ion torrent

Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GX

USER GUIDE

For use with the Genexus™ Integrated Sequencer

Catalog Number A51307

Publication Number MAN0024933

Revision B.0

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

ThermoFisher SCIENTIFIC

Life Technologies Corporation |
7335 Executive Way |
Frederick, Maryland 21704 USA

Products manufactured at this site: Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GX

Life Technologies Corporation |
200 Oyster Point Blvd |
South San Francisco, California 94080 USA

Products manufactured at this site: Genexus™ Software

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

Revision history: MAN0024933 B.0 (English)

Revision	Date	Description
B.0	29 January 2024	• Removed references to discontinued TaqMan™ 2019-nCoV Assay Kit v1 (A47532). • Removed references to discontinued TaqPath™ COVID-19 CE-IVD RT-PCR Kit (A51738/A48067). • Updates for Genexus™ Software version 6.8.
A.0	22 July 2021	New user guide for the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX.

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

Contents

1 Product information

Product description

The Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GX (Cat. No. A51307) is a next-generation sequencing (NGS) assay to enable research and surveillance of the SARS-CoV-2 virus, including variants of concern. The assay includes the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel, which contains variant-tolerant primers to broaden and improve the coverage for variants of concern and increase the sensitivity of the panel to enable detection from lower viral titer samples.

The assay enables complete genome sequencing of the SARS-CoV-2 virus on the Genexus™ Integrated Sequencer. The Genexus™ Integrated Sequencer performs library preparation, sequencing, analysis, and reporting in an automated sample-to-result workflow.

The Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX includes the following features.

- >99% coverage of the SARS-CoV-2 genome, covering all potential serotypes
- Internal human gene expression controls included to verify sample quality
- Samples with Ct values >28 can be used to prepare libraries
- Robust SARS-CoV-2 variant calling and reporting with plugin support in Genexus™ Software

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

- *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910)
- *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or *Genexus™ Software Help* (available in the software)

Software compatibility

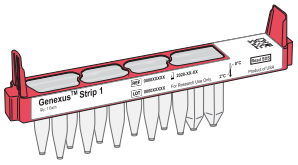
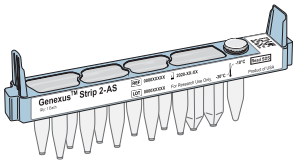
The Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel is compatible with Genexus™ Software 6.8 and later. We recommend that you update your software to the latest available version before using this kit.

Contents and storage

The Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GX (Cat. No. A51307) includes the 2-pool RNA panel (SARS-CoV-2 Insight Panel - Pool 1 and SARS-CoV-2 Insight Panel - Pool 2), Genexus™ Strip 1, and Genexus™ Strip 2-AS. The contents of each kit are sufficient for 16 samples. Each primer pool in the panel is provided in 8 pairs of tubes, where each tube pair contains one tube with primers in position 1 and one empty uncapped tube in position 2. One 8-strip pack containing Genexus™ Strip 1 and Genexus™ Strip 2-AS is provided with each kit.

The Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX consists of two primer pools that target 237 amplicons specific to SARS-CoV-2 and several human gene expression controls. With an amplicon length range of 125–275 bp, the panel provides >99% coverage of the SARS-CoV-2 genome (~30 kb), and covers all potential serotypes.

Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GX (Cat. No. A51307)

Contents	Carrier color	Number of samples	Amount	Part No.	Storage
Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX					
SARS-CoV-2 Insight Panel - Pool 1 (position 1, white cap)	Magenta	16 (4 samples/tube)	4 tubes	A51301	–30°C to –10°C
SARS-CoV-2 Insight Panel - Pool 2 (position 1, white cap)	Pale green	16 (4 samples/tube)	4 tubes		
Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252)					
Genexus™ Strip 1 	Light red	16	8 strips	A46812	2°C to 8°C
Genexus™ Strip 2-AS 	Light blue	16	8 strips	A46813	–30°C to –10°C

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

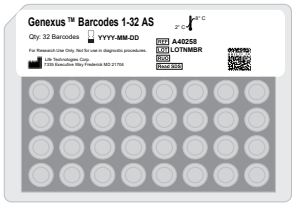
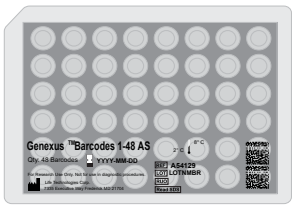
Genexus™ Integrated Sequencer reagents and supplies can be ordered as kits and starter packs. In addition, most consumables can also be ordered individually. This sectiion provides information about the various ordering options.

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

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

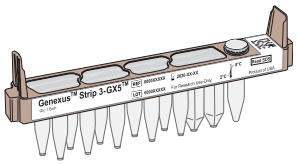
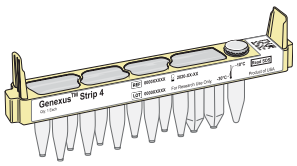
Genexus™ Barcodes AS

Ion Torrent™ Genexus™ Barcodes AS are supplied in plates containing 32 or 48 dual barcodes per plate. The barcodes can be ordered as a set of three 32-barcode 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™ Barcodes 1–48 AS 	Blue	A54129	1 plate	
Genexus™ Barcodes 49–96 AS	Blue	A54130	1 plate	

Genexus™ Templating Strips 3-GX5™ and 4

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

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

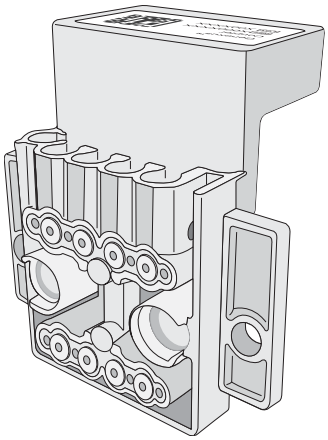
Genexus™ Pipette Tips

The Ion Torrent™ Genexus™ Pipette Tips (Cat. No. A40266) are ordered in packs of 12 racks each. The number of pipette tip racks that are required for your experiment depends on the number of samples included in the run.

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

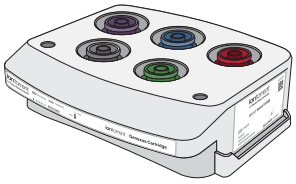
GX5™ Chip and Genexus™ Coupler

The GX5™ Chip and Genexus™ Coupler (Cat. No. A40269) are ordered as a set that contains 2 chips and 2 couplers, sufficient for up to 8 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 (Cat. No. A40271) provides reagents and solutions sufficient to sequence up to 2 full chips.

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

Genexus™ Conical Bottles

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

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

Genexus™ Integrated Sequencer

Components	Cat. No.
Genexus™ Integrated Sequencer	A45727

General laboratory supplies and reagents

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

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

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

Recommended materials

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

Item	Source
Recommended additional equipment	
One of the following Applied Biosystems™ real-time PCR instruments: • 7500 Real-Time PCR System • 7900HT Fast Real-Time PCR System[1] • StepOne™ Real-Time PCR System • StepOnePlus™ Real-Time PCR System • ViiA™ 7 Real-Time PCR System • QuantStudio™ 3 Real-Time PCR System • QuantStudio™ 5 Real-Time PCR System • QuantStudio™ 7 Flex Real-Time PCR System • QuantStudio™ 12K Flex Real–Time PCR System	Various
96-well plate centrifuge	**MLS**
Invitrogen™ Qubit™ 4 Fluorometer[2]	Q33238
Recommended for nucleic acid isolation	
Applied Biosystems™ MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit	A42352 or A48310
Recommended for nucleic acid quantification	
Invitrogen™ Qubit™ dsDNA HS Assay Kit (DNA)	Q32851, Q32854,
Invitrogen™ Qubit™ RNA HS Assay Kit (RNA)	Q32852, Q32855
Applied Biosystems™ TaqMan™ RNase P Detection Reagents Kit	4316831
Recommended for SARS-CoV-2 Quantification	
TaqPath™ COVID-19 Combo Kit	A47814
Recommended for RNA dilution	
THE RNA Storage Solution	AM7000

[1] Supported but no longer available for purchase.

[2] Invitrogen™ Qubit™ 2.0 Fluorometer or later are supported.

SARS-CoV-2 positive controls

SARS-Cov-2 positive controls are available from the following suppliers.

Supplier	Item	Cat. No.
AcroMetrix™/Thermo Fisher Scientific	AcroMetrix™ SARS-CoV-2 Control (RUO) AcroMetrix™ Coronavirus 2019 (COVID-19) RNA Control (RUO)	954517 954519
American Type Culture Collection **https://www.atcc.org**	SARS-CoV-2 External Control Kit	ATCC® MP-32™
BEI Resource Repository **https://www.beiresources.org**	SARS-CoV-2 isolates	Various catalog numbers
Twist BioScience **https://www.twistbioscience.com**	Twist Synthetic SARS-CoV-2 RNA Controls (1-7)	103086; various catalog numbers for individual controls

Workflow overview: nucleic acid sample to results

The following workflow outlines key steps when using the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX to analyze SARS-CoV-2 samples, from sample preparation to data analysis.

Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX

Create an assay (page 25)

System-installed assays that are specifically configured for low- and high-titer sample types are available in Genexus™ Software. You can use the system-installed assays in your run plan without change. If you want to modify any assay settings, contact support for help in editing a system-installed assay.

Enter samples (page 27)

Enter samples in Genexus™ Software to assign sample names and provide other information such as sample collection date.

Plan a run (page 36)

Run plans 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.

Dilute the samples and load the sample plate (page 42)

Dilute your samples, if needed, then load the sample plate.

Load the sequencer and start a run (page 44)

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

Review data and results in the Genexus™ Software (page 57)

View variant calls and visualize results in Genexus™ Software.

2 Before you begin

Before first use—Download plugins and assay files

IMPORTANT!

- If the computer running Torrent Suite™ Software is not connected to the network, you must manually upload the SARS-CoV-2 plugins and Run plan templates. Contact support for help.
- Do not restart your computer when downloading and installing plugin and assay files.

You must download or import the following objects.

- Plugin ZIP files
- Assay ZIP file

Download plugins

You must download the following files from Thermo Fisher™ Connect Platform.

1. Sign in to Thermo Fisher™ Connect Platform (**thermofisher.com/connect**).
2. In the Applications bar, click ⠿.
3. In the AppConnect screen, in the **Resource Libraries** pane, click **Plugins**.

Note: Plugins are downloaded from the Plugins store on Thermo Fisher™ Connect Platform and not the Genexus™ Software section of Thermo Fisher™ Connect Platform.

4. Find the following files in the list.

ZIP file	Files in ZIP file
SARS_CoV_2__Insight_Research_Plugin_Package_GenexusSoftware.zip[1]	• generateConsensus • SARS_CoV_2_annotateSnpEff • SARS_CoV_2_controlStat
SARS_CoV_2_lineageID_5.16.0.10-20210623.zip[1]	SARS_CoV_2_lineageID_5.16.0.10-20210623
GX_SARS_CoV_2_lineageID_20210623_dependency.zip[2]	Dependency packages for SARS_CoV_2_lineageID_5.16.0.10-20210623

[1] Available for Genexus™ Software version 6.2.1 and later.
[2] Available for Genexus™ Software 6.6.0 and later.

5. Click in the row of each plugin to download the plugin to your local storage.
6. Click « to return to the **AppConnect** screen.

Download assay definition files

You must download the following files from Thermo Fisher™ Connect Platform.

1. Sign in to Thermo Fisher™ Connect Platform (**thermofisher.com/connect**).
2. In the Applications bar, click .
3. In the AppConnect screen, in the **Resource Libraries** pane, click **Ion Torrent Genexus**.
4. Find **SARS-CoV-2 Insight Research Assay** in the list, then in the **Download** column, click .
   The following assays are included in the ZIP file for SARS-CoV-2 Insight Research Assay.
   - SARS-CoV-2 Insight Research Assay
   - SARS-CoV-2 Insight LowTiter Research Assay
5. Click in the row of each plugin to download the plugin to your local storage.
6. Click « to return to the **AppConnect** screen.

Import plugins and assay definition files

Contact local field support for assistance to import and upload plugins and assay definition files to Genexus™ Software.

Procedural guidelines

- Use only the reagents and supplies that have been verified for the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX. For a list of verified reagents and supplies, see "Required materials not supplied for use with the Genexus™ Integrated Sequencer" on page 9 and "Recommended materials" on page 13.
- Use good laboratory practices to minimize cross-contamination of products. Keep all tubes sealed until immediately before loading onto the Genexus™ Integrated Sequencer.
- Use controls to identify or rule out reagent contamination. For a list of verified controls, see "SARS-CoV-2 positive controls" on page 14.
- Minimize freeze-thaw cycles of the SARS-CoV-2 Insight Panel - Pool 1 and SARS-CoV-2 Insight Panel - Pool 2 tubes. Thaw only the number of panel tubes that are required for a given experiment and keep the thawed panels at 4°C until ready to use. Store unused panels at –30°C to –10°C.

  Note: One pair of primer pool tubes (SARS-CoV-2 Insight Panel - Pool 1 and SARS-CoV-2 Insight Panel - Pool 2) is sufficient for sequencing 4 samples.

- Do not store the SARS-CoV-2 Insight Panel - Pool 1 and SARS-CoV-2 Insight Panel - Pool 2, Genexus™ Strip 1, and Genexus™ Strip 2-AS on the Genexus™ Integrated Sequencer for more than 24 hours before starting an instrument run.

Before each use of the kit

Thaw assay reagents as indicated:

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

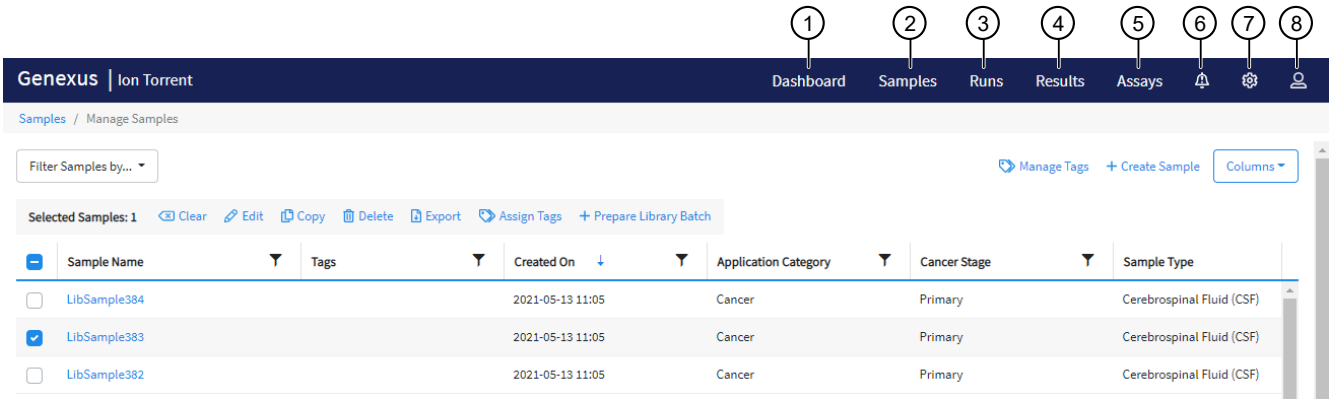
IMPORTANT! Ensure that the strip contents are completely thawed before installing in the sequencer.

When thawed, gently tap tubes and strips on the benchtop to remove any bubbles and collect the contents at the bottom of each tube. For information about preparing all reagents and consumables for loading onto the instrument, see "Before you begin" on page 44.

Note: If tapping fails to dislodge a bubble, you can dislodge large bubbles using the technique that is described in "Before you begin" on page 44.

About Genexus™ Software

Genexus™ Software provides menus to help you add, select, and manage samples, libraries, runs, and assays. You can also view and manage sequencing results, and manage software settings.



① **Dashboard**—View recent run history, and current sequencing run status.

② **Samples**—Add new samples, import samples, prepare library batches, import library batches and manage attributes.

③ **Runs**—Plan a run starting from a nucleic acid sample or a library. View, edit, and manage runs. View, edit, and manage runs.

④ **Results**—View sample results, run results, and verification results.

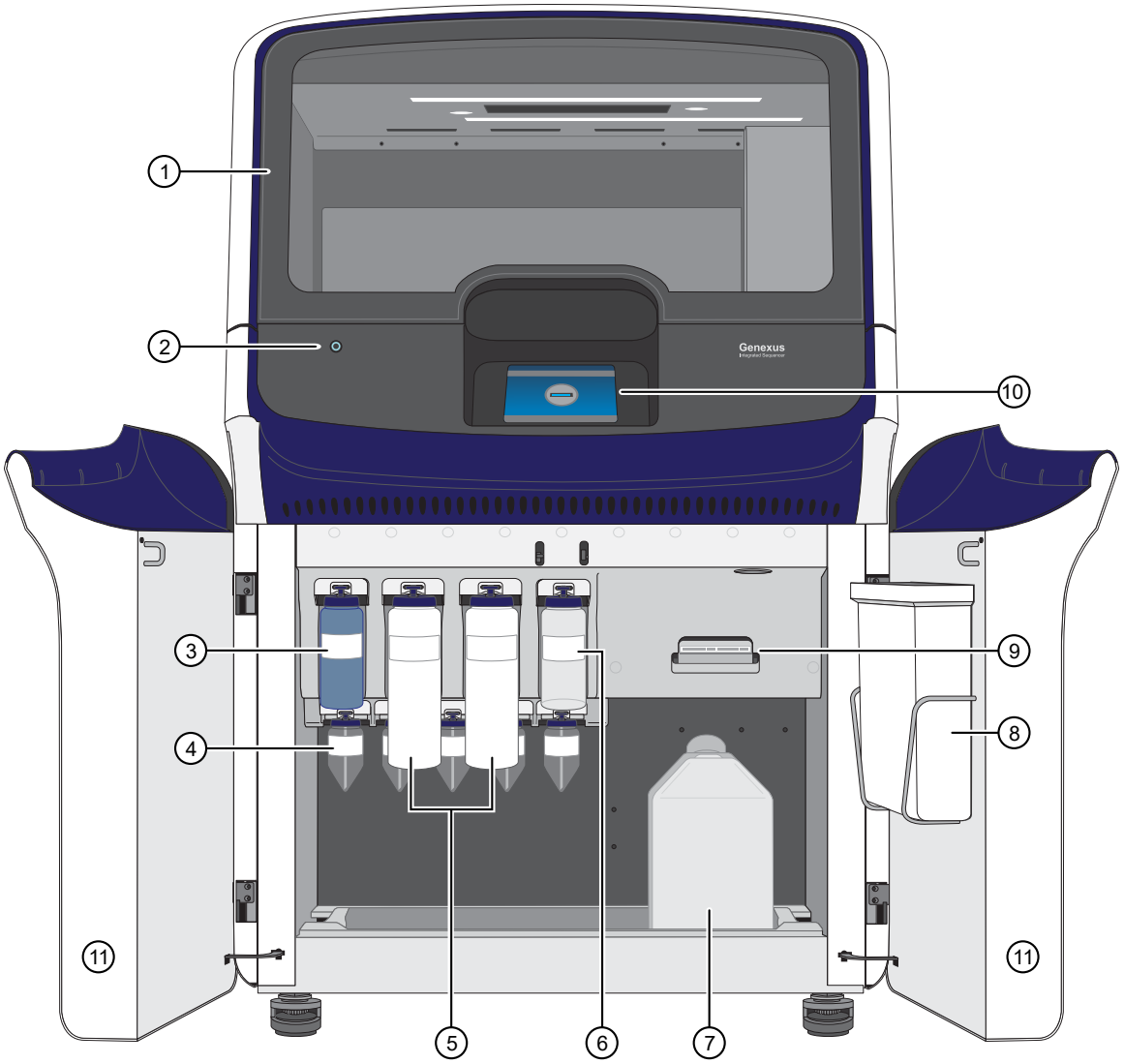
⑤ **Assays**—Manage, create, and import assays. Manage assay preset parameters and panels.

⑥ **Notifications**—Receive alerts and messages for password expiration, system critical service failures, system backup failures, available software updates, and full disk space.

⑦ **Settings**—Access audit records and run logs, configure network settings, manage backup settings, restore runs, manage gene lists, link to Connect user accounts and Ion Reporter™ Software accounts, check for software updates, and manage data archiving, disk space, and users. Field Service Engineers access verification templates during sequencer installation.

⑧ **Profile**—Access the Help system, manage and edit user profile settings, configure an SSH key (system administrator only), and sign out.

Genexus™ Integrated Sequencer components

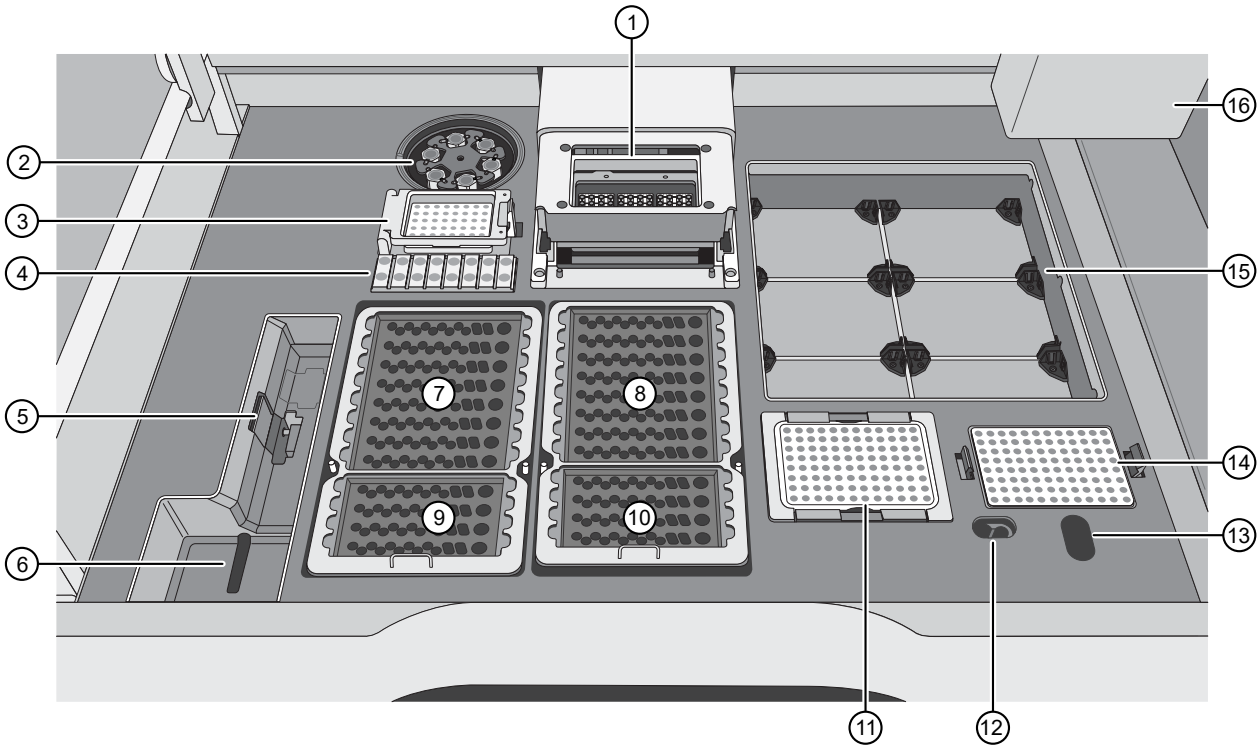


Major features and components of the exterior and sequencing reagents bay of the Genexus™ Integrated Sequencer

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

WARNING! This product contains very strong permanent magnets. People wearing a pacemaker or metallic prostheses should not use this product. A pacemaker or prostheses may be affected or damaged if it comes in close contact with a strong magnetic field.

Genexus™ Integrated Sequencer deck stations



Interior Genexus™ Integrated Sequencer deck components and stations

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

Precautions

Avoid nucleic acid contamination

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

Avoid chip damage

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

Avoid strong electromagnetic radiation

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

Protection by equipment

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

WARNING! This product contains very strong permanent magnets. People wearing a pacemaker or metallic prostheses should not use this product. A pacemaker or prostheses may be affected or damaged if it comes in close contact with a strong magnetic field.

3 Sample preparation guidelines

Guidelines for sample quality, viral RNA copy number, and variant calling

Use the following recommendations and guidelines for assessing the reliability of variant calls in a sample with a given viral RNA copy number and quality.

- The amount of viral RNA among samples should be approximately equivalent so that the target amplification conditions you select are optimal for all samples.
- Ensure that RNA samples were quantified using TaqPath™ COVID-19 Combo Kit (Cat. No. A47814).

Viral RNA copy number	Recommendations and guidelines
100 to 1,000,000 copies	Recommended range for optimal results.
>1,000,000 copies	A sample with more than 1,000,000 copies of viral RNA should be diluted to 1 to 10 for optimal sequencing results.
<100 copies	A sample input that is less than 100 copies of viral RNA may be used, provided that the samples are of high quality, are not degraded, and do not contain inhibitors. We recommend that you ignore heterozygous variants called for sample inputs that are less than 100 copies.

- To reliably sequence low quality samples, the samples must have a viral RNA copy number ≥100 copies. For partially degraded samples, which likely includes low titer samples, the effective copy number that can be amplified by the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX is lower than the viral RNA copy number detected by qPCR because the qPCR products are shorter than the 250 bp fragments generated by the panel.
- Even for samples with viral titer >100 copies, you may observe reverse transcription-derived false positives if you decrease the minimum allele frequency cutoff below 0.2 (20%). Reverse transcription-related errors occur randomly across the genome. To minimize calling false-positives, be certain to amplify a sufficient number of RNA molecules and set the minimum allele frequency to at least 20%.

Copy number determination by qPCR

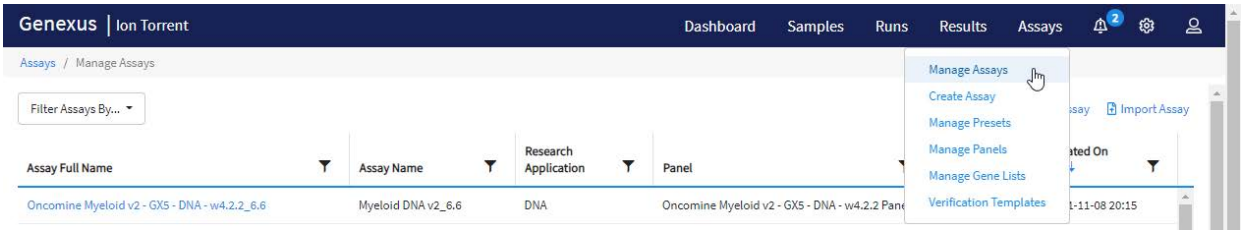
Note:

- If your qPCR data give a different relationship between C_t and copy number, this is likely a result of differences in the baseline or threshold selected. Determine the copy number of a sample according to the known copy number in control samples.
- We recommend that you base copy number on the N Protein C_t value.
- If the N Protein C_t value is not accurate, use the S Protein or ORF1ab C_t values to determine copy number.
- The copy number is only an estimate.
- The tier is used to select the correct system-installed assay (see step 3 of "Plan a library run" on page 39).

Approximate copy number to C_t conversion—TaqPath™ COVID-19 RT-PCR kits

Tier	Viral copy number	TaqPath™ C_t		
		N Protein	S Protein	ORF1ab
High	≥1,000 copies	≤25	≤24	≤24
Low	<1,000 copies	>25	>24	>24

4 Create and manage assays



Manager- and administrator-level users can create and manage assays in Genexus™ Software. This chapter describes how to copy and edit assays, including system-installed assays. If you are using a system-installed Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay without change, proceed to Chapter 5, “Samples and library batches”.

IMPORTANT! If you have updated the SARS_CoV_2_lineageID plugin, you must create a new assay (see “Copying an assay (manager/administrator)” on page 26).

System-installed assays for use with the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX

The following system-installed assays are available in Genexus™ Software 6.2.1 or later for use with the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX. Each system-installed assay is configured with settings that are optimized for viral RNA titer.

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

Assay name	Sample type
SARS-CoV-2 Insight LowTiter Research Assay	Low titer viral RNA
SARS-CoV-2 Insight Research Assay	High titer viral RNA

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

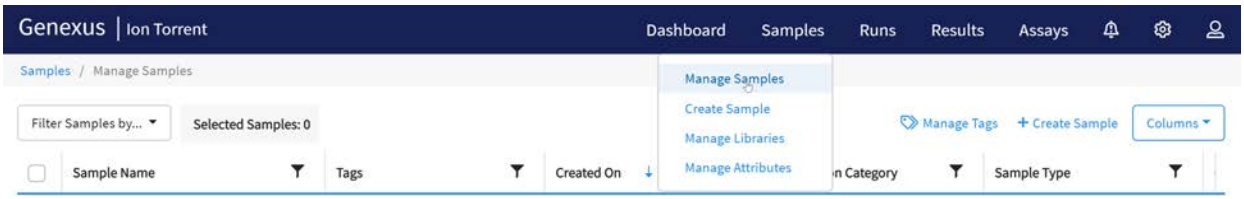
- If you are using a system-installed assay without changes, proceed directly to Chapter 5, "Samples and library batches".
- To modify a system-installed assay with custom settings, proceed to "Copying an assay (manager/administrator)" on page 26.

Copying an assay (manager/administrator)

Manager- and administrator-level users can create a new assay by copying an existing system-installed assay or other custom assay and modifying parameters if needed. Only locked assays can be copied. To copy or edit an assay, see "(Genexus™ Software version 6.6.0 and later) Copy and edit an assay" on page 99 or "(Genexus™ Software version 6.2.1 only) Copy and edit an assay" on page 99.

If you are using a new version of the SARS_CoV_2_lineageID plugin, you must copy the assay to use the new version of the SARS_CoV_2_lineageID. See "Create new copy assay for SARS_CoV_2_lineageID plugin updates" on page 101.

5 Samples and library batches



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

From the **Samples** menu, you can add samples in three ways. You can enter sample information for individual samples, you can import sample information from a file to create multiple samples, or you can upload a BAM file to create samples.

About samples and library batches

In Genexus™ Software, the data and attributes that characterize genomic data are called samples. A sample can be isolated nucleic acid, a specimen that requires nucleic acid isolation, or the sequencing data that are created from a BAM file that contains sample reads. Before you can plan a run to sequence or analyze a sample, you must add the information that characterizes each sample in the software.

A library batch is a group of samples that are sequenced in a Library to Result run. You can create library batches from samples you have previously added or uploaded to the software. During library batch preparation, you identify the barcode adapters that were used to prepare the libraries. After you create a library batch, you can plan and start a run to sequence and analyze the samples in the library.

Create a new sample

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

IMPORTANT! If you are using an NTC control, before proceeding, see "Create NTC sample" on page 100.

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 31.
4. Click **Save**.
   The new sample is listed in the **Manage Samples** screen and is available to use in your run.

Create multiple samples

You can create multiple new samples in Genexus™ Software.

If you are using an NTC control, before proceeding, see "Create NTC sample" on page 100.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, click + **Create Sample**.
3. In the **Create Sample** screen, click the **Sample Definition (.xls)** tab.
4. In **Application Category**, select the application category for the samples.
   A table with columns specific for the selected application category appears.
5. *(Optional)* Click **Columns** in the upper right corner of the screen to customize the optional attributes for the samples you would like to create.
   - Select the checkbox for a sample attribute to add it to the table so that you may enter information for that attribute.
   - Deselect the checkbox for a sample attribute to remove it from the table.
6. Enter the information for a sample.
   Attributes identified with a red asterisk (*) are required.
7. Click **Add Row**, then enter the information in the new row for each new sample.
8. Repeat step 6 for each new sample.

9. Select the checkbox in the row for each sample you would like to create. To select all samples, select the checkbox in the column heading row.

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

10. Click **Save**.
    The new samples are listed in the **Manage Samples** screen and are available to use in a run.

Import samples

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

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

You must create custom attributes before importing sample and run information for the attributes to be propagated through to output files. All attributes that are included in the file that you use to import samples must be either system-installed attributes or custom attributes that exist in the software. Other file content is not transferred with the sample.

You can use a file to import sample information into the software. You can create the file, or use a file that is exported from external LIMS software. Before you import a file from LIMS software, you must first map the sample attribute names that are named differently in the LIMS file to the attribute used in Genexus™ Software.

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

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

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

Option	Description
Download a template file, then edit it to create a new file.	For more information, see "Download a template file for sample creation" on page 31. Upload the edited file using the **Browse** button. **Note:** When you select **Other** as the **Application Category**, you must enter text in the **Application Category** text box, then click anywhere in the screen in order for the template file to be available.
Upload the sample data from an existing file.	a. Click **Browse**. b. Navigate to the file, then click **Open**. The data contained in the file populates the table in the screen.

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

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

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

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

7. Click **Save**.

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

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

Download a template file for sample creation

You can download a template file, add sample information to it, then use the file to import the sample data for multiple samples.

Template files contain two tabs. The **Instruction** tab in the spreadsheet lists and indicates required and optional attributes, which are the column headings in the **Sample Details** tab. Use the **Sample Details** tab to enter sample information.

Template files contain both the system-installed and the custom sample attributes as column headings.

1. In the menu bar, click **Samples** ▸ **Create Sample**.
2. In the **Create Sample** screen, click the **Multiple (.xls, .csv, .tsv)** tab.
3. In **Application Category**, select the application category for the samples.
   Template files are specific for each application category.
4. Click **Download Template** to download the Microsoft™ Excel™ template file.
   The template file contains default sample attributes as columns. If custom sample attributes have been configured in the software, the custom attributes are added to the template file.
5. Save the file to the computer, then open the file, enter sample data in the **Sample Details** tab.
6. When you are finished adding sample information to the file, save the file.

You can now import the file to Genexus™ Software.

System-installed sample attributes

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

(continued)

Sample attribute	Description
Collection Date	The date that the sample was collected. Click **Calendar** to select the date in the correct format.
Gender	The biological sex of the sample: **Female**, **Male**, or **Unknown**.
Sample Type	A term that describes the sample, for example, FFPE, DNA, DNA & RNA. You can also select **Other**, then enter a custom sample type such as ***Swab (Nasal)***.
Disease Category	The disease type of the sample. For SARS-CoV-2 samples, select **Other**, then enter ***SARS-CoV-2***.
Notes	An open-entry field for any additional sample information.

[1] Required attribute

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 28
- "Import samples" on page 29

When you plan the library run, you select the library batch. If you are planning a run starting from nucleic acid samples, skip this step and proceed to "Plan a sample run using the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX" on page 36.

Note:

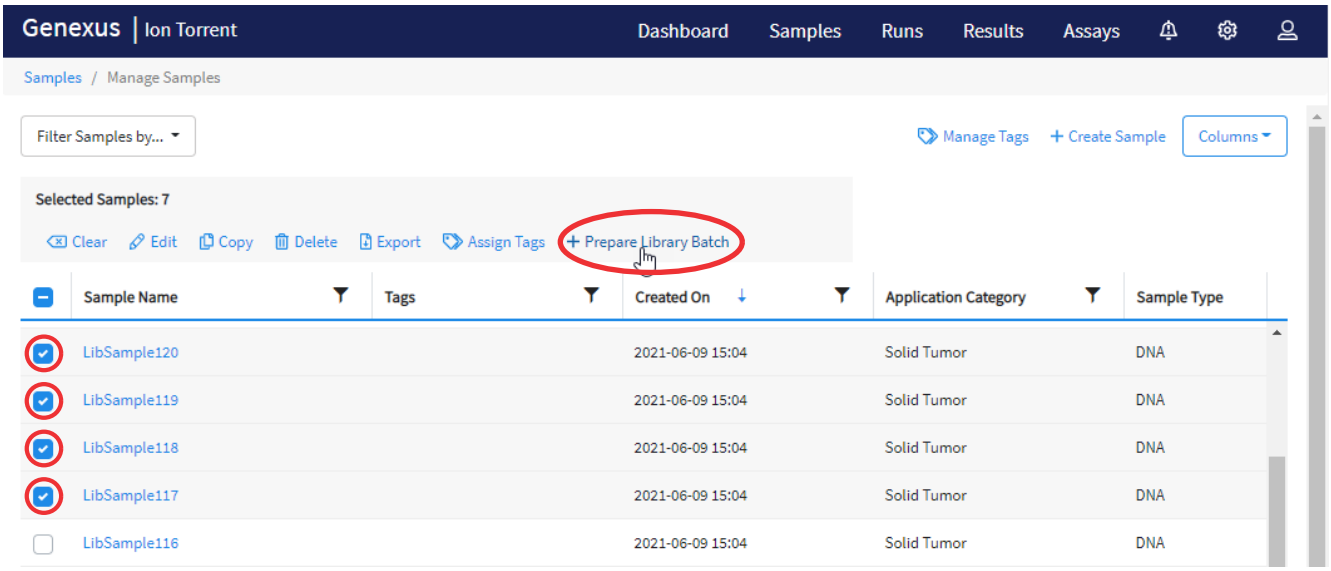
- Each library in a library batch must have a unique library name.
- Fields identified with a red asterisk (*) are required.

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 using the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX" on page 36.

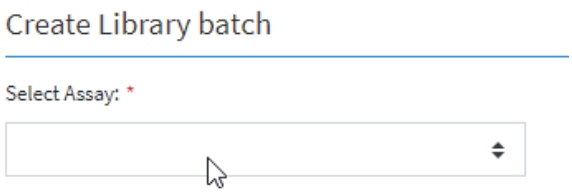
1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, in the **Filter Samples by...** dropdown menu, apply the **To Be Prepared** filter to limit the displayed samples to those samples that have not been placed in a library batch.

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.



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**.

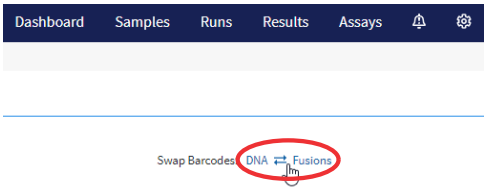
   Library Batch IDs can contain only alphanumeric characters (0–9, Aa–Zz), full stop/period (.), underscore (_), and hyphen (-). Required fields are indicated with a red asterisk (*).

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 Library Batches**.

2. In the **Manage Library Batches** 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.

   If the assay includes more than one library preparation type, a library preparation type dropdown list appears.

4. Select the library preparation type, such as for the library batch.

5. 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.

6. 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 5 (required).
Extraction Method Type	Auto-populated when assay and library prep type, if applicable, is selected in step 5 (required).
Library Batch ID	Must be alphanumeric (0–9, Aa–Zz), period (.), underscore (_), and hyphen (-) (required).
Library Kit Barcode	For example, Genexus™ Strip 1 and Genexus™ Strip 2-AS barcode (optional).
Panel Kit Barcode	For example, Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX barcode (optional).
Libraries tab	
Sample Name	Must be alphanumeric (0–9, Aa–Zz), period (.), underscore (_), and hyphen (-) (required). To Include a no-template control, add a row with ***NTC*** for the **Sample Name**.
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).
Control Kit Barcode	The barcode for the Positive Control and NTC kits used for Sample to Result and Nucleic Acid to Result runs (optional).
Extraction Kit Barcode	The barcode for the kit used for nucleic acid extraction of a sample (optional).

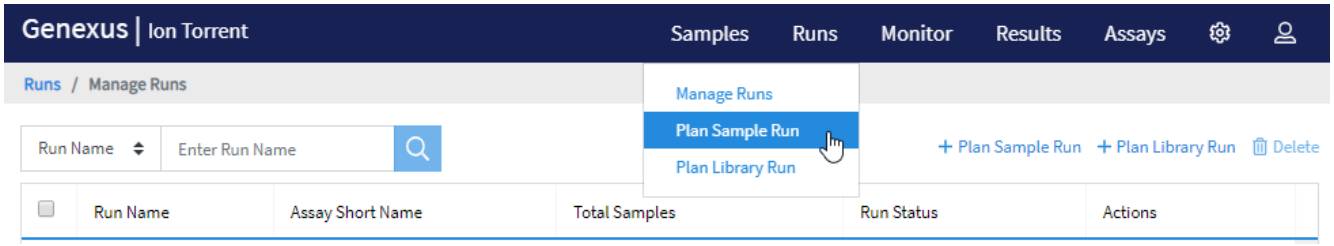
IMPORTANT! For DNA and Fusions assays, the DNA library and RNA libraries must be listed in sequential order per pool for each sample. For example, for a 1-pool DNA and Fusions assay, order should be DNA, RNA for sample 1, DNA, RNA for sample 2. For a 2-pool DNA and Fusions assay, library order should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

7. Save the file.

8. In the **Import Library Batch** dialog box, click **Select Library batch file**, navigate to the saved file, then select it.

9. Click **Upload**.
   A progress bar followed by an import report displays. If the import process fails, click **View errors** to review the reason for failure. The use of an invalid character is an example of an error. For more troubleshooting support, see “Library batch import fails” on page 83.

6 Plan a run



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.

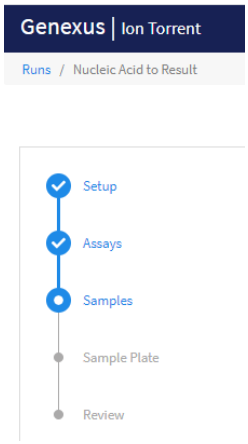
You can plan runs for sequencing runs that use either nucleic acid samples (sample run) or libraries that you have previously prepared manually (library run) as input. Genexus™ Software guides you step-by-step to set up a run that tells you what consumables are needed, and provides a printed run setup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

Ensure that you are using the latest version of the SARS_CoV_2_lineageID plugin (see “Download plugins” on page 16).

Plan a sample run using the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX

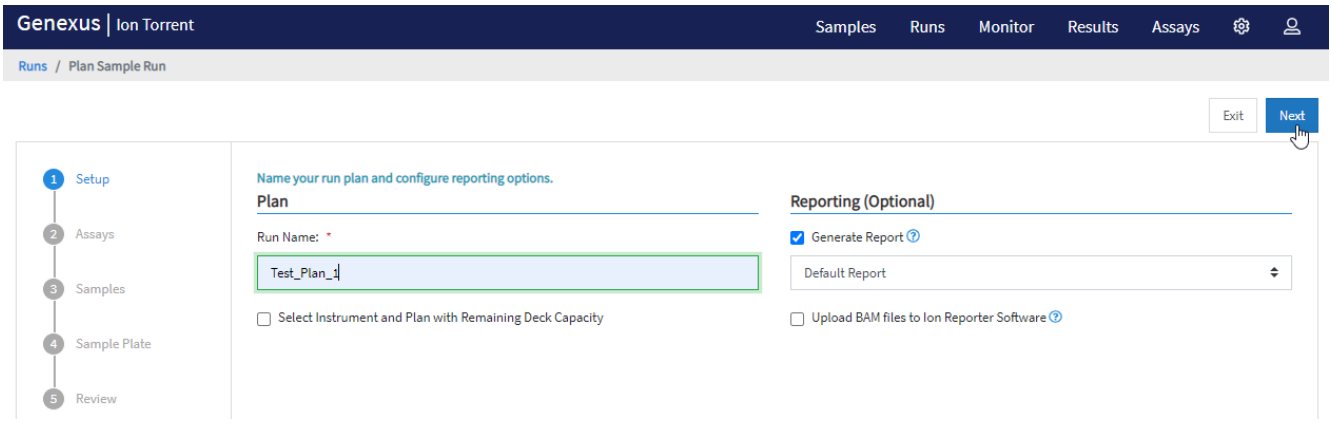
These instructions include specific settings and selections required for a run planned with the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX. For detailed instructions for planning sample runs, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910) or the *Genexus™ Software 6.2 User Guide* (Pub. No. MAN0018955). If you are planning a library run using manually prepared libraries, or using libraries recovered from a Genexus™ Integrated Sequencer sample run, proceed to “Plan a library run” on page 39.

Genexus™ Software guides you through the five steps of planning a sample run: **Setup**, **Assays**, **Samples**, **Sample Plate**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / Plan Sample Run** screen.



Ensure that you have downloaded and installed the appropriate plugins and assay development files. For details, see “Download assay definition files” on page 17.

1. In Genexus™ Software, click **Runs** ▸ **Plan Sample Run**.
2. In the **Setup** step, enter or make selections.
   a. In the **Run Name** field, enter a unique name.



   b. *(Optional)* In the **Reporting (Optional)** section, select **Generate Report** to generate a Lab Report using a report template that you specify in the dropdown list.

Note:
- To create a report template, click **Assays** ▸ **Manage Presets**, then in the **Report Templates** tab, click + **Add New**.
- The option **Upload BAM files to Ion Reporter™ Software** is not needed for analysis of Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX sequencing results.

   c. Click **Next**.

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

3. In the **Assays** step, select the system-installed assay based on the tier of the stock samples (determined in Chapter 3, "Sample preparation guidelines"), then click **Next**.

Tier	System-installed assay
Low (<1,000 copies)	SARS-CoV-2 Insight LowTiter Research Assay
High (≥1,000 copies)	SARS-CoV-2 Insight Research Assay

4. In the **Samples** step, select the samples from the list that you want to run with the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX, then click **Assign**.

   IMPORTANT! We recommend that viral RNA copy numbers differ between samples in a run no more than 1,000-fold (≤10 C_t) to ensure that read counts for lower titer samples are adequate.

   We recommend that you run up to 8 samples per lane (1,500,000 reads per sample). Depending on sample quality and the titer range of samples, up to 16 samples per lane can be run (750,000 reads per sample).

   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. We recommend 1.5 M reads per sample for the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX.

   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. In the **Sample Plate** step, review sample positions in the sample plate. Modify the concentration of samples, if needed, then click **Next**.

   IMPORTANT! If the viral RNA copy number is >1,000,000 copies, we recommend that you dilute the sample 1 to 10 using one of the following methods.

   - Dilute the sample manually. We recommend that you dilute the RNA with THE RNA Storage Solution (Cat. No. AM7000).
   - To have the sequencer dilute the sample for you, adjust the sample concentration in the **Conc. (ng/uL)** column of the run plan to 10 times higher than the default value.

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

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

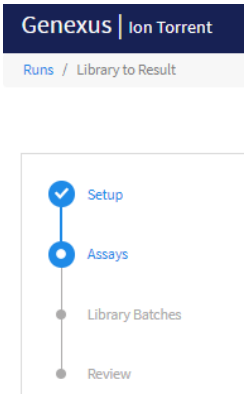
Plan a library run

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 that 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 “Copying an assay (manager/administrator)” on page 26, and “Prepare a library batch” on page 32.

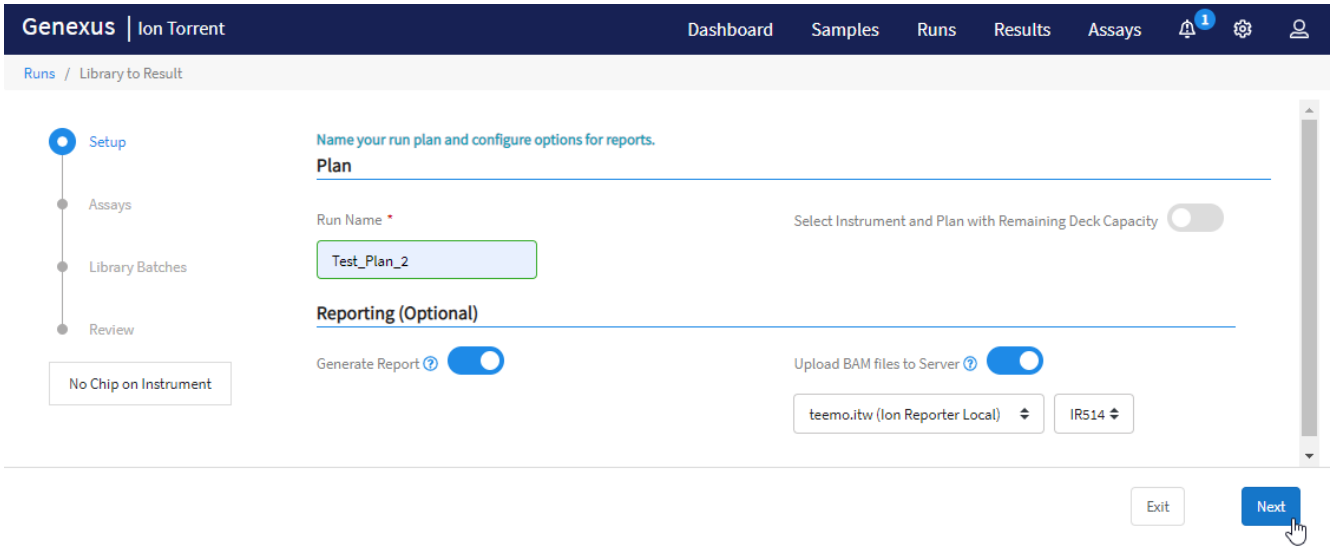
Genexus™ Software guides you through the four steps of planning a library run: **Setup**, **Assays**, **Library Batches**, and **Review**. Progression through the steps is tracked in the upper left corner of the **Runs / Plan Library Run** screen.



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

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

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



   b. *(Optional)* In the **Reporting (Optional)** section, select **Generate Report** to generate a Lab Report using a report template that you specify in the dropdown list.

Note:

- To create a report template, click **Assays** ▸ **Manage Presets**, then in the **Report Templates** tab, click + **Add New**.
- The option **Upload BAM files to Ion Reporter™ Software** is not needed for analysis of Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX sequencing results.

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   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 system-installed assay or custom assay based on the viral copy number in samples (see Chapter 3, "Sample preparation guidelines"), then click **Next**.

Tier	System-installed assay
Low (<1,000 copies)	SARS-CoV-2 Insight LowTiter Research Assay
High (≥1,000 copies)	SARS-CoV-2 Insight Research Assay

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 about preparing a library batch, see "Prepare a library batch" on page 32. The assay specifies the barcode set that was used to prepare the sample libraries.

IMPORTANT! Ensure that you select the assay that corresponds with the sample type to be used in the run. If you select a wrong assay when you plan a run, the instrument uses incorrect settings during the run, resulting in invalid sequencing results.

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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. We recommend 1.5M reads per sample for the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX.

   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.

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

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

7 Load the sample plate

Load the sample plate—sample run

Ensure that the SARS-CoV-2 samples that have been quantified by real-time qPCR (see Chapter 3, "Sample preparation guidelines").

1. Load sample plate wells with 25 µL of each sample as specified in the run setup guide.
2. Seal the plate with a sheet of Adhesive PCR Plate Foils (Thermo Fisher Scientific Cat. No. AB0626).
3. Keep the plate on ice until you are ready to load it in the sequencer.

Guidelines for library quantification—Library to Result 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.

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.

8 Load the sequencer and start a run

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

Before you begin

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

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

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

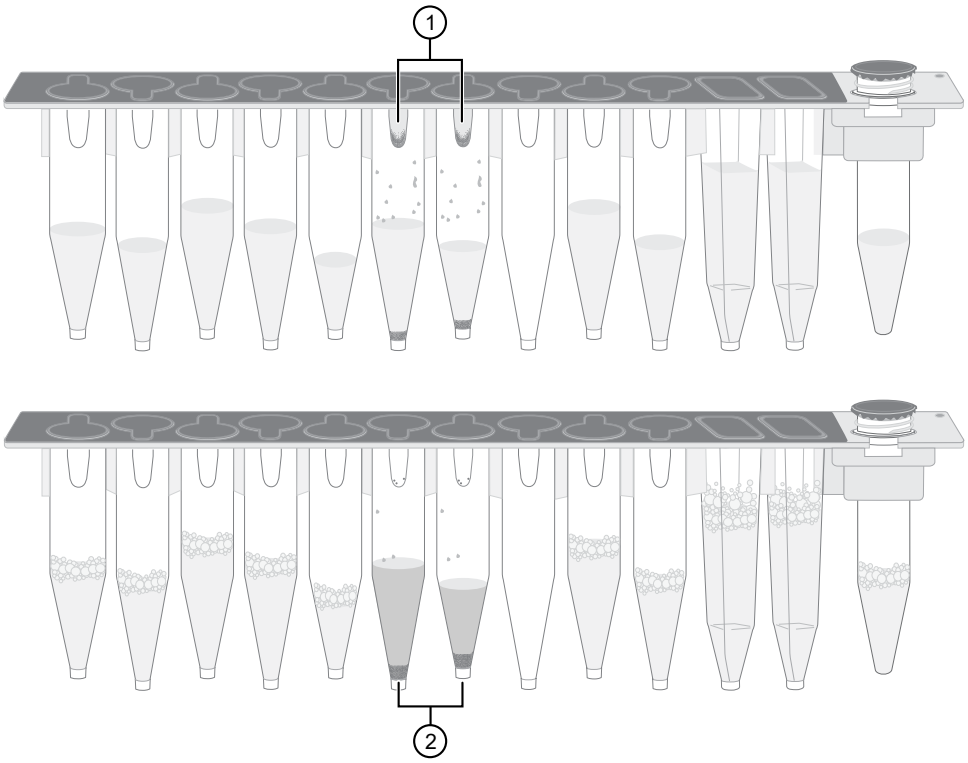
3. If you are installing a new Genexus™ Cartridge, thaw the cartridge at room temperature for 30 minutes before installing in the sequencer.

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

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

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

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



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

① Magnetic beads trapped in keyholes

② Magnetic beads dislodged from keyholes

Note:

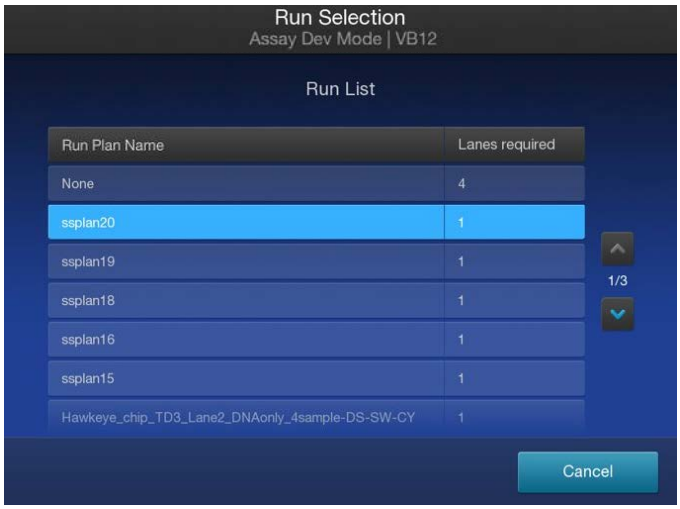
- It is not necessary to resuspend the magnetic beads completely—it is only necessary to dislodge most of the beads that may be trapped in the keyhole. The instrument resuspends the beads during the run when needed.
- Fine bubbles can form above the liquid in some tubes after inversion. These bubbles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

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

Load the sequencer and start a run

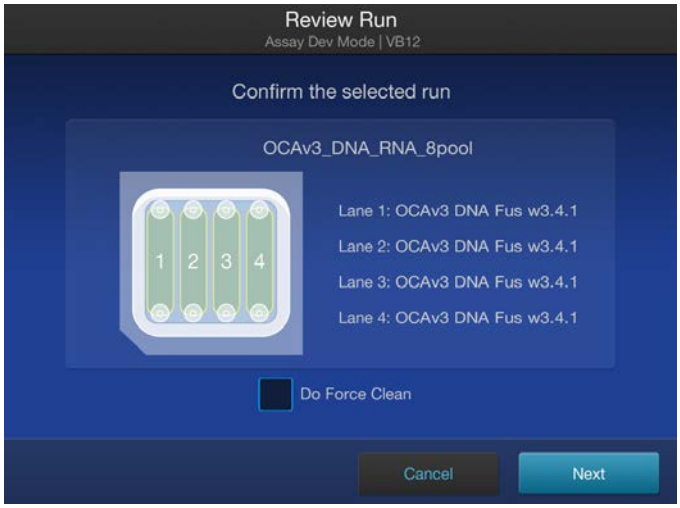
1. Tap **Run** on the sequencer home screen to start the loading procedure.

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



Note: If you select a run that requires more lanes than are available on a currently installed chip, a dialog appears giving you the option to install a new chip, or cancel. If you proceed with a new chip, a post-chip clean 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.

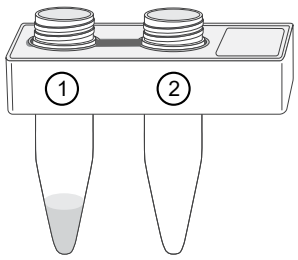
4. In the **Load Deck** screen, the sequencer instructs you step by step to load each required consumable in a highlighted position on the deck. The sequencer detects the loading of each consumable in real time and advances to the next component automatically.

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.

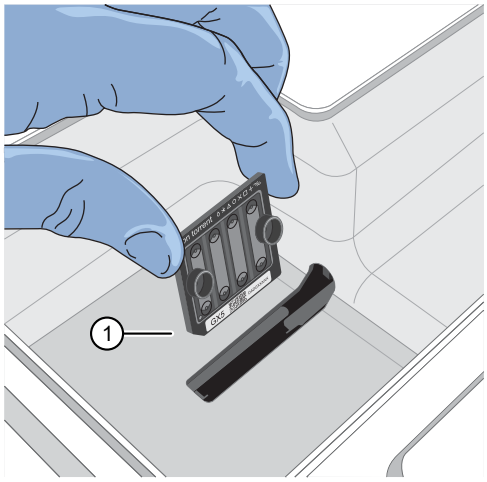
Note:

- 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.

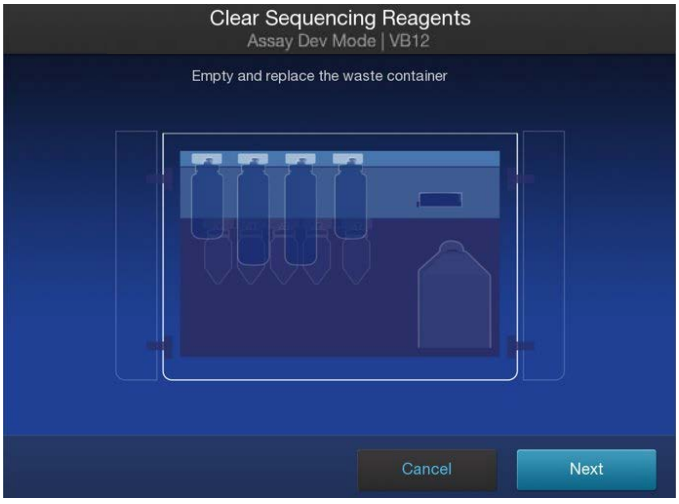
If a chip is detected and the strip latches are closed, the **Close Deck Door** screen appears.

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.

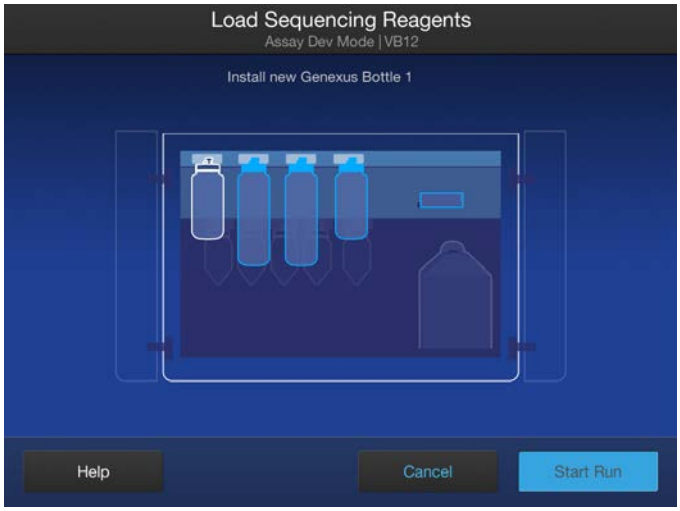
8. Follow on-screen instructions to empty the waste in the Waste carboy, remove waste pipette tips, remove the used Genexus™ Bottle 1, Genexus™ Bottle 2, Genexus™ Bottle 3, and Genexus™ Cartridge, then tap **Next**.



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.

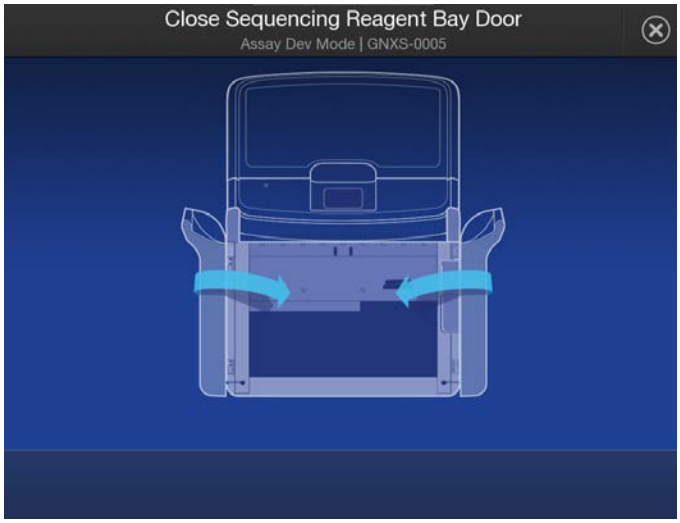
9. Install a new Genexus™ Bottle 1, Genexus™ Bottle 2 (two required), Genexus™ Bottle 3, and Genexus™ Cartridge.



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.

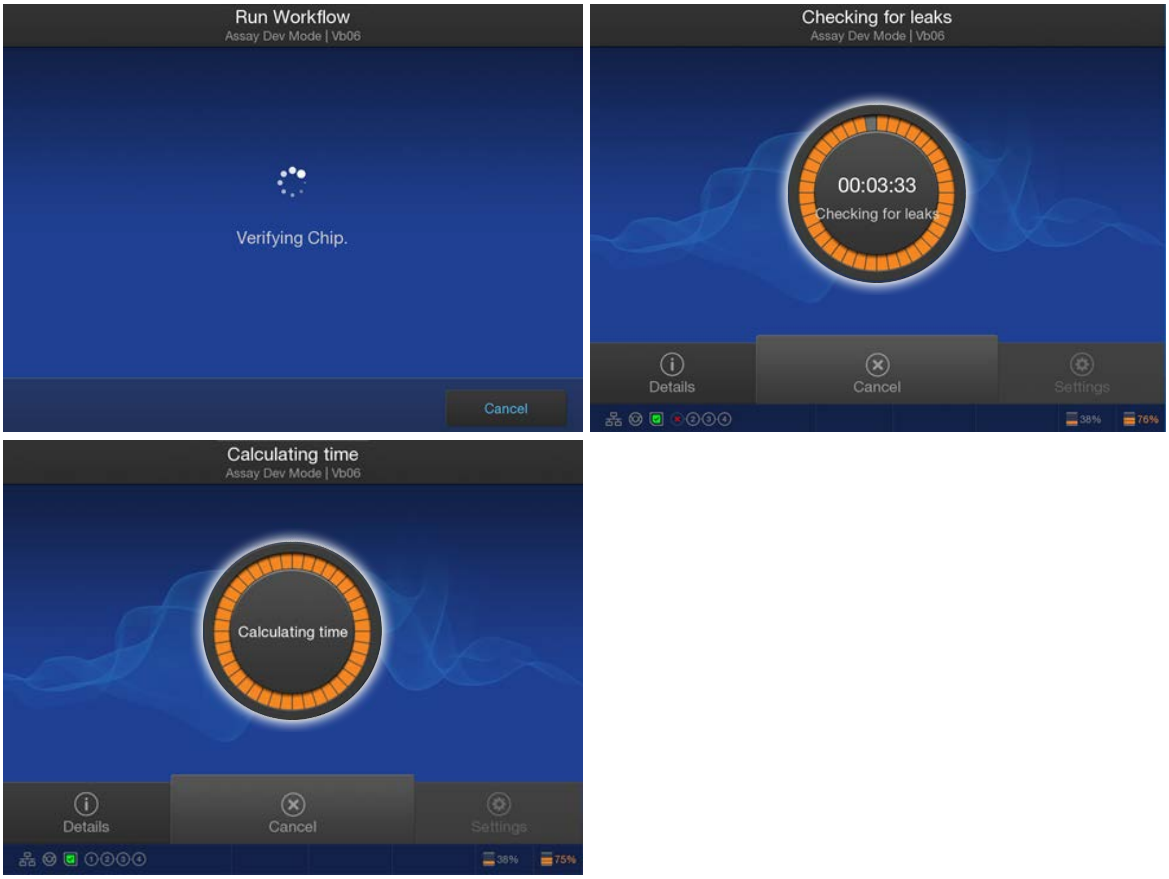
After reagents have been installed, the **Close Sequencing Reagent Bay Door** screen appears.

10. Close the sequencing reagents bay doors.



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

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.

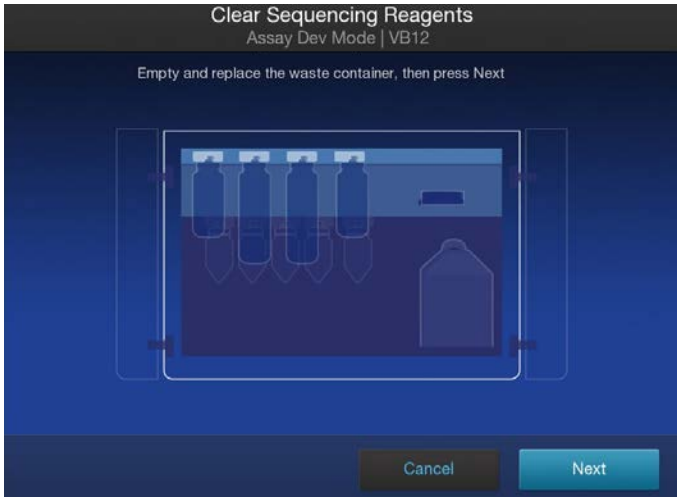
2. In the **Clear Deck** screen, the sequencer provides step-by-step instructions by highlighting the components to be removed. Unlock the library and templating strips by sliding the latches toward the front of the deck, then remove the used strips. Remove the remaining deck components specified by 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 the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).
   - If the Genexus™ Filter does not appear to be clogged, a line clog downstream of the filter is implicated. Contact Technical Support and report a possible deck liquid waste line clog.

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

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

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



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

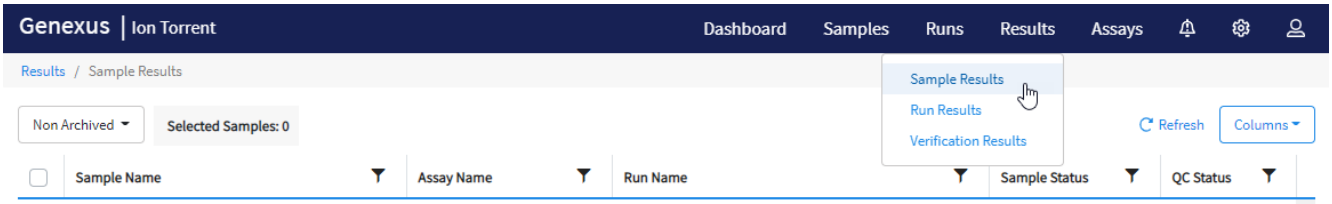
IMPORTANT! Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Genexus™ Integrated Sequencer consumables and liquid waste.

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

MISE EN GARDE ! Le Genexus™ Bottle 1 (petit flacon de récupération de déchets) contient de petites quantités de formamide. Éliminez ces déchets de façon appropriée.

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

9 Review data and results



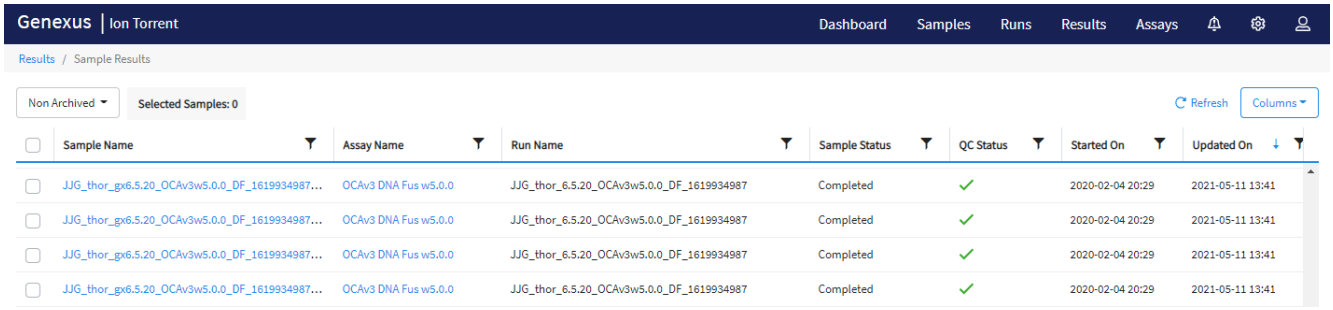
Use the **Results** menu to review results and data analysis, and to perform data management tasks. You can view results sorted by sample or by run.

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

Review 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 76.
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 that the sample passed all QC metrics. • ✘ (Failed) indicates that the sample failed a QC metric. • — (Not Calculated) indicates that 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.

Review run results

In the **Results / Run Results** screen, runs that are pending, running, failed, stalled, aborted, 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**, **Purification Complete**).
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 60.
Started On	The date and time when the run was started.
Updated On	The date and time when the last action was completed on the run.
Actions[1, 2]	Click the appropriate link. To see more actions, click ⋮ **(More Options)**. • **Delete**—Delete the run. • **Audit**—View the audit trail for the run. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting. • **Upload to IR**—Upload sample information to Ion Reporter™ Software for further analysis. • **Assign PCR Plate**—Enter a unique identifier for the 96-well plate used for library preparation and templating. For more information, see “Assign PCR Plate” on page 60.

(continued)

Column	Description
Actions[3]	• **View Plan**—View detailed run plan information. • **Review**—Review samples that do not have a concentration within a specified threshold after purification, but before library preparation. For more information, see the software help system. • **Abort**—Allows you to abort a run after purification, but before sequencing. This action is available when the run status is **Purification Review Required**, or when the run status is **Purification Completed** and some purification samples have been excluded from sequencing.

[1] The actions that are available depend on the type of run.
[2] **Upload to IR** is not available for BAM run results or for archived runs in which BAM files have been removed.
[3] More actions that are available for Sample to Result runs only.

Assign PCR Plate

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

Note: You should assign a PCR plate to a run if there are leftover libraries that you want to use at a later time to perform a Library to Result run. You can look up the PCR plate number on completed runs to get the library and barcode mapping, then enter the same Barcode information into the Library to Result run.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, place the pointer over the row of a run of interest, then click **Assign PCR Plate number**.
3. In the **Assign PCR Plate Number** dialog box, confirm, edit, or enter the **PCR Plate Number**.
   The PCR plate number must be between 1 and 10 characters. Only alphanumeric characters (numbers 0 to 9 and letters A to Z), period (.), underscore (_), or hyphen (-) are allowed. Spaces are not allowed.
4. Click **Submit** to associate the PCR plate with the run.

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 6, "Plan a run"). To generate a Lab Report for each sample after a run is complete, see "Generate a variant report" on page 89.

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

- A link is available in the **Results / Sample Results** screen in the **Actions** column for that sample. Click the link to download the PDF.
- In the **Summary** tab of the **Results** screen for a sample, click ··· **(More Options)** ▸ **Generate Report**.

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 76.

A Lab Report for an Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX assay contains the following sections and information.

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**.
Test Description	A description of the report or assay that was entered in the report template.
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
Reagents	Details about run consumables, including part numbers, expiration dates, and lot numbers.
Control QC Evaluation Metrics	• **Run QC Evaluation Metrics**—a summary of the run quality control metrics • **Sample QC Evaluation Metrics**—a summary of the RNA sample quality control metrics • **Templating Control QC**—a summary of the CF-1 quality control metrics

Download a variant report

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

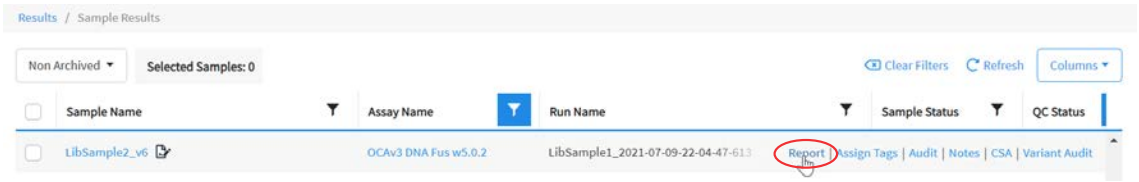
1. In the menu bar, click **Results** ▸ **Sample Results**.

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

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

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

3. In the **Sample Results** screen, place the pointer over the row of the sample of interest, then click **Report**.



   A ZIP file that contains the PDF report downloads automatically.

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

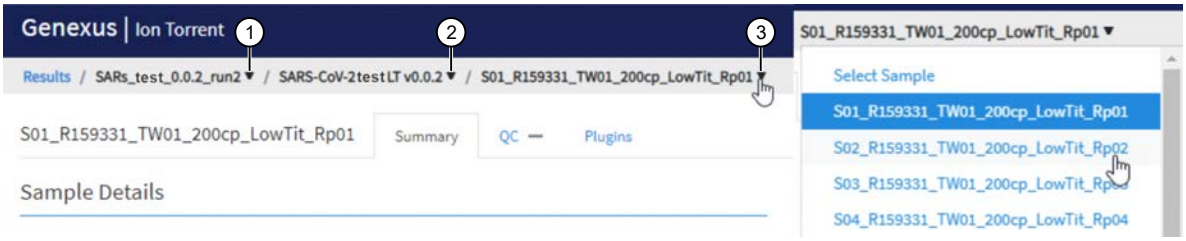
View sequencing results and assay metrics

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 the CustomerSupportArchive and generateConsensus plugin results. Sample-specific results include summary statistics, QC results, and sample-specific plugin results. The following run and sample-specific result information is available in each set of assay and sample result tabs.

Tab	Description
Assay-specific results	
Assay Metrics	Run metrics that apply to all of the samples in an assay, including lane loading, Ion Sphere™ Particle (ISP) statistics, and read statistics for each barcode. For more information, see "View assay results—Assay Metrics tab" on page 65.
Plugins	Results that apply to all of the samples in an assay, including the generateConsensus and CustomerSupportArchive (CSA) plugins. For more information, see "View generateConsensus plugin results" on page 70.
Sample-specific results	
Summary	An overview of the results for the sample, including **Sample Details**, and **Key Metrics**. For more information, see "View sample results—Summary tab" on page 73.
QC	The quality metrics for the sample sequenced in the run. For more information, see "View sample results—QC tab" on page 74.
Plugins	Results generated from sample-specific SARS-CoV-2 plugins used to analyze the sequenced sample. For more information, see "View sample results—Plugins tab" on page 76.

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

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. Click **Select Assay** to return to the **Run Summary** tab for the selected 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. Click **Select Sample** to return to the **Assay Metrics** tab for the selected assay.

Option	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 87.
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. Click **Select Sample** from the dropdown menu to open the **Assay Metrics** tab for the assay shown 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)** at the far right of the **Results** screen.

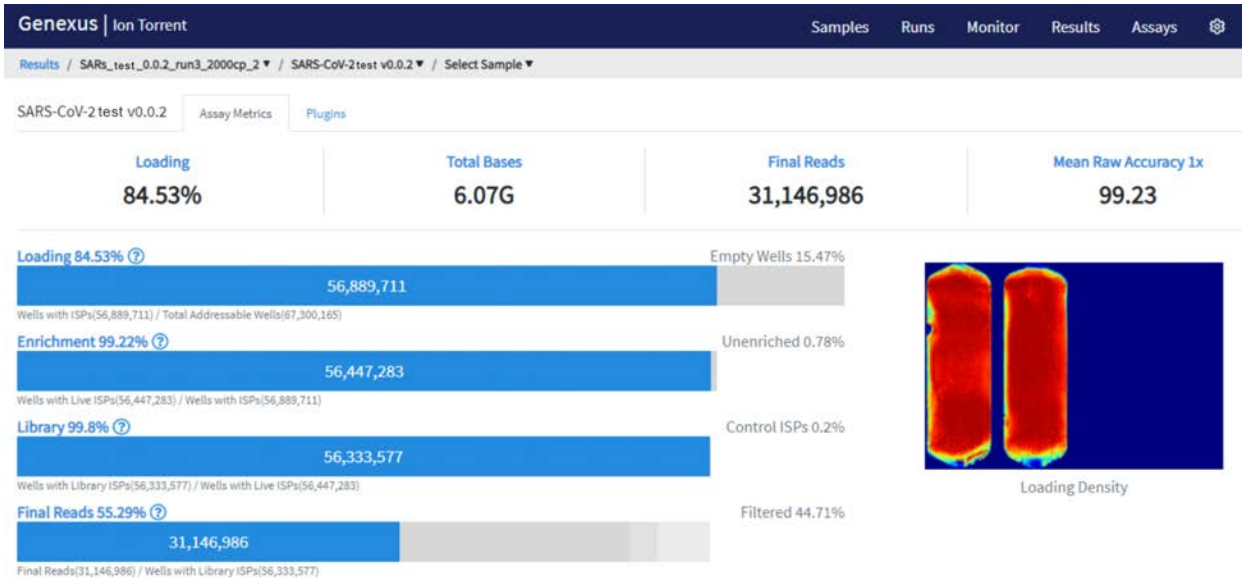
Option	Description
Reanalyze	Reanalyze a run with a new assay. For more information, see “Reanalyze a run” on page 87.
Run Plugin	Run plugins on your sequencing data after a sequencing run is complete.
Run Report	The run report includes assay metrics and the record of reagents that were used in a run. For more information, see “Download a run report” on page 69.
Download Files	Download sequencing results, plugin results, audit and logs, and lab report files. For more information, see “Results files” on page 78.
Generate Report	Generate a Lab Report for each sample in a sequencing run. Plugin results are not reported in the Lab Report. For more information, see “Lab Report” on page 61.
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 76.
CSA	Customer support archive (CSA) log files for the run to help with troubleshooting.

View assay results—Assay Metrics tab

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.

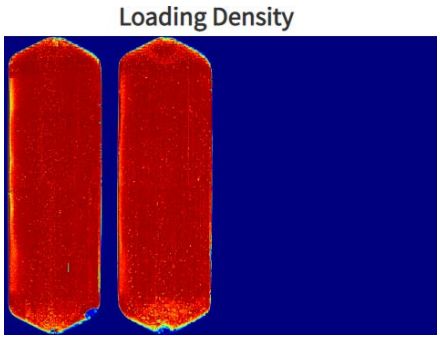


To view the assay metrics for a run, in the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest. In the **Run Summary** screen, select an assay from the **Select Assay** dropdown menu. To view the metrics for another assay in the run, select a different assay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

Assay Metrics

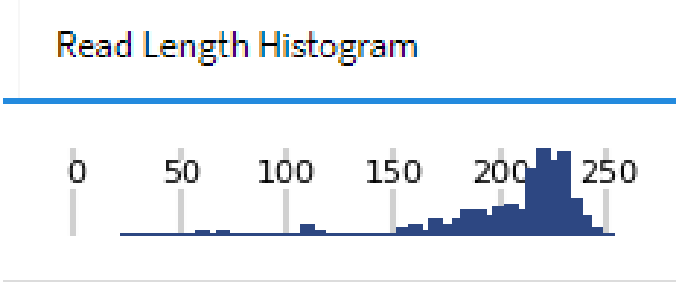
Metric	Description
Loading	The number and percentage of total addressable wells on the chip that contain an ISP.
Enrichment	The number and percentage of wells ISPs that contain live ISPs.
Library	The number and percentage of wells with live ISPs that contain Library ISPs.
Final Reads	Library reads passing 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.
Raw Read Accuracy	The raw read accuracy across each individual base position in a read calculated as, (1–[total errors in the sequenced reads]/[total bases sequenced]) × 100. Raw read accuracy is measured at each base across the length of the read and is based on 1x sequencing coverage; raw read accuracy is *not* based on consensus accuracy across multiple reads for the same base position.
Wells with ISPs	The number of wells that contain an ISP.
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.

Assay Metrics *(continued)*

Metric	Description
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 Out	The total percentage of filtered reads, or the sum of the percentages of polyclonal, low quality, and adapter dimer reads.
Adapter Dimer	Loaded wells with a library template of an insert size less than 8 bases.
Loading Density	A visual representation of chip loading. Red color indicates areas of higher density of loading. Blue color indicates areas of lower density of loading. The following example illustrates a sequencing experiment where two lanes on the chip are uniformly loaded with ISPs. 

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.  Click the **Read Length Histogram** to open an expanded view.

Barcodes with Reads Reported

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

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

Download a run report

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

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

View assay results—Plugins tab

The **Plugins** tab shows results of assay-level plugin results, including CustomerSupportArchive, generateConsensus, SARS_CoV_2_annotateSnpEff, and SARS_CoV_2_lineageID results, that are specified in the SARS-CoV-2 assay to run during the analysis stage of the run. Results for a plugin that is manually executed after completion of a run also appear here (see "View sequencing results and assay metrics", step 5) .

Review SARS_CoV_2_annotateSnpEff plugin results

The SARS_CoV_2_annotateSnpEff plugin generates an annotated list of variants. You can use this plugin to annotate variants and perform multi-sample comparisons.

Additional details, including definitions about parameters, can be found at **https://pcingola.github.io/SnpEff/se_outputsummary/**.

1. Click **Results** ▸ **Run Results** in the menu bar, then click a run name of interest.
2. In the **Run Summary** screen, select an assay from the **Select Assay** dropdown menu, then click the **Plugins** tab.
3. In the **SARS_CoV_2_annotateSnpEff** section, click a barcode name.
4. Click a barcode to see the variant lists with SnpEff annotations.
   The SnpEff summary contains statistics about the variants.

5. To see a SnpEff report for a barcode, click **Review** in the **SnpEff Report** column or click the barcode, then click **snpEff_summary.html**.

   An HTML file of the results opens in a new browser window.

6. To download a ZIP file with summary reports for all variants, scroll to the bottom of the section, then click **SARS_CoV_2_annotateSnpEff.zip**.

   The ZIP file contains annotated variants in VCF output files for each barcode.

View generateConsensus plugin results

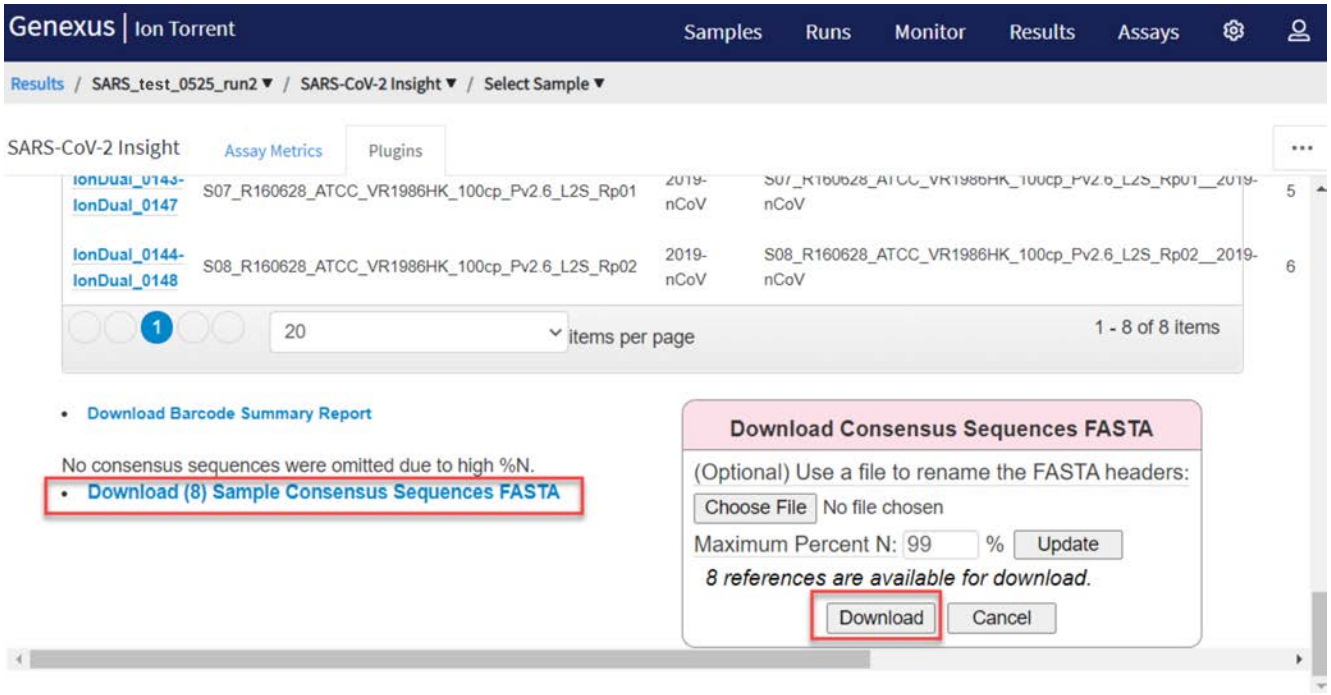
The generateConsensus plugin generates a consensus genome sequence from one or more selected samples using the variantCaller results and outputs multi-FASTA results for direct batch upload to GISAID website.

1. Click **Results** ▸ **Run Results** in the menu bar, then click a run name of interest.

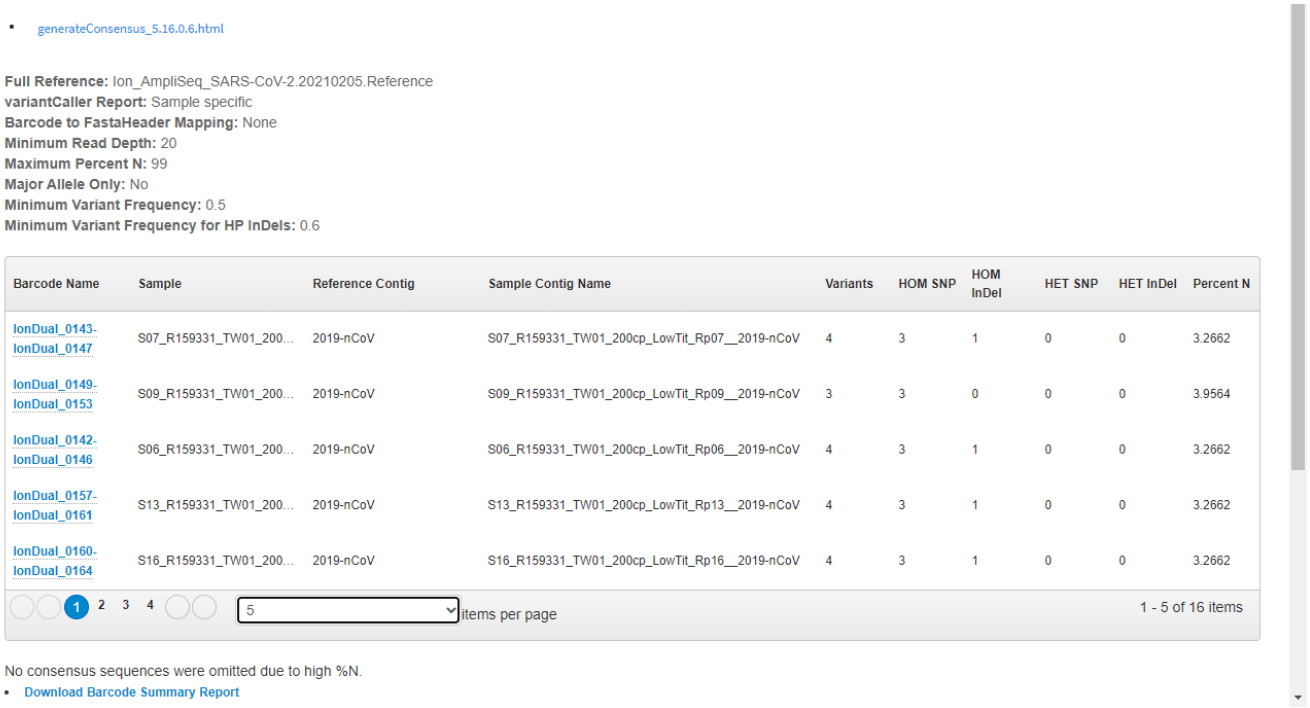
2. In the **Run Summary** screen, select an assay from the **Select Assay** dropdown menu, then click the **Plugins** tab.

3. In the generateConsensus section, click **Download Sample Consensus Sequences FASTA** to download the FASTA files. A dialog box opens that allows you to perform the following actions.
   - Upload a file to rename the FASTA headers.
   - Set a maximum percent N (% of no-call bases in the sample sequence) for the files that you want to download.

   Set an upper limit, if desired, click **Update**, then click **Download**.



4. Click the **generateConsensus.html** link to open a table of generateConsensus results for each sample listed by barcode name. Click **Download Barcode Summary Report** to download a table file for the data shown in the screen.



- generateConsensus_5.16.0.6.html

Full Reference: Ion_AmpliSeq_SARS-CoV-2.20210205.Reference
variantCaller Report: Sample specific
Barcode to FastaHeader Mapping: None
Minimum Read Depth: 20
Maximum Percent N: 99
Major Allele Only: No
Minimum Variant Frequency: 0.5
Minimum Variant Frequency for HP InDels: 0.6

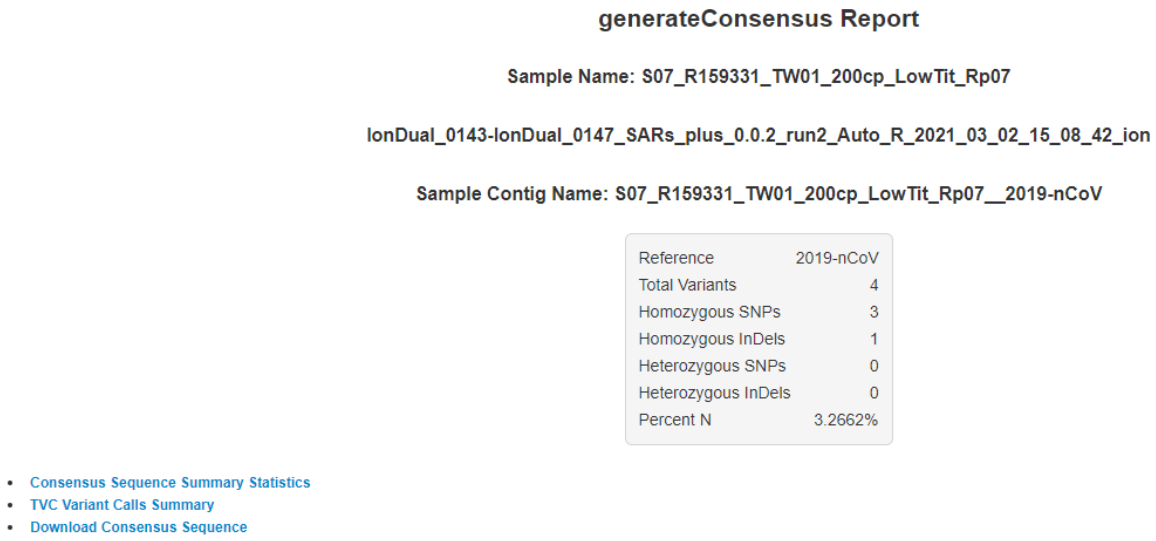
Barcode Name	Sample	Reference Contig	Sample Contig Name	Variants	HOM SNP	HOM InDel	HET SNP	HET InDel	Percent N
IonDual_0143-IonDual_0147	S07_R159331_TW01_200...	2019-nCoV	S07_R159331_TW01_200cp_LowTit_Rp07__2019-nCoV	4	3	1	0	0	3.2662
IonDual_0149-IonDual_0153	S09_R159331_TW01_200...	2019-nCoV	S09_R159331_TW01_200cp_LowTit_Rp09__2019-nCoV	3	3	0	0	0	3.9564
IonDual_0142-IonDual_0146	S06_R159331_TW01_200...	2019-nCoV	S06_R159331_TW01_200cp_LowTit_Rp06__2019-nCoV	4	3	1	0	0	3.2662
IonDual_0157-IonDual_0161	S13_R159331_TW01_200...	2019-nCoV	S13_R159331_TW01_200cp_LowTit_Rp13__2019-nCoV	4	3	1	0	0	3.2662
IonDual_0160-IonDual_0164	S16_R159331_TW01_200...	2019-nCoV	S16_R159331_TW01_200cp_LowTit_Rp16__2019-nCoV	4	3	1	0	0	3.2662

1 2 3 4 — 5 items per page — 1 - 5 of 16 items

No consensus sequences were omitted due to high %N.

- Download Barcode Summary Report

5. In the generateConsensus results table, click a barcode in the leftmost **Barcode Name** column to view the generateConsensus Report for that library.



generateConsensus Report

Sample Name: S07_R159331_TW01_200cp_LowTit_Rp07

IonDual_0143-IonDual_0147_SARs_plus_0.0.2_run2_Auto_R_2021_03_02_15_08_42_ion

Sample Contig Name: S07_R159331_TW01_200cp_LowTit_Rp07__2019-nCoV

Reference	2019-nCoV
Total Variants	4
Homozygous SNPs	3
Homozygous InDels	1
Heterozygous SNPs	0
Heterozygous InDels	0
Percent N	3.2662%

- Consensus Sequence Summary Statistics
- TVC Variant Calls Summary
- Download Consensus Sequence

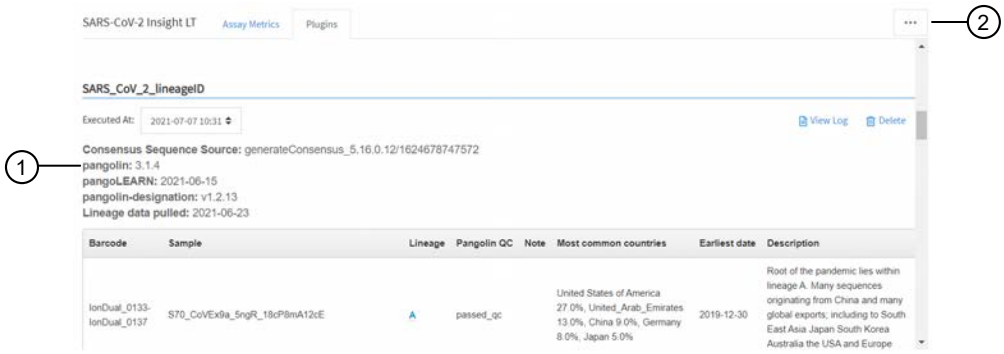
6. Click links for **Consensus Sequence Summary Statistics**, **TVC Variant Calls Summary**, and **Download Consensus Sequence** to review detailed sequence information for the sample.

Run the SARS_CoV_2_lineageID plugin and review results

The SARS_CoV_2_lineageID plugin assigns a lineage to a barcode using the Pangolin software (see **https://cov-lineages.org/**).

Ensure that you are using the latest version of the SARS_CoV_2_lineageID plugin (see “Download plugins” on page 16).

If you are using a new version of the SARS_CoV_2_lineageID plugin, you must copy the assay to use the new version of the SARS_CoV_2_lineageID. See “Create new copy assay for SARS_CoV_2_lineageID plugin updates” on page 101.



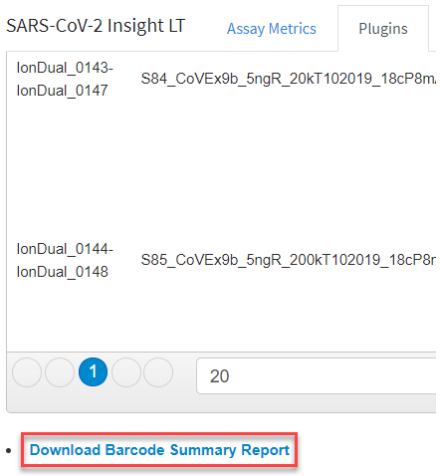
① Header
② More Options icon

The version of Pangolin software is displayed in the header of the SARS_CoV_2_lineageID section. The SARS_CoV_2_lineageID is regularly updated to align with updates to Pangolin software. To analyse results using another version of Pangolin software contact support.

To see the definition of a parameter, place the pointer over the column title.

1. Click **Results** ▸ **Run Results** in the menu bar, then click a run name of interest.
2. Click ··· **(More Options)**.
3. Click **Run Plugin**.
4. In the **Run Plugin** dialog box, select one or more assays in the run, then select the latest version of the SARS_CoV_2_lineageID plugin to run on those assays.
5. Click **Run**.
   While plugins are running, the **Run Plugin** link is unavailable for that run.
6. In the **Run Summary** screen, select an assay from the **Select Assay** dropdown menu, click the **Plugins** tab, then scroll to the **SARS_CoV_2_lineageID** section.
   A summary table of the coverage analysis, by barcode, is included in the SARS_CoV_2_lineageID summary pane.
7. To view Lineage results for a barcode, click the Lineage for that barcode.

8. Click **Download Barcode Summary Report** to download a CSV file with barcode summary results.



View sample results—Summary tab

The **Results** screen displays details about the sample and a summary of the metrics for the sample. You can view the **Results** screen for a sample starting from sample or run results, but navigating from sample results requires fewer steps.

To view sample results in the **Results** screen, click **Results** ▸ **Sample Results** in the menu bar, then click a sample name to open the **Results** screen.

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

Section	Description
Sample Details	
Sample Name	A unique identifier representing the sample. Click the **Sample Name** to open the **Sample Details** screen for the sample. Use the tabs above the Sample Details to view the run summary, assay metrics, quality control, detailed variant results, and results for plugins that are associated with the selected assay, if any.
Collection Date	The date that the sample was collected.
Gender	The biological sex of the sample: **Female**, **Male**, or **Unknown**.
Sample Type	A term that describes the sample, for example, DNA, DNA & RNA, RNA
Disease Category	The disease type of the sample.
Cancer Type	The type of cancer that is represented by the sample.[1]
Cancer Stage	The stage of the cancer from which the sample was collected.[1]
% Cellularity	The percentage of tumor cellularity in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is applicable to only FFPE samples.[1]

(continued)

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

[1] Not applicable to Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX.

View sample results—QC tab

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.

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.

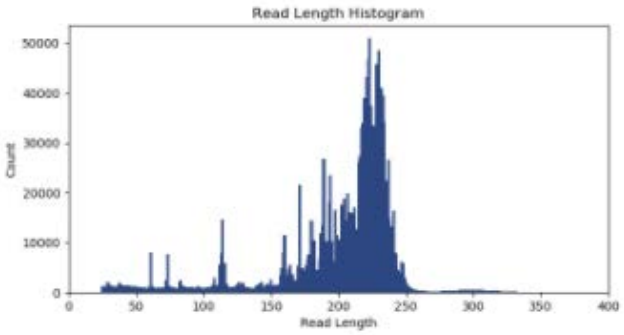
Note: If a sample fails to meet one or more QC parameters, you can reanalyze a run (see “Reanalyze a run” on page 87).

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, and Sample QC–RNA).

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))).

(continued)

Metric	Description
Templating QC—CF-1 Control	**Sequencing quality metrics of the control fragment. These metrics indicate templating success.**
Average Reads Per Lane	The number of CF-1 reads divided by the number of chip lanes used in the run.
Base Call Accuracy	The probability that a given base is called correctly. 1 – (total number of errors for all positions in CF-1) / (total number of CF-1 base reads).
Mean AQ20 Read Length (bp)	Average length, in base pairs, at which the accuracy rate is ≥99% for CF-1 reads.
Key Signal	The average signal after software processing for CF-1 ISPs that identically match the CF-1 key.
Sample QC – RNA	**Sequencing quality metrics of the sample RNA library.**
Uniformity of Base Coverage	The percentage of bases that are covered by at least 20% of the average base coverage depth reads.
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. 

View sample results—Plugins tab

The **Plugins** screen shows sample-level plugin results, including coverageAnalysis results, that are specified in the SARS-CoV-2 assay to run automatically during the analysis stage of the run.

To view sample-specific plugin results, click **Results** ▸ **Sample Results** in the menu bar, click a sample name to open the **Results** screen, then click the **Plugins** tab.

Sample-specific plugins forSARS-CoV-2 sequence analysis

Plugin	Description
SARS_CoV_2_controlStat	Provides QC metrics for the human expression control targets included in the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX, and metrics for the relative expression of SARS-CoV-2 and human control targets in a sample. Click the barcode link to view the report in a new browser tab. Hover on a column heading to see details.
coverageAnalysis	Generates a Coverage Analysis Report that includes read statistics and coverage charts specific to the sample. Click the barcode link to view the report in a new browser tab. For more information, see “coverageAnalysis plugin in Genexus™ Software” on page 93.

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 61. For information on how to create a report template, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

Multilanguage support for PDF report generation is provided. By default, reports are generated in the language that is selected in the **Report Template** used. When reports are generated in multiple languages, **Sign Off** occurs only in the report of the default language.

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, click **Report** to download the report.

Download results files for a sample result

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

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

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

2. In the **Download Files** dialog box, select the files to download, then click **Download**.

   For information about the files, see “Results files” on page 78.

The selected results files are downloaded in one ZIP folder.

Results files

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

For instructions to download results files, see "Download results files for a sample result" on page 77.

Option	File name	Description
Variants		
SmallVariants Filtered vcf File (.vcf)[1]	`SmallVariants.filtered.vcf`	Listing of variant results in variant call format (VCF).
VariantOutput filtered vcf file (.vcf)[1]	`VariantOutput.filtered.vcf`	Listing of filtered variant results in variant call format (VCF).
Sequencing results		
Unmapped Bam File (.bam)	`<barcode>_rawlib.basecaller.bam`	Unmapped barcode BAM file; output after mapping reads to reference.
Mapped bam file (.bam)	`merged.bam`	Mapped BAM file of combined barcode reads.
Mapped Bam Index File (.bai)	`merged.bam.bai`	Mapped BAM Index file.
Basecaller FASTAQ File (.fastq)	`<barcode>_rawlib.basecaller.fastq`	FASTQ file of the barcodes used.
Processed Bam File	`merged.bam.ptrim.bam`	Mapped BAM file of combined barcode reads.
Processed Bam Index	`merged.bam.ptrim.bam.bai`	Mapped BAM index file.
Test Fragment Basecaller FASTAQ File (.fastq)	`rawtf.basecaller.fastq`	FASTQ file for the test fragment.
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.

(continued)

Option	File name	Description
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 61.
SARS_CoV_2_ControlStat		
SARS_CoV_2_ControlStat (.xls)	`<run name>_ion.bc_summary.xls`	Provides QC metrics for the human expression control targets included in the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX, and metrics for the relative expression of SARS-CoV-2 and human control targets in a sample.
Coverage Analysis		
Coverage Statistics (.txt)	`<barcode>_<sample name>_ion.stats.cov`	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 contains a statistic title followed by a colon (:) and its value.
Chromosome Base Coverage Summary (.xls)	`<barcode>_<sample name>_ion.chr.cov`	Base reads per chromosome summary data used to create the default view of the Reference Coverage Chart.
Amplicon Coverage Summary (.xls)	`<barcode>_<sample name>_ion.amplicon.cov`	Coverage summary data used to create the Amplicon Coverage Chart.
Coverage Analysis Summary (.pdf)	`<barcode>_<sample name>_ion.summary`	PDF file showing tables for amplicon read coverage and target base coverage, charts for depth of coverage, amplicon coverage, and reference coverage, and representation plots
Base Depth Of Coverage (.xls)	`<barcode>_<sample name>_ion.base.cov`	Coverage summary data used to create the Depth of Coverage Chart.

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

A

Troubleshooting

Genexus™ Integrated Sequencer—general and QC troubleshooting

Observation	Possible cause	Recommended action
A consumable is not recognized by the sequencer after loading on the deck	The consumable (for example, a strip, pipette tip box) is correctly placed but is not completely inserted into its position, causing it to be misaligned with its expected position.	Ensure that the consumable is pressed completely into place. Apply firm pressure on the item so that it fits snugly into its deck position.
	The barcode of the consumable is not readable by the instrument.	Tap **Help** in the lower left corner of the **Load Instrument** screen and follow on-screen instructions to override the block manually. Note that the name of the consumable does not appear in the list of consumables in the run summary.
		If the behavior continues in subsequent runs, contact Technical Support.
	Consumable version does not match the Genexus™ Software version. For example, a consumable compatible with Genexus™ Software 6.6 is installed in a sequencer updated for Genexus™ Software 6.8.	Ensure that you are using consumables compatible with the software installed on the sequencer.
Run Status = **Failed** **Details:** In the Genexus™ Software **Run Result** screen, the **Run Status** for a completed run is listed as "**Failed**". In the **Sample Results** screen, the **Sample Status** is listed as "**BaseCallingActor FAILED**".	Chip calibration failed due to a chip problem, or an instrument problem.	Repeat the run with a new chip. If the problem persists, contact Technical Support.

Observation	Possible cause	Recommended action
A lane that has been used is not crossed out in the sequencer screen **Details:** After completion of a run, the lane used in the run was not crossed out, so that the next run could reuse the lane.	A chip problem caused a datacollect failure to read efuse.	In the sequencer screen, tap **Settings ▸ Clean instrument** to perform a clean instrument. For details, see See the *Genexus™ Integrated Sequencer User Guide*. After cleaning, start a new run.
The number of sample reads is low, CF-1 metrics pass QC, but read ratio of inline controls is low. **Details:** If CF-1 reads per lane, accuracy, and mean AQ20 read length are good, and read ratio of inline controls (endogenous vs. spike-in) is low (<< 3), a problem with sample input is indicated. For more information, see the *Genexus™ Software Help*.	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 the *Genexus™ Software Help*.	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 44.
	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 the *Genexus™ Software Help*.	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 44.

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 the *Genexus™ Software Help*. *(continued)*	Template strips were inadequately equilibrated to room temperature (Genexus™ Strip 3-GX5™), or incompletely thawed (Genexus™ Strip 4) before loading in the sequencer.	Ensure that Genexus™ Strip 3-GX5™ strips are fully equilibrated to room temperature, and Genexus™ Strip 4 strips are completely thawed before loading in the sequencer.
	The sequencing chip or coupler was defective or leaky.	Repeat the run with new chip and coupler. If low performance continues, contact Technical Support.
	The run was started >14 days after the last initialization was performed, or on an expired initialization.	Perform a Clean instrument procedure (**Settings ▸ Clean instrument**). For more information, see See the *Genexus™ Integrated Sequencer User Guide*. After the Clean instrument procedure, install new a chip, and new sequencing reagent bottles and cartridge in the sequencing reagents bay, then repeat the run. **Note:** Reagents are stable on the sequencer for 14 days, after which you may experience decreased performance. For more information, see *Appendix A: Troubleshooting* in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

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.

Observation	Possible cause	Recommended action
Batch sample import fails *(continued)*	The sample import spreadsheet contains a nonunique sample name.	Every sample name in the software must be unique. Ensure that the spreadsheet does not contain any duplicate sample names, then repeat the import. Note that the system check is not case-sensitive, so a sample name of ABC1 conflicts with abc1.
	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors. Map a sample attribute if needed.
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**.	Each **Library Batch ID** in the software must be unique. Ensure that the spreadsheet does not contain any duplicate IDs, then repeat the import. The system check is not case-sensitive. For example, 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 spreadsheet template that you used is from a previous software version.	New fields in the template file can be added with new software versions. Ensure that you download the template file from the current software version.

Observation	Possible cause	Recommended action
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. The down-sampling threshold for Genexus™ Software variant calling is 400.
Variants tab is missing hotspot entries **Details:** The remaining entries are present.	Hotspot BED file contains entries that are incorrectly formatted.	Check that BED file entry is correctly formatted. See the following examples: SNP entry: chr1 2337276 2337277 SVA_322 0 + REF=C;OBS=T;ANCHOR=G AMPL Deletion entry: chr1 201341175 201341180 SVA_497 0 + REF=AGAAG;OBS=;ANCHOR=C AMPL Insertion entry: chr1 236978992 236978992 SVA_621 0 + REF=;OBS=TCTG;ANCHOR=T AMPL
		Ensure that the REF values match the actual reference coordinate of hg19.
The results of the run do not appear in the **Results / Run Results** screen	The instrument disk space is full.	Clear disk space on the sequencer. For more information, see "*Manually delete run data*" in *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).
Cannot download run result files	The run failed.	Create an assay with the correct configuration for the samples, then reanalyze the samples.

B Supplemental information

Guidelines for Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX settings

In Genexus™ Software, the system-installed assays are configured with settings that are optimized for each sample type (see the Genexus™ Software Help). If needed, you can copy a system-installed assay, then modify settings (see "Copying an assay (manager/administrator)" on page 26). The following table provides guidelines on modifying key assay settings and recommendations for quality control metric threshold values.

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

Workflow step	Setting	Guidelines
Panel	**Minimum Read Count Per Sample**	This value determines the number of samples that are allowed on a chip. The Genexus™ Software uses this value to calculate how many lanes a run plan uses, then assigns samples accordingly. *Minimum Read Count Per Sample = (12,000,000 × number of lanes)÷(number of samples)* To increase or decrease the sequencing depth, adjust the **Minimum Read Count Per Sample** parameter. If higher sequencing depth is desired, decrease the number of samples per lane or increase the number of lanes used.
QC (Sample QC - RNA)	**Mapped Reads**	1,500,000
	Mean Read Length (bp)	>150

(continued)

Workflow step	Setting	Guidelines
Parameters	**Sample input Maximum (ng)**	By default, the target sample concentration for the Ion AmpliSeq™ SARS-CoV-2 Insight Research Panel – GX is set to a single value in the assay settings, where a sample input maximum is set to the same value as sample input minimum. You can modify the parameters to set a sample concentration range by adjusting the sample input maximum and minimum values as needed. The target concentration for a sample is the middle point of the range. When you modify the sample input maximum and minimum values, the **Sample Concentration Maximum (ng)** and **Sample Concentration Minimum (ng)** parameters are auto-adjusted to reflect the new maximum concentration values. **Note:** The sample volume that is required for library preparation is not adjustable. The volume depends on the number of primer pools in the assay, sample type, and library chemistry. For specific sample volumes to load onto the sample plate, see “Dilute and pool libraries, and load the sample plate—library run” on page 42. When you set a range for sample concentration in the assay settings, the instrument does not dilute the sample during the run as long as the concentration of a sample is within the designated range. If the sample concentration is outside of the range that is designated in the assay settings, the instrument dilutes the sample to the target concentration (the middle point of the range). For more information, see “Dilute and pool libraries, and load the sample plate—library run” on page 42.

Reanalyze a run

If a sequencing run fails to meet one or more QC parameters defined by the assay, you can adjust the assay parameters and reanalyze a run. For more information, see .

Reanalysis of runs can start from the alignment, basecalling, or signal processing steps. When you reanalyze a run, the reanalysis is applied to all samples in the assay.

Reanalysis from the	Description
Signal processing step	The option to reanalyze a run from the signal processing step is available only when the following conditions are met. • When a run fails during the basecalling step or earlier. • When the run has not already been successfully reanalyzed. Reanalysis at the signal processing step uses DAT files.

(continued)

Reanalysis from the	Description
Basecalling step	The option to reanalyze a run from the basecalling step is available only when a run or reanalysis successfully completes the signal processing step. Reanalysis at the basecalling step uses .wells files.
Alignment step	The option to reanalyze a run from the alignment step is available only when a run or reanalysis successfully completes the basecalling step. Reanalysis at the alignment step uses BAM files.

Note:

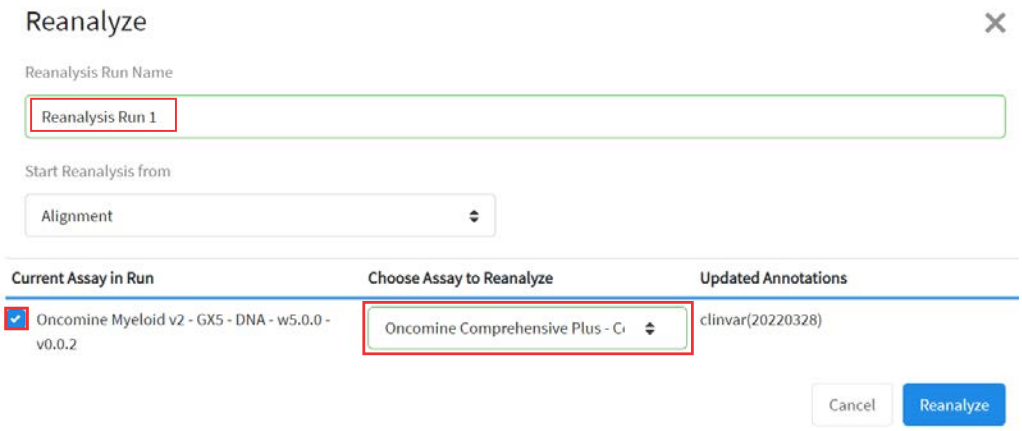
- Manager- and administrator-level users can reanalyze a sequencing run only if the run completed without any critical alarms or errors. If the run aborted or produced major alarms or errors, the run cannot be reanalyzed.

The files needed for the stage of reanalysis that you select must be present in the software. For example, if the .wells files for the run have been removed from the software, you cannot reanalyze from basecalling. Instead, reanalyze the BAM files at alignment. Administrator-level users can manage the settings and schedule to backup and delete files and data. For more information, see the software help system, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409).

You can reanalyze a run with any compatible assay that exists in the software. Alternatively, you can create a new assay or copy the original assay that was used in a run and modify assay parameters if needed. For more information, see Chapter 4, “Create and manage assays”.

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**, **Basecalling** or **Signal Processing**.

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** column. 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 ⟲ after the run name.

Generate a variant report

The Lab Report is generated in the language that is selected in the report template. You can customize this report by generating it in another language.

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

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

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

1. In the menu bar, click **Results ▸ Sample Results**.
2. In the **Results / Sample Results** screen, click the sample of interest in the **Sample Name** column.
3. Select the **Reports** tab, then click **+ Generate Variant Report**.

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

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

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

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

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

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

Configure Connect Platform accounts in Genexus™ Software (manager/administrator)

Manager- and administrator-level users can configure a link in Genexus™ Software to one or more Thermo Fisher™ Connect Platform accounts. This account type is called a Connect Platform account in Genexus™ Software.

When a Connect Platform account is configured and active, administrator-level users can perform tasks in the Genexus™ Software.

- Download the latest software updates
- Download more plugin software
- Download assay definition files
- Download software configuration packages

Before you configure a Thermo Fisher™ Connect Platform account you must have a valid Connect Platform account on the **thermofisher.com** website. If you do not have a Connect Platform account, create a new account at **thermofisher.com/connect**. Click **Sign up now**, then enter the information that is required to create a new account. Click **Create account** at the bottom of the screen to complete the registration.

1. In Genexus™ Software, 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, enter the information that is needed to create the account.

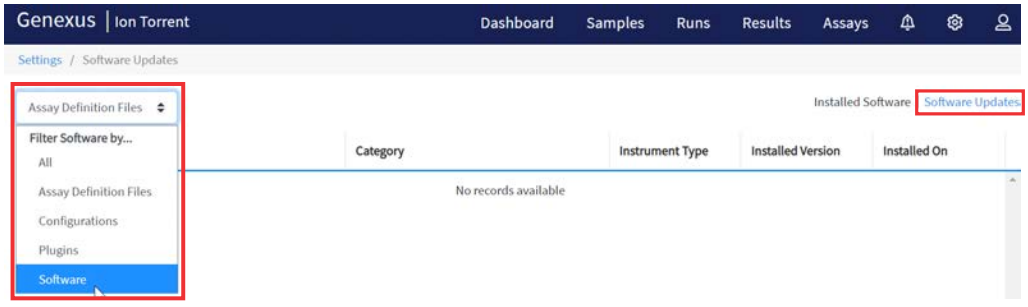
Item	Description
Account Type	Select **Genexus**.
Name	Enter a name to identify the account in the **Thermo Fisher Account Settings** screen in Genexus™ Software. The name can contain only alphanumeric characters (0-9, Aa-Zz), periods (.), underscores (_), or hyphens (-). For example, enter ***Lab_Admin***.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

Download software and assay package updates

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

- Configure a Thermo Fisher™ Connect Platform account. For instructions, see the Genexus™ Software Help.
- Ensure that the Genexus™ Integrated Sequencer is running on the latest Genexus™ Software version.
- Connect your Genexus™ Integrated Sequencer to the internet.

1. In the menu bar, click ⚙ **(Settings)** ▸ **Software Updates**, then click **Software Updates** in the upper right corner of the screen.
   The **Software Updates** screen opens with **App Store** enabled.



2. From the **Filter Software by...** dropdown list, select **Software** to view available software package updates or **Assay Templates** to view available assay package updates.
   The list of available updates is displayed.
3. In the **Actions** column, in the row of the package of interest, click **Download**.
   After completion of the download, the **Download** link changes to an **Install** link.
4. Click **Install**. When the confirmation window appears, click **Yes** to confirm that you want to install.

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

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. Place the pointer 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 nonduplicate filters, or 100% if no filters were specified. A read is considered on target if at least one aligned base overlaps at least one target region. A read that overlaps a targeted region but where only flanking sequence is aligned, for example, due to poor matching of 5' bases of the read, is not counted.

Amplicon read coverage statistics

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

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

(continued)

Statistic	Description
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 were specified, the whole genome is the targeted regions.
Average base coverage depth	The average number of reads of all targeted reference bases. This is the total number of base reads on target divided by the number of targeted bases, and therefore includes any bases that had no coverage.
Uniformity of base coverage	The percentage of bases in all targeted regions (or whole genome) that is covered by at least 20% of the average base coverage depth reads. Cumulative coverage is linearly interpolated between nearest integer base read depths.
Target base coverage at *N*x	The percentage of target bases covered by at least *N* reads.

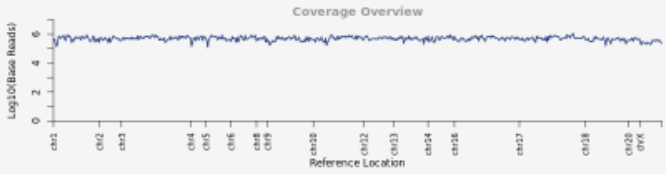
(continued)

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

Example 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.

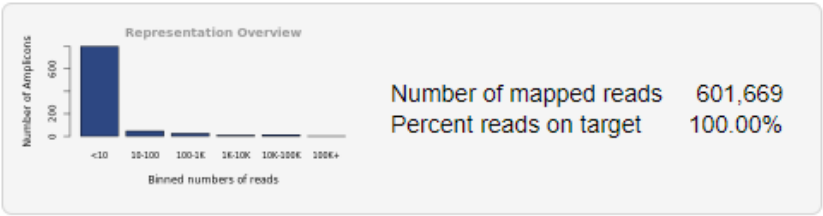
Library type: AmpliSeq DNA and Fusions
Reference: hg19 (DNA)
Target regions: OCAv3.20180426.designedUnmergedDetail



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



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.

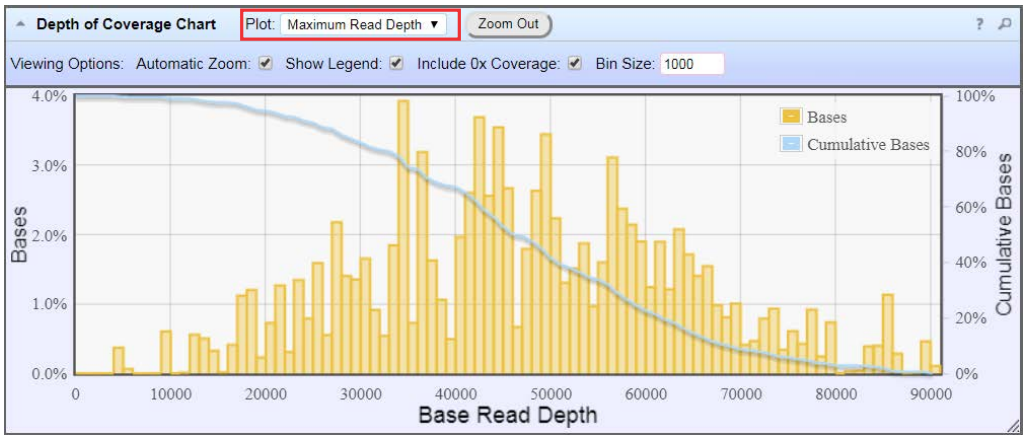


Figure 1 Representative Depth of Coverage Chart

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 region of interest file, this chart reflects only target regions (reads that fall within a region of interest). Use the **Plot** dropdown list to switch between **Maximum Read Depth**, **99.9% of All Reads**, and **Normalized Coverage** plots.

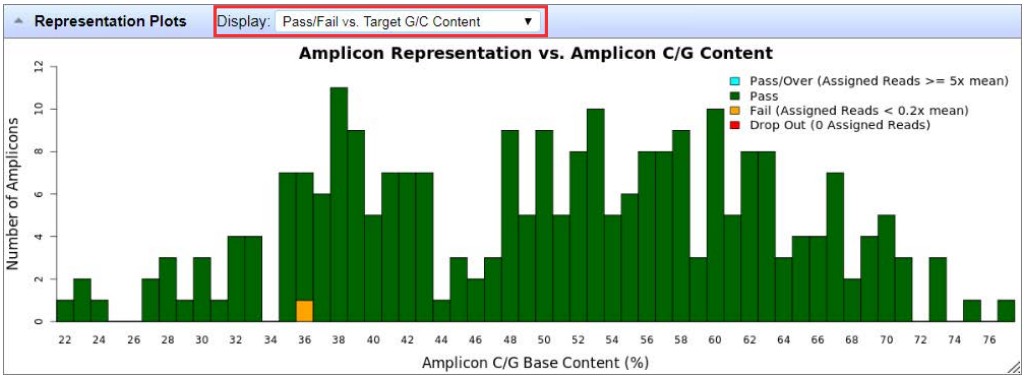


Figure 2 Representation Plots

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**, **Amplicon Coverage Chart**, **Mean Target Reads vs Pool**, **Reference Coverage Chart**, and **Representation vs. Target Length** plots. This figure shows an example **Pass/Fail vs. Target G/C Content** plot.

Output files generated by the coverageAnalysis plugin

You can download coverageAnalysis plugin results files from 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 contains a 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).

(Genexus™ Software version 6.6.0 and later) Copy and edit an assay

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

(Genexus™ Software version 6.2.1 only) Copy and edit an assay

1. In the menu bar, click **Assays** ▸ **Manage Assays**.
2. In the **Manage Assays** screen, in the **Actions** column in the row of an assay that you want to copy, click **Copy**.
3. In the **Copy Assay** screen, proceed through the workflow steps. When finished, enter a new name in the **Assay Name** field and a short name in the **Assay Short Name** field for the copied assay, then click **Save**.
4. In the **Manage Assays** screen, in the **Actions** column in the row of your copied assay, click **Edit**, then in the **Edit Assay**screen proceed to the **Parameters** step.
5. At the top of the screen in the **Parameters** step, select **Library Prep & Templating Parameters** from the dropdown list, then click **Download**.
   A ZIP folder that contains the RunParameters.json file for the assay is downloaded to your local storage.
6. Open the RunParameters.json file using a text editor, then search for “amplifyTargetPCRProfile”. The following text string should follow the instance of the matched text.

   ```
   value":"[[[990],[xxx],x],[[990,xxx],[xx,xxx],xx],[[xxx],[xx],x]]
   ```

7. Replace each instance of the 990 value with 980 in the text string (a total of two instances), then save the edited RunParameters.json file to your local storage.

8. Return to the **Edit Assay** screen in the Genexus™ Software, then at the top of the screen in the **Parameters** step, click **Upload**.

9. Under **Advanced Parameter Configuration**, click **Select files**, select the edited RunParameters.json file, then click **Open**.
   When upload is complete, **Parameter file uploaded successfully** message is displayed.

10. Proceed through the remainder of the workflow steps, then click **Save** to save the assay.

11. In the **Manage Assays** screen, in the **Actions** column in the row of your edited assay, click **Lock** to lock the assay.

12. In the **Actions** column, in the row of your edited assay, click **Download Parameters**.

13. Open the RunParameters.json file and verify that the parameter changes from step 7 have been saved.
    Your modified assay is now ready for use in a run.

Create NTC sample

If you are including an NTC sample, you must first create a NTC sample using the same procedure as any other viral RNA sample. The **Include NTC** checkbox is not functional for the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay.

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, in the **Sample Name** attribute, enter NTC_Sample, then complete the required fields.

   IMPORTANT! You must enter NTC_Sample in the **Sample Name** attribute.

   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 31.

4. Click **Save**.
   The NTC sample is listed in the **Manage Samples** screen and is available to use in your run. You can add the sample when planning a run. The results for the NTC sample may be viewed using the same procedure as any other sample included in the run.

Create new copy assay for SARS_CoV_2_lineageID plugin updates

1. In the menu bar, click **Assays** ▸ **Manage Assays**.
2. *(Optional)* Obsolete the copy assay created to include the previous version of the SARS_CoV_2_lineageID plugin.
3. In the **Manage Assays** screen, in the **Actions** column in the row of an assay that you want to copy, click **Copy**.
4. In the **Copy Assay** screen, proceed through the workflow steps, on **Plugins** tab, select the SARS_CoV_2_lineageID (only the latest installed version is available for selection). When finished, enter a new name in the **Assay Name** field and a short name in the **Assay Short Name** field for the copied assay, then click **Save**.

   The modified assay is now ready for use in a run.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Genexus™ Integrated Sequencer safety

For detailed safety information for use of the Genexus™ Integrated Sequencer, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020 **www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf**
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs) **www.who.int/publications/i/item/9789240011311**

D Documentation and support

Related documentation

Document	Publication number
Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GX Quick Reference	MAN0024940
Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GX Product Information Sheet	MAN0024941
Genexus™ Integrated Sequencer User Guide	MAN0017910
Genexus™ Software 6.8 User Guide	MAN0026409
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (manual extraction) User Guide	MAN0018072
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide	MAN0018073
Qubit™ RNA HS Assay Kits User Guide	MAN0002327

Customer and technical support

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

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

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

Limited product warranty

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