iontorrent

Oncomine™ Comprehensive Assay v3 GX

USER GUIDE

For use with the Genexus™ Integrated Sequencer

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Revision B.0

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

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Revision History: Pub. No. MAN0018512

Revision	Date	Description
B.0	09 July 2020	• Updated protocol for Genexus™ Software version 6.2. • Removed Ion Reporter™ Software Appendix. • Updated sample capacity for DNA-only workflow in "Guidelines for the usage of reagents and supplies, sample capacity, and total instrument run time" on page 10 and "Chip capacity by sample type" on page 35. • Updated guidelines for thawing reagents in "Before each use of the kit" on page 20 and "Before you begin" on page 39. • Updated ordering information and strip carrier colors in "Contents and storage" on page 9 and "Required materials not supplied" on page 12. • Added new instrument and software troubleshooting items in Appendix A, "Troubleshooting". • Added instructions on how to download software and assay package updates (see page 88).
A.0	09 January 2020	New user guide that documents the sample-to-result workflow for the Oncomine™ Comprehensive Assay v3 GX.

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About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of this guide

This user guide describes how to use the Oncomine™ Comprehensive Assay v3 GX to perform the sample-to-result next-generation sequencing (NGS) workflow on the Genexus™ Integrated Sequencer. The guide provides experienced-user procedures for assay creation and run planning in Genexus™ Software, loading samples onto the sequencer and starting an instrument run, and data analysis in Genexus™ Software.

If you are a new user, or you need detailed descriptions of all features of the Genexus™ Integrated Sequencer and Genexus™ Software, see the following resources available at **thermofisher.com**.

- *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910)
- *Genexus™ Software 6.2 User Guide* (Pub. No. MAN0018955) or *Genexus™ Software 6.2 Help* (available in the software)

Prerequisites

Category	Prerequisites
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" on page 12.
Software	• Genexus™ Software 6.2 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 6.2 or later

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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, using the Ion Torrent™ GX5™ Chip (see "Contents and storage" on page 9). Each chip can accommodate up to 6 DNA and RNA samples, or 5 samples and one no-template control. Additional Genexus™ Integrated Sequencer reagents and supplies must be purchased separately (see "Required materials not supplied" on page 12).

The Genexus™ Integrated Sequencer performs library preparation, sequencing, analysis, and reporting in an automated comprehensive sample-to-result workflow that uses as little as 10 ng of nucleic acid input per pool. Depending on the workflow, results can be obtained in as little as a single day (see "Guidelines for the usage of reagents and supplies, sample capacity, and total instrument run time" on page 10).

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

- Analysis of variants across 161 genes
- 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

The Oncomine™ Comprehensive Assay v3 GX includes the following configurations

- Ion AmpliSeq™ chemistry
- Automated and scalable sample-to-result sequencing workflow on the Genexus™ Integrated Sequencer
- System-installed assays pre-configured with settings that are optimized for specific application and sample type
- Optimized Oncomine™ informatics

Contents and storage

The Oncomine™ Comprehensive Assay v3 GX (Cat. No. A46296) includes the 2-pool DNA panel (OCAv3 DNA Pool 1 and OCAv3 DNA Pool 2), the 2-pool RNA panel (OCAv3 RNA Pool 1 and OCAv3 RNA Pool 2), Genexus™ Strip 1, and Genexus™ Strip 2-AS. The contents of each kit are sufficient for up to 16 × 4-pool reactions (16 × 2-pool DNA reactions and 16 × 2-pool RNA reactions). 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. Two 8-strip packs of the Genexus™ Strip 1 and Genexus™ Strip 2-AS (16 total of each strip) are provided with each kit. If needed, the Genexus™ Library Strips 1 and 2-AS combo kit (Cat. No. A40252) can be ordered separately.

Table 1 Oncomine™ Comprehensive Assay v3 GX (Cat. No. A46296)

Contents	Carrier color	Number of reactions	Amount	Part No.	Storage
Oncomine™ Comprehensive Assay v3 GX pools					
OCAv3 DNA Pool 1 (position 1, white cap)	Magenta	16 (4 reactions/tube)	4 tubes, 140 µL/tube	A40281	–30°C to –10°C
OCAv3 DNA Pool 2 (position 1, white cap)	Pale green	16 (4 reactions/tube)	4 tubes, 140 µL/tube		
OCAv3 RNA Pool 1 (position 1, white cap)	Pale orange	16 (4 reactions/tube)	4 tubes, 70 µL/tube	A44351	
OCAv3 RNA Pool 2 (position 1, white cap)	Blue	16 (4 reactions/tube)	4 tubes, 70 µL/tube		
Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252) × 2					
Genexus™ Strip 1	Light red	2 × 32 (4 single-pool reactions/strip)	2 × 8 strips	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	2 × 32 (4 single-pool reactions/strip)	2 × 8 strips	A46813	–30°C to –10°C

Guidelines for the usage of reagents and supplies, sample capacity, and total instrument run time

Table 2 provides guidelines for the usage of reagents and supplies, sample capacity, and total instrument run time for each sample-to-result Oncomine™ Comprehensive Assay v3 GX workflow. Table 3 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, and total instrument run time required for each workflow type

Assay component	DNA-only workflow	RNA-only workflow		DNA+RNA workflow
Samples per run	**7 samples**	**8 samples**	**16 samples**	**6 samples**
GX5™ Chip (Part No. 100081364)	1 chip (4 lanes)	¼ chip (1 lane)	½ chip (2 lanes)	1 chip (4 lanes)
Genexus™ Pipette Tips (Cat. No. A40266)	4 racks	3 racks	6 racks	6 racks
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™ 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[1] (Cat. No. A40257, A40258, A40259, or A40260)	12 barcodes	16 barcodes	32 barcodes	24 barcodes
Total run time (instrument run only)	~22.5 hrs	~18 hrs	~22.5 hrs	~30 hrs

[1] After a partially used barcode plate has been removed from the sequencer, it cannot be reused. If a barcode plate in a sequencer has unused barcodes, do not remove it unless you are sure that you want to replace it with a new barcode plate. If there are not enough barcodes left on the plate, insert a new plate.

Table 3 Guidelines for ordering reagents and supplies

Assay component	DNA-only workflow	RNA-only workflow		DNA+RNA workflow
Samples per run	**7 samples**	**8 samples**	**16 samples**	**6 samples**
Oncomine™ Comprehensive Assay v3 GX (Cat. No. A46296)	½ kit	½ kit	1 kit	1 kit
GX5™ Chip and Genexus™ Coupler (Cat. No. A40269)	½ set	⅛ set	¼ set	½ set
Genexus™ Templating Strips 3 and 4-GX5™ (Cat. No. A40263)	½ kit	⅛ kit	¼ kit	½ kit
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

IMPORTANT! The information provided in Table 3 is for estimation purposes only. Only full-sized kits are available for purchase. For ordering information, see "Contents and storage" on page 9 and "Required materials not supplied" on page 12.

Required materials not supplied

Genexus™ Integrated Sequencer reagents and supplies can be ordered in convenient combo kits and starter packs, but most consumables can also be ordered individually as your needs require. The following tables provide information on the various ordering options that are available.

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.

General laboratory supplies and reagents

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

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

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

Genexus™ Barcodes AS

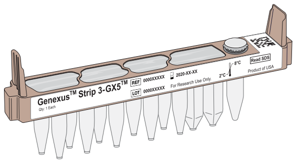
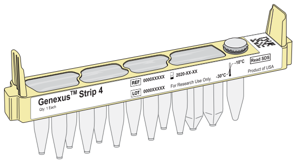
Ion Torrent™ Genexus™ Barcodes AS are supplied in plates containing 32 dual barcodes per plate. The barcodes can be ordered as a set of three plates (Cat. No. A40257), or ordered individually.

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 and 4-GX5™

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

Note: Genexus™ Templating Strips 3 and 4-GX5™ with part numbers listed in the following table are compatible only with Genexus™ Software 6.2 and later.

Component	Carrier color	Part No.	Quantity per kit	Storage
Genexus™ Strip 3-GX5™  Genexus™ Strip 3-GX5™ Read SDS For Research Use Only Product of USA	Brown	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4  Genexus™ Strip 4 Read SDS For Research Use Only Product of USA	Yellow	A46816	8 strips	–30°C to –10°C

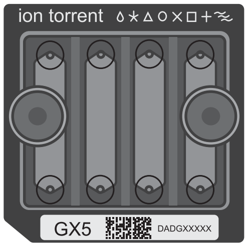
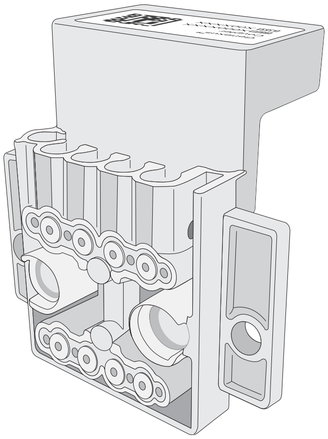
Genexus™ Pipette Tips

The 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, sample capacity, and total instrument run time” on page 10 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

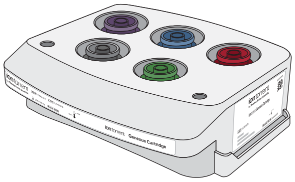
GX5™ Chip and Genexus™ Coupler

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, sample capacity, and total instrument run time” on page 10 to determine the number of chips required for your experiment.

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

Genexus™ Sequencing Kit

The Ion Torrent™ Genexus™ Sequencing Kit is ordered as a combo (Cat. No. A40271). Each combo kit is sufficient to sequence up to 2 full chips. The components of the combo are also orderable as listed.

Component	Cat. 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™ Conical Bottles

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

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

Genexus™ Integrated Sequencer

Components	Cat. No.
Genexus™ Integrated Sequencer	A45727

Recommended materials

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

Recommended materials for nucleic acid isolation and quantification

Item	Source
Nucleic acid isolation	
RecoverAll™ Multi-Sample RNA/DNA Workflow	A26069, A26135

(continued)

Item	Source
Nucleic acid quantification	
Qubit™ 4 Fluorometer[1] and one or more of the following kits:	Q33226
- Qubit™ dsDNA HS Assay Kit *(DNA samples)*	Q32851, Q32854
- Qubit™ RNA HS Assay Kit *(RNA samples)*	Q32852, Q32855

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

Recommended sample controls

Item	Source	Description
AcroMetrix™ Oncology Hotspot Control	969056	>500 somatic hotspot variations
Seraseq™ Tri-Level Tumor Mutation DNA Mix v2 HC	0710-0097 (SeraCare™)	40 variants across 28 genes at 4%, 7%, 10% allele frequency
Seraseq™ Lung & Brain CNV Mix, + 6 copies	0710-0415 (SeraCare™)	CNV: EGFR, MYCN, and MET at +6 copies (8 total copies)
Seraseq™ Breast CNV Mix, + 6 copies	0710-0412 (SeraCare™)	CNV: ERBB2, FGFR3, MYC at +6 copies (8 total copies)
Seraseq™ Fusion RNA Mix v3	0710-0431 (SeraCare™)	16 RNA fusions: ALK, RET, ROS1, NTRK1, FGFR3, PPARG1, FGFR3, NTRK3, BRAF, ERG, EGFR, METex14, EGFRvIII
Seraseq™ Fusion RNA Mix v4	0710-0497 (SeraCare™)	18 RNA fusions: RET, ROS1, EGFRvIII, EGFR, ALK, NTRK3, FGFR3, NTRK1, METex14, PPARG1, BRAF, ERG
Horizon™ ALK RET ROS RNA fusion	HD784 (Horizon™)	RNA fusions: EML4-ALK, CCDC6-RET, and SLC34A2-ROS1

Genexus™ Controls

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

Workflow overview: sample to results

The following workflow outlines the key steps in performing the Oncomine™ Comprehensive Assay v3 GX, from sample dilution to data analysis.

Oncomine™ Comprehensive Assay v3 GX

Create an assay (page 24)

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 your run plan without change. If you want to modify any assay settings, copy the system-installed assay that best represents your experiment, then edit the assay settings as needed.

Enter samples (page 27)

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

Create a run plan (page 31)

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

Dilute the samples and load the sample plate (page 36)

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

Load the sequencer and start a run (page 39)

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 46)

View variant calls and visualize results, including side-by-side sample comparison, in the Genexus™ Software.

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Before you begin

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Procedural guidelines

- Use only the reagents and supplies that have been validated for the Oncomine™ Comprehensive Assay v3 GX. For a list of validated reagents and supplies, see "Required materials not supplied" on page 12 and "Recommended materials" on page 15.
- Use good laboratory practices to minimize cross-contamination of products. Keep all tubes sealed until immediately before loading onto the Genexus™ Integrated Sequencer.
- Use controls to identify or rule out reagent contamination. For a list of validated controls, see "Genexus™ Controls" on page 17.
- 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.

Before each use of the kit

Thaw assay reagents as indicated:

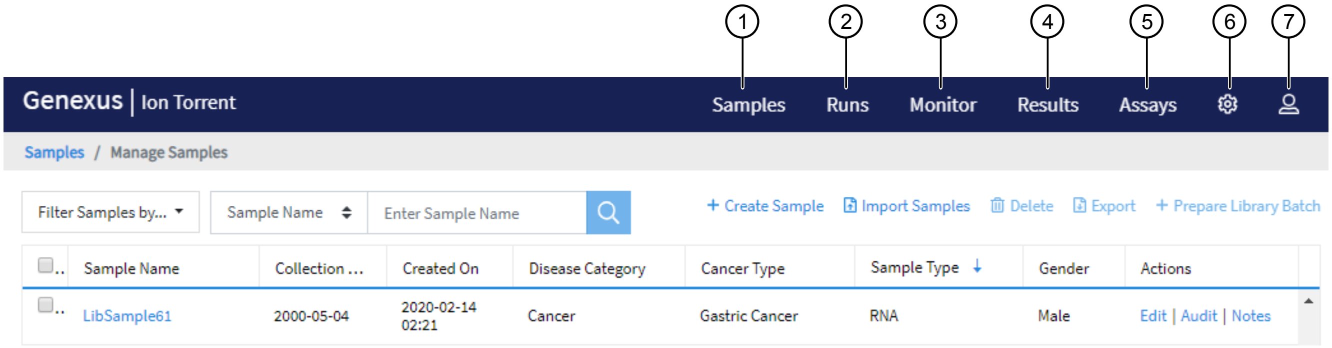
- Thaw OCAv3 DNA Pool 1, OCAv3 DNA Pool 2, OCAv3 RNA Pool 1, OCAv3 RNA Pool 2 on ice or at 4°C for at least 30 minutes and keep on ice until immediately before loading onto the Genexus™ Integrated Sequencer.
- Thaw Genexus™ Strip 2-AS at room temperature for 30 minutes, then place on ice until immediately before loading onto the Genexus™ Integrated Sequencer.
- Equilibrate Genexus™ Strip 1 at room temperature for at least 30 minutes before loading onto the Genexus™ Integrated Sequencer.

Once 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 on preparing all reagents and consumables for loading onto the instrument, see “Before you begin” on page 39.

Note: If tapping fails to dislodge a bubble, you can dislodge large bubbles using the technique that is described in substep 4b in “Before you begin” on page 39.

About the Genexus™ Software user interface

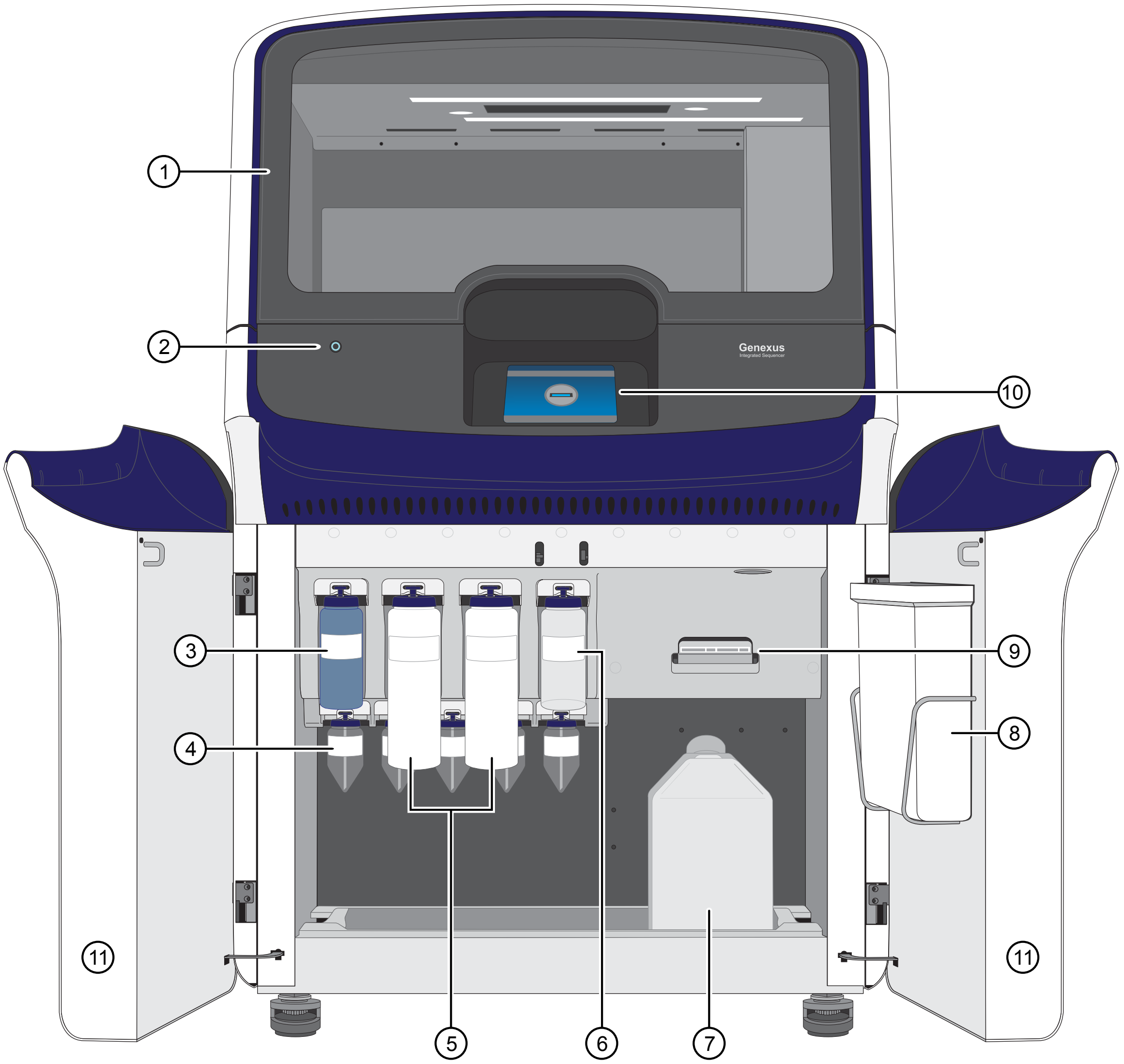
The Genexus™ Software user interface provides menus to help you add, select, and manage samples, libraries, runs, and assays. You can also view and manage your sequencing results, monitor Genexus™ Integrated Sequencer runs in progress, and manage software settings.



☐	Sample Name	Collection ...	Created On	Disease Category	Cancer Type	Sample Type	Gender	Actions
☐	LibSample61	2000-05-04	2020-02-14 02:21	Cancer	Gastric Cancer	RNA	Male	Edit | Audit | Notes

① **Samples**: add new samples, import samples, prepare library batches, import library batches and manage attributes.
② **Runs**: plan a run starting from a sample (Sample Run), or library (Library Run). View, edit, and manage runs.
③ **Monitor**: view a sequencer run in progress.
④ **Results**: view sample results, run results, and verification results.
⑤ **Assays**: manage, create, and import assays. Manage assay preset parameters and panels.
⑥ **Settings**: access audit records and run logs, configure network settings, and manage data archiving, disk space, and users.
⑦ **Profile**: access Help, manage and edit user profile settings and SSH key, sign out.

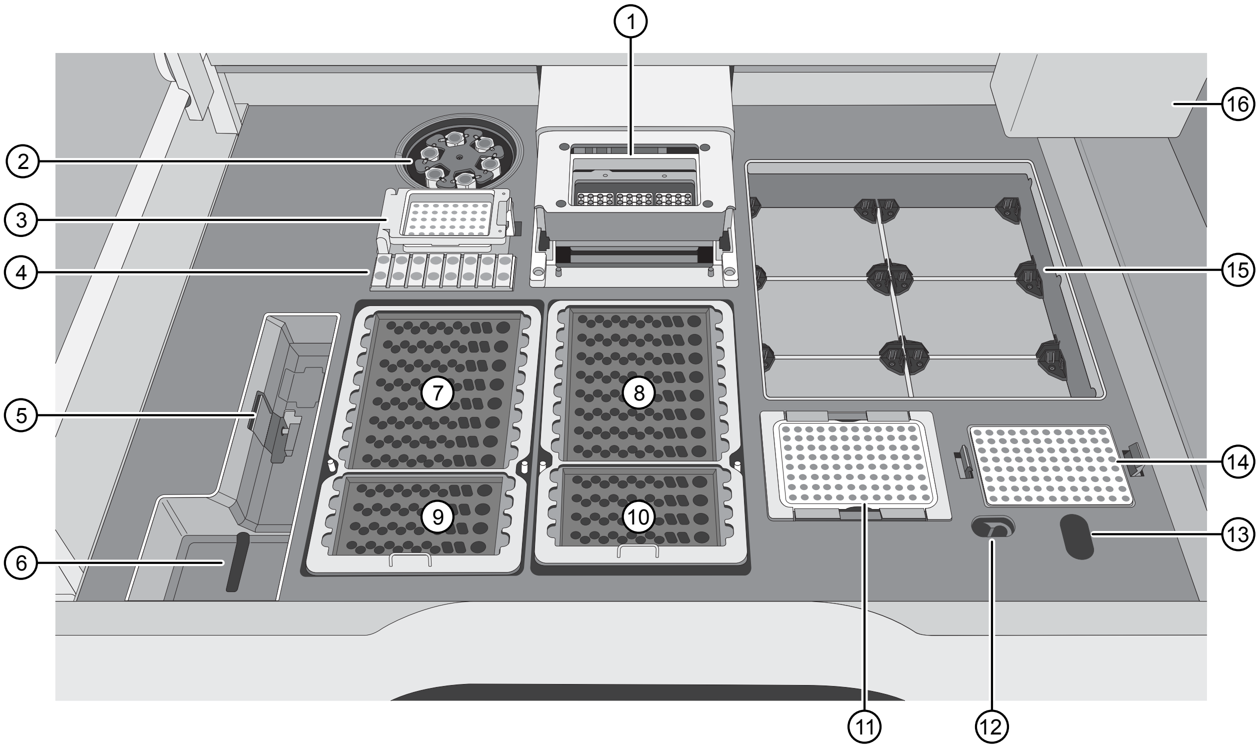
Genexus™ Integrated Sequencer components



Major features and components of the exterior and sequencing reagents bay of the Genexus™ Integrated Sequencer

① Door to deck chamber. The door is locked in the closed position during an instrument run.
② Power button
③ Genexus™ Bottle 1 (Chemical Waste)
④ Genexus™ Conical Bottles (Reusable conical bottles for Genexus™ Cartridge reagent dilution)
⑤ Genexus™ Bottle 2 (Sequencing Solution)
⑥ Genexus™ Bottle 3 (Cleaning Solution)
⑦ Waste Carboy
⑧ Waste pipette tip bin
⑨ Genexus™ Cartridge
⑩ Touchscreen
⑪ Sequencing reagents bay door. Doors are locked in the closed position during an instrument run.

Genexus™ Integrated Sequencer deck stations



Interior Genexus™ Integrated Sequencer deck components and stations

① PCR amplification station
② Microcentrifuge
③ Genexus™ Barcodes station
④ Genexus™ Primer Pool Tube station
⑤ Genexus™ Coupler station
⑥ Chip install station
⑦ Zone 1 station (Genexus™ Strip 1)
⑧ Zone 2 station (Genexus™ Strip 2-AS)
⑨ Zone 3 station (Genexus™ Strip 3-GX5™)
⑩ Zone 4 station (Genexus™ Strip 4)
⑪ Enrichment plate station
⑫ Liquid waste disposal port
⑬ Waste pipette tip disposal port
⑭ Sample plate station
⑮ Genexus™ Pipette Tips station
⑯ Robotic pipettor

Precautions

Avoid nucleic acid contamination

IMPORTANT! A primary source of contamination is spurious DNA 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.

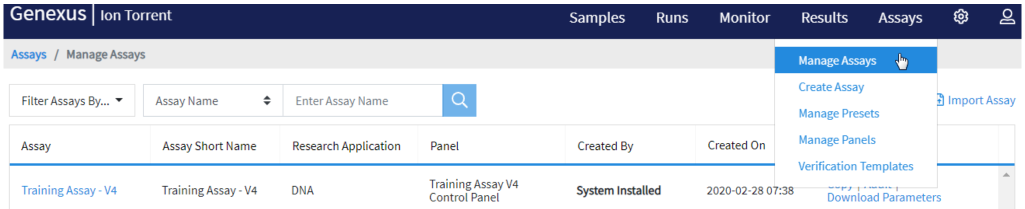
Protection by equipment

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).

3

Create and manage assays (manager/administrator)

- System-installed assays for use with the Oncomine™ Comprehensive Assay v3 GX 24
- Copy an assay (manager/administrator) 25



Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Assays / Manage Assays
Manage Assays
Create Assay
Manage Presets
Manage Panels
Verification Templates
Filter Assays By...
Assay Name
Enter Assay Name
Import Assay
Download Parameters

Assay	Assay Short Name	Research Application	Panel	Created By	Created On
Training Assay - V4	Training Assay - V4	DNA	Training Assay V4 Control Panel	System Installed	2020-02-28 07:38

Manager- and administrator-level users can create and manage assays in Genexus™ Software. This chapter describes how to copy and edit assays, including system-installed assays. If you are using a system-installed assay without change, proceed to Chapter 4, “Enter samples”.

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

The following system-installed assays are available in the Genexus™ Software 6.2 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: Make sure to update your software to the latest available versions of the system-installed assays. For more information, contact your field service representative. For information on how to perform software and assay package updates, see “Download software and assay package updates” on page 88.

Assay name	Sample type
Oncomine™ Comprehensive v3 - GX5 - DNA	DNA
Oncomine™ Comprehensive v3 - GX5 - Fusions	RNA
Oncomine™ Comprehensive v3 - GX5 - DNA and Fusions	DNA and RNA

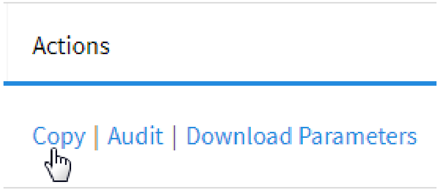
You can use the system-installed assays in your run plan without change. To modify any assay settings, you can copy the system-installed assay that best represents your sequencing experiment and sample type, then edit assay settings as needed.

- If you are using a system-installed assay without changes, proceed directly to Chapter 4, “Enter samples” and Chapter 5, “Plan and manage runs”.
- To modify a system-installed assay with custom settings, proceed to “Copy an assay (manager/administrator)” on page 25.

Copy an assay (manager/administrator)

Manager- and administrator-level users can create a new assay by copying an existing system-installed assay or other custom assay and modifying parameters as needed. Only locked assays can be copied.

1. In the menu bar, click **Assays ▸ Manage Assays**.
2. In the **Manage Assays** screen, in the **Actions** column in the row of an assay that you want to copy, click **Copy**.

   The **Copy Assay** screen opens to the **Panel** step.
3. Proceed through the workflow steps and modify assay settings as needed.

   When using high-quality nucleic acid samples, adjust the number of target amplification cycles as described in “Assay-specific settings” on page 26. For guidelines on modifying key assay settings, see “Guidelines for Oncomine™ Comprehensive Assay v3 GX settings” on page 80.

   For a description of assay settings in each workflow step, see *Genexus™ Software Help*.
4. When finished, enter a new name in the **Assay Name** field and a short name in the **Assay Short Name** field for the copied assay, then click **Save**.

   The newly created assay is added to the list of assays in the **Manage Assays** screen. The assay name is appended with ***(Draft)*** in the **Assay** column. The assay remains in the draft status until it is locked.
5. In the **Actions** column, in the row of the assay, click **Lock** to enable the assay for use in a run plan.

Assay-specific settings

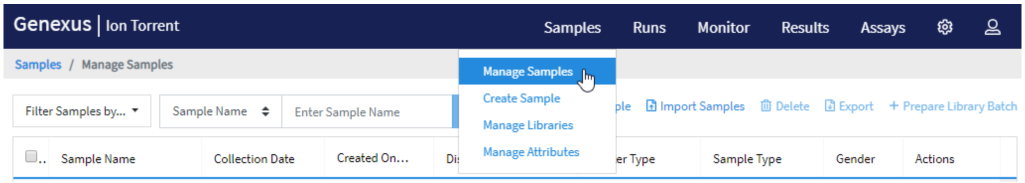
When using DNA controls, high-quality DNA, or high-quality RNA samples in a sequencing runs, make the following adjustments in the **Parameters** step of the assay creation workflow.

Workflow step	Setting	Default value	Recommended value
Parameters	Number of target amplification cycles DNA	17	15
	Number of target amplification cycles RNA	31	28
	Library Pooling Percent DNA	70	80
	Library Pooling Percent RNA	30	20

4

Enter samples

- Create a new sample 27
- Import samples 29
- Manage samples 30



Genexus | Ion Torrent
Samples
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Assays
Samples / Manage Samples
Manage Samples
Create Sample
Manage Libraries
Manage Attributes
Filter Samples by...
Sample Name
Enter Sample Name
ple
Import Samples
Delete
Export
+ Prepare Library Batch
Sample Name
Collection Date
Created On...
Di
er Type
Sample Type
Gender
Actions

Before creating a run plan in Genexus™ Software for a sample run, you must first enter samples in the software to assign names and attributes for each sample. From the **Samples** menu, samples can either be added by manually entering the sample information into the software or be imported into the software by uploading a data file.

Create a new sample

1. In the menu bar, click **Samples ▸ Manage Samples**.
2. In the **Manage Samples** screen, click + **Create Sample**.
3. In the **Create Sample** screen, complete the required fields.

   Note: Attributes identified with a red asterisk (*) are required. If attribute information is not available when adding a new sample, enter mock information to complete the required fields.

   For a description of the fields, see "System-installed sample attributes" on page 28.
4. Click **Save**.
5. Repeat step 2–step 4 for each additional sample.

The new sample is listed in the **Manage Samples** screen.

System-installed sample attributes

The following table lists and describes system-installed sample attributes. System-installed sample attributes cannot be edited. 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), full stops/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, make sure 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	The date that the sample was collected. Click 📅 **Calendar** to select the date in the correct format.
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. You can also select **Other**, then enter a custom sample type.
Disease Category	The disease type of the sample. **Note:** If you select **Cancer** in this list, the **Cancer Stage**, **Cancer Type**, **% Cellularity**, and **% Necrosis** attributes listed below become available in the **Add New Sample** dialog box.
Cancer Stage	The stage of the cancer from which the sample was collected. Select **Stage 0–IV**, or **Primary**, **Unknown**, or **Other**.
Cancer Type	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**.
% 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 to FFPE samples only. **IMPORTANT!** • If 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 "CNVs table" on page 64.

(continued)

Sample attribute	Description
% Necrosis	The percentage of cellular necrosis in the sample. This is a whole number between 1 and 100.
Notes	An open-entry field for any additional sample information.

[1] Required attribute

Import samples

Sample data files can be used to capture, manage, and edit sample data. You can import sample data files in the following formats: TXT, XLS, XLSX, or CSV. For a list of the sample attributes that are included in the import file, see “System-installed sample attributes” on page 28.

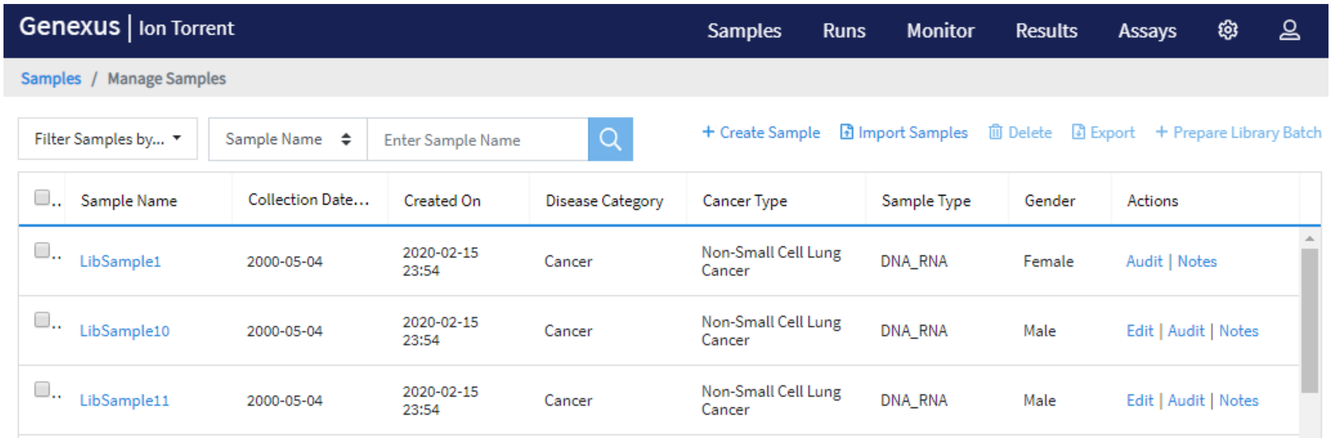
1. In the menu bar, click **Samples** ▸ **Manage Samples**, then click **Import Samples**.
2. In the **Import Samples** dialog box, click **Select samples file**.
3. Navigate to the file, then click **Open**.
4. Click **Upload**.

   A progress bar followed by an import report appears. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used).

Successfully imported samples are listed in the **Samples / Manage Samples** screen.

Manage samples

You can find tools for creating, searching, sorting, editing, deleting, and exporting samples, and for viewing the sample history in the **Samples** > **Manage Samples** screen. For more information, see the *Genexus™ Software Help*.

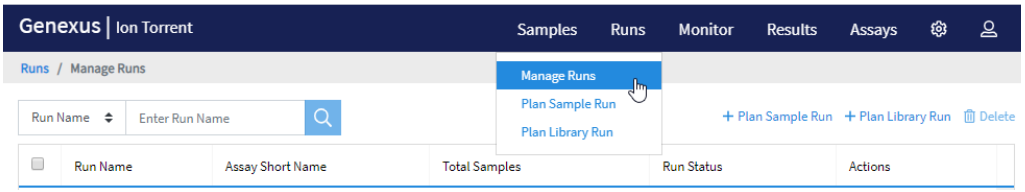

Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Samples / Manage Samples
Filter Samples by...
Sample Name
Enter Sample Name
+ Create Sample
Import Samples
Delete
Export
+ Prepare Library Batch

☐..	Sample Name	Collection Date...	Created On	Disease Category	Cancer Type	Sample Type	Gender	Actions
☐..	LibSample1	2000-05-04	2020-02-15 23:54	Cancer	Non-Small Cell Lung Cancer	DNA_RNA	Female	Audit | Notes
☐..	LibSample10	2000-05-04	2020-02-15 23:54	Cancer	Non-Small Cell Lung Cancer	DNA_RNA	Male	Edit | Audit | Notes
☐..	LibSample11	2000-05-04	2020-02-15 23:54	Cancer	Non-Small Cell Lung Cancer	DNA_RNA	Male	Edit | Audit | Notes

5

Plan and manage runs

- Create a run plan for a sample run 31
- Chip capacity by sample type 35


Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Runs / Manage Runs
Manage Runs
Plan Sample Run
Plan Library Run
Run Name
Enter Run Name
+ Plan Sample Run
+ Plan Library Run
Delete
Run Name
Assay Short Name
Total Samples
Run Status
Actions

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

You can plan runs for sequencing runs that use either nucleic acid samples (sample run) or libraries that you have previously prepared manually (library run) as input. Genexus™ Software guides you step-by-step to set up a run that tells you what consumables are needed, and provides a printed run setup guide to help you load the Genexus™ Integrated Sequencer with the required consumables.

Create a run plan for a sample run

Sample run planning is organized into the **Setup**, **Assays**, **Samples**, **Sample Plate**, and **Review** steps. Progress through the steps is tracked in the upper left corner of the **Runs / Plan Sample Run** screen.

Before planning a sample run (sample-to-results), perform the following tasks.

- Identify the assay to use in the run, system-installed or user-created (copied, modified, and locked). For more information, see Chapter 3, “Create and manage assays (manager/administrator)”.
- Enter sample information into Genexus™ Software. For more information, see “Create a new sample” on page 27, or “Import samples” on page 29.

1. In the menu bar, click **Runs** ▸ **Plan Sample Run**.

   Note: You can also click + **Plan Sample Run** in the **Runs / Manage Runs** screen.

2. In the **Setup** step, enter or make the following selections.
   a. In the **Run Name** field, enter a unique name.

b. *(Optional)* In the **Reporting (Optional)** section, select one or more options if needed.

Note: You can select both options, or leave both options deselected.

Reporting option	Description
Generate Report	Select this option to generate a Lab Report using a report template that you specify in the dropdown list. To create a report template, click **Assays ▸ Manage Presets**, then in the **Report Templates** tab, click + **Add New**.
Upload BAM files to Ion Reporter™ Software	Select this option to automatically upload data for secondary analysis with Ion Reporter™ Software. Select your Ion Reporter™ Software account and release version.

c. Click **Next**.

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

3. In the **Assays** step, select one or more assays that you want to use in the run.

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

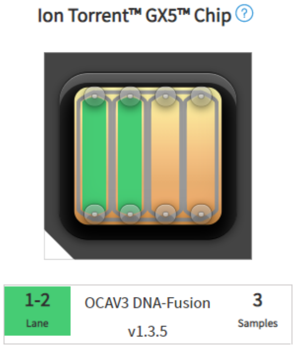
   IMPORTANT! Ensure that you select the assay that corresponds with the sample type that you will use in the run. If you select a wrong assay when you create a run plan, the instrument will use incorrect settings during the run, resulting in invalid sequencing results. To determine which system-installed assay to use for your sample type, see "System-installed assays for use with the Oncomine™ Comprehensive Assay v3 GX" on page 24.

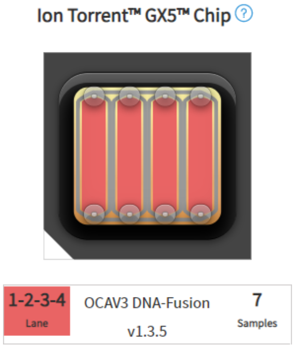
4. *(Optional)* In the **Include NTC** column for each assay that you select, select the checkbox to include a no template control for the assay.

5. Click **Next**.

6. In the **Samples** step, select the samples that you want to run with the assay from the list, then click **Assign**.

   The **Chip View** updates to show the lanes to be used in the run as green (left). Lane usage is calculated based on the number of samples (including a no template control, if selected), assay type, primer pools used, and minimum reads per sample entered at assay setup. For information on the GX5™ Chip lane usage for the Oncomine™ Comprehensive Assay v3 GX, see "Chip capacity by sample type" on page 35.

   If the minimum reads per sample × the 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 (right). In the chip view example on the right, the number of samples that were selected exceeds the chip capacity. The run is allowed, but you may not achieve the desired read count per sample. If needed, remove extra samples from the run as described in step 8.

7. If you selected more than one assay, repeat step 6 for each additional assay.

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

   - Click **View & Remove**, make your selections, then click **Update**.
   - Click **Remove All**, make your selections, then click **Assign**.

9. Click **Next**.

10. In the **Sample Plate** step, review sample positions in the sample plate. To modify the location of samples and controls, drag and drop the sample rows as needed.

11. *(Optional)* If needed, modify the concentration of samples.

    - To modify concentrations of each sample individually, in the row of each sample, enter the concentration value in the provided field in the **Conc. (ng/µl)** column.
    - To modify concentrations of all samples at one time, click ✎ **Bulk Edit**. In the **Add New Attribute** window, in the **Concentration** field, enter the concentration, then from the **Apply to** dropdown list, select **All Samples**. If you are running multiple assays and sample concentrations are different for each assay, select an assay name from the **Apply to** dropdown list, then if needed, select the library type. Click **Submit** to return to the **Sample Plate** screen.

    For assay-specific sample input requirements, see "Guidelines for nucleic acid isolation, quantification, and input amount" on page 36.

    IMPORTANT! Ensure that the sample concentration values are correct. Incorrect sample concentration entry results in incorrect sample dilution by the instrument, and subsequently, inaccurate sequencing results.

    In the **Sample Plate** screen, the **Target Conc. (ng/µl)** column displays the concentration range that is defined by the assay for each sample, with target concentration being the middle point of the range. 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 on sample dilution, see "Dilute the samples and load the sample plate" on page 37. If you want to modify the sample concentration range for an assay, see "Copy an assay (manager/administrator)" on page 25 and "Guidelines for Oncomine™ Comprehensive Assay v3 GX settings" on page 80.

    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 37.

12. Click **Next**.

13. 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 plan without printing.

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

After selecting the run plan and loading the sequencer, the run is started on the sequencer screen.

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. If needed, you can modify the number of samples that can be loaded onto a chip by copying a system-installed assay and adjusting the **Minimum Read Count Per Sample** parameter. For more information, see "Copy an assay (manager/administrator)" on page 25 and "Guidelines for Oncomine™ Comprehensive Assay v3 GX settings" on page 80.

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

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

Sample type	Number of available lanes	Capacity
DNA only	1	1 sample
	2	3 samples
	3	5 samples
	4	7 samples
DNA and RNA	1	1 sample
	2	3 samples
	3	4 samples
	4	6 samples
RNA only	1	8 samples
	2	16 samples

6

Dilute the samples and load the sample plate

- Guidelines for nucleic acid isolation, quantification, and input amount 36
- Quantify nucleic acid samples 37
- Dilute the samples and load the sample plate 37

Before starting a run on the instrument, you must quantify and dilute the samples, then load the sample plate.

Guidelines for nucleic acid isolation, quantification, and input amount

- For validated kits to use for isolation and quantification of nucleic acid samples, see “Recommended materials for nucleic acid isolation and quantification” on page 15.
- Use the RecoverAll™ Multi-Sample RNA/DNA Workflow for isolating DNA or RNA samples.
- Use the Qubit™ Fluorometer with the Qubit™ dsDNA HS Assay Kit or the Agilent™ 2100 Bioanalyzer™ with the Agilent™ High Sensitivity DNA Kit for quantifying DNA samples.
- Use the Qubit™ Fluorometer with the Qubit™ RNA HS Assay Kit for quantifying RNA samples.
- Quantification methods such as densitometry (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA or RNA. Use of these methods can lead to gross overestimation of the concentration of sample nucleic acid, under-seeding of the target amplification reaction, and low library yields.
- The recommended sample input amount for the assay is 20 ng at the concentration of 1.1 ng/µL for DNA samples or 0.95 ng/µL for RNA samples. At the recommended input amount and concentrations, the instrument requires 27.5 ng of input for DNA samples and 23.75 ng of input for RNA samples due to volume overage. For more information, see “Dilute the samples and load the sample plate” on page 37.

Quantify nucleic acid samples

Isolate nucleic acid samples using one of the validated procedures and kits that are listed in "Recommended materials for nucleic acid isolation and quantification" on page 15.

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 37.

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 37. If you want to run sample controls, select an appropriate control for your sample type as recommended in "Recommended sample controls" on page 16.

1. Use the run setup guide to dilute samples and sample controls, if used, to the required concentration using nuclease-free water (see "Create a run plan for a sample run" on page 31).

   IMPORTANT! Make sure to dilute samples to the concentration that was specified in step 11 in "Create a run plan for a sample run" on page 31.

   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.

7

Load the sequencer and start a run

- Before you begin 39
- Load the sequencer and start a run 41
- Clear the instrument deck and perform a UV Clean 44

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

Before you begin

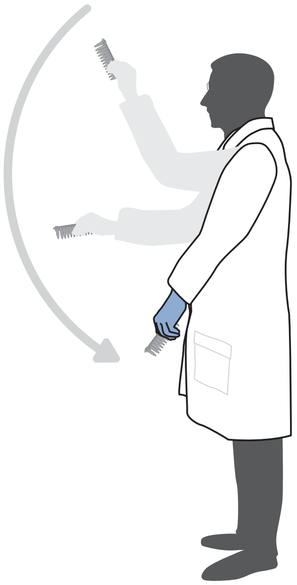
1. Remove the library and templating strips from their boxes in the refrigerator or freezer, and ready them for loading in the sequencer.
   - Genexus™ Strip 1 and Genexus™ Strip 3-GX5™: equilibrate to room temperature for 30 minutes.
   - Genexus™ Strip 2-AS and Genexus™ Strip 4: thaw at room temperature for 30 minutes. If you are delayed in loading, keep the thawed strips on ice or at 4℃ until you load them in the sequencer.

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

2. Remove primer pool tubes in tube carriers that are needed for the run from the freezer, then thaw for at least 30 minutes on ice or at 4℃. 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℃ until you load them in the sequencer.

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

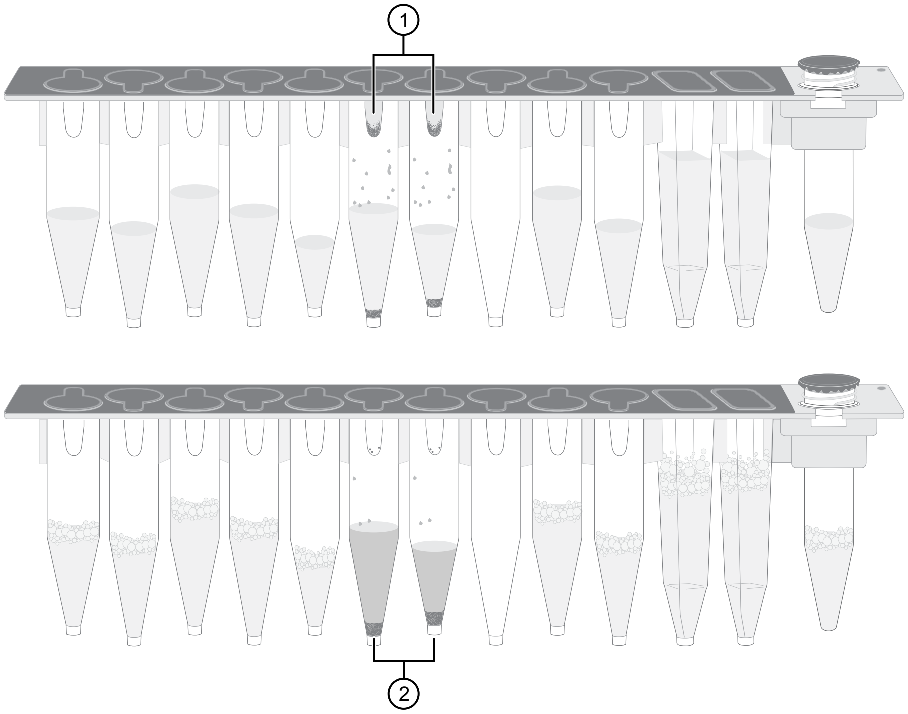
4. Genexus™ Strip 1 and Genexus™ Strip 3-GX5™ contain magnetic beads in one or two positions, yellow or brown in color, that sometimes get trapped in the upper "keyhole" of the tube. Dislodge these beads from the keyhole before installing the strip in the sequencer. Use the following procedure for each strip.
   a. Invert the strip 3–4 times to dislodge beads that are trapped in the keyholes.



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

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

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



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

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

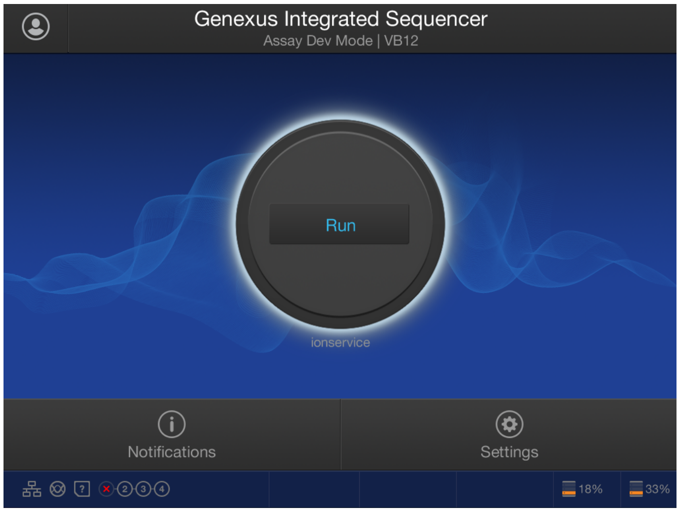
Note:

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

5. Inspect all strips for large bubbles lodged under the surface of the liquid or at the bottom of each tube or well. Gently tap the strips on a benchtop to dislodge any bubbles without splashing the contents onto the upper tube walls. If tapping fails to dislodge a bubble, use the technique that is described in substep 4b until large bubbles are dislodged.

Load the sequencer and start a run

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



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

   Note: If you select a run that requires more lanes than are available on a currently installed chip, a dialog appears giving you the option to install a new chip, or cancel. If you proceed with a new chip, a postChipClean is performed, then the sequencer prompts you to perform the Clear Deck, UV Clean, Load Deck, Clear Sequencing Reagents, and Load Sequencing Reagents steps.

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

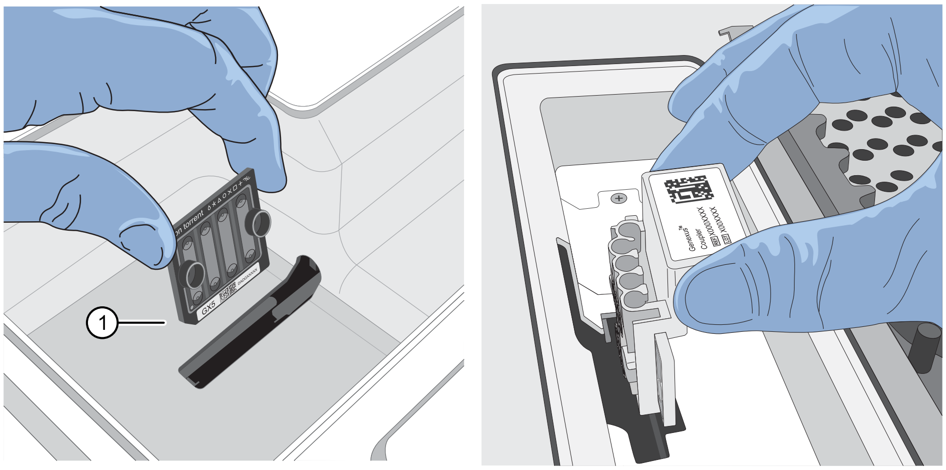
   The deck door opens automatically.

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.

   ---

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



① Notched corner of chip

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 prompts you to start the run.

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

   ---

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

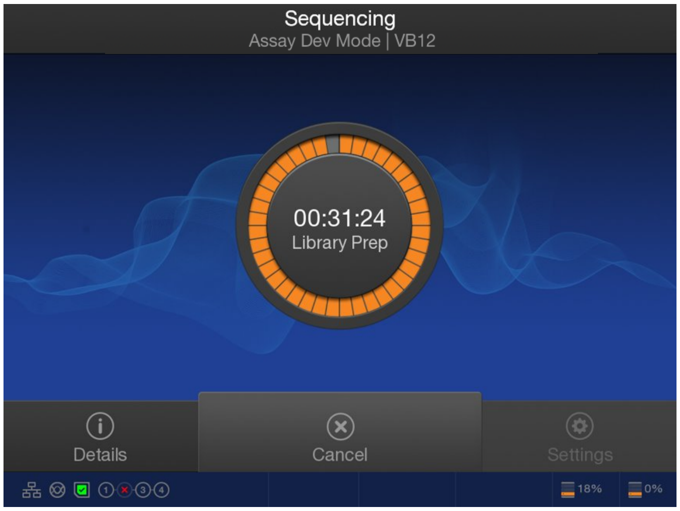
   IMPORTANT!
   - Ensure that you empty and replace the Waste Carboy and the waste pipette tip bin.
   - Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of consumables and liquid waste.

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

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

10. Close the sequencing reagents bay doors.

    After the doors are closed, the sequencer automatically starts the run, and shows the time remaining of the stage in progress on the touchscreen. A sequencing run encompasses the following stages:
    - Starting
    - Initializing
    - Library Prep
    - Templating
    - Pre-sequencing
    - Sequencing
    - Cleaning

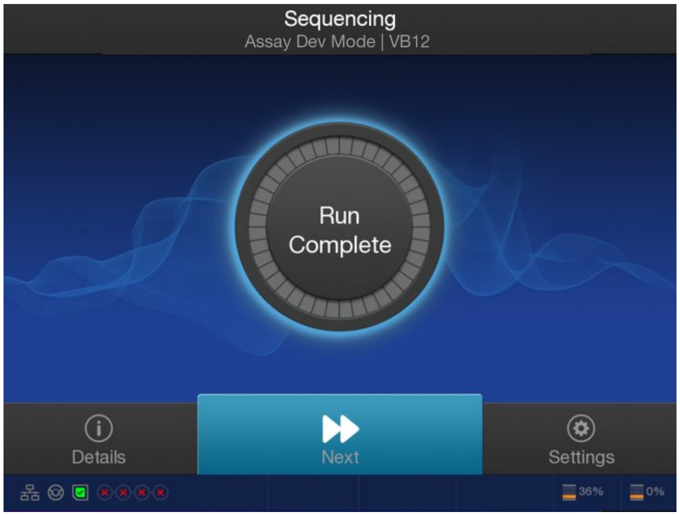


When the run finishes, the sequencer displays the **Run Complete** screen.

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.

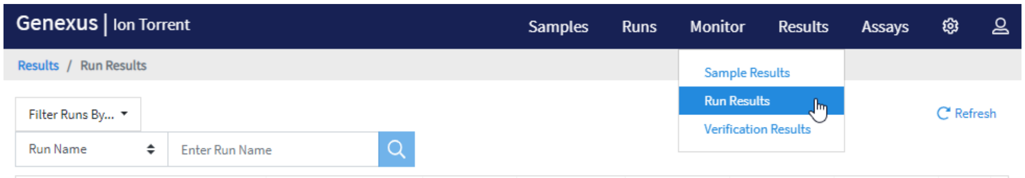
6. After removal of used components, close the sequencing reagents bay doors, then tap **Next**.

   The sequencer returns to the home screen.

8

Review data and results

- Review run results 46
- Lab Report 47
- View sequencing results and assay metrics 49
- Sign off on the run results (manager/administrator) 72


Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Results / Run Results
Sample Results
Run Results
Verification Results
Filter Runs By...
Run Name
Enter Run Name
Refresh

Use the **Results** menu to review run results and data analysis and perform data management tasks. In the menu bar, click **Results** ▸ **Sample Results** to review completed sample run results and reports.

Review run results

In the **Results / Run Results** screen, runs that are pending, running, or completed are listed.

You can search the list of results by run name or PCR plate number. Enter a search term, then click 🔍 **(Search)**.

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**.
Assay Short Name	The shortened unique identifier of an Assay name. You can view the complete list of **Assays** and **Assay Short Names** in the **Assays** ▸ **Manage Assays** screen.
Total Samples	The total number of samples in a run.
Run Status	The status of the run (for example: Not Started, Pending, Analysis Running, Executing Plugin, Completed, Terminated, Archival: In Progress).
PCR Plate Number	A unique identifier for the 96-well plate used for library preparation and templating. For more information, see “Assign PCR Plate” on page 47.
Started On	The date and time when the run analysis was started.

(continued)

Column	Description
Last Updated On	The date and time when the last action was completed on the run.
Actions	Click the appropriate link. To see more actions, click ⋮ **(More Options)**. • **Audit**—View the audit trail for the run. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting. • **Reanalyze**—Reanalyze the results of a completed run, starting from alignment or basecalling. Click **Reanalyze**, then select a new reanalysis assay that you have created with modified settings. For more information, see "Reanalyze a run" on page 85. • **Upload Samples to IR**—Upload sample results files to Ion Reporter™ Software for secondary analysis. • **Assign PCR Plate**—Enter a unique identifier for the 96-well plate used for library preparation and templating. For more information, see "Assign PCR Plate" on page 47.

Assign PCR Plate

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 (**PCR Plate Number**) to completed runs and runs in progress. The PCR plate number that you enter is generated on the Lab Report and if needed, can help you track libraries and troubleshoot.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, in the **Actions** column, click ⋮ **(More Options)** ▸ **Assign PCR Plate number** in the row of the run of interest.
3. In the **Assign PCR Plate** 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), periods, underscores, and hyphens are allowed. Spaces are not permitted.
4. Click **Submit** to associate the PCR plate with the run.

Lab Report

The Lab Report is a PDF report of the results for each sample in a sequencing run. The assay used in the run determines the data that is included in the report.

To automatically generate a Lab Report for each sample during data analysis of a run, select the **Generate Report** checkbox in the **Setup** step when you plan the run (for more information, see Chapter 5, "Plan and manage runs"). To generate a Lab Report for each sample after a run is complete, see “Generate customized reports” on page 86.

When a Lab Report has been generated for a sample, a link is available in the **Results / Sample Results** screen in the **Actions** column for that sample. Click the link to download the PDF.

Lab reports can be electronically signed by manager- and administrator-level users. Electronically signed reports have ***(Signed Off)*** after the sample name in the **Sample Results** screen. The electronic signature is included in the footer of the report. For more information, see “Sign off on the run results (manager/ administrator)” on page 72.

A Lab Report typically contains the following sections and information, depending on the assay used.

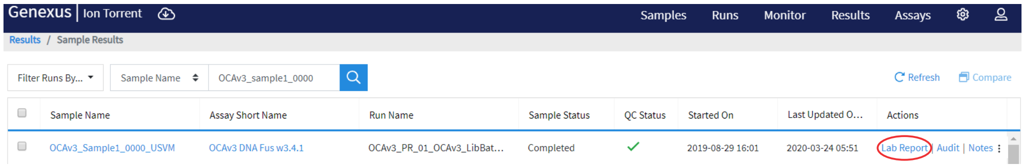
Section	Description
Sample Details	The sample information that is entered into the software. You can customize the format of the **Sample Details** section when you create a new report template. To create a new report template, click **Assays ▸ Manage Presets**, then in the **Report Templates** tab, click + **Add New**.
Sequence Variations: Detected	Variants and fusions detected in the sample, based on the targets defined by the assay. Allele frequencies are also reported.
Test Description	A description of the report or assay that was entered in the report template.
Sequence Variations: Not Detected	Variants and fusions not detected in the sample, based on the targets defined by the assay.
Comments	Laboratory comments entered in the report template.
Sequencing Run Details	Contains the following subsections: • **Assay**—the assay name and panel used • **Analysis**—the run date and name of the user who sent the run to the instrument • **Run Details**—the consumables used in the run • **Control QC Evaluation Metrics**—a summary of the CF-1 quality control metrics • **Run QC Evaluation Metrics**—a summary of the run quality control metrics • **Sample QC Evaluation Metrics**—a summary of the sample quality control metrics

Download the Lab Report

You can download the Lab Report for a sample of interest from the **Results / Sample Results** screen.

Note: The Lab 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 70.

1. In the menu bar, click **Results ▸ Sample Results**.
2. In the **Sample Results** screen, in the **Actions** column, click **Lab Report** in the row of the sample of interest.



OCAv3_sample1_0000

☐	Sample Name	Assay Short Name	Run Name	Sample Status	QC Status	Started On	Last Updated O...	Actions
☐	OCAv3_Sample1_0000_USVM	OCAv3 DNA Fus w3.4.1	OCAv3_PR_01_OCAv3_LibBat...	Completed	✓	2019-08-29 16:01	2020-03-24 05:51	Lab Report | Audit | Notes ⋮

A ZIP file that contains the PDF report downloads automatically.

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

View sequencing results and assay metrics

For every run, you can view assay-specific results and sample-specific results. Assay-specific results include assay metrics, such as final read data, and assay-level plugin information, such as execution of the customer support archive. For more information, see "Assay metrics" on page 51 and "Review plugin results in Genexus™ Software" on page 68.

The following sample-specific result information is available.

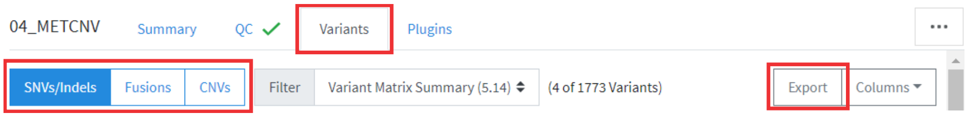
Tab	Description
Summary	An overview of the results for the sample, including **Sample Details**, **Key Metrics**, and the **Variant Summary**. For more information, see "Summary of the Sample Results" on page 58.
QC	The quality metrics for the sample sequenced in the run. For more information, see "QC results" on page 55.
Variants	Detailed variant results for **SNVs/Indels**, **Fusions**, and **CNVs**. For more information, see "View SNV/INDEL results" on page 59, "View Fusion results" on page 61, and "View CNV results" on page 64.
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 68.

1. To view sequencing results for a particular sample, click **Results ▸ Sample Results**. In the **Sample Name** column, click a sample name.

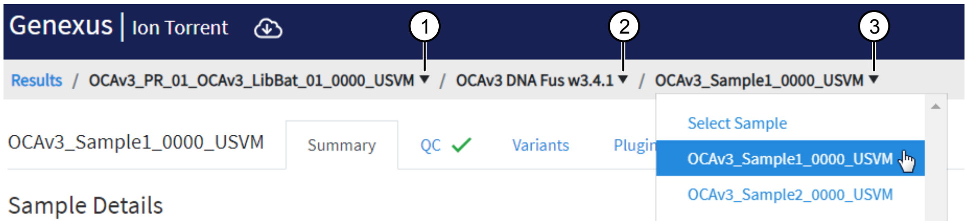
   The **Results** screen opens to the **Summary** tab, showing the **Sample Details**, **Key Metrics**, and **Variant Summary**.

2. In the **Results** screen, click the **QC**, **Variants**, **Plugins**, and **Summary** tabs to view the different types of sample-specific results and data.

   In the **Variants** tab, you can toggle between SNV/INDEL, Fusion, and CNV results. You can also export data in tabular format.


04_METCNV
Summary
QC ✓
Variants
Plugins
...
SNVs/Indels
Fusions
CNVs
Filter
Variant Matrix Summary (5.14)
(4 of 1773 Variants)
Export
Columns

3. Toggle between different assay-specific and sample-specific results for the run with the dropdown menus.


Genexus | Ion Torrent
1
2
3
Results / OCAv3_PR_01_OCAv3_LibBat_01_0000_USVM ▾ / OCAv3 DNA Fus w3.4.1 ▾ / OCAv3_Sample1_0000_USVM ▾
OCAv3_Sample1_0000_USVM
Summary
QC ✓
Variants
Plugin
Select Sample
OCAv3_Sample1_0000_USVM
OCAv3_Sample2_0000_USVM
Sample Details

① **Run Result**: The run name is listed.
② **Select Assay**: Click the assay name of interest to view the assay metrics for the run.
③ **Select Sample**: Click a sample name to view the sequencing results for the sample. Click a different sample name to view other sample results for the run and selected assay.

Options	Description
Run Results dropdown menu	The run name is listed. Multiple runs are listed only if the run has been reanalyzed. For more information, see "Reanalyze a run" on page 85.
Select Assay dropdown menu	Toggle between different assays used in the run. If only one assay is associated with the run, only one assay name is listed. To remove the assay selection, select **Select Assay** from the dropdown menu. Removing the assay selection opens the **Run Summary** tab for the run selected in the **Run Results** dropdown menu.
Select Sample dropdown menu	Toggle between different sample results for the selected run and assay. To remove the sample selection, select **Select Sample** from the dropdown menu. Removing the sample selection opens the **Assay Metrics** tab for the assay selected in the **Select Assay** dropdown menu.

All of the samples that were run with the same assay share the same assay metrics. All other results are sample-specific.

4. To view more options, click ··· **(More Options)**.

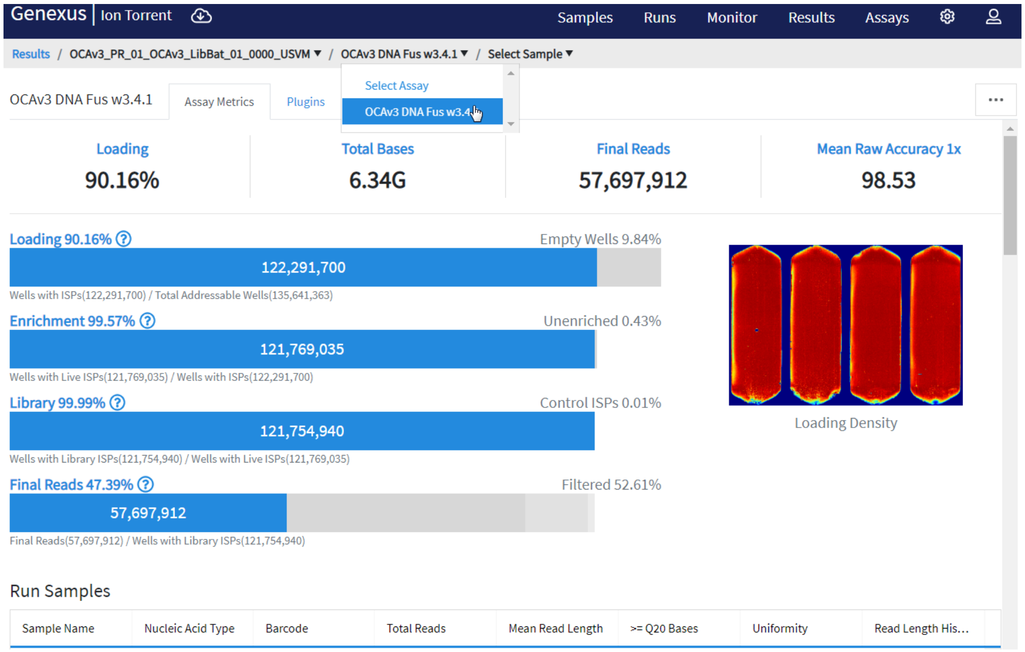
Options	Description
Reanalyze	Reanalyze a run with a new assay. For more information, see "Reanalyze a run" on page 85.
Run Plugin	Run plugins on your sequencing data after a sequencing run is complete.
Run Report	The run report includes assay metrics and the record of reagents that were used in a run. For more information, see "View or download a Run Report" on page 54.
Download Files	Download all or selected variant, results, audit and logs, and lab report files. For more information, see "Results files" on page 70.
Generate Report	Generate a Lab Report each sample in a sequencing run. For more information, see "Lab Report" on page 47.
Sign Off	Manager- and administrator-level users can provide their electronic signature on sample results for completed runs. For more information, see "Sign off on the run results (manager/administrator)" on page 72.
CSA	Customer support archive (CSA) log files for the run to help with troubleshooting.
Upload to IR	Upload results to Ion Reporter™ Software for further analysis.

Assay metrics

Assay metrics include various chip metrics for the run, such as well and Ion Sphere™ Particles (ISPs) statistics. For runs with multiple assays, metrics are provided for each assay in the run. Summary metrics are displayed at the top of the screen, followed by sample-specific metrics in the **Run Samples** table. Barcode-specific metrics are listed in the **Barcodes With Reads Reported** table. All barcodes with reads detected, even if unassigned or not planned in the run, are listed to allow you to readily identify the source of any barcode cross-contamination.

To view the assay metrics for a run, in the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest. In the **Run Summary** screen, select an assay from the **Select Assay** dropdown menu. To view the metrics for another assay in the run, select a different assay from the dropdown menu.

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


Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Results / OCAv3_PR_01_OCAv3_LibBat_01_0000_USVM ▼ / OCAv3 DNA Fus w3.4.1 ▼ / Select Sample ▼
OCAv3 DNA Fus w3.4.1
Assay Metrics
Plugins
Select Assay
OCAv3 DNA Fus w3.4
Run Samples
Sample Name
Nucleic Acid Type
Barcode
Total Reads
Mean Read Length
>= Q20 Bases
Uniformity
Read Length His...

Loading	Total Bases	Final Reads	Mean Raw Accuracy 1x
90.16%	6.34G	57,697,912	98.53

Loading 90.16% — Empty Wells 9.84%
122,291,700
Wells with ISPs(122,291,700) / Total Addressable Wells(135,641,363)

Enrichment 99.57% — Unenriched 0.43%
121,769,035
Wells with Live ISPs(121,769,035) / Wells with ISPs(122,291,700)

Library 99.99% — Control ISPs 0.01%
121,754,940
Wells with Library ISPs(121,754,940) / Wells with Live ISPs(121,769,035)

Final Reads 47.39% — Filtered 52.61%
57,697,912
Final Reads(57,697,912) / Wells with Library ISPs(121,754,940)

Loading Density

Table 6 Assay Metrics

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. This value can be different from the total number of reads due to technicalities associated with read trimming beyond a minimal requirement.
Total Bases	The number of filtered and trimmed base pairs that are reported in the output BAM file.
Mean Raw Accuracy 1x	The mean raw 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.
Total Addressable Wells	Wells on the chip that can be physically reached by a library.
Empty Wells	The percentage of total addressable wells on the chip that do not contain an ISP.
Wells with Live ISPs	Loaded wells with ISPs with a signal of sufficient strength and composition to be associated with the library or control fragment key.
Wells with Library ISPs	Loaded wells with live ISPs with a key signal that is identical to the library key signal.

Table 6 Assay Metrics *(continued)*

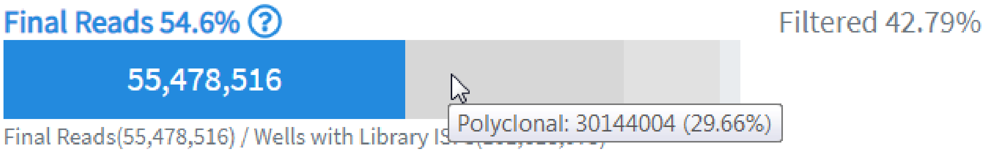
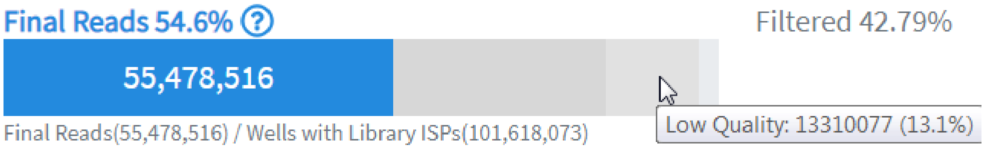
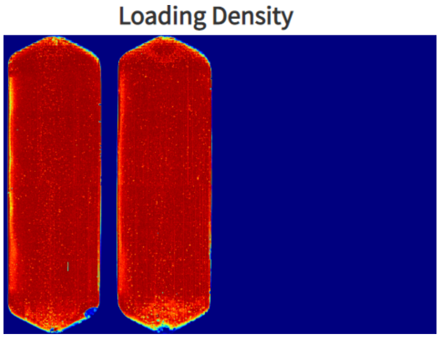
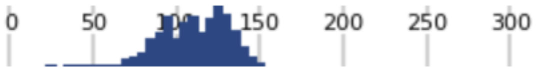
Metric	Description
Control ISPs	Loaded wells with live ISPs with a key signal that is identical to the control fragment key signal.
Polyclonal	Wells with a live ISP that carries clones from two or more templates. To view polyclonal metrics, mouse over the first low quality portion (gray) of the **Final Reads** bar plot.  Final Reads 54.6% Filtered 42.79% 55,478,516 Final Reads(55,478,516) / Wells with Library IS Polyclonal: 30144004 (29.66%)
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.  Final Reads 54.6% Filtered 42.79% 55,478,516 Final Reads(55,478,516) / Wells with Library ISPs(101,618,073) Low Quality: 13310077 (13.1%)
Filtered	The total percentage of filtered reads, or the sum of the percentages of polyclonal, low quality, and adapter dimer reads.
Adapter Dimer	Loaded wells with a library template of an insert size less than 8 bases.
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 illustrates a sequencing experiment where two lanes on the chip are uniformly loaded with ISPs. 

Table 7 Run Samples

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

Column	Description
Sample Name	The unique identifier created when the sample was entered in the software.
Nucleic Acid Type	The sample nucleic acid type, such as DNA, RNA, or TNA.
Barcode	The unique identifiers of the dual barcode pair assigned to the DNA and/or RNA library for a sample.
Total Reads	The total number of filtered and trimmed reads with the listed dual barcodes assigned to the sample. The reads are independent of length reported in the output BAM file.
Mean Read Length	The average length, in base pairs, of usable library reads for each sample.
≥Q20 Bases	The total number of called bases that have ≥99% accuracy (or less than 1% error rate) aligned to the reference for the sample.
Uniformity	The percentage of bases in all targeted regions (or whole genome) with a depth of coverage ≥20% of the mean read coverage.
Read Length Histogram	A histogram that presents all filtered and trimmed library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences. 

View or download a Run Report

You can view a run report or 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.

1. In the menu bar, click **Results ▸ Run Results**.
2. In the **Run Results** screen, in the **Run Name** column, click the run name of interest.
3. In the **Run Summary** screen, click ⋯ **(More Options) ▸ Run Report**.
   A **Run Report** window opens.
4. To download a run report summary in PDF format, click **Download as PDF**.

QC results

The **QC** screen displays quality metrics for each sample that was sequenced in a run. This information is also accessible through the **Monitor** menu within 72 hours of starting the run on the sequencer.

To view the **QC** screen, in the **Results / Sample Results** screen, click a sample name in the **Sample Name** column. In the **Results** screen, click the **QC** tab. The QC status for each metric is indicated beneath each QC test (Run QC, Templating Control QC–CF–1, Sample QC–DNA, and Sample QC–RNA).

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 broken down 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 a run (see “Reanalyze a run” on page 85).

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

Metric	Description
Run QC	General run quality control information.
Key Signal	The average signal after software processing for library ISPs that identically match the library key (TCAG).
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. If the MAPD QC threshold is not met, CNVs do not get called. The MAPD metric does not affect SNVs/INDEL calls.
Mapped Reads	The number of reads that are mapped to the reference file.
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.
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.
Median Mol Cov	The median number of functional molecule reads per amplicon calculated over all amplicons in the assay. This metric is applicable to Ion AmpliSeq™ HD library chemistry only.
Uniformity of Base Coverage	The percentage of reads with a depth of coverage ≥20% of the mean read coverage at each position. This metric is applicable to Ion AmpliSeq™ HD library chemistry only.
Read Length Histogram	The histogram presents all filtered and trimmed DNA library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences.
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 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 number of final library reads that are aligned to the reference file for the sample.
Mean Read Length (bp)	The average length, in base pairs, of the final library reads for the sample.
RNA Expression Ctrls Detected	The number of expression control genes detected for the sample. This metric measures the RNA input integrity, input amount, and the fidelity of the reverse transcriptase that was used in library preparation. Fusion panels include primer pairs that cover 7 control housekeeping genes.

(continued)

Metric	Description
Mean AQ20 Read Length	The average length, in base pairs, of the final library reads with no more than 1% error rate for the sample. The average length, in base pairs, at which the accuracy rate is ≥99% for all aligned reads of a library.
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). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences.
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of genomic sample reads to control reads for each inline control amplicon. You can use this metric to measure the level of contaminating genomic DNA in RNA libraries when you select the RNA checkbox for **Include Inline Controls** in assay set up. Using inline controls in RNA assays will reduce the total number of panel reads. **Note:** The **Read Ratio** for each inline control amplicon is expected to be 3 with 10 ng DNA input for both Ion AmpliSeq™ and Ion AmpliSeq™ HD chemistries.
NTC QC – DNA	**DNA Sequencing quality metrics of the no template control.**
Average Base Coverage Depth	The average number of DNA reads of all targeted reference bases.
Mean Read Length (bp)	The average length, in base pairs, of final DNA library reads for the no template control.
Read Length Histogram	The histogram presents all filtered and trimmed reads for the no template control that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis).
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of no template control sample reads to control reads for each DNA inline control amplicon.
NTC QC – RNA	**RNA Sequencing quality metrics of the no template control.**
Mapped Reads	The average number of RNA reads of all targeted reference bases.
Mean Read Length (bp)	The average length, in base pairs, of final RNA library reads for the no template control.
RNA Expression Ctrls Detected	The number of expression control genes detected for the no template control.
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.

Summary of the Sample Results

The **Results** screen displays a summary of the variant and fusion calls for each sample in a run, as well as details about the sample and a summary of the metrics for the run.

You can view the **Results** screen for a sample starting from sample or run results, but navigating from sample results requires fewer steps.

- In the menu bar, click **Results ▸ Sample Results**, then click a sample name to open the **Results** screen.
- In the menu bar, 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. Select a sample from the **Select Sample** dropdown list to open the **Results** screen.

The information that is displayed depends on the assay that was used in the run. You can toggle between different assays used in a run with the **Assays** dropdown list at the top of the screen.

Section	Description
Sample Details	
Sample Name	A unique identifier representing the sample. Click the **Sample Name** to open the **Sample Details** screen for the sample. Use the tabs above the Sample Details to view the run summary, assay metrics, quality control, detailed variant results, and results for plugins that are associated with the selected assay, if any.
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
Disease Category	The disease type of the sample.
Cancer Type	The type of cancer that is represented by the sample.
Cancer Stage	The stage of the cancer from which the sample was collected.
% Cellularity	The percentage of tumor cellularity in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is applicable to FFPE samples only.
Key Metrics	
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 are covered by at least 20% of the average base coverage depth reads. Cumulative coverage is linearly interpolated between the nearest integer base read depths.

(continued)

Section	Description
% Base Reads On Target	The percentage of filtered reads that are mapped to any targeted region relative to all reads mapped to the reference. A read is considered on target if at least one aligned base overlaps at least one target region. If no target regions (file) was specified, this value will be the percentage of reads passing uniquely mapped and/or non-duplicate filters, or 100% if no filters were specified.
Variant Summary	
SNVs/Indels	Lists and describes the SNV, MNV, and INDEL variants that are detected in the sample.
Fusions	Lists and describes the fusions that are detected in the sample.
CNVs	Lists and describes the copy number variants (CNVs) that are detected in the sample.

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. In the **Variants** tab, click **SNVs/Indels** to display the data. 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 table depend on the assay that was used in the run. Results in the table can be filtered using the filtering tools. For more information, see “Filter variant results” on page 67.

Column	Description
User Classifications	User-defined classification to selected from the list. For more information, see “Create and assign variant classifications” on page 83.
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.

(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.
Gene	The gene name. Click the link to open the **View Annotation Sources** window to view additional information. For more information, see "View annotation sources" on page 82.
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
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 70). • **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS) –** indicates a high confidence call that passes all filter thresholds at a given variant position. **Note:** • When the default filter chain is applied, **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** indicates the presence of the ALT (alternative) allele. • When the **No Filter** option is applied or when viewing the **Variants (.vcf)** file, **Present** does *not* imply the presence of the ALT (alternative) allele. To infer the presence of the ALT allele, refer to the **Alt** column. • **NO CALL –** while some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call. • **ABSENT –** indicates the presence of a variant that differs from the reference allele at a given position, however, this nucleotide is not an ALT allele that is targeted by this assay.

(continued)

Column	Description
No Call Reason	The reason why a variant is not reported.
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.
Variant ID	The name of the hotspot as defined in the Browser Extensible Data (BED) file.
Nuc Change	The position and identity of the nucleic acid change.
Allele Fraction	The number of variant read counts divided by the total number of read counts for the sample.

View Fusion results

The **Fusions** table lists the calls and other information for the fusions analyzed in 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. In the **Variants** tab, click **Fusions** to display the data. 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. Filter the results list in the table using the filtering tools. For more information, see “Filter variant results” on page 67.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see “Create and assign variant classifications” on page 83.
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)

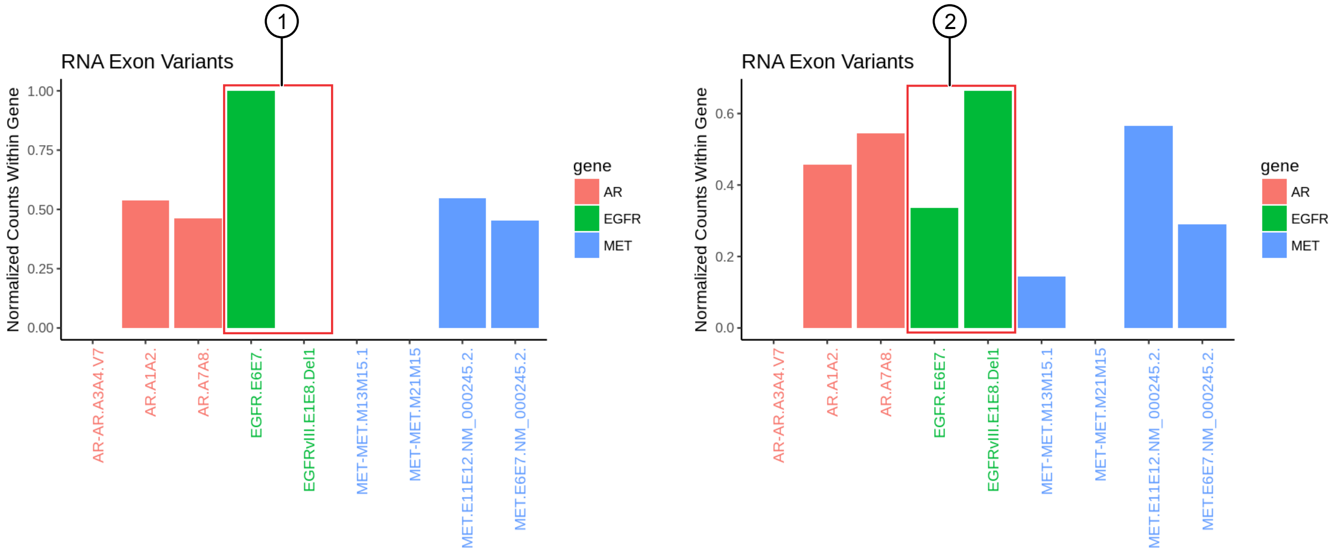
Column	Description
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function — the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • Loss-of-function — 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), RNAExon Tile, Proc Control).
Call	Indicates the presence or absence of a fusion or RNA exon variant. When the default filter chain is applied, only the fusion/RNA exon variant calls that are designated with **PRESENT** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants (.vcf)** file (see "Results files" on page 70). • **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 the variant call failing internal quality control. • **NO CALL –** while 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.
No Call Reason	The reason for reporting a fusion as **NOCALL**.
Failed Reason	The reason for reporting a fusion as **Absent.**
Variant ID	The name of the fusion target as defined in the BED file.
Read Counts Per Million	The number of fusion read counts detected per number of total reads (in millions).
Oncomine Driver Gene	The gene believed to be associated with increased oncogenic properties. The gene is inappropriately activated by the fusion.

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.
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 analysis where only the wild type EFGR (EFGR.E6E7) was detected.

② Example analysis 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. Results in the table can be filtered using the filtering tools. For more information, see "Filter variant results" on page 67.

IMPORTANT! *(FFPE samples only)* If **% Cellularity** value for a sample is set to <100, then the magnitude of copy number gain or loss can be decreased. For more information, see "System-installed sample attributes" on page 28.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see "Create and assign variant classifications" on page 83.
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, which provides a link to the **View Annotation Sources** dialog box with additional information about the **HotSpot ID**. For more information, see "View annotation sources" on page 82.
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 70). • **PRESENT –** indicates a high confidence call that passes all filter thresholds. • **PRESENT (GAIN) –** a CNV-positive call that indicates gene amplification; a high confidence variant call that passes all filter thresholds. • **PRESENT (LOSS) –** a CNV-positive call that indicates gene deletion; a high confidence variant call that passes all filter thresholds. • **ABSENT –** the absence of a variant; result is below detection threshold for a CNV call. • **NO CALL–** while some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call.
P-Value	The statistical significance of the CNV ratio measurement.
No Call Reason	The reason for reporting a CNV as No Call.
CNV Confidence	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.
Variant ID	The name of the hotspot as defined in the BED file.
CNV Ratio	The ratio of measured CNV gene locus coverage relative to coverage of non-CNV loci.
Med Read Cov Gene	The median read coverage of targeted CNV gene.
Med Read Cov Ref	The median read coverage of non-CNV reference loci.
Valid CNV Amplicons	The number of amplicons spanning the CNV call.

Compare sample results

In Genexus™ Software, you can compare variant results across samples and create customized comparison plots. If you want 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: At least 2 samples are required to be selected.

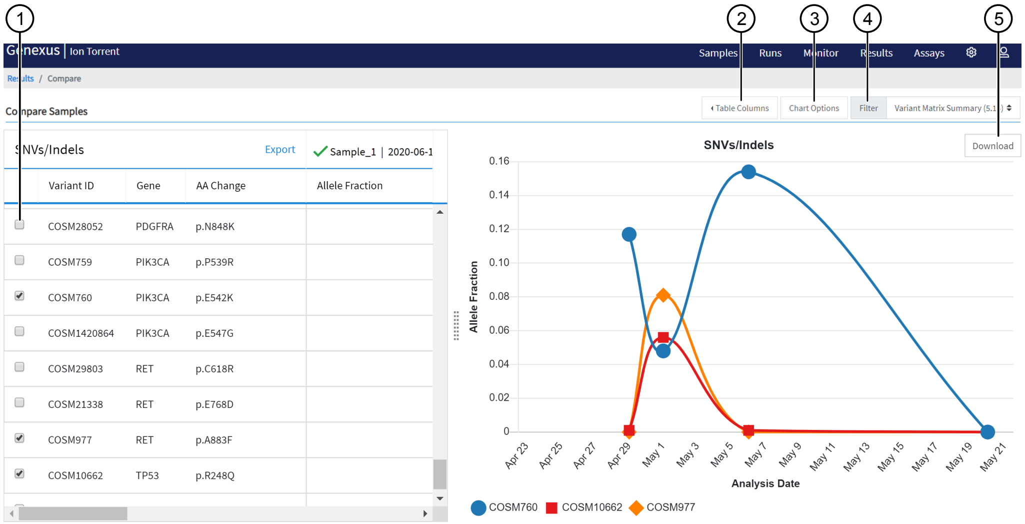
The **Results / Compare** screen opens, displaying a list of variants and variant information in the **Compare Samples** table.

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 as 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	From the **Filter** 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 68.

5. To download the variants plot in PNG format, click **Download** (see callout 5 in Figure 1).


①
②
③
④
⑤
Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Results / Compare
Compare Samples
Table Columns
Chart Options
Filter
Variant Matrix Summary (5.1)
SNVs/Indels
Export
✓ Sample_1 | 2020-06-1
SNVs/Indels
Download
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
Allele Fraction
Apr 23
Apr 25
Apr 27
Apr 29
May 1
May 3
May 5
May 7
May 9
May 11
May 13
May 15
May 17
May 19
May 21
Analysis Date
● COSM760
■ COSM10662
◆ COSM977

	Variant ID	Gene	AA Change	Allele Fraction
☐	COSM28052	PDGFRA	p.N848K	
☐	COSM759	PIK3CA	p.P539R	
☑	COSM760	PIK3CA	p.E542K	
☐	COSM1420864	PIK3CA	p.E547G	
☐	COSM29803	RET	p.C618R	
☐	COSM21338	RET	p.E768D	
☑	COSM977	RET	p.A883F	
☑	COSM10662	TP53	p.R248Q	

Figure 1 Compare variants

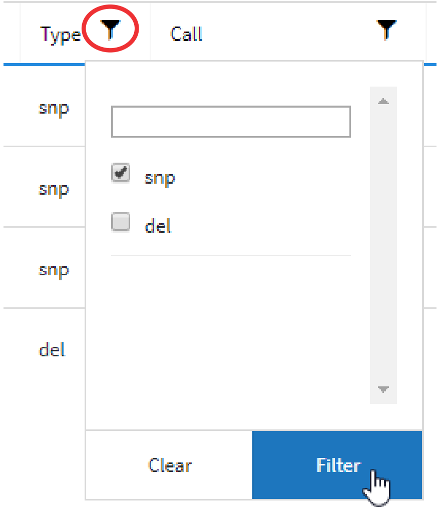
① Select one or more variants to compare in the variants plot.
② Customize columns that appear in the **Compare Samples** table.
③ Customize the X-axis and Y-axis that appear in the variants plot.
④ Select a filter chain, to filter the results that appear in the **Compare Samples** table.
⑤ Download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

Filter variant results

In the **Results** screen, in the **Variants** tab, click **SNVs/Indels**, **Fusions**, or **CNVs**, then filter the results tables in one of the following ways.

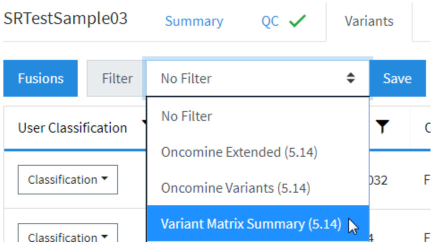
- Click ▼ **(Filter)** in the column header of interest, select the checkbox in the row of each filter that you want to apply, then click **Filter**.

  Note: If too many filter options are available, enter a keyword into the provided field to search the filters.


Type
Call
snp
snp
snp
del
☑ snp
☐ del
Clear
Filter

- From the **Filter** dropdown list in the upper left corner of the screen, select the desired filter chain.

  If the selected filter chain is not associated with the assay, and you want to apply the filter chain to all results that use the assay, click **Save**.


SRTestSample03
Summary
QC ✓
Variants
Fusions
Filter
No Filter
Save
User Classification
Classification
Classification
No Filter
Oncomine Extended (5.14)
Oncomine Variants (5.14)
Variant Matrix Summary (5.14)

  For more information about system-installed filters, see “System-installed filter chains” on page 68. For information on how to create a custom filter chain, see *Genexus™ Software Help*.

- Click **Columns** in the top left corner of the screen, then deselect the columns that you want to remove from the table.

The list of variants updates to reflect the new filter selections.

System-installed filter chains

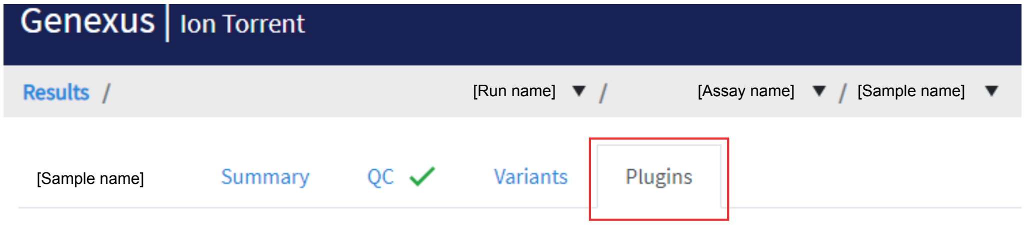
The following Genexus™ Software system-installed filter chains are available for use with the Oncomine™ Comprehensive Assay v3 GX results.

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

System-installed filter chain	Description
Oncomine™ Variants (5.14 or later)	Default filter chain that is optimized for reporting detected variants with the Oncomine™ Comprehensive Assay v3 GX.This filter chain returns results for INDELs and SNV variant types, and minor allele frequencies between 0.0 and 1.0E-6 based on 5000Exomes and ExAC annotation source databases that have homopolymer lengths less than or equal to 7 and allele frequencies between 0.05 and 1.0.
Oncomine™ Extended (5.14 or later)	This filter chain includes all Oncomine™-annotated variants and variants that are relevant to cancer due to their inclusion in one or more of the following classes. • Non-targeted fusions. • Likely somatic mutations based upon 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 filter chain to remove a previously applied filter chain and view all called variants.

Review plugin results in Genexus™ Software

In Genexus™ Software, you can review plugin results in the **Results** screen for a sample. Each plugin that was selected for an assay during assay creation is listed in the **Plugins** tab.



Review coverageAnalysis plugin results

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

The report summary lists the barcode, the sample, the number of mapped reads, the percentage of on target reads, mean base coverage depth, and base coverage uniformity. Microsoft™ Excel™-compatible reports are also generated, including differential expression tables. Additional details regarding read coverage are also provided on a per-barcode basis, along with a list of gene annotations for each sequenced region.

You can download coverageAnalysis plugin output files from the **Results** screen for a sample. For more information, see “Results files” on page 70 and “Output files generated by the coverageAnalysis plugin” on page 92.

For more information on how to review and interpret read statistics and output files, see Appendix C, “coverageAnalysis plugin in Genexus™ Software”.

1. In the menu bar, click **Results ▸ Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample of interest.
3. Click the **Plugins** tab.

   A summary table of the coverage analysis, by barcode, is included in the **coverageAnalysis** summary pane.
4. *(Optional)* From the **Executed At** dropdown list, select an alternate timestamp, if available, to view additional reports.
5. *(Optional)* Click **View Log** to view the coverageAnalysis log.
6. *(Optional)* Click **Delete** to delete the coverageAnalysis plugin output for the selected timestamp.

   IMPORTANT! If you click **Delete**, the report is deleted without the appearance of confirmation dialog window. Ensure that you intend to delete the report before clicking **Delete**.
7. 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.

Results files

The following files can be downloaded from the **View Results** screen for each sample. The files that are available for download vary depending on the assay used. For a list and descriptions of plugin output files, see "Output files generated by the coverageAnalysis plugin" on page 92.

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

Selection	File name	Description
Variants		
Filtered Variants (.vcf)	`<Filter name>_filtered.vcf`	Summary of filtered variant results in variant call format (VCF).
All Variants (.vcf)	`Oncomine_<LibPrepID>_<analysisID>.vcf`	Summary of variant results in variant call format (VCF).
Variant Summary (.tsv)	`Summary.tsv`	File that lists SNV/INDEL, copy number, and fusion results in tab-separated value format (TSV).
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.

(continued)

Selection	File name	Description
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.
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	`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		
Lab Report	`<language>_<sample name>_AD_Lab_Report_<assay name>_<date>.pdf`	A PDF report that contains sample-specific results. For more information, see “Lab Report” on page 47.

Sign off on the run results (manager/administrator)

Manager- and administrator-level users can provide their electronic signature on sample results for completed runs. In the **Results / Sample Results** screen, a sample name followed by ***(Signed Off)*** indicates that a manager- or administrator-level user has approved the sample results. The signature information appears in the Lab Report PDF file or a user-created report, if selected. For more information, see "Lab Report" on page 47. For information on how to create a report template, see *Genexus™ Software 6.2 Help*, or the *Genexus™ Software 6.2 User Guide* (Pub. No. MAN0018955).

A manager- or administrator-level user can update report template selections during sign off.

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

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, click the sample of interest in the **Sample Name** column.
3. In the upper right-hand corner of the screen, click ⋯ **(More Options)** ▸ **Sign Off**.
4. In the **Electronic Signature** dialog box, enter your user name, password, and comments.

   Items identified with a red asterisk (*) are required fields.
5. In the **Meaning of Signature** dropdown list, select **Approval**.
6. In the **Report Template** dropdown list, select the report template that you want to use.
7. In the **Report Customizations** section, in the **Lab Report** pane, select the types of variant calls that you want to include in each report. For assays that use Reporting Gene Lists, you can customize the variants by reporting category.
8. 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.
9. Click **Sign Off** to confirm your electronic signature.

10. In the menu bar, click **Results ▸ Sample Results** to return to the **Results / Sample Results** screen.
11. In the row of the sample of interest, in the **Actions** column, click **Lab Report** to download the report.

A

Troubleshooting

Genexus™ Integrated Sequencer—general and QC troubleshooting

Observation	Possible cause	Recommended action
Consumable is not recognized by instrument after loading on the deck	The consumable (for example, a strip, barcode plate, 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 6.0 is installed in a sequencer updated for Genexus™ Software 6.2.	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 for instrument service.
Used lane 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 write efuse.	In the sequencer screen, tap **Settings ▸ Clean instrument** to perform a clean instrument. After cleaning, start a new run.

Observation	Possible cause	Recommended action
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 77.	Nucleic acid input may have been insufficient, and/or the nucleic acid was degraded.	For a sample run, re-quantify nucleic acid samples and/or perform sample QC to ensure that the expected nucleic acid input and size was loaded.
		If needed, re-isolate and purify nucleic acid samples.
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 77.	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.
	One of 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 39.
	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.
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 77.	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 39.
	Template strips were inadequately equilibrated to room temperature (Genexus™ Strip 3-GX5™), or incompletely thawed (Genexus™ Strip 4) before loading in the sequencer.	Ensure that Genexus™ Strip 3-GX5™ strips are fully equilibrated to room temperature, and Genexus™ Strip 4 strips are completely thawed before loading in the sequencer.
	The sequencing chip or coupler was faulty or leaky.	Repeat the run with new chip and coupler. If low performance continues, contact Technical Support.

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 77. *(continued)*	The run was started >14 days after the last initialization was performed, or on an expired initialization.	Perform a Clean instrument procedure (**Settings ▸ Clean instrument**), install new a chip, and new sequencing reagent bottles and cartridge in the sequencing reagents bay, then repeat the run. Reagents are stable on the sequencer for 14 days, after which you may experience reduced performance. For further information, see *Appendix A: Troubleshooting* in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

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 of the **Create Assay** workflow, you include the inline control analysis in the post-run results analysis. Addition of a set of six control amplicons (covering a range of amplicon length) and spike-in nucleic acid into sample library preparation reactions helps you identify 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 inline 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 74.

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, and 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.
The assay I created does not appear in the menu when I plan a run	Forgot to lock your assay.	Go to **Assay tab** ▶ **Manage Assays** and make sure that the assay is locked.
Cannot upload my panel or hotspots	Issues with BED file format or files do not end in (.bed).	Ensure your file is in the correct BED format and has a (.bed) extension.
Variants tab is missing hotspot entries. **Details:** The remaining entries are present.	Hotspot BED file contains entries that are incorrectly formatted.	Check that BED file entry is correctly formatted. See the following examples: SNP entry: chr1 2337276 2337277 SVA_322 0 + REF=C;OBS=T;ANCHOR=G AMPL Deletion entry: chr1 201341175 201341180 SVA_497 0 + REF=AGAAG;OBS=;ANCHOR=C AMPL Insertion entry: chr1 236978992 236978992 SVA_621 0 + REF=;OBS=TCTG;ANCHOR=T AMPL
		Confirm 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.

Observation	Possible cause	Recommended action
The results of the run are not showing up in the Completed Runs & Reports 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).
Cannot download run result files	The run failed. Links to run files are not available for runs that fail QC.	Create a reanalysis assay with reduced QC parameters, then reanalyze your sample.
		Repeat the run.

B Supplemental information

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- Create and assign variant classifications 83
- Reanalyze a run 85
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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 24). If needed, you can copy a system-installed assay, then modify settings as needed (see "Copy an assay (manager/administrator)" on page 25). 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 validated. 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 descrease 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 35.

(continued)

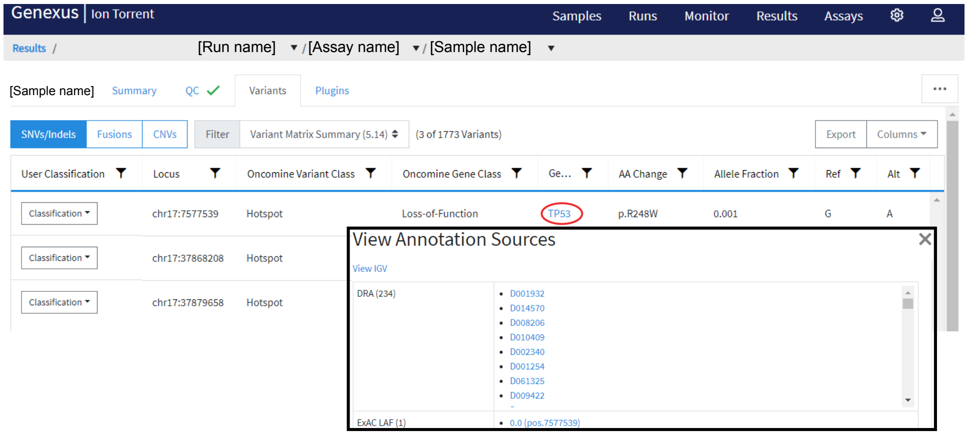
Workflow step	Setting	Guidelines
QC (Sample QC - DNA)	**MAPD**	0.5
	Mapped Reads	3,000,000
	Mean Read Length (bp)	75
	Uniformity Of Amplicon Coverage	85%
QC (Sample QC - RNA)	**Mapped Reads**	200,000
	Mean Read Length (bp)	60
Parameters	**Sample input Maximum (ng)** **Sample input Minimum (ng)**	By default, 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 as 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. **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 37. 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 "Create a run plan for a sample run" on page 31.

View annotation sources

You can view more information for each HotSpot ID in the sample **Results** screen in the **SNVs/Indels**, **Fusions**, and **CNVs** tables.

In the menu bar, click **Results ▸ Sample Results**, then click the sample name in the **Sample Name** column in the row of a sample of interest. In the **Variants** tab, click **SNVs/Indels**, **Fusions**, or **CNVs**.

1. In the **SNVs/Indels**, **Fusions**, or **CNVs** table, in the **Gene** column, click the gene symbol.


Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Results / [Run name] / [Assay name] / [Sample name]
[Sample name]
Summary
QC ✓
Variants
Plugins
SNVs/Indels
Fusions
CNVs
Filter
Variant Matrix Summary (5.14)
(3 of 1773 Variants)
Export
Columns
View Annotation Sources
View IGV

User Classification	Locus	Oncomine Variant Class	Oncomine Gene Class	Ge...	AA Change	Allele Fraction	Ref	Alt
Classification	chr17:7577539	Hotspot	Loss-of-Function	TP53	p.R248W	0.001	G	A
Classification	chr17:37868208	Hotspot						
Classification	chr17:37879658	Hotspot						

DRA (234)	D001932 D014570 D008206 D010409 D002340 D001254 D061325 D009422
ExAC LAF (1)	0.0 (pos.7577539)

The **View Annotation Sources** window opens, which displays information for the particular variant.

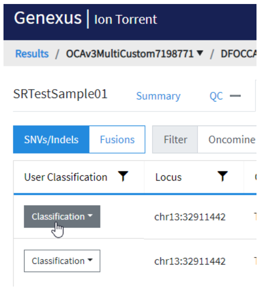
2. Review annotation information in the **View Annotation Sources** window.
   a. Click **View IGV** to download a JNPL file that can be opened in the Integrative Genomics Viewer (IGV) from the Broad Institute (see **software.broadinstitute.org/software/igv**).

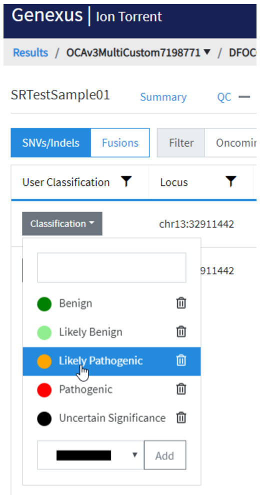
   b. If available, click a link in the row of an annotation source to navigate to the online annotation information.

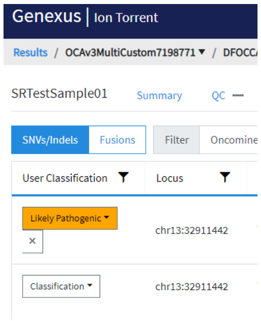
Create and assign variant classifications

You can create and assign user-defined variant classifications in the SNVs/Indels, Fusions, and CNV tables in the **Variants** tab of the **Results** screen.

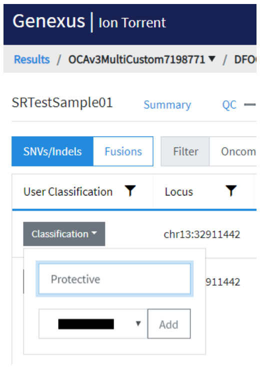
1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name in the **Sample Name** column in the row of a sample of interest to open the **Results** screen for the sample.
3. Click the **Variants** tab.
4. In the **Variants** table, click **SNVs/Indels**, **Fusions**, or **CNVs** to display the list of variants for the selected variant type.
5. In the **Variants** table, in the **User Classification** column, perform any of the following actions.
   - To assign an existing classification to a variant, select it from the list.

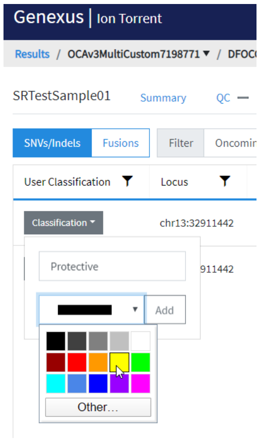

Genexus | Ion Torrent
Results / OCAv3MultiCustom7198771 ▾ / DFOCCA
SRTestSample01
Summary
QC
SNVs/Indels
Fusions
Filter
Oncomine
User Classification
Locus
Classification ▾
chr13:32911442
Classification ▾
chr13:32911442

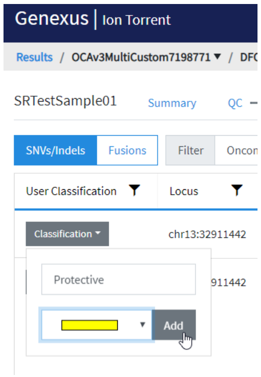

Genexus | Ion Torrent
Results / OCAv3MultiCustom7198771 ▾ / DFOC
SRTestSample01
Summary
QC
SNVs/Indels
Fusions
Filter
Oncomir
User Classification
Locus
Classification ▾
chr13:32911442
911442
● Benign
● Likely Benign
● Likely Pathogenic
● Pathogenic
● Uncertain Significance
Add


Genexus | Ion Torrent
Results / OCAv3MultiCustom7198771 ▾ / DFOCCA
SRTestSample01
Summary
QC
SNVs/Indels
Fusions
Filter
Oncomine
User Classification
Locus
Likely Pathogenic ▾
×
chr13:32911442
Classification ▾
chr13:32911442

- To create a new classification, enter a name for the classification in the text box, select a color for the new classification, then click **Add**.


Genexus | Ion Torrent
Results / OCAv3MultiCustom7198771 ▾ / DFO
SRTestSample01
Summary
QC
SNVs/Indels
Fusions
Filter
Oncom
User Classification
Locus
Classification ▾
chr13:32911442
Protective
911442
Add


Genexus | Ion Torrent
Results / OCAv3MultiCustom7198771 ▾ / DFOC
SRTestSample01
Summary
QC
SNVs/Indels
Fusions
Filter
Oncomir
User Classification
Locus
Classification ▾
chr13:32911442
Protective
911442
Add
Other...


Genexus | Ion Torrent
Results / OCAv3MultiCustom7198771 ▾ / DF
SRTestSample01
Summary
QC
SNVs/Indels
Fusions
Filter
Oncor
User Classification
Locus
Classification ▾
chr13:32911442
Protective
911442
Add

- To remove a classification from a variant, click ✕ **(Remove)**.
- To delete a classification from the list, click 🗑 **Delete** next to the classification name. The classification will be removed from all variants in all results.

Reanalyze a run

If a sequencing run fails to meet one or more QC parameters defined by the assay, you can relax the assay parameters and reanalyze a run. Runs can be reanalyzed starting from Alignment or Basecalling. When you reanalyze a run, the reanalysis is applied to all samples in the assay. For more information, see "View sequencing results and assay metrics" on page 49.

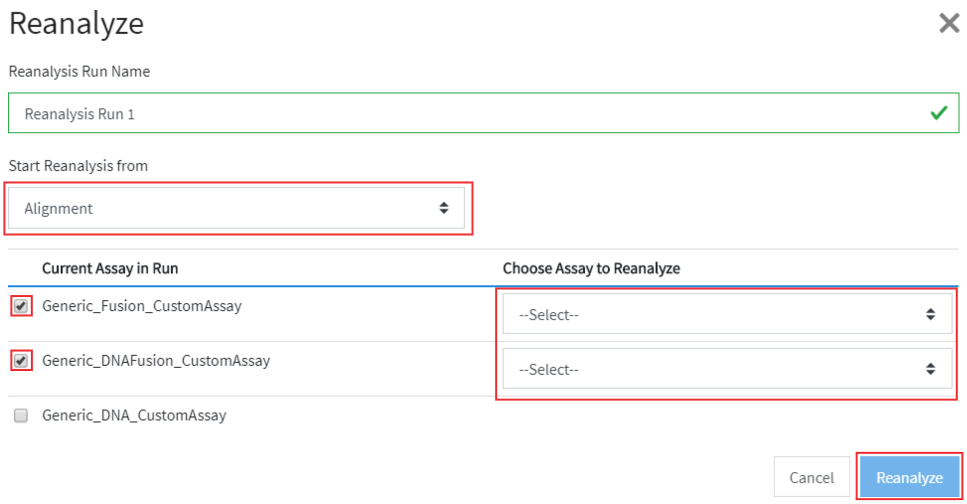
Note:

- Manager- and administrator-level users can reanalyze a sequencing run only if the run completed without any critical alarms or errors. If the run aborted or produced major alarms or errors, the run cannot be reanalyzed.
- QC parameters at the limits of stringency cannot be relaxed further. The sample library must be resequenced or a new library prepared.
- An assay can be used only once.

Before you begin, create a new assay or copy the original assay that was used in a run and modify assay parameters as needed. For more information, see Chapter 3, "Create and manage assays (manager/administrator)".

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest.
3. In the upper right corner of the screen, click ⋯ **(More Options)** ▸ **Reanalyze**.
4. In the **Reanalyze** dialog box, enter or select the following information.
    a. In **Reanalysis Run Name** field, enter a reanalysis run name.
    b. In **Start Reanalysis from** dropdown list, select **Alignment** or **Basecalling**.

c. In the **Current Assay in Run** column, select the checkbox in the row of each assay that you want to reanalyze, then in the **Choose Assay to Reanalyze** column, select an assay that you want to use for each reanalysis from the dropdown list.



Reanalyze

Reanalysis Run Name: Reanalysis Run 1

Start Reanalysis from: Alignment

Current Assay in Run	Choose Assay to Reanalyze
☑ Generic_Fusion_CustomAssay	--Select--
☑ Generic_DNAFusion_CustomAssay	--Select--
☐ Generic_DNA_CustomAssay	

5. Click **Reanalyze**.

Follow the progress of the reanalysis in the **Results / Run Results** screen in the **Run Status** column and in the **Results / Sample Results** screen in the **Sample Status** screen. When reanalysis is complete, the new results can be viewed by clicking the run name corresponding to the reanalysis assay in the **Results / Run Results** screen. Runs that have been reanalyzed are appended with ***(Reanalysis)*** after the run name.

Generate customized reports

When generating a customized report, you can update any report template selections. For more information on results files, see “Results files” on page 70.

1. In the menu bar, click **Results ▸ Sample Results**.
2. In the **Results / Sample Results** screen, click the sample of interest in the **Sample Name** column.
3. In the **Results** screen, click ⋯ **(More Options) ▸ Generate Report**.
4. In the **Generate Report** dialog box, select the report template and variants to include in the report.
5. Click **Generate**.
   A ZIP file that contains all the selected reports and other files is downloaded.

Configure Thermo Fisher Accounts in Genexus™ Software (administrator)

Administrator-level users can configure a link in Genexus™ Software to one or more Connect user accounts. This account type is called a **Thermo Fisher Connect** account in Genexus™ Software.

When a **Thermo Fisher Connect** account is configured and active, administrator-level users can perform the following tasks in the Genexus™ Software:

- Download the latest software updates
- Download additional plugin software
- Download assay configuration packages
- Download software configuration packages

Before you configure a **Thermo Fisher Connect** account you must have a valid Connect account on the **thermofisher.com** website.

1. Click ⚙ **(Settings)** ▸ **Thermo Fisher Account**.
2. If you do not have a Connect account, create a new Connect account. If you already have a Connect account, proceed to step 3.
   a. Navigate to **thermofisher.com/connect**.
   The internet browser opens to the Connect home page.
   b. Click **Sign up now** to navigate to the registration page, then enter the information that is required to create a new account.
   c. Click **Create account** at the bottom of the screen to complete the registration.
3. In the **Thermo Fisher Account Settings** screen, click + **Create Account**.
4. In the **Create Thermo Fisher Account** dialog box, enter the information that is required to create the account.

Item	Description
Account Type	Select **Thermo Fisher Connect**.
Name	Enter a name to identify the account in the **Thermo Fisher Account Settings** screen in Genexus™ Software. The name can contain only alphanumeric characters (0-9, Aa-Zz), periods (.), underscores (_), or hyphens (-). For example, enter ***Lab_Admin***.
User Name	The username is the email that you used to register for the Connect account.
Password	Enter the password for the Connect account.

The configured account is listed in the ⚙ **(Settings) / Thermo Fisher Account** screen. A successfully authenticated account has **Active** listed in the **Status** column.

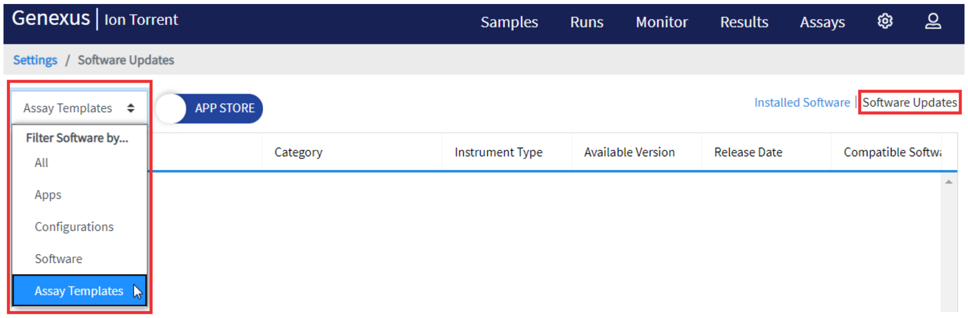
When the **Thermo Fisher Account** is active, software updates from the Connect App Store are automatically uploaded to the Genexus™ Software and administrator-level users can download the updates. For more information, see "Download software and assay package updates" on page 88.

Download software and assay package updates

In order to download and install software and assay package updates, you must first:

- Configure a Thermo Fisher Connect account. For instructions, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 87.
- Ensure that the Genexus™ Integrated Sequencer is running on the latest Genexus™ Software version.
- Connect your Genexus™ Integrated Sequencer to the internet.

1. 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.


Genexus | Ion Torrent
Samples
Runs
Monitor
Results
Assays
Settings / Software Updates
Assay Templates
APP STORE
Installed Software | Software Updates
Filter Software by...
All
Apps
Configurations
Software
Assay Templates
Category
Instrument Type
Available Version
Release Date
Compatible Softw

2. From the **Filter Software by...** dropdown list, select **Software** to view available software package updates or **Assay Templates** to view available assay package updates.
   The list of available updates is displayed.
3. 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.
4. Click **Install**. When the confirmation window appears, click **Yes** to confirm that you want to install.

When installation of the package is complete, click **Installed Software** in the upper right corner of the screen, then verify the installation of your package.

C

coverageAnalysis plugin in Genexus™ Software

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

Reads statistics

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

Table 8 General statistics

Statistic	Description
Number of mapped reads	The total number of reads mapped to the reference genome.
Percent reads on target	The percentage of filtered reads mapped to any targeted region relative to all reads mapped to the reference. If no target regions file is specified, this value will be the percentage of reads passing uniquely mapped and/or 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 9 Amplicon read coverage statistics

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

Statistic	Description
Number of amplicons	The number of amplicons that is specified in the target regions file.
Percent assigned amplicon reads	The percentage of reads that were assigned to individual amplicons relative to all reads mapped to the reference. A read is assigned to a particular (inner) amplicon region if any aligned bases overlap that region. If a read might be associated with multiple amplicons, it is assigned to the amplicon region that has the greatest overlap of aligned sequence.
Average reads per amplicon	The average number of reads assigned to amplicons.
Uniformity of amplicon coverage	The percentage of amplicons that had at least 20% of the average number of reads per amplicon. Cumulative coverage is linearly interpolated between nearest integer read depth counts.
Amplicons with at least *N* reads	The percentage of all amplicons that had at least *N* reads.
Amplicons with no strand bias	The percentage of all amplicons that did not show a bias towards forward or reverse strand read alignments. An individual amplicon has read bias if it has ≥10 reads and the percentage of forward or reverse reads to total reads is greater than 70%. Amplicons with <10 reads are considered to have no strand bias.
Amplicons reading end-to-end	The percentage of all amplicons that were considered to have a sufficient proportion of assigned reads (70%) that covered the whole amplicon target from 'end-to-end'. To allow for error, the effective ends of the amplicon region for read alignment are within 2 bases of the actual ends of the region.
Amplicon base composition bias	A number that represents the proportion of amplicons showing low representation (<0.2x mean reads) in the lower and/or upper quartiles of amplicons ordered by increasing G/C base pair content of their insert sequences. The value is relative to that in the center 50th percentile of amplicons and weighted by the standard deviation of representation over all amplicons. An RMS (root mean square) value is used so that a bias greater in either upper or lower quartiles produces a larger value than a mean bias seen more equally in both outer quartiles. The value is 0 if the uniformity of amplicon coverage metric is 100%, however, the value is not necessarily high at lower amplicon uniformity.

Table 10 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 was specified, the targeted regions is the whole genome.

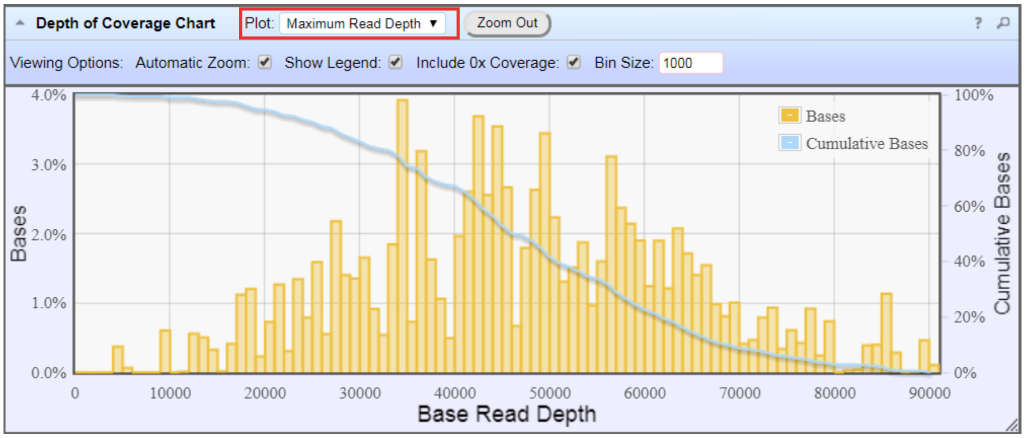
Table 10 Target base coverage statistics *(continued)*

Statistic	Description
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.
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.

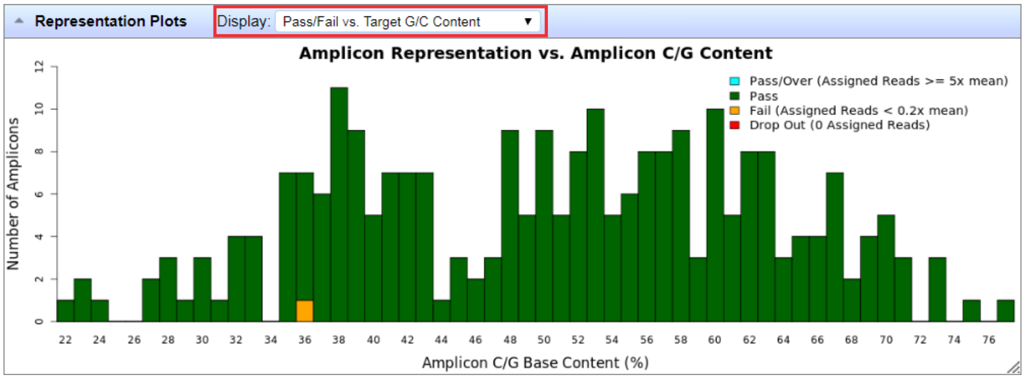


Plot: Maximum Read Depth

Viewing Options: Automatic Zoom: ☑ Show Legend: ☑ Include 0x Coverage: ☑ Bin Size: 1000

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 regions 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.



Display: Pass/Fail vs. Target G/C Content

Representation Plots
Amplicon Representation vs. Amplicon C/G Content
Pass/Over (Assigned Reads >= 5x mean)
Pass
Fail (Assigned Reads < 0.2x mean)
Drop Out (0 Assigned Reads)
Number of Amplicons
0
2
4
6
8
10
12
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
58
60
62
64
66
68
70
72
74
76
Amplicon C/G Base Content (%)

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**, 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 70.

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 11 File download selections in the Genexus™ Software

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

Table 11 File download selections in the Genexus Software *(continued)*

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

Table 12 File contents

File	Description
Coverage Statistics	A summary of the statistics presented in the tables at the top of the plugin report. The first line is the title. Each subsequent line is either blank or a particular statistic title followed by a colon (:) and its value.
Chromosome base coverage summary	Base reads per chromosome summary data that is used to create the default view of the Reference Coverage Chart. This file contains the following fields. • `chrom`: the name of the chromosome or contig of the reference. • `start`: the coordinate of the first base in this chromosome. This is always 1. • `end`: the coordinate of the last base of this chromosome. Also its length in bases. • `fwd_basereads`: the total number of forward strand base reads for the chromosome. • `rev_basereads`: the total number reverse strand base reads for the chromosome. • `fwd_trg_basereads` (if present): the total number of forward strand base reads that mapped over at least one target region. • `rev_trg_basereads` (if present): the total number of reverse strand base reads that mapped over at least one target region. • `total_reads`: the total number of sequencing reads that are mapped to individual contigs.
Base depth of coverage	Coverage summary data used to create the Depth of Coverage Chart. This file contains the following fields. • `read_depth`: the depth at which a (targeted) reference base has been read. • `base_cov`: the number of times any base was read (covered) at this depth. **Note:** Lines (read depths) for which `base_cov` is 0 are omitted to avoid excessively large files being produced in certain 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 12 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 4th 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 `cov500x`.
Coverage Analysis Summary (.pdf)	A PDF file that contains the Coverage Analysis Report, including read statistics and charts that are generated by the coverageAnalysis plugin.

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

Biological hazard safety

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

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Chemical safety

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

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

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

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

Documentation and support

Related documentation

Document	Publication number
Genexus™ Integrated Sequencer User Guide	MAN0017910
Genexus™ Software 6.2 User Guide	MAN0018955
Genexus™ Software 6.2 Help	MAN0018954 (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.

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

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

Limited product warranty

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

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9 July 2020