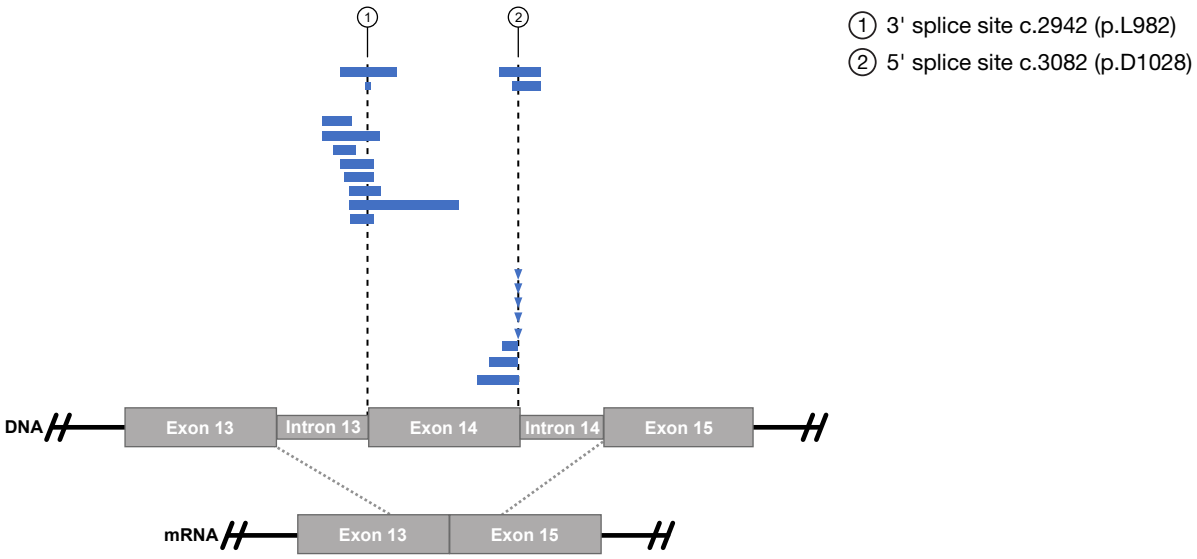
KIT exon 11 insertion and KIT exon 11 deletion

- **Background:** KIT, also known as CD117, is a proto-oncogene receptor tyrosine kinase made up of 976 amino acids located on chromosome 4q12 [PMID: 16689459]. KIT is a member of the PDGFR type III receptor tyrosine kinase family [PMID: 26579483; PMID: 26309392]. KIT has 21 exons— exons 1–9 encode the extracellular ligand-binding region containing five Ig-like domains, exon 10 encodes the transmembrane (TM) domain, and exon 11 encodes a cytoplasmic juxtamembrane region. The intracellular region of KIT is encoded by exons 12–21, wherein, exons 12–14 encode the tyrosine kinase domain 1, exons 15–16 encode the kinase insert (KI) region, and exons 17–19 encode the tyrosine kinase domain 2 [PMID: 24449920, PMID: 27536065, PMID: 31378162, PMID: 15342366, PMID: 17555444]. Recurrent somatic KIT alterations are observed in both solid and hematological cancers and include activating mutations such as single nucleotide variants (SNVs), small duplications, and complex in-frame insertions or deletions (indels). Mutations in KIT exons 8, 9, 11, and 17 disrupt the auto-inhibitory mechanisms and lead to constitutive activity [PMID: 27536065]. While mutations in exons 8 and 17 are common in myeloid cancers, 60–70% of somatic mutations (including SNVs and indels) in gastrointestinal stromal tumor (GIST) occur in exon 11, which encodes the juxtamembrane domain [PMID: 27536065, PMID: 22588877, PMID: 23678293; NCCN-GIST].
- **CBV Criteria:** The boundaries defined to capture KIT exon 11 insertions and deletions as a class-based variant start at codon 550 and end at codon 580.

MET exon 14 skipping

- **Background:** The MET proto-oncogene is a receptor tyrosine kinase made up of 1390 amino acids and is located on chromosome 7q31 [PMID: 15735036]. MET has 21 exons containing three main structural domains—an extracellular Sema domain in exon 2, a juxtamembrane domain in exon 14, and a tyrosine kinase domain in exons 15-21 [PMID: 28376232, PMID: 9380410]. Splice site mutations flanking exon 14 are observed in 4% of non-small cell lung cancer (NSCLC). These mutations include canonical splice site mutations affecting exon 14 and deletions that extend into the splicing motifs within intron 13 [PMID: 25971938, PMID: 27343443]. Such mutations disrupt splicing leading to the formation of an alternative transcript that joins exon 13 directly to exon 15 and skips exon 14 entirely. The MET exon 14 skipping transcript lacks the juxtamembrane domain that contains the recognition motif for ubiquitin-dependent proteolysis and thus leads to a marked increase in steady-state level of the MET protein [PMID: 28164087].

- **CBV Criteria:** The boundaries defined to capture MET exon 14 skipping as a class-based variant can be summarized into four individual rules: (a) has transcript = NM_001127500.3, exon = 14, and location = splicesite_3 (at c.2942) or splicesite_5 (at c.3082), (b) is an intronic deletion ≥ 4bp impacting 30 nucleotides preceding exon 14, (c) is a missense variant at c.3082 [PMID: 27343443, PMID: 26729443, PMID: 25971938, PMID: 25898962, PMID: 25898965, PMID: 31472177], (d) is one of the following variants with confirmed skipping defined as c.3082delG [PMID: 25971938, PMID: 27343443], c.3066_3081delAGCTACTTTTCCAGAA, c.3075_3082del [PMID: 31472177], or c.3080_3081delAA [PMID: 26729443].



PDGFRA exon 18 deletion

- **Background:** Platelet derived growth factor receptor alpha (PDGFRA) is a cell surface tyrosine kinase receptor made up of 1089 amino acids and located on chromosome 4q12. PDGFRA contains 23 exons –exons 1-2 encode the signaling peptide, exons 3-10 encode the extracellular domain, exon 11 encodes the transmembrane domain, exon 12 encodes the juxtamembrane domain, and exons 13-23 encode the kinase domain [PMID: 33449152]. Mutations in PDGFRA often target the activation loop located within exon 18. Additionally, mutations targeting the the juxtamembrane domain and the N-lobe of the kinase domain found within exons 12 and 14 to a lesser extent. [PMID: 30778083, PMID: 33449152, PMID: 15146165]. Activating PDGFRA exon 18 mutations such as V561D, N659K, D842V, and in-frame deletions are observed in 30-40% of KIT-negative gastrointestinal stromal tumors (GISTs) [PMID: 17193822, PMID: 15928335, PMID: 12522257, PMID: 23970477]. Specifically, PDGFRA in-frame deletions in codons 841-847 make up 23% of all exon 18 mutations [PMID: 15146165].
- **CBV Criteria:** The boundaries defined to capture PDGFRA exon 18 deletions as a class-based variant start at codon 841 and end at codon 847.

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).

Install software and assay definition file updates

Administrator-level users can select **(Settings) ▸ Software Updates** to view:

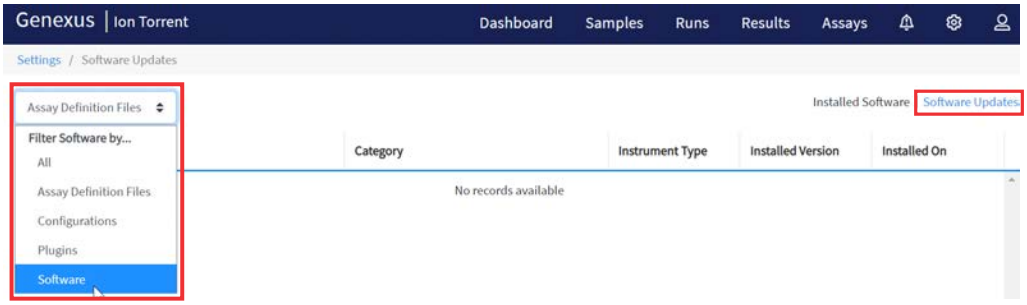
- The currently installed software versions for each software module, assay definition file, plugin, and configuration package installed on the system under the **Installed Software** link.
- Check for new software updates that are available to be downloaded and installed under the **Software Updates** link.

In order to download and install software and assay definition file (ADF) updates, you must first:

- Either, configure a Thermo Fisher™ Connect Platform account or format a USB drive for the software update. For instructions, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953). For users who have set up an account, **(Notification)** appears in the Genexus™ Software banner at the top of the screen upon login when updated software packages are available for download.
- Ensure that the Genexus™ Integrated Sequencer is running the latest Genexus™ Software version.

IMPORTANT! Do not reboot the system during installation.

- Download and install software updates directly.
  a. In the menu bar, click **(Settings) ▸ Software Updates**, then click **Software Updates** in the upper right corner of the screen.
  The **Software Updates** screen opens with **App Store** enabled.



  b. From the **Filter Software by** dropdown list, select **Software** to view available software package updates or **Assay Definition Files** to view available assay package updates.
  The list of available updates appear.

c. In the **Actions** column, in the row of the package of interest, click **Download**.
After completion of the download, the **Download** link changes to an **Install** link.

d. Click **Install**, then click **Yes** to confirm installation.

- Download and install software with a USB drive
  a. Sign in to the Thermo Fisher App Store on Connect at **apps.thermofisher.com**.

  b. Click **(AppConnect)**, then under **Resource Libraries**, click **Ion Torrent™ Genexus™**.

  c. Click **Software Update**, then in the row of the package of interest, click **(Download)**.

  d. Copy the downloaded package (.zip) to the `/media/usbinstall` folder in the USB drive, then insert the drive into one of the USB ports at the back of the Genexus™ Integrated Sequencer.

  e. In the menu bar, click **(Settings)** ▸ **Software Updates**, then click **Software Updates** in the upper right corner.

  f. Select **USB**.

  g. Click **Install**, then click **Yes** to confirm installation.

When installation of the package is complete, click **Installed Software** to verify the installation.

C

Release Notes

This appendix outlines major improvements in Oncomine™ Comprehensive Assay v3 GX, issues fixed and known issues to be fixed in subsequent releases.

Oncomine™ Comprehensive Assay v3 GX ADF 6.0.2

The Oncomine™ Comprehensive Assay v3 GX provides comprehensive genomic profiling with analysis of SNVs, INDELs, CNVs and gene fusions from 161 unique genes. The updated Assay Definition File (ADF) version 6.0.2 requires Genexus™ Software 6.8.2 or later and includes the following improvements:

- Assay performance improvements—workflow parameters are further optimized to increase assay robustness and reproducibility.
- Variant calling:
  - Ion Torrent™ Variant Caller (TVC) v5.20 module was updated to v5.20-17. The version number is visible in the header of the VCF file.
  - Sequence variant baseline, hotspots parameters were further optimized to better detect key variants in BRCA2, NOTCH2, PIK3CA, RNF4, BRAF, MET, RAD50, TERT, KIT, PDGFRA genes.
- Fusions—Fusion Reference and Property files updated to include additional EML4-ALK fusion isoforms.
- Variant filter chain—Updated to Oncomine Extended (6.8) Filter Chain to enable identification of additional variants.
- Oncomine™ Variant Annotator (OVAT) Ruleset—Updated to enable KIT and PDGFRA Class-Based Variants and incorporate updated hotspot variants. For a complete list of supported Class-Based Variants, see “Requirements for Oncomine™ Comprehensive Assay v3 GX variant annotation” on page 123.

Issues fixed in Oncomine™ Comprehensive Assay v3 GX ADF 6.0.2

- In the Oncomine™ Comprehensive Assay v3 GX w5.0.2 ADF file, the PCR amplification temperature was 99°C which may contribute to high MAPD, low uniformity, and low total AQ20 reads.

  To address this problem, the run parameter (`amplifyTargetPCRProfile`) value for PCR amplification temperature in library preparation is updated to 98°C in all three workflows.
- To track samples in Genexus™ Software analysis using the SampleID plugin, the assay had to be manually edited to select the plugin.

  In the new Oncomine™ Comprehensive Assay v3 GX ADF 6.0.2 the SampleID plugin (v5.16.0.3) is preconfigured for DNA only, DNA, and Fusion workflows.

D

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™ Purification System User Guide	MAN0018475
Genexus™ Software 6.8 User Guide	MAN0026409
Genexus™ Software 6.8 Help	Available in the software
Oncomine™ Reporter User Guide	MAN0018068
Qubit™ dsDNA HS Assay Kits User Guide	MAN0002326
Qubit™ RNA HS Assay Kits User Guide	MAN0002327

Note: For additional documentation, see “Customer and technical support”.

Customer and technical support

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

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

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

Limited product warranty

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