**ion**torrent

# Ion AmpliSeq™ & Ion AmpliSeq™ HD Custom Assay

## USER GUIDE

For sequencing on the Genexus™ Integrated Sequencer

for use with:
Ion AmpliSeq™ Community Panels
Ion AmpliSeq™ Made-to-Order Panels
Ion AmpliSeq™ On-Demand Panels
Custom Ion AmpliSeq™ & Ion AmpliSeq™ HD Panels

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

**ThermoFisher**
S C I E N T I F I C

**Revision history:  MAN0028005 B (English)**

| Revision | Date | Description |
|----------|------|-------------|
| B | 9 July 2024 | Updated HD library amplification cycle setting guidelines. See "Guidelines for using custom assays with the Genexus™ Integrated Sequencer" on page 32. |
| A.0 | 12 June 2023 | Initial release. |

# Contents

*Ion AmpliSeq™ & Ion AmpliSeq™ HD Custom Assay User Guide*

# Product information

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

## Ion AmpliSeq™ panels

Order Ion AmpliSeq™ panels using Ion AmpliSeq™ Designer. For more information, visit **AmpliSeq.com**.

### About Ion AmpliSeq™ Designer

Ion AmpliSeq™ Designer provides tools for designing and ordering next-generation sequencing (NGS) panels for targeted sequencing applications. Multiple design ordering options are available, from fixed pre-tested panels that are ready-to-order to fully customizable designs. Ion AmpliSeq™ Designer also provides a variety of public reference genomes for use in design creation as well as an option to upload a custom FASTA format reference sequence.

When your designs are finalized, Ion AmpliSeq™ Designer connects you directly to the Thermo Fisher Scientific ordering pipeline.

*Ion AmpliSeq™ Designer Help* (Pub. No. MAN0018937) provides information for using Ion AmpliSeq™ Designer to design and order panels.

### Ion AmpliSeq™ panels

Ion AmpliSeq™ panels provide pools of primers for the amplification of target regions. The primers contain proprietary modifications that enable removal of primer sequences during library preparation for efficient target assessment during sequencing. Multiple primer pools can be used to create overlapping amplicons that enable complete coverage of large targets. Panels are designed to be used with nucleic acid from various sources, including nucleic acid from formalin-fixed paraffin-embedded (FFPE) tissue and cell-free DNA (cfDNA).

Ion AmpliSeq™ panels are ordered using Ion AmpliSeq™ Designer. Users who are interested in inherited disease or germline research applications can design and order panels using a content selection engine or by uploading their own gene list. The gene designs in the On-Demand catalog have been optimized for high performance. For additional information, see "Ion AmpliSeq™ On-Demand Panel designs" on page 8 or visit **AmpliSeq.com**.

The following types of panels are compatible with the Genexus™ Integrated Sequencer.

| Product and description | In stock | Made–to–order | | Fixed panels | |
|---|---|---|---|---|---|
| | Ion AmpliSeq™ On-Demand Panels | Ion AmpliSeq™ Custom panels | Ion AmpliSeq™ HD Custom panels | Ion AmpliSeq™ Community Panels | Ion AmpliSeq™ Ready-to-Use Panels |
| Genome | Human | Any | Human | Human | Human |
| Nucleic Acid | DNA only | DNA/RNA | DNA/RNA | DNA/RNA | DNA/RNA |
| Sample type | Genomic DNA | Any | cfDNA/FFPE | Any | Any |
| Target type | Genes | Genes, regions, hotspots, fusions | Genes, regions, hotspots, fusions | Genes, regions, hotspots, fusions | Genes, regions, hotspots, fusions |

## Ion AmpliSeq™ Custom panel designs

Ion AmpliSeq™ Custom panel designs are customizable for any genome at the amplicon level. Panels can be designed against a number of pre-loaded reference genomes, or for any other organism by uploading a FASTA file reference sequence.

Custom panels are delivered within a few weeks in tubes of pre-pooled, multiplexed primers in ready-to-use concentrations.

| Custom panel type | # of oligos / pool | # of tubes / pool | Concentration | Storage[1] |
|---|---|---|---|---|
| DNA | ≤192 | 5 × 1.5 mL | 2X | –30°C to –10°C |
| | ≥193 | 20 × 1.5 mL | 2X | |
| RNA | — | 3 × 1.5 mL | 5X | |

[1] Shipped at ambient temperature. Store as indicated.

To determine the number of samples that can be sequenced per lane on either the GX5™ Chip or GX7™ Chip for a custom panel design, see Chapter 4, "Guidelines for setting up custom assays and sequencing runs on the Genexus™ Integrated Sequencer".

## Ion AmpliSeq™ On-Demand Panel designs

Ion AmpliSeq™ On-Demand designs are suitable for common disease research areas and are customizable at the gene level. The designs include optimized amplicons with verified performance for germline analysis. You can create designs by selecting from a catalogue of >5,000 pre-tested genes or browsing the Ion AmpliSeq™ Designer gene repository by research area of interest.

Each Ion AmpliSeq™ On-Demand Panel consists of 2 DNA primer pools, where the number of primer pairs per pool depends on the genes that are selected. The maximum number of genes that can be selected for design creation is 800. Each Ion AmpliSeq™ On-Demand panel *order* is limited to either 500 genes or a maximum of 15,000 amplicons. The minimum number of amplicons per design is 12 per pool.

You can add genes that are not in the On-Demand catalog to your panel as a Spike-in panel. Spike-in panels are high concentration Made-To-Order panels that are used to supplement On-Demand designs with genes that are not available On-Demand. Spike-in panels are created as 2-pool designs and are limited to 123 amplicons per pool.

Ion AmpliSeq™ On-Demand panels are available in 24 reaction packs for library preparation (in combination with 48 barcode plates on the GX5™ Chip and with any barcode plate on the GX7™ Chip) on the Genexus™ Integrated Sequencer. The panels support sequencing genomic targets from as little as 10 ng of DNA input.

## Ion AmpliSeq™ Community Panels

Ion AmpliSeq™ Community Panels are DNA and RNA panels that are predesigned for germline or somatic analysis. Designed with input from leading disease researchers and verified for performance, these panels are appropriate for a wide range of research areas, including inherited diseases, cancer research, human identification, infectious diseases, pharmacogenomics, developmental disorders, inflammation and immune response research, and more.

Ion AmpliSeq™ Community Panels are customizable, giving you the flexibility to add or remove genes and amplicons to fit your unique project requirements, and are available to ship within a few weeks.
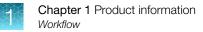
## Ion AmpliSeq™ HD Made-to-Order panels

Ion AmpliSeq™ HD Made-to-Order panels provide pools of primers for amplification of custom target regions to generate tagged targeted amplicon libraries for use as part of the Ion AmpliSeq™ HD sequencing workflow. Ion AmpliSeq™ HD technology enables scalable polymerase chain reactions (PCR) from 12- to 5000-plex (1 x 5000-plex in a 1-pool panel or 2 x 2500-plex in a 2-pool panel) for Unique Molecular Tag (UMT) targeted sequencing. The primers contain proprietary modifications that are required during library preparation for efficient target assessment during sequencing. Nucleic acid from various sources—including cell-free DNA (cfDNA), and formalin-fixed paraffin-embedded (FFPE) tissue—can be used as the starting material.

Visit **AmpliSeq.com** to design and order Ion AmpliSeq™ HD Made-to-Order panels using Ion AmpliSeq™ Designer. Panels are provided as 1 or 2 pools, each consisting of two subpools (forward (FWD) and reverse (REV) primers) at 10X concentration and enable sequencing: gene, region, hotspot, or fusion targets on the Genexus™ Integrated Sequencer.

| Primer pool panels | Volume | Storage[1] |
|---|---|---|
| **1-pool primer panel (DNA or RNA)** | | |
| 10X Pool 1 FWD (red cap) <br> 10X Pool 1 REV (yellow cap) | 6 × 1.5 mL each | −30°C to −10°C |
| **2-pool primer panel (DNA only)** | | |
| 10X Pool 1 FWD (red cap) <br> 10X Pool 1 REV (yellow cap) <br> 10X Pool 2 FWD (blue cap) <br> 10X Pool 2 REV (green cap) | 6 × 1.5 mL each | −30°C to −10°C |
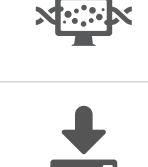
[1]  Shipped at ambient temperature. Store as indicated.

# Workflow

| **Custom assays run on the Genexus™ Integrated Sequencer** |
|---|

### Design your custom assay panel

Design and order your custom assay panel(s) at AmpliSeq.com

### Import your custom panel design files (page 41)

Import directly or download your custom panel files from AmpliSeq.com. Contact your field application scientist if you need help accessing the files.

### Create a custom assay (page 54)

To create a custom assay for your custom panel, copy an existing custom or system-installed assay, then edit the settings as needed. Alternatively, create your custom assay from scratch with the Create assay wizard .

### Enter samples (page 78)

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

### Plan a sequencing run (page 86)

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

### Dilute the samples and load the sample plate (page 95)

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

### Load the sequencer and start a run (page 98)

Follow the step-by-step instructions on the sequencer touchscreen to load the sample plate and consumables.

## Custom assays run on the Genexus™ Integrated Sequencer

### Review data and results (page 114)

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

# 2 Reagents, supplies, and required materials

This chapter lists the reagents, supplies, and materials needed to operate the Genexus™ Integrated Sequencer, and provides consumables ordering and storage information. Recommended products for nucleic acid isolation and purification are also provided.

## Genexus™ Integrated Sequencer

| Components | Cat. No. |
|---|---|
| Genexus™ Integrated Sequencer | A45727 |

## Reagents and supplies—Ion AmpliSeq™ library chemistry

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

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

# Genexus™ Library Strips 1 and 2-AS

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

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

# Genexus™ Barcodes AS

Ion Torrent™ Genexus™ Barcodes AS are supplied in plates containing 32 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 | |
| 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 | 15°C to 30°C |
| Genexus™ Barcodes 1–48 AS Kit | Blue | A54129 | 1 plate | |
| Genexus™ Barcodes 49–96 AS Kit | Blue | A54130 | 1 plate | |

# Genexus™ GX5™ Starter Pack-AS

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

**Note:** For custom assays, Genexus™ Primer Pool Tubes (Cat. No. A40262) must be ordered separately. See For more information, see page 18..

| Component | Part or Cat. No. | Quantity | Storage |
|---|---|---|---|
| Genexus™ Strip 1 | A46812 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 2-AS | A46813 | 8 strips | −30°C to −10°C |
| Genexus™ Strip 3-GX5™ | A46815 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 4 | A46816 | 8 strips | −30°C to −10°C |
| Genexus™ Barcodes 1–32 AS | A40258 | 1 plate | 15°C to 30°C |
| Genexus™ Pipette Tips | A40266 | 12 racks | |
| Genexus™ Cartridge | A40272 | 2 cartridges | −30°C to −10°C |
| Genexus™ Bottle 2 | A40273 | 4 bottles | 15°C to 30°C |
| Genexus™ Bottles 1 and 3 | A40274 | 2 bottles each | |

# Genexus™ GX7™ Starter Pack-AS

Ion Torrent™ Genexus™ GX7™ Starter Pack-AS (Cat. No. A50222) supplies the following components for Ion AmpliSeq™ library preparation and sequencing using a Genexus™-compatible assay.

**Note:** For custom assays, Genexus™ Primer Pool Tubes (Cat. No. A40262) must be ordered separately. See For more information, see page 18..

| Component | Part or Cat. No. | Quantity | Storage |
|---|---|---|---|
| Genexus™ Strip 1 | A46812 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 2-AS | A46813 | 8 strips | −30°C to −10°C |
| Genexus™ Strip 3-GX7™ | A46818 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 4 | A46816 | 8 strips | −30°C to −10°C |
| Genexus™ Barcodes 1–32 AS | A40258 | 1 plate | 15°C to 30°C |
| Genexus™ Pipette Tips | A40266 | 12 racks | |
| Genexus™ Cartridge | A40272 | 2 cartridges | −30°C to −10°C |
| Genexus™ Bottle 2 | A40273 | 4 bottles | 15°C to 30°C |
| Genexus™ Bottles 1 and 3 | A40274 | 2 bottles each | |

# Reagents and supplies—Ion AmpliSeq™ HD library chemistry

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

**Note:**

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

## Genexus™ Library Strips 1 and 2-HD

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

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

# Genexus™ Barcodes 1–32 HD

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

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

# Genexus™ GX5™ Starter Pack-HD

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

**Note:** For custom assays, Genexus™ Primer Pool Tubes (Cat. No. A40262) must be ordered separately. See For more information, see page 18..

| Component | Part or Cat. No. | Quantity | Storage |
|---|---|---|---|
| Genexus™ Strip 1 | A46812 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 2-HD | A46814 | 8 strips | −30°C to −10°C |
| Genexus™ Strip 3-GX5™ | A46815 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 4 | A46816 | 8 strips | −30°C to −10°C |
| Genexus™ Barcodes 1–32 HD | A40261 | 1 plate | 15°C to 30°C |
| Genexus™ Pipette Tips | A40266 | 12 racks | |
| Genexus™ Cartridge | A40272 | 2 cartridges | −30°C to −10°C |
| Genexus™ Bottle 2 | A40273 | 4 bottles | 15°C to 30°C |
| Genexus™ Bottles 1 and 3 | A40274 | 2 bottles each | |

# Genexus™ GX7™ Starter Pack-HD

Ion Torrent™ Genexus™ GX7™ Starter Pack-HD (Cat. No. A50223) supplies the following components for Ion AmpliSeq™ HD library preparation and sequencing using a Genexus™-compatible assay.

**Note:** For custom assays, Genexus™ Primer Pool Tubes (Cat. No. A40262) must be ordered separately. See For more information, see page 18..

| Component | Part or Cat. No. | Quantity | Storage |
|---|---|---|---|
| Genexus™ Strip 1 | A46812 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 2-HD | A46814 | 8 strips | −30°C to −10°C |
| Genexus™ Strip 3-GX7™ | A46818 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 4 | A46816 | 8 strips | −30°C to −10°C |

*(continued)*

| Component | Part or Cat. No. | Quantity | Storage |
|---|---|---|---|
| Genexus™ Barcodes 1–32 HD | A40261 | 1 plate | 15°C to 30°C |
| Genexus™ Pipette Tips | A40266 | 12 racks | |
| Genexus™ Cartridge | A40272 | 2 cartridges | −30°C to −10°C |
| Genexus™ Bottle 2 | A40273 | 4 bottles | 15°C to 30°C |
| Genexus™ Bottles 1 and 3 | A40274 | 2 bottles each | |

# Shared reagents and supplies

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

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

## Genexus™ Templating Strips 3-GX5™ and 4

Ion Torrent™ Genexus™ Templating Strips 3-GX5™ and 4 (Cat. No. A40263) are ordered as kits with 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™ Templating Strips 3-GX7™ and 4

Ion Torrent™ Genexus™ Templating Strips 3-GX7™ and 4 (Cat. No. A46817) are ordered as kits with 8 pairs of strips per kit.

| Component | Carrier color | Part No. | Quantity per kit | Storage |
|---|---|---|---|---|
| Genexus™ Strip 3-GX7™ | Green | A46818 | 8 strips | 2°C to 8°C |
| Genexus™ Strip 4 | Yellow | A46816 | 8 strips | −30°C to −10°C |

# Genexus™ Primer Pool Tubes and Pipette Tips

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

**IMPORTANT!** The catalog number listed for Genexus™ Pipette Tips is the only supported option for pipette tips. Use of pipette tips from third-party suppliers is not supported and can result in failed sequencing runs.

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

# GX5™ Chip and Genexus™ Coupler

The GX5™ Chip and Genexus™ Coupler (Cat. No. A40269) are ordered as a set that contains 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 | |

# GX7™ Chip and Genexus™ Coupler

The GX7™ Chip and Genexus™ Coupler (Cat. No. A46731) are ordered as a set that contains 2 chips and 2 couplers, sufficient for up to 8 sequencing runs.

| Component | Part No. | Quantity | Storage |
|---|---|---|---|
| GX7™ Chip  | 100093568 | 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* (Cat. No. MAN0017910).

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

## Genexus™ Filter

The Genexus™ Filter (Cat. No. A40302) is installed in the liquid waste disposal port on the instrument deck to prevent liquid waste line blockage. For information on installation, see the *Genexus™ Integrated Sequencer User Guide* (Cat. No. MAN0017910).

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

## Genexus™ Controls

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

---

**IMPORTANT!** Genexus™ Strip 2-AS is required for sequencing Genexus™ Controls. For ordering information, see "Genexus™ Library Strips 1 and 2-AS" on page 13.

---

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

---

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

# Required materials—general laboratory equipment and supplies

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

| Item | Source |
|---|---|
| MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode | 4483352, 4483354 |
| Adhesive PCR Plate Foils | AB0626 |
| 20-, 200-, and 1,000-µL pipettors and appropriate filtered tips | **MLS** |
| Microcentrifuge tubes, 1.5-mL or 1.7-mL (low retention for nucleic acids) | **MLS** |
| Vortex mixer with a rubber platform | **MLS** |
| Gloves, powder-free nitrile | **MLS** |
| Ice buckets and ice | — |
| Nuclease-free water, molecular biology grade | AM9932 |
| Isopropyl alcohol, 70% solution | **MLS** |
| Wipes, disposable lint-free | **MLS** |
| *(Optional)* Uninterruptible Power Supply (UPS)[1] | **MLS** |
| *(Optional)* Sorvall™ ST 8 Small Benchtop Centrifuge[2], with | 75007200 |
| Thermo Scientific™ M10 Microplate Swinging Bucket Rotor (or equivalent),[3] and | 75005706 |
| Sealed Bucket; Capacity: 4 Standard or 2 Midi-Deepwell plates (Set of 2) (or equivalent) | 75005721 |

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

[2] For centrifuging library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter. Centrifuge must achieve an RCF of 2000 × g, have a swinging bucket rotor and accommodate deepwell plates.

[3] Swinging bucket rotor must carry deepwell plate in the landscape orientation.

Chapter 2 Reagents, supplies, and required materials
*Recommended materials for nucleic acid isolation and quantification*

2

# Recommended materials for nucleic acid isolation and quantification

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

| Item | Source |
|---|---|
| **Genexus™ Purification System reagent kits for automated nucleic acid isolation and quantification[1]** | |
| Ion Torrent™ Genexus™ FFPE DNA and RNA Purification Kit | A45539 |
| Ion Torrent™ Genexus™ Cell-Free Total Nucleic Acid Purification Kit | A45542 |
| Ion Torrent™ Genexus™ Multisample DNA Purification Kit | A45540 |
| Ion Torrent™ Genexus™ Total RNA Purification Kit | A45541 |
| **Nucleic acid isolation—manual (Nucleic Acid to Result runs)** | |
| Ion AmpliSeq™ Direct FFPE DNA Kit | A31133, A31136 |
| RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE | AM1975 |
| RecoverAll™ Multi-Sample RNA/DNA Workflow | A26069 |
| MagMAX™ FFPE DNA/RNA Ultra Kit | A31881 |
| PureLink™ Genomic DNA Mini Kit | K1820-00 |
| MagMAX™ Cell-Free DNA Isolation Kit | A29319 |
| MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit | A36716 |
| RNase*Zap*™ RNase Decontamination Solution | AM9780 |
| DNA*Zap*™ PCR DNA Degradation Solutions | AM9890 |
| **Nucleic acid quantification—manual** | |
| TaqMan™ RNase P Detection Reagents Kit (Recommended for DNA only) | 4316831 |
| Qubit™ 4 Fluorometer[2] | Q33238 |
| One or more of the following kits for use with the Qubit™ 4 Fluorometer: | |
| · Qubit™ dsDNA HS Assay Kit (High-sensitivity DNA) | Q32851, Q32854 |
| · Qubit™ dsDNA BR Assay Kit (Broad range DNA) | Q32850, Q32853 |
| · Qubit™ RNA HS Assay Kit (High-sensitivity RNA) | Q32852, Q32855 |
| · Qubit™ RNA BR Assay Kit (Broad range RNA) | Q10210, Q10211 |

**Chapter 2** Reagents, supplies, and required materials
*Recommended materials for nucleic acid isolation and quantification*

2

*(continued)*

| Item | Source |
|---|---|
| **Library quantification (Library to Result runs only)** | |
| Ion Library TaqMan™ Quantitation Kit | 4468802 |

[1] Used with the Genexus™ Purification System. See the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475) for detailed information on the Genexus™ Purification System and its consumables.

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

# 3

# Before you begin

## Precautions

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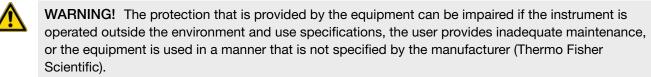
**IMPORTANT!** A primary source of contamination is spurious nucleic acid fragments from previous sample processing steps. Do not introduce amplified DNA into the work area where the instrument is located.

### Avoid strong electromagnetic radiation

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

### Protection by equipment

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

# Guidelines for panel and reagent use and handling

- Use only the reagents and supplies that have been recommended in "Required materials—general laboratory equipment and supplies" on page 22 and "Recommended materials for nucleic acid isolation and quantification" on page 23.

- Keep panel tubes capped until immediately before loading in the Genexus™ Integrated Sequencer.

- If using, thaw positive controls on ice for 30 minutes. After the positive controls are completely thawed, vortex the tubes, then centrifuge to collect tube contents. Return to ice before loading into sample plate.

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

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

- Thaw Genexus™ Strip 4 on ice for 30 minutes before loading in the sequencer.

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

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

  For information about obtaining and using the Genexus™ Strip Centrifuge Adapter, see "Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter" on page 180.

- Keep the sample plate, thawed panel, Genexus™ Strip 2-AS (or Genexus™ Strip 2-HD), and Genexus™ Strip 4 on ice or at 4°C until ready to load in the sequencer.

- Do not freeze-thaw the panel. Thaw only the number of panel tubes that are required for an instrument run. Discard unused panel tubes after they are thawed. Store panel tubes at –30°C to –10°C.

- If you are using an assay that uses Ion AmpliSeq™ HD chemistry, do not combine the contents of panel tubes. Forward and reverse primers must remain separate until they are combined by the sequencer as part of the on-instrument library preparation workflow.

- Do not store primer pool tubes or reagent strips on the sequencer for more than 24 hours before starting an instrument run.

# Guidelines for preventing contamination

We recommend following these guidelines to prevent cross-contamination of samples and controls between and within sequencing runs.

Personal protective equipment

- Wear a laboratory coat that is reserved for sequencing work and is laundered frequently. If possible, change to a fresh laboratory coat before setting up a run, or use new sleeve covers.

- Wear fresh gloves to load the instruments, including during the loading of the sequencing chip and coupler. Do not remove gloves to install the sequencing chip.

- Change gloves between dispensing positive controls and no-template control (NTC) in sample plate wells.

Equipment and instrument cleaning

- If you are preparing samples and sample plates in a hood (recommended), illuminate the hood with UV light for 15 minutes before use.

- Before preparing for a sequencing run, decontaminate surfaces of the hood or bench where samples or sample plates are handled, and other equipment such as vortexers, microcentrifuges, and pipettors. Use DNA*Zap*™ and either the RNase*Zap*™ RNase Decontamination Solution or the RNase *AWAY*™ Surface Decontaminant reagent. Follow manufacturer instructions for the proper storage and use of each decontamination reagent. Alternatively, a 10% solution of commercial bleach can be used. Follow with wiping of bench and equipment surfaces with wipes moistened with 70% isopropanol or 70% ethanol.

- Before and after a run, sequentially wipe instrument deck surfaces with lint-free wipes moistened with the two DNA*Zap*™ decontamination solutions, followed by wipes moistened with either 1) the RNase*Zap*™ RNase Decontamination Solution or 2) the RNase *AWAY*™ Surface Decontaminant reagent. Follow with wiping of deck surfaces with wipes moistened with 70% isopropanol or 70% ethanol. The robotic pipettor arm can also be cleaned in this manner.

  IMPORTANT! Do not spray decontamination solution or alcohol solution directly onto deck surfaces or into deck openings. Instead, use a lint-free wipe moistened with solution to clean surfaces. Do not use bleach to clean instrument surfaces.

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

Workflow tips

- After dispensing a positive control or sample (if applicable) in a sample plate well, do not pass the end of the used tip over wells intended for other samples or NTC. This practice minimizes the chance of depositing microdroplets in adjacent wells.

- If you dilute samples on the sample plate, apply the foil seal carefully between wells with an applicator before vortexing to ensure that the seal is complete and contamination between wells does not occur.

- If possible, reserve a pipettor and tips for dispensing only NTC.

- Before disposal, close or cap used sample and positive control tubes to avoid creation of aerosols.

- Avoid touching the foil seals of the reagent strips, barcode plate, and sample plate.

- When loading the sequencer deck for a run, install the sample plate last.
- After a run, seal the PCR amplification plate with a foil seal before removing the plate from the PCR amplification station. Sealing the plate before removal helps prevent contamination of libraries if libraries are recovered for reuse.

# Guidelines for Genexus™ Integrated Sequencer operation

- Follow guidance that is provided by Genexus™ Software when you plan a run to determine which consumables must be loaded and which consumables can be reused from a previous run.
- Follow guidance that is provided by the software when you plan a run to determine how many samples can be run with a given assay or assays in an instrument run. The number of samples that can be included in a sequencing run depends on multiple factors
  - The number of available barcodes
  - The sequencing chip, GX5™ Chip or GX7™ Chip
  - The number of available lanes on the sequencing chip
  - The sequencing chemistry being used, Ion AmpliSeq™ or Ion AmpliSeq™ HD
  - The targeted mean read depth needed to achieve the desired result

**Table 1   Ion AmpliSeq™ sequencing chemistry**

| Limiting factor | Description |
|---|---|
| The sequencing chip being run | GX5™ Chip or GX7™ Chip |
| The number of available barcodes in the barcode plate | The maximum number of available Ion AmpliSeq™ barcodes per run is 48. Ion AmpliSeq™ consumables are configured to support sample batch sizes in multiples of four to six samples depending on the barcode plate configuration (32 or 48) being used. The most efficient use of Ion AmpliSeq™ consumables occurs when samples are run in multiples of four or six.<br>**IMPORTANT!** When libraries are prepared on the Genexus™ Integrated Sequencer, each target amplification reaction for a sample requires a unique barcode. |
| Maximum number of target amplification reactions per run | One library strip pair has the reagents necessary for 6 target amplification reactions, or 6 barcodes. With a maximum of 8 library strip pairs loaded, a maximum of 48 samples can be run using an assay with one primer pool. |
| The number of primer pools per assay | Given the limits of 48 target amplification reactions, and 48 available barcodes, the number of samples in a run multiplied by the total number of primer pools in the assays that are used in a run cannot exceed 48. |
| The number of unused lanes on an installed chip | A maximum of 4 lanes are available on a single chip. |
| The minimum read count per sample for an assay | The minimum read count per sample parameter is set during assay creation. |

**Table 2  Ion AmpliSeq™ HD sequencing chemistry**

| Limiting factor | Description |
|---|---|
| The sequencing chip being run | GX5™ Chip or GX7™ Chip |
| The number of available barcodes in the barcode plate | The maximum number of available Ion AmpliSeq™ HD barcodes per run is 32. Ion AmpliSeq™ HD consumables are configured to support sample batch sizes in multiples of four samples. The most efficient use of Ion AmpliSeq™ HD consumables occurs when samples are run in multiples of four. **IMPORTANT!**  When Ion AmpliSeq™ HD libraries are prepared on the Genexus™ Integrated Sequencer, each target amplification reaction for a sample requires two unique barcodes. |
| Maximum number of target amplification reactions per run | One library strip pair has the reagents necessary for 4 target amplification reactions, or 4 barcodes. Given the limit of 32 Ion AmpliSeq™ HD barcodes, a maximum of 8 library strip pairs are needed for 32 target amplification reactions. |
| The number of primer pools per assay | Given the limits of 16 target amplification reactions, and 32 available barcodes, the number of samples in a run multiplied by the total number of primer pools in the assays that are used in a run cannot exceed 32. |
| The number of unused lanes on an installed chip | A maximum of 4 lanes are available on a single chip. |
| The minimum read count per sample for an assay | The minimum read count per sample parameter is set during assay creation. |

- Two assays cannot share a chip lane, so a maximum of 4 assays can be run per chip, one assay per lane.

- The assays that are used in a single run must use the same chemistry (Ion AmpliSeq™ or Ion AmpliSeq™ HD), and have compatible cycling parameters to allow amplification in the instrument thermal cycler. The thermal cycler has two independently controlled heating zones. After you select an assay, Genexus™ Software restricts the list of available assays to use in the run to those that are compatible with the selected assay or assays. For more information, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910), or see the "Guidelines for using custom assays with the Genexus™ Integrated Sequencer" .

- One library strip pair is needed for each primer tube position 1–8 that is filled in a run.

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

- If a chip installed in a sequencer has unused lanes, do not remove it unless you are sure that you want to replace it with a new chip. After a partially used chip has been removed from the sequencer, it cannot be reinserted and reused. The sequencer cannot track lane usage after chip removal.

- You can remove a chip in one of the following situations.
  - After all the lanes of a chip are used in a run, the chip shuttles to the install position and you are asked to remove the used chip.
  - When you select a run plan that requires more lanes than are available on the installed chip, you are asked to remove the partially used chip, and the sequencer performs a post-chip clean. In addition, you must clear consumables from the lower sequencing reagents bay, even if only a single lane of the chip was used.

- The Genexus™ Integrated Sequencer can track used and unused barcodes in barcode plates in Genexus™ Software 6.2.0 and later, enabling you to swap plates between runs if needed, and reload a partially used barcode plate for a run if a sufficient number of barcodes is available on the plate.

- After loading in the sequencer, reusable consumables, such as barcode plate, chips, and sequencing reagents bay components, must be used within 14 days for optimal results.

- An assay that is selected in a Library to Result run cannot include library batches that share a library with the same barcode. However, two different assays in a run can include a barcode in common, because assays are run in separate lanes of a chip.

- If desired, manually dilute samples to the needed concentration to avoid loss of sample due to the required volume overages during automated dilution.

# Guidelines for expired reagents and chips

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

- For all reagents, including the barcode plate, the instrument bypasses an expired reagent warning after you tap the **Help** button in the sequencer screen. However, the sequencer must be able to detect and read the 2D barcode on the expired reagent for the bypass to proceed.

- In Genexus™ Software 6.6 and later, if a chip is expiring in a given month, and you start a run in the next calendar month with the same chip installed, the sequencer allows the run to proceed. In addition, in Genexus™ Software 6.6 and later you can use expired chips for post-chip cleans or forced instrument cleans.

# Guidelines for nucleic acid isolation and quantification— Nucleic Acid to Result runs

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

- See "Recommended materials for nucleic acid isolation and quantification" on page 23 for recommended kits for isolating DNA and RNA.

- We recommend the TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831) for quantifying amplifiable human genomic DNA (see *Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan™ RNAse P Detection Reagents Kit* (Pub. No. MAN0007732) available at **thermofisher.com**).

- The Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851 or Q32854) can also be used for quantification, particularly for formalin-fixed, paraffin-embedded (FFPE) DNA, and highly degraded DNA samples. See "Quantify FFPE DNA with the Qubit™ Fluorometer" on page 179 for a detailed procedure for quantifying FFPE DNA.

- We recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32852 or Q32855) for quantifying RNA.

- Quantification methods such as densitometry (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA or RNA. Use of these methods can lead to gross overestimation of the concentration of sample nucleic acid, under-seeding of the target amplification reaction, and low library yields.

- The Ion AmpliSeq™ Direct FFPE DNA Kit bypasses nucleic acid isolation when preparing libraries from FFPE sections on slides. See the *Ion AmpliSeq™ Direct FFPE DNA Kit User Guide* (Pub. No. MAN0014881) for a protocol for using this kit to prepare gDNA from FFPE tissue.

- The Direct FFPE DNA preparation can be stored for up to 6 months at –30°C to –10°C before library preparation.

# Guidelines for setting up custom assays and sequencing runs on the Genexus™ Integrated Sequencer

## Guidelines for using custom assays with the Genexus™ Integrated Sequencer

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

**Ion AmpliSeq™ library chemistry:**

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

  Example: The panel has 500 amplicons (X) in each of two primer pools (Y).

  **Minimum Read Counts Per Sample** = (X × Y) × Z

  (500 amplicons × 2 pools) × 150 reads/amplicon/sample = 150,000

- For somatic Ion AmpliSeq™ assays, start with a coverage depth (Z) of about 2,500 reads per amplicon for calculating the **Minimum Read Count Per Sample** when you set up your assay.

  Example: The panel has 500 amplicons (X) in each of two primer pools (Y).

  **Minimum Read Counts Per Sample** = (X × Y) × Z

  (500 amplicons × 2 pools) × 2,500 reads/amplicon/sample = 2,500,000

- See the information in Table 3 for entering the number of target amplification cycles and anneal/extend time parameters for a custom Ion AmpliSeq™ panel.

Table 3  Target amplification parameters

| Primer pairs per pool | Recommended number of amplification cycles (10 ng high-quality DNA/RNA)[1] | Anneal/Extend time |
|---|---|---|
| RNA fusion panels | 28 | 4 minutes |
| DNA panels | | |
| 12–24 | 22 | 4 minutes |
| 25–48 | 21 | 4 minutes |
| 49–96 | 20 | 4 minutes |
| 97–192 | 19 | 4 minutes |
| 193–384 | 18 | 4 minutes |
| 385–768 | 17 | 4 minutes |

Chapter 4 Guidelines for setting up custom assays and sequencing runs on the Genexus™ Integrated Sequencer
*Guidelines for using custom assays with the Genexus™ Integrated Sequencer*

4

**Table 3 Target amplification parameters** *(continued)*

| Primer pairs per pool | Recommended number of amplification cycles (10 ng high-quality DNA/RNA)[1] | Anneal/Extend time |
|---|---|---|
| 769–1,536 | 16 | 8 minutes |
| 1,537–3,072 | 15 | 8 minutes |
| 3,073–6,144 | 14 | 16 minutes |
| 6,145–24,576 | 13 | 16 minutes |

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

**Ion AmpliSeq™ HD library chemistry:**

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

**Table 4 Minimum Read Count Per Sample at given target LOD**

| LOD | Minimum amplicon coverage |
|---|---|
| 5% | 1,400 |
| 1% | 7,000 |
| 0.2% | 35,000 |

For more information, see "Molecular tag sequencing technology—Important factors" on page 37 or the *Ion AmpliSeq™ HD Library Kit User Guide* (Pub. No. MAN0017392).

Examples:

- The panel has 200 amplicons (X) in each of two primer pools (Y), and you require 5% LOD and coverage depth (Z) of 1,400 reads/amplicon for a solid tumor sample.

  **Minimum Read Counts Per Sample** = (X × Y) × Z

  (200 amplicons × 2 pools) × 1,400 reads/amplicon/sample = 560,000

- The panel has 100 amplicons in each of two primer pools, and you require 1% LOD and 7,000 reads/amplicon for a cell-free sample.

  **Minimum Read Counts Per Sample** = (X × Y) × Z

  (100 amplicons × 2 pools) × 7,000 reads/amplicon/sample = 1,400,000

---

**Note:**

- These calculations are based on 100% uniformity and 100% on target reads. Depending on the actual uniformity of the panel, the quality of input sample, and panel on-target percentage, minimum read counts per sample may need adjustment.
- To achieve low LOD it can help to increase sample input to ≥20 ng and optimize parameters for low frequency detection.

---

- HD library amplification consists of 2 PCR cycling stages.
  - First stage consists of 5 cycles that are preset and cannot be modified.
  - Second stage varies depending on the number of primer pairs per pool in the panel. See Table 5 to determine the number of library target amplification cycles for a custom Ion AmpliSeq™ HD panel.

Accept the recommended default number of Library Amplification Cycles or adjust that number within the allowed range (12–25) of amplification cycles.



① Number of Library Amplification Cycles

**Table 5**

| Primer pairs per pool | Recommended number of target amplification cycles |
|---|---|
| 12–500 | 15 |
| 501–1,000 | 14 |
| 1,001–2,000 | 13 |
| 2,001–5,000 | 12 |

*Ion AmpliSeq™ & Ion AmpliSeq™ HD Custom Assay User Guide*

Chapter 4 Guidelines for setting up custom assays and sequencing runs on the Genexus™ Integrated Sequencer
*Planning sequencing runs for efficient use of consumables*

4

# Planning sequencing runs for efficient use of consumables

Genexus™ Integrated Sequencer consumables are used most efficiently when samples are grouped in multiples of 4 or 6, depending on the chip and barcode combination used. When using a GX5™ Chip with a 32 barcode plate, 4 reactions per sequencing strip are automatically used for custom assays. With a 48 barcode plate, 6 reactions are used. For GX7™ Chips, 6 reactions per sequencing strip are used for all runs regardless of the barcode plate loaded.

For example, running one sample uses the same number of primer pool tubes, library and template strips, and chip lanes as up to 6 samples when using a GX5™ Chip in combination with a 48 barcode plate or when using a GX7™ Chip with either a 32- or 48 barcode plate. In this setup, running 7 or 8 samples requires the same consumables as 12 samples. For the most economical use of reagents, therefore, you should try to group samples in multiples of 4 or 6, if possible.

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

Table 6   Ion AmpliSeq™ library chemistry consumable use

| Number of primer pools | Reads per sample | Samples per set of Genexus™ Library Strips 1 and 2-AS | Barcode plate | Link to consumable use table | |
|---|---|---|---|---|---|
| | | | | GX5™ Chip | GX7™ Chip |
| 1 | 500K | 4 | 32 | Table 39 | — |
| | | 6 | 48 | Table 40 | — |
| | 1M | 6 | 48 | — | Table 45 |
| 2 | 1M | 4 | 32 | Table 41 | — |
| | | 6 | 48 | Table 42 | Table 46 |
| 2 × 2 | 8M | 4 | 32 | Table 43 | — |
| | | | 48 | — | — |
| | | 6 | 32 | — | — |
| | | | 48 | Table 44 | Table 47 |

Table 7   Ion AmpliSeq™ HD library chemistry consumable use

| Number of primer pools | Reads per sample | Samples per set of Genexus™ Library Strips 1 and 2-HD | Barcode plate | Link to consumable use table | |
|---|---|---|---|---|---|
| | | | | GX5™ Chip | GX7™ Chip |
| 1 | 2M | 4 | 32 | Table 48 | Table 50 |
| 2 | 1M | 4 | 32 | Table 49 | Table 51 |
| 2 + 1 | 1M | 4 | 32 | — | Table 52 |

# Guidelines for chip selection

Use these guidelines to select the appropriate chip for sequencing custom panels. The nucleic acid type and amplicon size range is determined at the time of custom panel creation in Ion AmpliSeq™ Designer. For more information about designing and ordering custom panels, see the *Ion AmpliSeq™ Designer Help* (available at **AmpliSeq.com**).

| Chip | Reads per lane | Library type | Nucleic acid sample type[1] | Amplicon size range |
|------|---------------|--------------|-----------------------------|---------------------|
| GX5™ Chip | up to 15 M | Ion AmpliSeq | DNA | up to 275 bp |
| | | | RNA | |
| | | Ion AmpliSeq HD | cfDNA/FFPE ("short" designs) only | 75–125 bp |
| GX7™ Chip | up to 25 M | Ion AmpliSeq | DNA | up to 275 bp |
| | | | RNA | |
| | | Ion AmpliSeq HD | cfDNA/FFPE ("short" designs) only | 75–125 bp |

[1] Selected at the time of design creation in Ion AmpliSeq™ Designer (**AmpliSeq.com**).

# Ion AmpliSeq™ HD sequencing experiment design

| Experiment design workflow |
|:---:|

| ① | ② | ③ | ④ |
|:---:|:---:|:---:|:---:|
| **Decide on the desired target LOD** | **Determine the required DNA input amount based on the corresponding LOD target** | **Determine the required number of reads per sample based on DNA input amount and panel multiplexy (primer pairs per panel)** | **Determine the number of samples per chip based on the required number of reads per sample** |

**Chapter 4** Guidelines for setting up custom assays and sequencing runs on the Genexus™ Integrated Sequencer
*Molecular tag sequencing technology—Important factors*

4

# Molecular tag sequencing technology—Important factors

When designing a sequencing experiment, using either Ion AmpliSeq™ (non-molecular tag) or Ion AmpliSeq™ HD (molecular tag-based) sequencing, three factors must be taken into consideration: the targeted % variant allele frequency limit of detection (%VAF LOD), the quantity of input DNA, and the minimum read depth needed.

To detect a variant in a DNA sample, the physical presence of multiple copies of that variant containing molecule (nominally 6–9 copies) is needed to reliably capture target variant molecules for variant calling. Assuming about 300 molecules per ng of human DNA, theoretically to detect variants at 1% frequency, 2-3 ng human DNA sample input is needed to get the 6-9 variant containing molecules in the assay. For 0.1% VAF LOD detection, 20-30 ng human DNA sample input is required to get the 6-9 variant containing molecules. The direct tie between DNA sample input amount requirement and the %VAF LOD target in an assay applies for both Ion AmpliSeq™ (non-molecular tag) and Ion AmpliSeq™ HD (molecular tag-based) sequencing.

For assays that use Ion AmpliSeq™ chemistry, the required minimum DNA input amount and the minimum read depth are independently tied to the %VAF LOD target and the minimum reads depth requirement is not directly dependent on the DNA input amount but on the level of %VAF LOD target. Therefore, the lower the %VAF LOD target, the higher the required minimum read depth needed. To detect 1% variant allele frequency, 4,000X read depth is needed to get the 40 variant containing reads for variant calling.

In contrast, Ion AmpliSeq™ HD assays use molecular tag sequencing technology to achieve lower %VAF LOD targets. This relies on generating families of reads based on molecular tagging information before calling variants. So, the minimum reads depth requirement is directly dependent on the DNA input amount. That is, with increased DNA input, you must increase the minimum reads depth accordingly. This direct tie between DNA input amount and minimum reads depth needed is because of the requirement to build molecular tag families for variant calling. Therefore, increasing the DNA input amount and the concomitant increase in read depth results in low % VAF LOD detection (Figure 1).

In general, to build valid tag families at a given DNA input amount, the goal is to generate an average of 8 reads per amplicon for each input molecule (by default the minimum is 3 reads, with at least 1 read from each strand). For example, a human DNA sample with 20 ng input (~6,000 molecules) typically generates ~6,000 tag families per amplicon and can therefore theoretically achieve 0.1% LOD (6 / 6,000), when coverage depth is ≥50,000X (corresponding to an average of 8 reads per molecule per amplicon (8 × 6,000)). If the minimum coverage depth for a given input is not achieved, valid molecular tag families can be underrepresented, impairing the variant calling process. This also applies to DNA input amounts <20 ng. The reduction in the number of molecules in a sample results in the reduction of the number of tag families for higher %AF LOD targets, and consequently, a reduction of the reads depth requirement.

For 0.1%VAF LOD target with Ion AmpliSeq™ HD chemistry, we recommend at least 20–30 ng DNA input (50 ng for optimal performance). An optimal read coverage of 70,000X read depth per amplicon, which means 70,000 × (number of amplicons in the DNA panel) reads per library is recommended.
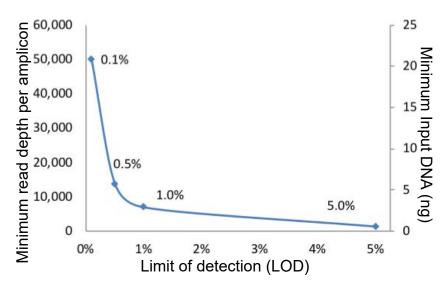
**Figure 1 Representative limit of detection for a custom Ion AmpliSeq™ HD panel, using human cfDNA input and the required per amplicon read depth.** The graph illustrates the theoretical relationship between the minimum human DNA input amount, and the minimum reads depth per amplicon requirement to achieve a given %VAF LOD target. For example, to achieve 1%VAF LOD target, you need 2–3 ng DNA input and 7,000 reads depth per amplicon assuming the sample contains 6–9 copies of variant containing molecules and the read depth generates sufficient tag families for variant calling. If you increase the DNA input amount, you must increase the minimum read depth accordingly even you are still targeting the same %VAF LOD. The read depth increase is to resolve the molecular tag families due to the increased number of DNA input molecules.

The number of samples that can be sequenced on a single chip is determined by the total reads needed for a given assay and the number of reads that are generated per chip. Because the total reads requirement increases with decreasing target %VAF, fewer samples can be sequenced per chip. By raising the target %VAF you can start with a lower DNA input amount, concomitantly reduce the needed total read depth, and therefore sequence a greater number of samples per chip.

# 5 Assays for use with custom panel files

Assays in Genexus™ Software contain the settings and parameters for library preparation, templating, sequencing, analyzing, and reporting the results. Assays also define the panels, kits, and chips that are used in a run, and specify the reference files and threshold values for quality control and variant detection. You can optionally add a filter chain to an assay, to narrow the list of variants that appear in a sample result.

An assay is a reusable experimental design that contains predefined settings appropriate for use with common types of research applications. An assay can be used to plan many runs and plays an important role in enabling rapid throughput. Assays help reduce variability and the chance of errors, because information is stored and then applied to runs instead of entered manually for each run. You can create a new assay for use with a custom Ion AmpliSeq™ or Ion AmpliSeq™ HD panel, copy and edit an existing assay, or import an assay.

Before you can create, copy or import an assay for use with a custom panel, you must first import a genome reference file for non-human DNA panels, design files and, optionally, the hotspots file that the panel includes, and a sequence variant baseline (SVB) file that you can create. Each file is available for selection in the software after files are imported to Genexus™ Software.

## Import or add a custom panel

Before you sequence samples on the Genexus™ Integrated Sequencer with a custom panel, you must import or add a ZIP file that contains files for the custom panel, or add individual custom panel files to Genexus™ Software. Manager- and administrator-level users can complete these procedures. If you will use a custom non-human genome reference file, ensure that you add the reference FASTA file to the software *before* you add the custom panel files (designed.bed or ZIP file) to Genexus™ Software.

Note: RNA gene expression is not currently supported in Genexus™ Software for custom Ion AmpliSeq™ panels.

The first time that you use a custom panel, we recommend that you complete one of the following procedures.

- Import the custom panel ZIP file directly from **AmpliSeq.com**. For more information, see "Import a custom panel file from Ampliseq.com to Genexus™ Software" on page 41. These custom panels must have a status of "ordered" or "quote requested" on AmpliSeq.com to be available for import.

- Add the custom panel ZIP file from your computer. For more information, see "Add a custom DNA panel file to Genexus™ Software" on page 43 and "Add a custom Fusions panel file to Genexus™ Software" on page 44. These custom panels can be designed with either a human or a non-human genomic reference.

The custom panel ZIP file that you import directly from AmpliSeq.com, or add from a directory on your computer, contains the panel target regions (designed.bed file) and other files that are related to the custom panel.

- For DNA panels, you can add the ZIP file that contains the panel file and optionally includes a hotspots file. To use versions of panel files that are not included in the Ion AmpliSeq™ custom panel, such as a custom hotspots (hotspot.bed) file, you must add the files after the genome reference FASTA file and the custom panel files (designed.bed or ZIP file) are added to Genexus Software.

- For fusion panels, the ZIP file from **AmpliSeq.com** contains the target region file (BED) and the fusion reference file (FASTA). The Ion AmpliSeq™ and Ion AmpliSeq™ HD custom fusion panel files are encrypted, so you cannot visualize or edit the file content or download file content after the fusion reference is uploaded in Genexus™ Software, but the files are used by the software only for analysis. Reverse transcription is included as part of the Genexus™ Software fusion panel workflow during library preparation.

  **Note:** For a DNA and Fusions combined assay workflow, contact your Field Application Scientist (FAS) or Field Bioinformatics Specialist (FBS).

- For custom panels that are not human DNA panels, you can create either a **Generic Sequencing Application** assay, which only performs sequencing and mapping but not variant calling, or a **Non-Human Reference** assay that includes both mapping and variant calling. You must first upload the genome reference FASTA file, and then upload the target regions (designed.bed file).

  For non-human custom panels, you can obtain the genome reference FASTA file from one of the following sources:

  – Use a reference that is included in the ZIP file for some non-human custom panels from **AmpliSeq.com**, along with a target regions BED file.

  – Use a reference from ionupdates.com.

  – Use your own genome reference file (for example, if you have uploaded a genome reference file to **AmpliSeq.com** for a custom design).

  **Note:**
  - If the reference FASTA file is in ZIP file format, the file must be extracted before it is uploaded to Genexus™ Software.
  - If a custom reference file was previously uploaded to Genexus™ Software, the custom reference is ready to use and can be selected from the reference dropdown menu when a panel is imported or added.

For more information, see "Add individual target regions files to Genexus™ Software" on page 46.

To improve specificity and sensitivity and to improve the limit of detection, you may want to create a Sequence Variant Baseline. You can also adjust parameters such as the Variant Finding parameters to lower limits of detection (LOD). For more information, see "Create a sequence variant baseline (manager/administrator)" on page 51.

## Import a custom panel file from Ampliseq.com to Genexus™ Software

Before you import panel files directly into Genexus™ Software, you must have the following:

- An account in Genexus™ Software.
- An access code that allows you to access **AmpliSeq.com** from Genexus™ Software. You can generate a randomized access code on the website, or create an access code of your choice. You can also use an access code that you generated or created previously.
- Custom panels with a status of "ordered" or "quote requested" on **AmpliSeq.com**.

1. To generate an access code for **AmpliSeq.com** do one of the following actions.

   Proceed to step 2 to use a previously saved access code for **AmpliSeq.com**.

| Option | Action |
|---|---|
| Generate a random access code on **AmpliSeq.com** for use in step 4. | a. Go to **AmpliSeq.com**, and sign into your account.<br>b. Click **My Account ▸ Manage Access Code**.<br>c. In the **Manage Access Code** dialog box, click **Generate** to receive an access code, then click **Save**.<br>d. Clip **Copy to Clipboard** to use the access code in step 4. |
| Create an access code of your choice for **AmpliSeq.com** for use in step 4. | a. Go to **AmpliSeq.com**, and sign into your account.<br>b. Click **My Account ▸ Manage Access Code**.<br>c. Enter the code that you want to use as your access code into the **Access Code** field. Select an **Access Code Expiration** in the dropdown menu, then click **Save**.<br>d. Click **Copy to Clipboard** to copy the access code for use in step 4.<br><br>If desired, you can remember the access code for future use. Alternatively, you can generate an access code each time that you sign into **AmpliSeq.com**. |

2. In Genexus™ Software, in the menu bar, click **Assays ▸ Manage Panels**.
   The **Panels** tab in the **Manage Panels** screen opens.

3. Click ⊡ **Import from AmpliSeq**.

4. In the **Import from AmpliSeq** dialog box, enter the required information.

| Panel Type | Options |
|---|---|
| **DNA and Fusion Panel** | **IMPORTANT!** This option is not supported for custom panel imports from **AmpliSeq.com**. |
| **DNA Panel** | • **Reference**—Select a reference genome.<br>• **Library Chemistry**—Select **AmpliSeq** or **AmpliSeqHD**<br>• **AmpliSeq Panels**—Select **Custom Panel**<br>• **AmpliSeq Username**—Enter your username for **AmpliSeq.com**.<br>• **AmpliSeq Access code**—Enter the access code that you created previously, or that you copied to your clipboard in step 1. |
| **Fusion Panel** | • **Library Chemistry**—Select **AmpliSeq** or **AmpliSeqHD**<br>• **AmpliSeq Panels**—Select **Custom Panel**<br>• **AmpliSeq Username**—Enter your username for **AmpliSeq.com**.<br>• **AmpliSeq Access code**—Enter the access code that you copied to your clipboard, or that you created previously. |
| **RNA Panel** | **IMPORTANT!** This option is not currently supported in Genexus™ Software. |

5. Click **List My Ampliseq panels**, then select the custom panel to import.

6. Click **Import**.

---

Note: Large files take time to import. You can see the file listed in the **Panels** tab only when the import is complete.

---

After the import is complete, the new panel is available to be associated with a new software assay that you create or copy in Genexus™ Software. Hotspots files for the panel are listed in the **Hotspots** tab and are also available to add to a custom assay. For ZIP file imports, the name of the hotspots is the name of the panel followed by _**Hotspot**. Fusion references are listed in the **Panels** tab with more details available in the **Fusion References** tab.

For more information, see "Create an assay in Genexus™ Software for use with a custom panel" on page 56 and "Copy an assay (manager/administrator)" on page 73

# Add a custom DNA panel file to Genexus™ Software

You must add the files for a custom panel to Genexus™ Software before you sequence samples on the Genexus™ Integrated Sequencer. Manager- and administrator-level users can add new panel files to the software.

Use this procedure if the custom panel ZIP file is available on your computer.

Custom DNA panel files can be added to Genexus™ Software in the following ways.

- You can upload the entire ZIP file for the Ion AmpliSeq™ custom panel to the software. This option can be completed successfully only for panels that are designed on ampliseq.com using the hg19 or hg38 genomic references. The panel file and related files for the Ion AmpliSeq™ custom panel are contained in the ZIP file that is downloaded from ampliseq.com. This file contains a target regions BED file, and can also optionally contain a hotspots file. We recommend that you use this procedure the first time that you add a custom DNA panel file.

- You can also upload panel files individually, including a DNA reference file and, optionally a hotspots file. Use the steps to upload individual BED files only for non-human custom panels, or edit panel files before the files are uploaded, if you want to use a version of a file that is not included in the Ion AmpliSeq™ custom panel file that you download. Individual design files in BED format can be uploaded only if a corresponding genomic reference is already present in the system. For more information, see "Add individual target regions files to Genexus™ Software" on page 46.

Use this procedure to upload the entire ZIP file for the Ion AmpliSeq custom panel.

---

**IMPORTANT!** If the reference genome is not available in the software, the reference genome must be imported into Genexus™ Software before you import the panel. For more information, see "Add reference genome files (manager/administrator)" on page 46.

---

1. In Genexus™ Software, in the menu bar, click **Assays ▸ Manage Panels**.
   The **Panels** tab in the **Manage Panels** screen opens.

2. Click ╋ **Add New**.

3. In the **Add Panel** dialog box, enter the following required information.
   a. Enter the following information.
      - **Name**—Enter a name for the new panel.
      - **Panel Type**—Select **DNA Panel**.
      - **Reference**—Select a reference genome.

   b. Under **File Type** select **Zip**, then click **Select Panel Zip**.

   c. Navigate to and select the Ion AmpliSeq™ custom panel ZIP file on your computer, then click **Open**.
      This is the ZIP file that you downloaded from Ampliseq.com.

    **d.** Click **Upload**. The custom panel ZIP file contains all required files.

           **Note:**  Large files take time to upload. You can see the file listed in the **Panels** tab only when the upload is complete.

The new panel is listed in the **Panels** tab and is available to add to a custom assay. For more information, see "Create an assay in Genexus™ Software for use with a custom panel" on page 56 and "Copy an assay (manager/administrator)" on page 73.

## Add a custom Fusions panel file to Genexus™ Software

You must add the files for an Ion AmpliSeq™ custom panel to Genexus™ Software before you sequence samples on the Genexus™ Integrated Sequencer. Manager- and administrator-level users can add new panel files to the software.

Use this procedure if a custom panel is available on your computer.

Fusion Ion AmpliSeq™ custom panel ZIP files from **AmpliSeq.com** contain the target region file (BED) and the fusion reference file (FASTA). Ion AmpliSeq™ fusion files are encrypted, so you cannot view the content of the files in Genexus™ Software, but the files can be used by the software. You must upload the entire ZIP file to the software. By including a fusion panel in an assay, reverse transcription is performed on the Genexus™ Integrated Sequencer as part of the fusion assay workflow during library preparation.

   **1.** In Genexus™ Software, in the menu bar, click **Assays ▸ Manage Panels**.
      The **Panels** tab in the **Manage Panels** screen opens.

   **2.** Click ✛ **Add New**.

   **3.** In the **Add Panel** dialog box, enter the following required information.

       **a.** Enter the following information.

           • **Name**—Enter a name for the new panel.

           • **Panel Type**—Select **Fusion Panel**.

       **b.** In the **File Type** section, select **Zip**, then in the **Fusion Panel** section, click **Select Panel Zip**.

       **c.** Find the Ion AmpliSeq™ custom panel ZIP file on your computer and click **Open** to upload the custom panel file that contains all required files in ZIP file format.
          This is the file that you downloaded from Ampliseq.com.

       **d.** Click **Upload** to upload the custom panel file that contains all required files in ZIP file format.

           **Note:**  Large files take time to upload. You can see the file listed only when the upload is complete. The fusion reference file is added to the list of references shown in the **Fusion References** tab.

Any new reference files you upload are available for use with the custom panel file when you create or copy an assay in the software. The files are listed **Fusion References** tab.

For more information, see "Create an assay in Genexus™ Software for use with a custom panel" on page 56 and "Copy an assay (manager/administrator)" on page 73.

*Ion AmpliSeq™ & Ion AmpliSeq™ HD Custom Assay User Guide*

## Obsolete panels (manager/administrator)

Manager- and administrator-level users can make panels unavailable that have been added or imported in Genexus™ Software. You cannot designate as obsolete system-installed panels.

1.  In the menu bar, click **Assays ▸ Manage Panels**.
    The **Panels** tab in the **Manage Panels** screen opens.

2.  Place the pointer over the row of the panel that you want to remove from the software, then click **Obsolete**.

The panel is listed in the **Panels** table with a **Status** of **Obsolete**. Panels that have been made obsolete cannot be added to a new assay. Manager- and administrator-level users can reactivate panels that have been made obsolete. For more information, see "Reactivate panels (manager/administrator)" on page 45.

## Reactivate panels (manager/administrator)

Manager- and administrator-level users can reactivate panels that have been made obsolete in Genexus™ Software.

1.  In the menu bar, click **Assays ▸ Manage Panels**.
    The **Panels** tab in the **Manage Panels** screen opens.

2.  Place the pointer over the row of the panel that you want to reactivate, then click **Reactivate**.

The panel is listed in the **Panels** table with a **Status** of **Locked** and is available to add to a new assay.

# Add individual custom panel files to Genexus™ Software

You can upload individual files for use with custom Ion AmpliSeq™ and Ion AmpliSeq™ HD panels, including hotspots and target regions files in BED format, and genomic reference files. You can also create a sequence variant baseline to improve the specificity of variant calling, which is especially useful when you want to use the software to detect somatic variants so that variants can be sensitively and specifically detected at lower LOD. You must add these files for custom panels to Genexus™ Software *before* you sequence samples on the Genexus™ Integrated Sequencer. When you add the files to Genexus™ Software, the files are available to select when you create or copy the assays that you will use for the sequencing runs, and in some cases, to ensure that the files are used correctly by the software.

Manager- and administrator-level users can add new panel files to the software.

# Add reference genome files (manager/administrator)

Manager- and administrator-level users can add new reference genome files in Genexus™ Software. The file must use FASTA format.

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

2. In the **Manage Panels** screen, click the **References** tab, then click ➕ **Add New**.

3. In the **Add Reference Genome** dialog box, enter the required and optional information, then select the FASTA file to upload the reference sequence information.
   Large files take time to upload and may not appear immediately.

4. Click **Upload** when you have finished.

The reference genome file is listed in the **References** table and is available to select when you add or import a custom panel.

# Add individual target regions files to Genexus™ Software

You can upload individual target regions files in BED format for use with custom DNA panels. You must add the files for an Ion AmpliSeq™ custom panel to Genexus™ Software before you sequence samples on the Genexus™ Integrated Sequencer.

To use a custom target regions file with your custom panel, ensure that you upload and add the reference FASTA file to Genexus™ Software *before* you import or add the custom panel files (designed.bed) to Genexus™ Software.

Manager- and administrator-level users can add new panel files to the software.

Use this procedure only if you want to upload reference and target region files separately, for example, to edit a target regions file, or use a version of a file that is not included in the Ion AmpliSeq™ custom panel that you download from **AmpliSeq.com**.

---

**IMPORTANT!**  Before you begin this procedure, the genome reference (FASTA file) that you will associate with the panel must be uploaded before a target regions file can be uploaded. For more information, see "Add reference genome files (manager/administrator)" on page 46.

---

1. In Genexus™ Software, in the menu bar, click **Assays ▸ Manage Panels**.
   The **Panels** tab in the **Manage Panels** screen opens.

2. Click ➕ **Add New**.

3.  In the **Add Panel** dialog box, enter the following required information.

    a.  Enter the following information.

        - **Name**—Enter a name for the new panel.

        - **Panel Type**—Select **DNA Panel** or **RNA Panel**.

          Note:  When you select **RNA Panel**, reverse transcription is performed on the Genexus™ Integrated Sequencer as part of the custom assay workflow during library preparation. This option can be used, for example, for viral RNA custom panels.

          **Reference**—Select the reference genome for the panel.

    b.  Under **File Type** select **BED File**, then click **Select Target Regions file**.

    c.  Find the target regions file to use with the Ion AmpliSeq™ custom panel file on your computer and click **Open**.

4.  Click **Upload**.

    Note:  Large files take time to upload. You can see the file listed only when the upload is complete.

The new target regions file is listed in the **Panels** tab and is available to add to a custom assay in Genexus™ Software.

## Hotspots files for use with Ion AmpliSeq™ and Ion AmpliSeq™ HD custom panels

All Ion AmpliSeq™ HD, and some Ion AmpliSeq™, custom panels include hotspots files. You can create a hotspots file or edit an existing hotspots file for any custom panel, if desired. You can create hotspots files to include variants for which you want to increase the likelihood of detection. For example, variants that have a lower LOD for complex alleles, or in the presence of noise.

To use a custom hotspots file or an edited hotspots file with your custom panel, you must first upload the hotspots (hotspot.bed) file to Genexus™ Software.

By default, the analysis pipeline in Genexus™ Software includes both hotspots calling and de novo calling for Ion AmpliSeq™ and Ion AmpliSeq™ HD custom panels. You can optionally change the settings to set up sequencing runs that call only hotspots. If no hotspots file is provided, only de novo variant calling is done. Default variant calling settings are slightly different for hotspots positions. For example, the settings can allow hotspots variants to be called at a lower LOD. You can modify the threshold for variant calling parameters to allow de novo variants and/or hotspots variants to be called at lower Variant Allele Frequency (VAF) thresholds in order to reduce the LOD after a sequence variant baseline is created. For more information, see "Guidelines to lower the limit of detection (LOD) with Variant Finding parameters" .

Manager- and administrator-level users add, view, and manage the hotspots BED files that are available in Genexus™ Software. Operators can view the hotspots files that are available in the software.

## View hotspot file details

Details about system-installed hotspots BED files and custom hotspots BED files are available to view in Genexus™ Software.

If you have operator-level permissions, you can view the hotspots files and details. Manager- and administrator-level users can view, add, and manage the hotspots files.

1. In the menu bar, click **Assays ▸ Manage Panels**.
   The **Panels** tab in the **Manage Panels** screen opens.

2. Click the **Hotspots** tab.

3. In the **Hotspots** table, view the details for the hotspot files, including the panel with which the file is associated, whether the file is **Locked**, if the file is **Active**, who created the file, and the date on which the file was created. You can also view the genomic coordinates and alleles that are included in the hotspot file.

## Add new hotspots files (manager/administrator)

Manager- and administrator-level users can add new hotspots files in Genexus™ Software.

To use a custom hotspots file or an edited a hotspots file with your custom panel, ensure that you upload and add the new hotspots (hotspot.bed) file to Genexus™ Software after you import or add the custom panel files (designed.bed or ZIP file) to Genexus™ Software.

1. In the menu bar, click **Assays ▸ Manage Panels**.
   The **Panels** tab in the **Manage Panels** screen opens.

2. Click the **Hotspots** tab.

3. Click ＋ **Add New**.

4. In the **Add Hotspot File** dialog box, enter a name, select a panel, then select the hotspots file in BED format that you want to upload.

5. Click **Upload**.
   Large files take time to upload and may not appear immediately.

The hotspots file is listed in the **Hotspots** table and is available to select when you create or copy and edit some custom assays.

### Export hotspot files (manager/administrator)

Manager- and administrator-level users can export hotspot files from Genexus™ Software.

1. In the menu bar, click **Assays ▸ Manage Panels**.
   The **Panels** tab in the **Manage Panels** screen opens.

2. Click the **Hotspots** tab.

3. Place the pointer over the row of the hotspot file that you want to export, then click **Export** to download the hotspot BED file.

## Sequence variant baselines

A sequence variant baseline is used to improve the specificity of variant calling. This is especially useful for workflows that are used to detect somatic variants so that variants can be sensitively and specifically detected at lower LOD.

Variant calling filters in Genexus™ Software suppress and remove most false positives caused by sequencing errors.

A sequence variant baseline is a BED file that contains a list of variant alleles that are identified by the application of an error model to reduce false positives. The baseline file includes detailed properties for each entry. The errors and properties are based on known germline normal samples. When added to an assay in Genexus™ Software, the baseline detects and filters out the errors in sequencing that could lead to false positive variant calls.

Custom Ion AmpliSeq™ panels and Ion AmpliSeq™ HD panels do not include sequence variant baselines. You can however create sequence variant baselines for custom panels to improve specificity and reduce the LOD.

Use of a sequence variant baseline will typically further improve specificity and reduce false positives. In addition, you might be able to set a lower LOD with high specificity when you create and upload a sequence variant baseline.

To create a sequence variant baseline, you must first sequence multiple samples that are amplified with your custom panel. You can then use the samples to create custom sequence variant baselines in Genexus™ Software .

You must create, copy, or import an assay then, to apply the sequence variant baseline to a custom panel, ensure that it is selected in the parameters step in the software when you create or copy the assay.

In addition, you can edit a sequence variant baseline and import it back into the software. You can then replace the existing sequence variant baseline in a newly copied assay, or an assay that is unlocked. Alternatively, you can add the edited sequence variant baseline to a newly created assay. The assay that references the new sequence variant baseline can then be used to reanalyze a sequencing run.

You can add the newly created or edited SVB file to an assay in Genexus™ Software, then reanalyze the run using the newly created assay. For more information, see "Reanalyze a run" .

You can also augment an existing sequence variant baseline by adding additional samples. For more information, see the software help system.

For more information about creating, copying and importing sequence variant baselines, see "Create a sequence variant baseline (manager/administrator)" , "Copy a sequence variant baseline (manager/administrator)" , and "Import a sequence variant baseline (manager/administrator)" . For more information about selecting the sequence variant baseline in the assay, see "Create an assay in Genexus™ Software for use with a custom panel" , and "Copy an assay (manager/administrator)" .

Add the custom sequence variant baseline to the assay that you create in Genexus™ Software. Using this custom sequence variant baseline will reduce false positives at low variant allele frequency, and therefore facilitate the optimization of this custom assay workflow with a lower variant allele frequency threshold. You can also customize parameters, for example, you can reduce the variant allele frequency threshold for SNVs, hotposts, indels, and so on, to lower the LOD. For more information, see "Guidelines to lower the limit of detection (LOD) with Variant Finding parameters" .

## View sequence variant baseline details (manager/administrator)

Manager- and administrator-lever users can view details for custom sequence variant baselines. You can view the name of a system installed sequence variant baseline file for an assay in the **Variant Finding** section of the **Parameters** step when you copy an assay.

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

2. In the **Assays / Manage Presets** screen, click the **Sequence Variant Baselines** tab.
   The **Sequence Variant Baselines** table lists custom sequence variant baselines.

3. Click the name of a sequence variant baseline.

Review the details in the **Sequence Variant Baselines** pane. The details that are shown depend on the setup of the sequence variant baseline. Parameters used to create sequence variant baselines are also shown, but are not listed in this table.

Table 8   Sequence variant baseline details

| Item | Description |
|---|---|
| Created By | Sign-in name of the person who created the baseline. |
| Created On | The date the baseline was created. |
| Number of Samples | The number of samples that are included in the baseline. |
| Amplification Type | Library amplification technology that is used in the baseline (Ion AmpliSeq™ or Ion AmpliSeq™ HD). |
| Panel Name | The name of the panel that is included in the baseline. |
| Chip Type | The semiconductor sequencing chip for the panel and baseline. |
| Reference | The reference genome for the panel and baseline. |

You can click ••• **(More Options)** to perform more actions, such as obsolete, export, copy, edit, or audit the sequence variant baseline.

The actions for each baseline are also available in the list of **Sequence Variant Baselines** when you place the pointer over the row of a sequence variant baseline.

## Create a sequence variant baseline (manager/administrator)

Manager- and administrator-level users can create a sequence variant baseline (SVB) if system-installed sequence variant baselines do not meet research needs. You can add the custom baseline to an assay to use as a baseline for a run.

---

**IMPORTANT!** Use the default parameter settings unless you are an advanced user. Contact a Field Bioinformatics Specialist (FBS) for help.

---

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

2. In the **Assays / Manage Presets** screen, click the **Sequence Variant Baselines** tab.

3. Click ➕ **Add New**.

4. Complete the **Panel** step.

   a. Enter a name in the **Sequence Variant Baseline Name** field.

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

   b. Select or leave **Augment an Existing Sequence Variant Baseline as Base** unselected.

   - Select **Augment an Existing Sequence Variant Baseline as Base**, then select an SVB from the dropdown list to use the existing sequence variant baseline as a starting point for the new baseline.

   - Leave **Augment an Existing Sequence Variant Baseline as Base** unselected to create a new baseline.

   c. Select the panel.

   d. *(Optional)* Enter information about the baseline into the **Description** field.

   e. Click **Next**.

5. In the **Parameters** step, accept the default settings or adjust the appropriate parameters, then click **Next**.

6. In the **Samples** step, select at least 40 DNA, DNA & RNA, or TNA samples for the baseline.

   The samples that you select must have the same library chemistry, chip type, and flow order.

7. In the **Review** step, review the selections, then click **Launch**.

   The new sequence variant baseline is added to the **Sequence Variant Baselines** list.

Sequence variant baselines with a **State** of **Completed** are available for selection when an assay is created or copied and edited. For assays that are compatible with the sequence variant baseline, you can select the sequence variant baseline file in the **Variant Finding** section of the **Parameters** step, in the **Analysis SVB File** dropdown list.

If the **State** of a sequence variant baseline is **Failed**, place the pointer over the row of the sequence variant baseline and click **View Log** to open the log in a separate browser tab. The log file can help with troubleshooting if the baseline creation job fails.

## Copy a sequence variant baseline (manager/administrator)

Manager- and administrator-level users can copy then edit a sequence variant baseline. You cannot copy sequence variant baselines that have been imported.

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

2. In the **Manage Assays** screen, place the pointer over the row of the sequence variant baseline that you want to copy, then click **Copy**.
   The **Copy Sequence Variant Baseline** screen opens to the **Panel** step with the panel and samples predefined. The settings and samples that are included can be modified for the new baseline.

3. Change the software-generated name of the baseline if needed, proceed through the workflow steps, and modify settings. For more information, see "Create a sequence variant baseline (manager/administrator)" on page 51.

4. In the **Review** step, review the selections, then click **Launch**.

The new sequence variant baseline is added to the **Sequence Variant Baseline** list. When the **State** of the SVB is **Completed** and the **Status** is **Locked**, the sequence variant baseline is available for selection when an assay is created or copied and edited.

For assays that are compatible with the sequence variant baseline, you can select the sequence variant baseline file in the **Variant Finding** section of the **Parameters** step, in the **Analysis SVB File** dropdown list. For more information, see "Create an assay in Genexus™ Software for use with a custom panel" on page 56.

## Import a sequence variant baseline (manager/administrator)

Manager- and administrator-level users can import a sequence variant baseline. Two types of files are supported:
- ZIP
- BED

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

2. In the **Assays / Manage Presets** screen, click the **Sequence Variant Baselines** tab.

3. Click ⬆ **Import Sequence Variant Baseline** in the top right of the screen.

| Option | Description |
|---|---|
| Import a ZIP file | a. In **File Type**, select **ZIP**.<br>b. Click **Select Sequence Variant Baseline file**.<br>c. Browse for and select the file.<br>d. Click **Upload**. |
| Import a BED file | a. In **File Type**, select **BED**.<br>b. Enter a name for the baseline.<br>c. In the **Panel** list, select the panel.<br>d. Click **Select Sequence Variant Baseline file**.<br>e. Browse for and select the file.<br>f. Click **Upload**. |

A message confirms that the file import was successful when the file import is complete.

The imported sequence variant baseline is added to the **Sequence Variant Baseline** list. When the **State** of the SVB is **Completed** and the **Status** is **Locked**, the sequence variant baseline is available for selection when an assay is created or copied and edited.

For assays that are compatible with the sequence variant baseline, you can select the imported sequence variant baseline file in the **Variant Finding** section of the **Parameters** step, in the **Analysis SVB File** dropdown list. For more information, see "Create an assay in Genexus™ Software for use with a custom panel" on page 56.

# Filter chains for use with Ion AmpliSeq™ custom panels

A filter chain is a set of filters that Genexus™ Software uses to narrow the list of variants that appear in a sample result.

When you view run results and the samples in the results, you can select a filter chain. The filter chains can be applied to dynamically change the view of the variants as you view results, or you can save the filter chain to be included as part of the assay.

System-installed filter chains are locked and cannot be changed. You cannot edit system-installed filter chains, but you can create custom filter chains to refine the results in the software. For example you can create a filter chain to remove common population variants from gnomAD or ExAC, using MAF

as a filter. You can remove variants below a specific variant allele frequency. You can remove intronic variants, and so on. For more information, see the software system help.

For Ion AmpliSeq™ or Ion AmpliSeq™ HD somatic oncology panels, you can use a filter chain for use with Oncomine™ assays, such as the Oncomine™ Extended or Oncomine™ Variants filter chain, to show the most clinically relevant variants.

If you are interested in filtering variants in a different way, you can create a custom filter chain that filters for variants in public databases such as gnomAD, Clinvar, and ClinGen, variants applicable only to Exons, and so on. In addition, you can create a custom gene list that shows only variants in selected genes. For more information, see the software system help.

Filter chains and gene lists allow you to first view the variants that are likely to be most interesting and important in the sample, then you can remove the filter chain or gene list to see all variants in the sample, if desired.

**Table 9   System-installed filter chains available for use with Ion AmpliSeq™ custom panels**

| System-installed filter chain [1] | Description |
|---|---|
| Oncomine™ Extended | This filter chain includes all Oncomine™-annotated variants and variants that can be relevant to cancer due to their inclusion in one or more of the following classes: <ul><li>Non-targeted fusions.</li><li>CNV variants with **FILTER** value of **GAIN** or **LOSS**.</li><li>Likely somatic mutations based on **dbSNP**, **5000Exomes**, **ExAC**, and **UCSC Common SNPs** annotation source databases. The minor allele frequencies range lies between 0.0 and 1.0E-6. Mutations must also be nonsynonymous and occur in exonic or splice-site regions.</li><li>Variants with **ClinVar** annotations of pathogenic or likely pathogenic.</li></ul> |
| Oncomine™ Variants | This filter chain includes all Oncomine™-annotated variants. |
| Variant Matrix Summary | Returns variant types in a visualization. Variant types returned are **SNV/INDEL**, **CNV**, **fusions**, and **RNAExonVariants**. |
| No Filter | Select this option to remove a previously applied filter chain and view all called variants. |

[1]   The version depends on the assay parameter set that is installed in the software.

# Custom assay creation

**Note:**  You must import a custom panel, into Genexus™ Software before you use the procedures in this section. For more information, see "Import or add a custom panel" on page 39.

Before you can use an Ion AmpliSeq™ custom panel in a sequencing run, you must create an assay in Genexus™ Software. Assays contain the settings and parameters for library preparation, templating, sequencing, analyzing, and reporting the results. Assays also define the panels, kits, and chips that are used in a run, and specify the reference files and threshold values for quality control and variant detection.

Manager- and administrator-level users can create a new assay from a system-installed template in Genexus™ Software. Each assay template allows you to select kits, chips, primer panels, and adjustable Quality Control (QC) thresholds and other parameters that are appropriate for the custom panel. The assay is then available to select when you plan a run.

To create a custom assay:

- Use an assay template to create a custom assay that contains preconfigured settings. The template includes settings for various genetic research analysis applications. For more information, see "Create an assay in Genexus™ Software for use with a custom panel" on page 56.

- Copy an existing system-installed assay or a locked custom assay, then edit the settings of the assay to create a new custom assay. For more information, see "Copy an assay (manager/administrator)" on page 73.

After you create an assay, you can optimize the assay configuration if needed. You can plan a run with a draft assay, view the results, then edit the assay and plan new runs before you lock the draft assay. This is useful if you want to further optimize analysis parameters for the custom Ion AmpliSeq™ panel.

---

**IMPORTANT!** Use the default settings for system-installed assays unless you are an advanced user. Contact your Field Bioinformatics Specialist (FBS) for assistance.

---

# Create an assay in Genexus™ Software for use with a custom panel

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

   The **Create Assay** screen opens.

2. Click the assay type for the template that provides the appropriate kits, chips, primer panels, and adjustable QC thresholds and analysis parameters.

| Assay type | Description |
|---|---|
| **Generic Sequencing Application** | Use this template to sequence samples with or without mapping to reference files. Creates an unmapped (unaligned) BAM file, and optionally an (mapped) aligned BAM file if a panel is selected in the software. The BAM file can be exported for use in other applications, such as the analysis of gene expression data, analysis of viral and microbial sequencing and assembly, and so on.<br><br>With this assay type, if a panel is selected in Genexus™ Software, the software uses the target regions BED file for the following information.<br><br>• the number of pools<br>• the chemistry type (Ion AmpliSeq™ or Ion AmpliSeq™ HD)<br><br>This assay type is typically used to sequence samples that are not human or for non-DNA or non-Fusion panels, for example, RNA gene expression panels, viral or microbial panels. Assays that are based on RNA panels will include the reverse-transcription step before target amplification. |
| **Non-Human Reference** | Use this template to sequence samples that are mapped to a non-human reference, and perform variant calling. Typically, run results from the **Non-Human Reference** assay type are not filtered by default with filter chains, and do not include any annotations, or automatic report generation.<br><br>Assays that are based on RNA panels will include the reverse-transcription step before target amplification during library preparation.<br><br>For example, sequencing an RNA virus with the goal of mapping and variant calling will use the **Non-Human Reference** assay type.<br><br>With this assay type, Genexus™ Software creates a mapped BAM file after mapping, and a VCF file after variant calling.<br><br>IMPORTANT! Ensure that you add the reference (FASTA file) to the software before the custom panel file (designed.bed) is added to the software. Then, create an assay after the panel is added. |
| **DNA and Fusions** [1] | Use this template to create a single assay that detects and annotates somatic DNA variants and RNA fusion targets in paired DNA and RNA samples from the same source. |
| **DNA Somatic** [1] | Use this template to create an assay that detects and annotates somatic variants in DNA samples, and to use Genexus™ Software to analyze liquid biopsy samples. For more information, see "Create a liquid biopsy assay in Genexus™ Software" on page 72. |
| **DNA Germline** [1] | Use this template to create an assay that detects and annotates germline variants in DNA samples. |

| Assay type | Description |
|---|---|
| **Fusions** [1] | Use this template to create an assay that detects and analyzes fusions in RNA samples. |

[1] Automatically detects chemistry (for standard Ion AmpliSeq™ or Ion AmpliSeq™ HD) based on the panel file format.

3. In the **Panel** step, select the **Panel** that applies to your custom assay.

   If you select **None** for use with the **Generic Sequencing Application**, an unmapped (unaligned) BAM file is created.

   If you selected **Non-Human Reference** as the assay type, you can create both DNA- and RNA sequencing runs, depending on which **Panel** you select in this step. Assays that are based on RNA panels include the reverse transcription step before target amplification during library preparation.

4. Select other options for the assay. For descriptions of the panel information that are appropriate for the assay, see "Panel step assay options" on page 60.

5. Click **Next**.

6. In the **Reagent** step, select the kits and chips that you are using. Ensure that the reagents are appropriate for the panel that you are using.

   ---
   **Note:** The Genexus™ Templating Strip 3B GX5™ is not supported for use with Ion AmpliSeq™ Custom panels.

   ---

   For descriptions of the reagent information that is available for the assay, see "Reagent step assay options" on page 61.

7. Click **Next**.

8. In the **Parameters** step, accept the recommended default values, or adjust the values within the allowed ranges. Refer to the following guidelines to maximize the use of your custom panels.

**Table 10  Recommended parameter settings for Ion AmpliSeq™ custom panels**

| Assay type | Setting description |
|---|---|
| DNA | **Resequencing**—This option is set by default based on chemistry type. **AmpliSeqHD** = True, **AmpliSeq** = False. |
| DNA and Fusions | By default, the **Library Pooling Percent DNA** parameter is set to 80 percent and the **Library Pooling Percent RNA** parameter is set to 20 percent. Both parameters are set to 50 percent by default for Ion AmpliSeq™ HD custom panels. If you have a panel with a small number of fusion amplicons, and a large number of DNA amplicons, you can change **Library Pooling Percent DNA** parameter to help ensure sufficient amplicon coverage. When you change this parameter, the **Library Pooling Percent RNA** parameter changes automatically to add to a total of 100 percent. |
| Fusions | To help ensure that results for Fusions custom panels are robust, adjust the Fusion QC parameters. For more information, see "Guidelines to adjust Fusion parameters" on page 64. |

To improve specificity and sensitivity and to reduce the limit of detection, you might want to create a Sequence Variant Baseline (SVB). You can also adjust parameters such as the **Variant Finding** parameters to lower limits of detection (LOD). For more information, see "Guidelines to lower the limit of detection (LOD) with Variant Finding parameters" on page 66 and "Sequence variant baselines" on page 49.

9. *(Optional)* You can set the parameters with an advanced parameters configuration file in JSON format to override the default values. First, download the template that is used with the assay, then edit the JSON file if needed, and upload the edited file.

   a. At the bottom of the **Parameters** step, click **Download** to download a ZIP file of the JSON template that is appropriate for the assay.

   b. Edit the file with custom settings, then save the file to the computer.

   c. At the bottom of the **Parameters** step, click **Upload**.

   d. Click **Select files**, navigate to the file on the computer, then click **Open**. Click **Upload** to upload the file and override the default values in the template.

10. *(Optional)* To use parameters from another assay or panel, you can download and export assay parameters that are locked. For more information, see "Download assay parameters" on page 77.

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

    b. In the **Manage Assays** screen, place the pointer over the row of a locked assay, then click **Download Parameters**.

       The assay parameter files are downloaded to the computer as a ZIP file that contains JSON files. The assay parameter files are available to use in a custom assay.

    c. Edit the parameter values in the file and save the file.

    d. Click **Select File**.

    e. Navigate to the edited file.

    f. Click **Open**.

    g. In the **Upload Parameters** dialog box, click **Upload** to upload the file.

11. *(Optional)* To apply a sequence variant baseline (SVB) file to the assay, do the following.

    a. In the **Variant Finding** section, in the **Analysis SVB File** dropdown list, select an SVB.

       Sequence variant baselines that are compatible with the assay and have a **State** of **Completed** are available for selection when an assay is created or copied and edited.

12. Click **Next**

13. In the **QC** step, enter QC metric values, then click **Next**.

    For descriptions of the QC information for the assay, see "QC step assay options" on page 68.

14. In the **Plugins** step, plugins to run with this assay are selected by default.

   - Ion AmpliSeq™ panels — Ensure that CoverageAnalysis and Customer Support Archive are selected. Select Sample ID, if you have added the SampleID amplicons. You can deselect MolecularCoverageAnalysis, because this plugin applies only to Ion AmpliSeq™ HD panels.

   - Ion AmpliSeq™ HD panels — Ensure that CoverageAnalysis, Customer Support Archive and MolecularCoverageAnalysis are selected.

   For more information about plugin results, see "Review coverageAnalysis plugin results" on page 166, "Review molecular Coverage Analysis plugin results" on page 170, and "Customer Support Archive" on page 172.

   - For more information about spike-in panels and use of the Sample ID panel, see "Ion AmpliSeq™ Sample ID Panel" on page 176.

15. Click **Next**.

16. In the **Save** step, enter an assay name, an assay full name, and an optional description, then click **Save**.

   The types of runs that the assay is compatible with are listed in the **Save** step. The types of assays listed depends on the selections in the **Reagent** step. For example, if the Ion Torrent™ Genexus™ Library Strips 1 and 2-AS library kits are selected, then **Nucleic Acid to Report** and **Library to Results** run types are shown. If other AmpliSeq library kits are selected, then only **Library to Results** run type is shown.

The new assay is listed in the **Manage Assays** screen. The **Assay Full Name** is followed by Draft to indicate that the assay is in draft status. You can plan a run with the draft assay and the assay is available to lock, edit, or delete, and audit. You can also download parameters from assays in **Draft** or **Locked** statuses.

## Panel step assay options

Manager- and administrator-level users can use the **Panel** step to select the appropriate reference files and other settings for use with an assay in Genexus™ Software.

In the **Panel** step, you can select the appropriate reference files to use in an assay.

The following table lists options that are available in the **Panel** step. The available options depend on the type of assay.

IMPORTANT!  Use the assay default settings unless you are an advanced user. Contact your Field Bioinformatics Specialist (FBS) for assistance.

| Option | Description |
|---|---|
| Panel | The panel to use in the assay. The remaining options are appropriately populated based on the selected panel. |
| | The panel based on a tab-delimited BED file that defines the coordinates of the regions of interest to be amplified with the selected primer set. |
| Hotspots | *(Optional)* The hotspots file to use in the assay. The hotspots file tab-delimited BED file that indicates the positions where a DNA variant is expected to occur. It also annotates these positions with the reference allele, variant allele, and anchor base. |
| Target Regions | The target regions BED file that is appropriate for the sample. Depending on the application, you may need to select a separate DNA and Fusions Target Regions file. |
| Genome Reference | The genome reference file for the panel that is selected is displayed. When you create or upload a panel, you select the genome reference. |
| | The hg19 and GRCh38 genome references are installed with the system. |
| Library Chemistry | The type of library chemistry to use in the assay. |
| Extraction Method Type | The method used to extract the DNA, RNA, or DNA and RNA from the sample, or as a single library TNA from the sample. |
| | Available options depend on the panel that is selected. |
| Minimum Read Count Per Sample | The minimum number of reads per sample that are needed to achieve the coverage depth required in an assay. |
| | This value, usually associated with the panel, determines the number of samples that can be run per lane on a chip. The system uses this value to calculate how many lanes a run uses and assigns samples according to the calculation. For example, a minimum read count per sample of 1,000,000 allows twice as many samples on the same lane as that of 2,000,000. |
| Fusion Reference | The FASTA file to use in the assay that indicates the reference sequence of the targeted RNA fusion isoform and other RNA variants. These files are encrypted. |

*(continued)*

| Option | Description |
|---|---|
| Fusion Panel | The tab-delimited Fusion BED file to use in the assay that defines the coordinates of the regions of interest to be amplified with the selected primer set. It also annotates these positions. These files are encrypted. |
| Primer Pools | The number of primer pools used in the assay. |
| Annotation Sets | The annotation set to use in the assay. For example, dbSNP, IGSR, ClinVar, COSMIC, and so on.<br><br>Some annotation sets are system-installed. Custom annotation sets can be created as required. For more information, see the Genexus™ Software help system. |
| Apply Oncomine Variant Annotations | If you select the **Apply Oncomine™ Variant Annotations** option in the **Panel** step to create or edit an assay, analysis results data from more than 24,000 exomes across solid tumor and hematological cancer types is used as a reference to annotate variants that are relevant to cancer. The annotations are shown in results in the Oncomine™ Gene class and Oncomine™ Variant class columns. For more information, see the Genexus™ Software help system. or contact a support representative, Field Bioinformatics Specialist (FBS), or Clinical Account Consultant (CAC). |
| Use Latest Annotations | When enabled, this setting instructs the assay to use the most recent version of the annotation source in the software. The version of the annotation source that is used with the run depends on installation of newer versions of the annotation source. After this setting is enabled, any new run plan uses the latest version of the annotation source that is available in the software. This means that run plans with the same assay can use a different version of the annotation source. |

## Reagent step assay options

Manager- and administrator-level users can use the **Reagent** step to select options that are related to the reagents for use with an assay in Genexus™ Software.

In the **Reagent** step, you select the kits and chips to use in an assay. Ensure that the kits and chips that you select are appropriate for the primer panel that you are using.

The following tables list options that can be available in the **Reagent** step. The available options depend on the type of assay.

---

**IMPORTANT!** Use the assay default settings unless you are an advanced user. Contact your Field Bioinformatics Specialist (FBS) for assistance.

---

Table 11 Purification

| Option | Description |
|---|---|
| Purification Kit | The purification kit that is used for nucleic acid purification. |

Table 12   Library Preparation

| Option | Description |
|---|---|
| Library Kit | The library kit that is used to prepare the library. |
| Barcode Kit | The barcode kit to use in the assay. |
| Include Inline controls | You can select **DNA** or **RNA**, or both **DNA** and **RNA**, depending on the assay template that is used. For example, you might want to ensure that both **DNA** and **RNA** are selected if you use the DNA/RNA template. |
| | By default, only **DNA** is enabled and **RNA** is disabled. RNA is available for selection only if the assay template includes it. |

Table 13   Templating

| Option | Description |
|---|---|
| Sequencing Chip | The sequencing chip to use in the assay. |
| | When you change the **Sequencing Chip** option, other assay parameters are changed to the default settings that are associated with the chip. Review the settings in the **Parameters** step after you make this change. |
| Templating Kit | The templating kit to use in the assay. |
| | When you change the **Templating Kit** option, other assay parameters are changed to the default settings that are associated with the templating kit. Review the `runparameter.json` after you make this change. |
| | **Note:**  The Genexus™ Templating Strip 3B GX5™ is not supported for use with Ion AmpliSeq™ Custom panels. |
| Template Size (bp) | The template size, measured in base pairs, to use in the assay. |
| | • **200** |
| | **Note:**  400 bp template size is not supported for use with Ion AmpliSeq™ Custom panels. |

Table 14   Sequencing

| Option | Description |
|---|---|
| Sequencing Kit | The sequencing kit to use in the assay. |
| Sequencing Flows | The number of flows performed by the sequencer during the sequencing stage of the run. |

## Parameters step assay options

Manager- and administrator-level users can use the **Parameters** step to select options related to parameters for use with an assay in Genexus™ Software. In the **Parameters** step, you can accept the default settings for the assay parameters, or adjust the settings if needed to further optimize performance.

---

**IMPORTANT!** Use the default parameter settings for assays unless you are an advanced user. Contact your Field Bioinformatics Specialist (FBS) for assistance.

---

When you click the field of a parameter, the software shows the default value and the range that is allowed for the parameter. You can see a description of most parameters when you place the pointer over ⑦ **(Learn More)** next to a parameter.

The options that are available depend on the type of assay and the selections that are made in the **Reagent** step.

- **Library Prep & Templating**

- **Primary Analysis**

  **Advanced Command Line Arguments** in the **Primary Analysis** section can only be adjusted when you click **Enable customization**.

- **Annotation**

- **Fusions**

  To help ensure that results for Fusions custom panels are robust, adjust the Fusion QC parameters. For more information, see "Guidelines to adjust Fusion parameters" on page 64.

- **Read Mapping**

- **Variant Finding**

  To improve specificity and sensitivity and to reduce the limit of detection, you may want to create an Sequence Variant Baseline. Adjust parameters such as the **Variant Finding** parameters to lower limits of detection (LOD). For more information, see "Guidelines to lower the limit of detection (LOD) with Variant Finding parameters" on page 66.

## Guidelines to adjust Fusion parameters

The sensitivity and specificity of fusion sample quality control (QC) results can be optimized with slight adjustments to the settings for the fusion quality control parameters. The recommended settings in this table can be used to optimize QC parameters for fusion custom panels.

| Parameter name in Parameters screen | Parameter name in JSON file | Description | Recommended setting |
|---|---|---|---|
| Fusions sensitivity | fusions.sensitivity | The fusions sensitivity parameter requires 60%, 70%, or 80% overlap of alignment with insert and 50%, 66.66%, or 75% exact matches depending on low/medium/high sensitivity. | Use default setting of **MEDIUM**. |
| Estimate max crosstalk | fusions.estimate.max.crosstalk | Maximum percentage of spill-over reads that can be seen in any sample due to reasons such as barcode crosstalk. | 0.5 (This is also used for Oncomine™ panels.) |
| Minimum family coverage per strand | fusions.min.fam.per.strand.cov | Minimum required coverage of reads on each strand in a bi-directional molecular tag family. If the number of reads on each strand is greater than or equal to (>=) this value, the family is counted as a functional family. | 0 |
| Minimum mean read length for valid sampleQC | fusions.min.mean.read.length.sampleQC | Minimum mean read length in order to qualify a sample as **PASS** and proceed with the analysis. | Samples with average library read length well below 50bp typically indicate poor sample quality or poor library quality. |
| Minimum molecular family consensus size | fusions.min.tag.fam.size | Minimum number of reads with same tag required to form a functional family. If the number of reads in a family is greater than or equal to (>=) this value, the family is counted as a functional family. | Use a value of 2 to 7. **Note:** Higher values make variant calls less sensitive but more specific. |
| Minimum molecular family count | fusions.min.tag.fam.count | Minimum number of variant supporting functional families that are required to make a call. If the number of functional families of a target is greater than (>) this value, the target is called as present. | Use a value of 2 to 10. **Note:** Higher values make variant calls less sensitive but more specific. |

*(continued)*

| Parameter name in Parameters screen | Parameter name in JSON file | Description | Recommended setting |
|---|---|---|---|
| **Minimum Normalized Read Counts for Fusions** | fusions.min.normalized.read.count | Minimum normalized read counts threshold to call fusion isoform as present.<br><br>By default, this parameter is set at 0, so is not used. | Some fusions, such as FGFR3-TACC3, can have improved specificity with a setting for a normalized cutoff value to 0.001, that is, 0.1% of mapped reads. |
| **Minimum Number Of PC Amplicons Required To Not Fail QC** | fusions.var.max.control.detected.for.fail | Minimum number of process (or expression) control amplicons that contain greater than or equal (>=) the number of families than **Minimum Molecular Family Count** required to not fail quality control (QC). | Use a low value of 1-3, which can prevent samples from failing. |
| Minimum Number Of PC Amplicons Required To Pass QC | fusions.var.min.control.detected.for.pass | Minimum number of process (or expression) control amplicons containing equal or more families than **Minimum Molecular Family Count** required to pass quality control. | Set a value >2 the number of PC amplicons in the panel. |
| **Minimum number of valid pools for SampleQC** | fusions.min.valid.pools.for.sampleQC.pass | Minimum number of valid pools in order to qualify a sample QC as **PASS** and proceed with software analysis. | Set this value to the number of pools in the assay. |
| **Minimum percentage of read counts for a non-targeted fusion in case where another fusion involving same gene pair is reported with high read counts** | fusions.nontargeted.min.read.count.percent | If a non-targeted fusion is detected in a sample where another fusion involving same gene pair is also detected, the non-targeted fusion will be flagged as secondary fusion isoform if the percentage of read counts relative to the other targeted fusion with high read counts is lower than this threshold. | 5 |
| **Minimum Read Counts for Controls** | fusions.min.read.count.controls | Minimum number of valid reads aligned to specific fusion isoform sequence in order to call the isoform as **Present**. If the number of valid reads assigned to a target is greater than this threshold (>), the target is called as **Present**. | In most cases, use the same value as the cutoff the fusion calling parameter (fusions.min.read.count). For example, >20 |
| **Minimum Read Counts for Non-Targeted Fusions** | fusions.min.read.count.non.targeted | The criteria used to set this parameter should be more stringent than targeted fusion calling. | 20 to 100 |

*(continued)*

| Parameter name in Parameters screen | Parameter name in JSON file | Description | Recommended setting |
|---|---|---|---|
| Minimum Total Valid mapped reads | fusions.min.total.valid.mapped.reads | Minimum number of total valid mapped reads in order to qualify a Sample as **Valid** and proceed with the analysis. If the total valid mapped reads in a sample is greater than or equal to this threshold (>=), the sample is qualified as **PASS**. | 5000 |
| Use either family counts only or read counts only to make calls | fusions.use.family.or.read.counts | If the flag is true, the software uses either family counts only or read counts only to make calls. This parameter is used only for sequencing runs that use Molecular Tags, or Unique Molecular Indices (UMIs). | 0 |

## Guidelines to lower the limit of detection (LOD) with Variant Finding parameters

The following guidelines explain how to change the values for **Variant Finding** parameters to lower the limit of detection (LOD) in sequencing run results.

Table 15   Variant Finding parameters guidelines for reduced LOD

| Parameter | Description | Recommended setting |
|---|---|---|
| Hotspot Min Allele Freq | Used to define the allele frequency (AF) threshold for statistically testing the presence of a hotspot above the AF. Hotspots are known relevant variant alleles present in the hotspot bed file. Range: 0.0-1.0. | To detect hotspots present in the sample at 5% allele frequency, set this threshold at 3%. |
| SNP Min Allele Freq | Used to define the allele frequency (AF) threshold for statistically testing the presence of a de novo SNP above the AF. De novo SNPs are single nucleotide variants not present in the hotspot file. Range: 0.0-1.0. | To detect SNPs present in the sample at 5% allele frequency, set this threshold to 3%. |
| Indel Min Allele Freq | Used to define the allele frequency (AF) threshold for statistically testing the presence of a de novo InDel above the AF. De novo InDels include insertions and deletions not present in the hotspot file. Range: 0.0-1.0. | To detect InDels present in the sample at 5% allele frequency, set this threshold to 3%. |
| MNP Min Allele Freq | Used to define the allele frequency (AF) threshold for statistically testing the presence of a de novo MNP above the AF. De novo MNPs are multiple nucleotide variants not present in the hotspot file. Range: 0.0-1.0. | To detect MNPs present in the sample at 5% allele frequency, user may want to set this threshold at 3%. |

**Table 15  Variant Finding parameters guidelines for reduced LOD**  *(continued)*

| Parameter | Description | Recommended setting |
|---|---|---|
| Generate Min Alt Allele Freq | Used to search for possible SNPs and non-homopolymer insertions and deletions, which are then statistically evaluated using the Min Allele Freq parameter. The Generate Min Alt Allele Freq parameter is the cutoff of candidate SNPs and non-homopolymer insertions and deletions to be generated in de novo variant calling. It is not used in hotspot variant calling. Range: 0.0-1.0. | The Generate Min Alt Allele Freq threshold should usually be lower than the desired LOD, for example, 40% lower than the LOD in the sample. However, setting this threshold too low may result in false positives. False positives can be reduced by creating and using a sequence variant baseline (SVB). |
| Generate Min Indel Alt Allele Freq | Used to search for possible homopolymer insertions and deletions, which are then statistically evaluated using the Min Allele Freq parameter.The Generate Min Indel Alt Allele Freq parameter is the cutoff of candidate homopolymer insertions and deletions to be generated in de novo variant calling. It is not used in hotspot variant calling. Range: 0.0-1.0. | The Generate Min Indel Alt Allele Freq threshold should usually be greater than the Generate Min Alt Allele Freq, for example, twice as high. Setting this threshold too low can result in false positives. False positives can be reduced by creating and using a sequence variant baseline (SVB). |

For more information about the **Variant Finding** parameters, see the Genexus™ Software help system.

## Edit assay parameters in a file

Manager- and administrator-level users can create assays in Genexus™ Software.

You can edit assay parameters in the **Parameters** step. Not all parameters appear in the software. You can download a JSON file that contains the parameters that appear in the software and more parameters that do not appear in the software. If you are an advanced user and understand how the changes can affect results, you can edit the parameters file and then upload the edited file to create an assay that captures the values of the JSON file.

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

2. Select the type of assay you want to create.

3. In the **Panel** step, select a panel and other options, then click **Next**.

4. In the **Reagent** step, select the kits and chips, and then click **Next**.

5. In the **Parameters** step:

   a. In the **Filter Parameters by** dropdown list at the bottom of the screen, select the section for the parameters you want to change or select **All** to download a file that contains all parameters.

   b. Click **Download** to download a JSON file for the parameters.

6. Edit the parameter values in the file and save the file.

7. Click **Upload**.

   a. Click **Select File**.

  b. Navigate to the edited file.

  c. Click **Open**.

  d. In the **Upload Parameters** dialog box, click **Upload** to upload the file.

The parameter values in the uploaded file are added to the assay.

## QC step assay options

Manager- and administrator-level users can create assays in Genexus™ Software.

You can specify Quality Control (QC) metric values to use in an assay in the **QC** step.

The following tables list options that are available in the **QC** step. The available options depend on the type of assay. For any custom assay, QC failure does impact variant reporting. If detected value thresholds are not met, the QC metric value is **Failed**. If the QC threshold is not set, the QC metric value is **Pass**.

Table 16   CF-1 Control

| Option | Description |
|---|---|
| **Min Average Reads Per Lane** | The number of average reads per chip lane.<br><br>You can specify the minimum threshold value required for the CF-1 (templating Control Fragment-1) to pass the QC metric. |
| **Min Base Call Accuracy** | The total number of errors for all positions in CF-1/ total number of CF-1 base reads.<br><br>You can specify the minimum threshold value required for CF-1 to pass the QC metric. |
| **Min Mean AQ20 Read Length (bp)** | The average length, in base pairs, at which the accuracy rate is ≥99% for reads of a library.<br><br>You can specify the minimum threshold value required for CF-1 to pass the QC metric. |

Table 17   NTC QC - DNA

| Option | Description |
|---|---|
| **Max Average Base Coverage Depth** | The average number of reads of all targeted reference bases.<br><br>You can specify the maximum threshold value required for the DNA no template control to pass the QC metric. |
| **Max Mean Read Length (bp)** | The average length, in base pairs, of called reads.<br><br>You can specify the maximum threshold value required for the DNA no template control to pass the QC metric. |

Table 18   NTC QC - RNA

| Option | Description |
|---|---|
| Max Mapped Reads | The total number of reads that are mapped to the reference genome in Genexus™ Software.<br><br>You can specify the maximum threshold value required for the RNA no template control to pass the QC metric. |
| Max Mean Read Length (bp) | The average length, in base pairs, of called reads.<br><br>You can specify the maximum threshold value required for the RNA no template control to pass the QC metric. |

Table 19   Run QC

| Option | Description |
|---|---|
| Min Key Signal | The average signal after software processing for all library ISPs that identically match the library key (TCAG). This value is a measure of the efficiency of template amplification.<br><br>You can specify the minimum threshold value required for a run to pass QC for the key signal metric. |
| Min Percent Loading | The percentage of addressable wells on a chip lane that are loaded with an ISP. The percentage is derived from the number of wells with ISPs divided by the number of the total addressable wells in a run.<br><br>You can specify the minimum threshold value required for a run to pass QC for the percent loading metric. |
| Min 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))).<br><br>You can specify the minimum threshold value required for a run to pass QC for the raw read accuracy metric. |

Table 20   Sample QC - DNA

| Options | Description |
|---|---|
| Min Deamination score | Deamination is reported as the estimated SNP proportion consistent with deamination (low allele frequency C:G>T:A SNVs). The deamination score can be used to determine the quality of the FFPE sample.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |
| Min Mapped Reads | The number of reads aligned to the reference genome.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |
| Min Mean AQ20 Read Length (bp) | The average length, in base pairs, at which the accuracy rate is ≥99% for reads of a library.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |

**Table 20   Sample QC - DNA**  *(continued)*

| Options | Description |
|---|---|
| **Min Mean Read Length (bp)** | The average length, in base pairs, of called reads.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |
| **Min Number Of Bases Used In Calculating TMB** | The number of exonic bases or all of the genomic bases that are covered by the panel. Only bases with sufficient base coverage are used in the calculation, as defined in the workflow parameters. By default, the TMB calculation uses total exonic bases with ≥60 bp coverage. In the TMB parameter settings, you can select between exonic bases or all of the genomic bases. You can also modify the sufficient coverage value.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |
| **Min Mean Red Cov** | The mean molecular coverage of targeted CNV gene.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |
| **Min Median Mol Cov** | The median molecular coverage of non-CNV reference loci.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |
| **Min Number of variant calls** | The number of somatic variants that are identified in the sample. This value is reported in the statistic.txt file as Total Somatic Filtered Variants Count (numerator for TMB calculation) and Variant Count.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |
| **Min Uniformity of Base Coverage** | The percentage of reads showing a depth of coverage ≥20% of the mean base coverage.<br><br>You can specify the minimum threshold value required for a DNA sample to pass the QC metric. |
| **Max MAPD** | MAPD is a quality metric that estimates coverage variability between adjacent amplicons in CNV analyses. A MAPD value of <0.5 indicates an acceptable level of coverage variability. High MAPD value typically translates to a lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. The MAPD metric does not affect SNVs/INDEL/CNV calls.<br><br>You can specify the minimum threshold value required for an DNA sample to pass the QC metric.<br><br>This option is available only when a **Copy Number Baseline for CNV Calling** is selected in the panel step. |

Table 21  Sample QC - RNA

| Option | Description |
|---|---|
| Min Mapped Fusion Reads | The number of reads aligned to the reference genome.<br><br>You can specify the minimum threshold value required for an RNA sample to pass the QC metric. |
| Min Mean AQ20 Read Length (bp) | The average length, in base pairs, at which the accuracy rate is ≥99% for reads of a library.<br><br>You can specify the minimum threshold value required for an RNA sample to pass the QC metric. |
| Min Mean Read Length (bp) | The average length, in base pairs, of called reads.<br><br>You can specify the minimum threshold value required for an RNA sample to pass the QC metric. |
| Min RNA Expression Ctrls Detected (For AmpliSeq HD assays only.) | The number of expression control genes detected for the sample. This metric measures the RNA input integrity, input amount, and the fidelity of the reverse transcriptase that was used in library preparation. Fusion panels include primer pairs that cover seven control housekeeping genes.<br><br>You can specify the minimum threshold value required for an RNA sample to pass the QC metric. |

## Edit the QC parameters of a locked assay (manager/administrator)

Manager- and administrator-level users can edit the QC parameters of a locked assay. The QC parameters can be adjusted according to research needs that can change over time. You can edit the QC parameters of any locked assay, including system-installed assays, if needed. When the QC parameters are changed, the edited QC parameters are applied to any new run that uses the assay. Edits to QC parameters do not affect runs that use the assay and are completed before edits are made.

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

2. In the **Manage Assays** screen, place the pointer over the row of a locked assay, then click **Edit QC**.
   The **Edit QC** dialog window opens.

3. Edit the QC parameters as desired.
   To reset the QC parameters to the original values in a subsection, such as **Run QC** or **Sample QC – DNA**, click **Reset** in the upper right corner of the subsection.

4. Click **Save**.

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

# Create a liquid biopsy assay in Genexus™ Software

Liquid biopsy samples are typically used to look for variants that occur at very low frequencies. To support such low Levels of Detection (LOD), modifications need to be made to the variant calling method in the software. In addition to the creation of an assay, you must also edit the thresholds for variant calling parameters related to LOD. For more information, see "Guidelines to lower the limit of detection (LOD) with Variant Finding parameters" on page 66. For best results, we highly recommend that you also create a sequence variant baseline to reduce false positives when the software analyzes liquid biopsy samples. For more information, see "Sequence variant baselines" on page 49.

The analysis of Ion AmpliSeq™ HD custom panels use sequencing data from cfDNA samples that use Unique Molecular Tags (UMTs), or Unique Molecular Indices (UMIs), to remove systematic and homopolymer errors when calling SNV and Indel variants. Alternative allele variant calls are supported by families/molecules, resulting in a very low limit of detection. For more information, see "Molecular tag sequencing technology—Important factors" on page 37.

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

2. Select **DNA Somatic** for the assay type.

3. In the **Panel** step, select the Ion AmpliSeq HD **Panel** that applies to your custom assay. Then, select other options for the assay. For descriptions of the panel information appropriate for the assay, see "Panel step assay options" on page 60.

4. Click **Next**.

5. In the **Reagent** step, select the kits and chips that you are using. Ensure that the reagents are appropriate for the panel that you are using.

   For descriptions of the reagent information that is available for the assay, see "Reagent step assay options" on page 61.

6. Click **Next**.

7. In the **Parameters** step, click **Variant Finding** and adjust the parameter settings as follows.

   | Parameter name | Setting for molecular-based variant calling |
   |---|---|
   | **Additional advanced variant caller arguments** | Add "tvc --suppress-mol-tags **off** --do-indel-assembly off" |
   | **Min Family Size** | 3 |
   | **Minimum Family Coverage per Strand** | 1 |
   | **Down sample to coverage** | 50,000 |

8. Click **Primary Analysis** and change the setting of the **Analyze as single Barcode** parameter to **False**.

9. Click **Next**.

10. In the **Save** step, enter a new assay name, an assay full name, and an optional description, then click **Save**.

    The newly created assay is added to the list of assays in the **Assays** / **Manage Assays** screen. The **Assay Full Name** is followed by `Draft` to indicate that the assay is in draft status. You can plan a run with the draft assay. The assay remains in draft status until it is locked. You can lock the draft assay to ensure that the assay cannot be edited. You can plan a run with the draft assay and the assay is available to lock, edit, or delete, and audit. You can also download parameters from assays in **Draft** or **Locked** statuses.

11. Reduce variant allele thresholds. For more information, see "Parameters step assay options" on page 63.

12. Create a Sequence Variant Baseline (SVB) to improve specificity and sensitivity and to reduce the limit of detection. For more information, see "Sequence variant baselines" on page 49.

Analyze a sequencing run with the new assay.

## Copy an assay (manager/administrator)

You can copy and edit the assay that you create for the custom panel. For example, you might want to start with the current assay as a template, then adjust parameter settings to optimize the settings to use the assay in a different sequencing run plan.

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

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

2. In the **Manage Assays** screen, place the pointer over the row of the assay that you want to copy, then click **Copy**.

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

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

4. In the **Save** step, enter a new assay name, an assay full name, and an optional description, then click **Save**.

    The newly created assay is added to the list of assays in the **Assays** / **Manage Assays** screen. The **Assay Full Name** is followed by `Draft` to indicate that the assay is in draft status. You can plan a run with the draft assay. The assay remains in draft status until it is locked. You can lock the draft assay to ensure that the assay cannot be edited.

## Manage assays

You can view assay details, view the audit trail of an assay, and download assay parameters in Genexus™ Software.

In addition, manager- and administrator-level users can create or import an assay, export a locked assay, edit, lock, or delete a draft assay, edit the QC parameters of a locked assay, and obsolete a locked assay in the software.

5

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

The following tools are available in this screen.

| To | Do the following |
|---|---|
| Review and download the assay audit trail | 1. In the **Assays / Manage Assays** screen, place the pointer over the row of an assay of interest, then click **Audit**.<br><br>2. In the **Audit Trail** screen, click ⬇ **Download** to download an audit details PDF file to your local drive. |
| Edit a draft assay | When a manager- or administrator-level user creates an assay, the status of the assay is draft. While the assay is in draft status, it can be edited.<br><br>1. Place the pointer In the row of a draft assay, then click **Edit**.<br>  The **Edit Assay** workflow opens.<br><br>2. Edit the options on each assay step as needed, then click **Save**. |
| Lock a draft assay | When a manager- or administrator-level user creates an assay, the status of the assay is draft.<br>Place the pointer In the row of a draft assay, then click **Lock**.<br>Locked assays cannot be edited or deleted. |
| Copy an assay to create a new assay | Only locked assays can be copied.<br><br>1. Place the pointer In the row of a locked assay, then click **Copy**.<br>  The **Copy Assay** workflow opens.<br><br>2. Edit the options at each assay step as needed, enter a new name for the assay, then click **Save**. |
| Delete an assay | Only draft assays can be deleted. When an assay is locked, it can be removed from use in the software by designating it obsolete.<br>Place the pointer In the row of a draft assay, click **Delete**, then confirm the deletion. |
| Remove a locked assay | A manager-or administrator-level user can remove a locked assay from use in the software by designating it obsolete. The assay is not deleted and a record of it is maintained in the audit trail. The results for any runs already performed with the assay remain on the sequencer.<br><br>1. Place the pointer In the row of a locked assay, then click **Obsolete**.<br><br>2. Click **Yes** to confirm the operation. |
| Export an assay | An assay can be exported, for example if you want to use that assay in another Genexus™ Integrated Sequencer in your lab. Only locked assays can be exported.<br>Place the pointer in the row of a locked assay, then click **Export**. The assay parameter files are downloaded to your local drive as a ZIP folder and are available for import to another sequencer.<br>Panel reference files are not included in the exported folder. |
| Download parameters | Place the pointer In the row of the assay, then click **Download Parameters**. Assay parameter files are downloaded to your local drive as a ZIP folder containing assay parameter JSON files. |

# View assay details

You can view assay details in Genexus™ Software.

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

2. In the **Manage Assays** screen, click the assay name of interest.

The assay details are shown.

If needed, you can click ••• **(More Options)** to perform more actions, such as export, copy, edit, lock, delete, obsolete, edit QC, download parameters, or audit the assay. The options that are available depend on whether the assay is system-installed or is a custom assay. For custom assays, the options that are available also depend on whether or not the assay is locked.

The actions for each assay are also available in the **Manage Assays** screen when you place the pointer over the row of an assay. For more information, see "Manage assays" on page 73, "Export a custom, locked assay (manager/administrator)" on page 77, "Copy an assay (manager/administrator)" on page 73, "Edit the QC parameters of a locked assay (manager/administrator)" on page 71, "Download assay parameters" on page 77 and the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

## Assay details

When you view assay details in Genexus™ Software, you can view information about the components of the assay. The information that is available depends on the type of assay.

You can view the following assay creation details.

- **Research Application**—The research application for the assay, such as **DNA** or **DNA and Fusions**.

- **Created By**—The sign-in name of the person who created the assay.

  Assays that are included with the software are labeled as **System Installed**.

- **Analysis Pipeline Version**—The version of the analysis pipeline used for the assay.

- **Created On**—The date on which the assay was created.

- **Assay Name**—The name and version of the assay.

  Note:  For system-installed assays or assays that you install in the software, the end of the assay name, for example w5.0.0, is

the version number of the assay. The assay version number is not the same as the **Version** that is shown in the assay creation details.

- **Version**—Tracks revisions made to unlocked assays. For example, when you edit an assay for the first time, the version changes from **1** to **2**.

| Assay detail[1] | Description |
|---|---|
| Panel | In addition to other components, the panel section lists the reference files that are used in an assay. |
| | One item that is listed is the **Minimum Read Count Per Sample**, which is the minimum number of reads per sample that are needed to achieve the coverage depth required for the assay. The minimum read counts per sample is used to calculate the number of samples that are allowed in a chip lane when you plan a run. |
| | For more information and a complete list of components in the panel section, see "Panel step assay options" on page 60. |
| Reagents | In addition to other components, the reagents section lists the kits and chips for the assay. |
| | For more information and a complete list of components in the reagents section, see "Reagent step assay options" on page 61. |
| • NTC QC – RNA<br>• CF-1 Control<br>• NTC QC – DNA<br>• Run QC<br>• Sample QC | The QC sections list the Quality Control (QC) metric values for the assay.<br><br>For more information and a complete list of components in the QC section, see "QC step assay options" on page 68. |
| Plugins | The plugins sections lists the plugins that are associated with the assay. |
| | For more information about plugins, see "Plugins for data analysis" on page 166. |
| Description | A short description of the assay, similar to the assay full name. |

[1]  The details that are shown depend on the assay.

# Import an assay (manager/administrator)

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

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

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

3. In the **Import Assay** dialog box, click **Select Assay file**, then go to the folder on the computer that contains the exported assay and select the ZIP file for the assay.

4. Click **Upload**.

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

# Export a custom, locked assay (manager/administrator)

An assay can be exported, for example to use that assay in another Genexus™ Integrated Sequencer. You cannot export system-installed assays. Only custom assays that are locked can be exported.

If you want to view or edit assay parameters, download only parameter files. For more information, see instead "Download assay parameters" .

If you want to edit the hotspots file, or the target regions BED file, first export the assay, then edit the files.

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

2. In the **Assays ▸ Manage Assays** screen, place the pointer over the row of a locked, custom assay, then click **Export**.

The assay parameter files are downloaded to the computer as a ZIP file and are available for import to another sequencer.

Panel reference files are not included in the exported file.

# Download assay parameters

Assay parameters can be downloaded and exported, for example to use that assay in another Genexus™ Integrated Sequencer.

Panel reference files are not included in the export.

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

2. In the **Manage Assays** screen, place the pointer over the row of a locked assay, then click **Download Parameters**.

The assay parameter JSON files are downloaded to the computer in a ZIP file and are available to import to another sequencer.

# Plan a run

Before you can sequence samples on the Genexus™ Integrated Sequencer, you must create a run plan to specify the assay (or assays), the samples, and any additional parameters needed for the run. In Genexus™ Software 6.4 and later, there are three types of runs that you can plan for use custom assays on the Genexus™ Integrated Sequencer:

- Nucleic Acid to Result—start from purified nucleic acid samples.

- Library to Result—start from prepared libraries, either prepared manually or prepared previously on the Genexus™ Integrated Sequencer.

- BAM to Result—start from a BAM sequence file and analyze using the assay you specify.

## Before you plan a run

The following conditions must be met before you plan a run in Genexus™ Software.

- Sample information is accurate in the software and the name assigned to each sample is unique. For more information, see "Enter samples in the Genexus™ Software" on page 78.

- Ensure that the following conditions are met for a **Library to Result** run.
  - Library batches are prepared and each batch uses a unique library batch ID.
  - Each sample library in a library batch is prepared with and assigned a unique barcode or barcode pair.

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

The software returns an error message when any of the conditions are not met.

## Enter samples in the Genexus™ Software

In Genexus™ Software, the data and attributes that characterize a purified nucleic acid—or a specimen that requires nucleic acid purification and quantitation—to be sequenced are called samples.

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

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

# Find samples

You can sort, search, and filter information to refine and narrow the samples that are listed in the **Samples / Manage Samples** screen in Genexus™ Software. The sort, search, and filter tools help you organize samples and find a samples of interest.

For information to search for a sample by a unique identifier, such as the sample name, see "Search for samples" on page 79.

For information to filter samples by a common trait, such as the date the sample was created, see "Filter the list of samples by the date of sample creation" on page 81.

## Search for samples

Samples are listed in the **Samples / Manage Samples** screen in Genexus™ Software.

You can search for samples with keywords or text that are included in the sample attributes, such as sample name, tags, or application category. You can also search for a specific date or date range for the **Created On** attribute.

IMPORTANT!  Search terms must adhere to the following rules.

- An asterisk (*) is not allowed in the search field for use as a wildcard.
- Searches are case-insensitive, that is, both upper-case and lower-cases letters are found regardless the case of search term letters.
- Searches find every occurrence of a continuous string. For example, a search for *demo* in analysis names returns a list of all analyses with a name that includes *demo*. For example, demo1, demo2, and so on.
- Spaces are removed during searches, and therefore are not recommended. For example, a search for *demo 1* would return results only for data that includes the string *demo1*.

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

2. In the **Manage Samples** screen, use the options to filter the list of sample attributes.

| Option | Steps |
|---|---|
| Limit the list of sample attributes that is shown. | Click ▼ **(Filter)** in in the column heading for a sample attribute of interest, then do one or move of the following.<br>• In the search field, enter 3 or more characters for the sample attribute.<br>• Select a checkbox next to one or more sample attributes.<br>• Click **Select All** to select all sample attributes.<br>• Click **Clear** to close the filter dialog box. |
| Limit the list of samples based on a creation date, or a range of dates. | Click ▼ **(Filter)** in the **Created On** column heading, and select **Is equal to**, **Is before or equal to**, or **is after or equal to** to set the criteria for the date or date range.<br>Select a date in the calendar.<br>*(Optional)* Select **And**, or **Or** to include a second set of criteria and further narrow the search.<br>For more details, see "Filter the list of samples by the date of sample creation" on page 81. |

3. Click **Filter**.
   In the column heading, ▼ changes color to indicate that a filter is applied.

The filtered list of samples is shown in the **Manage Samples** screen.

Click ⟨×⟩ **Clear Filters** at the top of the **Samples / Manage Samples** screen to remove all filters and view the full list of samples.

## Sort the list of samples

You can sort the list of samples to make it easier to find a sample in Genexus™ Software.

By default, samples are listed in the **Samples / Manage Samples** screen by the date that the sample was created. The most recently created sample is at the top of the list.

You can change the order that the samples are listed in the table.

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

2. In the **Manage Samples** screen, click a column heading of interest.
   The list of samples is sorted by the sample attribute, in alphabetical or chronological order of the attribute, depending on the heading.

3. Click the column heading again to reverse the order.

## Filter the list of samples by the date of sample creation

You can apply filters to narrow search results, or shorten the list of samples when you view the list of samples in Genexus™ Software.

In addition to the filter tools in the column headings, you can also filter the list of samples with a dropdown list and search by the sample name or other column headings. For more information, see "Refine the list of samples" and "Search for samples" .

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

2. In the **Manage Samples** screen, select one or more filters.

| Option | Description |
|---|---|
| List only samples created on an exact date. | a. In the **Created On** column heading, click ▼ **(Filter)**.<br>b. In the first dropdown list, select **Is equal to**.<br>c. In the calendar that appears, select the date of interest.<br>d. Click **Filter**. |
| List samples created within a date range. | a. In the **Created On** column heading, click ▼ **(Filter)**.<br>b. In the first dropdown list, select **Is after or equal to**.<br>c. In the calendar that appears, select the earliest date that the samples of interest were created.<br>d. In the Boolean operator dropdown list, select **And**.<br>e. In the second dropdown list, select **Is before or equal to**.<br>f. In the calendar that appears, select the latest date that the samples of interest were created.<br>g. Click **Filter**.<br><br>Records are listed for samples created on dates that meet both conditions. |
| List samples according to a date that meets either one condition or a second condition. | a. In the **Created On** column heading, click ▼ **(Filter)**.<br>b. In the first dropdown list, select **Is after or equal to**.<br>c. In the calendar that appears, select the earliest date that the samples of interest were created.<br>d. In the Boolean operator dropdown list, select **Or**.<br>e. In the second dropdown list, select **Is before or equal to**.<br>f. In the calendar that appears, select the latest date that the samples of interest were created.<br>g. Click **Filter**.<br><br>Records are listed for samples created on dates that meet at least one of the conditions. |

| Option | Description |
|---|---|
| List only samples that were created before a date that you indicate. | a. In the **Created On** column heading, click ▼ **(Filter)**.<br>b. In the first dropdown list, select **Is before or equal to**.<br>c. In the calendar that appears, select the date of interest.<br>d. Click **Filter**. |
| List only samples that were created after a date that you indicate. | a. In the **Created On** column heading, click ▼ **(Filter)**.<br>b. In the first dropdown list, select **Is after or equal to**.<br>c. In the calendar that appears, select the date of interest.<br>d. Click **Filter**. |
| Remove all selections and close the filter dialog box. | In the filter dialog box, click **Clear**. |

3. In the filter dialog box, click **Filter** to apply the filtered selections.

   In the column heading, ▼ changes color to indicate that a filter is applied.

The list of samples is filtered according to the selections.

Click ⟨×⟩ **Clear Filters** to remove all filters and view the full list of samples.

## Refine the list of samples

You can shorten the list of samples that are shown in Genexus™ Software.

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

2. In the **Manage Samples** screen, in the **Filter Samples by** dropdown list, select the type of sample you want to view.

| Option | Description |
|---|---|
| List only the samples that have not been prepared as a library batch. | Select **To Be Prepared**. |
| List samples created from BAM files. | Select **BAM Samples**. |
| List all samples. | Select **All**. |

Only the selected type of samples are listed.

# Create a new sample

You can enter information individually for each sample that you add in the software. Complete this procedure to make a sample available for use in a run, or to add the sample to a library batch. If you have many samples to add, see instead "Download a template file for sample creation" on page 85

and the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

1. In the menu bar, click **Samples ▸ Create Sample**.

2. In the **Samples/Create Sample** screen, enter a name for the sample, select an **Application Category**, then complete the required fields, and optional fields if needed.

   - In the **Tags** field, enter a full or partial tag name of at least 3 characters, then select one or more tags of interest to assign a tag to the sample.

   - If the tag that you want to assign to the sample does not exist in the software, a manager-or administrator-level user can enter a new tag name in the **Tags** field to create a new tag and assign it to the sample.

   The fields for sample attributes change based on the **Application Category**. Attributes identified with a red asterisk (*) are required.

   If you select **Other** as the **Sample Type**, you must enter a name for the custom sample type in the text box that is shown. For example, enter *Nasopharyngeal Swab*.

   

3. Click **Save**.

   The new sample is listed in the **Manage Samples** screen and is available to use in a run plan.

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

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 85. Upload the edited file using the **Browse** button.<br><br>**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**.<br>b. Navigate to the file, then click **Open**.<br><br>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. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

# Upload a BAM file to create a sample or samples

You can upload BAM files to create samples in Genexus™ Software. You can also redefine existing BAM files as new samples. That is, you can upload a BAM file and create a new sample name for the BAM file and enter the same or different values for the sample attributes that are associated with the BAM file.

If the BAM file is not on the Genexus™ Integrated Sequencer, you can upload a BAM file from the computer that is used to access Genexus™ Software. You can upload the BAM file directly from the computer or from a USB drive connected to the computer.

BAM files can be analyzed in the software. For more information, see "Plan a BAM to Result run" on page 94.

You can upload BAM files from a USB drive, from the hard drive, or from a connected Genexus server, or from a connected Ion Reporter™ Software server. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

1. In the menu bar, click **Samples ▸ Create Sample**.

2. In the **Create Sample** screen, click the **From Sequence Files (.bam)** tab.

3. In **Application Category**, select the application category for the samples.

4. Click **Browse**, then navigate to the file.

| Option | Description |
|---|---|
| Find the file on the instrument. | Click ▤ **Server**, then navigate to the file. |
| Find the file on the computer used to access the software. | Click 💻 **My Computer**, then in the **Browse** dialog box that appears, navigate to the file. |

5. Navigate to the file, then click **Select**.

   The file is now available to select and assign to a sample. The BAM file name is visible in the dropdown lists in the **DNA BAM File**, **RNA BAM File**, and **TNA BAM File** columns.

6. Enter the information for the sample.

   Attributes identified with a red asterisk (*) are required.

7. In one or more of the **DNA BAM File**, **RNA BAM File**, and **TNA BAM File** dropdown lists for the sample, select the BAM file that you want to assign to the sample.

8. To add more samples, click **Add Row**.

9. Repeat step 4 to step 8 for each extra BAM sample you want to create.

10. Select the checkbox in the row for each sample that you want to create. To select all samples, select the checkbox in the column heading row.

11. Click **Save**.

The new samples are listed in the **Manage Samples** screen, are denoted with **BAM** after the sample name, and are available to assign to a BAM to Result run plan.

# Plan a Nucleic Acid to Result run

You can plan a run for sequencing that starts with nucleic acid samples. A run that starts with nucleic acid samples is called a **Nucleic Acid to Result** run.

Planning a **Nucleic Acid to Result** run is organized into steps: **Setup**, **Assays**, **Samples**, **Sample Plate**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / Nucleic Acid to Result** screen.

Ensure that the following prerequisites are complete before you plan a **Nucleic Acid to Result** run.

- Enter sample information into Genexus™ Software. For more information, see "Create a new sample" on page 82, or the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

- Ensure that the assay that you want to use in the run exists in the software. For more information, see Chapter 5, "Assays for use with custom panel files" .

- Quantify sample concentration, if you do not intend to dilute sample concentrations manually to the target concentration of the assay. For more information, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

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

   Note: Alternatively, you can click ╋ **Nucleic Acid to Result** in the **Runs / Manage Runs** screen.

2. In the **Setup** step, enter or make the following selections.

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

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

   b. *(Optional)* In the **Reporting (Optional)** section, ensure that **Generate Report** is enabled to generate a variant report using the default report template.

   c. *(Optional)* In the **Reporting (Optional)** section, enable **Upload BAM files to Server** to upload BAM files to another Genexus™ Integrated Sequencer or Ion Reporter™ Software.

   | Option | Description |
   |---|---|
   | Upload BAM files to another Genexus™ Integrated Sequencer. | In the dropdown lists that appear, select the Genexus™ Software account and the software version.<br>To configure a Genexus™ Software account that you can use for data uploads, click **Set up Account**. For more information, see *Genexus™ Software 6.8 User Guide*. |
   | Automatically upload data for further analysis with Ion Reporter™ Software. | In the dropdown lists that appear, select the Ion Reporter™ Software account and software version.<br>To configure an Ion Reporter™ Software account that you can use for data uploads, click **Set up Account**.<br>**Note:** To transfer data to Ion Reporter™ Software, you must add one or more Ion Reporter™ Software accounts in Genexus™ Software. To configure an Ion Reporter™ Software account that you can use for data uploads, see *Genexus™ Software 6.8 User Guide* and *Genexus™ Software 6.8 User Guide*. |

   Alternatively, you can upload BAM files to another Genexus™ Integrated Sequencer or Ion Reporter™ Software after a run is successfully completed. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

   d. In the **Reason for Change** field, enter the reason for the new run plan.

   e. Click **Next**.

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

   a. Use the ▼ **(Filter)** tools in table column headings to find assays of interest, if desired.

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

    **b.** In the **Research Application** column for the assay of interest, select one or more research applications, such as **DNA and Fusions** or **DNA**, to include the research application for the assay in the run plan.

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

---

    **Note:** To create a new assay, see "Create an assay in Genexus™ Software for use with a custom panel" on page 56.

---

    **c.** If more assays are included in the run, repeat substep 3b for each extra assay.

    **d.** Click **Next**.

4. In the **Samples** step, select the samples that you want to run with each research application of each assay.

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

    The **Chip View** updates to show the lanes used in the run. Lane usage is calculated based on the number of samples (including a no template control, if selected) and the minimum read counts per sample for the assay. Green denotes a chip lane in the run containing assigned samples within lane capacity.

    If the *minimum reads per sample × number of samples* exceeds the chip or lane well capacity, a dialog box appears after you click **Assign** asking you to confirm that you want to continue. After confirmation, the **Chip View** updates and shows the lane color as red instead of green. The run is allowed if the lane capacity is exceeded, but you might not achieve the reads needed per sample to pass QC metrics.



① Green lane color denotes lane usage and sample assignment within lane capacity.

② Red lane color denotes sample assignment that exceeds lane capacity.

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

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

- Click **View & Remove**, make the selections, then click **Update**.
- Click **Remove All**, to remove all sample assignments for all assays.

d. If you selected the **Upload BAM files to Server** reporting option in the **Setup** step, make the following selections from the **Genexus Workflow** or **Ion Reporter Workflow** dropdown list for each assay that you selected.

- Select **Upload Only** to upload sample data to the selected server automatically after run completion.

- Select the desired Genexus™ Software assay or Ion Reporter™ Software analysis workflow to upload sample data *and* perform an analysis in the target software automatically after run completion.

---

**Note:** In order for the Ion Reporter™ Software analysis workflow to appear in the list, you must tag the analysis workflow for use with the IonReporterUploader plugin. For more information, see the software help system or the *Ion Reporter™ Software 5.18 User Guide* (Pub. No. MAN0024776).

---

e. If desired, for each research application of each assay in the run plan, select **NTC** to include a no template control.

The **Chip View** updates to show the lanes used in the run for the included no template controls.

f. Click **Next**.

5. In the **Sample Plate** step, review position assignments in the sample plate. Drag-and-drop samples and no template controls to edit the location of samples and controls, if applicable.

a. If desired, enter the extraction kit barcode for one or more samples or controls. For a single sample, in the row of the sample of interest, in the **Kit Barcodes** column, enter the extraction kit barcode or control kit barcode, if applicable. For multiple samples or controls, select the samples and controls, then click ☑ **Assign Kit Barcodes**. In the **Assign Kit Barcodes** dialog box, enter the extraction kit barcode for the samples, and if applicable, enter the barcode for the no template control.

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

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

The sequencer automatically dilutes the sample to the target concentration during the run if either of the following is true:

- The sample concentration is less than or equal to 1,024.00X the target concentration for an assay that performs 4 reactions per library.

- The sample concentration is less than or equal to 40.00X the target concentration for an assay that performs 6 reactions per library strip.

If a sample concentration is greater than 1,024.00X or 40.00X the target concentration, depending on the assay, you must manually dilute the sample to the target concentration, or to a value within range for automated dilution before loading on the sample plate.

---

**IMPORTANT!**  A warning symbol (⚠) with a message that states "Sample concentration out of range" can be shown in error for automated sample dilution in the **Run Plan** screen when you plan a run, or view the **Run Summary** screen, in Genexus™ Software.

- If the sample concentration is below the required minimum concentration and shows a **Dilution Factor** of 1.00 (that is, no dilution), the message is valid.
- If a sample that includes the warning message shows a **Dilution Factor** >1.00 and <1,024.00 (4 reactions per strip) or >1.00 and <40.00 (6 reactions per strip), the message is not valid and can be ignored.
- When you see the maximum **Dilution Factor** of 1,024.00 (4 reactions per strip) or 40.00 (6 reactions per strip), multiply the maximum value of the target concentration range by the maximum dilution factor (that is, 1,024.00 or 40.00). If the product of the calculation is greater than the sample concentration, the message is not valid and can be ignored.

---

**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.

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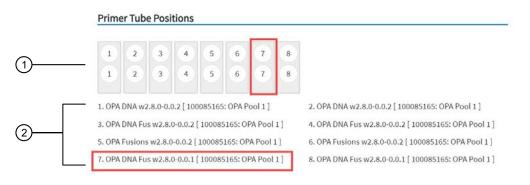
c. Ensure that sample plate information is correct, then click **Next**.

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

---

**Note:**  To print the run setup guide, you must allow pop-ups in the browser.

---

The run plan summary lists the following details.

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

---

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

**Primer Tube Positions**



1. OPA DNA w2.8.0-0.0.2 [ 100085165: OPA Pool 1 ]   2. OPA DNA w2.8.0-0.0.2 [ 100085165: OPA Pool 1 ]

3. OPA DNA Fus w2.8.0-0.0.2 [ 100085165: OPA Pool 1 ]   4. OPA DNA Fus w2.8.0-0.0.2 [ 100085165: OPA Pool 1 ]

5. OPA Fusions w2.8.0-0.0.2 [ 100085165: OPA Pool 1 ]   6. OPA Fusions w2.8.0-0.0.2 [ 100085165: OPA Pool 1 ]

7. OPA DNA Fus w2.8.0-0.0.1 [ 100085165: OPA Pool 1 ]   8. OPA DNA Fus w2.8.0-0.0.1 [ 100085165: OPA Pool 1 ]

① The position of the primer tubes.

② The legend for the image.

In the example outlined in red, primer tube 7 contains OPA Pool 1 for the OPA DNA Fusion assay, in the seventh position from the left.

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

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

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

# Plan a Library to Result run

You can plan runs for sequencing that start with libraries. This is called a **Library to Result** run. Before you can plan a **Library to Result** run, you must first enter sample information and prepare a library batch that associates an assay with the library batch. The library batch must be created with the assay to be used in the run. The assay specifies the barcode set that was used to prepare the sample libraries.

If the sample libraries were prepared using a barcode set that is not specified in the assay you want run, you must do the following:
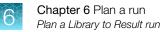
- Create a new assay, or copy an existing assay, and specify the new barcode set in assay setup.
- Prepare a new library batch that selects the new assay.

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

Genexus™ Software guides you through the four steps of planning a **Library to Result** run: **Setup**, **Assays**, **Library Batches**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / Library to Result** screen.

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

   **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 **Run Name**, enter a unique name.

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

   b. *(Optional)* In the **Reporting (Optional)** section, ensure that **Generate Report** is enabled to generate a variant report using the default report template.

   c. *(Optional)* In the **Reporting (Optional)** section, enable **Upload BAM files to Server** to upload BAM files to another Genexus™ Integrated Sequencer or to Ion Reporter™ Software.

| Option | Description |
|---|---|
| Upload BAM files to another Genexus™ Integrated Sequencer. | In the dropdown lists that appear, select the Genexus™ Software account and the software version.<br>To configure a Genexus™ Software account that you can use for data uploads, click **Set up Account**. For more information, see *Genexus™ Software 6.8 User Guide* or the Genexus™ Software system help. |
| Automatically upload data for further analysis with Ion Reporter™ Software. | In the dropdown lists that appear, select the Ion Reporter™ Software account and software version.<br>To configure an Ion Reporter™ Software account that you can use for data uploads, click **Set up Account**.<br>**Note:** To transfer data to Ion Reporter™ Software, you must add one or more Ion Reporter™ Software accounts in Genexus™ Software.<br>For more information, see *Genexus™ Software 6.8 User Guide* or the Genexus™ Software system help. |

   Alternatively, you can upload BAM files to another Genexus™ Integrated Sequencer or to Ion Reporter™ Software after a run is successfully completed.

   d. 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 one or more assays that you want to include in the run.

   For the assay to be selectable at this step, you must have prepared a library batch that assigns the assay to the batch. The assay specifies the barcode set that was used to prepare the sample libraries.

   a. Use the ▼ **(Filter)** tools in table column headings to find assays of interest, if desired.

   b. In the **Research Application** column for the assay of interest, select one or more research applications, such as **DNA and Fusions**, to include each selected research application for the assay in the run plan.

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

   **Note:** To create a new assay, see "Create an assay in Genexus™ Software for use with a custom panel" on page 56.

   c. If more assays are included in the run, repeat substep 3b for each extra assay.

    d. Click **Next**.

4. In the **Library Batches** step, select the library batch that you want to use in the run, then click **Next**.

---

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

---

    a. If you selected the **Upload BAM files to Server** reporting option in the **Setup** step, make the following selections from the **Genexus Workflow** or **Ion Reporter Workflow** dropdown list.

- Select **Upload Only** to upload sample data to the selected software account automatically after run completion.

- Select the desired Genexus™ Software assay or Ion Reporter™ Software analysis workflow to upload sample data *and* perform an analysis in the target software automatically after run completion.

---

Note:  In order for the Ion Reporter™ Software analysis workflow to appear in the list, you must tag the analysis workflow for use with the IonReporterUploader plugin. For more information, see the software help system or the *Ion Reporter™ Software 5.18 User Guide* (Pub. No. MAN0024776).

---

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 and minimum reads per sample entered at assay setup.



① Green lane color denotes lane usage and sample assignment within lane capacity.
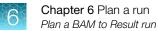
② Red lane color denotes sample assignment that exceeds lane capacity.

If the *minimum reads per sample × 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 four. The run is allowed if the lane capacity is exceeded, but you may not achieve the required reads per sample to pass QC metrics.

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

- Click **Save and Print** to print the run setup guide.
- Click **Save** to save the run without printing.

---

Note:  To print the run setup guide, you must allow pop-ups in the browser.

---

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

The run appears in the run list on the **Manage Runs** screen with the name you specified.

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

# Plan a BAM to Result run

Before you can plan a **BAM to Result** run, you must enter BAM samples into Genexus™ Software. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

Planning a run to analyze BAM samples is organized into steps: **Assays**, **Samples**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / BAM to Result** screen.

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

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

2. In the **Assays** step, enter or make the following selections.
   a. Enter a unique name.

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

   b. Use the ▼ **(Filter)** tools in table column headings to find assays of interest, if desired.

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

   c. In the **Research Application** column for the assay of interest, select the research application, such as **DNA and Fusions** or **DNA**, to include the selected research application for the assay in the run plan.

      You can select only one research application of an assay per run.

   d. Click **Next**.

3. In the **Samples** step, select the BAM samples from the list that you want to analyze, then click **Assign**.

   Note:  Only BAM samples that are compatible with the selected assay are listed. Compatibility is determined by the sample type, such as DNA or DNA and Fusion.

   a. If needed, click ⊗ in an assigned sample tile to remove the sample from the plan.

   b. Click **Next**.

4. In the **Review** step, review the samples in the run plan summary, then click **Save and Launch**.

   The run is listed in the **Results / Run Results** screen. The run is launched or the run is queued if other runs are in progress.

# Load the sample plate

Before starting a Nucleic Acid to Result or Library to Result run on the instrument, you must quantify and dilute the samples or sample libraries, if needed, then load the sample plate.

If you are using the Genexus™ Purification Instrument in the standalone configuration, you must manually transfer quantified samples from the 48-Well Nucleic Acid Archive Plate to the sequencer sample plate, following the run setup guide in the Nucleic Acid to Result run plan.

## Dilute or concentrate the samples, if needed, then load the sample plate—Nucleic Acid to Result run

Isolate DNA and RNA samples using one of the procedures and kits that are recommended in "Recommended materials for nucleic acid isolation and quantification" on page 23.

Nucleic acid samples with concentrations up to 1,024X of the target concentration for an assay (displayed as default values in the **Sample Plate** step screen in run planning) are in range for automated dilution and require no manual dilution. Enter the concentrations during run planning at the **Sample Plate** step (see step 5 on page 89).

Note:  If desired, manually dilute samples to the required concentration to avoid loss of sample due to the required volume overages during automated dilution.

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

| Sample concentration | Action |
|---|---|
| <0.04 ng/µL | Concentrate the sample to greater than or equal to the target concentration. |
| ≥0.04 ng/µL, but less than the target concentration | Run is allowed but sample concentration may not be optimal for library preparation. Concentrate the sample to greater than or equal to the target concentration. |
| ≤1,024X of the target concentration | No manual dilution is necessary. The sequencer dilutes the sample to the target concentration automatically during the run. |
| >1,024X of the target concentration | Manually dilute to the target concentration based on assay type, or to a concentration in range for automated dilution by the sequencer. |

**Note:**

- For samples with less than the minimum required volume, manually dilute an aliquot to the target concentration and minimum required volume.
- If you enter a concentration <0.04 ng/μL or >10,000 ng/μL, a warning that the concentration is out of range appears, and you are not allowed to proceed to the next step.
- If the concentration is ≤10,000 ng/μL, but >1,024X of the target concentration, you can proceed, but because the instrument cannot dilute samples more than 1,024-fold, the diluted sample concentration will be greater than the target concentration.

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

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

   | Sample type | Number of primer pools | Volume |
   |---|---|---|
   | Ion AmpliSeq™ chemistry | | |
   | DNA | 1 | 15 μL |
   | DNA | 2 | 25 μL |
   | RNA | 1 | 15 μL |
   | RNA | 2 | 25 μL |
   | Ion AmpliSeq™ HD chemistry | | |
   | DNA | 1 | 20 μL |
   | DNA | 2 | 40 μL |
   | RNA | 1 | 20 μL |

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

   **Note:** The use of other plate seals can affect performance.

   **Note:** We recommend that you use Corning™ Microplate Aluminum Sealing Tape (Fisher Scientific Cat. No. 07-200-684), or Adhesive PCR Plate Foils (Thermo Fisher Scientific Cat. No. AB0626). The use of other plate seals has not been tested and could affect performance.

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

# Dilute and pool libraries, then load the sample plate— Library to Result run

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

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

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

   **Note:** For information on combining DNA and RNA libraries recovered from Sample to Result or Nucleic acid to Result runs using assays that include DNA and fusions, see *Genexus™ Software 6.8 User Guide*.

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.

   **Note:** We recommend that you use Corning™ Microplate Aluminum Sealing Tape (Fisher Scientific Cat. No. 07-200-684), or Adhesive PCR Plate Foils (Thermo Fisher Scientific Cat. No. AB0626). The use of other plate seals has not been tested and may affect performance.

5. 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.
- Preprepared 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)

# 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

Before setting up a sequencing run, review general procedural guidelines for handling panels, reagents, and samples to minimize the chance of contamination. For more information, see "Guidelines for panel and reagent use and handling" on page 26 and "Guidelines for preventing contamination" on page 27.

1. Remove the library and templating strips from their packaging in the refrigerator or freezer, and prepare them for loading in the sequencer.

   • Equilibrate or thaw the following reagent strips at room temperature for 30 minutes.

     – Genexus™ Strip 1

     – Genexus™ Strip 2-AS. If you are delayed in loading, keep the thawed strips on ice or at 4°C until you load them in the sequencer.

     – Genexus™ Strip 3-GX5™ (or Genexus™ Strip 3-GX7™)

   • Thaw Genexus™ Strip 4 by laying the strips on ice for 30 minutes, or incubating at 4°C for 30 minutes. If you are delayed in loading, keep the thawed strips on ice or at 4°C until you load them in the sequencer.

   ---

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

   ---

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

---

**Note:**  You can also vigorously vortex the strip on a platform vortexer to dissolve precipitate if you have a Genexus™ Strip Centrifuge Adapter to hold the strip during the centrifugation. Centrifugation is needed after you vortex a strip. For more information about vortexing the strips, and obtaining and using the adapter, see .

---

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

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

5. Genexus™ Strip 1, Genexus™ Strip 3-GX5™, and Genexus™ Strip 3-GX7™ contain magnetic beads in one or two positions, yellow or brown in color, that sometimes get trapped in the upper "keyhole" of the tube. Dislodge these beads from the keyhole before installing the strip in the sequencer. Use the following procedure for each strip.

---
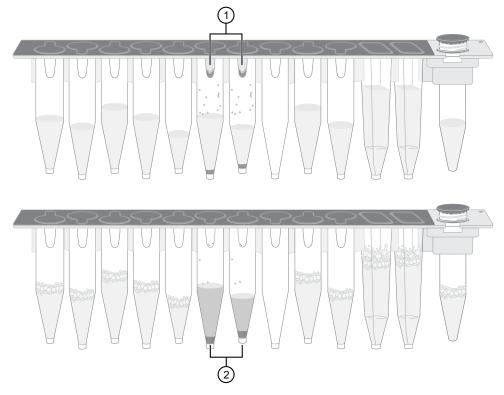
**Note:**  If you have a Genexus™ Strip Centrifuge Adapter, vortex the strips on a platform vortexer to dislodge air bubbles and magnetic beads or to dissolve precipitate, then briefly centrifuge the strips using the adapter to collect the contents at the bottom of the tubes. For more information, see .

---

   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.

8

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



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

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

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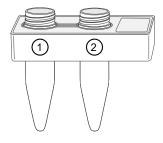
**Note:**
- It is not necessary to resuspend the magnetic beads completely—it is only necessary to dislodge most of the beads that can be trapped in the keyhole. The instrument resuspends the beads during the run when needed.
- Fine bubbles can form above the liquid in some tubes after inversion. These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

---

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

# Fill Genexus™ Primer Pool Tubes

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

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

② Position 2 tube: Contains Ion AmpliSeq™ HD REV primer pool

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

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

**Table 22  Ion AmpliSeq™ DNA assays**

| Chip | Panel concentration[1] | Barcode kit configuration | Number of reactions | Volume in position 1 | Volume in position 2 |
|---|---|---|---|---|---|
| GX5™ Chip | 2X | 32 | 4 | 140 µL | — |
| | 2X | 48 | 6 | 168 µL | — |
| GX7™ Chip | 2X | 32 | 6 | 168 µL | — |
| | 2X | 48 | 6 | 168 µL | — |

[1] Custom DNA panels can be delivered at 5X concentration on request for specific purposes (for example, the need for increased sample volume per reaction in manual library preparation). Panels at 5X concentration must be manually diluted to 2X concentration before loading into Genexus™ Primer Pool Tubes.

**Table 23  Ion AmpliSeq™ RNA assays**

| Chip | Panel concentration | Barcode kit configuration | Number of reactions | Volume in position 1 | Volume in position 2 |
|---|---|---|---|---|---|
| GX5™ Chip | 5X | 32 | 4 | 75 µL | — |
| | 5X | 48 | 6 | 88.5 µL | — |
| GX7™ Chip | 5X | 32 | 6 | 88.5 µL | — |
| | 5X | 48 | 6 | 88.5 µL | — |

Table 24   Ion AmpliSeq™ HD assays

| Primer pool type | Panel concentration | Volume in position 1 | Volume in position 2 |
|---|---|---|---|
| Ion AmpliSeq™ HD FWD | 10X | 50 µL | — |
| Ion AmpliSeq™ HD REV | 10X | — | 50 µL |

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

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

# Load the sequencer and start a run

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

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



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

- **Clear Deck**
- **UV Clean**
- **Load Deck**

- **Clear Sequencing Reagents**
- **Load Sequencing Reagents**

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



The deck door opens automatically.

**Note:**

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

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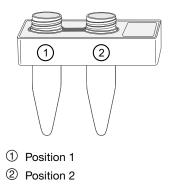


**IMPORTANT!**

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

*Ion AmpliSeq™ & Ion AmpliSeq™ HD Custom Assay User Guide*
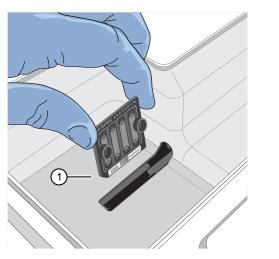
**Note:**

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

① Position 1
② Position 2

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

① Notched corner of chip

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

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

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

**Note:** We recommend that you load the sample plate last.

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

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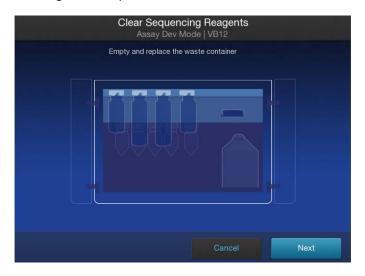


- If you installed a new chip in the sequencer, the sequencer prompts you to open the sequencing reagents bay doors to empty the waste and remove used sequencing reagents bay consumables. Proceed to step 8.

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

IMPORTANT! The cartridge and bottles in the sequencing reagents bay must be replaced every time that a new chip is installed, regardless of how many lanes were used in the previous chip.
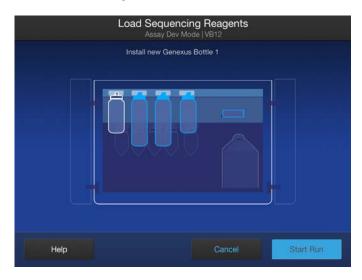
8. Follow on-screen instructions to empty the waste in the Waste carboy, remove waste pipette tips, remove the used Genexus™ Bottle 1, Genexus™ Bottle 2, Genexus™ Bottle 3, and Genexus™ Cartridge, then tap **Next**.



**IMPORTANT!**

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

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

---
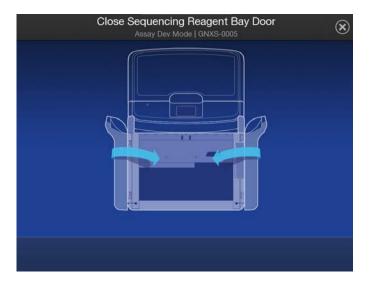
**IMPORTANT!**

- Before installing, *gently* invert each Genexus™ Bottle 2 five times to mix—avoid vigorous mixing. Inspect the plastic nozzles for any pinches or deformations. To avoid pinching or folding of the plastic nozzle, install the bottles straight-on, not at an angle.
- The installed reagents can be used for up to 14 days on the sequencer with full performance. After 14 days, you may observe reduced performance.

---

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

10. Close the sequencing reagents bay doors.



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

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

A sequencing run encompasses the following stages.

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

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

---

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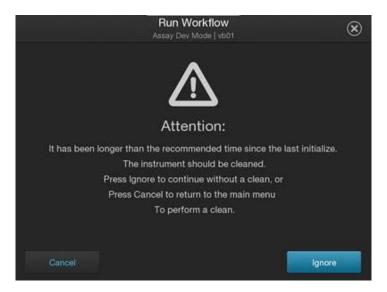
⚠ **MISE EN GARDE !** Le Genexus™ Bottle 1 (petit flacon de récupération de déchets) contient de petites quantités de formamide. Éliminez ces déchets de façon appropriée.

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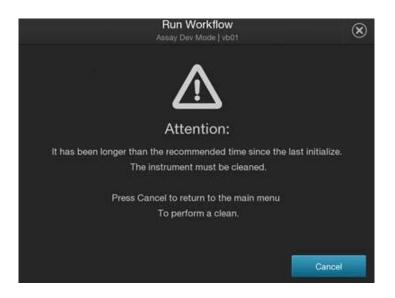
# Options for an expired sequencer initialization

A sequencer initialization is defined by the installation of a new chip, chip coupler, sequencing bottles, and reagent cartridge on the sequencer before a run. Reagents are stable on the sequencer for 14 days, after which you can experience reduced performance. After 14 days, the following on-instrument warning appears:



Users have the option to ignore the warning and proceed with an expired initialization by tapping **Ignore**, or to perform an instrument clean by tapping **Cancel**. After tapping **Cancel**, select **Settings ▸ Clean instrument** to clean the instrument before starting a run with a new chip. Lane assignment for a new run starts with lane 1.

After 28 days, you can no longer proceed with an expired initialization. The following message appears:

Tap **Cancel** to return to the main menu to perform an instrument clean.

# 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. |

# Example results

**Note:** The following examples are from sequencing runs that include a control sample.



**Figure 2  Example results from a custom DNA and Fusions sequencing run**

① Run Name - The unique name of the run given when it was created in the software. For more information, see "Review run results" on page 127 and "View the run summary" on page 123.

② Assay Name - The name of the assay used in the run plan.

③ Sample Name - The unique identifier created when the sample was entered into the software. For more information, see "Review sample results" on page 118 and "View sequencing results" on page 134.

④ **Variants**—Click to see a list of all variants in the results. For more information, see "View SNV/INDEL results" on page 144, "View fusion results" on page 148, and "Review the variant pileup view" on page 152.

⑤ **Plugins**—Click to view results for plugins that are included in the sequencing run. For more information, see "Review molecularCoverageAnalysis plugin results" on page 170 and "Review coverageAnalysis plugin results" on page 166.

⑥ **Reports**—Click to open the screen from which you can create a report. For more information, see "View assay metrics and the run report" on page 129 and "Generate a variant report" on page 163.

⑦ **Edit Filters**—Click **Edit Filters** and select another filter chain from the list , or select **No Filter Chain**, to remove all filter chains, then click **Save** to apply the filter chain selection to the list of results that are shown. Or, Click **Edit Filters** and select another gene list, or select **No Gene List**, then click **Save** to apply the gene list selection to the list of results. If you do not apply a gene list, variants in all genes will be shown. But if you do apply a gene list, the list of variants will show only variants for the genes that are included in the gene list. For more information, see the Genexus™ Software help system.

⑧ **Export** —Click to download the information for the variants in XLS or TSV format.

⑨ **Columns**—Click **Columns** to change the columns in the sample results table. Select a checkbox in the list of columns to add a column to the table, or deselect a checkbox to remove a column from the table. For more information, see the Genexus™ Software help system.

⑩ ••• **(More Options)** - Use this menu to reanalyze the run, download a Customer Service Archive (CSA) file, download files, view a history of the variant classifications, view the run report tab, or run plugins after a sequencing run is complete. For more information, see "Reanalyze a run" on page 164, "Customer Support Archive" on page 172 or the Genexus™ Software.

⑪ Columns currently available in the list of results. You can change the columns that are shown in the sample results table. For more information, see "Customer Support Archive" on page 172 or the Genexus™ Software.

⑫ Variant class - Select an option to limit the types of variants that are shown and toggle between different variant results: **All** variants, **SNVs/Indels** or **Fusions**. For more information, see "Create and assign variant classifications" on page 159.

⑬ **QC** - The QC status of a completed run.

- ✅ (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.

⑭ **Key Findings**—The **Key Findings** tab shows details about the sample, a summary of key metrics for the run, and key variants for genes assayed in the run.

Note: The **Key Findings** tab is by default the first view that is first shown for sample results. For more information, see "View key findings" on page 139.

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



Figure 3   Example results from a custom Fusions sequencing run

# Results navigation bar overview

After you select a run result or a sample result, a results navigation bar appears and allows you to easily toggle between different results screens for each run. You can quickly switch between run-level results, assay-level results, and sample-level results for the run. In addition, you can easily find samples or assays in the run with the search field that appears in each dropdown list.

Assay-level results include assay metrics, such as final read data.

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

| Callout | Dropdown menu | Description |
|---------|---------------|-------------|
| 1 | **Run name** | The run name is listed. Multiple runs are listed only if the run has been reanalyzed. For more information, see "Reanalyze a run" on page 164. |
| 2 | **Select Assay** | You can click the assay name of interest to view assay metrics for the run. |
| | | You can search for or toggle between different assays used in the run. If only one assay is associated with the run, only one assay name is listed. |
| | | Some results from a plugin that are included in the run, such as the customer support archive plugin, apply to all of the samples for an assay in a run plugin results are comprised of a consolidation of all of the samples. Results from such assay-level plugins are shown in the **Plugins** tab when you select an assay but do not select any samples for the run. For more information about plugins, see "Plugins for data analysis" on page 166. |
| | | You can select **Select Assay** from the dropdown menu to remove the assay selection. If you remove the assay and sample selections, the **Run Summary** tab opens with a summary of results for the run selected in the **Run Results** dropdown menu. Assay metrics are also shown in the **Run Report** tab. For more information, see "View assay metrics and the run report" on page 129. |
| 3 | **Select Sample** | You can click a sample name to view the sequencing results for the sample. You can click a different sample name to view other sample results for other samples in the run for the assay that is selected in **Select Assay**. The search field allows you to find samples in the run. |
| | | For **Sample to Result** runs that include a positive control, results for the positive control are shown when you select **Control Sample**. |
| | | You can select **Select Sample** from the dropdown menu to remove the sample selection. If you remove the sample selection, the **Assay metrics** tab for the selected assay opens. If you select **Select Assay** with **Select Sample** shown, the **Run Summary** tab opens. Assay metrics are also shown in the **Run Report** tab. For more information, see "View assay metrics and the run report" on page 129. |

# Review sample results

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

See the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system for information about how to find sample results of interest.

The following information appears in the **Sample Results** screen. You can add columns to the Sample Results table to view more information. For example, you can add a column for **Electronic Signatures** to view signature and lock information for variant reports. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

| Column | Description |
|---|---|
| **Sample Name** | The unique identifier created when the sample was entered into the software. Click the **Sample Name** to open the **Sample Results** screen for the sample. |
| **Assay Name** | The name of the assay used in the run plan. |
| **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.<br>• ✔ (Passed) indicates the sample passed all QC metrics.<br>• ✗ (Failed) indicates the sample failed a QC metric.<br>• — (Not Calculated) indicates a sample did not undergo QC analysis. |
| **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. |
| **Tags** | Tags that are assigned to the sample are listed. |
| **Updated Annotations** | The information that is shown depends on the type of the assay and whether the option to use the latest annotation is configured in the assay.<br>• The name and version of the annotation source that is applied to the sample result is shown when an assay is configured to use the latest annotations but the annotation that is applied to the results is not the latest annotation source.<br>• No information is shown in the column (the value is blank) when the latest annotation source version is applied to the result, regardless of whether the assay is configured to use the latest annotation source.<br>• No information is shown in the column (the value is blank) when an assay is configured to not use the latest annotation source, and instead uses a fixed version of an annotation source.<br>• **N/A** is shown when an assay does not use an annotation set. |

You can also perform the following actions in the **Sample Results** screen.

| Option | Description |
|--------|-------------|
| Actions | Available action links for a sample are shown when you place the pointer over the row of a sample.<br><br>• **Report**—Download the report (available only for samples with a sample status of completed).<br>• **Assign Tags**—Add or remove the tags assigned to a sample result.<br>• **Audit**—View the audit trail for the run.<br>• **Notes**—View, add, or delete notes for sample result.<br>• **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting.<br>• **Reanalyze**—Reanalyze a sample from the alignment step when a sample or reanalysis successfully completes the basecalling step.<br>• **Variant Audit**—View the history of variant classifications. |
| Actions that you can perform when you select samples | More actions are shown when you select one or more samples.<br><br>• **Assign Tags**—Assign a tag or tags to selected sample results in one action. The ◇ **Assign Tags** command appears above the **Sample Results** table after you select one or more samples.<br>• **Compare**—Compare variant results between multiple samples, or from a single sample over time. The ▱ **Compare** command is not shown until you select at least two samples. |

## 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.<br><br>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**. |

*(continued)*

| Option | Selection |
|---|---|
| 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.<br>2. Click the run or assay of interest from the results navigation bar.<br>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 the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

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 "Plugins for data analysis"

For instructions to download results files, see "Download results files for a sample result"

| Option | File name | Description |
|---|---|---|
| **Variant and QC Metrics (.txt)** | `statistic.txt` | Contains tumor mutational burden statistics. |
| **Substitution Type and Context Of Somatic Mutations (.png)** | `somatic_mutation_plot1.png` | A histogram that shows the class of somatic mutations. Somatic mutations can be divided into 6 base substitution classes (that is, C>A, C>G, C>T, T>A, T>C, T>G) based on their substitution type. After incorporating information about the bases immediately 5′ and 3′ to each mutated base, 96 possible mutation types are in this classification. These 96 mutation types are represented on the x-axis, and variant frequency for mutation type on the y-axis. Bars for each substitution class are grouped and displayed with different color. |
| **Somatic Mutations Across Substitution Type (.png)** | `somatic_mutation_plot2.png` | A pie chart dividing somatic mutations into 6 base substitution classes (that is, C>A, C>G, C>T, T>A, T>C, T>G) based on their substitution type. |
| **Germ-line and Somatic Variants (.png)** | `before_AFDist.png` | A histogram that shows the frequency distribution of allele ratio for total called germline and somatic variants. |

*(continued)*

| Option | File name | Description |
|---|---|---|
| **Signature Pattern Of Somatic Mutations (.png)** | `somatic_mutation_plot3.png` | A pie chart dividing somatic mutations in groups consistent with specific mechanisms. |
| **Variants** | | |
| **Filtered Variants (.vcf)**[1] | `<Filter name>_filtered.vcf` | Summary of filtered variant results in variant call format (VCF). |
| **All Variants (.vcf)**[1] | `Oncomine_<LibPrepID>_<analysisID>.vcf` | Summary of variant results in variant call format (VCF). |
| **Variant Summary (.tsv)** | `Summary.tsv` | File that lists SNV/INDEL, copy number, and fusion results in tab-separated value format (TSV). |
| **Snvindel (.tsv)** | `Snvindel.tsv` | File that lists SNV/INDEL variant results in tab-separated value format (TSV). |
| **Fusion (.tsv)** | `Fusion.tsv` | File that lists fusion results in tab-separated value format (TSV). |
| **CNV (.tsv)** | `Cnv.tsv` | File that lists copy number variant results in tab-separated value format (TSV). |
| **Sequencing Results** | | |
| **DNA Unmapped Bam File (.bam)** | `<barcode>_rawlib.basecaller.bam` | Unmapped DNA barcode BAM file; output after mapping reads to reference. |
| **DNA mapped bam file (.bam)** | `merged.bam` | Mapped BAM file of combined barcode reads. |
| **DNA Mapped Bam Index File (.bai)** | `merged.bam.bai` | Mapped BAM Index file. |
| **DNA Basecaller FASTAQ File (.fastq)** | `<barcode>_rawlib.basecaller.fastq` | FASTQ file of the DNA barcodes used. |
| **DNA Processed Bam File** | `merged.bam.ptrim.bam` | Mapped BAM file of combined barcode reads. |
| **DNA Processed Bam Index** | `merged.bam.ptrim.bam.bai` | Mapped BAM index file. |
| **RNA Unmapped Bam File (.bam)** | `<barcode>_rawlib.basecaller.bam` | Unmapped RNA BAM file; output of base calling, contains unmapped reads. |
| **RNA Mapped Bam File (.bam)** | `<barcode>_rawlib.basecaller_alignments.bam` | Mapped BAM file of combined barcode reads. |
| **RNA Mapped Bam Index File (.bai)** | `<barcode>_rawlib.basecaller_alignments.bam.bai` | Mapped BAM index file. |

*(continued)*

| Option | File name | Description |
|---|---|---|
| **RNA Basecaller FASTAQ File (.fastq)** | `<barcode>_rawlib. basecaller.fastq` | FASTQ file generated from the unmapped BAM file of the RNA barcodes used. |
| **Test Fragment Basecaller FASTAQ File (.fastq)** | `rawtf.basecaller.fastq` | FASTQ file for the test fragment. |
| **Audit and Log** | | |
| **Analysis Log File** | `analysis.log` | Analysis log file. |
| **Run Summary**[2] | `Info.csv` | Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary. |
| **Run Audit** | `PlannedRun-AuditTrail.pdf` | Contains all audit records pertaining to the run. |
| **Reports** | | |
| **Report** | `<language>_<sampleName>_<mode> _<templateName>_<assayName>_ <date>.pdf` | A PDF report that contains sample-specific results. For more information, see "Variant report" on page 162. |
| **Sample Summary**[3] | `Info.csv` | Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary. |
| **Troubleshooting Files** | | |
| **Log Files**[4] | `analysis.log` | Analysis log file. |
| | `summary.<timestamp>.log` | Start and end time for each time an assay module is executed for the analysis. |
| | `various.err` `various.out` | Analysis pipeline logs used by field service engineers for troubleshooting. |
| | `Info.csv` | Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary. |

*(continued)*

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

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

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

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

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

# View the run summary

The run summary provides an overview of the run. The information that is displayed includes a list of the assays used in the run, sample locations, information about the reagents used in the run, primer tube positions, and instrument information.

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

2. In the **Run Name** column, click the run name of interest.
   The **Run Summary** tab opens.

3. Review the run summary.

| Action | Procedure |
|---|---|
| View the assay metrics. | In the **Assays** section, click the assay name of interest. |
| View the sample locations in an image of a 96-well sample plate. | In the **Sample Locations** section, click **PCR Plate View**. |
| Reanalyze a run with a new assay. | In the upper right corner of the screen, click ⋯ (**More Options**) ▸ **Reanalyze**.<br><br>For more information, see "Reanalyze a run" on page 164. |
| Run plugins on the sequencing data after a sequencing run is complete. | In the upper right corner of the screen, click ⋯ (**More Options**) ▸ **Run Plugin**.<br><br>For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system. |
| Download customer support archive (CSA) log files for the run to help with troubleshooting. | In the upper right corner of the screen, click ⋯ (**More Options**) ▸ **CSA**.<br><br>For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system. |

*(continued)*

| Action | Procedure |
|---|---|
| Upload results to another Genexus™ Integrated Sequencer or to Ion Reporter™ Software for further analysis. | In the upper right corner of the screen, click ••• **(More Options)** ▸ **BAM Uploader.**<br><br>For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system. |
| View the history of variant classifications. | In the upper right corner of the screen, click ••• **(More Options)** ▸ **Variant Audit.**<br><br>The information is available only for results that include variant classifications. |
| View the run report. | Click the **Run Report** tab. |

## The run summary

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

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

Table 25   Run information

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

Table 26   Sequencing instrument information

| Item | Description |
|---|---|
| **Instrument Name** | The name of the sequencer. |
| **Instrument Serial Number** | The serial number of the sequencer. |
| **Status** | The status of the sequencing portion of the run. |

Table 26   Sequencing instrument information  *(continued)*

| Item | Description |
|---|---|
| Operator | The name of the person who was signed in the Genexus™ Integrated Sequencer when the sequencing run was performed. |
| Start Date | The date and time when the run was started on the sequencer. |
| Completion Date | The date and time when the run was completed on the sequencer. |
| Sequencing Flows | The number of flows performed by the sequencer. |

Table 27   Assays table

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

Table 28   Sample Locations table

| Item | Description |
|---|---|
| PCR Plate View | Click to view the sample locations in an image of a 96-well sample plate. |
| Well Pos. | The well position indicates the location of the sample on the plate. |

Table 28   Sample Locations table  *(continued)*

| Item | Description |
|---|---|
| Library Batch ID | The unique identifier created for the library batch. This information is available only for Library to Result runs. |
| Sample Name | The unique identifier created when the sample was entered into the software. |
| Nucleic Acid Type | The sample nucleic acid type, such as DNA, RNA, or TNA. |
| Vol. (µl) | The volume of the sample. |
| Conc. (ng/µl) | The concentration of the sample. |
| Dilution Factor | The dilution factor of the sample. |
| Kit Barcodes | The barcode for the kit used for nucleic acid extraction, if applicable. |
| Barcode | The name of the barcode adapter or adapters that are associated with the sample, for example, **IonHDdual_0101**. |
| Assay Name | The assay that was used to sequence the sample for the indicated well position. |
| Library Position | The plate location of libraries. For completed runs that were planned using samples, the **Library Position** indicates the location of unused libraries that can be sequenced by planning and initiating a library run. |

Table 29   Reagents table

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

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

# Review run results

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

See the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system for information about how to find run results of interest.

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**. For more information, see "The run summary" on page 124. |
| | Runs that are reanalyzed are listed with ↺ **(Reanalysis)** after the run name. For more information, see "Reanalyze a run" on page 164. |
| Assay Name | The name of the assay selected for the run. You can view the **Assay Name** and corresponding **Assay Full Name** for all assays in the **Assays ▸ Manage Assays** screen. |
| Run Status | The status of the run. For example, **Analysis In Progress**, **Executing Plugins**, **Analysis Completed**, **Terminated**, **Archival: In Progress**). |
| Total Samples | The total number of samples in a run. |
| PCR Plate Number | A unique identifier for the 96-well plate used for library preparation and templating. For more information, see "Assign PCR Plate" on page 128. |
| Started On | The date and time when the run was started. |
| Updated On | The date and time when the last action was completed on the run. |

You can also perform the following actions in the **Results / Run Results** screen.

| Option | Description |
|---|---|
| Actions | Available action links for a run are shown when you place the pointer over the row of a run. The actions that are available depend on the type of run.<br><br>• **Delete**—Delete the run.<br><br>• **BAM Uploader**—Upload run information to another Genexus™ Integrated Sequencer or to Ion Reporter™ Software for further analysis.<br><br>**BAM Uploader** is not available for BAM run results or for archived runs in which BAM files have been removed.<br><br>For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.<br><br>• **Audit**—View the audit trail for the run.<br><br>For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.<br><br>• **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting.<br><br>For more information, see "Download a Customer Support Archive" on page 172.<br><br>• **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 128. |
| Actions | Available action links for a run are shown when you select the checkbox to the left of the **Run Name**.<br><br>• **Clear**—Deselect the selected run or runs.<br><br>• **Retain Data**—Click to keep run data regardless of disk cleanup settings. To undo, select **Release Data**.<br><br>• **Archive Data**—Click to archive run data. Select run data options (**Sequencing Output**, **Intermediate Files**) in the **Archive Confirmation** dialog box. **Analysis Results** are selected by default and are not optional.<br><br>• **Delete**—Delete the run data of the selected run or runs. |

## 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.

For more information to find a run with a search of the PCR Plate Number, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

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

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

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

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

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

# View assay metrics and the run report

The run report provides detailed information about a run, such as the **Total Bases** and **Final Reads**. For runs with multiple assays, metrics are provided for each assay in the run. The run report contains the assay metrics for all assays in the run.

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

1. Click **Results ▸ Run Results** to view sequencing results for a particular sample.

2. In the **Run Name** column, click the run name of interest.

| Action | Procedure |
|---|---|
| View the run report | Click the **Run Report** tab. |
| View the assay metrics for a single assay | In the **Select Assay** dropdown list at the top of the screen, select the assay of interest. |

For more information, see "View assay metrics and the run report" .

## Assay metrics and the run report

The run report provides detailed information, such as chip metrics for the run, well, and Ion Sphere™ Particles (ISPs) statistics. For runs with multiple assays, metrics are provided for each assay in the run. Sequencing metrics are shown at the top of the screen, followed by sample-specific metrics in the **Run Samples** table. Read data for individual samples for the assay are listed in the **Run Samples** table.

Barcode-specific metrics for barcodes that are included in the run are listed in the **Barcodes With Reads Reported** table, which follows the **Run Samples** table. The CSA file for the run contains information for barcodes that are not assigned to samples in the run. Information in the CSA file can

help you troubleshoot results, if needed. For more information, see "Download a Customer Support Archive" on page 172.

Table 30  Run Report

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

**Table 30   Run Report**  *(continued)*

| Metric | Description |
|---|---|
| Polyclonal | Wells with a live ISP that carries clones from two or more templates.<br><br>To view polyclonal metrics, mouse over the first low quality portion (gray) of the **Final Reads** bar plot.<br><br>Final Reads 58.39% ⑦    Filtered out 41.61%<br>30,735,120<br>Final Reads(30,735,120) / Wells with Library ISPs(52,635,124)    Polyclonal: 15935106 (30.27%) |
| Low Quality | Loaded wells with a low or unrecognizable signal.<br><br>To view polyclonal metrics, mouse over the second low quality portion (gray) of the **Final Reads** bar plot.<br><br>Final Reads 58.39% ⑦    Filtered out 41.61%<br>30,735,120<br>Final Reads(30,735,120) / Wells with Library ISPs(52,635,124)    Low Quality: 3208258 (6.1%) |
| Adapter Dimer | Loaded wells with a library template of an insert size less than 8 bases.<br><br>To view adapter dimer metrics, mouse over the lightest gray portion of the **Final Reads** bar plot.<br><br>Final Reads 43.57% ⑦    Filtered out 56.44%<br>52,494,934<br>Final Reads(52,494,934) / Wells with Library ISPs(120,492,758)    Adapter Dimer: 10862794 (9.02%)<br><br>**Note:**  In assays using Ion AmpliSeq™ HD library chemistry, adapter dimer reads represent a small proportion of total reads and can be seen by hovering the pointer over the right end of the **Final Reads** bar. |
| Loading Density | A visual representation of chip loading. Red color indicates areas of higher density of loading. Blue color indicates areas of lower density of loading. The following example shows a sequencing experiment where two lanes on the chip are uniformly loaded with ISPs.<br><br>Loading Density |

9

### Table 31   Run Samples

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

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

### Table 32   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. |

Table 32   Barcodes with Reads Reported  *(continued)*

| Column | Description |
|--------|-------------|
| 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).<br><br><br><br>Click the **Read Length Histogram** to open an expanded view. |

## View resequencing metrics

For assays that include resequencing, you can view resequencing metrics when a run is complete.

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

2. In the **Run Results** screen, in the **Run Name** column, click the run name of interest.

3. In **Select Assay**, select the assay name of interest.
   The assay metrics for the sequencing run are shown.

4. Click **Resequencing Metrics**.
   The resequencing assay metrics are shown. For more information, see "View assay metrics and the run report" <span>on page 129</span>.

## Download a run report

You can download a run report summary in PDF format. The run report includes assay metrics and the record of reagents that were used in a run. For information about the contents of the run report, see "View assay metrics and the run report" <span>on page 129</span>. 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 sequencing results

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

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

3. In the **Results** screen, click the tabs to view the different types of sample-specific results and data.

| Tab | Description |
|---|---|
| QC | The quality metrics for the sample sequenced in the run. For more information, see "QC results" on page 135. |
| Key Findings | An overview of the results for the sample, including **Sample Details**, **Key Metrics**, **Genes**, and **Coverage** graphs. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system. |
| Variants | Detailed variant results for **SNVs Indels**, **Fusions**, and **CNV**. For more information, see "View SNV/INDEL results" on page 144 and "View fusion results" on page 148. |
| Plugins | Results generated from the plugins associated with the assay used to analyze the sequenced sample. For more information, see "Plugins for data analysis" on page 166. |
| Reports | You can download and generate summaries of run results. There are two types of reports:<br>• Run reports – include assay metrics and the record of reagents that were used in a run.<br>• Variant reports – include the variant results for each sample in a sequencing run, reagents used, and QC evaluation metrics.<br>For more information, see "Download a run report" on page 133. |

## View the QC results

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

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

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

3. Click the **QC** tab.
   The QC metrics are shown.

4. Review the QC metrics.
   For more information, see "QC results" on page 135.

## QC results

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

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

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

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

The data shown in the screen depend on the assay that was used in the run. Not all metrics are shown for every assay.

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

*(continued)*

| Metric[1] | Description |
|---|---|
| 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—DNA** | **Sequencing quality metrics of the sample DNA library.** |
| Deamination score | Deamination is reported as the estimated SNP proportion consistent with deamination (low allele frequency C:G>T:A SNVs). The deamination score can be used to determine the quality of the FFPE sample. |
| MAPD | MAPD (Median of the Absolute values of all Pairwise Differences) is a quality metric that estimates coverage variability between adjacent amplicons in CNV analyses. A MAPD value of 0.4 indicates an acceptable level of coverage variability. High MAPD value typically translates to a lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. If the MAPD QC threshold is not met, CNVs are not called. The MAPD metric does not affect SNVs/INDEL calls. |
| Mapped Reads | The total valid mapped reads. |
| Mean AQ20 Read Length (bp) | The average length, in base pairs, at which the accuracy rate is ≥99% for all aligned reads of a library. |
| Mean Read Cov | The number of average reads per amplicon. |
| Molecular Uniformity | Uniformity of molecular coverage for all amplicons. |
| Mean Read Length (bp) | The average length, in base pairs, of final library reads for the sample. |
| Mean Read Cov | The average number of reads per amplicon calculated over all amplicons in the assay. |
| Number of bases used in calculating TMB | |
| Number of variant calls | |
| Uniformity of Amplicon Coverage | The percentage of amplicons that had at least 20 percent of the average number of reads per amplicon. Cumulative coverage is linearly interpolated between nearest integer read depth counts. |
| Median Mol Cov | The median number of functional molecule reads per amplicon calculated over all amplicons in the assay. This metric is applicable to Ion AmpliSeq™ HD library chemistry only. |
| Uniformity of Base Coverage | The percentage of reads with a depth of coverage ≥20% of the mean read coverage at each position. This metric is applicable to Ion AmpliSeq™ HD library chemistry only. |

*(continued)*

| Metric[1] | Description |
|---|---|
| **Read Length Histogram** | The histogram presents all filtered and trimmed DNA library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences. |
| **Read Ratio for Inline Controls (Endogenous to Spike-ins)** | The ratio of genomic sample reads to control reads for each inline control amplicon.<br><br><br><br>**Note:** The **Read Ratio** for each inline control amplicon is expected to be approximately 3 with 10 ng DNA input for both Ion AmpliSeq™ and Ion AmpliSeq™ HD chemistries. |
| **Sample QC – RNA** | Sequencing quality metrics of the sample RNA library. |
| **Mapped Fusion Reads** | The total valid mapped reads. That is, the mapped reads that pass the thresholds and filters across all of the RNA targets in the reference file. This number is a subset of the mapped reads shown in the **Run Samples** table in the **Assay Metrics**. |
| **Mean Read Length (bp)** | The average length, in base pairs, of the final library reads for the sample. |
| **RNA Expression Ctrls Detected** | The number of expression control genes detected for the sample. This metric measures the RNA input integrity, input amount, and the fidelity of the reverse transcriptase that was used in library preparation.<br><br>Fusion panels include primer pairs that cover control housekeeping genes. |
| **Mean AQ20 Read Length** | The average length, in base pairs, at which the accuracy rate is ≥99% for all aligned reads of a library. |
| **Read Length Histogram** | The histogram presents all filtered and trimmed library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). |
| **Read Ratio for Inline Controls (Endogenous to Spike-ins)** | The ratio of genomic sample reads to control reads for each inline control amplicon. You can use this metric to measure the level of contaminating genomic DNA in RNA libraries when you select the RNA checkbox for **Include Inline Controls** in assay set up. Using inline controls in RNA assays will reduce the total number of panel reads.<br><br>**Note:** The **Read Ratio** for each inline control amplicon is expected to be approximately 3 with 10 ng DNA input for both Ion AmpliSeq™ and Ion AmpliSeq™ HD chemistries. |

*(continued)*

| Metric[1] | Description |
|---|---|
| NTC QC – DNA[2] | DNA Sequencing quality metrics of the no-template control. |
| Average Base Coverage Depth | The average number of DNA reads of all targeted reference bases. |
| Mean Read Length (bp) | The average length, in base pairs, of final DNA library reads for the no-template control. |
| Read Length Histogram | The histogram presents all filtered and trimmed reads for the no-template control that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). |
| Read Ratio for Inline Controls (Endogenous to Spike-ins) | The ratio of no-template control sample reads to control reads for each DNA inline control amplicon. |
| NTC QC – RNA[2] | RNA Sequencing quality metrics of the no-template control. |
| Mapped Fusion Reads | The total number of valid mapped reads for the NTC. |
| Mean Read Length (bp) | The average length, in base pairs, of final RNA library reads for the no-template control. |
| Read Length Histogram | The histogram presents all filtered and trimmed reads for the no-template control that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). |
| Read Ratio for Inline Controls (Endogenous to Spike-ins) | The ratio of no-template control sample reads to control reads for each RNA inline control amplicon. |

[1] For **BAM to Result** runs, only the **Sample QC—DNA** or **Sample QC—RNA** metrics are shown.
[2] Metrics are shown only if NTC is included in the run plan.

## MAPD copy number QC metric

The Median of the Absolute values of all Pairwise Differences (MAPD) score is reported on Aneuploidy run results and other runs that detect CNVs.

MAPD is one of the metrics that we define to assess whether the panel data are useful for copy number variation (CNV) run results.

MAPD is defined as the Median of the Absolute values of all Pairwise Differences between log2 ratios per tile for a given run. Tiles roughly correspond to amplicons in an Ion AmpliSeq™ assay. Each pair is defined as adjacent in terms of genomic distance. Tiles corresponding to copy number amplicons and other amplicons are being treated equally as no differences in variability are seen between these types. Then, any two log2 ratios that are adjacent on the genome are a pair. Except at the beginning and the end of a chromosome, every log2 ratio belongs to two pairs.

Formally, if `xi` is the log2 ratio at position `i`, with `i` ordered by genomic position:

```
MAPD = median ( | x(i - 1) - x(i) | )
```

MAPD is a per-sequencing run estimate of copy number variability, similar to standard deviation (SD). If one assumes the log2 ratios are distributed normally with mean 0 and a constant SD, then MAPD/0.95 is approximately equal to SD. However, unlike SD, using MAPD is robust against high biological variability in log2 ratios induced by known conditions such as cancer.

Regardless of the source of the variability, increased variability decreases the quality of CNV calls.

# View key findings

You can view **Sample Details**, a summary of the metrics for the run, and the **Key Variants** matrix for genes assayed in the run in Genexus™ Software.

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

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

Review the key findings. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

## Key findings

The **Key Findings** tab shows details about the sample, a summary of key metrics for the run, and key variants for genes assayed in the run.

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

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

Table 33   Sample details

| Section | Description |
|---|---|
| Sample Name | A unique identifier representing the sample. |
| Collection Date | The date that the sample was collected. |
| Gender | The biological sex of the sample: **Female**, **Male**, or **Unknown**. |
| Sample Type | A term that describes the sample, for example, FFPE, DNA, DNA & RNA. |
| Application Category | The application type of the sample. |
| Cancer Type | The type of cancer that is represented by the sample. |
| Cancer Stage | The stage of the cancer from which the sample was collected. |
| %Cellularity | The percentage of tumor cellularity in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is entered when a sample is created. This attribute applies only to FFPE samples. |

**Table 34  Key metrics**

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

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

## The Key Variants matrix

The **Key Variants** matrix provides a color-coded visual representation of the variant results. When the application category of the sample is **Cancer** or **Cancer (Germline)** the genes that are shown in the **Key Variants** matrix are determined by the filter chain and the gene list applied to the results. You can change the filters that are applied to expand or restrict the genes that are shown. For more information, see "View the Key Variants matrix" on page 140.

Each tile in the **Key Variants** matrix represents one gene and indicates whether a variant is detected or not. Note that a single gene is represented with multiple tiles when there are multiple mutations detected in that gene.

## View the Key Variants matrix

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

Each tile in the **Key Variants** matrix represents one gene and indicates whether a variant is detected or not. Note that a single gene is represented with multiple tiles when there are multiple mutations detected in that gene.

You can view a color-coded visual summary of variant results in Genexus™ Software.

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

2. Click a sample name.
   The **Results** screen opens to the **Key Findings** tab, which shows the **Sample Details**, **Key Metrics**, the **Key Variants**, and the **Coverage Graphs**.

3. Refine the genes that are shown in the **Key Variants** matrix.

| Action | Procedure |
|---|---|
| Hide genes that were not included in an assay but are in a gene list. | Select or deselect the **Not Assayed** checkbox. |
| Refine the genes that are shown in the **Key Variants** matrix. | a. Click **Edit Filters**.<br><br>b. To refine or expand the genes, change one or both of the filters.<br><br>   • Select a different filter chain or no filter chain.<br>     For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.<br>   • Select a different gene list or no gene list.<br>     For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.<br><br>c. Click **Save**. |

Example **Key Variants** matrix

① Color coded legend to the variant tile matrix.

**Key Variants Detected** —A gene is present in the gene list, variants are called by the Oncomine™ Variant Annotator, and the variants are **Key Variants**. Genes that are called are listed in the Variants tab with **Present** in the **Call** column. **Key Variants** are listed in the **Variants** tab, with a value of **Yes** in the **Key Variant** column. For more information about gene lists, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

**Other Variants Detected** —A gene is present in the gene list and variants are called by the Oncomine™ Variant Annotator, but the variants are not **Key Variants**. Genes that are called are listed in the Variants tab with **Present** in the **Call** column. Variants that are not **Key Variants** are listed in the **Variants** tab, with a value of **No** in the **Key Variant** column.
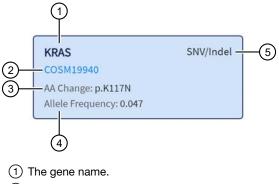
**None Detected** —A gene is present in the gene list but no variants are called by the Oncomine™ Variant Annotator.

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

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

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

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

## Gene tiles in the Key Variants matrix

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



① The gene name.

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

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

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

⑤ The variant type.

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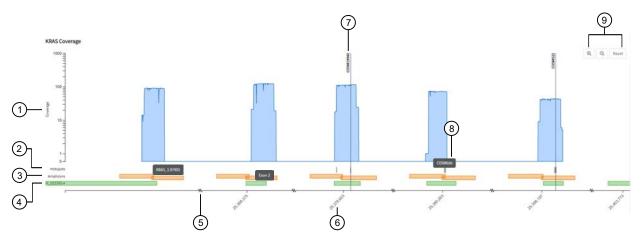
You can view DNA exon amplicon coverage in Genexus™ Software to help you determine whether the sequencing reads across a gene are uniform and sufficient. The default genes that are shown are determined by the gene list that is designated as default for the cancer type of the sample. You can also view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

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

2.  Click a sample name to open the **Key Findings** tab in the **Results** screen.

3.  Scroll to the amplicon **Coverage Graphs**.

4.  Review the coverage graphs.

5.  To show coverage graphs for more genes, scroll to the bottom of the screen. Click **Show Coverage for Gene**, then select the gene of interest.

6.  You can adjust the amplicon coverage graph with the pointer and the buttons in the upper right corner of each graph. The coverage graphs change to show coverage for the selected gene.

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

### Example amplicon coverage graph

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



① Base coverage is shown on the y-axis on a logarithmic scale.

② The location of the known hotspots are denoted with gray bars.

③ The location and span of the amplicons are represented with orange bars. You can hover the mouse over an orange bar to view the amplicon name.

④ The location and span of the transcript track is denoted with green bars. You can hover the mouse over the green bar to view the exon number.

⑤ The line along the x-axis is broken to indicate that the graph is discontinuous to show exons.

⑥ The genome coordinate position is shown on the x-axis.

⑦ Called variants are indicated with a vertical line above the blue coverage plot. The variants noted in the graph depend on the filter chain applied to the results in the **Key Variants** matrix. For more information, see "View the Key Variants matrix" on page 140.

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

⑨ You can adjust the amplicon coverage graph. For more information, see "View the amplicon coverage" on page 143.

## View SNV/INDEL results

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

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

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

   For more information about the data shown in the table, see "SNVs/Indels table" on page 145.

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

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

- The file in XLS format contains the information that is shown in the SNV/Indels variant table.

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

## SNVs/Indels table

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

You can filter the results list in the table using filtering tools and filter chains. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

| Column | Description |
|---|---|
| User Classifications | User-defined classification to select from the list. |
| | For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system. |
| Variant ID | The name of the hotspot as defined in the Browser Extensible Data (BED) file. |
| | Click the link to view more annotation information. For more information, see "View more annotations and annotation sources" on page 151. |
| Variant Name | The name of the variant. |
| Key Variant | Indicates whether the variant is a key variant. |
| | Possible values are **Yes** or **No**. |
| | This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run. |
| Locus | The chromosome and position of the detected variant. |
| Oncomine Variant Class | The type of SNV or INDEL at the locus based on Oncomine™ annotations. |
| | This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run. |
| Oncomine Gene Class | The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: |
| | • **Gain-of-function**—The altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene |
| | • **Loss-of-function**—The altered gene product lacks the molecular function of the wild-type gene |
| | This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run. |

*(continued)*

| Column | Description |
|---|---|
| Gene | The gene name. |
| AA Change | Identification of the amino acid change using Human Genome Variation Society (HGVS) nomenclature. |
| Ref | The reference base or bases at that locus. |
| Alt | The alternate base or bases at that locus. |
| Type | The type of variant that is detected.<br>• **snp** (single nucleotide polymorphism)<br>• **mnp** (multi-nucleotide polymorphism)<br>• **ins** (insertion)<br>• **del** (deletion)<br>• **complex**<br>• **FLT3-ITD** |
| Call | Indicates the presence or absence of an SNV/Indel variant. When the default filter chain is applied, only the variant calls that are designated with **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants** (VCF) file (see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system).<br>• **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)**—Indicates a high confidence call that passes all filter thresholds at a given variant position.<br>  – When the default filter chain is applied, **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** indicates the presence of the ALT (alternative) allele.<br>  – When the **No Filter** option is applied or when viewing the **Variants** (VCF) file, **Present** does not imply the presence of the ALT (alternative) allele. To infer the presence of the ALT allele, see the **Alt** column.<br>• **NO CALL**—Although some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call.<br>• **ABSENT**—Indicates that the variant is unlikely to be present in the sample. |
| Call Details | The reason why a variant is reported as **NO CALL**. |
| Phred QUAL Score | The relative probability of either the "reference" hypothesis interval [0,cutoff], or the "variant" hypothesis interval [cutoff,1], Phred-scaled (-10*log10). A higher score means more evidence for the variant call. |
| Raw Read Depth | Total read coverage across amplicon containing SNV/Indel hotspot locations. Count of chip-level reads aligned at this locus that participate in variant calling. |
| Effective Read Depth | The number of reads covering the position. |
| Alt Allele Read Counts | The number of reads containing the alternate allele. |
| Coverage | The number of total reads at a locus, wild-type + alt allele. |

*(continued)*

| Column | Description |
|---|---|
| Nuc Change | The position and identity of the nucleic acid change. |
| Allele Frequency | The number of variant read counts divided by the total number of read counts for the sample. |
| Allele Frequency (%) | The allele frequency, represented as a percentage. |
| Allele Ratio | The relative frequency of each allele. |
| Mut/WT | The ratio of mutant allele to wild-type allele. |
| Zygosity | Describes whether the variant is homozygous (0) or heterozygous (1). |
| Filtered Read Coverage | Coverage at the position considering only filtered reads. |
| Allele read Count | The number of reads detected for the allele. The Allele Read Count filter sets the minimum count of the genotype alleles. |
| Raw Alt Allele Read Counts | The number of unfiltered reads containing the alternate allele. |
| PPA | Possible Polyploidy Alleles (PPA). A value of **Yes** indicates variants that are PPA alleles. A value of **No** indicates variants that are not PPA alleles. |
| P-Value | The probability value for the detection of variant calls. |
| Mol Depth[1] | The reports number of interrogated DNA molecules containing target. This metric defines the limit of detection at a hotspot position in a particular run and sample. For reference calls, molecular depth provides a measurable metric that serves as confirmation of variant absence among a large number of interrogated molecules. For instance, if molecular depth is ≥1,500, you can have high confidence that no variant is present at ≥0.2% variant allele frequency. If molecular depth is ≥2,500, you can have high confidence that no variant is present at ≥0.1% variant allele frequency. |
| WT Mol Counts[1] | The number of detected molecules containing the wildtype allele. |
| Alt Allele Mol Counts[1] | The number of detected molecules containing the alternate allele. |
| Mol Freq %[1] | Molecular frequency percentage. The percentage of alternate allele reads over total reads at the locus. |
| % LOD[1] | the limit of detection (LOD) of a variant allele expressed as a percentage of the WT allele. LOD is the lowest possible variant frequency in the sample that can be detected by the system with a true positive rate greater than 98% for FFPE samples or 95% for cfTNA samples. LOD is dependent on the molecular read depth at the locus. %LOD is reported when there are no variant calls for the gene. |

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

# View fusion results

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

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

4. Click **Fusions** to view the **Fusions** table.

   For more information about the data displayed in the table, see "Fusions table" <span style="color:gray">on page 148</span>.

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

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

   - The file in XLS format contains the information that is shown in the Fusions variant table.

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

## Fusions table

The data displayed in the **Fusions** table depends on the assay that was used in the run.

You can filter the results list in the table using filtering tools and filter chains. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

| Column | Description |
|---|---|
| User Classifications | A user-defined classification selected from the list. |
|  | For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system. |
| Variant ID | The name of the fusion target as defined in the BED file. |
|  | Click the link to view more annotation information. For more information, see "View more annotations and annotation sources" <span style="color:gray">on page 151</span>. |
| Variant Name | The name of the variant. |
|  | For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system. |
| Key Variant | Indicates whether the variant is a key variant. |
|  | Possible values are **Yes** or **No**. |
|  | This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run. |

*(continued)*

| Column | Description |
|---|---|
| Locus | The chromosome positions in the reference genome that define the fusion junction. |
| Oncomine Variant Class | Oncomine variant class annotation that indicates fusion type based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run. |
| Oncomine Gene Class | The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations:<br><br>• **Gain-of-function**—the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene<br><br>• **Loss-of-function**—the altered gene product lacks the molecular function of the wild-type gene<br><br>This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run. |
| Genes (Exon) | The name of fusion target and representative acceptor and donor exons. |
| Read Counts | The frequency that the fusion was detected in the sample. |
| Type | Assay type (for example, Fusion, RNA exon variant (exon skipping), RNAExon Tile, Proc Control). |
| Call | Indicates the presence or absence of a fusion or RNA exon variant. When the default filter chain is applied, only the fusion/RNA exon variant calls that are designated with **PRESENT** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants** (VCF) file (see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system).<br><br>• **PRESENT**—indicates a high confidence call that passes all filter thresholds at a given variant position.<br><br>• **ABSENT**—indicates the absence of a fusion due to a variant call that falls below thresholds.<br><br>• **NO CALL**—although some evidence for the presence of a fusion exists, the call does not pass one or more filters that are required for a high confidence fusion call. |
| Call Details | The reason for reporting a fusion as **NO CALL** or **ABSENT**. |
| Read Counts Per Million | The number of fusion read counts detected per million total reads. |
| Oncomine Driver Gene | The gene believed to be associated with increased oncogenic properties. The gene is inappropriately activated by the fusion. |
| Gene Isoform | The name of the fusion target as defined in the BED file. |
| Mol Cov. Mutant[1] | The median molecular coverage across a fusion amplicon. |

*(continued)*

| Column | Description |
|---|---|
| Imbalance Score[2] | Each fusion gene exhibits a characteristic **Imbalance Score** threshold. Scores that exceed the threshold value indicate a high likelihood of the presence of the fusion in the sample. Imbalance score calculation starts with the normalization of reads in the exon-tiling amplicons of the gene, followed by correction with a baseline that represents expression values in normal samples. Reads for observed imbalance scores come from samples. Baseline scores come from normal, fusion-negative samples and are stored in the exon tile assay baseline file for the assay. <br><br> Imbalance score = Observed imbalance (in samples) / Expected imbalance (in the baseline) <br><br> where <br><br> Observed imbalance = 3' of breakpoint (in samples) / 5' of breakpoint (in samples) <br> • 3' of breakpoint is the sum of the normalized reads of amplicons 3' of a predicted breakpoint for the gene in the sample. <br> • 5' of breakpoint is the total normalized reads of the amplicons for the gene in the sample. <br><br> Expected imbalance = 3' of breakpoint (in the baseline) / 5' of breakpoint (in the baseline) <br> • 3' of breakpoint is the sum of normalized baseline values of amplicons 3' of the breakpoint for the gene. <br> • 5' of breakpoint is the total normalized baseline values of the amplicons for the gene. |
| Imbalance P-Value[2] | The statistical significance of measure of imbalance relative to a control gene. |
| Predicted Break-point Range[2] | The exonic range for predicted fusion break point in exon tiling assays. |
| Ratio To Wild Type[2] | The molecular ratio for exon skipping assays relative to wild type control amplicons. |
| Norm Count Within Gene[2] | Exon skipping assay coverage normalized to molecular coverage of wild type (WT) MET control amplicons. |

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

[2] Column appears only in analyses that use the exon tiling fusion detection method.

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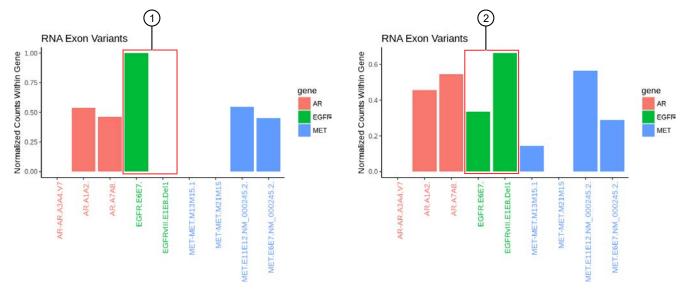
The **RNA Exon Variant** data view displays a bar graph summary of intragenic exon rearrangements or fusions. The displayed RNA exon variants are defined in the BED file that is associated with an assay. The **RNA Exon Variant** data view is available for all RNA and fusion assays.

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

2. In the **Sample name** column, click the name of the sample of interest.

3. Click the **Variants** tab, then click **Fusions**.

The **Fusions** table opens to display fusions results. For more information, see "Fusions table" on page 148.

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

**Representative RNA Exon Variant plots**



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

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

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

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

## View more annotations and annotation sources

In addition to the annotations that are included with Genexus™ Software, you can view annotations and annotation source information from public websites for each variant.

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

2. Click the sample name in the **Sample Name** column in the row of a sample of interest.

3. Click the **Variants** tab.

4. In the **Variants** table, click the **Variant ID** of the variant of interest.

You can click the row even if a **Variant ID** is not listed.

The **Pileup** view is shown below the variants table. For more information, see "Review the variant pileup view" on page 152.

5. Click the **Annotations** tab.

6. Review annotation information in the **Annotations** tab, and if available, click a link in the row of an annotation to open a website with information about the annotation.

# Review the variant pileup view

You can review variant tracks with the pileup view in Genexus™ Software. The pileup view is a figure that the software generates to show each BAM track represented by a single bar, with the sequence variation or variations denoted in each track. The BAM tracks are aligned against a reference track. The variant pileup view can be used to identify potential variants.

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

The pileup view is shown below the variants table.

## Change the view of the variant pileup

You can zoom and pan the variant pileup view and add or remove guides and track labels to more easily understand the data. You can also adjust the view of the individual pileup tracks. For more information, see "Adjust pileup tracks" on page 153.

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

| Action | Procedure |
|---|---|
| Track the position as you place the pointer over the variant pileup. | Click **Cursor Guide**. |
| Mark the center of the variant pileup. | Click **Center Line**. |
| Remove the track labels. | Click **Track Labels**. |
| Zoom in to increase the magnification of the image. | Click ⊕ **(Zoom in)** one or more times, or double-click the image. |
| Zoom out to decrease the magnification of the image. | Click ⊖ **(Zoom out)** one or more times. |
| Pan across the image to move the view to another location on the screen with its current magnification. | Click and drag the pointer over the image. |

## Adjust pileup tracks

You can adjust the BAM read coverage track, the reference track, hotspot tracks, the target region track, and annotation tracks in Genexus™ Software to make it easier to view the data. When you adjust a track, it changes the amount of data that is shown in the plot. For example, you can see more tracks when you use the **Squish** setting.

Note: For best results, click the **Expand** option to view details about variants and base calls that are shown in each read coverage track

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

5. In the **Variants** tab, in the **Pileup** subtab, click ⚙ (Actions) next to a track, then select an option to adjust the view of the track.

| Option | Description |
|---|---|
| **Expand** | Select this option to view the tracks with the maximum visible height. |
| **Squish** | Select this option to view the tracks with the minimal height for each track. |
| **Collapse** | Select this option to view the tracks with overlapping transcripts shown along a single line. When **Collapse** is selected for BAM tracks, the BAM tracks are not shown; only the coverage density is shown. |

## Adjust the view of the reference track

You can adjust the view of the reference track in Genexus™ Software to view or hide the three-frame translation of the reference track sequence. You can also view the forward or reverse reference track sequence.

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

5. In the **Variants** tab, in the **Pileup** subtab, click ⚙ (Actions) next to the reference track.

| Option | Description |
|---|---|
| View the reverse strand | Click **Reverse**.<br>This option is available only when the forward strand is shown. |
| View the forward stand | Click **Forward**.<br>This option is available only when the reverse strand is shown. |
| View the reference sequence translation | Click **Three-Frame Translate**.<br>This option is available only when the amino acid sequence is not shown. |
| Hide the reference sequence translation | Click **Close Translation**.<br>This option is available only when the amino acid sequence is shown. |

## Adjust the view of the BAM tracks

You can adjust the view of the BAM tracks in Genexus™ Software to dynamically customize the view of the data.

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

5. In the **Variants** tab, in the **Pileup** subtab, click ⚙ (Actions) next to the BAM tracks.
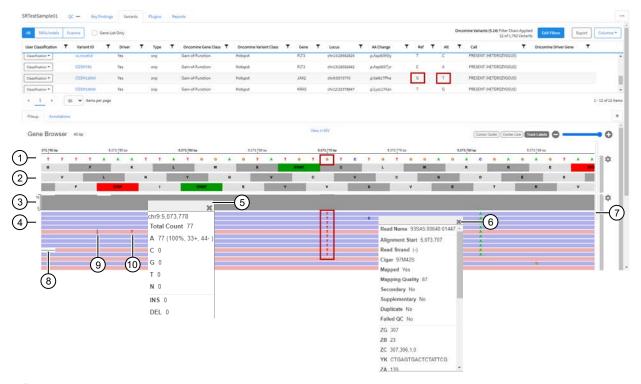
| Option | Description |
|---|---|
| View all BAM tracks in monochrome gray | In **Color By**, deselect the checkmark next to **Read Strand**.<br>This option is available only when the BAM tracks are colored by read strand. |
| Distinguish between the direction of the BAM tracks with color | In **Color By**, click **Read Strand**.<br>This option is available only when the BAM tracks are not colored by read strand. |
| Expand, squish or collapse the BAM tracks | For more information, see "Adjust pileup tracks" on page 153. |
| Show all the bases in the BAM tracks | Click **Show all bases**.<br>This option is available only when all bases in the BAM tracks are not shown. |
| Change the minimum threshold for the visibility of BAM tracks | a. Click **Set visibility window**.<br>b. In the **Set visibility window** dialog box, enter the minimum number of bases needed to show the BAM tracks.<br>When you zoom in and out to view the variant pileup, the scale of the tracks shown in the screen changes. For more information, see "Change the view of the variant pileup" on page 152. You can adjust the visibility window according to the scale shown in the screen. |

## Examples of a variant pileup view
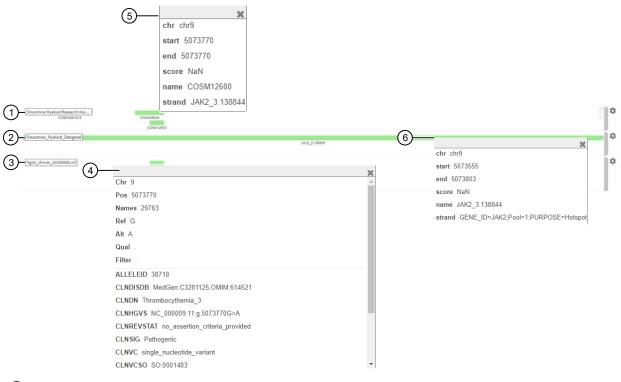
The following figure is an example of a variant pileup view, shown in the **Gene Browser** in the **Variant** tab. The variant pileup view graphically represents the sequencing tracks, the reference track, and the annotation tracks, and can help you understand the variant data and analyze results. The reference allele and the alternate base for the selected variant are indicated in the image, both in the variant table and the in the pileup view. You can customize your pileup view. Reorder the tracks displayed by clicking a gray bar on the right edge of a track, then dragging the track to a different location. You can also click-drag the cursor at any position in the image to move the image left or right in the screen. For more information, see "Adjust pileup tracks" on page 153.

① The reference track. When zoomed out, the nucleotide sequence is no longer displayed, but the sequence is represented by colored bars.

② The three-frame translation is shown in this example (not by default). For more information, see "Adjust the view of the reference track" on page 153.

③ The coverage track.

④ The BAM track.

⑤ Click the coverage track to see detailed information, such as the total count, the number of reads, and the number of molecules.

⑥ Click a BAM track to see detailed information, such as the **Mapping Quality** and **Base Quality**.

⑦ Use the sliders to view more tracks.

⑧ Deletion.

⑨ Insertion.

⑩ Alternate base in the BAM read coverage track.

The following figure is an example of the reference tracks and annotation tracks shown beneath the BAM tracks in the **Gene Browser**.

chr chr9
start 5073770
end 5073770
score NaN
name COSM12600
strand JAK2_3.138844

Chr 9
Pos 5073770
Names 29763
Ref G
Alt A
Qual .
Filter .
ALLELEID 38718
CLNDISDB MedGen:C3281125,OMIM:614521
CLNDN Thrombocythemia_3
CLNHGVS NC_000009.11:g.5073770G>A
CLNREVSTAT no_assertion_criteria_provided
CLNSIG Pathogenic
CLNVC single_nucleotide_variant
CLNVCSO SO:0001483

chr chr9
start 5073555
end 5073803
score NaN
name JAK2_3.138844
strand GENE_ID=JAK2;Pool=1;PURPOSE=Hotspot

① The hotspot track.

② The target region track.

③ Annotation tracks (COSMIC and ClinVar). In this example, only ClinVar is shown.

④ Click the annotation track for additional details; for example the CLNHGVS (the top-level genomic HGVS expression for the variant).

⑤ Click a hotspot track for additional details, such as the start and end positions.

⑥ Click the target region track for additional details, such as the start and end positions.

## Coverage histogram
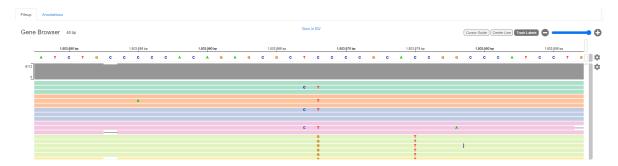
When you zoom in on the variant pileup view, you see the coverage histogram and reads from the .bam track. The histogram in gray shows read depth at that location and reads from the .bam track show read direction and location of variants.



① Read depth

② Read location and direction of variants

## View the variants from an Ion AmpliSeq™ HD-Library Chemistry run

You can view a summary of data about the identified variants, and toggle to other views that provide more details about the same variants.

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

5. Review detailed variant data and read coverage tracks.



**Example visualization**

Ion AmpliSeq™ HD sequencing runs group consensus reads into families. A family is a group of reads that are associated with the same DNA molecule before library amplification. Each family is identified using the molecular tags, and consensus reads with the same molecular tags are grouped into the same family. The color of the consensus reads is used to indicate a family.

Within each read track, each nucleotide variant is indicated by a different color. T, A, C, and G are red, green, blue, and orange, respectively. An "I" indicates insertion, and white color with a dash indicates deletion.
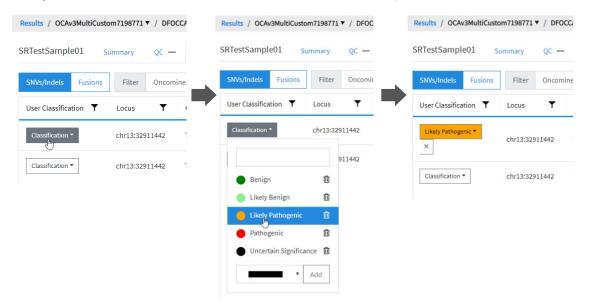
6. You can sort, adjust, and view details about variants and base calls that are visualized in each read coverage track.

| Option | Description |
|---|---|
| View the read coverage tracks sorted by family | Click ⚙ **(Actions)** next to the read coverage track, then select **Color ▸ Molecule**. |
| Adjust the BAM tracks | Click ⚙ **(Actions)** next to the read coverage track, then select an option to adjust the view of the track. For more information, see "Adjust pileup tracks" on page 153. |
| Review detailed data about a BAM read | Single-click the read track to obtain information such as the mapping quality, the strand, and the read base. |
| Review distribution of base calls at a selected position | Click the density plot (the gray bar at the top of the read coverage tracks) to view information about the total count, total reads, and total number of molecules, the distribution of single nucleotides at that position, and the number of insertions and deletions. |
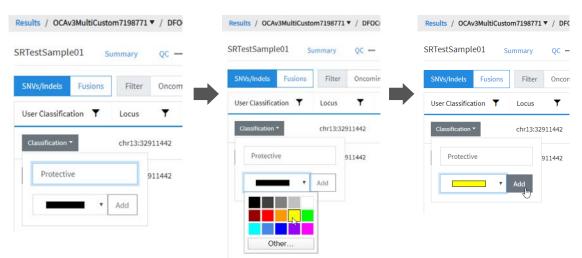
# Create and assign variant classifications

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

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

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

3. Click the **Variants** tab.

4. To refine the list of variants shown in the table, select a variant type to display: **SNVs Indels**, **Fusions**, or **CNV**.

5. In the **Variants** table, in the **User Classification** column, perform any of the following actions.

   - To assign an existing classification to a variant, select it from the list. The **Classification** menu changes to the name of the classification, as shown in this example.

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



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

## Filter results

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

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

### Search and filter the list of variants

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

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

2. Click a sample name in the **Sample Name** column.

3. In the **Results** screen, click the **Variants** tab.

4. Select the variant class to display the results: **SNVs/Indels**, **Fusions**, or **CNVs**.

5. In the table of variants, in the column heading of interest, click ▼ **(Filter)**.

   - In the search field, enter at least 3 characters, then click **Filter**.

   - Select the checkbox in the row of each filter that you want to apply, then click **Filter**.

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

6. Click ⟨×⟩ **Clear Filters** to remove all filters and view the full list of run results.

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

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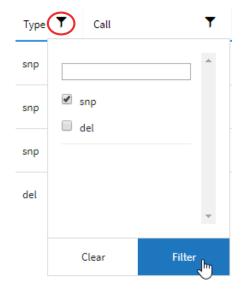
You can filter the results that are listed in the **Results** screen with a system-installed or a custom-designed filter chain. A filter chain is a set of filters that Genexus™ Software uses to narrow the list of variants that are included in results.

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

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

For information about system-installed filter chains, and how manager- and administrator-level users can create custom filter chains, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

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

2. In the **Sample Results** screen, in the **Sample Name** column, select a sample of interest.

3. In the **Results** screen, click the **Variants** tab.

4. To refine the list of variants shown in the table, select a variant type.

   - **SNVs/Indels**
   - **Fusions**
   - **CNVs**

5. Above the variant table in the **Results** screen, click **Edit Filters**, then select a filter chain.

   - Click ✎ **Edit** to make changes to a draft filter chain, if needed.
   - Click ⧉ **Copy** to copy, then edit the selected filter chain, if needed.

The list of results changes to reflect the selected filter chain. If you made edits to the filter chain, all sample results to which the edited filter chain is applied are also updated with the changes.

6. Click **Save** if you want the filter chain to be applied to the results when the results are later opened. The filter chain is selected and applied to the results when the results are reopened.

# Variant report

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

To make a variant report available for each sample result upon completion of a sequencing run, enable **Generate Report** in the **Setup** step when you plan the run. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system). To generate a variant report after a run is complete, see "Generate a variant report" on page 163.

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

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

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

For information about the sections that can be included in a variant report, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

## View a variant report

You can view a variant report in Genexus™ Software. A variant report includes details of sample results. The results depend on the filter chain applied to the results.

A variant report must be generated before you can view the report. If a report template is set as the default template for the assay associated with the run, one or more variant reports are generated after a run completes successfully. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system. Alternatively, you can generate one or more variant reports after a run completes successfully. For more information, see "Generate a variant report" on page 163.

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

2. In the **Sample Results** screen, click the sample name of interest in the **Sample Name** column.

3. Click the **Reports** tab.

   A pane for the run report and each variant report that has been generated is shown. The pane for a variant report includes the following information.

   - Report name
   - Report template name
   - Date that the report was generated
   - Username for the user who generated the report
   - Language used for the report
   - If a variant report has been electronically signed at least once, the signature information is listed in the pane for the variant report. If multiple users have signed the report, you can place the pointer over the electronic signature information to view more details. If the electronic signature that is used is associated with a lock-level signature type, the report is locked.
   - If a variant report is locked, 🔒 **(Lock)** is shown in the upper right corner of the pane.

4. In the pane of the report of interest, click ⋮ **(More)** ▸ **Download Report**.

   The report opens in a new browser tab. You can download variant reports as PDF files.

## 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.

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.

   Report templates that are associated with the assay that was used in the run are included in the **Report Template** list. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

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.

# 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 the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

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. <ul><li>When a run fails during the basecalling step or earlier.</li><li>When the run has not already been successfully reanalyzed.</li></ul> Reanalysis at the signal processing step uses DAT files. |
| 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. <br> 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. <br> 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.
- QC parameters at the limits of stringency cannot be adjusted further. If samples fail QC and you cannot adjust the QC parameters further, you can sequence the sample library again. For information to find, recover, and purify the leftover library preparations, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910). Alternatively, you can prepare a new library for sequencing.

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

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 "Create an assay in Genexus™ Software for use with a custom panel" on page 56.

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.

# Reanalyze a sample

If a sample fails to meet one or more QC parameters defined by the assay, you can adjust the assay parameters and reanalyze a sample. A sample can be reanalyzed starting only from the alignment step. You can reanalyze all samples in a run from the basecalling or signal processing steps. For more information, see "Reanalyze a run" on page 164.

Reanalysis at the alignment step uses BAM files.

---

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

---

The option to reanalyze a sample from the alignment step is available only when a sample or reanalysis successfully completes the basecalling step.

You can reanalyze a sample 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 "Create an assay in Genexus™ Software for use with a custom panel" on page 56.

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

2. In the **Sample Results** screen, place the pointer over the row of a sample of interest, then click **Reanalyze**.

   Alternatively, you can reanalyze a sample when you click a sample name, then click
   ••• **(More Options)** ▸ **Reanalyze**.

3. In the **Reanalyze** dialog box, enter or select the following information.

   a. In **Reanalysis Run Name** field, enter a reanalysis run name.

   b. 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.

4. Click **Reanalyze**.

Follow the progress of the reanalysis in the **Results / Sample Results** screen in the **Sample Status** column. When reanalysis is complete, the new results can be viewed by clicking the sample name corresponding to the reanalysis assay in the **Results / Sample Results** screen. Samples that have been reanalyzed are listed in the table of sample results with the run name appended with ⟳.

# Plugins for data analysis

You can expand the analysis capabilities of Genexus™ Software with plugins that are preinstalled with the software. The plugin results are added to the report summary and can be used for a various purposes.

An administrator-level user can download and install more plugins from the Thermo Fisher™ Connect Platform.

## Review coverageAnalysis plugin results

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

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

You can download coverageAnalysis plugin output files from the **Results** screen for a sample. For more information, see "Output files generated by the coverageAnalysis plugin" on page 167.

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

2. In the **Sample Results** screen, in the **Sample Name** column, click the sample of interest.

3. Click the **Plugins** tab.

   A summary table of the coverage analysis, by barcode, is included in the **coverageAnalysis** summary pane.

4. *(Optional)* From the **Executed At** dropdown list, select an alternate timestamp, if available, to view additional reports.

5. *(Optional)* Click 📄 **View Log** to view the coverageAnalysis log.

6. *(Optional)* Click 🗑 **Delete** to delete the coverageAnalysis plugin output for the selected timestamp.

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

   ---

7. Click ⋯ **(More)** ▸ **Download Files** to download coverageAnalysis plugin results files.

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

   ---

8. In the **coverageAnalysis** summary pane, in the **Barcode Name** column, click the link in the row of the barcode of interest.

   The detailed **Coverage Analysis Report** for the barcode opens in a separate window.

## Output files generated by the coverageAnalysis plugin

You can download coverageAnalysis plugin results files from the **Results** screen for a sample. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

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

---

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

**Table 35  File download selections in the Genexus™ Software**

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

**Table 35 File download selections in the Genexus Software** *(continued)*

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

**Table 36 File contents**

| File | Description |
|---|---|
| Coverage Statistics | A summary of the statistics presented in the tables at the top of the plugin report. The first line is the title. Each subsequent line is either blank or a particular statistic title followed by a colon (:) and its value. |
| Chromosome base coverage summary | Base reads per chromosome summary data that is used to create the default view of the Reference Coverage Chart. This file contains the following fields.<br><br>• `chrom`: the name of the chromosome or contig of the reference.<br>• `start`: the coordinate of the first base in this chromosome. This is always 1.<br>• `end`: the coordinate of the last base of this chromosome. Also its length in bases.<br>• `fwd_basereads`: the total number of forward strand base reads for the chromosome.<br>• `rev_basereads`: the total number reverse strand base reads for the chromosome.<br>• `fwd_trg_basereads` (if present): the total number of forward strand base reads that mapped over at least one target region.<br>• `rev_trg_basereads` (if present): the total number of reverse strand base reads that mapped over at least one target region.<br>• `total_reads`: the total number of sequencing reads that are mapped to individual contigs. |

**Table 36   File contents** *(continued)*

| File | Description |
|------|-------------|
| Base depth of coverage | Coverage summary data used to create the Depth of Coverage Chart. This file contains the following fields.<br><br>• `read_depth`: the depth at which a (targeted) reference base has been read.<br>• `base_cov`: the number of times any base was read (covered) at this depth.<br><br>**Note:**  Lines (read depths) for which `base_cov` is 0 are omitted to avoid excessively large files being produced in specific situations.<br><br>• `base_cum_cov`: the cumulative number of reads (coverage) at this read depth or greater.<br>• `norm_read_depth`: the normalized read depth (depth divided by average base read depth).<br>• `pc_base_cum_cov`: same as base_cum_cov but represented as a percentage of the total base reads. |

**Table 36   File contents**  *(continued)*

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

## Review molecular Coverage Analysis plugin results

The molecular Coverage Analysis plugin generates a Molecular Coverage Analysis Report. This report includes molecule statistics and several charts. The report summary lists the barcode, the sample, the median molecular coverage, the molecular uniformity, the median reads per functional molecule, and the median percentage of functional reads. Additional details regarding molecular coverage are also provided on a per-barcode basis, along with amplicon information that is included in the BED file.

You can download molecular Coverage Analysis plugin output files from the **Results** screen for a sample. For more information, see "Output files generated by the molecular Coverage Analysis plugin" on page 171.

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

2. In the **Sample Results** screen, in the **Sample Name** column, click the sample of interest.

3. Click the **Plugins** tab.
   A summary table of the coverage analysis, by barcode, is included in the **molecular Coverage Analysis** summary pane.

4. *(Optional)* From the **Executed At** dropdown list, select an alternate timestamp, if available, to view additional reports.

5. *(Optional)* Click 📄 **View Log** to view the molecular Coverage Analysis log.

6. *(Optional)* Click 🗑 **Delete** to delete the molecular Coverage Analysis plugin output for the selected timestamp.

   ---

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

   ---

7. Click ⋮ **(More) ▸ Download Files** to download coverageAnalysis plugin results files.

   ---

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

   ---

8. In the **molecular Coverage Analysis** summary pane, in the **Barcode Name** column, click the link in the row of the barcode of interest.
   The detailed **Molecular Coverage Analysis Report** for the barcode opens in a separate window.

## Output files generated by the molecular Coverage Analysis plugin

You can download plugin results files from the **Results** screen for a sample. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

---

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

---

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

# Customer Support Archive

The Customer Support Archive is a downloadable archive that a technical support representative can use to troubleshoot and diagnose issues with sequencing runs or with Genexus™ Software. The archive contains log files and other technical data about the software and other files from sequencing runs on a Genexus™ Integrated Sequencer.

For more information, see "Download a Customer Support Archive" .

## Download a Customer Support Archive

When you download a Customer Support Archive (CSA) in Genexus™ Software, files are included for all samples for a specific assay. If the run includes only one assay, files for all samples are included. If the run includes more than one assay, you can specify the assay of interest when you download the CSA.

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

2. In **Sample Results** screen, place the pointer over the row of the sample result of interest, then click **CSA**.

   Alternatively, you can download CSA files three other ways.

   - Click **Results ▸ Sample Results**. Click the sample name of interest. Then, click ⋯ **(More Options) ▸ CSA.**

   - Click **Results ▸ Run Results**. Click the run name of interest. Place the pointer over the run of interest, then click **CSA**. In the **Download CSA** dialog box that appears, select the assay of interest, then click **Download**.

   - Click **Results ▸ Run Results**. Click the run name of interest. Then, click ⋯ **(More Options) ▸ CSA**. In the **Download CSA** dialog box that appears, select the assay of interest, then click **Download**.

An XZ compressed TAR archive (TXZ) file is downloaded to the folder that you specified to download files from the browser. This location depends on the browser settings. You can attach the archive to an email to send to Customer Support.

# Upload results files to another Genexus™ Integrated Sequencer

When a run completes successfully in Genexus™ Software, you can upload the results (BAM files) to another Genexus™ Integrated Sequencer.

---

**Tip:** To automatically upload BAM files when a run is complete, select the **Upload BAM files to Server** checkbox in the **Setup** step of planning a run.

---

Before you can upload results files to another Genexus™ Integrated Sequencer, you must link the accounts that you can use for data uploads. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

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 **BAM Uploader**.

3. In the **Upload Samples to Server** dialog box, make the following selections.

   a. From the **Configured Account** list, select the Genexus™ Software account and the software version.

   b. In the **Select** column, select one or more assays from the list to upload the results files from the selected assays.

   c. In the **Genexus Workflow** column, select whether you want analysis to start on the selected Genexus™ Integrated Sequencer immediately.

      • Select **Upload Only** to upload the sample results (BAM files) to the software.

      • Select an assay of interest to upload the sample results (BAM files) and automatically start analysis with the selected assay on the Genexus™ Integrated Sequencer.

   d. Click **Upload**.

The results are uploaded to another Genexus™ Integrated Sequencer.

# A  Troubleshooting and FAQs

Visit our online Support Centers and FAQ database for tips and tricks for conducting your experiment, troubleshooting information, and FAQs. The online FAQ database is frequently.

- For the Next–Generation Sequencing Support Center: **thermofisher.com/ngssupport**
- To browse the FAQ database and search using keywords: **thermofisher.com/faqs**

# B

# Supplemental procedures

## Expand any Ion AmpliSeq™ panel by adding a spike-in panel

Any Ion AmpliSeq™ Custom panel can be modified by using a spike-in panel. Spike-in panels enable addition of new content, control assays, and improvements in coverage uniformity (for example, by adding the same primer pair to two or more primer pools). Spike-in panels come as pooled amplicons at 50X (2500 nM) concentration.

1. If needed, prepare a 50X (2,500 nM) spike-in panel with individual primer pairs from a panel provided in 384-well plate format.

2. Add the spike-in panel (at 2,500 nM) to a primary Ion AmpliSeq™ panel using the following guidelines.

| Number of primer pairs/pool | | | Target Concentration (Combined) | Instructions |
|---|---|---|---|---|
| Primary panel | Spike-in panel | Combined panel | | |
| Ion AmpliSeq™ On-Demand Panels[1] | | | | |
| Add 6.4 µL of the spike-in panel directly into the Ion AmpliSeq™ On-Demand Panel in the Ion Chef™ Library Sample Tube. | | | | |
| All other types of primary panels | | | | |
| ≤96 | ≤123 | ≤96 | 400 nM | Add 16 µL of the spike-in panel per 100 µL of the primary panel |
| ≤96 | ≤123 | >96 | 100 nM | Dilute parent panel from 400 nM to 100 nM, then add 4 µL spike-in panel per 100 µL of primary panel |
| >96 | ≤123 | >96 | 100 nM | Add 4 µL spike-in panel per 100 µL primary panel |

[1] Each Ion AmpliSeq™ On-Demand Panel is provided in Ion Chef™ Library Sample Tubes and must be transferred into Genexus™ Primer Pool Tubes for loading on the instrument.

**Table 37  Examples of spike-in volumes for different categories of parent and spike-in panel**

| Number of primer pairs/pool | | | Concentration (nM) | | | Volumes for spiking (µL) | | Final concentration (nM) | |
|---|---|---|---|---|---|---|---|---|---|
| Parent panel | Spike-in panel | Combined panel | Parent | Spike-in | Target (combined) | Parent | Spike-in | Parent | Spike-in |
| 50 | 20 | 70 | 400 | 2,500 | 400 | 160 | 25.6 | 345 | 345 |
| 80 | 60 | 140 | 100 | 2,500 | 100 | 160 (diluted) | 6.4 | 96.2 | 96.2 |
| 3,000 | 120 | 3,120 | 100 | 2,500 | 98.5 | 160 | 6.4 | 96.2 | 96.2 |

3. Mix thoroughly by vortexing, then centrifuge briefly to collect contents.

4. Pipet the needed volume of combined panel into a Genexus™ Primer Pool Tube.

   - 4 reactions ( GX5™ Chip)—add 140 µL
   - 4 reactions (GX7™ Chip)—add 168 µL
   - 6 reactions (GX5™ or GX7™ Chip)—add 168 µL

   For more information, see "Fill Genexus™ Primer Pool Tubes" .

# Ion AmpliSeq™ Sample ID Panel

The Ion AmpliSeq™ Sample ID Panel is a versatile, cost effective, and easy-to-use human SNP genotyping panel containing 9 specially designed primer pairs that generate a unique ID during post-sequencing analysis of human gDNA research samples. The panel can be spiked into any Ion AmpliSeq™ Custom panel.

| Panel | Concentration | Approx. library size (with adapters) | Amount | Storage[1] |
|---|---|---|---|---|
| Ion AmpliSeq™ Sample ID Panel (Cat. No. 4479790) | 20X | 150–220 bp | 1 tube (96 rxns) | –30°C to –10°C |

[1] Shipped at ambient temperature. Store as indicated.

## Add the Ion AmpliSeq™ Sample ID Panel to a DNA Custom panel

The Ion AmpliSeq™ Sample ID Panel can be added to any Ion AmpliSeq™ Custom DNA panel.

**Note:** The Ion AmpliSeq™ Sample ID Panel is provided at 20X concentration and must be diluted 1:10 into the primary custom DNA panel at 2X concentration for use.

1. Add the Ion AmpliSeq™ Sample ID Panel to each DNA panel pool using the following guidelines.

   - 4 reactions—add 15 µL Ion AmpliSeq™ Sample ID Panel to 135 µL of the main Ion AmpliSeq™ Custom panel
   - 6 reactions—add 17 µL Ion AmpliSeq™ Sample ID Panel to 153 µL of the main Ion AmpliSeq™ Custom panel

2. Vortex the combined panel to mix thoroughly, then centrifuge briefly to collect the contents.

3. Pipet the needed volume of combined panel into a Genexus™ Primer Pool Tube.
   - 4 reactions ( GX5™ Chip)—add 140 µL
   - 4 reactions (GX7™ Chip)—add 168 µL
   - 6 reactions (GX5™ or GX7™ Chip)—add 168 µL

   For more information, see "Fill Genexus™ Primer Pool Tubes" .

# sampleID plugin

The Ion AmpliSeq™ Sample ID Panel is a human genotyping panel consisting of 9 primer pairs that identify the gender and eight high-frequency noncoding SNPs of human samples. The panel can be added to any Ion AmpliSeq™ Custom panel.

The sampleID plugin is used to analyze sequencing results from any panel containing the 9 primer pairs of the Ion AmpliSeq™ Sample ID Panel to generate a unique identification code (**SampleID**) for each sample.

Use the **SampleID** to monitor samples and identify any misassignments or mix-ups between samples and barcodes during a sequencing run.

The sampleID plugin does not run by default. You must select the sampleID plugin in the **Plugins** step when you create an assay to have the sampleID plugin run during analysis. For more information, see "Create an assay in Genexus™ Software for use with a custom panel" .

## Review sampleID plugin results in Genexus™ Software

After the sequencing run completes, review the plugin results.

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

2. In the **Sample Results** screen, in the **Sample Name** column, click the sample of interest.

3. Click the **Plugins** tab.
   A summary table of the **sampleID** results is shown.

4. Click a barcode name to open the **Sample ID Report**.

5. To return to Genexus™ Software, click back in the browser.

## Sample ID Report

The **Sample ID Report** that is available when you select a barcode in the **SampleID** plugin summary for a run includes a variety of read statistics.

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 a tooltip with definitions. Place the pointer over a heading or description in the report to view the tooltip.

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

## Output files generated by the sampleID plugin

You can download sampleID plugin results files from the **Results** screen for a sample. For more information, see the *Genexus™ Software 6.8 User Guide* (Pub. No. MAN0026409) or the Genexus™ Software help system.

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

Table 38 describes the files that can be generated by the sampleID plugin. The list of files depends on the application type that was selected during assay creation.

Table 38   File download selections in the Genexus™ Software

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

# Quantify FFPE DNA with the Qubit™ Fluorometer

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

1. Set up the needed number of 0.5-mL Qubit™ Assay tubes for standards and samples. The Qubit™ dsDNA HS Assay requires 2 standards.

2. Prepare sufficient Qubit™ working solution for all samples and standards by diluting Qubit™ dsDNA HS Reagent 1:200 in Qubit™ dsDNA HS Buffer.

3. Combine 2 µL of the FFPE DNA sample with 198 µL (200-µL final volume) of working solution, mix well, then incubate for at least 2 minutes.

4. Prepare each Qubit™ standard as directed in the user guide.

5. Measure the concentration of each sample and standard on the Qubit™ Fluorometer.

6. *(Qubit™ 2.0 Fluorometer only.)* Calculate the concentration of the undiluted sample by multiplying by the dilution factor. Alternatively, use the **Calculate Stock Conc.** feature on the instrument.
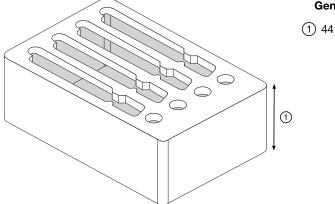
Proceed to "Dilute or concentrate the samples, if needed, then load the sample plate—Nucleic Acid to Result run" .

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

You can use the Genexus™ Strip Centrifuge Adapter as a holder for centrifuging library and templating strips to collect contents after vortexing the strips. Vortexing and centrifuging strips is recommended to decrease errors in the workflow due to air bubbles in strip wells or beads trapped near the foil seal. Users can request this part from a Thermo Fisher Scientific Field Service Engineer.

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

Use of the adapter requires a centrifuge with buckets that support the height of the adapter at 44 mm so that buckets swing freely in the centrifuge rotor when loaded with strips. For more information, see "Required materials—general laboratory equipment and supplies" .



**Genexus™ Strip Centrifuge Adapter**

① 44 mm height

To use the Genexus™ Strip Centrifuge Adapter, follow these steps.

1. After thawing, vortex each strip at maximum speed for 5–10 seconds while rocking the strip from side to side.

2. Load the strips in the adapters in a balanced orientation, then place each adapter loaded with strips in the centrifuge. The centrifuge buckets must support the height of the adapters loaded with strips.

3. Centrifuge the strips at 300 × *g* for 15 seconds.

4. Remove strips from the adapters, then inspect the strips to ensure that contents have been collected and air bubbles are not present.

5. If brown magnetic beads are still visible in the tube keyhole near the foil seal, invert the strip to resuspend the contents, then repeat step 3.

---

Note: It is not necessary to dislodge all the beads trapped in a keyhole—dislodging most beads is sufficient.

---

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

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

# Genexus™ consumables use tables

Table 39    GX5™ Chip One-pool assay, 4 reactions per strip (32 barcode plate), requiring 500K reads per sample

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16 | 18 | 20 | 24 | 28 | 30 | 32[1] | 36 | 42 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool 1 | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | 7 | 8 | | N/A | |
| Sets of strips 1 and 2-AS | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | 7 | 8 | | N/A | |
| Sets of strips 3 and 4 | | | | | 1 | | | | | | | 2 | | | N/A | |
| Lanes used (12–15 M reads per lane) | | | | | 1 | | | | | | | 2 | | | N/A | |

[1]  Maximum of 32 samples, limit of 8 Genexus™ Library Strips 1 and 2-AS.

Table 40    GX5™ Chip One-pool assay, 6 reactions per strip (48 barcode plate), requiring 500K reads per sample

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16 | 18 | 20 | 24 | 28 | 30 | 32 | 36 | 42 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool 1 | | 1 | | | 2 | | | 3 | | 4 | | 5 | | 6 | 7 | 8 |
| Sets of strips 1 and 2-AS | | 1 | | | 2 | | | 3 | | 4 | | 5 | | 6 | 7 | 8 |
| Sets of strips 3 and 4 | | | | | | 1 | | | | | | | 2 | | | |
| Lanes used (12–15 M reads per lane) | | | | | | 1 | | | | | | | 2 | | | |

**Table 41** **GX5™ Chip Two-pool assay, 4 reactions per strip (32 barcode plate), requiring 1 M reads per sample**

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16[1] | 18 | 20 | 24 | 28 | 30 | 32 | 36 | 42 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool 1 | 1 | | 2 | | 3 | | 4 | | | | N/A | | | | | |
| Tube carriers with primer pool 2 | 1 | | 2 | | 3 | | 4 | | | | N/A | | | | | |
| Sets of strips 1 and 2-AS | 2 | | 4 | | 6 | | 8 | | | | N/A | | | | | |
| Sets of strips 3 and 4 | 1 | | | | | | 2 | | | | N/A | | | | | |
| Lanes used (12–15 M reads per lane) | 1[2] | | | | | | 2 | | | | N/A | | | | | |

[1] Maximum of 16 samples, limit of 8 Genexus™ Library Strips 1 and 2-AS and 32 barcodes.

[2] Limit of 12 samples per lane due to 1 M read per sample requirement.

**Table 42** **GX5™ Chip Two-pool assay, 6 reactions per strip (48 barcode plate), requiring 1 M reads per sample**

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16 | 18 | 20 | 24[1] | 28 | 30 | 32 | 36 | 42 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool 1 | 1 | | | 2 | | | 3 | | | 4 | | | N/A | | | |
| Tube carriers with primer pool 2 | 1 | | | 2 | | | 3 | | | 4 | | | N/A | | | |
| Sets of strips 1 and 2-AS | 2 | | | 4 | | | 6 | | | 8 | | | N/A | | | |
| Sets of strips 3 and 4 | 1 | | | | | | | 2 | | | | | N/A | | | |
| Lanes used (12–15 M reads per lane) | 1[2] | | | | | | | 2 | | | | | N/A | | | |

[1] Maximum of 24 samples, limit of 8 Genexus™ Library Strips 1 and 2-AS and 48 barcodes.
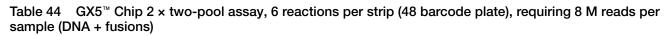
[2] Limit of 12 samples per lane due to 1 M read per sample requirement.

**Table 43** **GX5™ Chip 2 × two-pool assay, 4 reactions per strip (32 barcode plate), requiring 8 M reads per sample (DNA + fusions)**

| Sample number | 1 | 2 | 3 | 4 | 5 | 6[1] | 7 |
|---|---|---|---|---|---|---|---|
| Tube carriers with DNA primer pool 1 | | | 1 | | | 2 | N/A |
| Tube carriers with DNA primer pool 2 | | | 1 | | | 2 | N/A |
| Tube carriers with RNA primer pool 1 | | | 1 | | | 2 | N/A |
| Tube carriers with RNA primer pool 2 | | | 1 | | | 2 | N/A |
| Sets of strips 1 and 2-AS | | | 4 | | | 8 | N/A |
| Sets of strips 3 and 4 | 1 | 2 | | 3 | 4 | | N/A |
| Lanes used (12–15 M reads per lane) | 1 | 2 | | 3 | 4 | | N/A |

[1] Limit of 6 samples due to 8 M read per sample requirement.

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

**Table 44  GX5™ Chip 2 × two-pool assay, 6 reactions per strip (48 barcode plate), requiring 8 M reads per sample (DNA + fusions)**

| Sample number | 1 | 2 | 3 | 4 | 5 | 6[1] | 7 |
|---|---|---|---|---|---|---|---|
| Tube carriers with DNA primer pool 1 | 1 | | | | | | N/A |
| Tube carriers with DNA primer pool 2 | 1 | | | | | | N/A |
| Tube carriers with RNA primer pool 1 | 1 | | | | | | N/A |
| Tube carriers with RNA primer pool 2 | 1 | | | | | | N/A |
| Sets of strips 1 and 2-AS | 4 | | | | | | N/A |
| Sets of strips 3 and 4 | 1 | 2 | | 3 | | 4 | N/A |
| Lanes used (12–15 M reads per lane) | 1 | 2 | | 3 | | 4 | N/A |

[1] Limit of 6 samples due to 8 M read per sample requirement.

**Table 45  GX7™ Chip One-pool assay, 6 reactions per strip, requiring 1 M reads per sample**

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16 | 18 | 20 | 24 | 28 | 30[1] | 32 | 36 | 42 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool 1 | 1 | | | 2 | | | 3 | | 4 | | 5 | | 6 | | 7 | 8 |
| Sets of strips 1 and 2-AS | 1 | | | 2 | | | 3 | | 4 | | 5 | | 6 | | 7 | 8 |
| Sets of strips 3 and 4 | 1 | | | | | | | | | | 2 | | | | | |
| Lanes used (20–25 M reads per lane) | 1[2] | | | | | | | | | | 2 | | | | | |

[1] When using a 32 barcode plate, barcode 31 & barcode 32 cannot be used and are discarded after the run.
[2] Limit 24 samples per lane due to 1 M read per sample requirement.

**Table 46  GX7™ Chip Two-pool assay, 6 reactions per strip, requiring 2 M reads per sample**

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16 | 18 | 20 | 24[1] | 28 | 30 | 32 | 36 | 42 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool 1 | 1 | | | 2 | | | 3 | | 4 | | N/A | | | | | |
| Tube carriers with primer pool 2 | 1 | | | 2 | | | 3 | | 4 | | N/A | | | | | |
| Sets of strips 1 and 2-AS | 2 | | | 4 | | | 6 | | 8 | | N/A | | | | | |
| Sets of strips 3 and 4 | 1 | | | | | | 2 | | | | N/A | | | | | |
| Lanes used (20–25 M reads per lane) | 1 | | | | | | 2 | | | | N/A | | | | | |

[1] Maximum of 24 samples, limit of 8 Genexus™ Library Strips 1 and 2-AS and 48 barcodes.

**Table 47  GX7™ Chip 2 × two-pool assay, 6 reactions per strip, requiring 16 M reads per sample (DNA + fusions)**

| Sample number | 1 | 2 | 3 | 4 | 5 | 6[1] | 7 |
|---|---|---|---|---|---|---|---|
| Tube carriers with DNA primer pool 1 | 1 | | | | | | N/A |
| Tube carriers with DNA primer pool 2 | 1 | | | | | | N/A |
| Tube carriers with RNA primer pool 1 | 1 | | | | | | N/A |
| Tube carriers with RNA primer pool 2 | 1 | | | | | | N/A |
| Sets of strips 1 and 2 | 4 | | | | | | N/A |
| Sets of strips 3 and 4 | 1 | 2 | | 3 | | 4 | N/A |
| Lanes used (20–25 M reads per lane) | 1 | 2 | | 3 | | 4 | N/A |

[1]  Limit of 6 samples due to 16 M read per sample requirement.

# Ion AmpliSeq™ HD consumables use tables

**Table 48  GX5™ Chip One-pool assay, 4 reactions per strip (32 HD barcode plate), requiring 1.5 M reads per sample**

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16 | 18 | 20 | 24 | 28 | 30 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool[1] | 1 | | 2 | | 3 | | 4 | | 5 | 6 | | 7 | 8 |
| Sets of strips 1 and 2-HD | 1 | | 2 | | 3 | | 4 | | 5 | 6 | | 7 | 8 |
| Sets of strips 3 and 4 | 1 | | | | 2 | | | 3 | | | 4 | | |
| Lanes used (12–15 M reads per lane) | 1[2] | | | | 2 | | | 3 | | | 4 | | |

[1]  Forward (FWD) primer in tube position 1, reverse (REV) primer in tube position 2.
[2]  Limit of 8 samples per lane due to 1.5 M reads per sample requirement.

**Table 49  GX5™ Chip Two-pool assay, 4 reactions per strip (32 HD barcode plate), requiring 1 M reads per sample**

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16[1] | 18 | 20 | 24 | 28 | 30 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool 1[2] | 1 | | 2 | | 3 | | 4 | N/A | | | | | |
| Tube carriers with primer pool 2[2] | 1 | | 2 | | 3 | | 4 | N/A | | | | | |
| Sets of strips 1 and 2-HD | 2 | | 4 | | 6 | | 8 | N/A | | | | | |
| Sets of strips 3 and 4 | | | 1 | | | | 2 | N/A | | | | | |
| Lanes used (12–15 M reads per lane) | | | 1 | | | | 2 | N/A | | | | | |

[1]  Genexus™ Barcodes 1–32 HD plate limits the run to a maximum of 16 samples. Each pool requires a unique barcode.
[2]  Forward (FWD) primer in tube position 1, reverse (REV) primer in tube position 2.

**Table 50  GX7™ Chip One-pool assay, 4 reactions per strip (32 HD barcode plate), requiring 2 M reads per sample**

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16 | 18 | 20 | 24 | 28 | 30 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool[1] | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | 7 | 8 |
| Sets of strips 1 and 2-HD | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | 7 | 8 |
| Sets of strips 3 and 4 | | | | 1 | | | | | 2 | | | | 3 |
| Lanes used (20–25 M reads per lane) | | | | 1 | | | | | 2 | | | | 3 |

[1]  Forward (FWD) primer in tube position 1, reverse (REV) primer in tube position 2.

**Table 51  GX7™ Chip Two-pool assay, 4 reactions per strip (32 HD barcode plate), requiring 1.5 M reads per sample**

| Sample number | 1 | 4 | 6 | 8 | 10 | 12 | 16[1] | 18 | 20 | 24 | 28 | 30 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with primer pool 1[2] | 1 | | 2 | | 3 | | 4 | N/A | | | | | |
| Tube carriers with primer pool 2[2] | 1 | | 2 | | 3 | | 4 | N/A | | | | | |
| Sets of strips 1 and 2-HD | 2 | | 4 | | 6 | | 8 | N/A | | | | | |
| Sets of strips 3 and 4 | | | | 1 | | | | N/A | | | | | |
| Lanes used (20–25 M reads per lane) | | | | 1 | | | | N/A | | | | | |

[1]  Genexus™ Barcodes 1–32 HD plate limits the run to a maximum of 16 samples. Each pool requires a unique barcode.
[2]  Forward (FWD) primer in tube position 1, reverse (REV) primer in tube position 2.

**Table 52  GX7™ Chip Two-pool DNA + 1-pool RNA assay, 4 reactions per strip (32 HD barcode plate), requiring 3 M reads per sample**

| Sample number | 1 | 4 | 6 | 8[1] | 10 | 12 | 16 | 18 | 20 | 24 | 28 | 30 | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tube carriers with DNA primer pool 1[2] | 1 | | 2 | | N/A | | | | | | | | |
| Tube carriers with DNA primer pool 2[2] | 1 | | 2 | | N/A | | | | | | | | |
| Tube carriers with RNA primer pool 1[2] | 1 | | 2 | | N/A | | | | | | | | |
| Sets of strips 1 and 2-HD | 3 | | 6 | | N/A | | | | | | | | |
| Sets of strips 3 and 4 | | | 1 | | N/A | | | | | | | | |
| Lanes used (20–25 M reads per lane) | | | 1 | | N/A | | | | | | | | |

[1]  Maximum of 8 tube carriers limits run to 8 samples. Four reactions per set of three tube carriers.
[2]  Forward (FWD) primer in tube position 1, reverse (REV) primer in tube position 2.

# 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**.

# 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**

# Documentation and support

## Customer and technical support

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

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

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

## Limited product warranty

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