ion torrent

Genexus™ Integrated Sequencer

USER GUIDE

Catalog Number A45727

Publication Number MAN0017910

Revision D.0

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

ThermoFisher SCIENTIFIC

Manufacturer:
Life Technologies Holdings Pte Ltd |
Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Product:
Genexus™ Integrated Sequencer

Manufacturer:
Life Technologies Corporation |
200 Oyster Point Blvd |
South San Francisco, CA 94080 | USA

Product:
Genexus™ Software

Manufacturer:
Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

Products:
GX5™ Chip and Genexus™ Coupler
Genexus™ Library Strips 1 and 2-AS
Genexus™ Library Strips 1 and 2-HD
Genexus™ Templating Strips 3-GX5™ and 4
Genexus™ Barcodes 1–96 AS
Genexus™ Barcodes 1–32 HD
Genexus™ Primer Pool Tubes
Genexus™ Pipette Tips
Genexus™ Sequencing Kit
Genexus™ Controls
Genexus™ Conical Bottles
Genexus™ Filter
Genexus™ GX5™ Starter Pack-AS
Genexus™ GX5™ Starter Pack-HD
Oncomine™ GX assays

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

Revision history: Pub. No. MAN0017910

Revision	Date	Description
D.0	25 November 2020	Corrected an error in Genexus™ Software version number in “Software compatibility and requirements” on page 11.
C.0	29 October 2020	• Updated the contents of the Genexus™ Installation and Training Kit. See “Genexus™ Integrated Sequencer”. • Corrected guidance on reuse of Genexus™ Barcodes plates. See “Guidelines for Genexus™ Integrated Sequencer operation” on page 26. • Added the topic “Guidelines for expired reagents and chips” on page 27. • Changed the recommended concentration for manually prepared libraries from 125 pM to 200 pM. • Clarified use of "Do Force Clean" checkbox. See “Load the sequencer and start a run” on page 83. • Added screenshots for chip verification and leak checking in “Load the sequencer and start a run” on page 83. • Updated “Options for an expired sequencer initialization” and moved to Chapter 7. • Updated recommended actions for instrument error and warning messages in “Genexus™ Integrated Sequencer error and warning messages” on page 140. • Performance qualification results table updated with removal of MAPD metric to align with a new version of the Performance Qualification Assay (v1.4.0). See “Performance Qualification results” on page 135.
B.0	7 July 2020	• Updated for Genexus™ Software 6.2.0. • Updated for library and template strip part numbers. • Updated the components of the Genexus™ Installation and Training Kit. • Added guidance for using inline controls for troubleshooting. • Added the troubleshooting section “Genexus™ Integrated Sequencer error and warning messages”. • Added the topic “Replace the Genexus™ Conical Bottles” on page 165. • Added the section “Library QC Archive: recover library preparations from the Genexus™ Integrated Sequencer for reuse” on page 172.
A.0	13 November 2019	New user guide for the Genexus™ Integrated Sequencer

Contents

1 Product information

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

Product description

The Ion Torrent™ Genexus™ Integrated Sequencer is a next-generation (NGS) sequencing system that integrates library preparation, template preparation, and sequencing into a single-day, single-instrument automated run. The instrument supports both sample-to-results and library-to-results sequencing runs (up to 32 DNA or RNA samples/run). Ion Torrent™ Genexus™ Software streamlines the NGS workflow by integrating the setup-to-report workflow within a single software system. Key features include:

- Go from nucleic acid to report in a single day
- Flexible and cost-effective run planning making use of a multi-lane, multi-run sequencing chip: the Ion Torrent™ GX5™ Chip
- Automated library preparation, including cDNA synthesis, for up to 400 base-read libraries using either standard Ion AmpliSeq™ or Ion AmpliSeq™ HD library chemistry
- Automated Ion Sphere™ Particle (ISP)-loading and template preparation with on-chip amplification
- Support for up to four compatible assays on a GX5™ Chip in a single run, with an output of 12–15 million reads from each lane.
- As little as five minutes total hands-on time required per run
- Real-time consumables tracking by the instrument to guide consumable loading during run setup

- Consumables that are usable for up to 14 days after loading
- On-instrument sequencing data analysis requiring no external server

Genexus™ Integrated Sequencer

The Genexus™ Integrated Sequencer includes the following components.

Components	Cat. No.
Genexus™ Integrated Sequencer	A45727
Genexus™ Installation and Training Kit	A40278[1]

[1] Not available for separate purchase.

The Ion Torrent™ Genexus™ Installation and Training Kit (Cat. No. A40278) is available to first-time owners of a Genexus™ Integrated Sequencer and is shipped with the instrument. The kit contains the following reagents, supplies, and controls that are used during the installation, training, and operation of the instrument.

Genexus™ Installation and Training Kit

Contents	Part No.	Quantity	Storage
Genexus™ Controls	A40267	1 kit	–30°C to –10°C
Genexus™ Strip 1	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-AS	A46813	8 strips	–30°C to –10°C
Genexus™ Barcodes 1–32 AS	A40258	1 plate	15°C to 30°C
Genexus™ Strip 3-GX5™	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4	A46816	8 strips	–30°C to –10°C
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	
Genexus™ Pipette Tips	A40266	12 racks	
Genexus™ Conical Bottles	A40275	2 sets of 5 bottles	
Genexus™ Filter	A40302	1 filter	
GX5™ Chip and Genexus™ Coupler	A40269	2 each	
Adhesive PCR Plate Foils	AB0626	1 box (100 foils)	

Software compatibility and requirements

The procedures in this guide are designed for use with Genexus™ Software 6.2.0 or later. Version-specific information is provided in the software release notes for your version of the software. An administrator-level user can view the software version in the ⚙ **(Settings) / Software Updates** screen.

Genexus™ Software is supported on Google™ Chrome™ browser version 64 and later and is best viewed with 1440 × 900 screen resolution. Google™ Chrome™ browser is recommended for use with the software.

The operating system of the sequencer is Ubuntu™ 18.041.

For more information on using the software, see the online *Genexus™ Software 6.2 Help*.

Reagents and supplies—Ion AmpliSeq™ library chemistry

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 for Ion AmpliSeq™ library chemistry.

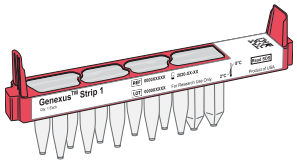
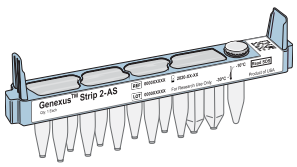
Note:

- Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.
- Reagents that are specific to Ion AmpliSeq™ library chemistry have an AS suffix.

Genexus™ Library Strips 1 and 2-AS

Ion Torrent™ Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252) for standard Ion AmpliSeq™ library-based chemistry are ordered as kits with eight pairs of strips/kit.

Note: Genexus™ Library Strips 1 and 2-AS with part numbers listed in the following table are compatible only with Genexus™ Software 6.2.0 and later.

Component	Carrier color	Part No.	Quantity per kit	Storage
Genexus™ Strip 1 	Light red	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-AS 	Light blue	A46813	8 strips	–30°C to –10°C

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™ GX5™ Starter Pack-AS

Ion Torrent™ Genexus™ GX5™ Starter Pack-AS (Cat. No. A40279) supplies the following components for Ion AmpliSeq™ library preparation and sequencing using a Genexus™-ready assay.

Note: For custom assays, Genexus™ Primer Pool Tubes (Cat. No. A40262) must be ordered separately.

Component	Part or Cat. No.	Quantity	Storage
Genexus™ Strip 1	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-AS	A46813	8 strips	–30°C to –10°C
Genexus™ Strip 3-GX5™	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4	A46816	8 strips	–30°C to –10°C
Genexus™ Barcodes 1–32 AS	A40258	1 plate	15°C to 30°C
Genexus™ Pipette Tips	A40266	12 racks	
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	

Reagents and supplies—Ion AmpliSeq™ HD library chemistry

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 for Ion AmpliSeq™ HD library chemistry.

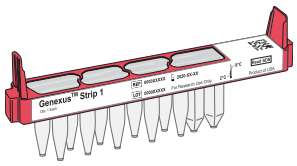
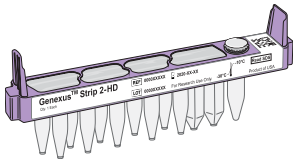
Note:

- Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.
- Reagents that are specific to Ion AmpliSeq™ HD library chemistry have an HD suffix.

Genexus™ Library Strips 1 and 2-HD

Ion Torrent™ Genexus™ Library Strips 1 and 2-HD (Cat. No. A40255) for Ion AmpliSeq™ HD library-based chemistry are ordered as kits with eight pairs of strips/kit.

Note: Genexus™ Library Strips 1 and 2-HD with part numbers listed in the following table are compatible only with Genexus™ Software 6.2.0 and later.

Component	Carrier color	Part No.	Quantity per kit	Storage
Genexus™ Strip 1 	Light red	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-HD 	Violet	A46814	8 strips	–30°C to –10°C

Genexus™ Barcodes 1–32 HD

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

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

Genexus™ GX5™ Starter Pack-HD

Ion Torrent™ Genexus™ GX5™ Starter Pack-HD (Cat. No. A40280) supplies the following components for Ion AmpliSeq™ HD library preparation and sequencing using a Genexus™-ready assay.

Note: For custom assays, Genexus™ Primer Pool Tubes (Cat. No. A40262) must be ordered separately.

Component	Part or Cat. No.	Quantity	Storage
Genexus™ Strip 1	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-HD	A46814	8 strips	–30°C to –10°C
Genexus™ Strip 3-GX5™	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4	A46816	8 strips	–30°C to –10°C
Genexus™ Barcodes 1–32 HD	A40261	1 plate	15°C to 30°C
Genexus™ Pipette Tips	A40266	12 racks	
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	

Shared reagents and supplies

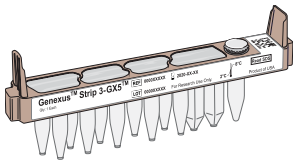
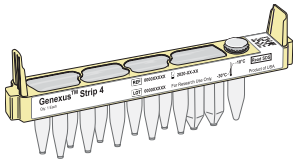
The following reagents and supplies are used in both Ion AmpliSeq™ library chemistry and Ion AmpliSeq™ HD library chemistry runs.

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

Genexus™ Templating Strips 3-GX5™ and 4

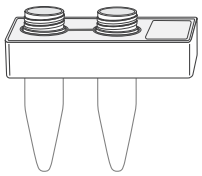
Ion Torrent™ Genexus™ Templating Strips 3-GX5™ and 4 (Cat. No. A40263) are ordered as kits with eight pairs of strips/kit.

Note: Genexus™ Templating Strips 3-GX5™ and 4 with part numbers listed in the following table are compatible only with Genexus™ Software 6.2.0 and later.

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

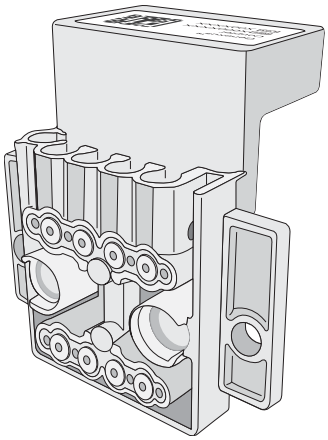
Genexus™ Primer Pool Tubes and Pipette Tips

Genexus™ Primer Pool Tubes and Pipette Tips can be ordered individually. Genexus™ Primer Pool Tubes are required for custom assays.

Item	Cat. No.	Quantity	Storage
Genexus™ Primer Pool Tubes 	A40262	50 assemblies (2 tubes/assembly) Bag of 100 caps	15°C to 30°C
Genexus™ Pipette Tips	A40266	12 racks	

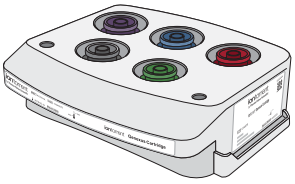
GX5™ Chip and Genexus™ Coupler

The GX5™ Chip and Genexus™ Coupler (Cat. No. A40269) are ordered as a set that contains two chips and two couplers, sufficient for up to eight sequencing runs.

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 "Replace the Genexus™ Conical Bottles" on page 165.

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

Genexus™ Filter

The Genexus™ Filter (Cat. No. A40302) is installed in the liquid waste disposal port on the instrument deck to prevent liquid waste line blockage. For information on installation, see "Replace the Genexus™ Filter" on page 164.

Component	Quantity	Storage
Genexus™ Filter	2 filters	15°C to 30°C

Genexus™ Controls

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

IMPORTANT! Genexus™ Strip 2-AS is required for sequencing Genexus™ Controls. For ordering information, see "Genexus™ Library Strips 1 and 2-AS" on page 11.

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	

Oncomine™ GX assays

Ion Torrent™ Oncomine™ GX assays are Genexus™-ready assays sufficient for 32 reactions, and are supplied in pre-measured ready-to-load Genexus™ Primer Pool Tubes. Assays are provided with Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252) or Genexus™ Library Strips 1 and 2-HD (Cat. No. A40255) in the amount listed.

Contents	Carrier color	Pool	Carriers per kit	Part No.	Storage
Oncomine™ Comprehensive Assay v3 GX (Cat. No. A46296)					
Oncomine™ Comprehensive Assay v3 DNA GX	Magenta	DNA Pool 1	4	A40281	–30°C to –10°C
	Pale green	DNA Pool 2	4		
Oncomine™ Comprehensive Assay v3 RNA GX	Pale orange	RNA Pool 1	4	A44351	
	Blue	RNA Pool 2	4		
Genexus™ Strip 1	Light red	—	2 × 8	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	—	2 × 8	A46813	–30°C to –10°C
Oncomine™ Precision Assay GX (Cat. No. A46291)					
Oncomine™ Precision Assay GX (panel only)	Magenta	OPA Pool 1 (FWD and REV primers)	8	A44350	–30°C to –10°C
Genexus™ Strip 1	Light red	—	8	A46812	2°C to 8°C
Genexus™ Strip 2-HD	Violet	—	8	A46814	–30°C to –10°C
Oncomine™ TCR Beta-LR Assay GX (Cat. No. A46297)					
Oncomine™ TCR Beta-LR Assay GX (panel only)	Magenta	RNA Pool 1	8	A40282	–30°C to –10°C
Genexus™ Strip 1	Light red	—	8	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	—	8	A46813	–30°C to –10°C

Note:

- Assays using Ion AmpliSeq™ chemistry have primer pools that are loaded in capped tubes in position 1 of the primer pool tube carriers. Empty uncapped tubes are loaded in position 2.
- Assays using Ion AmpliSeq™ HD chemistry have FWD and REV primer pools that are loaded in capped tubes in both positions of the primer pool tube carrier.

Required materials not supplied

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

Item	Source
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	4483352, 4483354
Adhesive PCR Plate Foils	AB0626
20-, 200-, and 1,000-µL pipettors and appropriate filtered tips	MLS
Microcentrifuge tubes, 1.5-mL or 1.7-mL (low retention for nucleic acids)	MLS
Vortex mixer with a rubber platform	MLS
Gloves, powder-free nitrile	MLS
Ice buckets and ice	—
Nuclease-free water, molecular biology grade	AM9932
Isopropyl alcohol, 70% solution	MLS
Wipes, disposable lint-free	MLS
(Optional) Uninterruptible Power Supply (UPS)[1]	MLS

[1] 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.

Recommended materials for nucleic acid isolation and quantification

Unless otherwise indicated, all materials are available through **thermofisher.com**. Catalog numbers that appear as links open the web pages for those products.

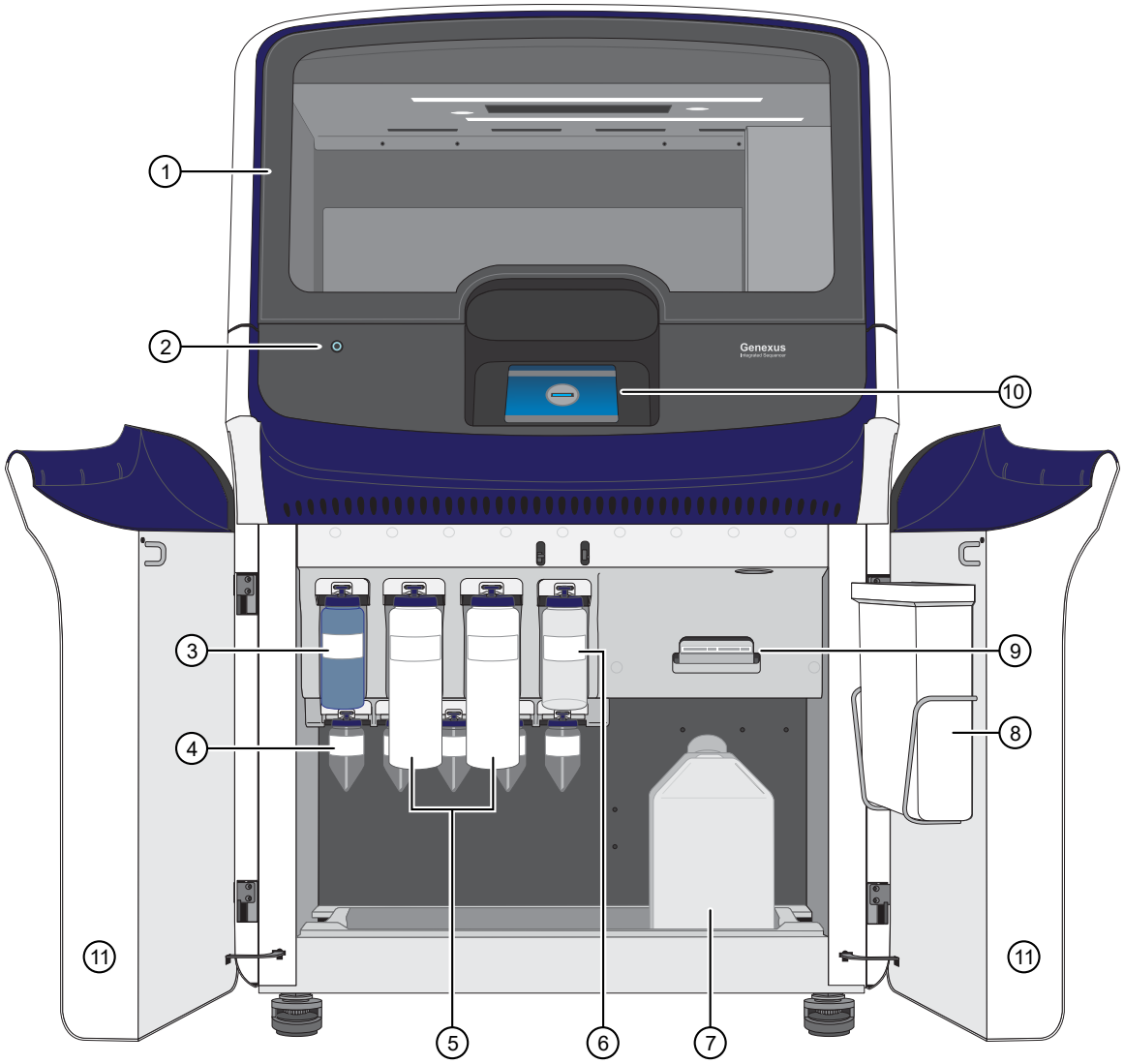
Item	Source
Nucleic acid isolation	
Ion AmpliSeq™ Direct FFPE DNA Kit	A31133, A31136
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
RecoverAll™ Multi-Sample RNA/DNA Workflow	A26069
MagMAX™ FFPE DNA/RNA Ultra Kit	A31881
PureLink™ Genomic DNA Mini Kit	K1820-00
MagMAX™ Cell-Free DNA Isolation Kit	A29319

(continued)

Item	Source
MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit	A36716
RNase*Zap*™ RNase Decontamination Solution	AM9780
Nucleic acid quantification	
TaqMan™ RNase P Detection Reagents Kit *(Recommended for DNA only)*	4316831
Qubit™ 4 Fluorometer[1]	Q33238
One or more of the following kits for use with the Qubit™ 4 Fluorometer: · Qubit™ dsDNA HS Assay Kit (High-sensitivity DNA) · Qubit™ dsDNA BR Assay Kit (Broad range DNA) · Qubit™ RNA HS Assay Kit (High-sensitivity RNA) · Qubit™ RNA BR Assay Kit (Broad range RNA)	 Q32851, Q32854 Q32850, Q32853 Q32852, Q32855 Q10210, Q10211
Library quantification (library runs only)	
Ion Library TaqMan™ Quantitation Kit	4468802

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

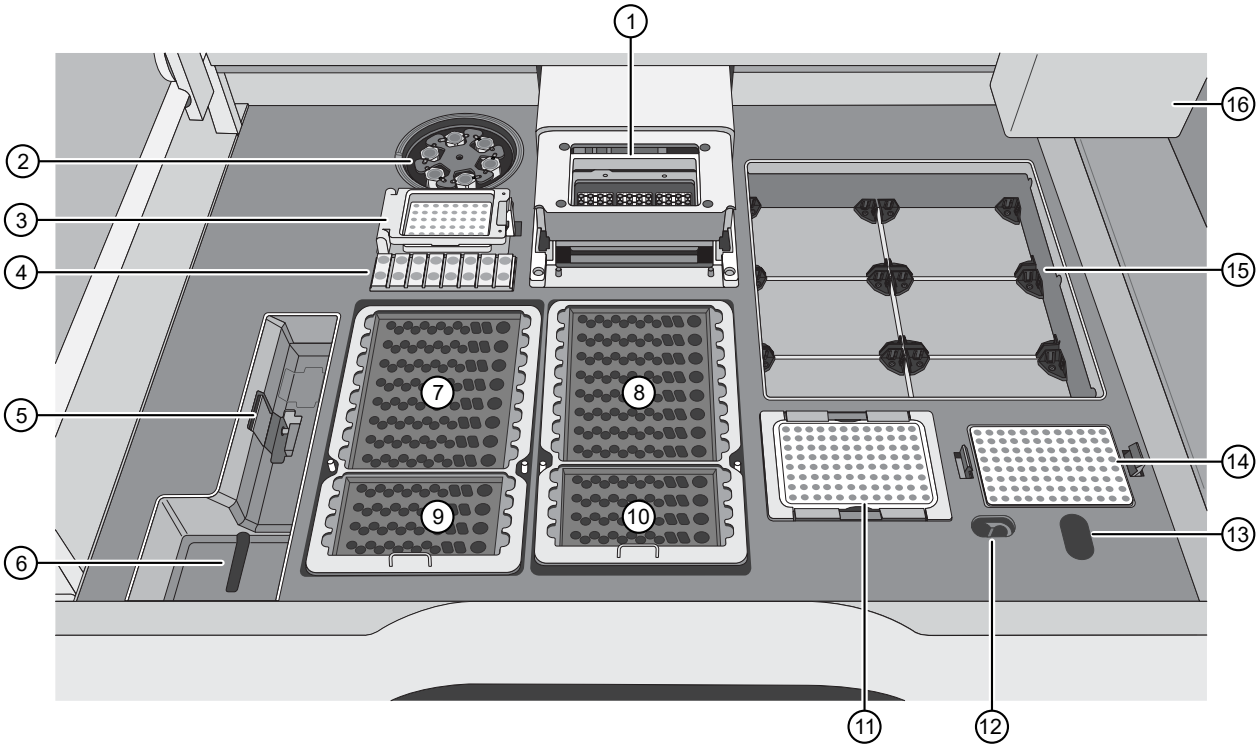
Genexus™ Integrated Sequencer components



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

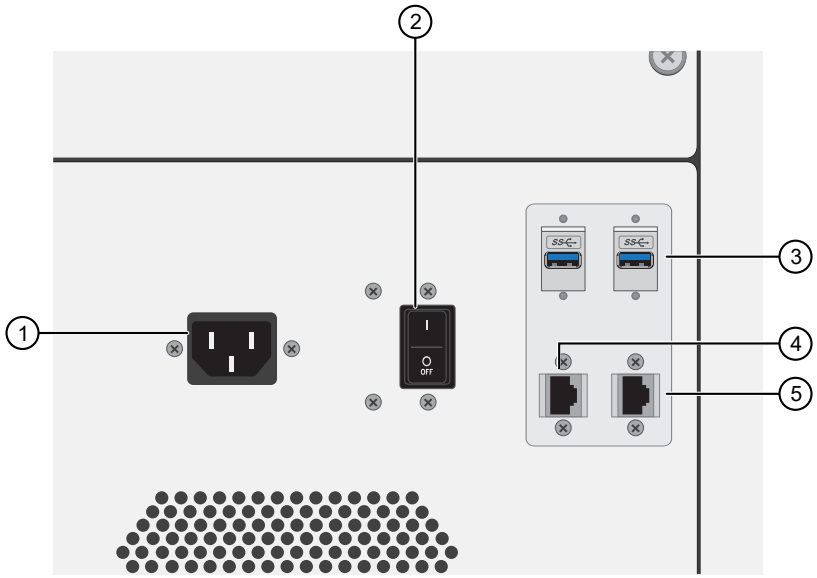


Interior Genexus™ Integrated Sequencer deck components and stations

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 or Genexus™ Strip 2-HD, depending on your assay,)
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

Genexus™ Integrated Sequencer input and output connections

The connection panel, power port, and an on/off switch are located on the right side of the rear panel of the instrument.



1. Power port—100–240VAC port that provides power to the instrument.
2. On/off switch—Power switch, where the states are on (|) or off (O).
3. USB ports—Connects a USB device to the instrument.
4. Ethernet port—An RJ45 port that provides Ethernet (Gigabit) communication between the sequencer and a local area network.
5. Ethernet port—An RJ45 port that provides Ethernet (Gigabit) communication between the sequencer and an accessory instrument.

Workflow

Sample-to-results sequencing run using the Genexus™ Integrated Sequencer

Create an assay (page 32)

System installed assays that are specifically configured for each sample type are available in Genexus™ Software. You can use the system-installed assays in your run 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 46)

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

Plan a sample run (page 62)

Runs planned 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.

Dilute the samples and load the sample plate (page 75)

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

Load the sequencer and start a run (page 79)

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

Monitor the run (page 94)

Monitor the run in Genexus™ Software in real time.

Review data and results in Genexus™ Software (page 95)

Review data and results in Genexus™ Software, or analyze data in Ion Reporter™ Software using an Ion Reporter™ analysis workflow.

2 Before you begin

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 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 deck surface or one of the metal deck stations.

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).

Guidelines for Genexus™ Integrated Sequencer operation

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

Limiting factor	Description
The number of available barcodes in the barcode plate	The maximum number of available barcodes per run is 32. **IMPORTANT!** When libraries are prepared on the Genexus™ Integrated Sequencer, each target amplification reaction for a sample requires a unique barcode.
Maximum number of target amplification reactions per run	One library strip pair has the reagents necessary for 4 target amplification reactions, or 4 barcodes. With a maximum of 8 library strip pairs loaded, a maximum of 32 samples can be run using an assay with one primer pool.
The number of primer pools per assay	Given the limits of 32 target amplification reactions, and 32 available barcodes, the number of samples in a run multiplied by the total number of primer pools in the assays that are used in a run cannot exceed 32. For one single-pool assay, a maximum of 32 samples can be run on a single chip. If you are using 2 assays with two primer pools each, you can sequence a maximum of 8 samples in a run. Similarly, for one assay with 4 primer pools, you can sequence a maximum of 8 samples in a run, if the minimum read count per sample allows it.
The number of unused lanes on an installed chip	A maximum of 4 lanes are available on a single GX5™ Chip.
The minimum read count per sample for an assay	The minimum read count per sample parameter is set during assay creation.

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

- You can remove a chip in one of the following situations.
  - After all the lanes of a chip are used in a run, the chip shuttles to the install position and you are asked to remove the used chip.
  - When you select a run plan that requires more lanes than are available on the installed chip, you are asked to remove the partially used chip, and the sequencer performs a post-chip clean. In addition, you must clear consumables from the lower sequencing reagents bay, even if only a single lane of the chip was used.
- The Genexus™ Integrated Sequencer can track used and unused barcodes in barcode plates in Genexus™ Software 6.2.0 and later, enabling you to swap plates between runs as needed, and reload a partially used barcode plate for a run if a sufficient number of barcodes are available on the plate.
- After loading in the sequencer, reusable consumables, such as barcodes, chips, and sequencing reagents bay components, must be used within 14 days for optimal results.
- An assay that is selected in a library run cannot include library batches that share one or more samples. However, two different assays in a run can include shared samples, because assays are run in separate lanes of a chip.

Guidelines for expired reagents and chips

Follow these guidelines for using reagents and sequencing chips that are at or near expiration. We do not recommend using components past the expiration date, but under certain circumstances, a sequencer warning for expired reagents can be overridden to allow a sequencing run to proceed.

- For all reagents except for Genexus™ barcode plates, the instrument bypasses an expired reagent warning after you tap the **Help** button in the sequencer screen.
- If a barcode plate has expired, a run cannot proceed, even if you tap **Help**.
- If a GX5™ Chip is expiring in a given month, ensure that you use up all lanes of the chip within that calendar month. If you start a run in the next calendar month with the same chip installed, the sequencer does not allow the run to proceed. The sequencer prompts you to perform a Clean instrument procedure and install a new chip and sequencing consumables, even if the initialization has not expired. For further information, see “Perform a Clean instrument procedure” on page 152.

Power the Genexus™ Integrated Sequencer on or off

Note: If the Genexus™ Integrated Sequencer is powered on, and the touchscreen is blank, touch the screen to "wake" the touchscreen.

Power on

If the touchscreen is unresponsive, check the power switch on the back of the instrument to ensure that the switch is in the on (|) position. If the power switch is in the off (O) position, proceed with step 1. If the power switch is already in the on position, proceed to step 2.

1. Turn the power switch on the back of the instrument to the on (|) position.
2. Press the power button on the front of the instrument.
   The button illuminates.
3. In the **Sign In** screen, enter the username and password created by the field service engineer when the instrument was set up.
   When the instrument home screen appears, the instrument is ready for use.

Power off

It is not necessary to power off the instrument overnight or over the weekend. If the instrument or Genexus™ Software will not be used for more than 3 days, power off the instrument as follows:

1. In the home screen, tap **Settings** ▸ **System Tools** ▸ **Shut down**.
2. Select either **Shutdown** or **Reboot**.
3. If you select **Shutdown**, a confirmation message appears. Select **Yes** to power off the instrument.

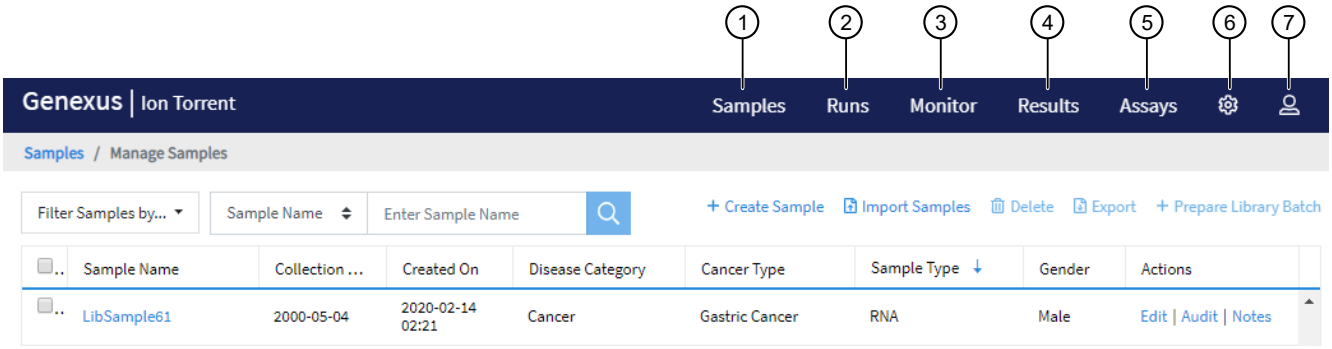
   Note: If you power off the instrument with a partially used chip installed, the chip and consumables status is saved. When you power back on, the saved chip and consumable information enables you to use the chip for up to 14 days after the chip was installed.

IMPORTANT! Do *not* press the power button during a run. Interrupting power to the instrument during a run can result in sequencing run failure and loss of sample.

Get started with Genexus™ Software

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.



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.

User-access levels

Users at this level...	Can...
Operator	• Add and import samples • Prepare library batches • Plan and save runs • Monitor runs • View results and reports
Manager	Operator functions plus: • Create and edit sample attributes • Delete runs • Create and import assays • Manage reference sequences and panel, hotspot, and other sequence files • Access services information
Administrator	Operator and manager functions plus: • View, export, and print audit records • Configure network settings • View and manage software updates • Configure data archive and storage settings • Manage instrument and software log files • Add and manage users

System tracking

The system tracks and checks user, sample, workflow, reagents and QC metrics for auditable records. If the software detects an error at any step—for example, a scanned barcode is inconsistent with the information given for the run—the software alerts the user and does not proceed with the run.

Request and sign in to a new account

Only administrator-level users can create user accounts.

After account creation, the Genexus™ Integrated Sequencer automatically sends an email to the new user with the username and password information.

- To request a new account, contact your local administrator.
- To sign in to a new account for the first time:
  a. Open the Genexus™ Software, then enter your username and password.
  b. Press **Enter**, or click **Sign In**.
  c. Click **Accept** to accept the End User Software License Agreement.

d. In the **Change Password** screen, enter your temporary password in the **Current Password** field. Type a new password in the **New Password** field, then confirm the password.
   - Passwords must be between 6 and 10 alphanumeric characters (0–9, Aa–Zz) with no spaces or special characters.
   - Passwords must contain at least one alphabetic character (Aa–Zz).
   - Passwords must contain at least one numeric character (0–9).
   - Passwords are case-sensitive.

e. Click **Change**.

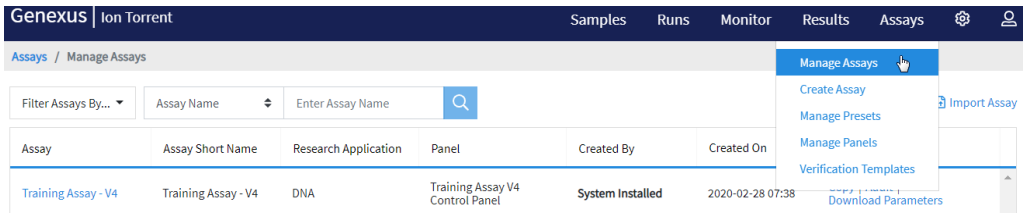
Sign in

1. Open the software home page.
2. Select your preferred language from the dropdown list in the upper right corner of the page.
3. Enter your username and password, then press **Enter** or click **Sign In**.

   IMPORTANT! Your username and password must be unique and not shared with other users.

   The software opens to the **Manage Samples** screen.

3 Create and manage assays (manager/administrator)



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

About assays in Genexus™ Software

Assays contain the settings and parameters for library preparation, templating, controlling the sequencing run, and analyzing the results. Assays also define the panels, kits, and chips that are used in a run, and specify the reference files and threshold values for quality control and variant detection. Assays can be created from system-installed templates or assays, or from custom assays that are copied and edited.

An assay is a reusable experimental design that contains predefined settings appropriate for use with common types of research applications. An assay can be used to plan many runs and plays an important role in enabling rapid throughput across the sequencing instrument. Assays help reduce the chance of errors, because information is stored and then applied to runs instead of entered manually for each run.

An assay can be copied and edited for a different use. Before you can create a custom assay, you must add a panel file, and hotspot and copy number baseline files (if needed for your assay), to the software. Custom assays are for advanced users. For assistance, contact your local Field Service Engineer.

Assays can contain system-installed annotation sets, filter chains, copy number baselines and report templates. You can also create custom versions of each of these presets.

The software provides to tools to:

- Create, import, and manage assays.
- Create and manage annotation sets, report templates, filter chains, and copy number baselines (**Manage Presets**).
- Add and manage panels (**Manage Panels**).

System-installed assays

Genexus™ Software includes system-installed assays that are preconfigured for use with Oncomine™ GX assays. System-installed assays are available for download at **Software Updates** in the ⚙ **(Settings)** menu. System-installed assays are locked and cannot be changed, but the assays can be copied, then edited.

Manage assays (manager/administrator)

Manager- and administrator-level users create and manage assays for use in Genexus™ Software.

In the menu bar, click **Assays** ▸ **Manage Assays** to open the **Manage Assays** screen.

The following tools are available in this screen.

To...	Do the following...
Review and export the assay audit trail	1. In the row of an assay, in the **Actions** column, click **Audit**. 2. In the **Audit Trail** dialog box, in the **Record** column, click **(Audit Trail Details)** in the row of an action to view the details of that action. 3. In the **Audit Record Details** dialog box, click **Export** to export a PDF file of the record.
Lock a draft assay	When a manager- or administrator-level user first creates an assay, in the **Assay** column, the assay is listed as **assay name (Draft)**. To use the assay in a run, you must lock it. In the row of a draft assay, in the **Actions** column, for an assay with a status of **Draft**, click **Lock**. Locked assays cannot be edited or deleted.
Edit a draft assay	When a manager- and administrator-level user first creates an assay, the assay name is followed by **(Draft)** in the **Assay** column. While the assay is in draft status, it can be edited. A locked assay cannot be edited. 1. In the row of a draft assay, in the **Actions** column, click **Edit**. The **Create Assay** workflow reopens. 2. Edit the options on each assay step as desired, then click **Save**.

(continued)

To...	Do the following...
Copy an assay to create a new assay	Only locked assays can be copied. 1. In the row of an assay, in the **Actions** column, click **Copy**. The **Create Assay** workflow reopens. 2. Edit the options on each assay step as desired, enter a new name for the assay, then click **Save**.
Delete an assay	Only draft assays can be deleted. When an assay is locked, it can be removed from use in the software by designating it obsolete. In the row of a draft assay, in the **Actions** column, click **Delete**, then confirm the deletion.
Remove a locked assay	A manager-or administrator-level user can remove a locked assay from use in the software by designating it obsolete. The assay is not deleted and a record of it is maintained in the audit trail. The results for any runs already performed with the assay remain on the sequencer. 1. In the row of a locked assay, in the **Actions** column, click **Obsolete**. 2. Click **Yes** to confirm the operation.
Export an assay	An assay can be exported, for example if you want to use that assay in another Genexus™ Integrated Sequencer in your lab. Only locked assays can be exported. In the row of a locked assay, in the **Actions** column, click **Export**. The assay parameter files are downloaded to your local drive as a ZIP folder and are available for import to another sequencer. Panel reference files are not included in the exported folder.
Download parameters	In the row of an assay, in the **Actions** column, click ··· **(More Options)**. Assay parameter files are downloaded to your local drive as a ZIP folder containing assay parameter JSON files.

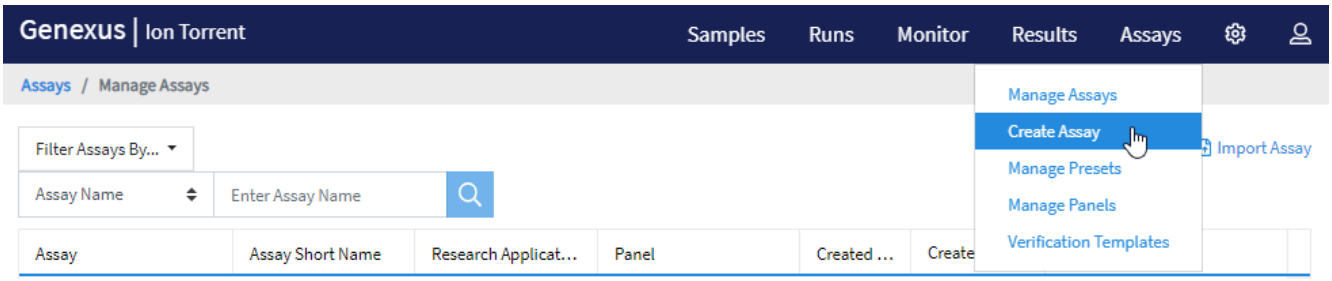
Create a new assay (manager/administrator)

Manager- and administrator-level users can create a new assay.

Assays can be copied from an existing system-installed assay or other assay, then modified as needed. For more information, see “Copy an assay (manager/administrator)” on page 43.

To create a new assay from an assay template, follow these steps.

1. In the menu bar, click **Assays** ▸ **Create Assay** .

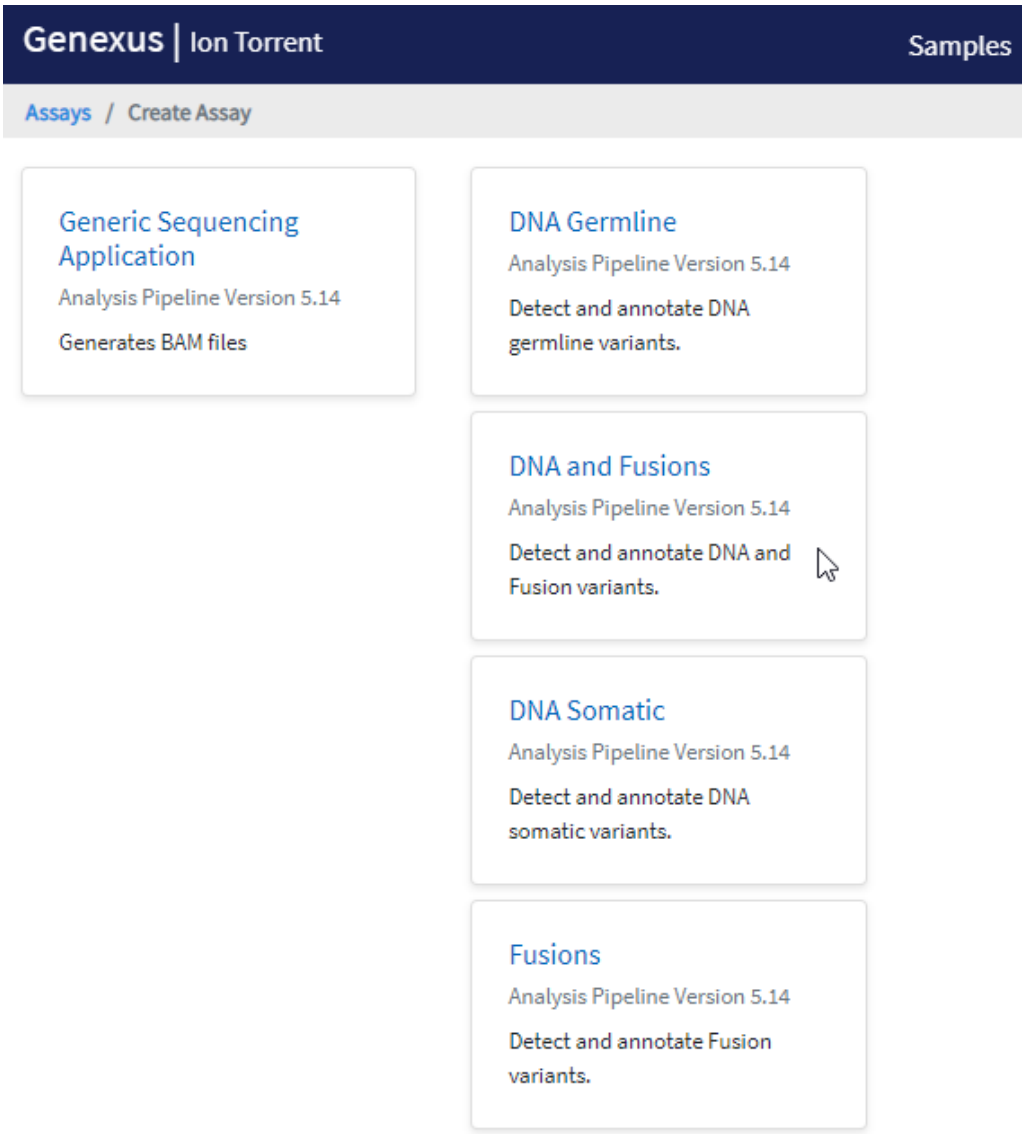


The **Create Assay** screen opens. Four oncology and one generic sequencing assay templates are available. The templates have assay-specific configuration steps that are prepopulated with default settings and parameters.

- Generic Sequencing Application
- DNA Germline
- DNA and Fusions
- DNA Somatic
- Fusions

Note: Clicking **+ Create Assay** takes you to the same screen.

2. Select the assay template that you want to use, then click anywhere inside its box.

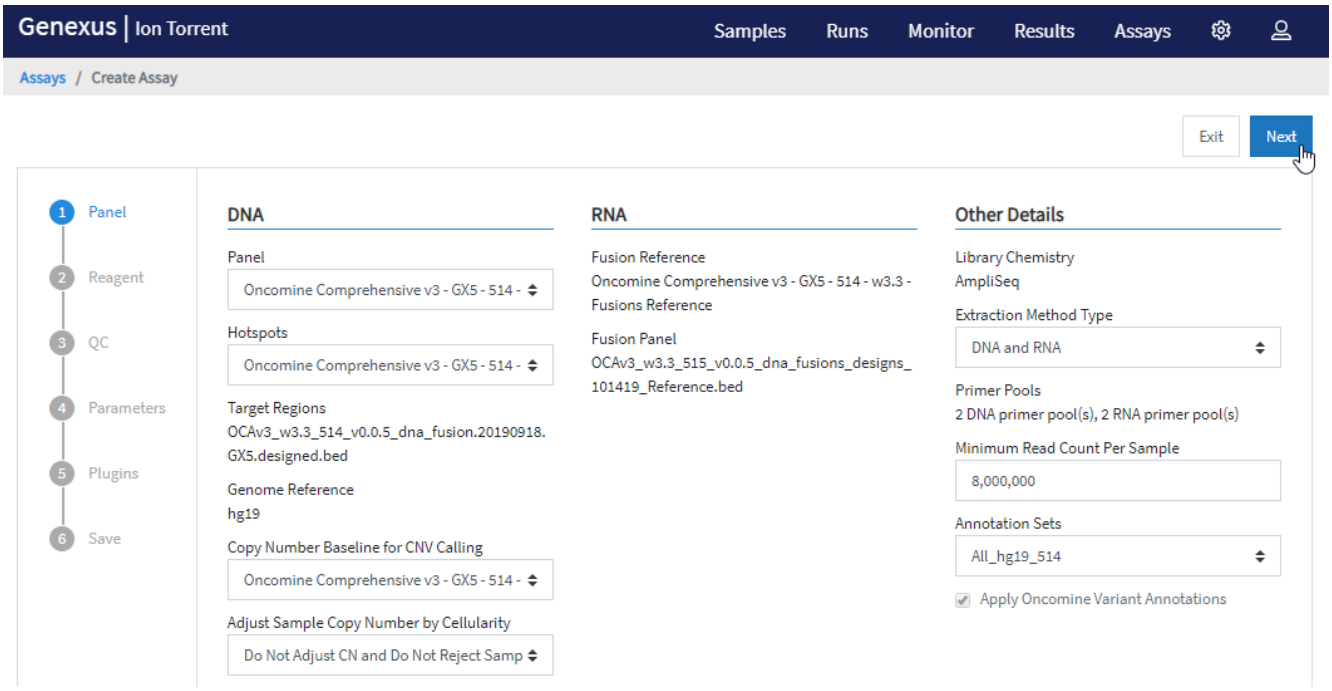


Note:

- Although each assay template has a specific set of steps in the setup wizard, the setup procedures for all are similar. The following is an example of assay creation after selecting the **DNA and Fusions** template.
- If you are using a custom assay, see "Guidelines for using custom assays with the Genexus™ Integrated Sequencer" on page 176 for guidelines for setting parameters for Minimum Read Count Per Sample and target amplification in the **Panel** and **Parameters** steps.

3. In the **Panel** step, make the following selections or entries, then click **Next**.
   a. The panel for the assay from the **Panel** list.

   Note: To add a new panel, click **Assays** ▸ **Manage Panels**, then click **+ Add New**.



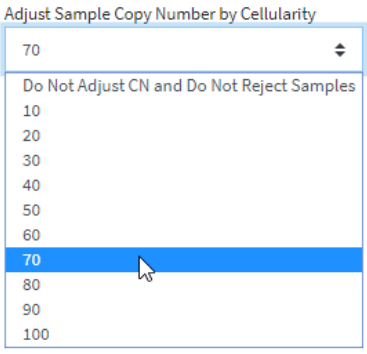
   b. The hotspot file for the panel.

   Note: To add a hotspot file, click **Assay** ▸ **Manage Panels** ▸ **Hotspots**, then click **+ Add New**.

   c. The CNV baseline for CNV calling, if used in the assay. Select **Do Not Run CNV Algorithm** if you are not performing CNV (copy number variant) analysis.

      To add a CNV baseline, click **Assay** ▸ **Manage Presets** ▸ **Copy Number Baselines**, then click **+ Create New** or **Import Copy Number Baseline**.

   d. *(Optional)* If you selected a CNV baseline, select a value to copy number based on tumor cellularity, if you want the CNV analysis to adjust for heterogenous tumor content in your samples. Samples with % cellularity below this value when created are rejected. If you do not want to make this adjustment, select **Do Not Adjust CN and Do Not Reject Samples**.



   e. The extraction method type.

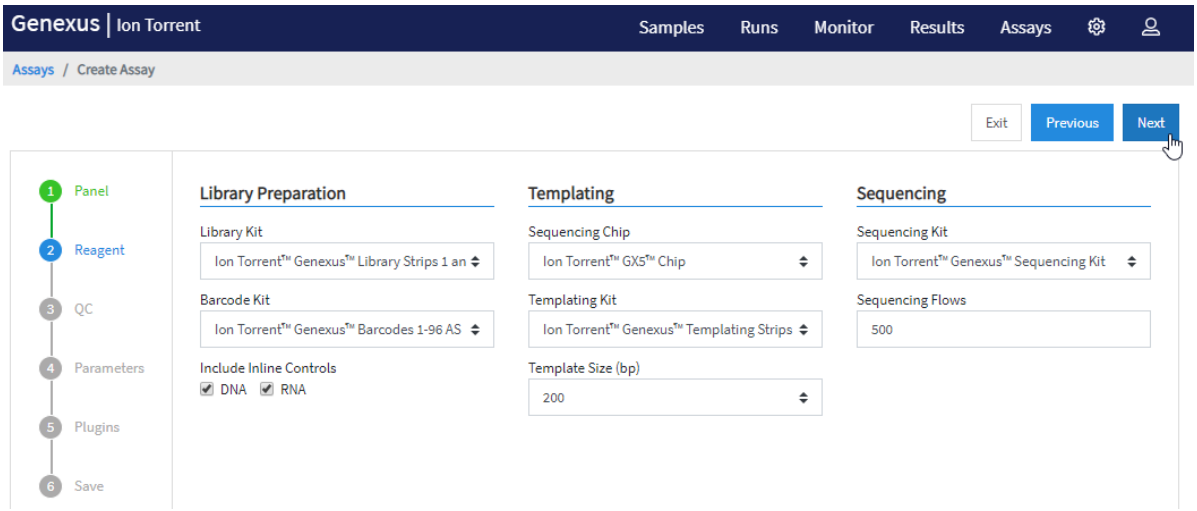
   f. The minimum read count per sample coverage setting (required).

g. The annotation set to use in variant reporting.

   To add an annotation set, click **Assays** ▸ **Manage Presets** ▸ **Annotation Sets**, then click **+ Add New**.

4. In the **Reagent** step, make the following selections or entries, then click **Next**. confirm or select names of library, barcode, templating, and sequencing kits, select the sequencing chip, and inline control check boxes, then select or enter values for template size and sequencing flows, if different from the prepopulated values. Click **Next**.

   a. Confirm or select names of library, barcode, templating, and sequencing kits.



Note: If you select **Other AmpliSeq** from the **Library Kit** dropdown list, the **Barcode Kit** dropdown list includes barcodes sets, such as IonCode™ and Ion Xpress™ barcodes, that are compatible with manually or Ion Chef™-prepared libraries.

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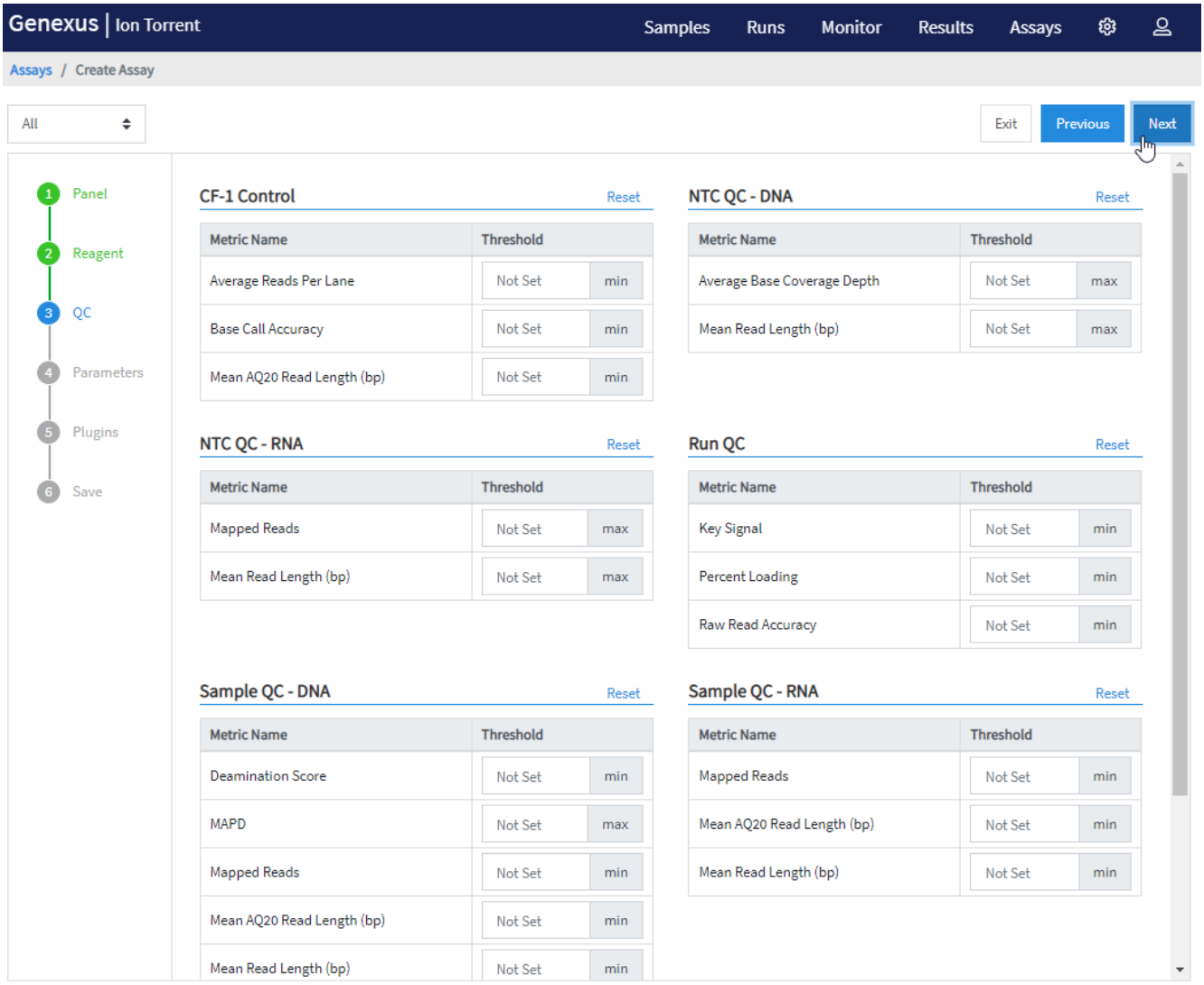
   b. Select the sequencing chip.

   c. Select one or both of the DNA and RNA inline control check boxes if you want to include inline controls in the quality control analysis.

   d. Select or enter values for template size and sequencing flows, if different from the prepopulated values.

5. In the **QC** step, enter parameters in the **CF-1 Control** and **Run QC** sections, and in the NTC (no template control) QC and Sample QC fields that appear appropriate to the sample types specified in the assay: **NTC QC - DNA**, **NTC QC - RNA**, **Sample QC - DNA**, and **Sample QC - RNA**. Leave parameters that are not applicable to your assay as **Not Set**. Click **Next** when finished.



Parameters for QC pass/fail thresholds

Parameter	Description
CF-1 Control	
Average Reads Per Lane	The average number of CF-1 reads per chip lane.
Base Call Accuracy	The percentage of accuracy of the CF-1 calls aligned to the CF-1 reference.
Mean AQ20 Read Length	The mean length of CF-1 reads having ≥99% accuracy at each position.
Run QC	
Key Signal	The average signal after software processing for all ISPs that identically match the library key (TCAG). A measure of the efficiency of template amplification.

Parameters for QC pass/fail thresholds *(continued)*

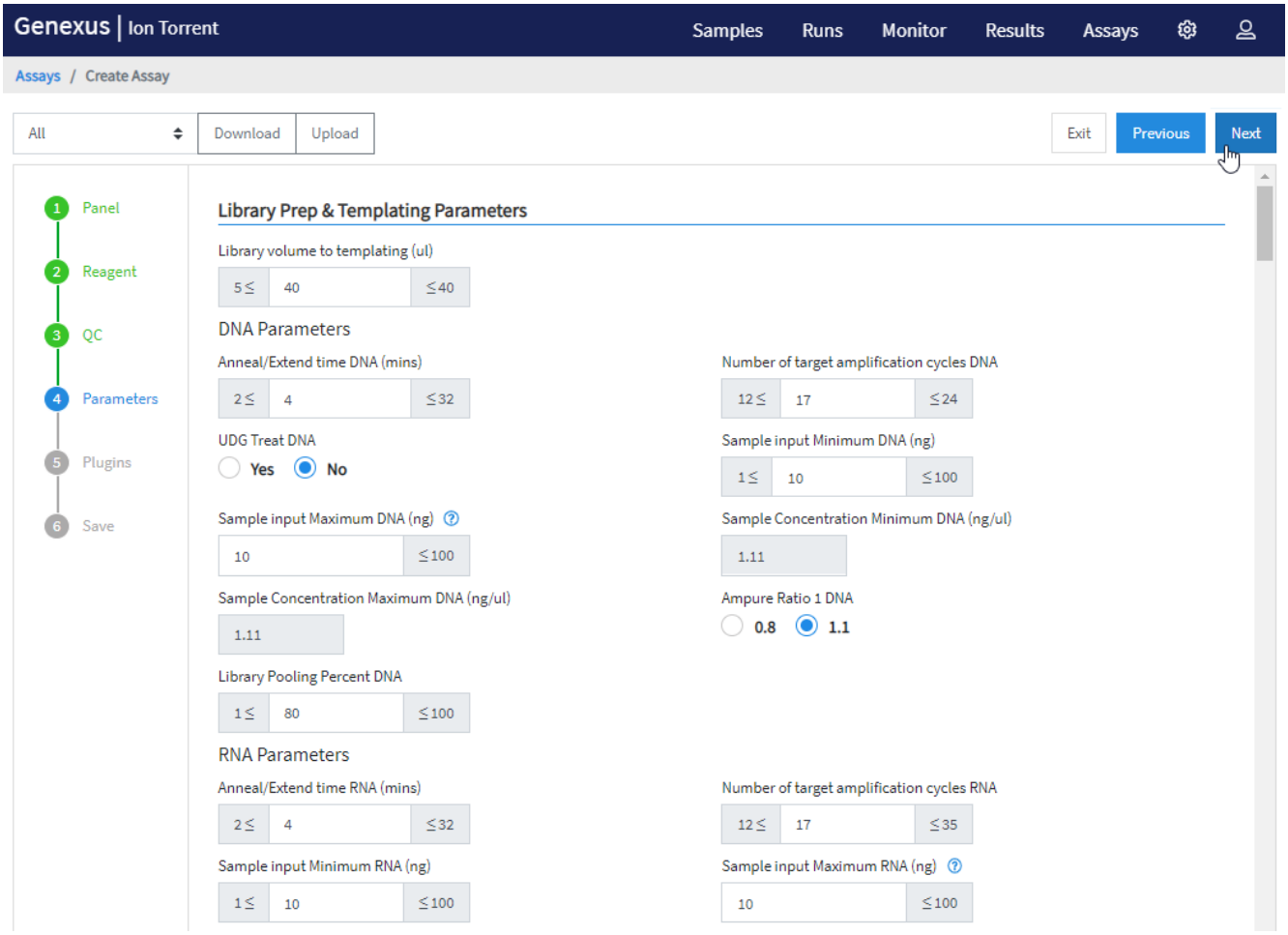
Parameter	Description
Percent Loading	The percentage of addressable wells on a chip lane that are loaded with an ISP.
Raw Read Accuracy	The percentage of raw reads mapping to the reference sequence.
Sample QC - DNA	
Deamination score	Deamination is reported as the estimated SNP proportion consistent with deamination (low allele frequency C:G>T:A SNVs). The deamination score can be used to determine the quality of an FFPE sample.
MAPD	The Median of the Absolute values of all Pairwise Differences; a quality metric that estimates coverage variability between adjacent amplicons in copy number variant (CNV) analyses. A MAPD value ≤0.5 generally indicates an acceptable level of coverage variability in the DNA Library or DNA Control.
Mapped Reads	The total number of bases mapped to target amplicons.
Mean AQ20 Read Length (bp)	The mean length of sample reads aligned to a reference sequence that have ≥99% accuracy at each position.
Mean Read Length (bp)	The mean length of all sample reads.
Uniformity of Amplicon Coverage	The percentage of reads showing a depth of coverage ≥20% of the mean base coverage.
Sample QC - RNA	
Mapped Reads	The total number of reads mapping to a fusion reference sequence.
Mean AQ20 Read Length (bp)	The mean length of called fusion reads that have ≥99% accuracy at each position.
Mean Read Length (bp)	The mean length of all sample reads.
NTC QC- DNA	
Average Base Coverage Depth	Average base coverage depth of reads aligned to a reference sequence in the no template control.
Mean Read Length (bp)	The mean length of reads in the no template control.
NTC QC - RNA	
Mapped Reads	The total number of reads in the no template control mapping to a fusion reference sequence.
Mean Read Length (bp)	The mean length of reads in the no template control.

6. In the **Parameters** step, review the prepopulated analysis settings, then modify if needed. Click **Next** when finished.

Note:

- Not all parameters are adjustable. To modify primary analysis parameters, select the **Customize Parameters** checkbox.
- Select **Yes** under **UDG Treat DNA** in the **Library Prep & Templating Parameters** section to include uracil DNA glycosylase (UDG) treatment of DNA during library preparation. Removal of uracil residues can increase sequencing quality for FFPE samples that have undergone significant cytosine deamination.

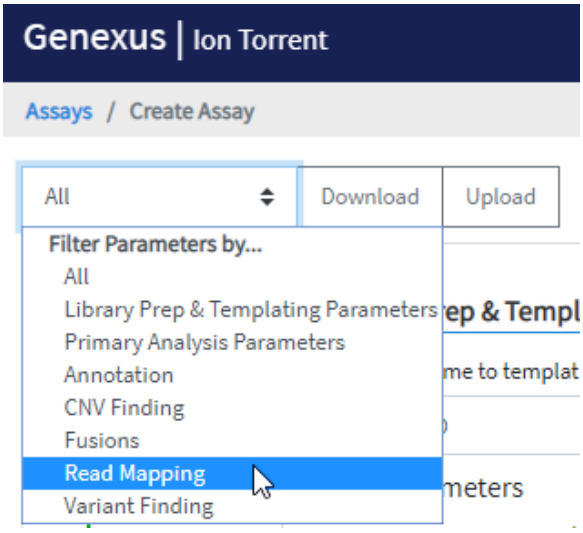
  We recommend using multiple samples in runs that include UDG treatment. Single sample runs can result in low read number.



Scrolling from the top, the settings are grouped in the following categories:

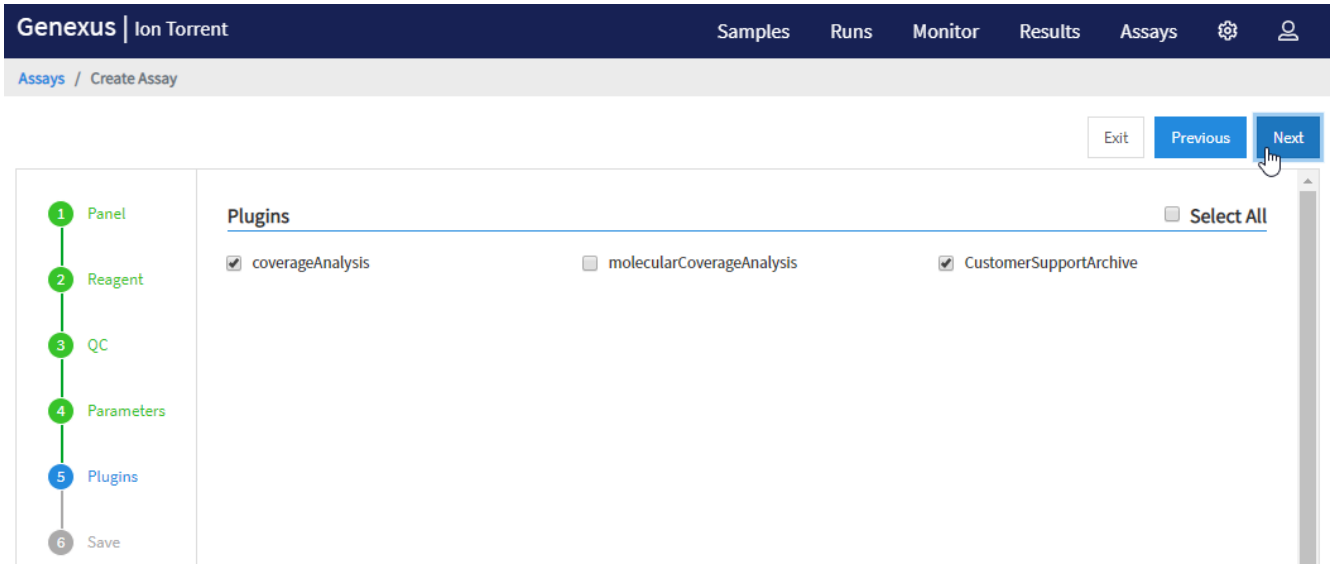
- **Library Prep & Templating Parameters**
  If needed, change the default settings for the cycling and input parameters used in library and template preparation.
- **Primary Analysis Parameters**
- **Annotation**
- **CNV Finding**
- **Fusions**
- **Read Mapping**
- **Variant Finding**

A parameter category can be quickly brought to the top of the screen by selecting it in the **Filter Parameters by...** list at the upper left.

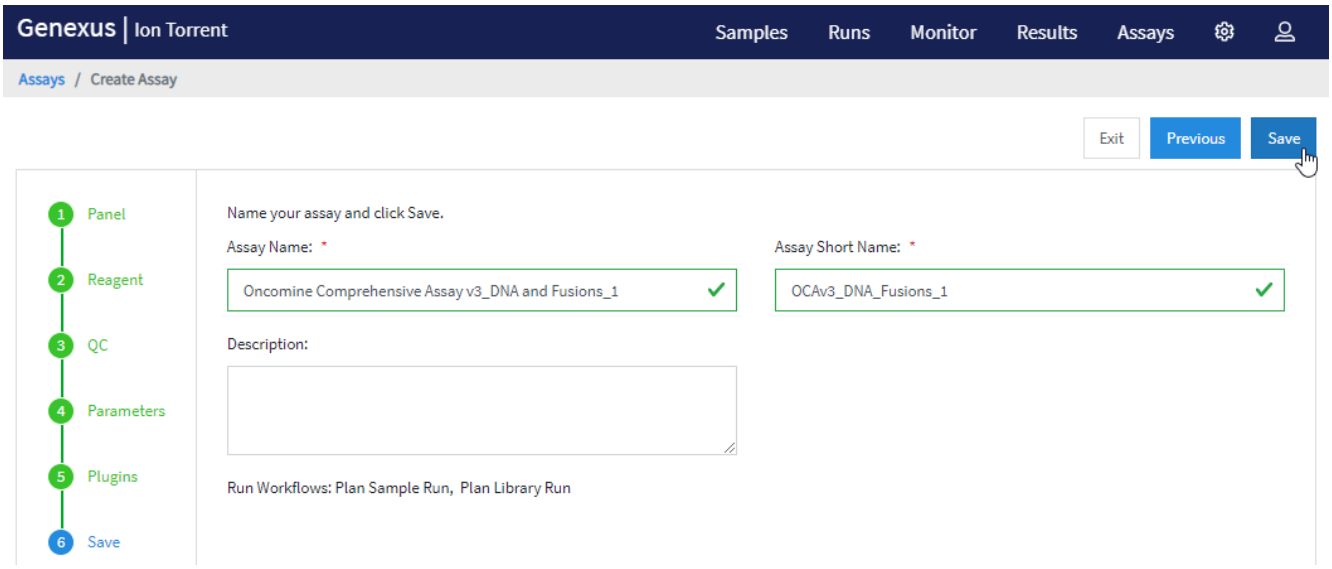


Parameters can also be set by uploading an **Advanced Parameter Configuration** file, which overrides default settings. Click **Upload** , then click **Select files...** to navigate to this file on your drive and upload. Click **Download** to download parameters settings as a JSON file to your hard drive.

7. In the **Plugins** step, select plugins that you want to include in the sequencing data analysis from the plugin list, then click **Next**.



8. In the **Save** step, enter a name and a short name for the assay, an optional description, then click **Save**.



The assay appears in the **Manage Assays** screen with the name you entered.

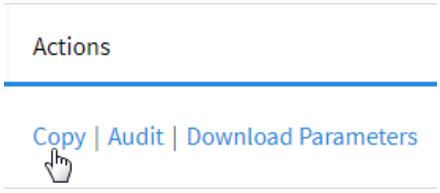
9. In the **Manage Assays** screen, click **Lock** in the **Actions** column of the assay to prevent changes to the assay.

   The assay must be locked to be available for use when planning a run.

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 it if 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 for the assay that you want to copy, click **Copy**.



The **Copy Assay** screen opens to the **Panel** step. The assay settings can be modified for the new assay.

3. Proceed through the workflow steps, and modify assay settings if needed.

4. When finished, enter a new name and short name for the copied assay in the **Assay Name** field, then click **Save**.
   The newly created assay is added to the list of assays in the **Assays** / **Manage Assays** screen. The assay name is followed by (Draft) in the Assay column. The assay remains in draft status until it is locked.

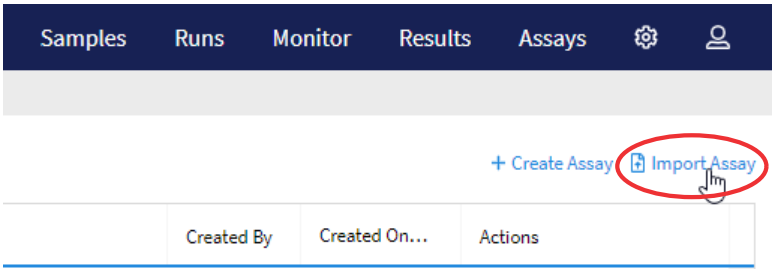
5. In the **Actions** column, in the row of the assay, click **Lock** to enable its use in a run.

Import an assay (manager/administrator)

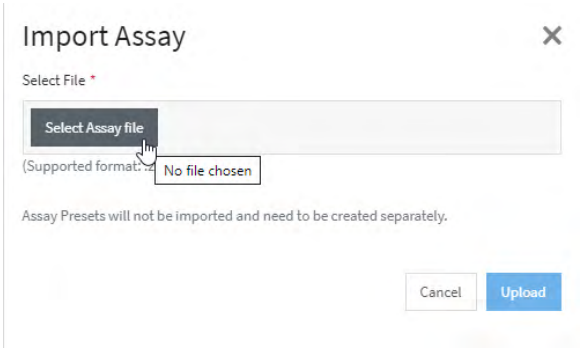
A manager- or administrator-level user can import an assay from another Genexus™ Integrated Sequencer if the assay has been first exported to a local drive.

1. In the menu bar, click **Assays** ▸ **Manage Assays**.

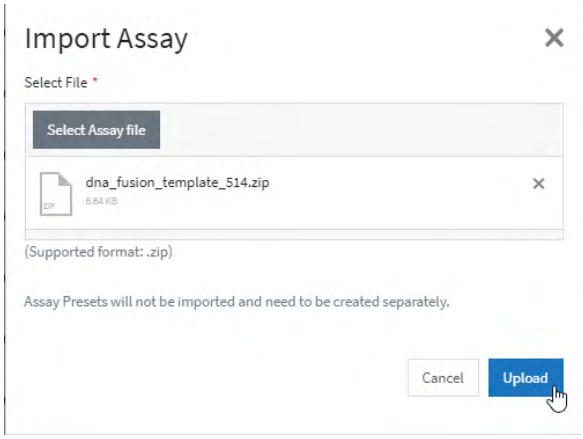
2. In the **Manage Assays** screen, click **Import Assay**.



3. In the **Import Assay** dialog box, click **Select Assay file**, navigate to the exported assay ZIP folder on your drive, then select it.

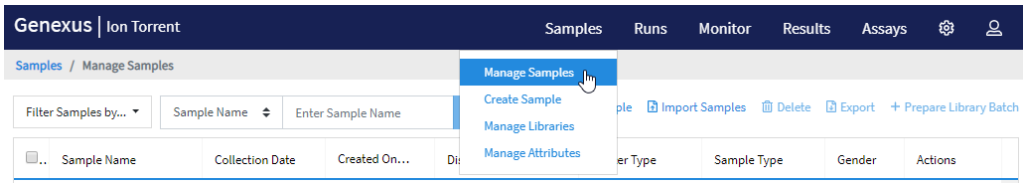


4. Navigate to the exported assay ZIP folder on your drive, select it, then click **Upload**.



The assay appears in the list of assays in the **Assays ▸ Manage Assays** screen.

4 Enter samples and libraries

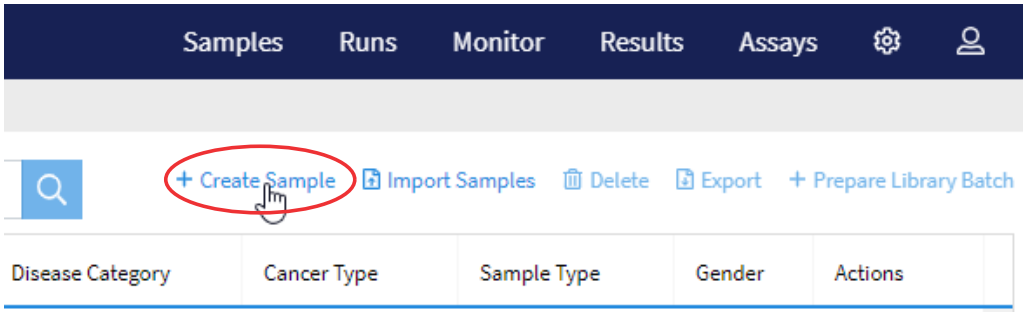


Before planning a run in Genexus™ Software for either a sample run or a library run, you must first enter sample information in the software to assign sample names and provide other information. From the **Samples** menu, you can add samples in two ways. You can manually enter sample information or import sample information from a file.

Create a new sample

You can add a new sample in the **Samples / Manage Samples** screen. The new sample will be available to use in your run.

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



3. In the **Create Sample** dialog box, complete the required fields.

   Attributes identified with a red asterisk (*) in the **Create Sample** dialog box are required when adding a new sample. If attribute information is not available when adding a new sample, substitute mock information to complete the required fields.

For more information, see “System-installed sample attributes” on page 47.

4. Click **Save**.
   The new sample is listed in the **Manage Samples** screen and will be available to use in your run.

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[1]	The date that the sample was collected. Click **Calendar** to select the date in the correct format.
Gender[1]	The biological sex of the sample: **Female**, **Male**, or **Unknown**. **IMPORTANT!** **Male** or **Female** must be selected for proper measurement of AR CNV.
Sample Type[1]	A term that describes the sample, for example, FFPE, DNA, DNA & RNA. You can also select **Other**, then enter a custom sample type.
Disease Category[1]	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 Type[1]	The type of cancer that is represented by the sample. Select the type of solid or hematologic cancer. If cancer type is unknown, select **Unknown Primary Origin**.
Cancer Stage[1]	The stage of the cancer from which the sample was collected. Select **Stage 0–IV**, or **Primary**, **Unknown**, or **Other**.

(continued)

Sample attribute	Description
% 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 119.
% 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

Create a custom sample attribute (manager/administrator)

A manager- or administrator-level user can create custom sample attributes. Sample attributes, such as the date of collection and type of cancer, characterize and define a nucleic acid sample. A sample attribute can be made mandatory, in which case you are required to enter the attribute information for each new sample. Custom attributes can be used to further characterize samples.

Custom samples attributes cannot be edited or deleted after the samples that use them have been added to a library. To remove a custom sample attribute from use, see "Remove a sample attribute (administrator)" on page 49.

1. In the menu bar, click **Samples** ▸ **Manage Attributes**.
2. In the **Manage Attributes** screen, click + **Add New Attribute**.
3. Complete the fields in the **Add New Attribute** dialog box.
   a. In **Attribute Name**, enter the name of the attribute.
      Attribute names are limited to ≤20 alphanumeric characters (0–9 and Aa–Zz), full stops/periods (.), underscores (_), or hyphens (-).
   b. In **Data Type**, specify whether the attribute is text or a number.
   c. To require users to select the new attribute when adding or importing samples, click the toggle button to designate the attribute as **Required**.
   d. Click **Submit**.

The new sample attribute is listed in the **Attribute Name** column and is available when you add a new sample.

The new sample attribute is available in the **Create Sample** dialog box and in the **Edit Sample** dialog box, even for samples that were created before the new sample attribute was created. If the new sample attribute is a required attribute, it must be specified in the **Edit Sample** dialog box in order to save the changes. For more information, see "Edit a sample (manager/administrator)" on page 52.

Remove a sample attribute (administrator)

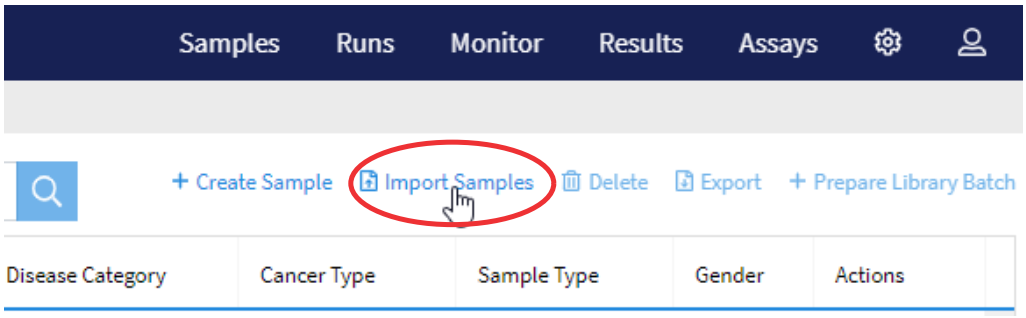
An administrator-level user can remove user-created sample attributes from use in the software. Custom sample attributes that have been made obsolete can be reactivated. A record of how the attribute is used, removed, or changed, and by whom, is maintained in the audit trail of samples that are created using that attribute. You cannot designate as obsolete system-installed sample attributes.

1. In the menu bar, click **Samples** ▸ **Manage Attributes**.
2. In the **Manage Attributes** screen, select a custom sample attribute to remove.
3. Click **Obsolete** in the **Actions** column, then confirm the action.
   Reactivate replaces **Obsolete | Edit** in the **Actions** column.
4. *(Optional)* To reactivate an attribute, click **Reactivate** in the **Actions** column.
   Active sample attributes are listed in the **Add New Sample** dialog box.

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 47. For ease of use, you can download a Microsoft™ Excel™ example file to create an import file. For more information, see "Download an example samples file" on page 50.

1. In the menu bar, click **Samples** ▸ **Manage Samples**, then click **Import Samples**.



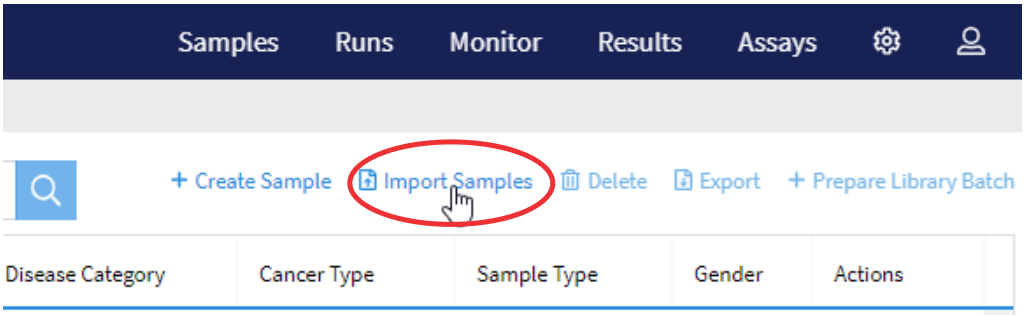
2. In the **Import Samples** dialog box, click **Select samples file**.
3. Navigate to the file, then click **Open**.
4. Click **Upload**.
   A progress bar followed by an import report appears. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For troubleshooting, see "Batch sample import fails" on page 144.

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

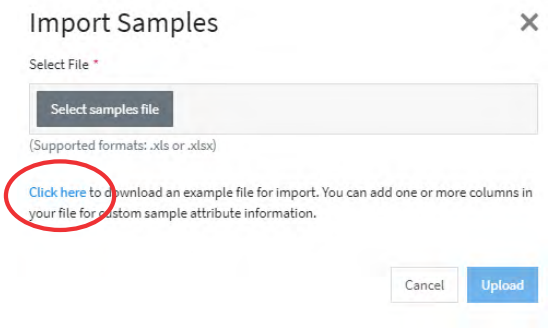
Download an example samples file

Download a Microsoft™ Excel™ example file to create a sample import file. The example file contains two tabs. The **Instruction** tab in the spreadsheet lists and indicates mandatory and optional attributes, which are the column headings in the **Specimen Format** tab. Use the **Specimen Format** tab to enter samples.

1. In the menu bar, click **Samples** ▸ **Manage Samples**, then click **Import Samples**.



2. In the **Import Samples** dialog box, click **Click here** to download the Microsoft™ Excel™ example file.



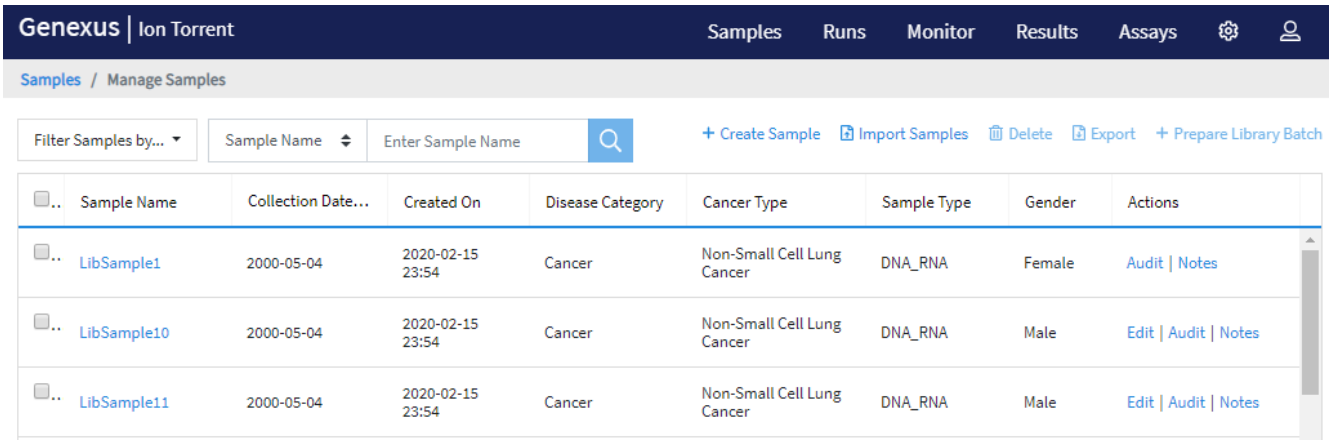
The example file contains default sample attributes as columns. If custom sample attributes have been configured in the software, add these attributes as columns to the example file.

3. Save the file.

   You can now import the file in the **Import Samples** dialog box. For more information, see “Import samples” on page 49.

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.



Sort, search, and filter samples

Use the **Samples / Manage Samples** screen to sort, search, and filter the list of samples. The samples are listed by name with the most recently created sample at the top of the list.

- Use the **Filter Samples by** list and the **Sample Name** search box to search, sort, and filter the list of samples.

To	Do this
List only the samples that have not been extracted.	In **Filter Samples by**, select **To Be Extracted**.
List only the samples that have not been prepared as a library.	In **Filter Samples by**, select **To Be Prepared**.
List all samples.	In **Filter Samples by**, select **All**.
Sort the samples list.	1. Click a column heading to sort the list by the entries in that column. 2. Click the column heading again to reverse the order.
Search for samples of interest.	1. In the **Sample Name** search box, enter the full or partial sample name. 2. Click Q **(Search)** or press **Enter**. The sample or samples that match the sample name search entry are listed. 3. Click ✕ **(Remove)** to return to the complete list of samples.

Export samples

The **Export** function generates an XLS file that contains details about the selected sample.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, select the checkbox in the row of each sample that you want to export. To select all samples, select the checkbox in the column heading row.
3. Click **Export**.
   An XLS file is created that contains the details of the selected samples. Depending on your browser settings, the software automatically downloads the file or prompts you to open or save the file.
4. Open the XLS file in an appropriate viewer to review or print.

View notes or add a note to a sample

You can add notes to a specific sample or view existing notes. Use **Notes** to capture time-stamped written comments and observations for a sample. A sample can contain multiple notes from different users.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the row of the sample of interest, in the **Actions** column, click **Notes**, then review existing notes or add a new note.

Action	Description
View existing notes for a sample.	In the **Notes** dialog box, notes that are associated with the sample are listed by user, date, and time.
Add a new note to a sample.	1. In the **Notes** dialog box, click **Add Notes**. 2. In **Add Notes**, enter the note, then click **Save**.

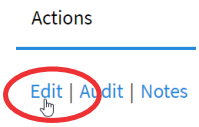
Edit a sample (manager/administrator)

A manager- or administrator-level user can edit samples. In the **Samples / Manage Samples** screen, samples that can be edited are identified by the presence of the **Edit** link in the **Actions** column.

Samples can be edited at any point before a sample run that uses that sample is complete using the **Edit** link. After the sample is used in a run, the **Edit** link is no longer available. To edit a sample after a run is complete, use the **Edit Sample & Amend Report** link instead. For more information, see “Edit a sample and amend a report after a run (manager/administrator)” on page 53.

To add a custom sample attribute to a sample, see “Create a custom sample attribute (manager/administrator)” on page 48.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, in the **Actions** column, click **Edit** in the row of the sample of interest.



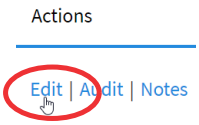
3. In the **Edit Sample** dialog box, edit the sample attributes.

   See “System-installed sample attributes” on page 47 for more information about the system-installed sample attributes.
4. Click **Save**.

Edit a sample and amend a report after a run (manager/administrator)

After a sequencing run and its analysis have completed, a manager- or administrator-level user can edit a sample and amend the Lab Report for up to 30 days. Editing a sample after a run triggers an automatic update of the report and other files associated with the sample. In the **Samples / Manage Samples** screen, editable samples are identified by the presence of the **Edit** link in the **Actions** column. After 30 days, the **Edit** link is unavailable, only the **Audit** link is available.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, in the **Actions** column, click **Edit** in the row of the sample of interest.



3. In the **Edit Sample & Amend Report** dialog box, edit sample attributes as necessary.

   See “System-installed sample attributes” on page 47 for more information about system-installed sample attributes.
4. Click **Save**.

   The **Edit Sample and Amend Report** confirmation dialog box opens.
5. Click **Yes** to confirm the changes and continue.

   The sample information is edited, and all runs and reports associated with that sample are updated. Any test report, lab report, tab files and info.csv file that is associated with the sample will be updated with the changes.

Review sample edit history

The entire history of defining a sample is available to review and export using the audit feature. The history shows the original sample values and any new values or activities. The following actions generate an audit record:

- Create a new sample
- Edit a sample
- Obsolete a sample
- Delete a sample

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 **Samples / Manage Samples** screen, in the **Actions** column, click **Audit** in the row of the sample of interest.
   The **Audit Trail Details** window opens, listing each action that was performed for the sample, such as sample creation or edits.
2. *(Optional)* Click **Download** to export the audit record to a PDF file.
3. Click **Back** to close the **Audit Trail Details** window and return to the list of samples.

Delete samples

You can delete samples that have not been assigned to a run. Samples that are assigned to a run are locked and cannot be edited or deleted. Locked samples display **Audit** in the **Actions** column.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, select the checkbox in the row of each sample that you want to delete. To select all samples, select the checkbox in the column heading row.
3. Click **Delete**.
   The **Delete Sample** dialog box opens with the message Are you sure you want to delete selected sample(s)?
4. Click **Yes** to delete the selected samples.

The sample is removed from the **Manage Samples** screen.

Prepare or import a library batch

A library batch is a group of prepared libraries that are sequenced in the same library run. If you are planning a run starting from libraries that you have already prepared manually, you must first create a library batch in Genexus™ Software from samples. You can enter samples in the software or import a sample file. For more information see:

- “Create a new sample” on page 46
- “Import samples” on page 49

Select the library batch when you plan the run. If you are planning a run starting from nucleic acid samples, skip this step and proceed to “Plan a sample run” on page 62.

Note:

- Each library in a library batch must have a unique library name.
- When combining libraries in the same run, each library must have a unique barcode.
- Fields identified with a red asterisk (*) are required.

Prepare a library batch

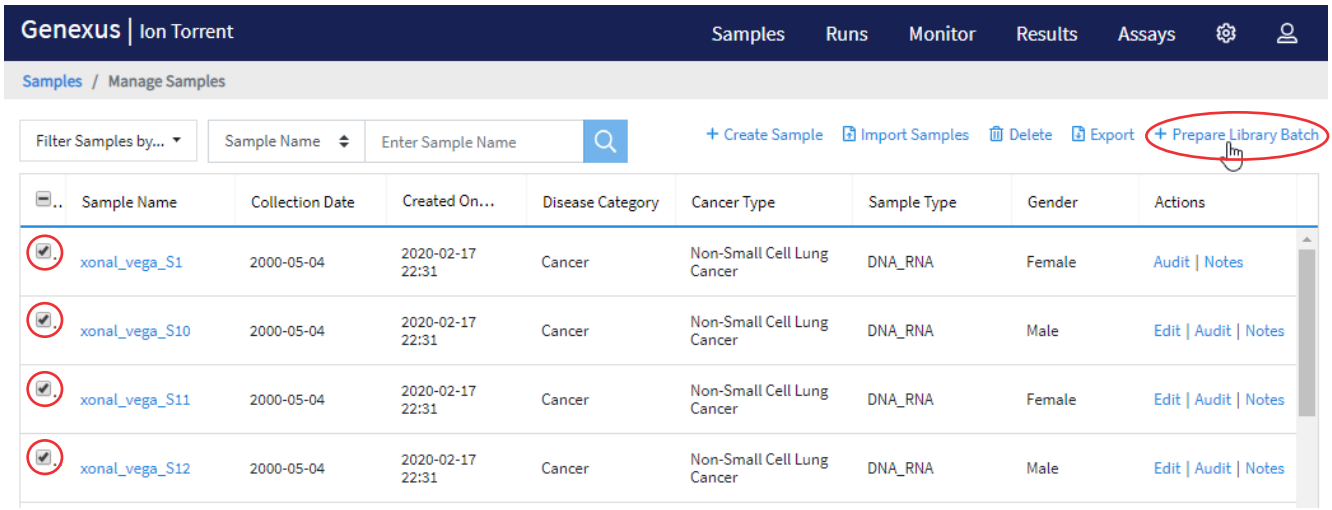
A library batch is a group of prepared libraries that are sequenced in the same library run. If you are planning a run starting from libraries that you have already prepared manually, you must first create a library batch in Genexus™ Software from samples that you have added. If you are planning a run starting from nucleic acid samples, skip this step and proceed to “Plan a sample run” on page 62.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.

2. In the **Manage Samples** screen, in the **Filter Samples by...** dropdown menu, apply the **To Be Prepared** filter to limit the displayed samples to those samples that have not been placed in a library batch.

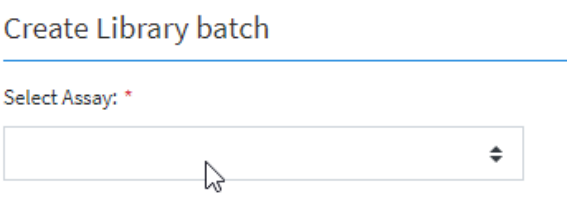
   See “Sort, search, and filter samples” on page 51 for more information on the **Filter samples** feature.

3. Select samples in the list by clicking the checkbox to the left of each sample, then click + **Prepare Library Batch**.



4. In the **Create Library batch** screen, in **Select Assay**, select the assay that you want to run.

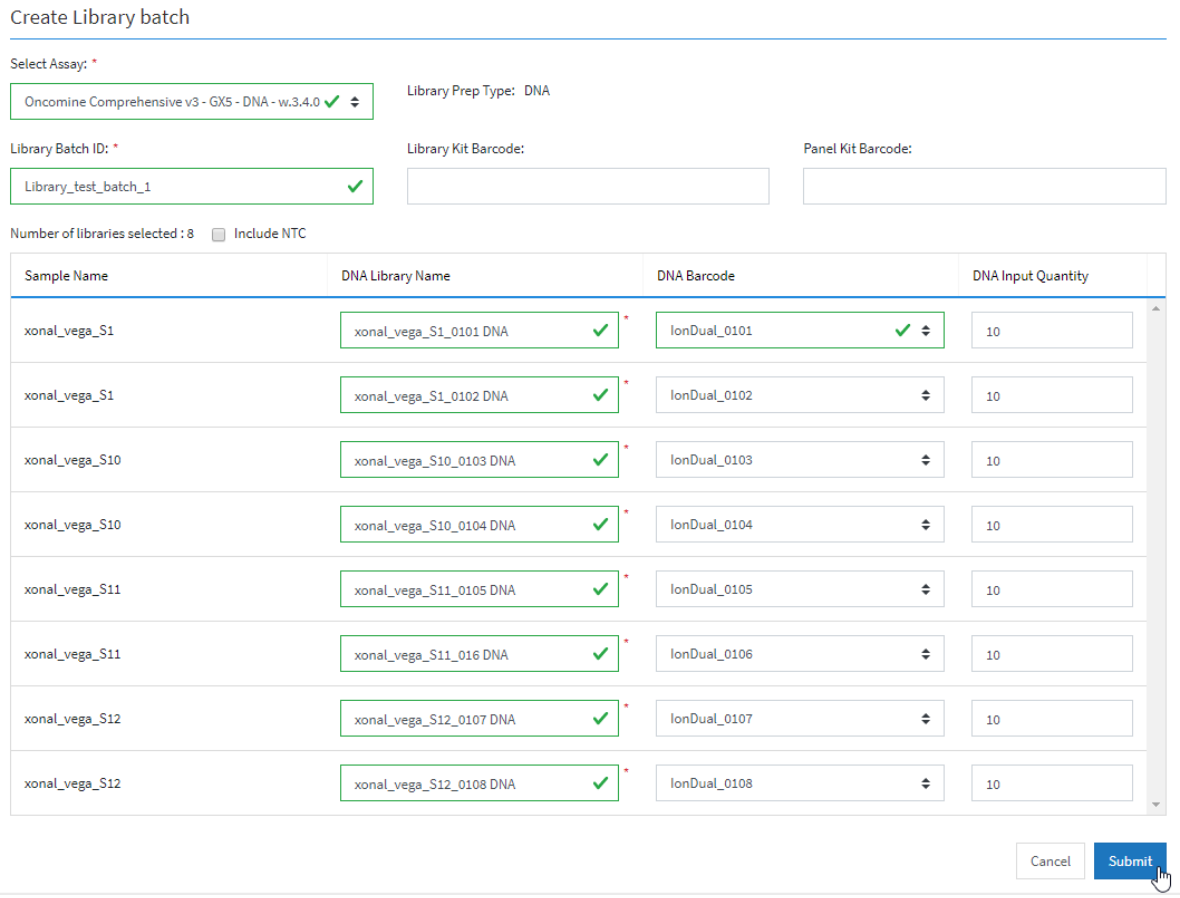
   The assay determines specific parameters of the run, including any required controls and post-run data analysis settings. Only locked assays appear in the **Select Assay** dropdown menu. For instructions to lock an assay, see “Manage assays (manager/administrator)” on page 33.



5. In the expanded screen, in **Library Batch ID**, enter a unique identifier for the library batch.

Note: Library Prep Type: automatically fills for the nucleic acid type specified by the assay you selected: **DNA, RNA, DNA+RNA, or TNA**.

Library Batch IDs can contain only alphanumeric characters (0–9, Aa–Zz), full stop/period (.), underscore (_), and hyphen (-). Required fields are indicated with a red asterisk (*).



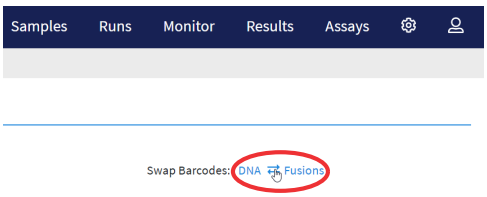
6. Select the barcodes from the kit boxes into the appropriate fields.

7. Select the **Include NTC** checkbox to add no template control sample processing and reporting to the library batch.

8. Type a unique library name for each DNA and/or RNA library in the appropriate field.
   - Library names can contain only alphanumeric characters (0–9, Aa–Zz), full stop/period (.), underscore (_), and hyphen (-).
   - If your assay requires specific controls, they are automatically listed in the dialog box. These controls each require a unique barcode ID within the library batch, but do not require library names.

9. Select the barcode ID of the adapter used to prepare each library. If appropriate, swap the default barcodes in the dialog box between DNA, RNA, and Fusions by clicking the **Swap Barcodes** swap image.

   For example, click the **DNA** and **Fusions** swap button.



Each library in a library batch must have a different barcode ID. When preparing the physical libraries, best practice is to swap barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. The barcodes that are listed in the **DNA Barcode** or **RNA Barcode** dropdown list belong to the barcode set that was selected when the assay was created.

IMPORTANT! Ensure that the actual barcodes that you used to create the libraries match the barcodes that you enter in the **Create Library batch** screen.

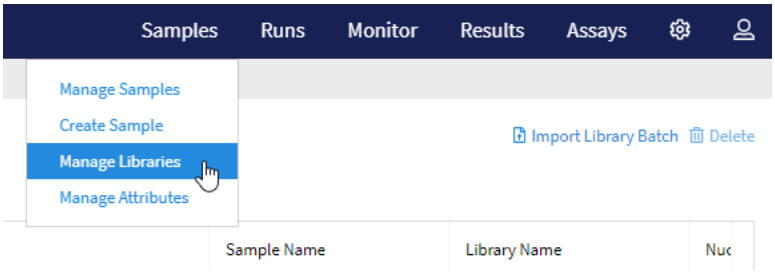
10. Enter the **Input Quantity** for each library.

11. Click **Submit** to save and submit your selections.
    The **Manage Libraries** screen opens, listing the library batch that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

Import a library batch

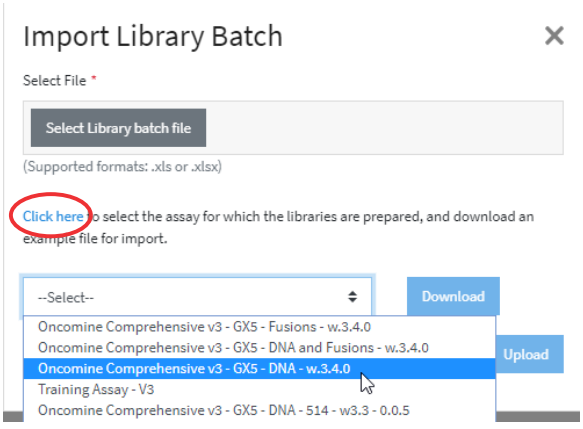
You can import library batch information in the form of an .XLS or .XLSX file. The import file must include all required library and kit information.

1. In the menu bar, click **Samples** ▸ **Manage Libraries**.



2. In the **Manage Libraries** screen, click **Import Library Batch**.

3. In the **Import Library Batch** dialog box, click **Click here** to select an assay for which the libraries are prepared, and to download an example file for import.



4. Select an assay from the list, then click **Download**.
   The assay name is auto-populated in the Microsoft™ Excel™ template file that downloads to your drive.

5. In the template file, enter or confirm the library batch information.

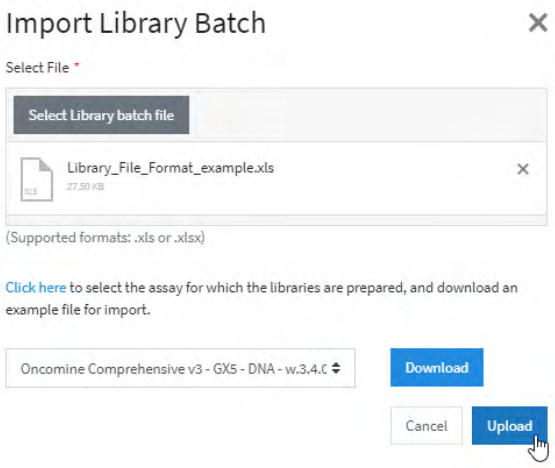
Template item	Description
Reagents tab	
Assay Name	Auto-populated when assay is selected in step 4 (required)
Library Batch ID	Must be alphanumeric (0–9, Aa–Zz), full stop/period (.), underscore (_), and hyphen (-) (required)
Library kit barcode	For example, Genexus™ Library Strips 1 and 2-AS barcode (optional)
Panel kit barcode	For example, Oncomine™ Comprehensive Assay v3 DNA GX barcode (optional)
Libraries tab	
Sample Name	Same character requirement as Library Batch ID (required)
Library Name	Same character requirement as Library Batch ID (required)
Barcode	Barcodes used for each sample and control library preparation (required)
Nucleic Acid Type	DNA, RNA, or TNA (required)
Input Quantity	Library input quantity (optional)
No Template Control	To Include a no template control, add a row with **Sample Name** as NTC, **Library Name** as NA, **Barcode** and **Nucleic Acid Type** similar to sample rows (optional)

IMPORTANT! For DNA+Fusions assays, the DNA library and RNA libraries must be listed in sequential order per pool for each sample. For example, for a 1-pool DNA+Fusions assay, order should be DNA, RNA for sample 1, DNA, RNA for sample 2. For a 2-pool DNA+Fusions assay, library order should be DNA, RNA (pool 1), DNA, RNA (pool 2) for sample1, then DNA, RNA (pool 1), DNA, RNA (pool 2) for sample 2.

6. Save the file.

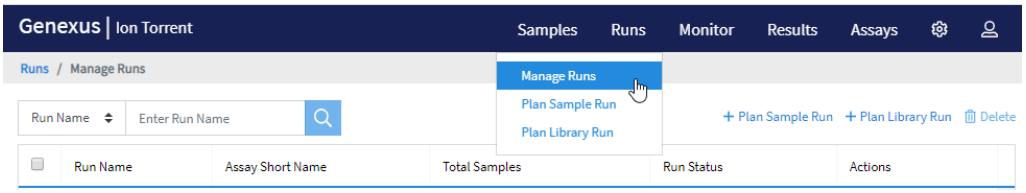
7. Click **Browse**, navigate to the saved file, then select it.

8. Click **Upload**.



A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting support, see “Library batch import fails” on page 145.

5 Plan and manage runs



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.

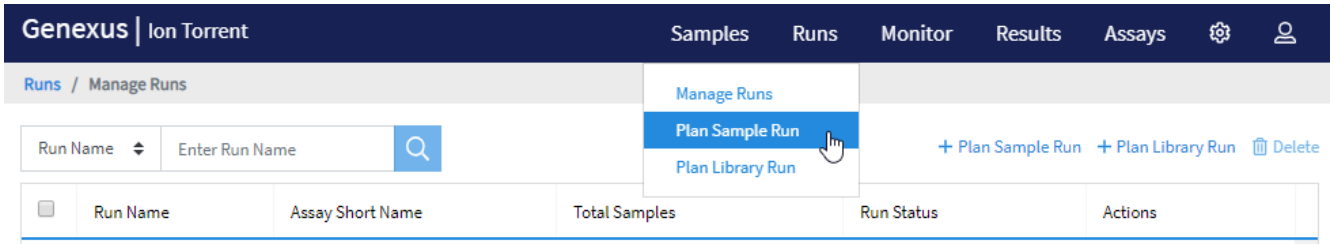
Plan a sample run

You can plan a run starting from isolated nucleic acid samples.

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

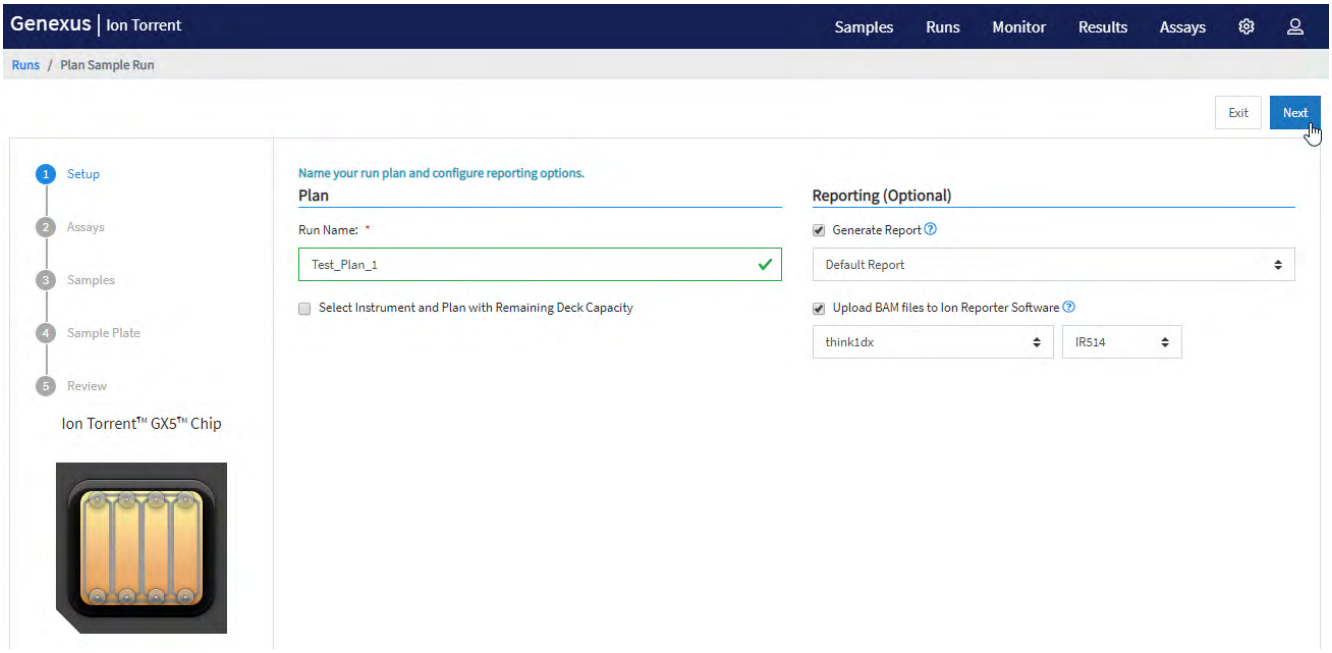
Before planning a run for a sample run, you must enter sample information into Genexus™ Software. For more information, see “Create a new sample” on page 46, or “Import samples” on page 49.

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 **Plan** section, enter a unique name.



   b. *(Optional)* In the **Reporting (Optional)** section, select one or more options if needed. You can select both options, or leave both options deselected.

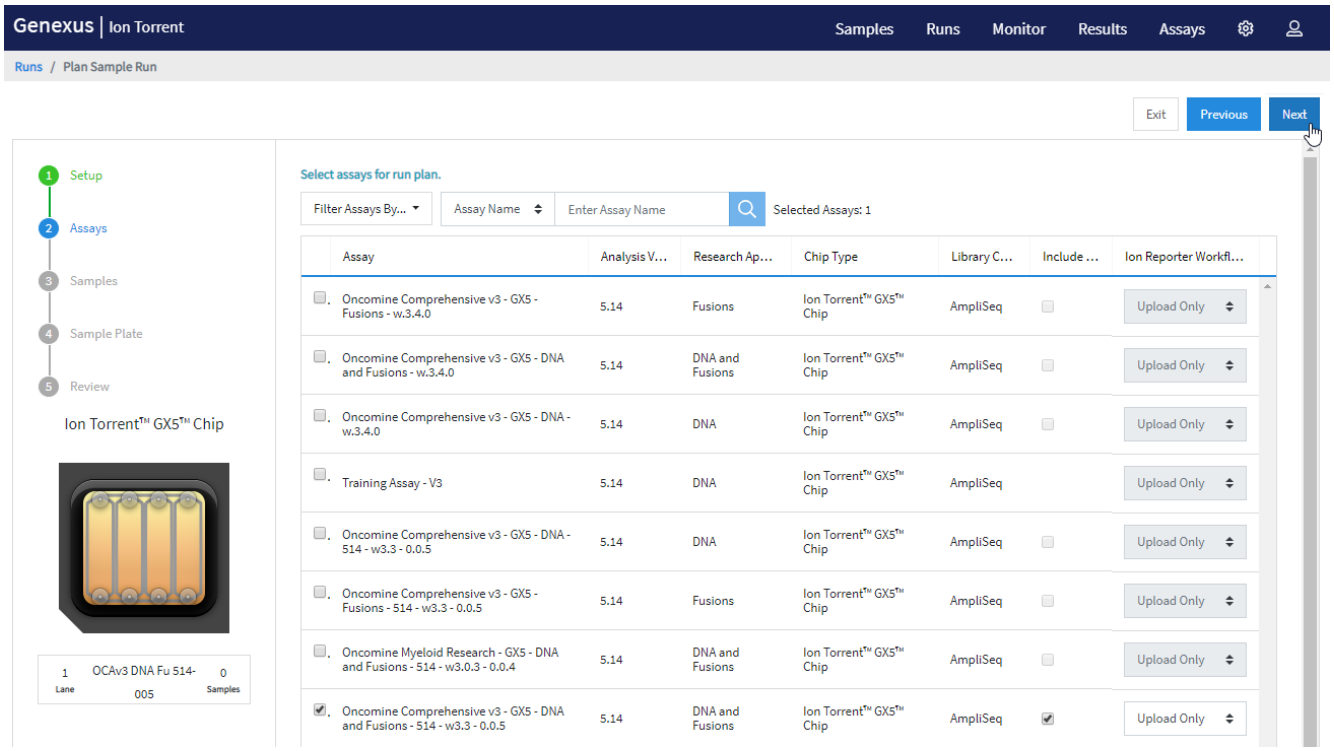
Reporting option	Description
Generate Report	Select this option to generate a Lab Report using a report template that you specify in the list. To create a report template, click **Assays** ▸ **Manage Presets**, then in the **Report Templates** tab, click + **Add New**.
Upload BAM files to Ion Reporter™ Software	Select this option to automatically upload data for further analysis with Ion Reporter™ Software. You can also upload BAM files after a run if you leave this option unselected. Select your Ion Reporter™ Software account and release version. To configure an Ion Reporter™ Server account, see "Configure an Ion Reporter™ Server account (administrator)" on page 179. To configure an Ion Reporter™ Software on Connect account, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 182.

   c. Click **Next**.

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

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

   Use the **Filter Assays By** list and the **Assay Name** search box to search, sort, and filter the list of assays.



Note:

- After selecting an assay, the list is filtered to show compatible assays that can be selected and run at the same time.
- To create a new assay, see Chapter 3, “Create and manage assays (manager/administrator)”.

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

If you selected the **Upload BAM files to Ion Reporter™ Software** reporting option in substep 2b, make the following selections from the dropdown list in the **Ion Reporter™ Workflow** column in the row of each assay that you selected.

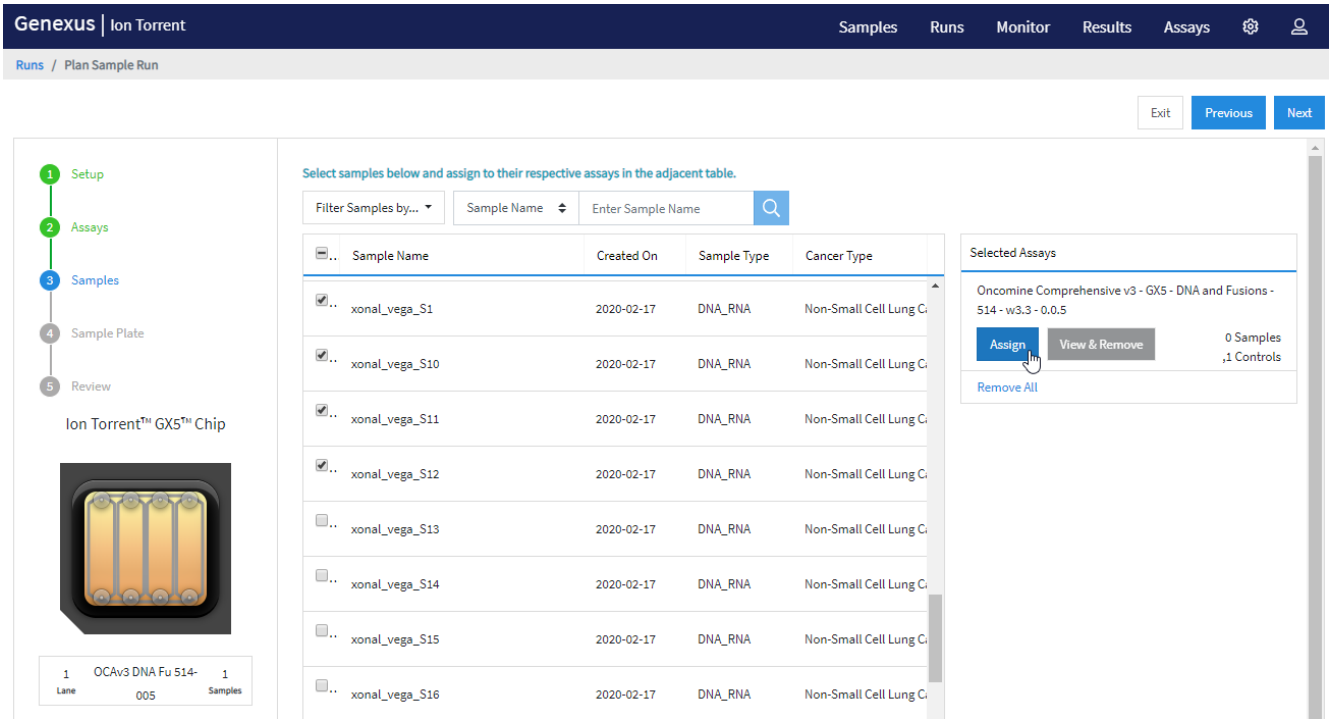
- Select **Upload Only** to upload sample data to Ion Reporter™ Software automatically upon run completion.
- Select the desired Ion Reporter™ analysis workflow to upload sample data *and* launch an analysis in Ion Reporter™ Software automatically upon run completion.

Note: In order for the Ion Reporter™ analysis workflow to appear in the list, you must tag the analysis workflow for use with the IonReporterUploader plugin. For more information, see "Tag an Ion Reporter™ Software analysis workflow for use with the IonReporterUploader plugin" on page 181.

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

5. After you select an assay (or assays) and make the appropriate Ion Reporter™ Software selections (if applicable), click **Next**.

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



The **Chip View** updates to show the lanes to be used in the run. Lane usage is calculated based on the number of samples (including a no template control, if selected), assay type, primer pools used, and minimum reads per sample entered at assay setup. Green denotes a chip lane that will be used in the run containing assigned samples within lane capacity. If the minimum reads per sample × the number of samples exceeds the chip or lane well capacity, a dialog box appears

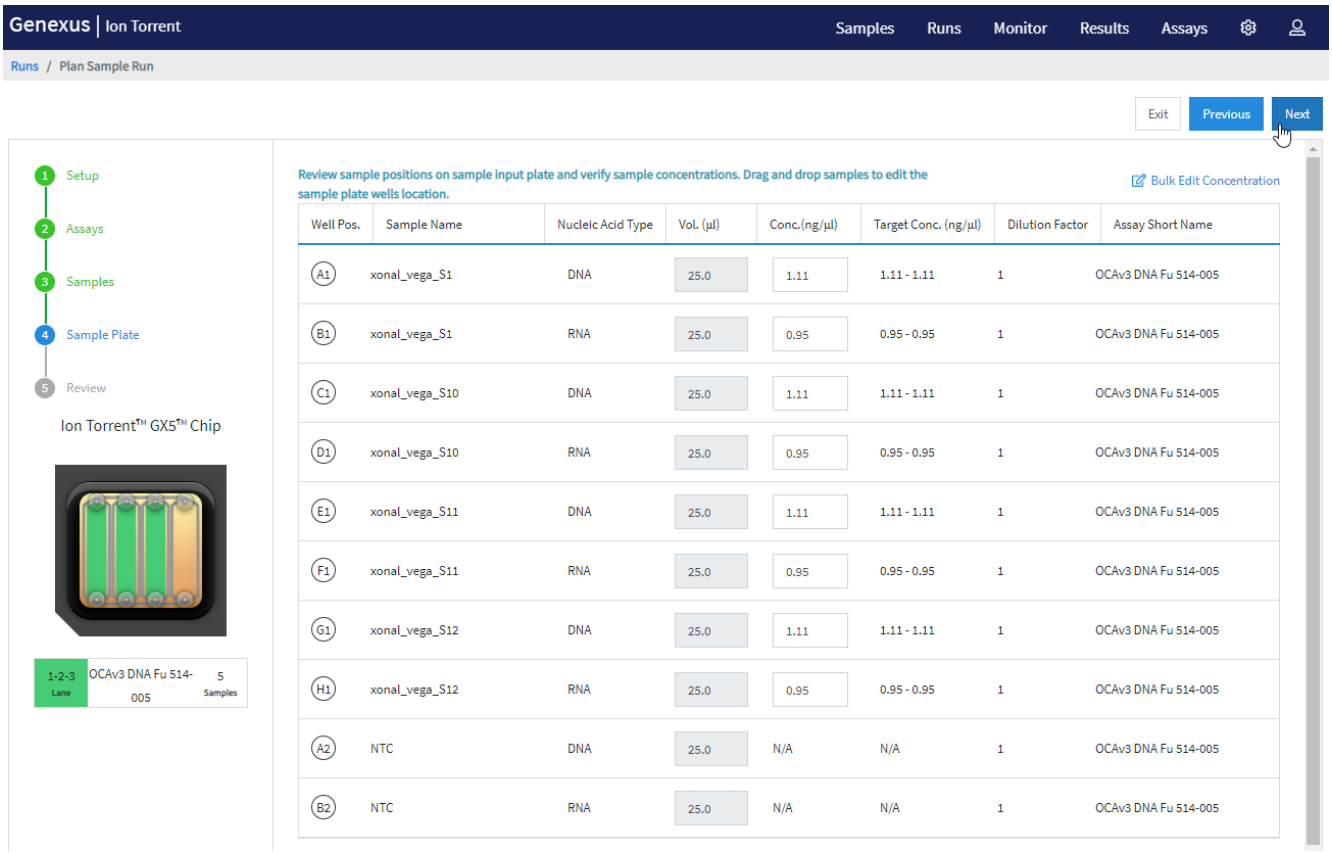
after you click **Assign** asking you to confirm that you want to continue. After confirmation, the **Chip View** updates and shows the lane color as red instead of green. The run is allowed, but you may not achieve the required reads per sample to pass QC metrics.

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

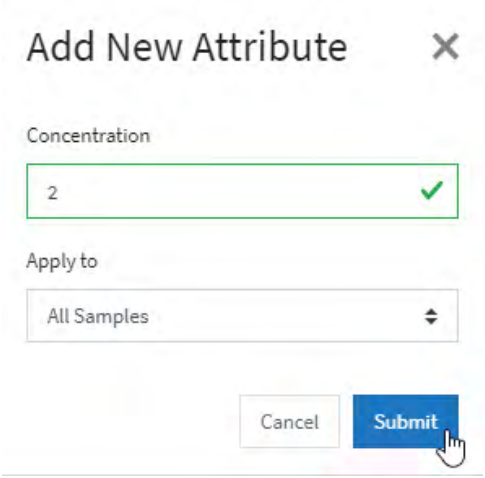
Red lane color denotes sample assignment that exceeds lane capacity.

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, then click **Next**.
   - Click **View & Remove**, make your selections, then click **Update**.
   - Click **Remove All**, make your selections, then click **Assign**.
9. In the **Sample Plate** step, review sample positions in the sample plate. Drag and drop samples and no template controls to edit the location of samples and controls, if desired.

10. Modify the concentration of samples, if needed.



Click **Bulk Edit** to modify sample concentration of all samples at one time, then click **Submit** to return to the **Sample Plate** screen.



If a sample concentration is ≤1,024X of the target concentration for the assay, which is displayed as a default value for each sample in the **Sample Plate** screen, the sequencer automatically dilutes the sample to the target concentration during the run. If a sample concentration is greater than this value, you must manually dilute the sample to the target concentration, or to a value within

range for automated dilution before loading on the sample plate. For more information, see “Dilute or concentrate the samples (if needed) and load the sample plate—sample run” on page 76.

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 more information, see “Dilute or concentrate the samples (if needed) and load the sample plate—sample run” on page 76 for specific sample volumes to load on the sample plate.

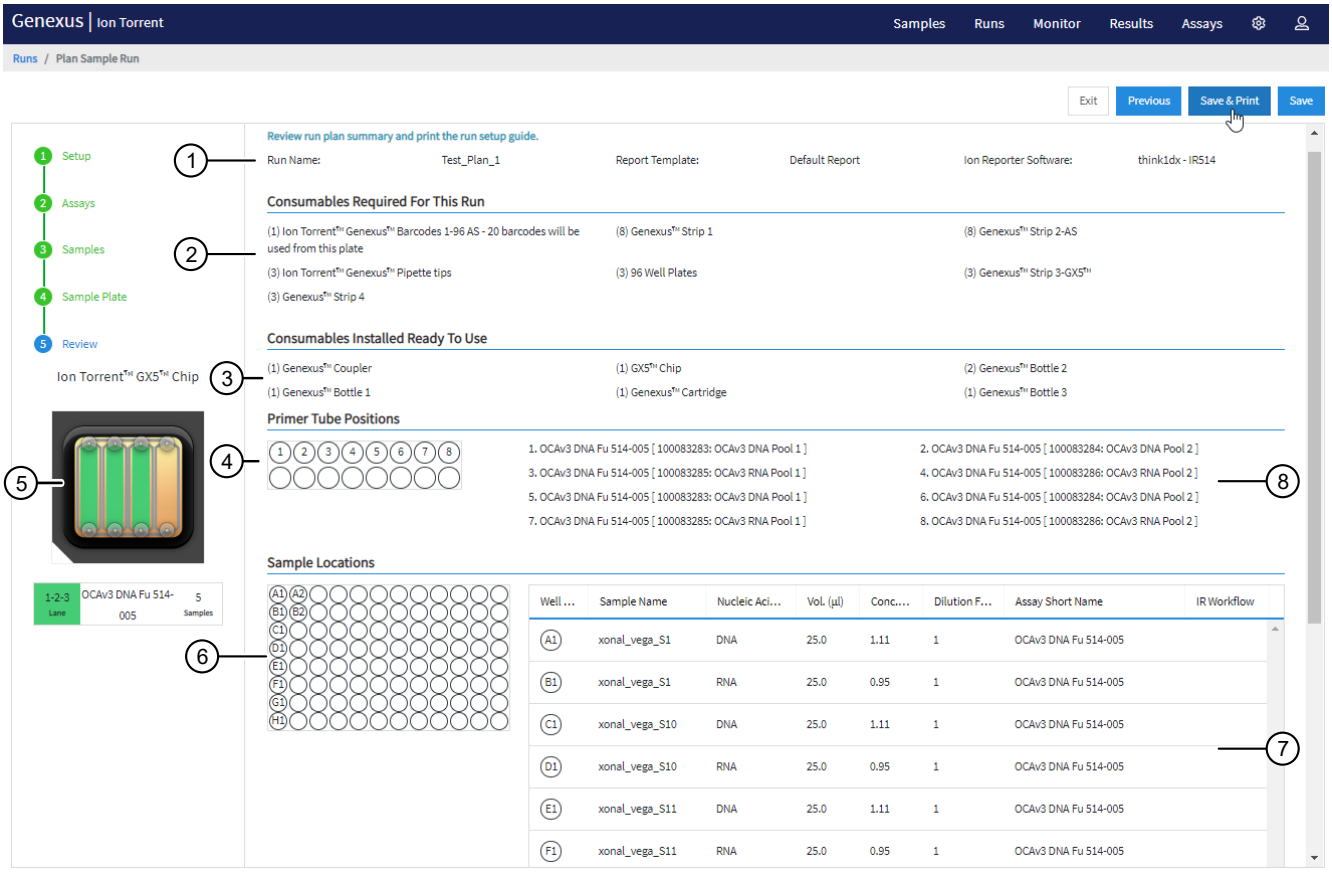
---

11. If sample plate information is correct, click **Next**.

12. In the **Review** step, review the run plan summary, then click **Save & Print** to print the run setup guide, if desired. Click **Save** to save the run without printing.

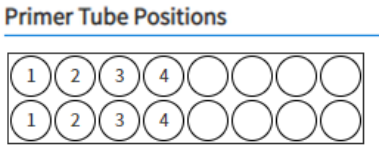
    The run plan summary lists the following details:

    - the consumables that are required for this run
    - how much sample volume to load
    - where to load samples and primer pool tubes
    - the sample concentration.



1. Run information
2. List of consumables required for the run
3. List of consumables that are installed on the sequencer and available for the run
4. Positions to load primer pool tubes
5. Chip view showing the lanes to be used in the run
6. Positions in the sample plate to load the samples
7. Table listing the sample plate position, sample type, volume to load, concentration, dilution factor, and assay for each sample
8. Description of each primer pool and its position

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



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

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

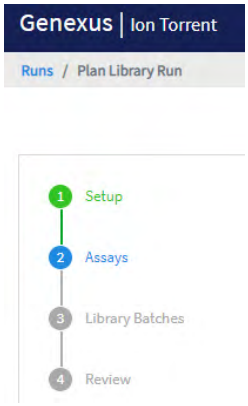
Plan a library run

Before planning a library run, you must enter sample information and prepare a library batch in Genexus™ Software. The library batch selects the assay to be used in the run, and the assay in turn specifies the barcode set that was used to prepare the sample libraries. If your sample libraries were prepared using a barcode set not specified in an assay you want to use in the run, you must

1. create a new assay, or copy an existing assay, and specify the new barcode set in assay setup.
2. prepare a library batch that selects the new assay.

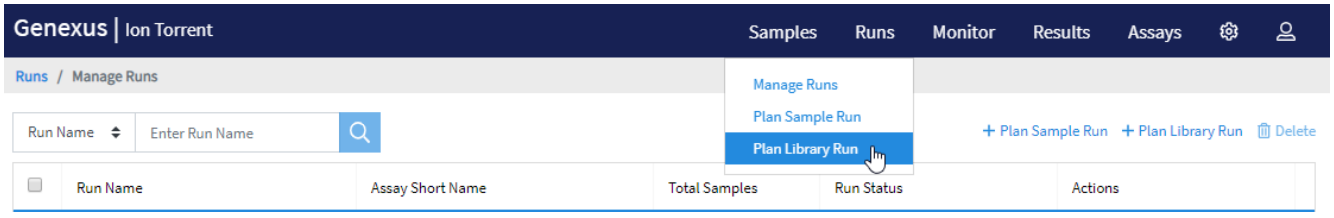
For more information, see “Create a new assay (manager/administrator)” on page 35, “Copy an assay (manager/administrator)” on page 43, and “Prepare a library batch” on page 55.

Genexus™ Software guides you through the four steps of planning a library run: **Setup**, **Assays**, **Library Batches**, and **Review**. Progress through the steps is tracked in the upper left corner of the **Runs / Plan Library Run** screen.

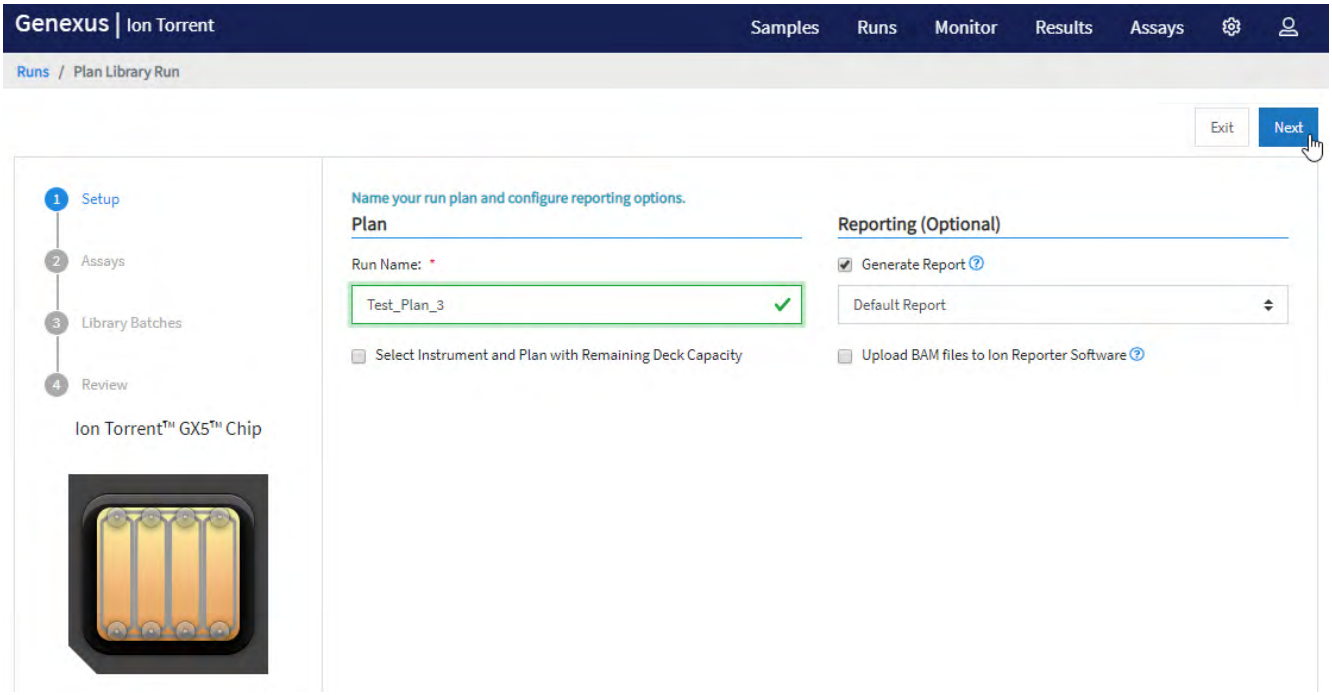


1. In the menu bar, click **Runs** ▸ **Plan Library Run**.

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



2. In the **Setup** step, enter a name for the run, then configure the reporting options.
   a. In **Run Name**, enter a unique name.



   b. *(Optional)* In the **Reporting (Optional)** section, select one or more options if needed. You can select both options, or leave both options deselected.

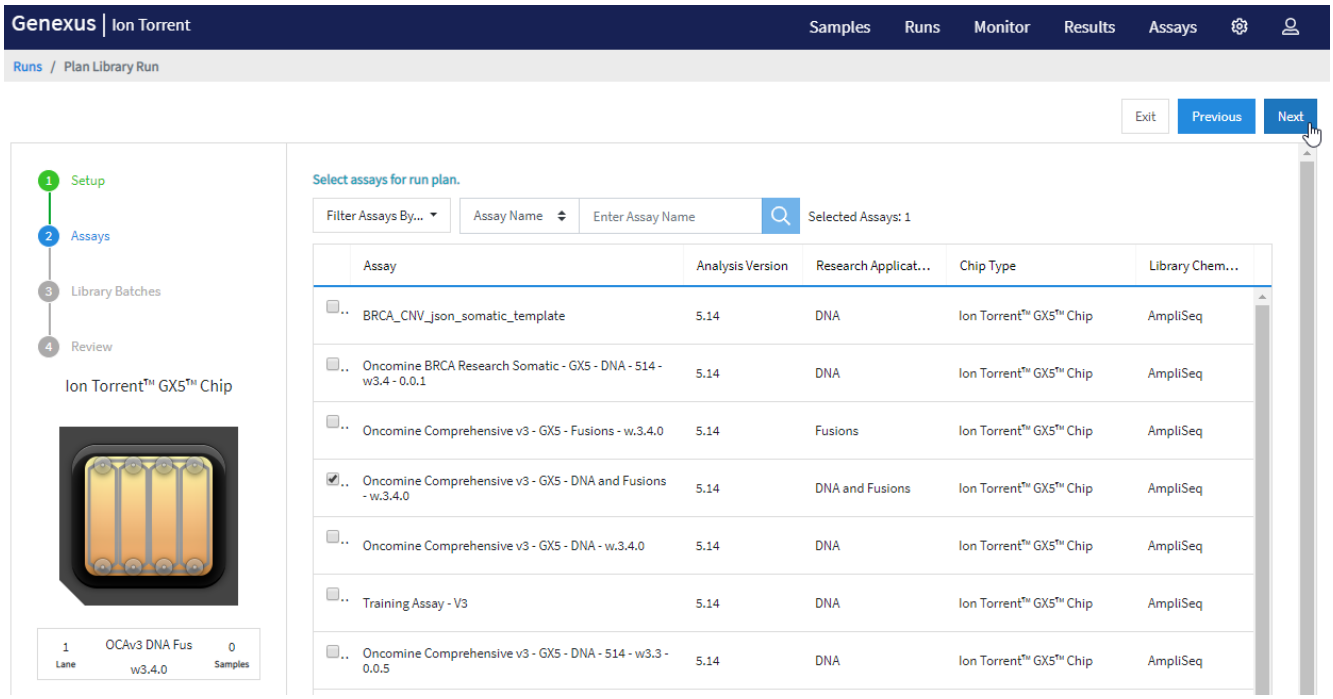
Reporting option	Description
Generate Report	Select this option to generate a Lab Report using a report template that you specify in the list. To create a report template, click **Assays** ▸ **Manage Presets**, then click + **Add New**.
Upload BAM files to Ion Reporter™ Software	Select this option to upload data for further analysis with Ion Reporter™ Software. You can also upload BAM files after a run if you leave this option unselected. Select your Ion Reporter™ Software account and release version. To configure an Ion Reporter™ Server account, see "Configure an Ion Reporter™ Server account (administrator)" on page 179. To configure an Ion Reporter™ Software on Connect account, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 182.

   c. Click **Next**.

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

3. In the **Assays** step, select the assay or assays that you want to use in the run, then click **Next**.

   Use the **Filter Assays By** list and the **Assay Name** search box to search, sort, and filter the list of assays.



Note: For the assay to be selectable at this step, you must have prepared a library batch that assigns the assay to the batch. For more information on preparing a library batch, see “Prepare a library batch” on page 55. The assay specifies the barcode set that was used to prepare the sample libraries. To create a new assay, or copy an existing assay, see “Create a new assay (manager/administrator)” on page 35, or “Copy an assay (manager/administrator)” on page 43.

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 plan a run, the instrument will use incorrect settings during the run, resulting in invalid sequencing results. Available assays are listed in the **Assays / Manage Assays** screen.

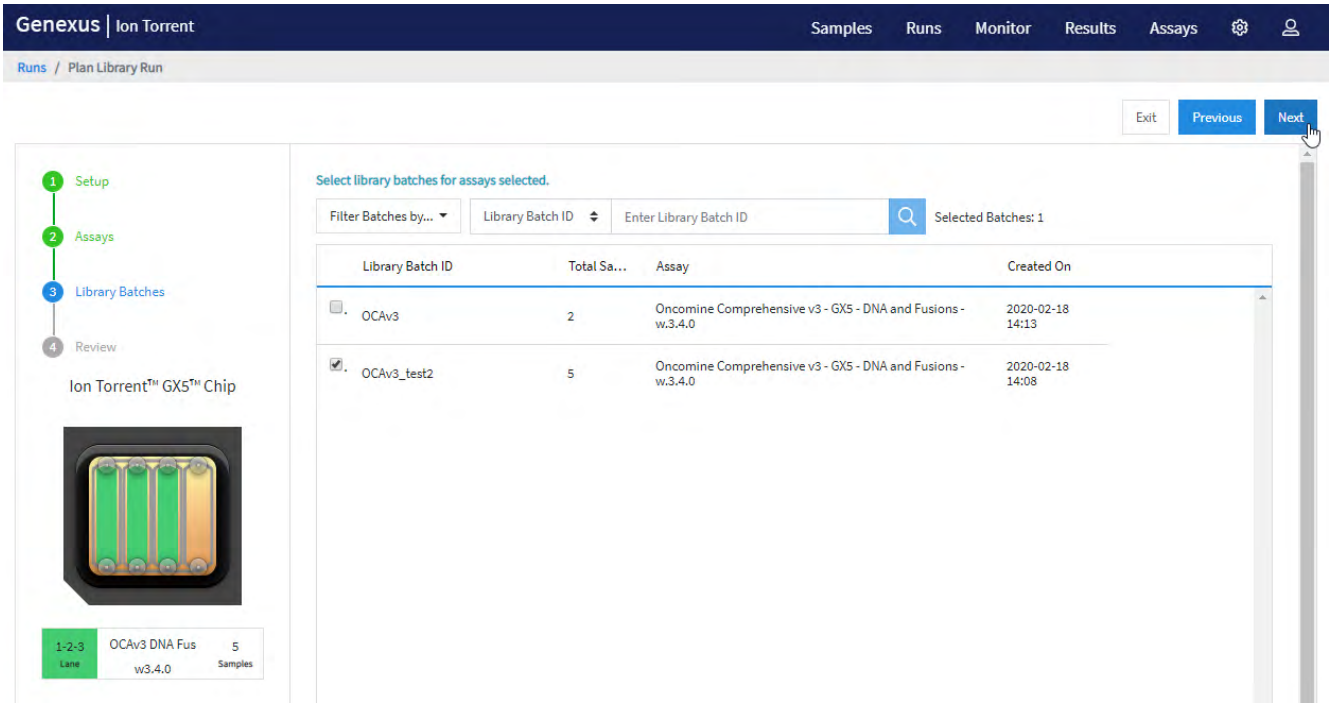
If you selected the **Upload BAM files to Ion Reporter™ Software** reporting option in substep 2b, make the following selections from the dropdown list in the **Ion Reporter™ Workflow** column in the row of each assay that you selected.

- Select **Upload Only** to upload sample data to Ion Reporter™ Software automatically upon run completion.
- Select the desired Ion Reporter™ analysis workflow to upload sample data *and* launch an analysis in Ion Reporter™ Software automatically upon run completion.

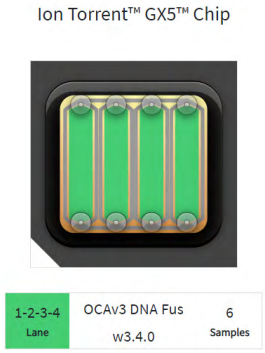
  Note: In order for the Ion Reporter™ analysis workflow to appear in the list, you must tag the analysis workflow for use with the IonReporterUploader plugin. For more information, see “Tag an Ion Reporter™ Software analysis workflow for use with the IonReporterUploader plugin” on page 181.

4. In the **Library Batches** step, select the library batch or batches that you want to use in the run.

Note: Only one library batch can be selected per assay. However, you can plan a multi-assay library run if you select multiple, different assays in the **Assays** step.



The **Chip View** updates to show the lanes to be used in the run as green. Lane usage is calculated based on the number of samples, assay type, primer pools used, and minimum reads per sample entered at assay setup.



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

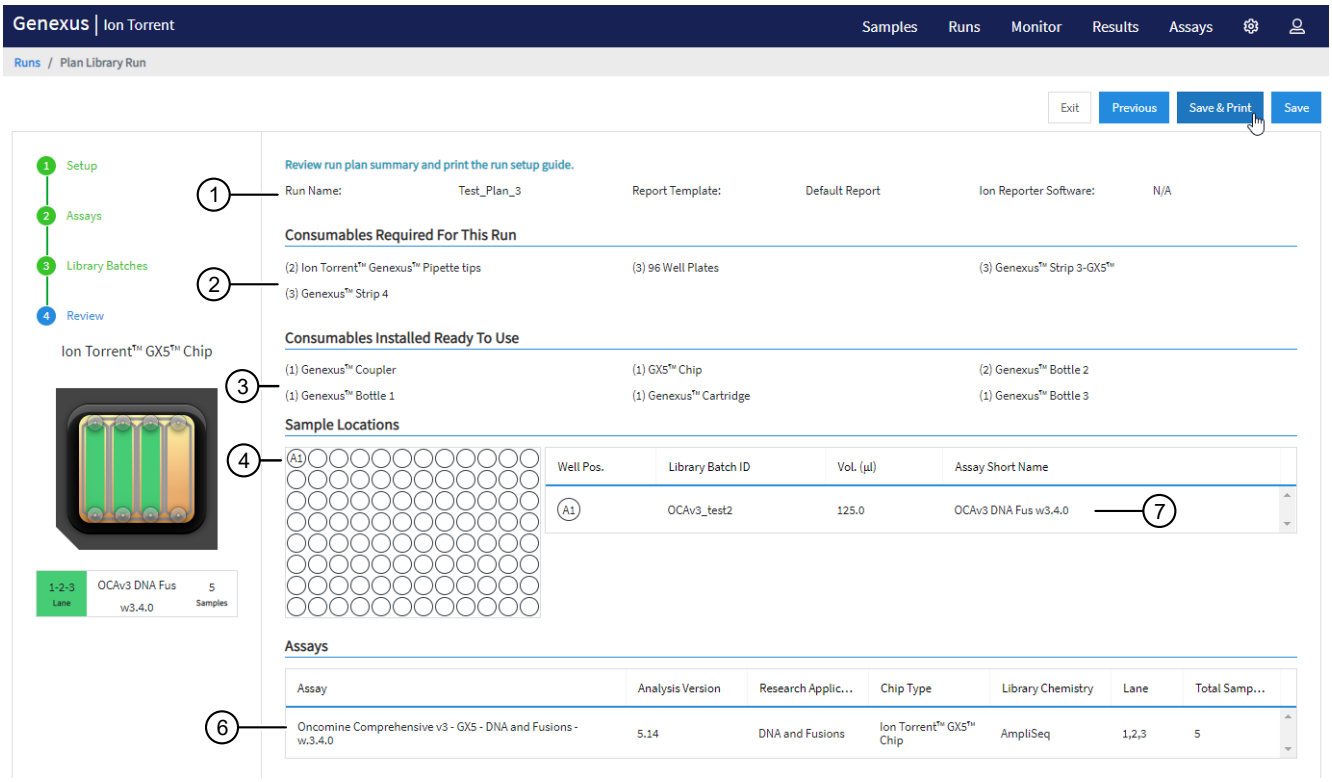
Red lane color denotes sample assignment that exceeds lane capacity.

If the minimum reads per sample × the number of samples exceeds the chip or lane well capacity, a dialog box appears after you click **Next** asking you to confirm that you want to continue. After clicking **Yes**, the **Chip View** updates and shows the lane color as red instead of green. In the example shown at right, seven samples were included in a library batch instead of six. The run is allowed, but you may not achieve the required reads per sample to pass QC metrics.

5. After you select a library batch (or batches), click **Next**.

6. In the **Review** step, review the run plan summary, then click **Save and Print** to print the run setup guide, if desired. Click **Save** to save the run without printing.

   The run plan summary lists the consumables that are required for the run, where to load the library batch on the sample plate, and how much library volume to load.



1. Run information
2. List of consumables required for the run
3. List of consumables installed on the sequencer and available for the run
4. Position(s) in the sample plate to load the library batch
5. Chip view showing the lanes to be used in the run
6. Table listing assay and run information
7. Table listing the well position, library batch ID, volume to load, and assay for each library batch

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

The run is started on the sequencer screen after selecting a run and loading the sequencer.

6 Dilute the samples and load the sample plate

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

Guidelines for nucleic acid isolation and quantification—sample runs

These are general guidelines for isolating and quantifying DNA and RNA. For assay-specific guidelines, see the assay user guide.

- See "Recommended materials for nucleic acid isolation and quantification" on page 19 for recommended kits for isolating DNA and RNA.
- We recommend the TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831) for quantifying amplifiable human genomic DNA (see *Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan™ RNAse P Detection Reagents Kit* (Pub. No. MAN0007732) available at **thermofisher.com**).
- The Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851 or Q32854) can also be used for quantification, particularly for FFPE DNA, and highly degraded DNA samples. See "Quantify FFPE DNA with the Qubit™ Fluorometer" on page 171 for a detailed procedure for quantifying FFPE DNA.
- We recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32852 or Q32855) for quantifying RNA.
- 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 Ion AmpliSeq™ Direct FFPE DNA Kit bypasses nucleic acid isolation when preparing libraries from FFPE sections on slides. See the *Ion AmpliSeq™ Direct FFPE DNA Kit User Guide* (Pub. No. MAN0014881) for a protocol for using this kit to prepare gDNA from FFPE tissue.
- The Direct FFPE DNA preparation can be stored for up to 6 months at –30°C to –10°C before library preparation.

Dilute or concentrate the samples (if needed) and load the sample plate—sample run

Isolate DNA and RNA samples using one of the procedures and kits that are recommended in "Recommended materials for nucleic acid isolation and quantification" on page 19.

Samples with concentrations up to 1,024X of the target concentration for an assay (displayed as default values in the **Sample Plate** step screen in run planning) are in range for automated dilution and require no manual dilution. Enter the concentrations during sample run planning at the **Sample Plate** step (see step 9 on page 66).

1. For samples with concentrations that are out of range for automated dilution, manually dilute the sample with nuclease-free water, or concentrate the sample to a concentration ≤1,024X of the target concentration. For samples that are in range, go to step 2.

If the sample concentration is…	Then…
<0.11 ng/µL	Concentrate the sample to greater than or equal to the target concentration.
≥0.11 ng/µL, but less than the target concentration	Run is allowed but sample concentration may not be optimal for library preparation. Concentrate the sample to greater than or equal to the target concentration.
≤1,024X of the target concentration	No manual dilution is necessary. The sequencer dilutes the sample to the target concentration automatically during the run.
>1,024X of the target concentration	Manually dilute to the target concentration based on assay type, or to a concentration in range for automated dilution by the sequencer.

Note:

- If you enter a concentration <0.11 ng/µL or >10,000 ng/µL target concentration, a warning that the concentration is out of range appears, and you are not allowed to proceed to the next step.
- If the concentration is ≤10,000 ng/µL, but >1,024X of the target concentration, you can proceed, but because the instrument cannot dilute samples more than 1,024-fold, the diluted sample concentration will be greater than the target concentration.

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

   The sample volume is not adjustable and depends on sample type, the number of primer pools in the assay, and library chemistry. The following table also provides loading volume.

Sample type	Number of primer pools	Volume
Ion AmpliSeq™ chemistry		
DNA	1	15 µL
DNA	2	25 µL
RNA	1	15 µL
RNA	2	25 µL

(continued)

Sample type	Number of primer pools	Volume
Ion AmpliSeq™ HD chemistry		
DNA	1	20 µL
RNA	1	20 µL
TNA	1	20 µL

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

   Note: The use of other plate seals may affect performance.

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

Guidelines for library quantification—library runs

- We recommend that you use libraries that are freshly quantified and diluted before pooling in a library batch.
- Pre-prepared libraries can be quantified by one of the following three methods:
  - Quantification using the Agilent™ 2100 Bioanalyzer™ instrument
  - Quantification using the Qubit™ Fluorometer
  - Quantification by qPCR using the Ion Library TaqMan™ Quantitation Kit

  See one of the following guides for specific procedures.
  - *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735)
  - *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003)
  - *Ion AmpliSeq™ HD Library Kit User Guide* (Pub. No. MAN0017392)

Dilute and pool libraries, and load the sample plate—library run

1. Dilute each manually prepared and quantified sample library to 200 pM with nuclease-free water.

   Note: Each library must be barcoded with a unique barcode or barcode pair. Use this concentration as a starting point, then titrate up or down based on sequencing results, if needed.

2. Add equal volumes of each library to a new 1.5-mL low DNA retention tube so that the total volume is greater than the volume specified in the run setup guide provided by the software.

   Note: For information on combining DNA and RNA libraries recovered from sample runs using assays that include DNA and fusions, see “Combine libraries” on page 175.

3. Mix well by pipetting up and down five times, then transfer the specified volume of each library batch to the sample plate position specified in the run setup guide.

4. Seal the plate with a sheet of Adhesive PCR Plate Foils (Thermo Fisher Scientific Cat. No. AB0626).

   Note: The use of other plate seals may affect performance.

5. Keep the plate on ice until you are ready to load it in the sequencer.

7 Load the sequencer and start a run

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

Before you begin

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 or Genexus™ Strip 2-HD, depending on your assay, 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°C until you load them in the sequencer.

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

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

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

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

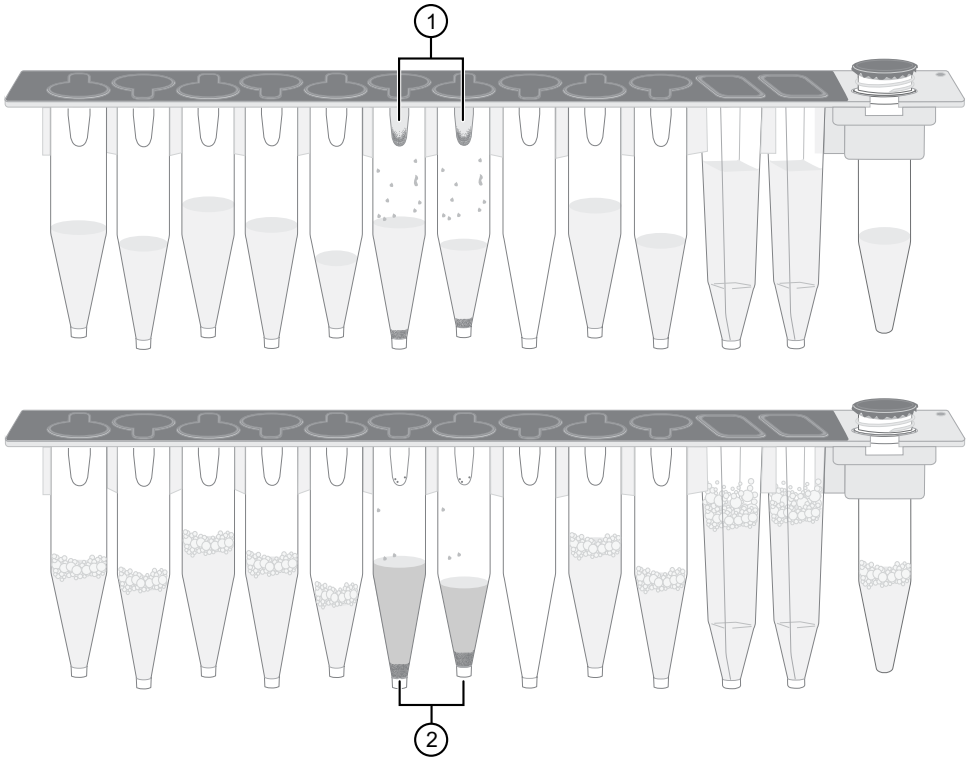
5. Genexus™ Strip 1 and Genexus™ Strip 3-GX5™ contain magnetic beads in one or two positions, yellow or brown in color, that sometimes get trapped in the upper "keyhole" of the tube. Dislodge these beads from the keyhole before installing the strip in the sequencer. Use the following procedure for each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

   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

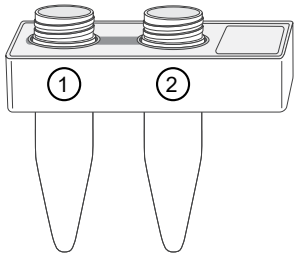
Note:

- 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.

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

Fill Genexus™ Primer Pool Tubes *(custom assays only)*

If you are using a custom assay, Genexus™ Primer Pool Tubes must be manually filled with the custom Ion AmpliSeq™ or Ion AmpliSeq™ HD panels at the appropriate volume and in the correct primer pool tube positions. For Ion AmpliSeq™ library panels, use one carrier per DNA or RNA assay primer pool. The two positions in the primer pool tube carrier are designated as shown in the following figure:



① Position 1 tube: Contains Ion AmpliSeq™ DNA, Ion AmpliSeq™ RNA, or Ion AmpliSeq™ HD FWD primer pool
② Position 2 tube: Contains Ion AmpliSeq™ HD REV primer pool

Note: When you order assays from Ion AmpliSeq™ Designer (**AmpliSeq.com**), be sure to order a sufficient amount of panel for your needs, and request the tube format, not the plate format. Library preparation on the Genexus™ Integrated Sequencer requires greater panel volume per sample than manual library preparation, or library preparation on the Ion Chef™ System.

1. Add primer pool at the indicated volume, appropriate to your assay type, to the Genexus™ Primer Pool Tubes using the following tables as a guide. Fill the number of tubes specified by the run plan summary.

Ion AmpliSeq™ DNA assays

Number of primer pairs per pool	Concentration	Volume in position 1	Volume in position 2
12–96	2X (400 nM)	140 µL	—
97–3,072	2X (100 nM)	140 µL	—
>3,072	2X ([3,072 / Number of primer pairs per pool] × 100 nM)[1]	140 µL	—

[1] For example, if a panel pool has 3,500 primer pairs, the 2X concentration is (3,072 / 3,500) × 100 nM = 87.8 nM.

Ion AmpliSeq™ RNA assays

Number of primer pairs per pool	Concentration	Volume in position 1	Volume in position 2
12-1,228	5X (250 nM)	75 µL	—
>1,228	5X ([1,228 / Number of primer pairs per pool] × 250 nM)[1]	75 µL	—

[1] For example, if a panel pool has 1,500 primer pairs, the 5X concentration is (1,228 / 1,500) × 250 nM = 205 nM.

Ion AmpliSeq™ HD assays

Primer pool type	Concentration	Volume in position 1	Volume in position 2
Ion AmpliSeq™ HD FWD	10X	50 µL	—
Ion AmpliSeq™ HD REV	10X	—	50 µL

IMPORTANT!

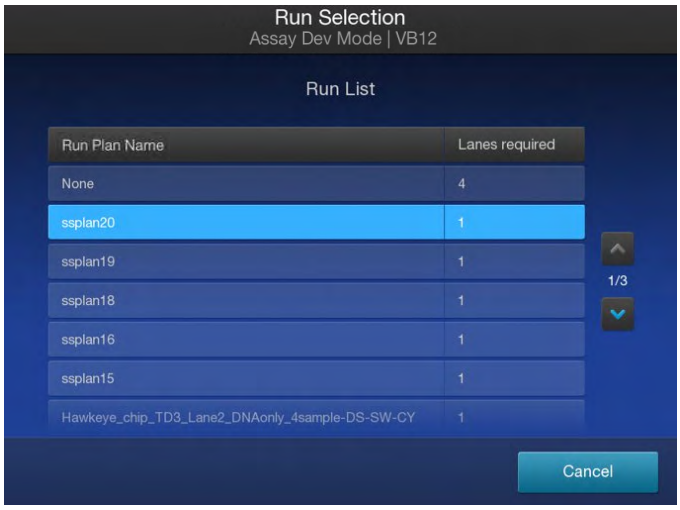
- If you are using Ion AmpliSeq™ library chemistry, leave the tube in position 2 empty and uncapped, but do not remove the tube from the carrier before loading in the sequencer. Do not add a second Ion AmpliSeq™ primer pool to the position 2 tube.
- If you are using Ion AmpliSeq™ HD library chemistry, add the FWD and REV primer pools to the appropriate tubes in the same carrier.
- Ensure that no bubbles are introduced at the bottom of the tube when adding the primer pool.

2. If you do not install the primer pool tube carriers in the sequencer immediately, cap the tubes that contain primer pools, then store the tube carriers on ice. Remember to uncap all tubes before installing.

Load the sequencer and start a run

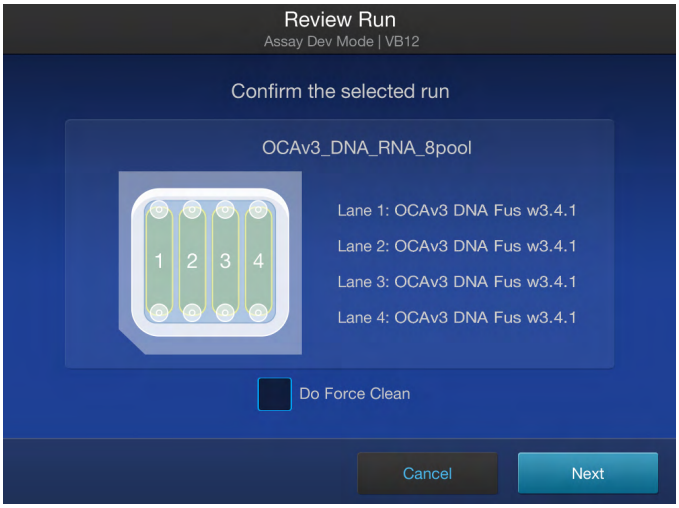
1. Tap **Run** on the sequencer home screen to start the loading procedure.

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.

Note:

- If the instrument vision system detects consumables loaded on the deck, the sequencer prompts you to remove the consumables, then starts a UV Clean.
- Select the **Do Force Clean** checkbox if there will be an unused lane or lanes on the installed chip after the run, but you want to start your next run on a new chip after the current run. A force clean automatically cleans the instrument after the run, eliminating the need for an operator to execute the cleaning procedure between the completion of the current run and the next run. Selecting **Do Force Clean** renders all lanes of the installed chip unusable after the run.

---

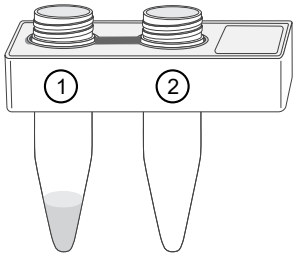
4. In the **Load Deck** screen, the sequencer instructs you step by step to load each required consumable in a highlighted position on the deck. The sequencer detects the loading of each consumable in real time and advances to the next component automatically.

IMPORTANT!

- Ensure that you remove the primer pool tube cap or caps before installing the tube carrier on the deck.
- Ensure that you load the correct type of barcode plate and library strip 2 for the type of run you are setting up. The sequencer displays a warning if you have installed consumables that are incompatible with the run you have selected, for example, a Genexus™ Barcodes AS plate or Genexus™ Strip 2-AS in an HD run.

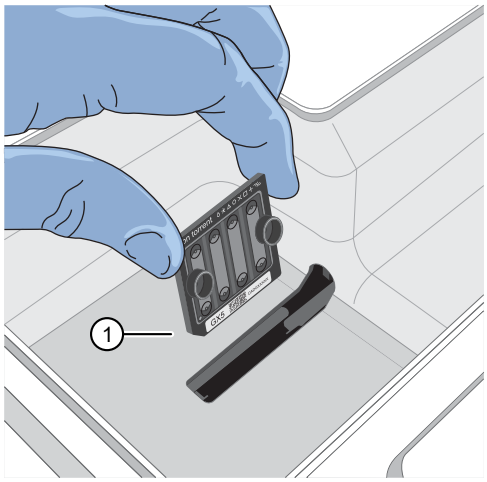
Note:

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



① Position 1
② Position 2

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

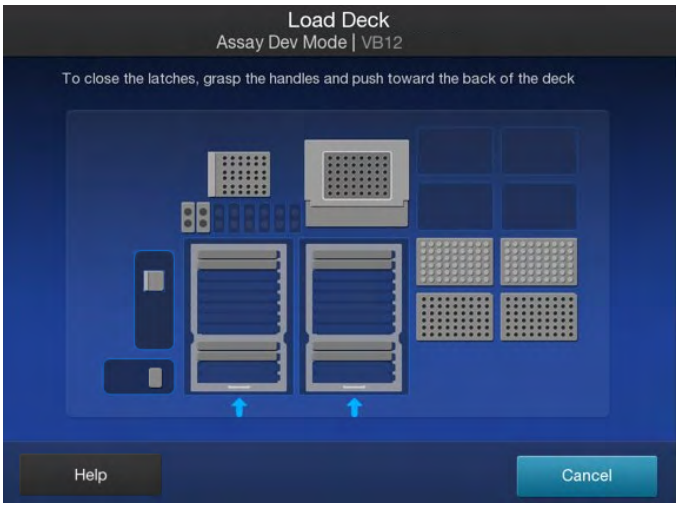


① Notched corner of chip

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

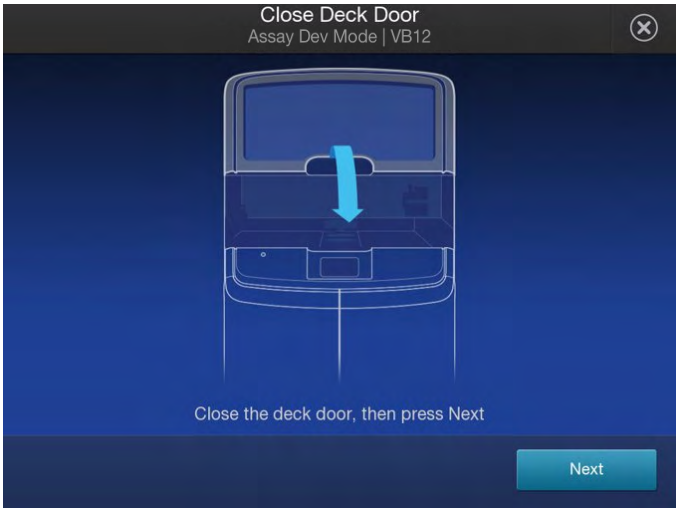
IMPORTANT! Insert the Genexus™ Coupler so that it is level to ensure it will properly align with the GX5™ Chip. A coupler that is installed at an angle or is not level will not align properly to the chip and can result in a failed run.

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



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

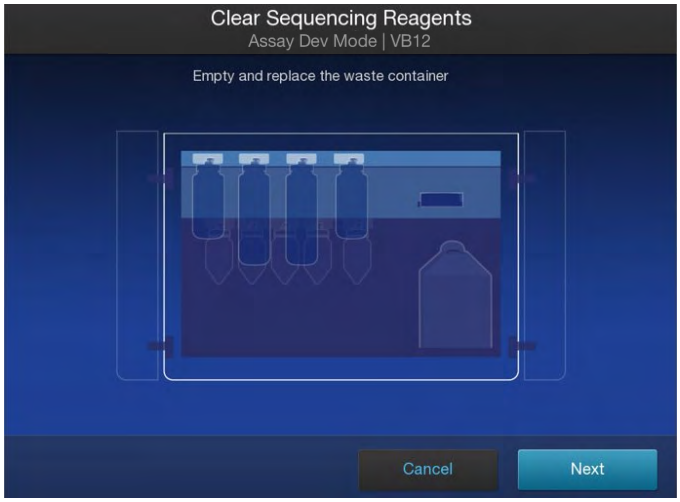
7. Close the deck door, then tap **Next**.



- If you installed a new chip in the sequencer, the sequencer prompts you to open the sequencing reagents bay doors to empty the waste and remove used sequencing reagents bay consumables. Proceed to step 8.
- If you are using a chip that was previously installed and has sufficient lane capacity for the run, the sequencer prompts you to start the run.

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

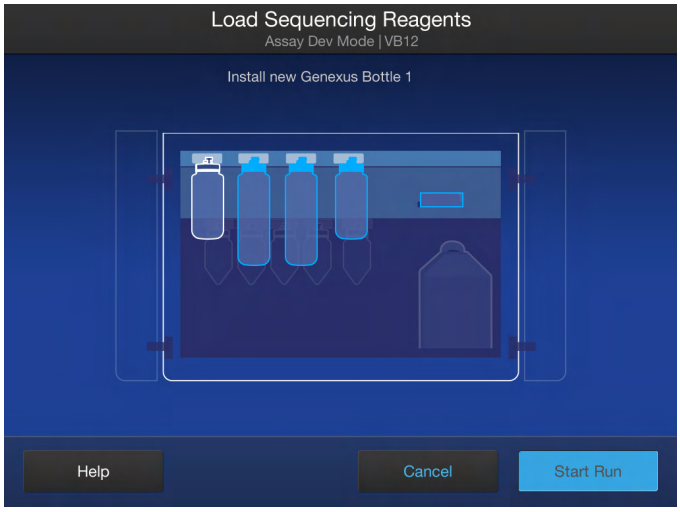
8. Follow on-screen instructions to empty the waste in the Waste Carboy, remove waste pipette tips, remove the used Genexus™ Bottle 1, Genexus™ Bottle 2, Genexus™ Bottle 3, and Genexus™ Cartridge, then tap **Next**.



IMPORTANT!

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

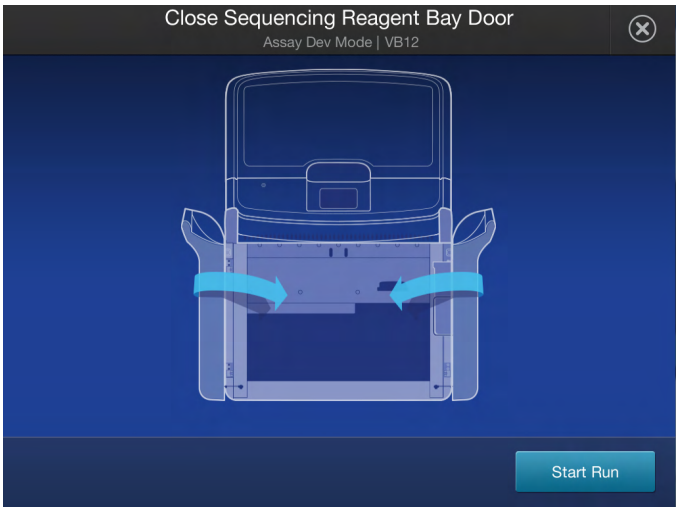
9. Install a new Genexus™ Bottle 1, Genexus™ Bottle 2 (two required), Genexus™ Bottle 3, and Genexus™ Cartridge.



Note: The installed reagents can be used for up to 14 days on the sequencer with full performance. After 14 days, you may observe reduced performance.

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

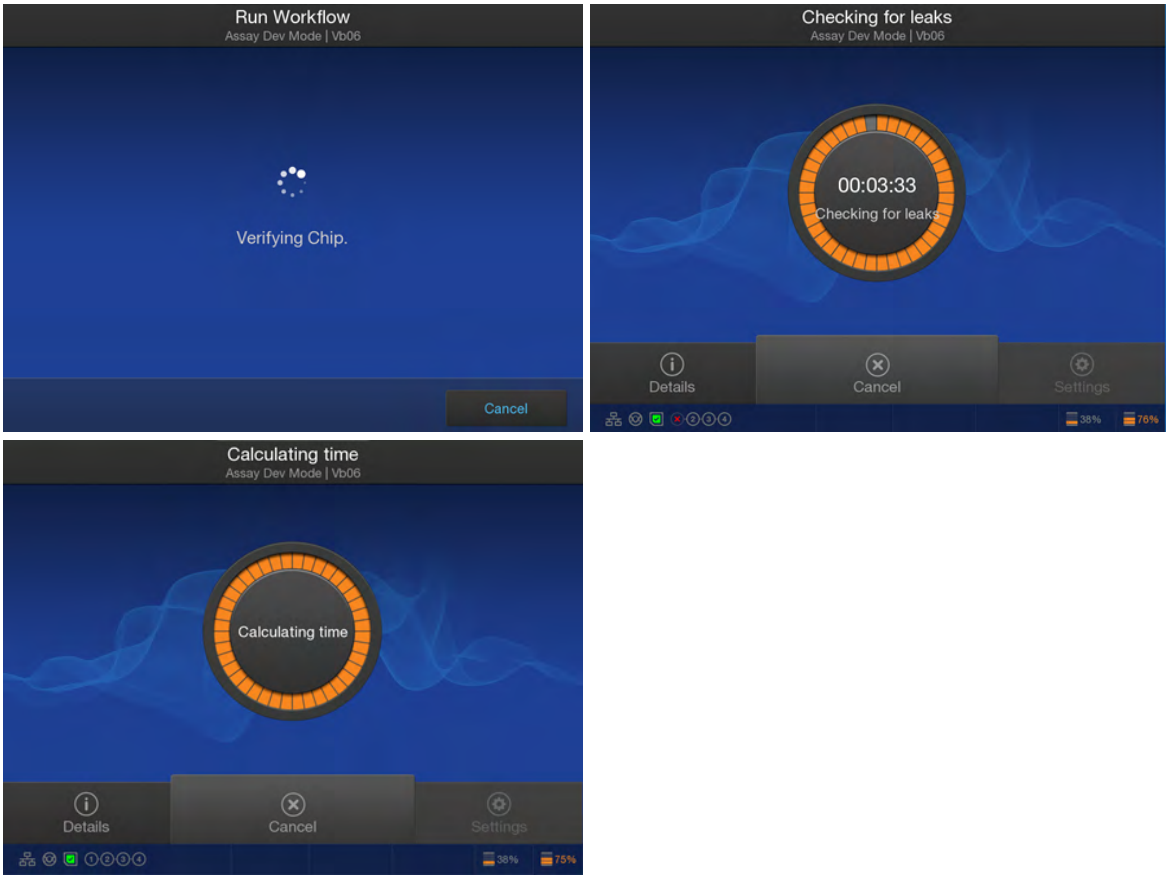
10. Close the sequencing reagents bay doors.



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

IMPORTANT! Do not tap **Start Run**. Tapping **Start Run** can cancel the run.

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



A sequencing run encompasses the following stages:

- Starting
- Initializing
- Library Prep
- Templating
- Pre-sequencing
- Sequencing
- Cleaning

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

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

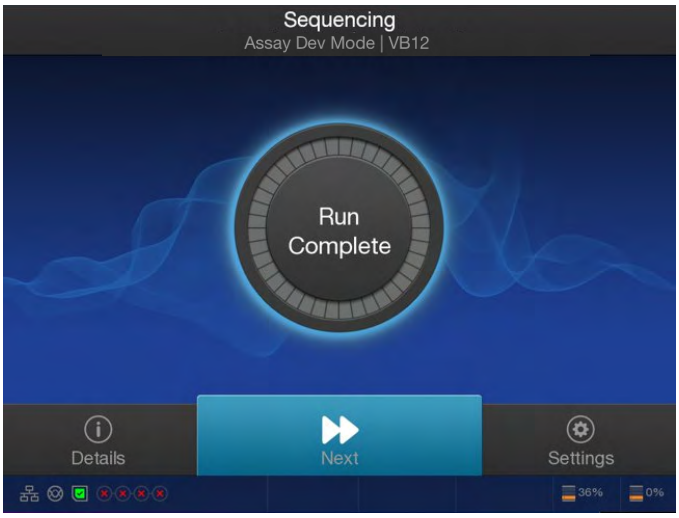
When the run finishes, the sequencer displays the **Run Complete** screen.

Note: If all the lanes of a chip are used, the chip shuttles to the install position. You are asked to remove the chip and coupler, and clear the sequencing reagents.

Clear the instrument deck and perform a UV Clean

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

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

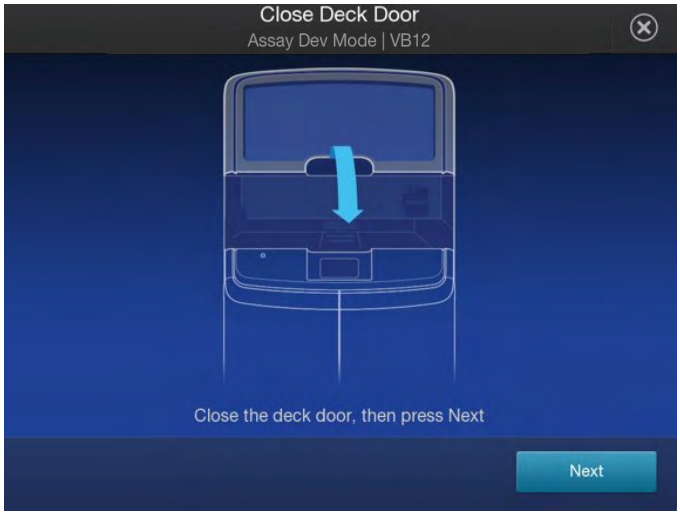


The deck door opens.

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

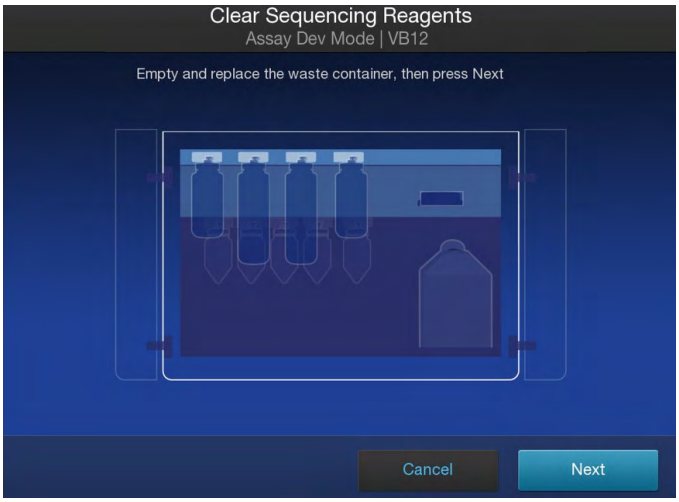
3. Inspect the Genexus™ Filter in the liquid waste disposal port and verify that no standing liquid is present. If standing liquid is present, manually remove the liquid with a pipette, then pull out the filter. Test the filter with water to determine if a clog is present.
   - If the Genexus™ Filter is clogged, replace it with a new filter. For more information, see "Replace the Genexus™ Filter" on page 164.
   - 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 "Replace the Genexus™ Conical Bottles" on page 165.

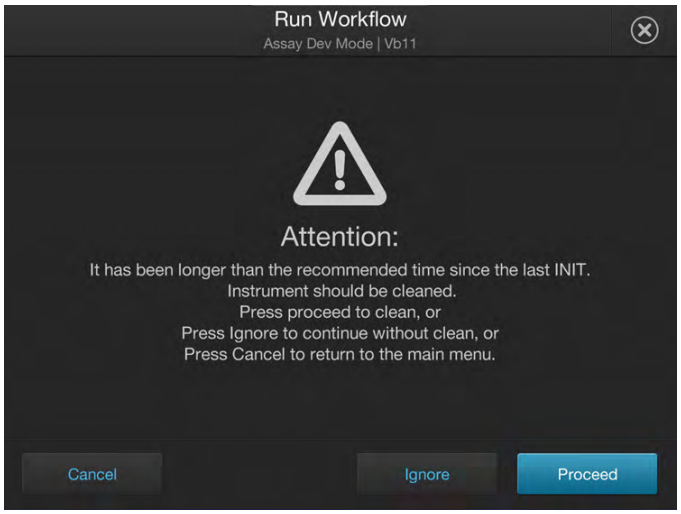
IMPORTANT! Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Genexus™ Integrated Sequencer consumables and liquid waste.

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.

Options for an expired sequencer initialization

A sequencer initialization is defined by the installation of a new chip, chip coupler, sequencing bottles, and reagent cartridge on the sequencer before a run. Reagents are stable on the sequencer for 14 days, after which you may experience reduced performance. An initialization expires after 28 days, after which the following warning appears. Three options are available: **Cancel**, **Ignore**, and **Proceed**.

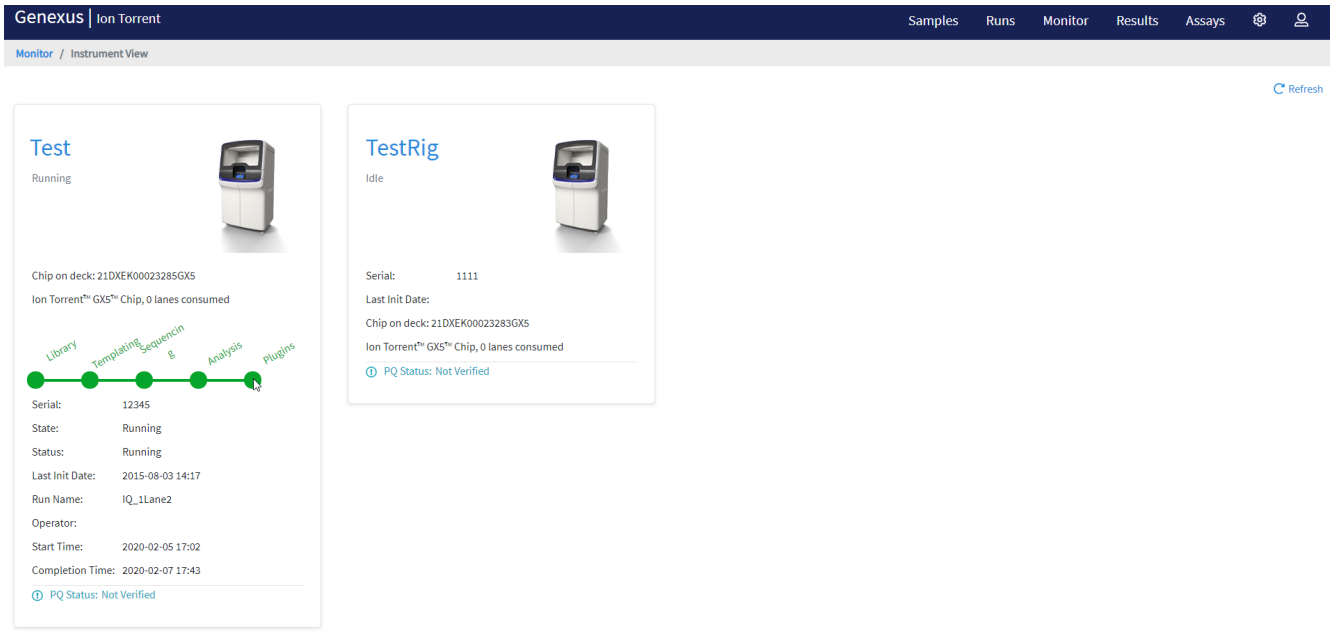


The outcome of each option is described in the following table.

Option	Outcome
Cancel	The sequencer returns to the home screen and the run is canceled. The expired initialization warning appears again if a run is started on the same initialization. After tapping **Cancel**, select **Settings** ▸ **Clean instrument** to clean the instrument before starting a run with a new chip. Lane assignment for a new run starts with lane 1.
Ignore	The instrument moves forward with the run and ignores the expired initialization warning. The warning appears for each run that uses the expired initialization until the instrument is cleaned and ready for a new initialization. Troubleshooting efforts are not supported on runs that start 28 days after an initialization or later.
Proceed	The instrument starts a clean before run setup. After cleaning, you are asked to remove the used chip and coupler, and to load the deck for a new run. You are also asked to remove the used sequencing bottles and cartridge and install new consumables. A new run starts. **IMPORTANT!** We strongly recommend that you **not** tap **Proceed**, and instead use **Cancel** or **Ignore**. After tapping **Proceed**, the lane assignment for the new run after the clean remains the originally assigned chip lane before the clean (not necessarily lane 1). Subsequent runs will assign the next available lane. For example, if the originally assigned chip lane was lane 3, lane 3 will be assigned in the newly installed chip and you will lose the use of lanes 1 and 2.

8 Monitor the run

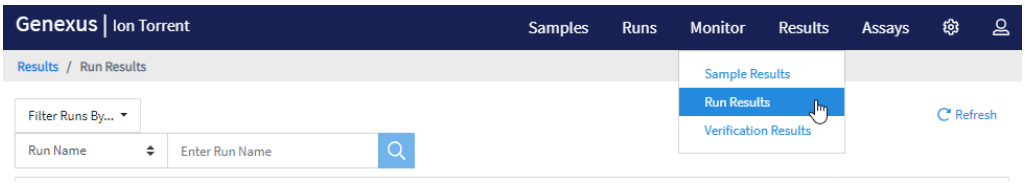
In the **Monitor** menu, you can view the status of the sequencer in an idle condition, or the status of a run in progress.



View run progress on the instrument

1. In the menu bar, click **Monitor** ▸ **Instrument View**.
2. In the **Instrument View** screen, view the status of a run in progress, or the status of an instrument. The following information is provided:
   - Sequencer, operator, and run names
   - PQ status of the instrument
   - Instrument status at various stages of the run and post-run
   - Status of the results analysis
   - Run start and completion times
3. Click **Refresh** to update the information.

9 Review data and results



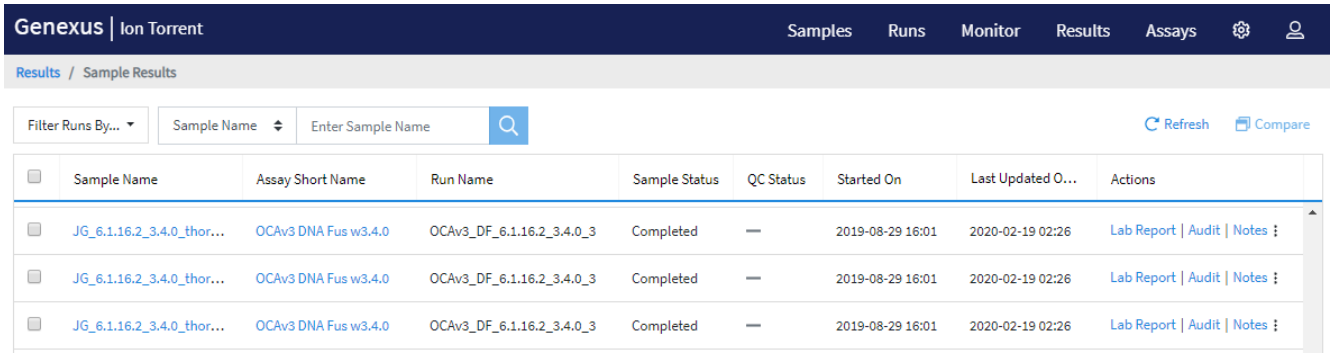
Use the **Results** menu to review results and data analysis and perform data management tasks. You can view results sorted by sample or by run.

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

Review sample results

In the **Results / Sample Results** screen, samples that have been sequenced are listed by sample name.

You can search the list of results by sample name. Enter a search term, then click **(Search)**.



The following information appears in the **Sample Results** screen.

Column	Description
Sample Name	The unique identifier created when the sample was entered into the software. 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.
Sample Name followed by ***(Signed Off)***	Manager- and administrator-level users can provide their electronic signature on sample results for completed runs. 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 “Sign off on the run results (manager/administrator)” on page 127.
Assay Short Name	A shortened version of the assay name you imported or created.
Run Name	The unique name of the run given when it was created in the software.
Sample Status	The status of the run or sample (for example: Completed, Running, Failed, Terminated, Pending, Stalled).
QC Status	The QC status of a completed run. **Note:** • ✔ (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.
Started On	The date and time when the run analysis was started.
Last Updated On	The date and time when the last action was completed on the run.

(continued)

Column	Description
Actions	Click the appropriate link. To see more actions, click ⋮ **(More Options)**. • **Lab Report**—Download the **Lab Report** (available only for samples with a sample status of completed. • **Audit**—View the audit trail for the run. • **Notes**—View or add notes to a run. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting.

Click the **Compare** button in the upper right corner of the **Results / Sample Results** screen to compare variant results between multiple samples, or from a single sample source over time. For detailed information, see *Genexus™ Software 6.2 Help*, or the *Genexus™ Software 6.2 User Guide* (Pub. No. MAN0018955).

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 98.
Started On	The date and time when the run analysis was started.
Updated On	The date and time when the last action was completed on the run.

(continued)

Column	Description
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 126. • **Upload Samples to IR**—Upload sample results files to Ion Reporter™ Software for secondary analysis. For more information, see "Upload sample results files to Ion Reporter™ Software" on page 132. • **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 98.

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 128.

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 127.

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

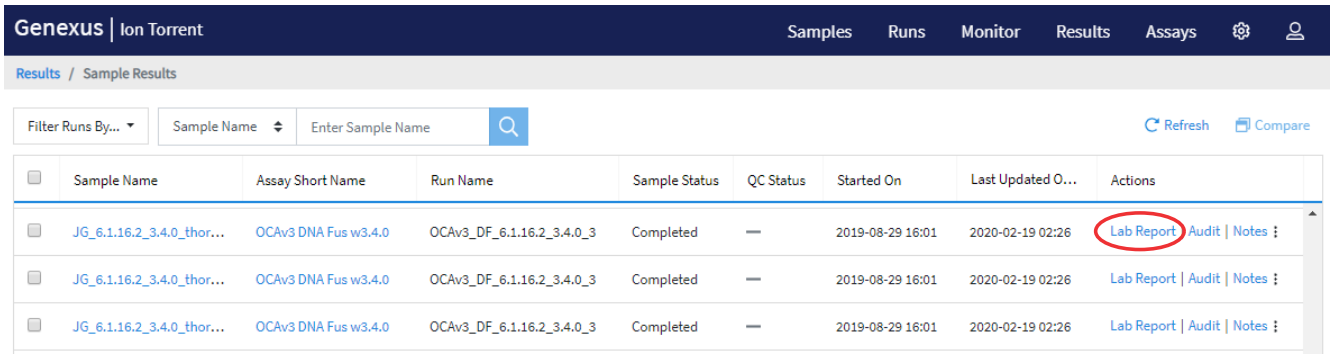
Section	Description
Sample Details	The sample information that is entered into the software. You can customize the format of the **Sample Details** section when you create a new report template. To create a new report template, click **Assays** ▸ **Manage Presets**, then in the **Report Templates** tab, 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 130.

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.



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 103 and "Review coverageAnalysis plugin results" on page 132.

The following sample-specific result information is available.

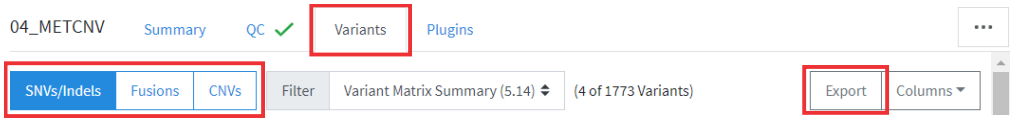
Tab	Description
Summary	An overview of the results for the sample, including **Sample Details**, **Key Metrics**, and the **Variant Summary**. For more information, see "Summary of the Sample Results" on page 102.
QC	The quality metrics for the sample sequenced in the run. For more information, see "QC results" on page 107.
Variants	Detailed variant results for **SNVs/Indels**, **Fusions**, and **CNVs**. For more information, see "View SNV/INDEL results" on page 112.
Plugins	Results generated from the plugins associated with the assay used to analyze the sequenced sample. For more information, see "Review coverageAnalysis plugin results" on page 132.

1. To view sequencing results for a particular sample, including variant and fusion calls, click **Results ▸ Sample Results**.

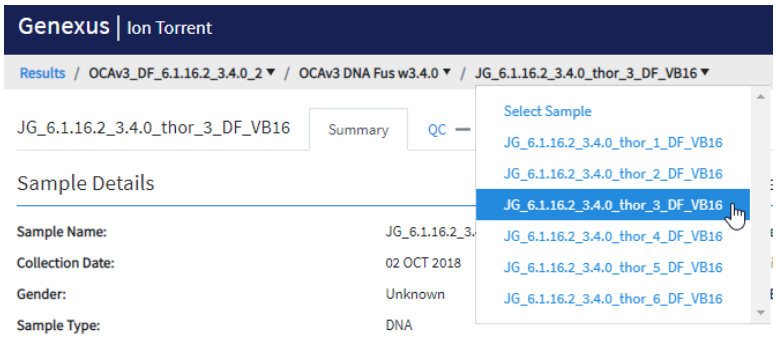
2. In the **Sample Name** column, click a sample name.

3. 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 variant types. You can also export data in tabular format.



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



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 126.
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.

5. 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 126.
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 107.
Download Files	Download all or selected variant, results, audit and logs, and lab report files. For more information, see "Results files" on page 130.
Generate Report	Generate a Lab Report each sample in a sequencing run. For more information, see "Lab Report" on page 99.
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 127.
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. For more information, see "Upload sample results files to Ion Reporter™ Software" on page 132.

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.

(continued)

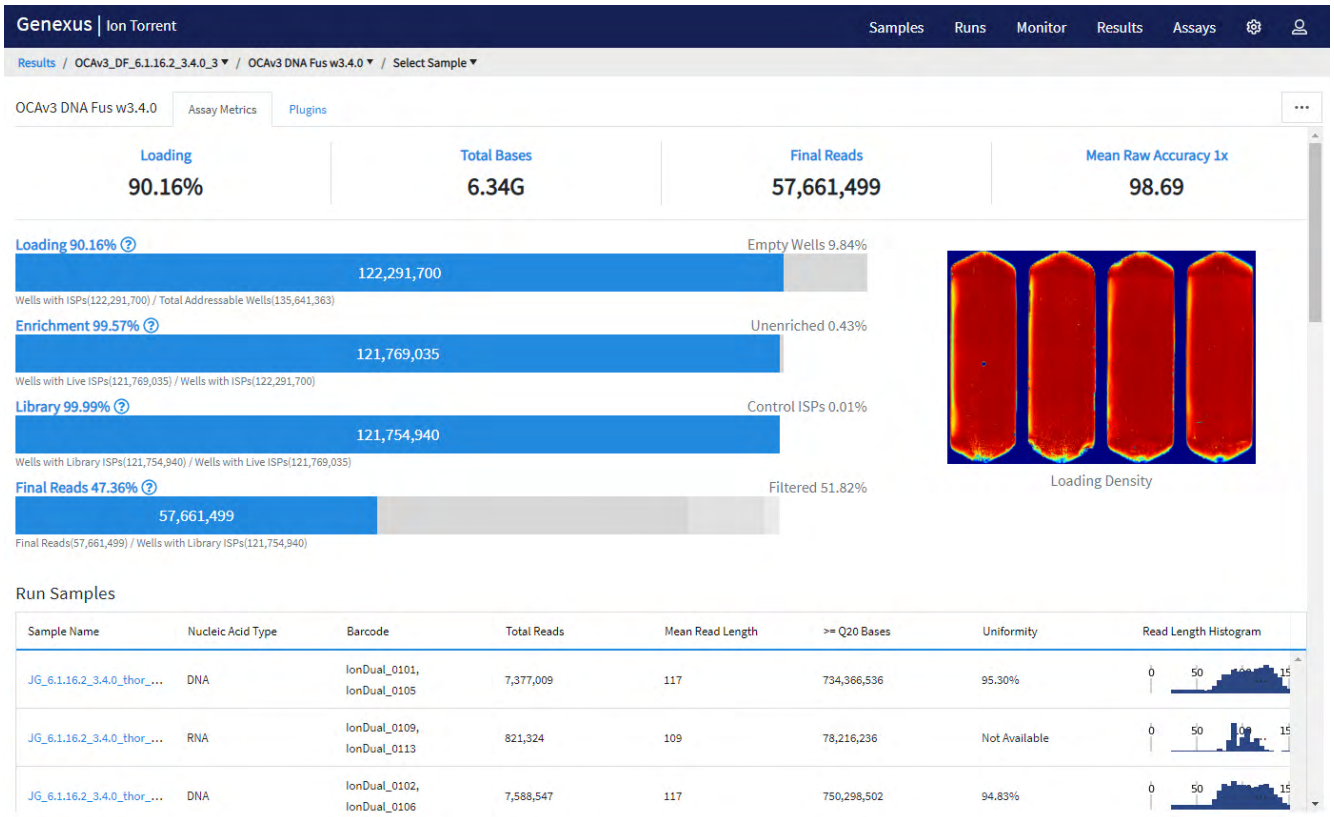
Section	Description
Collection Date	The date that the sample was collected.
Gender	The biological sex of the sample: **Female**, **Male**, or **Unknown**.
Sample Type	A term that describes the sample, for example, FFPE, DNA, DNA & RNA
Disease Category	The disease type of the sample.
Cancer Type	The type 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.
% 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.

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.

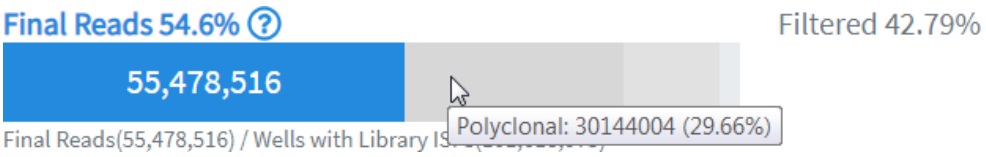
Assay metrics are assay-specific and cannot be viewed within the sample results screens. To view assay metrics, ensure that **Select Sample** is selected in the **Select Sample** dropdown menu.



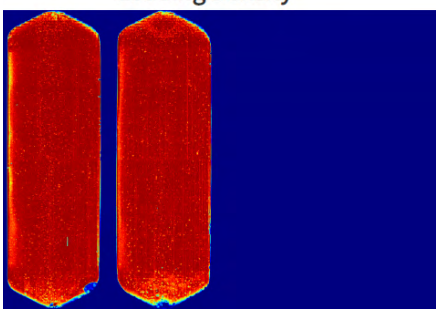
Assay Metrics

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.

Assay Metrics *(continued)*

Metric	Description
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.
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. 
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. 
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.

Assay Metrics *(continued)*

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

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.

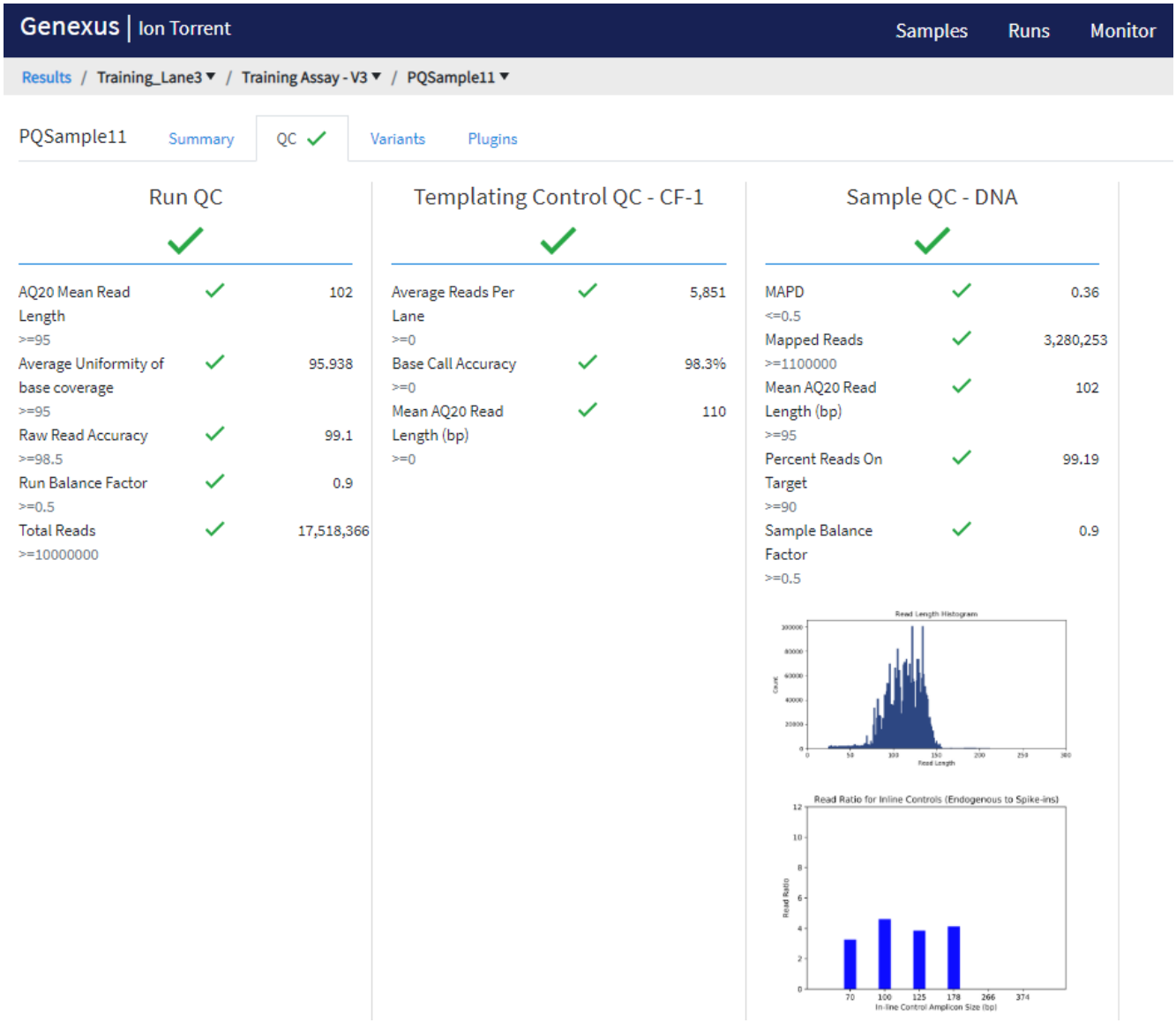
To view the **QC** screen, in the **Results / Sample Results** screen, click a sample name in the **Sample Name** column. In the **Results** screen, click the **QC** tab. The QC status for each metric is indicated beneath each QC test (Run QC, Templating Control QC–CF–1, Sample QC–DNA, and Sample QC–RNA).

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 126).

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

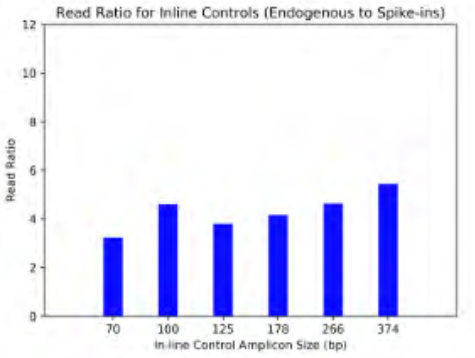


Metric	Description
Run QC	**General run quality control information.**
Key Signal	The average signal after software processing for library ISPs that identically match the library key (TCAG).
Percent Loading	The number of wells with ISPs divided by the number of the total addressable wells in a run.
Raw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

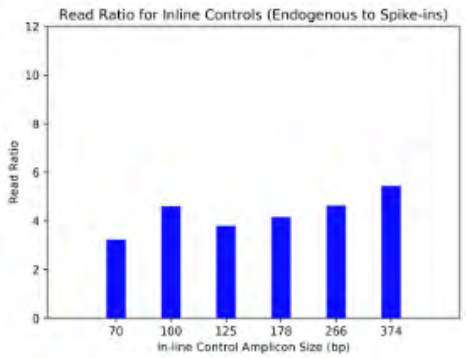
(continued)

Metric	Description
Base Call Accuracy	The probability that a given base is called correctly. 1 – (total number of errors for all positions in CF-1) / (total number of CF-1 base reads).
Mean AQ20 Read Length (bp)	Average length, in base pairs, at which the accuracy rate is ≥99% for CF-1 reads.
Sample QC—DNA	**Sequencing quality metrics of the sample DNA library.**
MAPD	MAPD (Median of the Absolute values of all Pairwise Differences) is a quality metric that estimates coverage variability between adjacent amplicons in CNV analyses. A MAPD value of ≤0.5 indicates an acceptable level of coverage variability. High MAPD value typically translates to a lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. If the MAPD QC threshold is not met, CNVs 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.

(continued)

Metric	Description
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of genomic sample reads to control reads for each inline control amplicon.  **Note:** The **Read Ratio** for each inline control amplicon is expected to be 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 5–7 control housekeeping genes.
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.

(continued)

Metric	Description
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 total number of final library reads that are aligned to the reference file for the NTC.
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.

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 results” on page 122.

Column	Description
User Classifications	User-defined classification to selected from the list. For more information, see “Create and assign variant classifications” on page 121.
Locus	The chromosome and position of the detected variant.
Oncomine Variant Class	The type of SNV or INDEL at the locus based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function — the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • Loss-of-function — the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Gene	The gene name. Click the link to open the **View Annotation Sources** window to view additional information. For more information, see “View annotation sources” on page 121.
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

(continued)

Column	Description
Call	Indicates the presence or absence of an SNV/Indel variant. When the default filter chain is applied, only the variant calls that are designated with **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants (.vcf)** file (see "Results files" on page 130). • **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.
No Call Reason	The reason why a variant is reported as **No Call**.
Phred QUAL Score	The relative probability of either the "reference" hypothesis interval [0,cutoff], or the "variant" hypothesis interval [cutoff,1], Phred-scaled (-10*log10). A higher score means more evidence for the variant call.
Raw Read Depth	Total read coverage across amplicon containing SNV/INDEL hotspot locations. Count of chip-level reads aligned at this locus that participate in variant calling.
Effective Read Depth	The number of reads covering the position.
Alt Allele Read Counts	The number of reads containing the alternate allele.
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.
Mol Depth[1]	Reports number of interrogated DNA molecules containing target. It defines limit of detection at hotspot position in a particular run and sample. For instance, if molecular depth is ≥1,500, you can have high confidence that no variant is present at 0.2% LOD. If molecular depth is ≥2,500, you can have high confidence that no variant is present down to 0.1% LOD. For reference calls, Molecular Depth provides measurable metric that serves as confirmation for variant absence among a large number of interrogated molecules.
WT Mol Counts[1]	Number of detected molecules containing the wildtype allele.

(continued)

Column	Description
Alt Allele Mol Counts[1]	Number of detected molecules containing the alternate allele.
Mol Freq %[1]	Molecular frequency percentage. The percentage of mutant reads over total reads at the locus.
% LOD[1]	Limit of detection (LOD) of a variant allele expressed as a percentage of the WT allele. LOD is the lowest possible variant frequency in the sample that can be detected by the system with a true positive rate greater than 98% for FFPE samples or 95% for cfTNA samples. LOD is dependent on the molecular read depth at the locus. %LOD is reported when there are no variant calls for the gene.

[1] Column appears in analyses of Ion AmpliSeq™ HD sequencing data only.

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 results” on page 122.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see “Create and assign variant classifications” on page 121.
Locus	The chromosome positions in the reference genome that define the fusion junction.
Oncomine Variant Class	Oncomine variant class annotation that indicates fusion type based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function — the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • Loss-of-function — the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Genes (Exon)	The name of fusion target and representative acceptor and donor exons.

(continued)

Column	Description
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 130). • **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.
Mol Cov. Mutant[1]	The median molecular coverage across a fusion amplicon.
Imbalance Score[2]	Each fusion gene exhibits a characteristic **Imbalance Score** threshold. Scores that exceed this threshold value indicate a high likelihood of the presence of the fusion in the test sample. • **Observed** = (sum of read count coverage of amplicons downstream (3') of a predicted breakpoint in a target gene) / (sum of read count coverage of all amplicons of the gene) [Read counts from test sample] • **Expected** = (sum of baseline value for amplicons downstream of the breakpoint/sum of baseline values) / (sum of baseline values of all amplicons of the gene) [Baseline values computed from normal samples]
Imbalance P-Value[2]	The statistical significance of measure of imbalance relative to a control gene.
Predicted Break-point Range[2]	The exonic range for predicted fusion break point in exon tiling assays.

(continued)

Column	Description
Ratio To Wild Type[1]	The molecular ratio for exon skipping assays relative to wild type control amplicons.
Norm Count Within Gene[1]	*(Lung panel only)* Exon skipping assay coverage normalized to molecular coverage of wild type (WT) MET control amplicons.

[1] Column appears in analyses of Ion AmpliSeq™ HD sequencing data only.
[2] Column appears in analyses that use the exon tiling fusion detection method.

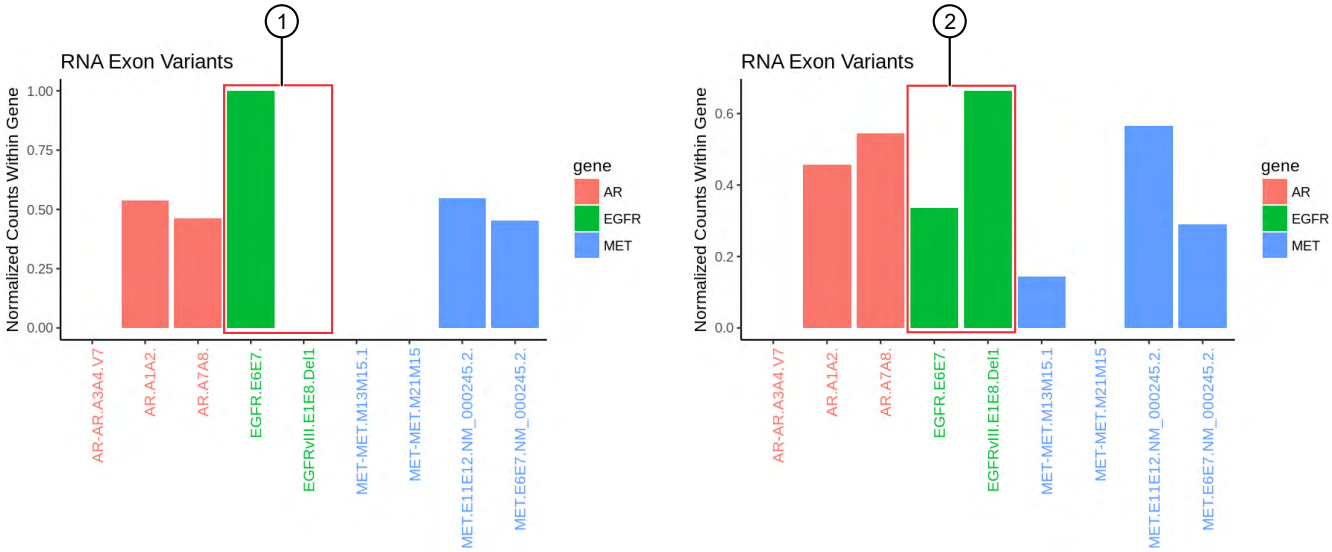
View RNA Exon Variants

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

Note: To review RNA Exon Tile Fusion Imbalance analysis plots, see the user guide for your assay.

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).

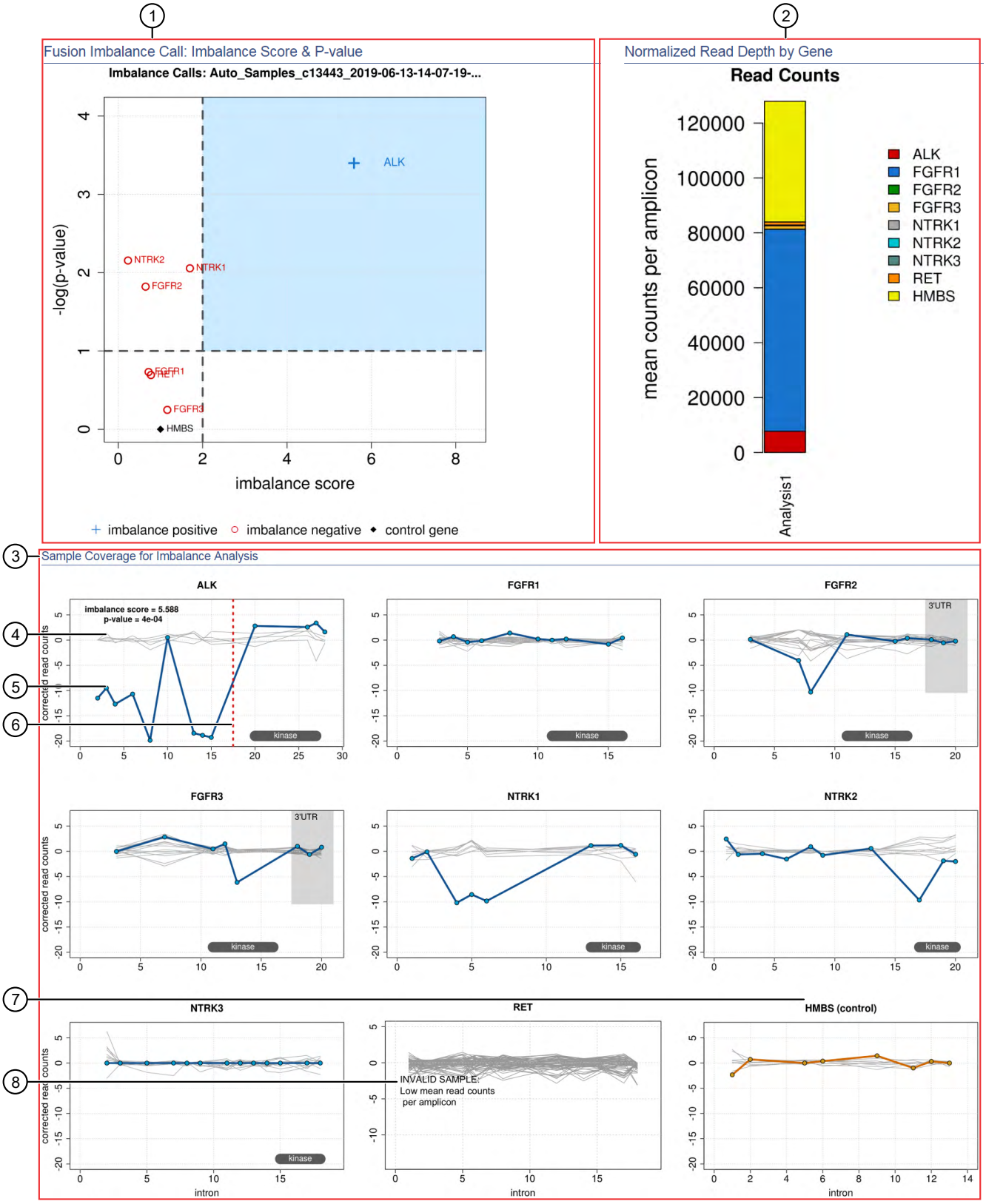
To return to the table view of fusions, click ✕ **(Remove)** next to the **Visualization** dropdown menu.

View RNA Exon Tile Fusion Imbalance

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

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 Tile Fusion Imbalance**, then review the RNA Exon Tile Fusion Imbalance plots.

Representative RNA Exon Tile Fusion Imbalance plots



① The **Fusion Imbalance Call: Imbalance Score & P-value** plot shows the imbalance scores and p-values for all the genes in the selected sample. The dashed gray lines mark the threshold for an imbalance call, which is applied to all genes across all samples. Points that fall within the blue shaded area of the plot represent fusion-positive genes (✚). All other points that are outside of the blue shaded area represent fusion-negative genes (◯). Control genes are marked with ◆.

② The **Normalized Read Depth by Gene** plot shows the mean read counts of each gene that is captured on the chip for the selected sample. For each gene, the read counts are normalized to the number of amplicons.

③ The **Sample Coverage for Imbalance Analysis** plots show the expression profile for each exon-exon tiling amplicon for each gene. The Y-axis represents the corrected molecular counts. The X-axis represents individual exon-exon junctions, which are listed from 5′ to 3′. The **imbalance score** and **p-value** are listed in the panel of each gene that was called positive for fusion.

④ Baseline (a cluster of gray lines), generated from a fusion-negative sample.

⑤ Test sample corrected read coverage (blue line), normalized to the baseline. Each point on the line represents a unique exon-exon junction that was covered by the assay and normalized to the baseline.

⑥ Predicted range for the fusion break point for a fusion-positive gene (dashed red line).

⑦ Sample coverage profile for the control gene (orange line).

⑧ If the collected data are insufficient to determine an imbalance score, the **INVALID SAMPLE** message appears in the panel for that gene.

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

View CNV results

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

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

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 results” on page 122.

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 47.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see “Create and assign variant classifications” on page 121.
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.

(continued)

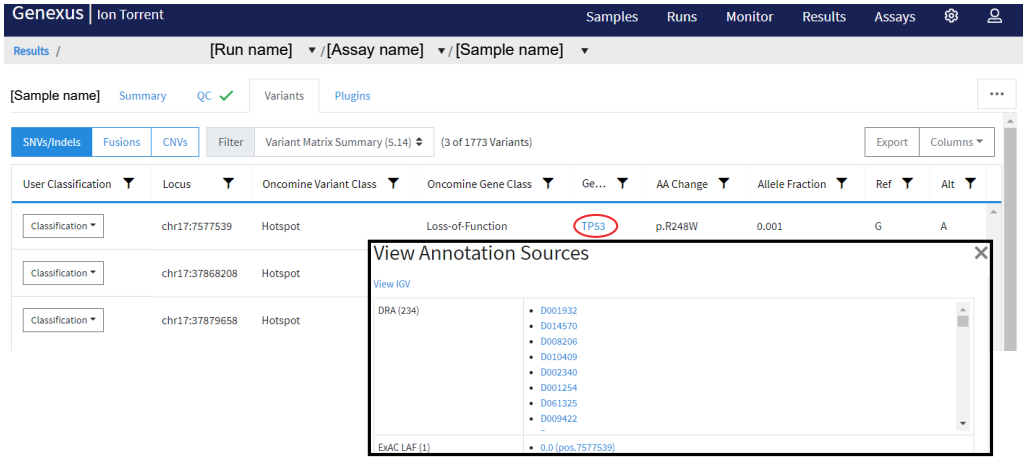
Column	Description
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function — the altered gene product has a new molecular function or pattern of gene expression, compared to the wild-type gene • Loss-of-function — 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, 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 121.
Copy Number	The copy number of a CNV gene locus per genome. This column is available when a positive call is made.
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 130). • **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.

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.



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 CNVs tables in the **Variants** tab of the **Results** screen.

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

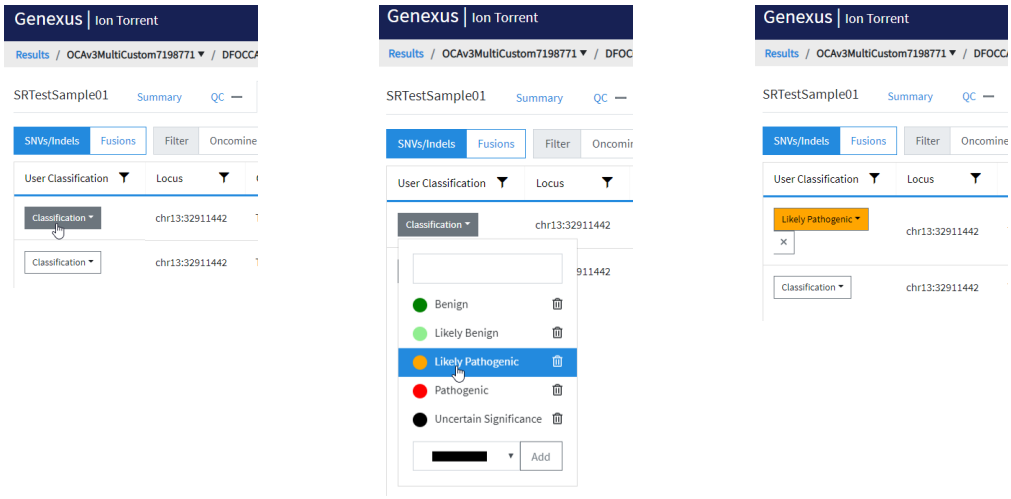
2. Click a sample name in the **Sample Name** column in the row of a sample of interest to open the **Results** screen for the sample.

3. Click the **Variants** tab.

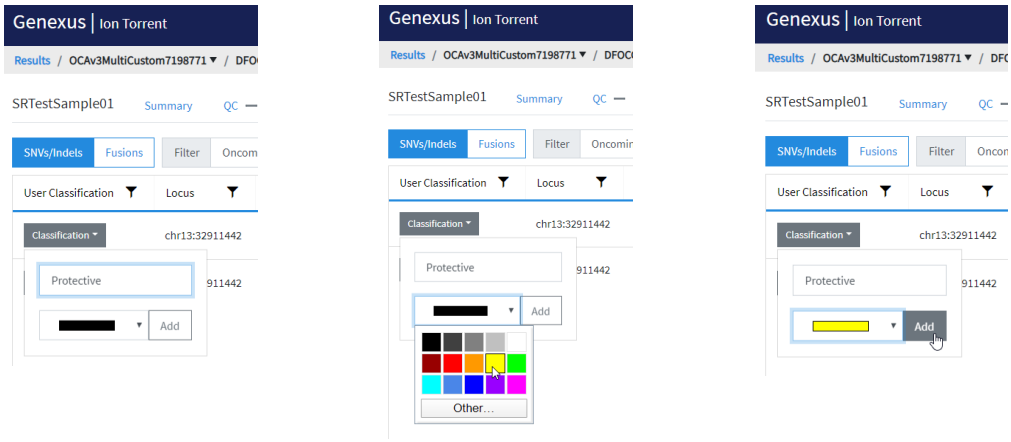
4. In the **Variants** table, click SNVs/Indels, Fusions, or CNVs to display the list of variants for the selected variant type.

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.



   - 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**.



   - 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 results.

Filter results

You can filter results in the SNVs/Indels, Fusions, and CNV tables in the **Variants** tab of the **Results** screen in two ways. You can apply filters to columns of information that appear in the screen. The filters, available at the top of each column, immediately narrow the list of information in any columns to which filters are applied.

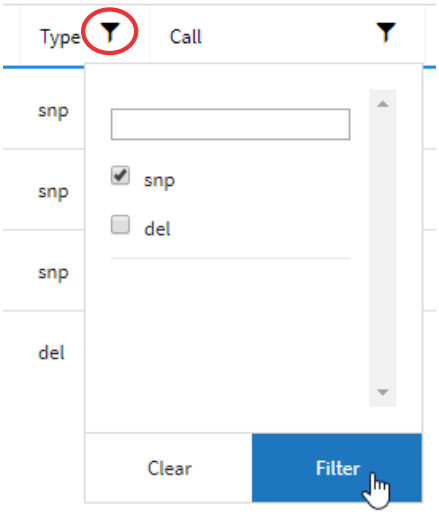
You can also apply a filter chain, a set of filters that Genexus™ Software uses to narrow the list of variants that are included in results. A manager-or administrator-level user creates filter chains from system-installed filters.

Filter the view of results in the Results screen

You can filter results to immediately narrow the list of results that is shown in columns in **Results** screen. Filters are available for each column in the list of results.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name in the **Sample Name** column.
3. In the **Results** screen, click the **Variants** tab.
4. Select the variant class to display the results: **SNVs/Indels**, **Fusions**, or **CNVs**.
5. In the results table, click **(Filter)** in the column heading of interest, enter filter options, then click **Filter**.

   The options that are available depend on the column and variant class. For example, you can filter data in the **Type** column to show one specific variant type.



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

Filter results using a filter chain

You can filter the results that are listed in the **Results** screen with a system-installed or custom filter chain. A filter chain is a set of filters that Genexus™ Software uses to narrow the list of variants that are included in results.

Select a filter chain to change the list of variants that are included in the results. You can apply the filter chain temporarily, then review the results before you decide whether to save the updated results, or discard the changes.

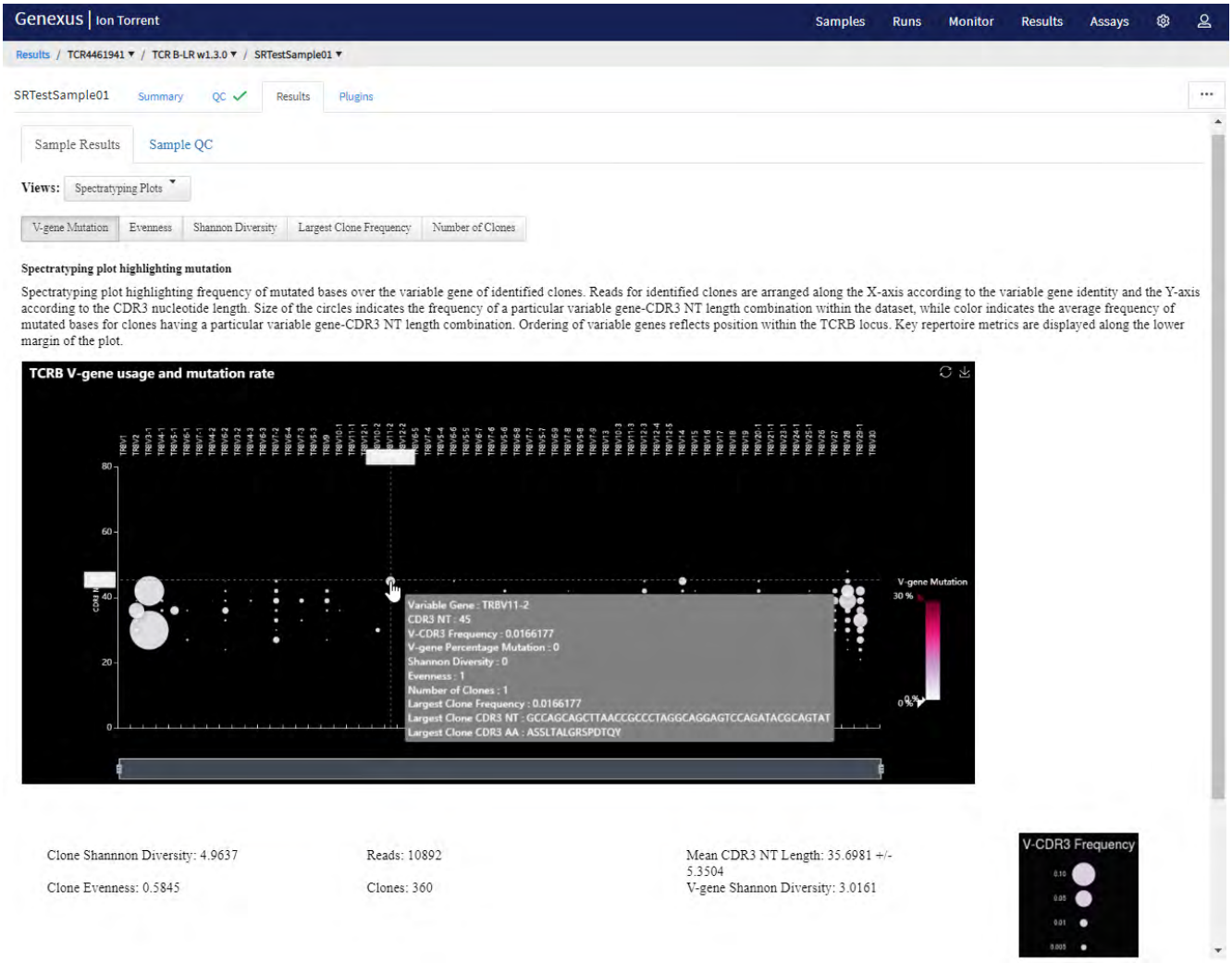
If you save the filter chain to a result, the variants that are included reflect the filtered results when the results are later opened.

For information about system-installed filter chains, and how manager- and administrator-level users can create custom filter chains, see *Genexus™ Software 6.2 Help*, or the *Genexus™ Software 6.2 User Guide* (Pub. No. MAN0018955).

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, select a sample of interest.
3. In the **Results** screen, click the **Variants** tab.
4. Select the variant type to display the results: **SNVs/Indels**, **Fusions**, or **CNVs**.
5. Above the variant table in the **Results** screen, in the **Filter** dropdown menu, select a filter chain.
   The list of results changes to reflect the selected filter chain.
6. *(Optional)* Click **Save** next to the filter chain name if you want the filter chain to be applied to the results when the results are later opened.
   The filter chain is selected in, and applied to the results when the results are reopened.

View Oncomine™ TCR Beta-LR Assay GX run results

- To view the analysis of Oncomine™ TCR Beta-LR Assay GX expression data, in the **Results / Sample Results** screen, click the sample name in the **Sample Name** column, then click the **Results** tab. Results are viewed in two tabs: **Sample Results** and **Sample QC**.
- In the **Sample Results** tab, select from the following options in the **Views** dropdown menu.
  - **Spectratyping Plots**
  - **V-gene usage**
  - **VJ-gene usage**
  - **Clones detected per variable gene**
  - **CDR3 histogram**
  - **Clone Summary**



- In the **Sample QC** tab, select from the following options in the **Views** dropdown menu.
  - **Read classification**
  - **Proportion of full length, quality-trimmed, and reads lacking P1key, by read classification**
  - **Base composition of overcalled and undercalled homopolymers**
  - **Downsampling analysis**
  - **QC metrics**

For detailed information on viewing and analyzing Oncomine™ TCR Beta-LR Assay GX results, see the *Oncomine™ TCR Beta-LR Assay GX User Guide* (Pub. No. MAN0018513).

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 100.

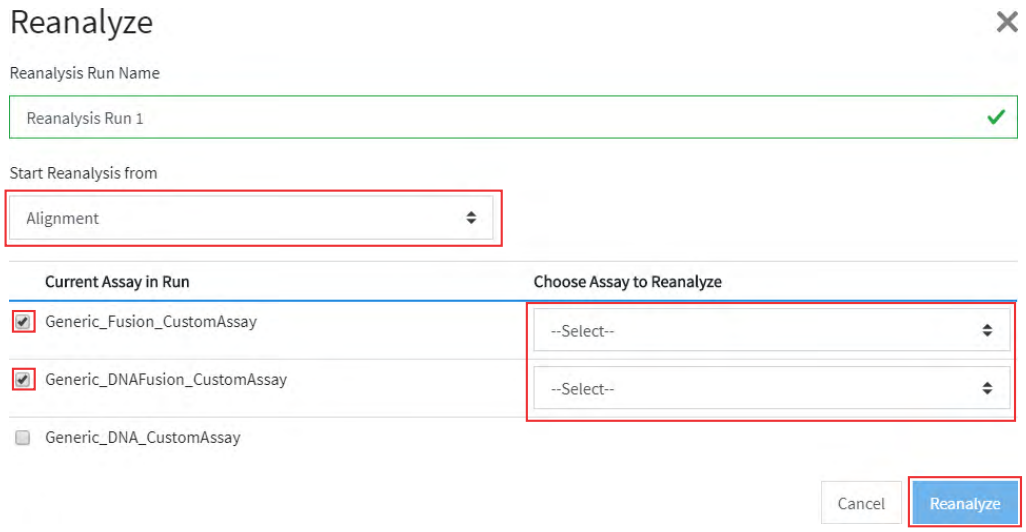
Note:

- 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)”.

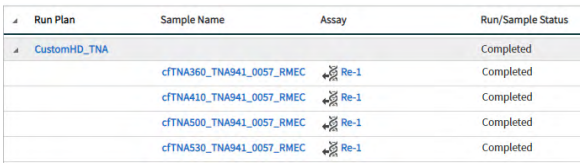
1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest.
3. In the upper right corner of the screen, click ··· **(More Options)** ▸ **Reanalyze**.
4. In the **Reanalyze** dialog box, enter or select 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.



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.



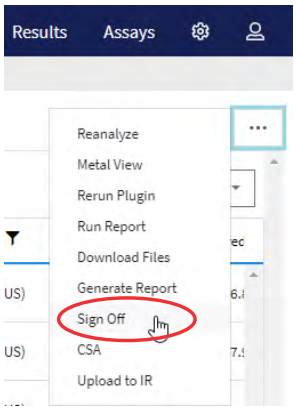
Sign off on the run results (manager/administrator)

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 99. 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.

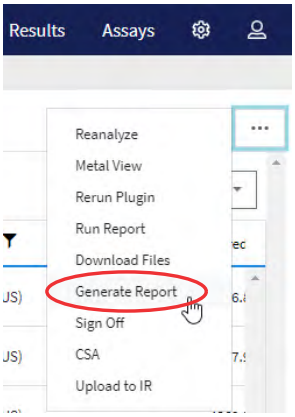
   If you entered footer information in the **Footer Field** when you created a report template, the same footer information appears in the **Electronic Signature** dialog box. You can enter new footer information to override the report template.
9. Click **Sign Off** to confirm your electronic signature.
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.

Generate customized reports

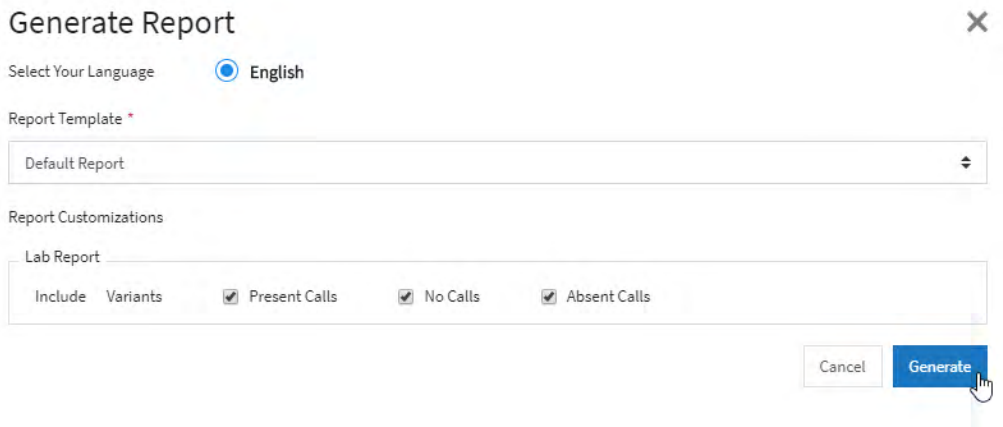
When generating a customized report, you can update any report template selections. For more information on results files, see “Results files” on page 130.

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.

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 190.

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 99.

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 130 and “Output files generated by the coverageAnalysis plugin” on page 190.

For more information on how to review and interpret read statistics and output files, see Appendix D, “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.

Upload sample results files to Ion Reporter™ Software

When a run completes successfully in Genexus™ Software, and the results were not automatically uploaded to Ion Reporter™ Software, you can upload the sample results (BAM files) to Ion Reporter™ Software for further analysis.

Note: To automatically upload BAM files at the completion of a run, select the **Upload BAM files to Ion Reporter™ Software** checkbox in the **Setup** step of planning a run.

Before you can upload sample results files to Ion Reporter™ Software, you must configure your Ion Reporter™ Software account.

- To configure an Ion Reporter™ Server account, see "Configure an Ion Reporter™ Server account (administrator)" on page 179.
- To configure an Ion Reporter™ Software on Connect account, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 182.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, in the **Actions** column, click **Upload to IR** in the row of a run of interest.
3. In the **Upload Samples to Ion Reporter Software** dialog box, make the following selections.
   a. From the **Ion Reporter™ Software Account** list, select your Ion Reporter™ Server account.
   b. In the **Assay** column, select the appropriate assay from the list.
   c. In the **Ion Reporter Workflow** column, do one of the following:
      - Select **Upload Only** to upload the sample results (BAM files) Ion Reporter™ Software. You will need to sign in to Ion Reporter™ Software to launch an analysis.
      - Select an appropriate Ion Reporter™ Software analysis workflow to upload the sample results (BAM files) and automatically launch the selected analysis workflow in Ion Reporter™ Software. This results in an analysis in Ion Reporter™ Software.
   d. Click **Upload**.

The sample results are uploaded to Ion Reporter™ Software.

Verification runs

The following information is available in the **Results / Verification Results** screen.

Column	Description
Run Name	The name of the run, created when the run was planned. Click the name to open the **View Sample Run** dialog box.
Assay Name	The name of the assay used in the run.
Field Engineer Name	The name of the support specialist who performed the run.
Instrument Name	The name of the sequencer that was verified.
Run Status	The current status of the full sequencing run, including analysis.
QC Status	Indicates whether a sequencing run passed or failed, based on the sequencing QC metrics selected for the assay.
Last Updated on	The date and time that the sequencing run was last updated.

(continued)

Column	Description
Actions	• **Audit**—Click this link to display the list of users who created/edited the run. You can export and print information from the list from the **Audit Trail** dialog box. • **CSA**—Customer Support Archive. Click this link to download all of the sequencer log files. Log files contained within the CSA may be useful when troubleshooting issues with the sequencer. For more information, see the *Genexus™ Software 6.2 User Guide* (Pub. No. MAN0018955).

View and sign off on the verification run results

Manager- and administrator-level users can view verification run results, but we recommend that a qualified Thermo Fisher Scientific support specialist signs off on verification results.

1. In the menu bar, click **Results** ▸ **Verification Results**.
2. In the **Verification Results** screen, click a run name in the **Run Name** column to view verification results for a verification run.
3. Click **Sign Off** in the upper right corner of the screen to open the **Electronic Signature** dialog box, then enter your user name, password, and comments. Use the **Meaning of Signature** dropdown list to indicate approval.
4. In **Footer Field**, enter any text that you want to appear in the footer of the PDF report pages.
5. Click **Sign Off** to confirm your electronic signature.

System Install Qualification results

For a System Install Qualification run, basic information about the verification run is provided, and the pass/fail QC status for the following metrics are reported for the controls that are used in the run:

QC metric	Description	Pass threshold
Run QC		
AQ20 Mean Read Length	Average read length, in base pairs, at which the error rate is ≤1% for all aligned reads.	≥90
Average Uniformity of base coverage	The percentage of bases having a depth of coverage ≥20% of the mean coverage at each position.	≥90
Raw Read Accuracy	The percentage of raw reads mapped to the reference genome.	≥98.5
Run Balance Factor	The minimum Sample Balance Factor in a set of four tested samples A–D.	≥0.5
Total Reads	The total number of filtered and trimmed reads with the listed barcodes assigned to the samples.	≥10,000,000

(continued)

QC metric	Description	Pass threshold
Templating Control QC		
Average Reads Per Lane	Average number of CF-1 reads per GX5™ Chip lane.	≥0
Base Call Accuracy	1 – (total number of errors for all positions in the control / total number of aligned bases).	≥0
Mean AQ20 Read Length (bp)	Average length, in base pairs, at which the error rate is ≤1% for all aligned reads of the CF-1 control fragment.	≥0
Library QC		
AQ20 Mean Read Length	Average read length, in base pairs, at which the error rate is ≤1% for all aligned reads.	≥90
Percent Reads On Target	The percentage of reads mapped on target to total reads.	≥90
Sample Balance Factor	For a given sample in a system install qualification run, Sample Balance Factor = Number of mapped reads / Mean number of mapped reads for four tested samples A–D.	≥0.5

Performance Qualification results

For a Performance Qualification (PQ) run, basic information about the verification run is provided, and the reference range and pass/fail QC status for the following metrics are reported for the samples and controls that are used in the run.

Manager- and administrator-level users can sign off on the PQ Report, at which point it becomes a locked PQ Report. However, we recommend that only qualified support specialists sign off on a PQ Report.

QC metric	Description	Pass threshold
Run QC		
AQ20 Mean Read Length (bp)	Average read length, in base pairs, at which the error rate is ≤1% for all aligned reads.	≥90
Average Uniformity of base coverage	The percentage of bases having a depth of coverage ≥20% of the mean coverage at each position.	≥90
Raw Read Accuracy	The percentage of raw reads mapped to the reference genome.	≥98.5
Run Balance Factor	The minimum Sample Balance Factor in a set of four tested samples A–D.	≥0.5
Total Reads	The total number of filtered and trimmed reads with the listed barcodes assigned to the samples.	≥10,000,000

(continued)

QC metric	Description	Pass threshold
Templating Control QC		
Average Reads Per Lane	Average number of CF-1 reads per GX5™ Chip lane.	≥0
Base Call Accuracy	1 – (total number of errors for all positions in the control / total number of aligned bases).	≥0
Mean AQ20 read length (bp)	Average read length, in base pairs, at which the error rate is ≤1% for all aligned reads of the CF-1 control fragment.	≥0
Control QC		
COSM12558	Detection of variant yields call of PRESENT and QC Status = Pass	Present
COSM28747		
COSM476		
COSM521		
COSM6223		
COSM6224		
COSM760		
Library QC		
Mapped Reads	The total number of reads mapped to the reference sequence.	≥1,100,000
Mean AQ20 Read Length (bp)	Average length, in base pairs, at which the error rate is ≤1% for all aligned reads of the sample DNA library.	≥90
Percent Reads On Target	The percentage of reads mapped on target over total reads.	≥90
Sample Balance Factor	For a given sample in a PQ run, Sample Balance Factor = Number of mapped reads / Mean number of mapped reads for four tested samples A–D.	≥0.5

A

Troubleshooting

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 (see "Create a new assay (manager/administrator)" on page 35), 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 138.

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

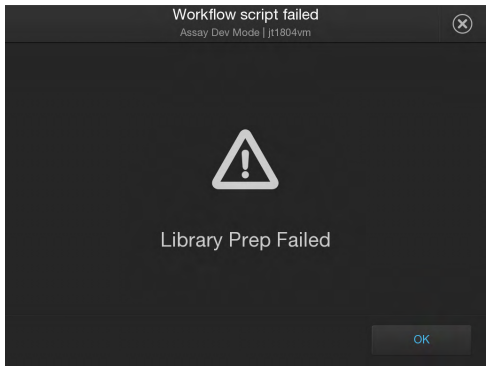
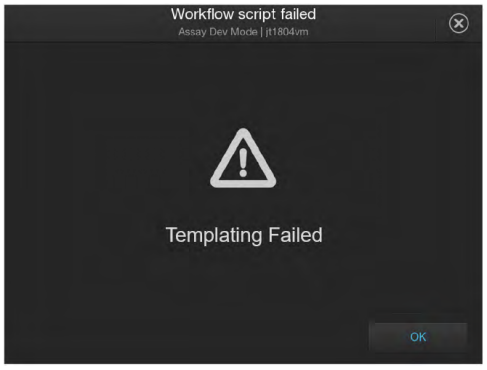
Genexus™ Integrated Sequencer—general and QC troubleshooting

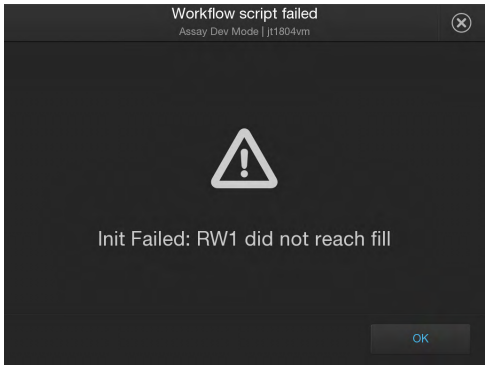
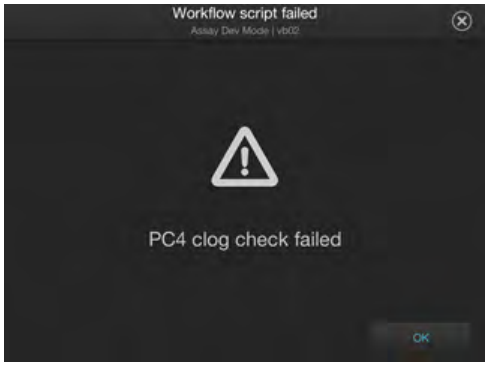
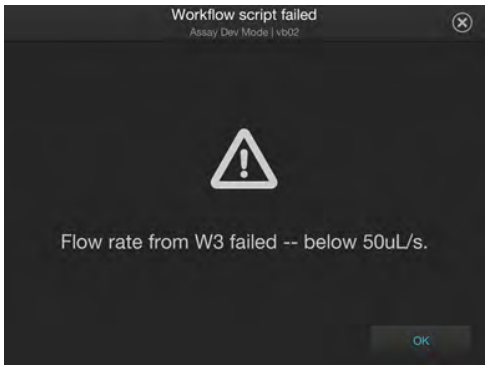
Observation	Possible cause	Recommended action
Consumable is not recognized by instrument after loading on the deck	The consumable (for example, a strip, barcode plate, pipette tip box) is correctly placed but is not completely inserted into its position, causing it to be misaligned with its expected 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 6.2.0.	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.
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. For details, see "Perform a Clean instrument procedure" on page 152. 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 137.	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 137.	One or more of the Genexus™ Strip 1 strips used in the run had magnetic beads trapped in the tube 5 keyhole.	Repeat the run with strips that you have verified have no trapped beads. For more information, see "Before you begin" on page 79.
	An incorrect assay was selected for the run, or library amplification parameters were not optimal.	Ensure that you have selected the correct assay and reviewed assay parameters.
	Library strips were inadequately equilibrated to room temperature (Genexus™ Strip 1), or incompletely thawed (Genexus™ Strip 2-AS or Genexus™ Strip 2-HD) before loading in the sequencer.	Ensure that Genexus™ Strip 1 strips are fully equilibrated to room temperature, and Genexus™ Strip 2-AS strips are completely thawed before loading in the sequencer.
The number of sample reads is low, and CF-1 metrics fail QC **Details:** If CF-1 metrics failed QC, a problem in templating or sequencing is indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 137.	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 79.
	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 137. *(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. **Note:** Reagents are stable on the sequencer for 14 days, after which you may experience reduced performance. For further information, see "Options for an expired sequencer initialization" on page 93.

Genexus™ Integrated Sequencer error and warning messages

Observation	Possible cause	Recommended action
Error message: **Library Prep Failed** 	• The sequencer failed xy homing, *or* • The sequencing chip failed to engage at pipette position.	Confirm that no analyses are in progress, then reboot the instrument. Repeat the run. If the failure continues, contact Technical Support.
Error message: Templating Failed 	• The sequencer failed xy homing, *or* • The Genexus™ Coupler was misaligned.	Confirm that no analyses are in progress, then reboot the instrument. Repeat the run, and ensure that the coupler is installed with proper alignment. If the failure continues, contact Technical Support.

Observation	Possible cause	Recommended action
Error message: **Init Failed: XXX did not reach fill** 	Genexus™ Bottle 2 flow was restricted, or a flow sensor malfunctioned. **Note:** XXX is the name of the conical tube that did not reach fill.	Allow the run to complete, then select **Settings ▸ Clean instrument** to do a clean. After the clean, reboot the instrument, then start a new run. If the error message appears again, call Technical Support for service. **Note:** After the run completes, you can recover libraries from the amplification plate, if desired. For more information, see "Library QC Archive: recover library preparations from the Genexus™ Integrated Sequencer for reuse" on page 172.
Error message: **XXX clog check failed** 	Clog check following Clean Instrument failed. Incomplete fluid flush or debris has caused a clog. **Note:** XXX refers to the failed waste lines, chip, or main 1-4.	Contact Technical Support for recommended action.
Error message: **Flow rate from W3 failed – below 50 µL/s** 	During the post-chip clean the Genexus™ Bottle 3 was empty, or Genexus™ Bottle 3 flow was restricted.	Select **Settings ▸ Clean instrument** to do a clean. Ensure that the Genexus™ Bottle 3 is installed correctly, and the bottle contains sufficient volume. Replace as needed. For details, see "Perform a Clean instrument procedure" on page 152. If the failure persists, contact Technical Support.

Observation	Possible cause	Recommended action
Error message: **PostChipClean failed**	PostChipClean aborted due to a chip error.	In the sequencer screen, tap **Settings ▸ Clean instrument** to re-do a Clean instrument procedure. For details, see "Perform a Clean instrument procedure" on page 152. You may or may not need to replace the chip during deck set up.
Warning message: **Pressure Leak Detected in vac pressure drop leakrate.** **Press Ok to open doors and fix the leak.**	Genexus™ Bottle 1 leak was detected.	Remove the Genexus™ Bottle 1 and ensure that the cap is tight, then replace the bottle securely on the instrument. Continue with the run. If the pressure leak message appears again, replace the Genexus™ Bottle 1 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in vac time to pressure.** **Press Ok to open doors and fix the leak.**	A Genexus™ Bottle 1 leak or over-fill was detected.	Ensure that a new Genexus™ Bottle 1 is installed, and the cap is tight and the bottle is secure on the instrument. Continue with the run. If the pressure leak message appears again, replace the Genexus™ Bottle 1 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in vac pressure baseline.** **Press Ok to open doors and fix the leak.**	Genexus™ Bottle 1 leak was detected.	Remove the Genexus™ Bottle 1 and ensure that the cap is tight, then replace the bottle securely on the instrument. Continue with the run. If the pressure leak message appears again, replace Genexus™ Bottle 1 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.

Observation	Possible cause	Recommended action
Warning message: **Pressure Leak Detected in W2P.** **Press Ok to open doors and fix the leak.**	A leak was detected in the Genexus™ Bottle 2 at the left position.	Remove Genexus™ Bottle 2 installed at the left position, ensure that the cap is tight, then reinstall the bottle securely. Continue with the run. If the pressure leak message is seen again, replace the left Genexus™ Bottle 2 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in W22P.** **Press Ok to open doors and fix the leak.**	A leak was detected in the Genexus™ Bottle 2 installed at the right position.	Remove the Genexus™ Bottle 2 installed at the right position, ensure that the cap is tight, then reinstall the bottle securely. Continue with the run. If the pressure leak message appears again, replace the right Genexus™ Bottle 2 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in W3P.** **Press Ok to open doors and fix the leak.**	A leak was detected in the Genexus™ Bottle 3.	Remove the Genexus™ Bottle 3, ensure that the cap is tight, then reinstall the bottle securely. Continue with the run, or repeat the clean if this message appears in a postChipClean. If the pressure leak message appears again, replace the Genexus™ Bottle 3 with a new bottle. Continue with the run, or repeat the clean if this message appears in a postChipClean. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in Conicals & Reagent Cartridge.** **Press Ok to open doors and fix the leak.**	A leak was detected in one or more of the Genexus™ Conical Bottles or the Genexus™ Cartridge.	Tighten all Genexus™ Conical Bottles one bottle at a time to prevent accidental position changes. Inspect the Genexus™ Cartridge for defects. Continue with the run. If the failure persists, contact Technical Support.

Observation	Possible cause	Recommended action
Warning message: **Liquid detected in conicals. Press Ok to open doors and fix the issue.**	The previous postChipClean did not remove all of the liquid in the Genexus™ Conical Bottles, causing failure of the Conicals empty test and Conical volume test.	Replace the Genexus™ Conical Bottles with new bottles, then tap **Settings** ▸ **Clean instrument** to clean the instrument. After cleaning, start a new run.

Note: If a leak check fails in a run starting with lanes 2–4, tap **Help** in the lower left corner of the screen to allow use of the remaining sequencing reagents in the next run.

Genexus™ Software

Observation	Possible cause	Recommended action
Cannot sign in to Genexus™ Software	You either entered an incorrect password or you are signed out due to several failed login attempts.	Contact the Genexus™ Software system administrator.
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique sample name.	Every sample name in the software must be unique. Ensure that the spreadsheet does not contain any duplicate sample names, 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.

Observation	Possible cause	Recommended action
Library batch import fails	One or more entries in the library batch 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 library batch 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 library batch import template file, and be careful to copy only those rows that contain actual data.
	The library batch import spreadsheet contains a nonunique Library Batch ID.	Every Library Batch ID in the software must be unique. Ensure that the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Library Batch ID of ABC1 conflicts with abc1.
	A sample name entered in the library batch import spreadsheet does not match a sample name listed in the **Manage Samples** screen.	Ensure that the sample names entered into the spreadsheet are correct and match an existing sample name added to the software.
	The Barcode ID name format does not exactly match the format that is used in the **Prepare Library Batch** dialog box.	Use the name format following the Barcode ID name format found in the Barcode Set reference lists (**Settings** ▸ **References** ▸ **Barcode Set**), for example: IonDual_0101 through IonDual_0196, or IonHDdual_0101 to IonHDdual_0132.
	An invalid library, control, or panel kit barcode has been entered in the spreadsheet.	Ensure that you have correctly entered a valid kit barcode in the appropriate cell of the spreadsheet.
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.
Allele coverage does not match hotspot coverage	The coverage value reported under the Variants tab and Allele Coverage tab can be different.	No action required. The coverage value reported under the Variants tab is the coverage after down-sampling, while the Allele Coverage tab reports the raw coverage without down-sampling. Down-sampling can speed up variant calling for some over-sampled positions.

Observation	Possible cause	Recommended action
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.
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” on page 159.
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

Touchscreen reference

Touchscreen icons

Number	Icon	Description
1		Network connectivity – connected
		Network connectivity – not connected
2		Instrument idle
		Sequencing in progress
		Instrument ready
		Error
3		Chip status – Absent

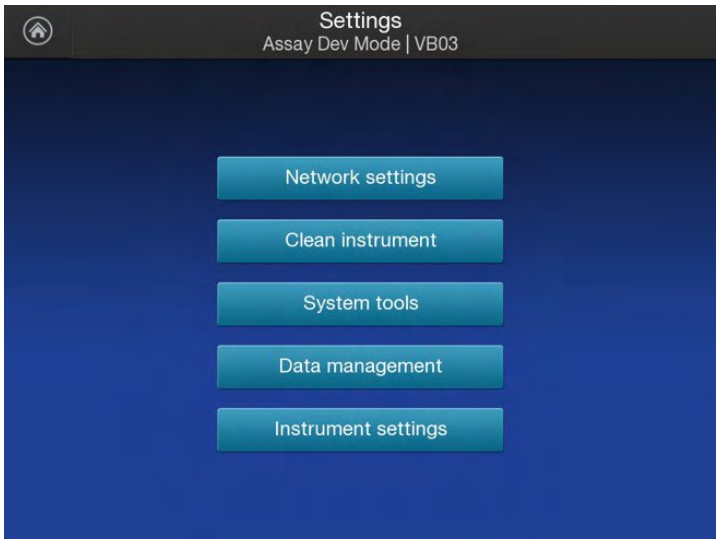
(continued)

Number	Icon	Description
3		Chip status – Standby
		Chip status – Connecting
		Chip status – Ready
		Chip status – Imaging
		Chip status – Error
4		Chip lane status – 4 lanes available
		Chip lane status – 1 lane in use or used, 3 lanes available
		Chip lane status – 2 lanes in use or used, 2 lanes available
		Chip lane status – 3 lanes in use or used,1 lane available
		Chip lane status – 4 lanes in use or used, 0 lanes available
5		Instrument File System Space, the percent of file space used is indicated.[1] The instrument checks for sufficient disk space before each run and notifies the user if there is not enough.
6		Instrument Server File System Space, the percent of file space used is indicated. **Note:** If the indicator turns red, archive data from the server to free up disk space. Refer to the Genexus™ Software Help for more information on archiving data.

[1] Indicator turns yellow when disk space is ≥67% full, indicator turns red when ≥90% full.

Settings

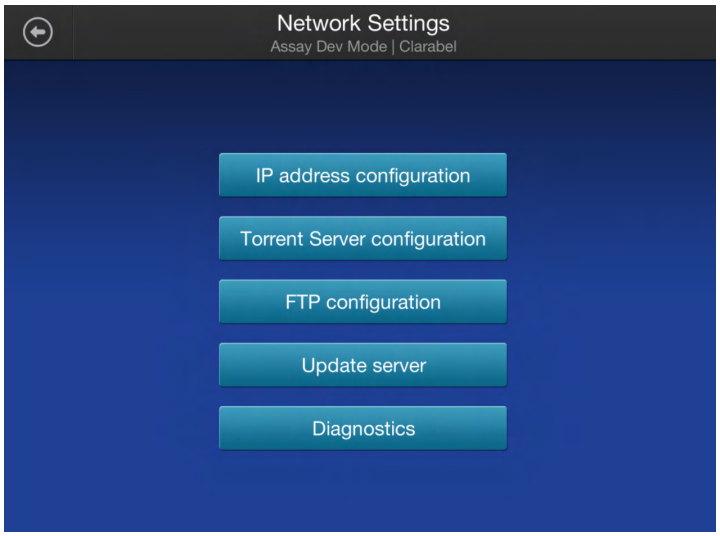
In the **Settings** menu, users can view and/or change instrument settings, manage data and network configurations, and clean the instrument.

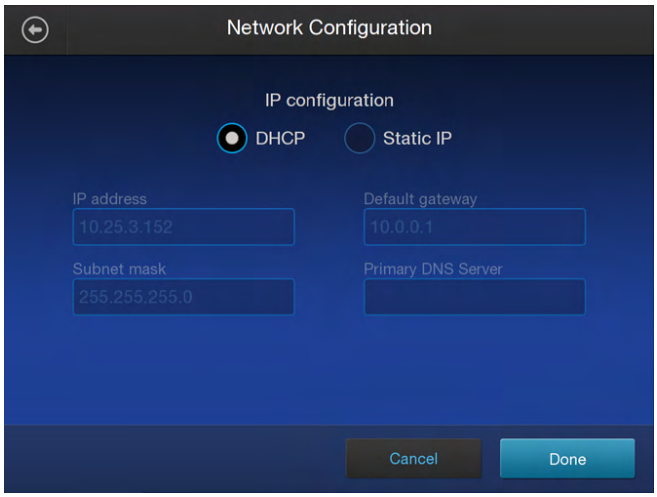
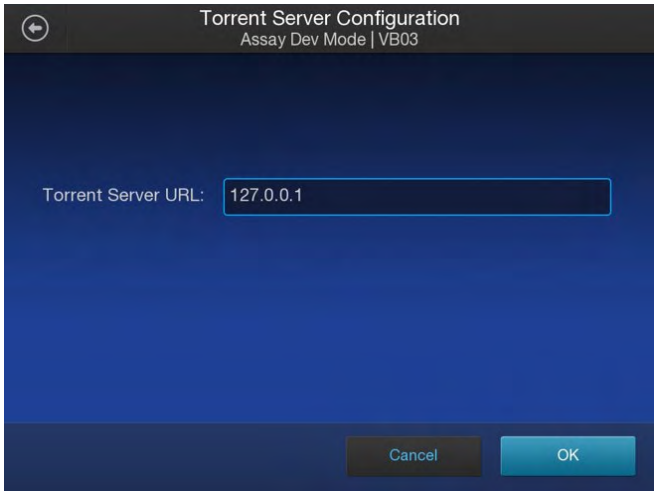


Note: The **System tools** option is for use by trained service personnel only.

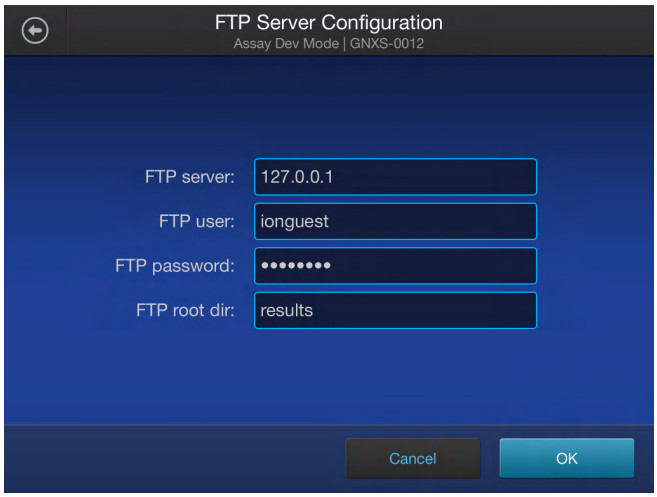
Network Settings

The **Network Settings** menu allows you to configure IP address, Ion Torrent™ Server, and FTP settings.



Item	Description	When/How to use
IP Address Configuration	Allows users to set or change the IP configuration (DHCP or Static IP). 	1. Select either the **DHCP** or **Static IP** radio button. 2. Touch the screen in the field you want to edit to activate the field. A virtual keyboard appears. 3. Enter the new information, then press **Done**.
Ion Torrent™ Server Configuration	Allows users to change the Ion Torrent™ Server IP address and user information.  Enter the following IP address in the **Torrent Server URL** field for your instrument: 127.0.0.1	When a change to the Ion Torrent™ Server IP address or user information is required. **Note:** Do not change address unless directed to do so by a Thermo Fisher Scientific field service engineer.

(continued)

Item	Description	When/How to use
FTP Server Configuration	Allows users to change the FTP Server IP address and user information.  Enter the following IP address in the **FTP server** field for your instrument: 127.0.0.1	When a change to the FTP Server IP address or user information is required. **Note:** Do not change address unless directed to do so by a Thermo Fisher Scientific field service engineer.

Perform a Clean instrument procedure

Cleaning is normally performed automatically at the completion of the previous sequencing run. Perform a **Clean instrument** procedure if the sequencing run:

- was aborted or had a power failure during a run, or
- the normal post-sequencing run cleaning was not completed.

The **Clean instrument** command initiates the following cleans:

- UV clean—irradiation of the deck surface with UV light (see “Clear the instrument deck and perform a UV Clean” on page 90)
- Vacuum clean—cleaning of the four chip vacuum lines and robotic waste line.
- postChipClean—cleaning of sequencer fluidic lines with conical flow rate test.

IMPORTANT! The **Clean instrument** procedure renders remaining sequencing reagents and unused lanes on the installed chip unusable after the cleaning. Tap **Help** in the lower left corner of the screen to allow use of remaining sequencing reagents in the clean procedure.

---

1. In the **Settings** menu, tap **Clean instrument**.
2. Follow the on-screen instructions, then tap **Next** to go to the following sequence of screens: **Clear Deck** if there are items to be cleared, **UV Clean**, **Load Deck**, **Clear Sequencing Reagents**, and **Load Sequencing Reagents**. For more information, see “Clear the instrument deck and perform a UV Clean” on page 90.
3. After cleaning is complete, tap **Next**. Follow the on-screen instructions in the **Clear Deck** and **Clear Sequencing Reagents** screens. After sequencing reagents are cleared, tap **Next**.

The user interface returns to the home screen.

Replace Genexus™ Conical Bottles during a Clean instrument

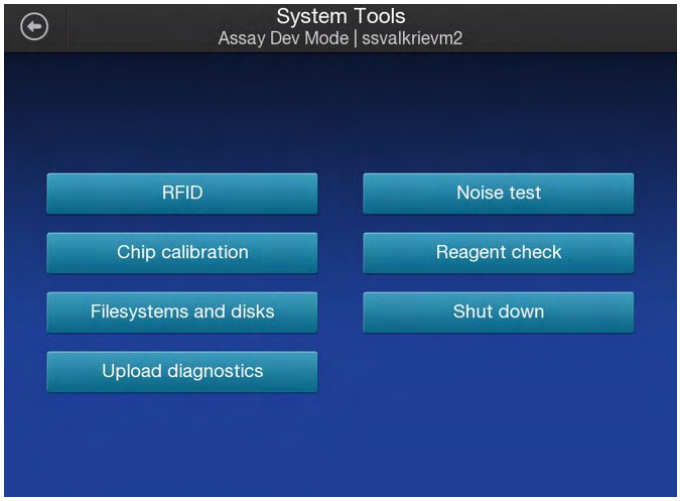
If the sequencer detects restricted flow from the Genexus™ Conical Bottles during a **Clean instrument** procedure, the following alert is displayed.

If you see this alert, follow these steps to replace the five Genexus™ Conical Bottles.

1. Tap **OK**. The screen returns to the run screen.
2. Tap **Settings** ▸ **Clean instrument** again.
3. Follow the screen instructions to clear the deck, then load the deck.
4. When the sequencing reagent bay doors open, do the following.
   a. Remove, but do not discard, the Genexus™ Bottle 1, the two Genexus™ Bottle 2 bottles, and the Genexus™ Bottle 3 to gain easier access to the Genexus™ Conical Bottles.
   b. Remove the five used Genexus™ Conical Bottles, then install five new conical bottles.
   c. Replace the four reagent bottles that you removed in their original positions.
   d. Tap **Help** in the lower left corner of the screen to ignore the reagent bottles and Genexus™ Cartridge as used so they can be reused in the next clean.
5. Close the sequencing reagent bay doors.
   The **Clean instrument** procedure begins automatically.
6. After the cleaning finishes, follow the screen instructions to clear the deck and remove the sequencing reagents.
   For further information, see “Replace the Genexus™ Conical Bottles” on page 165.

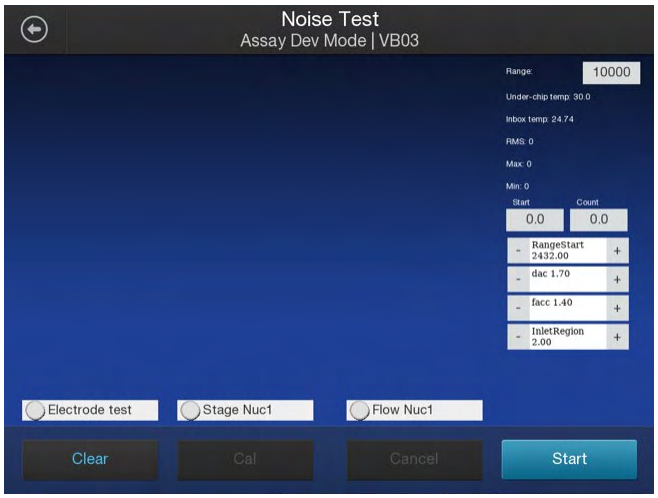
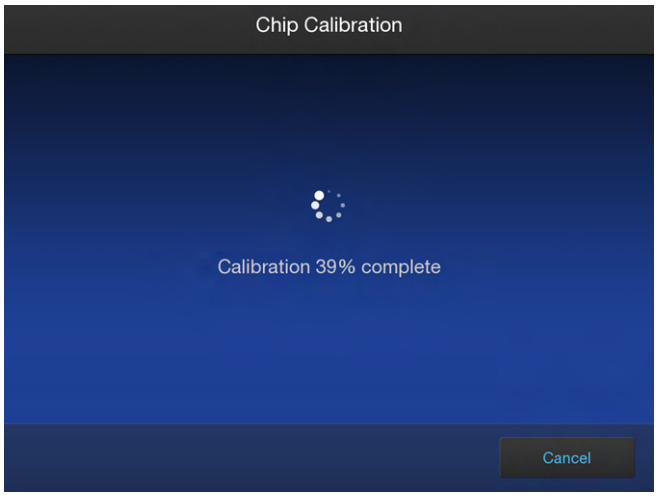
System Tools

The **System Tools** menu enables you to upload instrument diagnostics, manage data, and shut down or reboot the instrument.

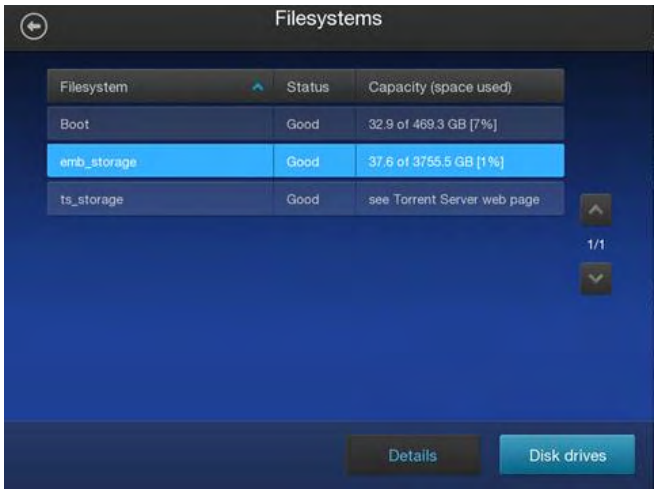
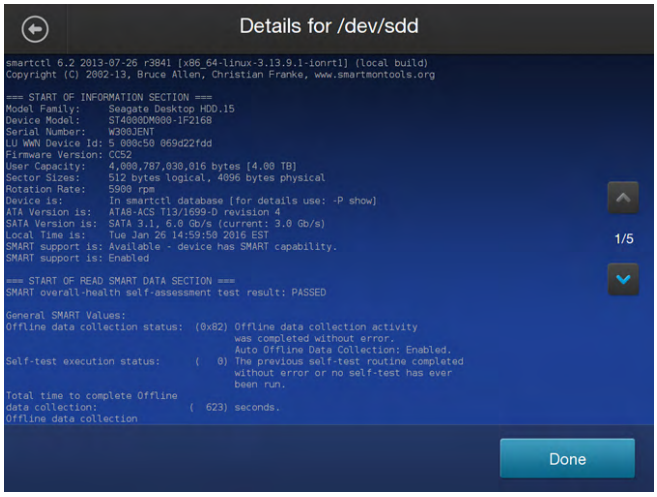


Item	Description	When/How to use
RFID	Lists the product, product expiration date, and remaining uses of the product. 	To determine if reagents that were left on an instrument during a period of instrument shutdown have expired. 1. Tap the screen within the row of the product to view an **RFID Tag Detail** page. 2. Tap **Done** to return. **Note:** The RFID screen can also be used to track remaining days of reagent use before a sequencer initialization expires. We recommend that you use reagents within 14 days of initialization. After 28 days, the sequencer displays a warning at run start with options to proceed to a clean and new initialization, ignore to continue without clean, or cancel the run. For further information, see “Options for an expired sequencer initialization” on page 93.

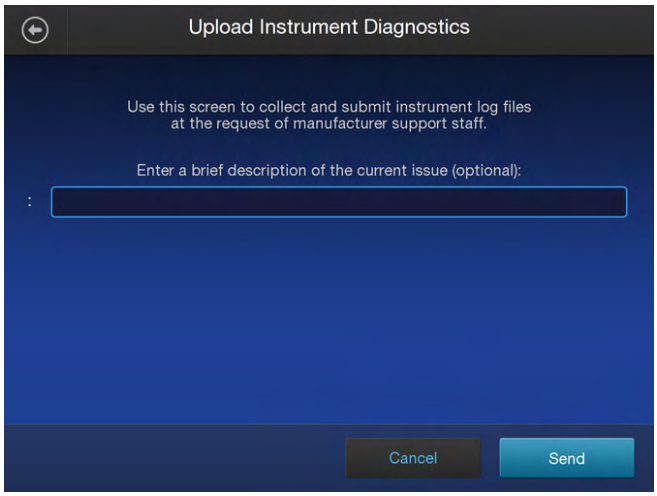
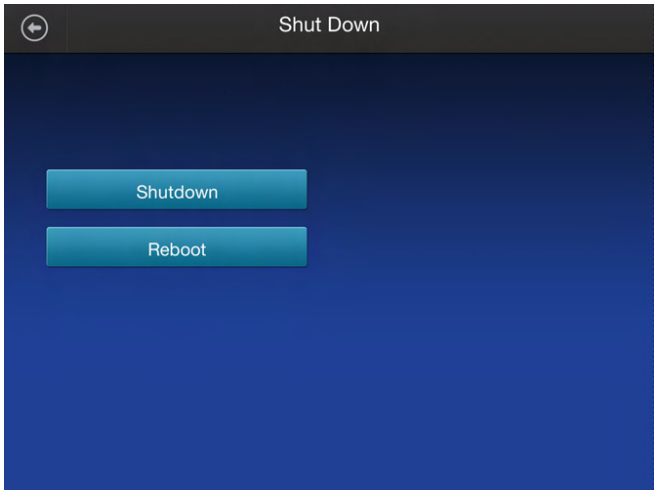
(continued)

Item	Description	When/How to use
Noise Test	Provides real-time measurement of electrical noise readings on the chip. 	For troubleshooting if directed to do so by Technical Support.
Chip Calibration	Checks the status of a chip. **IMPORTANT!** Do not perform a chip calibration unless instructed to do so by a field service engineer. 	1. Insert a chip into the chip install slot. 2. Tap **Chip Calibration** to start the chip calibration. 3. In the dropdown lists, select the **Image** to display and the type of **Calibration** to perform. 4. After a chip calibration, user should perform a **Clean instrument** before removing the chip. **Note:** Tap **Cancel** to quit the chip calibration.

(continued)

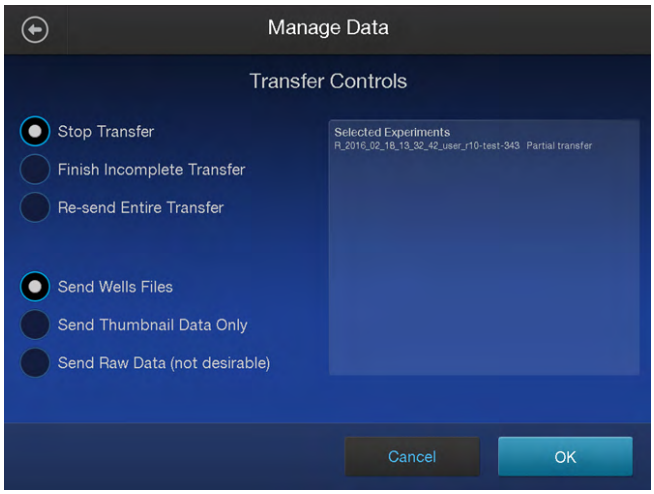
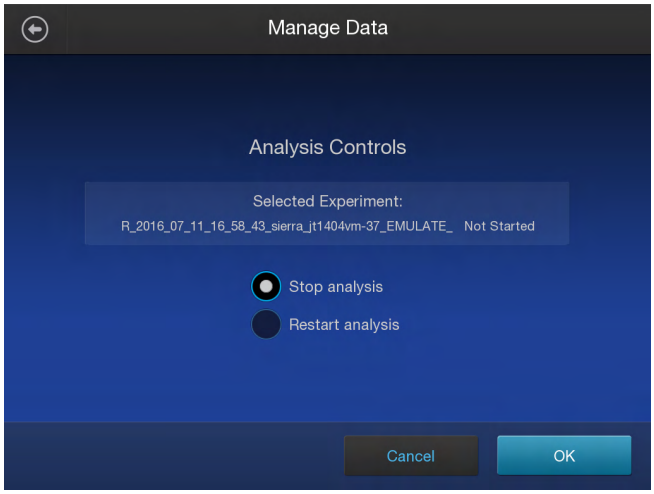
Item	Description	When/How to use
Filesystems and disks	Provides real-time drive activity status and disk space used.   	Use if directed to do so as part of a troubleshooting procedure: 1. In the System Tools menu, press **Filesystems and disks**. The **Filesystems** screen opens. 2. Tap the screen within the row of the **Filesystem** you want to view, then press **Details**. The **Disk Drives in <Filesystem>** screen opens. 3. Tap the screen within the row of the **Drive** to view details of the disk drive. The **Details for <Drive>** screen opens. 4. Tap **Done** to return to the previous screen.

(continued)

Item	Description	When/How to use
Upload Diagnostics	Allows upload of instrument diagnostics files for review by support personnel. 	Upload an instrument diagnostics file after an aborted run, or if otherwise directed to do so by Technical Support. **Note:** The file is pushed to Genexus™ Software. To retrieve the file using Genexus™ Software, navigate to ⚙ **(Settings) ▸ About**, click **Instrument Diagnostics**, then follow the on-screen instructions.
Shut Down	Access to "Shut Down" and "Reboot" commands. 	If directed to do so by Technical Support as part of a troubleshooting procedure, or if the instrument will not be used for more than three days. **Note:** It is not necessary/recommended to shut down the instrument overnight or over the weekend. To power on the instrument after a shut down, see “Power the Genexus™ Integrated Sequencer on or off” on page 28.

Data Management

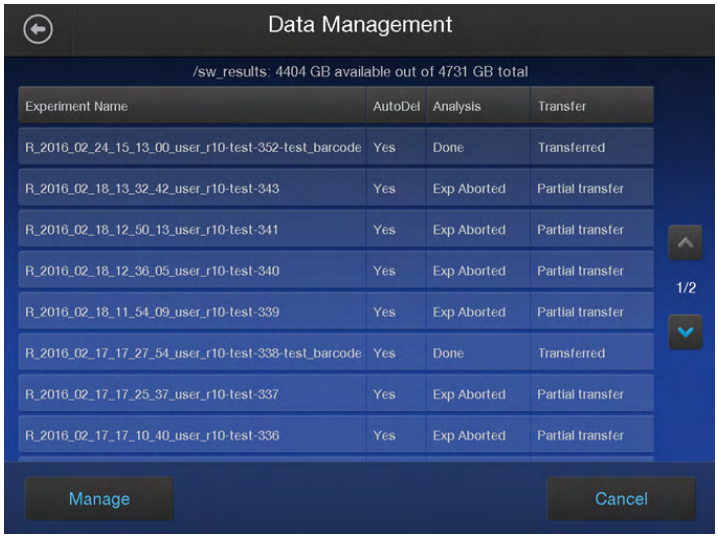
The **Data Management** function allows users to delete run data manually, or transfer data in the event of failure of automatic transfer. Under normal conditions, run data are automatically transferred to the analysis server, then deleted from the instrument hard drive.

Item	Description	When/How to use
Delete data	Manually delete run data from the instrument.	If the instrument hard drive becomes full, see “Manually delete run data” on page 159.
Transfer data		Transfer run data from the instrument hard drive to the Ion Torrent™ Server. 1. Tap the screen in the **Experiment Name** row of the individual experiment to be transferred. 2. Tap **Transfer**. 3. Select the radio button of the action to be performed. 4. Select the radio button of the files to be transferred. 5. Tap **OK**.
Analysis Controls	Allows the user to stop analysis the data of individual runs. 	1. Tap the screen in the **Experiment Name** row of the individual experiment to be analyzed, then tap **Analysis**. 2. Select the **Stop Analysis** radio button, then tap **OK**. **Note: Restart analysis** is currently not supported.

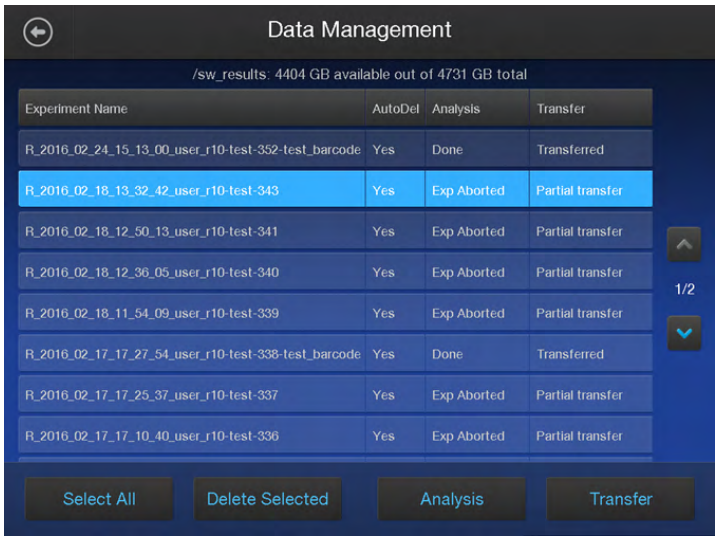
Manually delete run data

To troubleshoot data management problems the **Data Management** function allows users to delete run data manually or transfer the data to an external server.

1. In the **Settings** menu, tap **Data Management** to access the **Data Management** screen, then tap **Manage**.



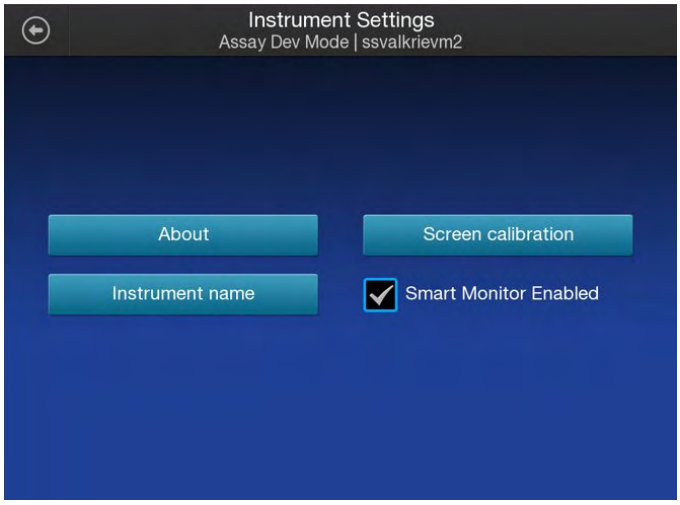
2. Tap **Select All** to select all the available experiments, or tap the screen in the **Experiment Name** row of the individual experiment to be managed.

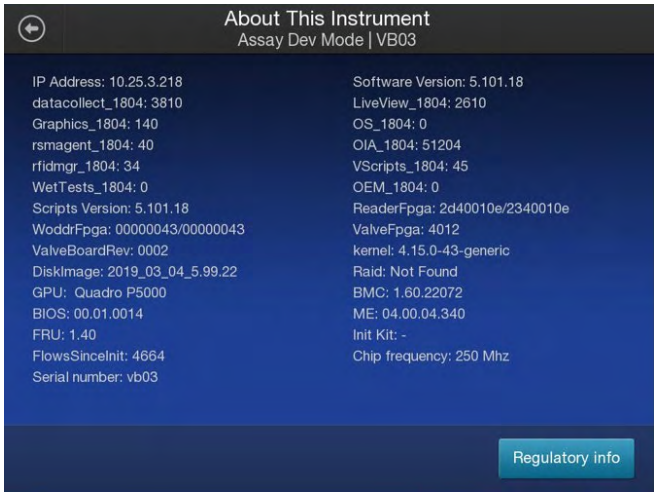


3. Tap **Delete Selected**.

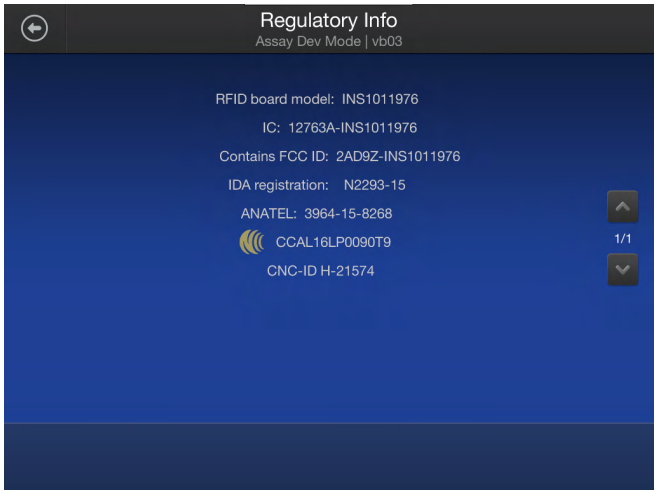
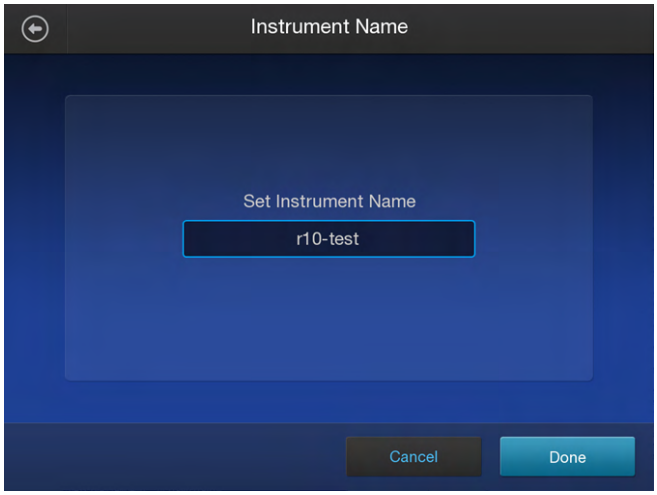
Instrument settings

The **Instrument Settings** menu provides information about the instrument and allows you to set the instrument name and calibrate the touchscreen.

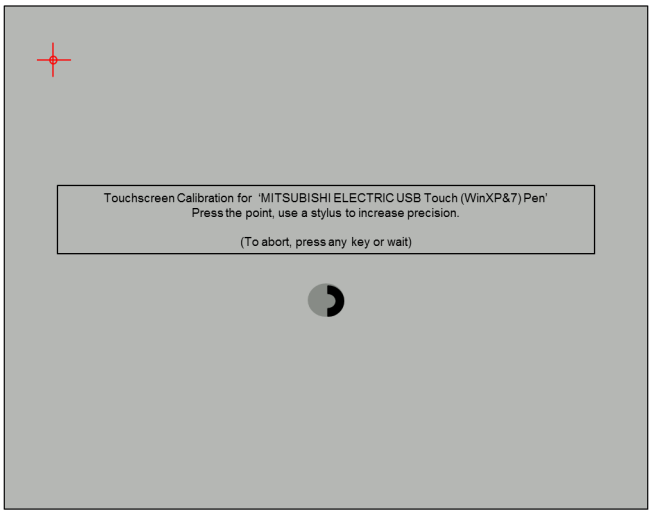


Item	Description	When/How to use
About	Provides instrument details. 	To view instrument reference information or access regulatory information. Click **Regulatory info** to view the following screen.

(continued)

Item	Description	When/How to use
Regulatory Info	Lists instrument-specific regulatory information. 	To view instrument RFID regulatory information. See “Radio compliance standards” on page 201 for compliance information.
Instrument Name		To change the instrument name.

(continued)

Item	Description	When/How to use
Screen Calibration		For troubleshooting if directed to do so by Technical Support. Touch the red cross with your finger or a stylus each time it appears. In total, you touch the screen 4 times, one time in each corner.
Smart Monitor Enabled	—	Select the checkbox to enable remote monitoring of instrument runs by Thermo Fisher Scientific for troubleshooting.

C Supplemental information

Maintain the sequencer

Materials required

Cleaning

- Lint-free wipes
- 70% isopropanol
- *(Optional)* 10% bleach solution

Liquid waste filter replacement

- Genexus™ Filter (ordered separately [Cat. No. A40302])

Conical bottle replacement

- Genexus™ Conical Bottles (from the Genexus™ Installation and Training Kit, or ordered separately [Cat. No. A40275])

Clean or decontaminate the sequencer

If a spill or leak occurs inside the instrument, perform the following steps to clean or decontaminate the sequencer.

Note: Dispose of all waste in appropriate liquid or solid waste containers.

1. Remove each Genexus™ Bottle 2, then remove and empty the Genexus™ Bottle 1 and Waste Carboy.
2. Remove the Genexus™ Cartridge.
3. Inspect the floor of the sequencing reagents bay and Genexus™ Cartridge port for liquid.
4. Using absorbent paper, soak up as much liquid as possible, then wash the affected area with 10% bleach solution.
5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.

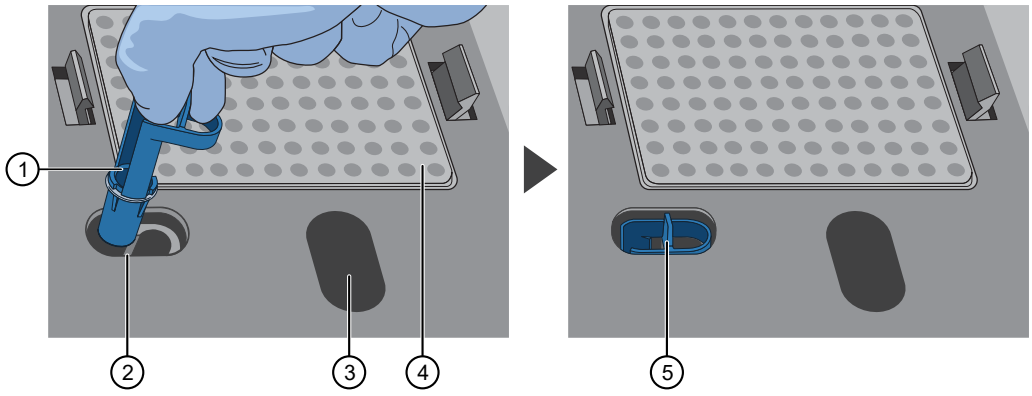
Replace the Genexus™ Filter

The Genexus™ Filter captures particulate matter in the liquid waste to prevent blockage of the waste line over time. The filter needs to be replaced with a new filter after one year of regular instrument use.

1. Remove the old filter from the liquid waste disposal port on the instrument deck by grasping the filter firmly and pulling up. Dispose of the filter as regular waste.

   Note: If an interim filter is installed in the port, use a straight needle probe to pierce the filter, then lift out the filter using the probe.

2. Insert a new Genexus™ Filter into the liquid waste disposal port, then press firmly to seat the filter O-ring securely.



① Genexus™ Filter
② Liquid waste disposal port
③ Used pipette tip disposal port
④ Sample plate station
⑤ Installed filter

Replace the Genexus™ Conical Bottles

Genexus™ Conical Bottles need to be replaced when filters in the bottles become partially clogged, resulting in reduced flow rate. The Genexus™ Integrated Sequencer measures flow rate in a Conical Flow Rate test that is performed during every postChipClean. If the conical flow rate test fails, the sequencer automatically redirects to the conical bottle replacement procedure. During a normal run, a postChipClean is performed in three situations:

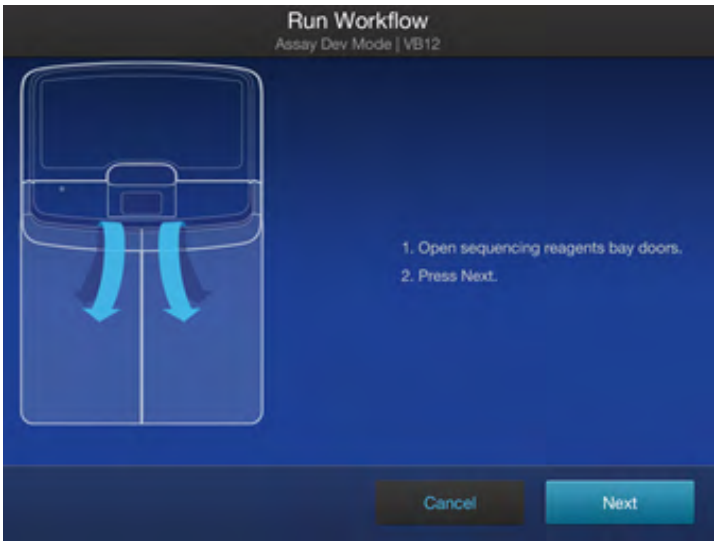
- At the end of the run if all four lanes have been used.
- At the end of the run if you selected **Do Force Clean** at run setup.
- At run setup when a run is selected but insufficient unused lanes in the installed chip are available for the run. If you continue with the run, a postChipClean is needed before the run can proceed.

Follow these steps to replace the Genexus™ Conical Bottles.

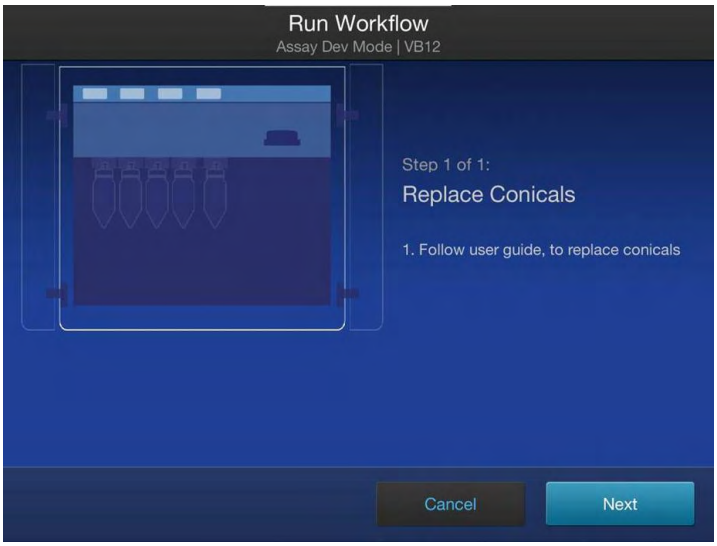
1. After a run, In the **Run Complete** screen, tap **Next**.

If the Conical Flow Rate test failed, a screen appears directing you to open the sequencing reagents bay doors.

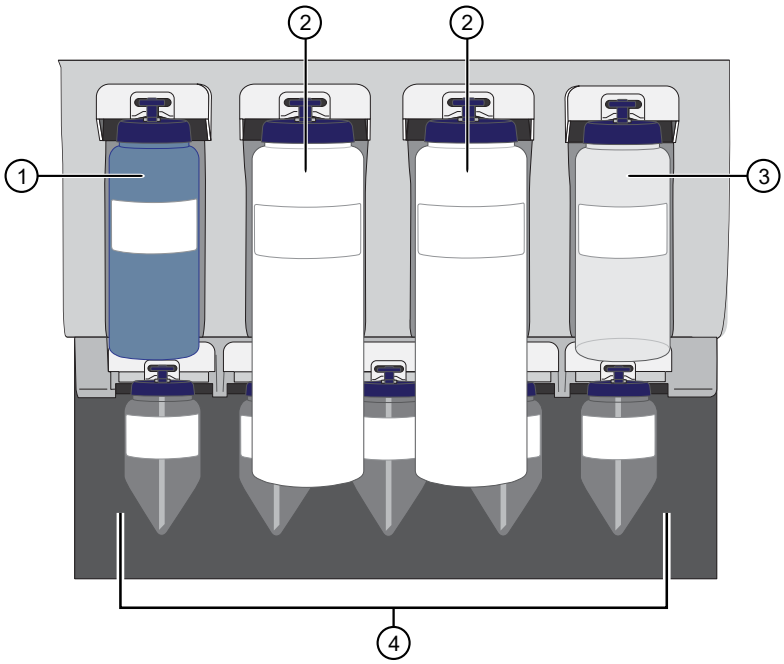
2. Open the sequencing reagents bay doors, then tap **Next**.



The **Replace Conicals** screen appears.



3. Remove, but do not discard, the Genexus™ Bottle 1, the two Genexus™ Bottle 2 bottles, and the Genexus™ Bottle 3 to gain easier access to the Genexus™ Conical Bottles.



① Genexus™ Bottle 1
② Genexus™ Bottle 2
③ Genexus™ Bottle 3
④ Genexus™ Conical Bottles

4. Remove the five used Genexus™ Conical Bottles, then install five new conical bottles.

Note: Discard the used conical bottles appropriately.

5. Replace the four reagent bottles that you removed in step 3 in their original positions.

6. After replacing the bottles, close the sequencing reagents bay doors, then tap **Next** in the **Replace Conicals** screen.
   After you tap **Next**, the sequencer starts a **Leak Test**.

- If the sequencer passes the **Leak Test**, no action is needed. The sequencer proceeds to a cleaning cycle.

- If the sequencer fails the **Leak Test**, a notification appears. Tap **OK** to return to step 2 to fix the leak. Open the sequencing reagents bay doors, check the tightness of each conical bottle that you installed, then ensure that the four reagent bottles are installed correctly. Close the sequencing reagents bay doors, then tap **Next** in the **Replace Conicals** screen to repeat the **Leak Test**.

After completion of a successful **Leak Test** and cleaning, the sequencer moves to a normal workflow.

- If the sequencer is at the end of the run, the next screen is a **Clear Deck** screen, followed by a **Clear Sequencing Reagents** screen.
- If the sequencer is at the start of the run, the next screen is a **Clear Deck** screen, followed by a **Load Deck** screen.

Genexus™ Integrated Sequencer power off and power on before and after a long-term shutdown

Follow these procedures to power off the sequencer if it will not be used for more than 28 days, and power on after a long-term shutdown.

Power off before a long-term shutdown

- If all four lanes of a chip have been used, and a postchip clean has completed successfully, follow these steps to power off the sequencer, as described in “Power off” on page 28.
  a. In the home screen, tap **Settings** ▸ **System Tools** ▸ **Shut down**.
  b. Select **Shutdown**.
  A confirmation message appears. Select **Yes** to power off the instrument.
  c. Turn the power switch located at the back of the instrument to the off (O) position.
- If a partially used chip is installed on the instrument, follow these steps to perform a Clean instrument procedure as described in page 152, then power off the instrument.
  a. In the home screen, tap **Settings** ▸ **Clean Instrument**.
  b. Follow the on-screen instructions to start the cleaning.

  Note: If an alarm appears, follow the on-screen prompts, or contact technical support for further help. Do not continue until the problem is resolved.

  c. After the cleaning completes, power off the system in the home screen by tapping **Settings** ▸ **System Tools** ▸ **Shut down**.
  d. Select **Shutdown**.
  A confirmation message appears. Select **Yes** to power off the instrument.
  e. Turn the power switch located at the back of the instrument to the off (O) position.

Power on after a long-term shutdown

After a long-term shutdown (>28 days), follow these steps to power on the sequencer, as described in “Power on” on page 28.

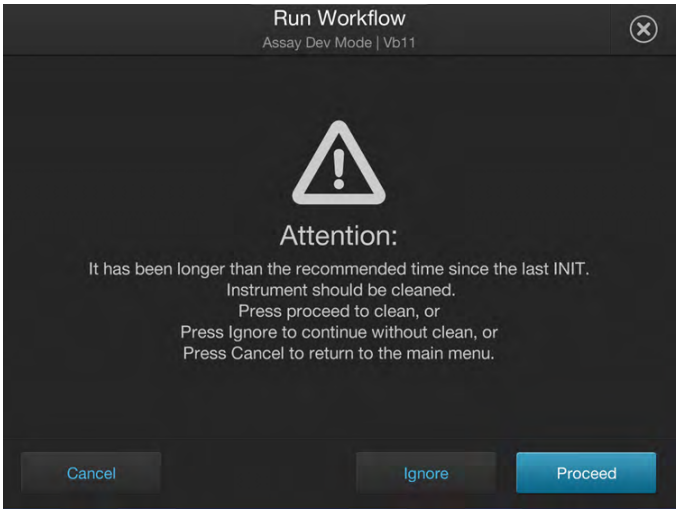
1. Turn the power switch on the back of the sequencer to the on (|) position.
2. Press the power button on the front of the instrument.
The button illuminates.
3. Sign in with your user name and password.
After the home screen appears, the sequencer is ready for use. Check for instrument alarms, if any. If a postchip clean was performed before the system was powered off, the instrument is ready to use.

4. If a postchip clean was not performed before the sequencer was powered off, perform a Clean instrument procedure as described in "Perform a Clean instrument procedure" on page 152.
   a. In the home screen, tap **Settings** ▸ **Clean Instrument**.
   b. Follow the on-screen instructions to start the cleaning.

      Note: If an alarm appears, follow the on-screen prompts, or contact technical support for further help. Do not continue until the problem is resolved.

   c. After the cleaning is complete, the instrument is ready to use.

5. If the instrument was in an initialized state before the system was powered off, and shows the following alarm for an expired initialization, perform the following steps to clean the instrument as described in "Options for an expired sequencer initialization" on page 93.



   a. Select **Cancel** to return to the home screen, then tap **Settings** ▸ **Clean Instrument**.
   b. Follow the on-screen instructions to start the cleaning.

      Note: If an alarm appears, follow the on-screen prompts, or contact technical support for further help. Do not continue until the problem is resolved.

   c. After the cleaning is complete, the instrument is ready to use.

Quantify FFPE DNA with the Qubit™ Fluorometer

When using the Ion AmpliSeq™ Direct FFPE DNA Kit, the DNA concentration can be estimated using a Qubit™ Fluorometer and the Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851). See the *Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. MAN0002326) for more information.

1. Set up the required number of 0.5-mL Qubit™ Assay tubes for standards and samples. The Qubit™ dsDNA HS Assay requires 2 standards.
2. Prepare sufficient Qubit™ working solution for all samples and standards by diluting Qubit™ dsDNA HS Reagent 1:200 in Qubit™ dsDNA HS Buffer.
3. Combine 2 µL of the FFPE DNA sample with 198 µL (200-µL final volume) of working solution, mix well, then incubate for at least 2 minutes.
4. Prepare each Qubit™ standard as directed in the user guide.
5. Measure the concentration of each sample and standard on the Qubit™ Fluorometer.
6. *(Qubit™ 2.0 Fluorometer only.)* Calculate the concentration of the undiluted sample by multiplying by the dilution factor. Alternatively, use the **Calculate Stock Conc.** feature on your instrument.

Proceed to “Dilute or concentrate the samples (if needed) and load the sample plate—sample run” on page 76.

Library QC Archive: recover library preparations from the Genexus™ Integrated Sequencer for reuse

After a sample run completes on the Genexus™ Integrated Sequencer, a variable volume of library prepared during the run is left over, and can be manually recovered from Zone 2 wells (columns 5–8) of the amplification plate. The leftover volume depends on the number of samples used per library strip. If one sample is used per strip, 7 µL is present; if four samples are used per strip, 55 µL is present. The recovered pre-pooled libraries can be purified, quantified, pooled, and reused in a library run in the Genexus™ Integrated Sequencer, or in a sequencing run in another Ion Torrent™ sequencer after template preparation. The recovered and purified libraries can also be archived for later use.

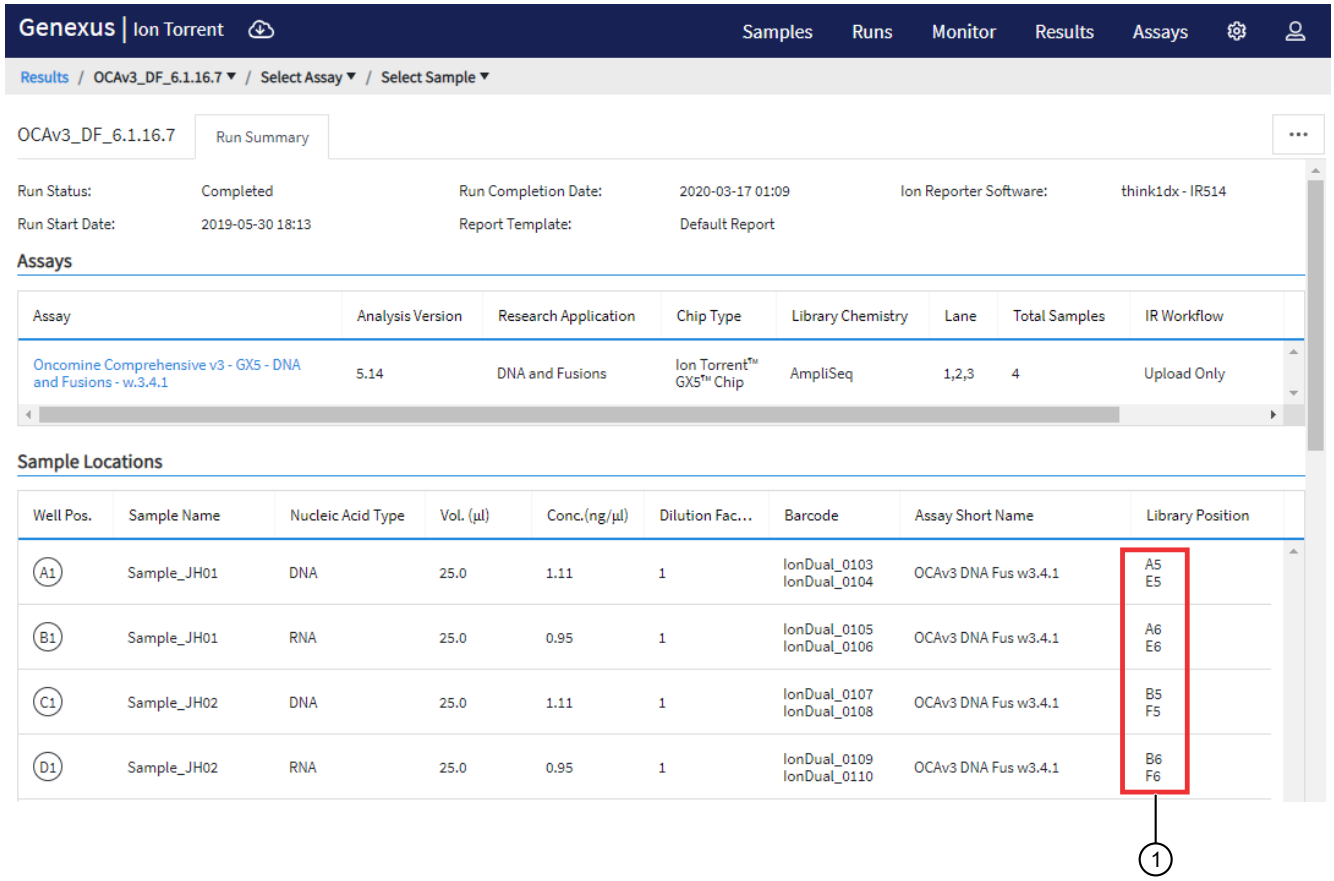
Required materials and equipment

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

Item	Source
Agencourt™ AMPure™ XP Reagent	NC9959336, NC9933872 (**fisherscientific.com**)
DynaMag™–96 Side Magnet, or equivalent	12331D
Ethanol, Absolute, Molecular Biology Grade	BP2818500 (**fisherscientific.com**)
Nuclease-free Water	AM9932
Low TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)	—
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes, 1.5-mL	13-698-791 (**fisherscientific.com**)
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
MicroAmp™ Clear Adhesive Film	4306311
Ion Library TaqMan™ Quantitation Kit	4468802
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument, or equivalent	4351106

Recover libraries from the sequencer and purify

After a sequencing run, leftover volume from each library prepared from a sample and primer pool (7–55 µL, depending on the number of samples used per library strip) is present in the plate loaded in the PCR amplification station. The library order in the plate is provided in the **Run Summary** tab (**Results** ▸ **Run Results** ▸ **Run name**). The following figure shows the library positions for two samples in a run using the Oncomine™ Comprehensive Assay v3 GX with two primer pools each for DNA and RNA.



① Library position in PCR plate

The aqueous library layer is overlayed with approximately 25 µL of mineral oil. Use the following procedure to recover and purify the libraries.

Before you start the procedure, do the following.

- Prepare sufficient 70% ethanol to have 400 µL for each library to be recovered and purified.
- Warm the Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the suspension slowly.

IMPORTANT! Do not substitute a Dynabeads™-based purification reagent for the Agencourt™ AMPure™ XP Reagent.

1. Remove the PCR amplification plate from the sequencer deck, then transfer 30 µL of the lower aqueous layer to a new PCR plate

   Note:
   - If a well contains less than 30 µL, transfer the entire aqueous volume.
   - It is not critical if you transfer some of the oil overlay. The oil does not interfere in the purification procedure.

2. Add 30 µL of Nuclease-free Water to each well with library.

3. Add 60 µL (1X sample volume) of Agencourt™ AMPure™ XP Reagent to each library, then pipet up and down 5 times (with the pipettor set to 60 µL) to mix the bead suspension with the DNA thoroughly. Visually inspect each well to ensure that the mixture is homogeneous. Use a new pipette tip for each library.

4. Incubate for 5 minutes at room temperature.

5. Place the plate in a magnetic rack such as the DynaMag™-96 Side Magnet, then incubate for 2 minutes, or until the suspension clears.

6. Carefully remove the supernatant without disturbing the pellet, then discard the supernatant.

7. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet for 30 seconds to wash the beads.

   Note: If your magnet does not have two positions for shifting the beads, remove the plate from the magnet and gently pipet up and down 5 times (with the pipettor set at 100 µL), then return the plate to the magnet and incubate for 2 minutes or until the solution clears.

8. Carefully remove the supernatant without disturbing the pellet, then discard.

9. Repeat step 7 and step 8 for a total of two 70% ethanol washes.

10. Ensure that you remove all ethanol droplets from the wells. Keeping the plate in the magnet, air-dry the beads for 5 minutes at room temperature.

    IMPORTANT! Residual ethanol can inhibit later reactions. If needed, centrifuge the plate and remove remaining ethanol before air-drying the beads. Under conditions of low relative humidity the beads air-dry rapidly. Do not overdry.

11. Remove the plate from the magnet, then add 50 µL of Low TE to each pellet to disperse the beads.

12. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.

13. Incubate the plate for at least 2 minutes at room temperature.

14. Place the plate on the magnet for at least 2 minutes.

15. Remove the supernatant, which contains your purified library, and transfer it to a new labeled Eppendorf LoBind™ tube.

Proceed to “Quantify the purified libraries”.

Quantify the purified libraries

1. Dilute 2 µL of each purified library with 198 µL Nuclease-free Water for a 100-fold dilution.
2. Use the dilution to quantify your libraries with the Ion Library TaqMan™ Quantitation Kit. For detailed procedures, see the *Ion Library TaqMan™ Quantitation Kit User Guide* (Pub. No. MAN0015802), available at **thermofisher.com**.

Quantified libraries can be combined with sample libraries of similar panel and barcode type (Ion Torrent™ Dual Barcode) and used in library runs on the Genexus™ Integrated Sequencer, or in templating reactions for sequencing on other Ion Torrent™ platforms. For details, see "Combine libraries".

Combine libraries

After quantification, combine libraries that were prepared with different barcodes according to the assay used.

IMPORTANT! Be careful not to combine libraries barcoded with the same barcode adapter.

- For single primer pool assays, and for multiple primer pool DNA *or* fusion assays, adjust sample library concentration to 200 pM, then combine an equal volume of each library so that the total volume is ≥125 µL, or the volume specified in the setup guide for the library run that you plan.
- For DNA and fusion assays, adjust sample library concentration to 200 pM, then follow the recommendations in the table below to combine the DNA and RNA libraries prepared for a given sample in the appropriate ratio. Prepare sufficient volume so that the total volume of combined libraries is ≥125 µL.

Assay	DNA:RNA library ratio[1]	
	FFPE sample	High-molecular weight sample
Oncomine™ Comprehensive v3 - GX5 - DNA and Fusions	70:30	80:20

[1] DNA:RNA library pooling ratio in system-installed assays can be found in the Library Pooling Percent DNA and Library Pooling Percent RNA assay parameters.

Note: For DNA or RNA panels with two primer pools, the fraction of each pool should be equal.

Proceed to "Plan a library run" on page 70 to use the purified and combined libraries in a library run on the Genexus™ Integrated Sequencer. To achieve the required number of reads per sample specified in the assay that is used, follow guidance during library run planning on how many samples you can combine in a single run with the sequencing chip that is loaded.

Store libraries

You can store libraries at 4–8°C for up to 1 month. For longer lengths of time, store at –30°C to –10°C.

Guidelines for using custom assays with the Genexus™ Integrated Sequencer

Follow these guidelines for setting up assays on the Genexus™ Integrated Sequencer if you are using a custom Ion AmpliSeq™ or Ion AmpliSeq™ HD assay.

Ion AmpliSeq™ library chemistry:

- For germline Ion AmpliSeq™ assays, start with a coverage depth of 150 reads per amplicon for calculating the **Minimum Read Count Per Sample** that you enter in the **Panel** step of assay setup.

  Example: Your panel has 500 amplicons in each of two primer pools.

  Minimum Read Counts Per Sample =

  (500 amplicons × 2 pools) × 150 reads/amplicon/sample = 150,000
- For somatic Ion AmpliSeq™ assays, start with a coverage depth of 2,500 reads per amplicon for calculating the **Minimum Read Count Per Sample** when you set up your assay.

  Example: Your panel has 500 amplicons in each of two primer pools.

  Minimum Read Counts Per Sample =

  (500 amplicons × 2 pools) × 2,500 reads/amplicon/sample = 2,500,000
- Refer to the information in the following table for entering the number of target amplification cycles and anneal/extend time parameters for a custom Ion AmpliSeq™ panel.

Primer pairs per pool	Recommended number of amplification cycles (10 ng high-quality DNA/RNA)[1]	Anneal/Extend time[2]
RNA fusion panels	28	4 minutes
12–24	22	4 minutes
25–48	21	4 minutes
49–96	20	4 minutes
97–192	19	4 minutes
193–384	18	4 minutes
385–768	17	4 minutes
769–1,536	16	8 minutes
1,537–3,072	15	8 minutes
3,073–6,144	14	16 minutes
6,145–24,576	13	16 minutes

[1] Add 3 cycles for low quality (FFPE) samples.

[2] For Ion AmpliSeq™ panels using a 375-bp amplicon design, add 4 minutes to the anneal/extend time recommended in the table.

Ion AmpliSeq™ HD library chemistry:

- To calculate the **Minimum Read Count Per Sample** parameter that you enter during assay setup, estimate the coverage depth that you require to achieve the limit of detection (LOD) needed for your samples. The limit of detection depends on coverage depth and amount of input material.

  For more information, see the *Ion AmpliSeq™ HD Library Kit User Guide* (Pub. No. MAN0017392).

LOD	Minimum amplicon coverage
5%	1,400
1%	7,000
0.5%	14,000
0.1%	50,000

  Examples:

  - Your panel has 200 amplicons in each of two primer pools, and you require 5% LOD and 1,400 reads/amplicon for a solid tumor sample.

    Minimum Read Counts Per Sample =

    (200 amplicons × 2 pools) × 1,400 reads/amplicon/sample = 560,000
  - Your panel has 100 amplicons in each of two primer pools, and you require 0.1% LOD and 50,000 reads/amplicon for a cfDNA sample.

    Minimum Read Counts Per Sample =

    (100 amplicons × 2 pools) × 50,000 reads/amplicon/sample = 10,000,000

  Note: These calculations are based on 100% uniformity and 100% on target reads. Depending on the actual uniformity of the panel, the quality of input sample, and panel on-target percentage, minimum read counts per sample may need adjustment.

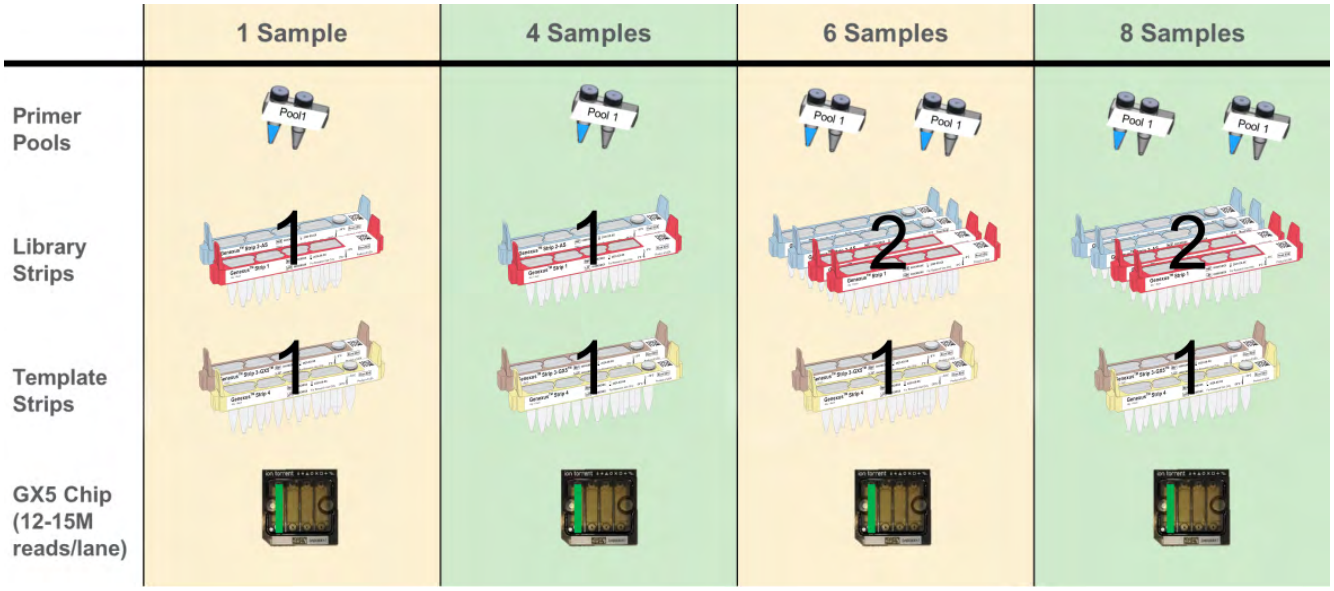
- Refer to the information in the following table for entering the number of library amplification cycles for a custom Ion AmpliSeq™ HD panel.

Primer pairs per pool	Recommended number of cycles
12–500	20
501–1,000	19
1,001–2,000	18
2,001–5,000	17

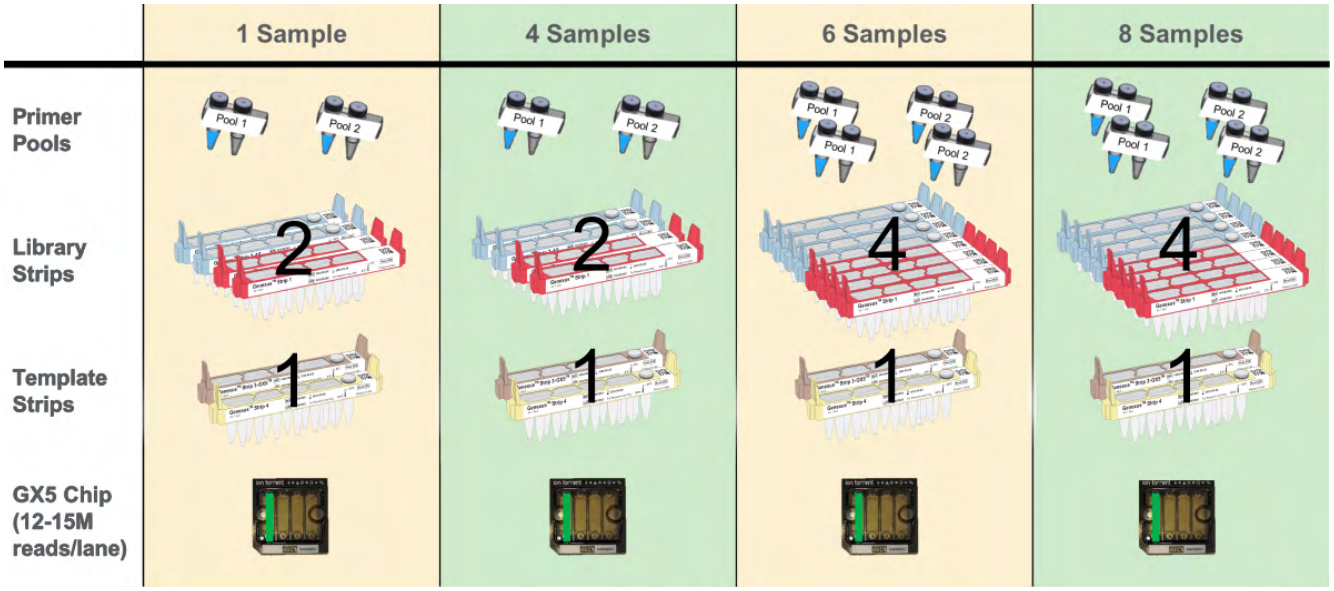
Planning sequencing runs for efficient use of consumables

Genexus™ Integrated Sequencer consumables are designed to be used most efficiently when samples are grouped in multiples of four. For example, running one sample uses the same number of primer pool tubes, library and template strips, and chip lanes as four samples. Running five or six samples requires the same consumables as eight samples. For the most economical use of reagents, therefore, you should try to group samples in your runs in multiples of four, if possible.

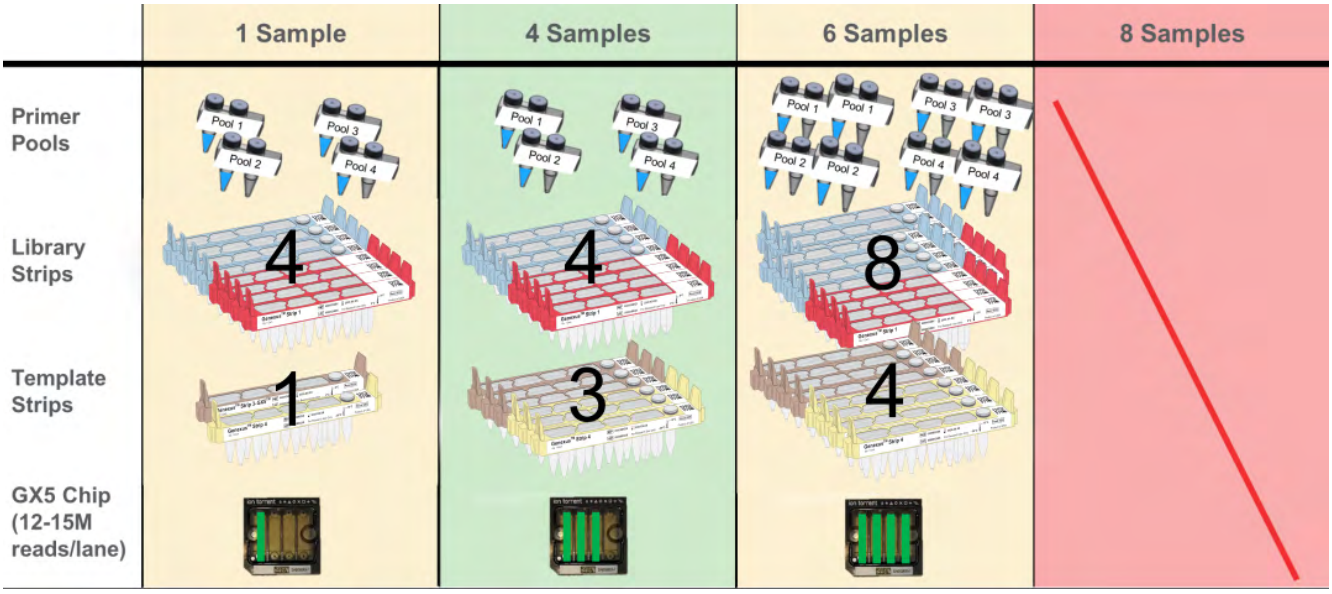
The following figures illustrate the relationship between sample number, the number of pools in an assay, the required number of reads per sample, and the amount of deck consumables required for a sequencing run.



One-pool assay, requiring 500K reads per sample



Two-pool assay, requiring 1M reads per sample



Four-pool assay, requiring 8M reads per sample

Note: Eight samples at 8M reads per sample exceed the capacity of the GX5™ Chip. You can proceed with the run using eight pairs of library strips and four pairs of template strips, but you may not achieve the minimum reads per sample set for the assay.

Configure an Ion Reporter™ Server account (administrator)

An administrator-level user can configure a link to an Ion Reporter™ Server account in Genexus™ Software. When the server is configured, you can upload sample results files from Genexus™ Software directly to the Ion Reporter™ Server and use Ion Reporter™ Software for further data analysis, annotation and reporting. The uploads contain unmapped BAM files.

1. Click ⚙ **(Settings)** ▸ **Thermo Fisher Account**.
2. In the **Thermo Fisher Account Settings** screen, click + **Create Account**.

3. In the **Create Thermo Fisher Account** dialog box, complete the information required to configure the account.

Option	Description
Account Type	Select **IonReporter Local**
Name	A name to identify the account in the list of **Thermo Fisher Account Settings**
User Name	This must a username for a valid Ion Reporter™ Server account.
Password	Enter the password for the Ion Reporter™ Server account.
Server	Enter the name of the Ion Reporter™ Server that you will use to upload sample results files.
Version	Click **Get Ion Reporter Software Versions**, then select the software version.
Port	Enter the port of the Ion Reporter™ Server.
(Optional) **Set as Default Account**	Enable the toggle switch if you want to make this account a default account to upload sample results files.

4. Click **Submit**.

The configured Ion Reporter™ Server account is added to the **Thermo Fisher Account Settings** screen. The account can be selected in the **Setup** step of a run plan to automatically upload sample results files and launch analyses in Ion Reporter™ Software. Completed sample results files can also be uploaded to Ion Reporter™ Software from the **Actions** column in the **Results / Run Results** screen.

The configured account is listed in the **(Settings) / Thermo Fisher Account** screen. A successfully authenticated account has **Active** in the **Status** column. The software version for the Ion Reporter™ Software is listed in the **Version** column.

Tag an Ion Reporter™ Software analysis workflow for use with the IonReporterUploader plugin

If you configure Genexus™ Software for use with Ion Reporter™ Software, you can select an Ion Reporter™ Software analysis workflow to transfer sample results files to and launch an analysis automatically in Ion Reporter™ Software. To limit the number of analysis workflows that are listed as available in the Genexus™ Software, you can tag workflows for use with the IonReporterUploader plugin.

IMPORTANT! If you use the **Tag for IRU** option to tag analysis workflows for use with the IonReporterUploader plugin, you must tag *all* analysis workflows that you want to be available in Genexus™ Software. After you use the tag for the first time, only analysis workflows that include the tag are visible.

1. Sign in to Ion Reporter™ Software.
2. In the **Workflows** tab, click the row of the workflow of interest.
3. In the **Details** pane, click ⚙ **(Actions)** ▸ **Tag for IRU**.

   Note: To remove a previously tagged workflow from the list of workflows that are available for selection in the Genexus™ Software, click ⚙ **Actions** ▸ **Untag from IRU**.

The analysis workflows are available for selection when you perform one of the following actions.

Action	Description
Plan a run	1. In the **Setup** step of run planning, select **Upload BAM files to Ion Reporter™ Software**, then select an Ion Reporter™ Software account and software version. 2. In the **Assays** step, in the row of each assay, the workflow that was tagged for use with the IonReporterUploader plugin is available for selection from the dropdown list in the **Ion Reporter™ Workflow** column.
Upload sample results files to Ion Reporter™ Software after the sequencing run is complete	1. In the **Results / Run Results** screen, in the **Actions** column, click **Upload to IR** in the row of a run. 2. In the **Upload Samples to Ion Reporter™ Software** window, the workflow that was tagged for use with the IonReporterUploader plugin is available for selection from the dropdown list in the **Ion Reporter™ Workflow** column.

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.

   If you are configuring your Ion Reporter™ Software on Connect account, you can also create an account by clicking **Ion Reporter Software on Connect**. This action opens your internet browser to the Connect sign-in page where you can click **Create Account** to navigate to the registration page.

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 Empty.

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.

D coverageAnalysis plugin in Genexus™ Software

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

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.

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.

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.

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.

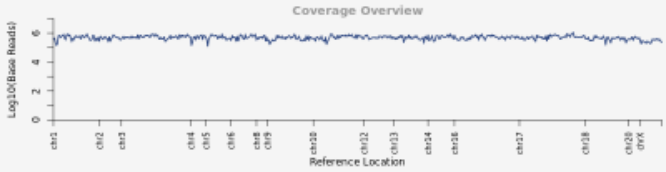
Target base coverage statistics *(continued)*

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 Coverage Analysis Report

The following is an example of a Coverage Analysis Report generated by the coverageAnalysis plugin for an Ion AmpliSeq™ DNA and fusions run. For a DNA and fusions run, the DNA and fusion coverage results are viewable by clicking the respective links in the **Barcode Name** column of the coverageAnalysis results table.

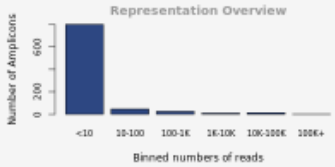
Library type: AmpliSeq DNA and Fusions
Reference: hg19 (DNA)
Target regions: OCAv3.20180426.designedUnmergedDetail



Number of mapped reads	3,248,826
Percent reads on target	94.23%
Average base coverage depth	869.9
Uniformity of base coverage	96.05%

Amplicon Read Coverage		Target Base Coverage	
Number of amplicons	3,781	Bases in target regions	349,355
Percent assigned amplicon reads	94.23%	Percent base reads on target	92.05%
Average reads per amplicon	809.7	Average base coverage depth	869.9
Uniformity of amplicon coverage	96.54%	Uniformity of base coverage	96.05%
Amplicons with at least 1 read	99.95%	Target base coverage at 1x	99.88%
Amplicons with at least 20 reads	99.34%	Target base coverage at 20x	99.31%
Amplicons with at least 100 reads	97.86%	Target base coverage at 100x	97.85%
Amplicons with at least 500 reads	74.50%	Target base coverage at 500x	72.74%
Amplicons with no strand bias	95.29%	Target bases with no strand bias	94.84%
Amplicons reading end-to-end	95.69%	Percent end-to-end reads	91.08%
Amplicon base composition bias	0.714		

Library type: AmpliSeq DNA and Fusions
Reference: OCAv3_designs_022619_Reference (RNA)
Target regions: OCAv3_designs_022619_Reference



Number of mapped reads	601,669
Percent reads on target	100.00%

Amplicon Read Coverage

Number of amplicons	896
Amplicons with at least 1 read	189
Amplicons with at least 10 reads	94
Amplicons with at least 100 reads	48
Amplicons with at least 1000 reads	23
Amplicons with at least 10K reads	14
Amplicons with at least 100K reads	1
Amplicons with no strand bias	882
Amplicons reading end-to-end	49
Amplicon base composition bias	0.036

Example charts generated by the coverageAnalysis plugin

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

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

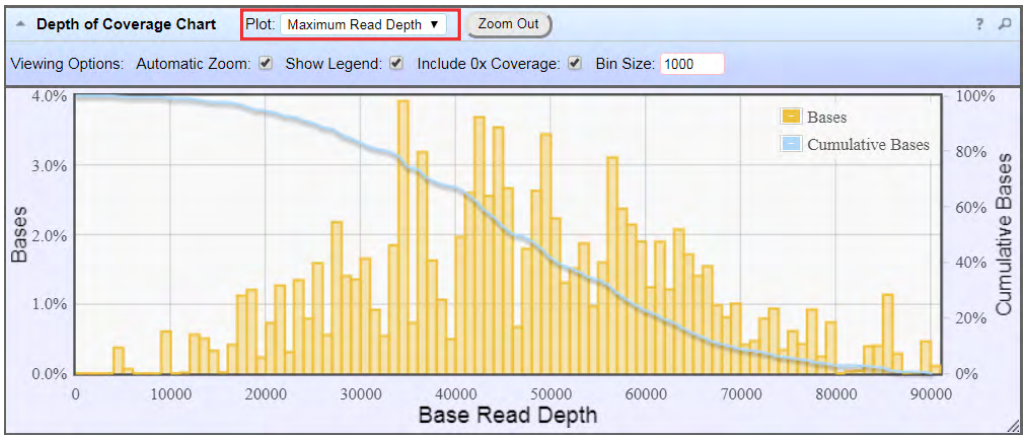


Figure 1 Representative Depth of Coverage Chart

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.

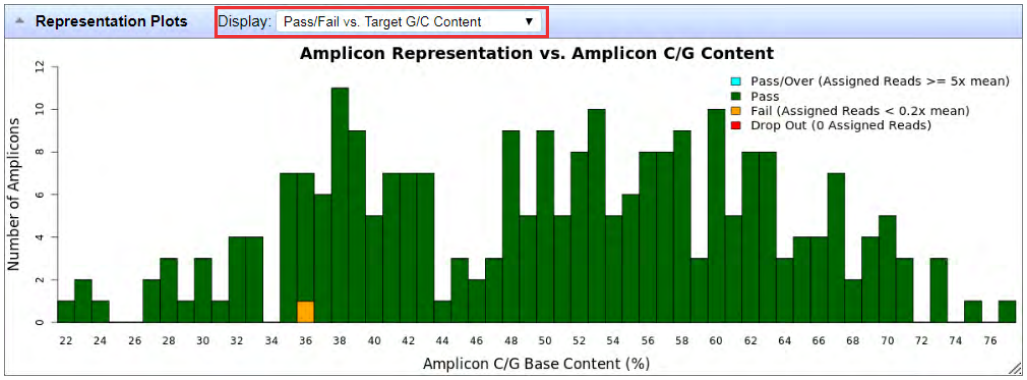


Figure 2 Representation Plots

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 links that are contained in the **File Links** section.

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.

Click ⓘ **(Help)** next to the file to open a description of the file.

The following is an example of the content of a results file that is generated by the coverageAnalysis plugin.

The list of files depends on the application type selected.

File	Description
Coverage statistics summary	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.
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. • `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.

(continued)

File	Description
Amplicon coverage summary	Coverage summary data used to create the Amplicon Coverage Chart. This file contains these 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. 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`: the gene symbol as given as the last field of the targets BED file. • `gc_count`: the number of G and C bases in the target region. %GC = 100% * gc / tlen. • `overlaps`: the number of times this target was overlapped by any read by at least one base. 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 assigned to this amplicon. • `rev_reads`: the number of reverse strand reads assigned to this amplicon. • `cov20x`: the number of bases of the amplicon target that had at least 20 reads. • `cov100x`: the number of bases of the amplicon target that had at least 100 reads. • `cov500x`: the number of bases of the amplicon target that had at least 500 reads.
Chromosome base coverage summary	Base reads per chromosome summary data used to create the default view of the Reference Coverage Chart. This file contains these 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_reads`: the total number of forward strand base reads for the chromosome. • `rev_reads`: the total number reverse strand base reads for the chromosome. • `fwd_ontrg` (if present): the total number of forward strand base reads that were in at least one target region. • `seq_reads`: the total sequencing (whole) reads that are mapped to individual contigs.

(continued)

File	Description
Aligned reads BAM file	Contains all aligned reads that are used to generate this report, in BAM format. This is the same file that can be downloaded from the main report (for the specific barcode). See the current SAM tools documentation for more file format information.
Aligned reads BAI file	Binary BAM index file as required by some analysis tools and alignment viewers such as IGV. This is the same file that can be downloaded from the main report (for the specific barcode).

E

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.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Risk of electrical shock.
CAUTION! Hot surface.

(continued)

Symbol and description
CAUTION! Potential biohazard.
CAUTION! Ultraviolet light.

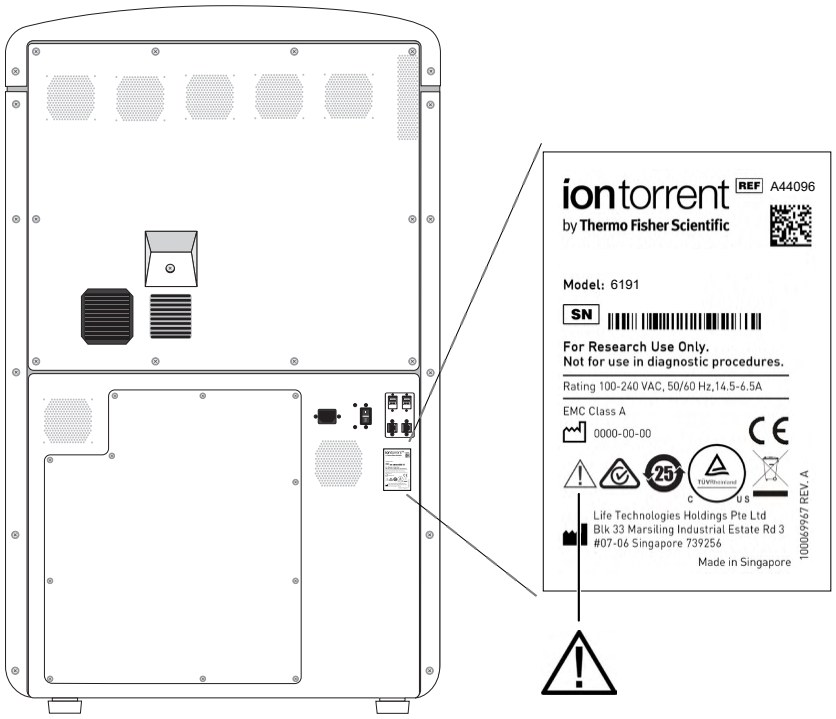
Symbole et description
MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
MISE EN GARDE ! Risque de choc électrique.
MISE EN GARDE ! Surface chaude.
MISE EN GARDE ! Danger biologique potentiel.
MISE EN GARDE ! Rayonnement ultraviolet.

Additional safety symbols

Symbol and description
CAUTION! Moving parts.
CAUTION! Piercing hazard.

Symbole et description
MISE EN GARDE ! Parties mobiles.
MISE EN GARDE ! Danger de perforation.

Location of safety labels

Label and location
 Rear panel

Control and connection symbols

Symbols	Descriptions
	On (Power)
	Off (Power)
	Protective conductor terminal (main ground)
	Alternating current

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with China RoHS requirements.
	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.
	Indicates conformity with the WEEE Directive 2012/19/EU. **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before moving the instrument:

- Depending on the weight, moving may require two or more persons.
- If you decide to move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC 61010-2-020 EN 61010-2-020	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 61010-2-101 EN 61010-2-101	*Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment*

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
EN 61326-2-6 IEC 61326-2-6	*Electrical equipment for measurement, control and laboratory use. EMC requirements. Particular requirements. In vitro diagnostic (IVD) medical equipment*
FCC Part 18 (47 CFR)	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*

(continued)

Reference	Description
ICES-001, Issue 4	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case, you may need to take measures to mitigate the interference. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these can interfere with proper operation.

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
Directive 2011/65/EU	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	“China RoHS” Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at **www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html**.

Radio compliance standards

Reference	Description
Directive 2014/53/EU	European Union “RE Directive”—Radio equipment
RFID	FCC Notice (for U.S. Customers): This device complies with Part 15 of the FCC Rules: Operation is subject to the following conditions: 1. This device may not cause harmful interference, and 2. This device must accept any interference received, Including interference that may cause undesired operation. Changes and modifications not expressly approved by Thermo Fisher Scientific can void your authority to operate this equipment under Federal Communications Commissions rules.

(continued)

Reference	Description
RFID	Canada (English): This device complies with Industry Canada license-exempt RSS standard(s). Operation is subject to the following two conditions: (1) this device may not cause interference, and (2) this device must accept any interference, including interference that may cause undesired operation of the device.
RFID	Canada (Français): Le présent appareil est conforme aux CNR d'Industrie Canada applicables aux appareils radio exempts de licence. L'exploitation est autorisée aux deux conditions suivantes : (1) l'appareil ne doit pas produire de brouillage, et (2) l'utilisateur de l'appareil doit accepter tout brouillage adioélectrique subi, même si le brouillage est susceptible d'en compromettre le fonctionnement.

Chemical safety

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

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

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

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

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

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

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

Biological hazard safety

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

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

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

Documentation and support

Related documentation

Document	Publication number
Genexus™ Software 6.2 User Guide	MAN0018955
Genexus™ Integrated Sequencer Quick Reference	MAN0017912
Oncomine™ Precision Assay GX User Guide	MAN0018508
Oncomine™ Comprehensive Assay v3 GX User Guide	MAN0018512
Oncomine™ TCR Beta-LR Assay GX User Guide	MAN0018513
Genexus™ Integrated Sequencer Site Preparation Guide	MAN0017918
Genexus™ Integrated Sequencer IT Checklist	MAN0018466

Customer and technical support

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

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- 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**.