ion torrent

Genexus™ Software 6.2

USER GUIDE

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

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

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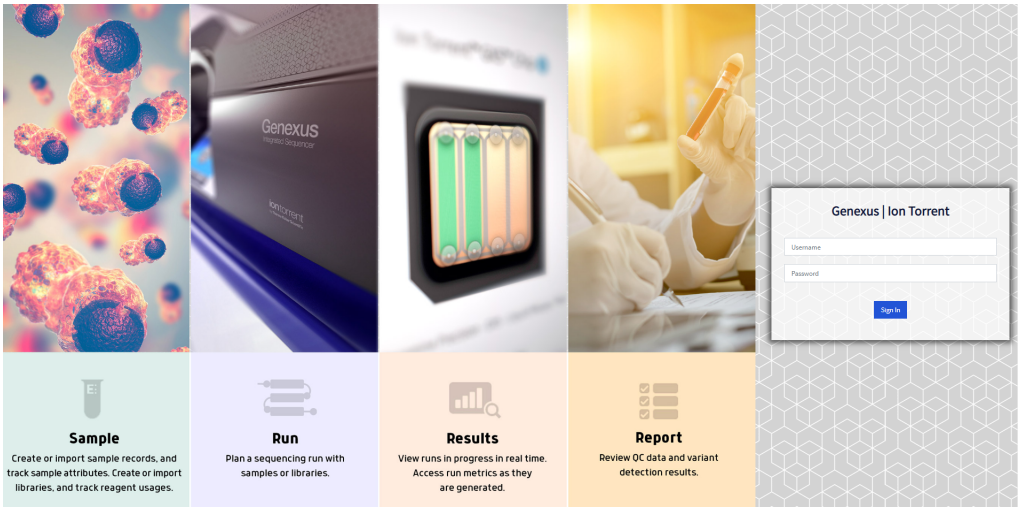
Revision	Date	Description
A.0	20 March 2020	New reference user guide for Ion Torrent™ Genexus™ Software 6.2.

Contents

About Genexus™ Software 6.2



- **Sample**—Create and manage sample records and libraries.
- **Run**—Plan a sequencing run by selecting samples or libraries, and assay parameters.
- **Results**—View the progress of the run in real time and evaluate the assay metrics as they are generated.
- **Report**—Review run results and variant detection and sign off on reports.

Software overview

Ion Torrent™ Genexus™ Software supports the Ion Torrent™ Genexus™ Integrated Sequencer workflow for research use purposes from sample preparation through library preparation, template preparation, and sequencing. During and after sequencing, the software generates base calls, trims reads, and determines quality values (primary analysis), then aligns reads, calls variants, and generates reports (secondary analysis).

This documentation provides instructions for using the software to perform the following functions:

- Create and manage samples, libraries, assays, and runs in the software.
- Monitor the status of runs on the Genexus™ Integrated Sequencer, which provides all-in-one library preparation, chip loading, template preparation, and sequencing.
- View data, results, and quality control (QC) reports after a run.
- Download data files.
- Export and print audit records.
- Create and manage annotation sets, genome references, report templates, baseline files, primer sets, plugins, and reference files.
- Create and manage user accounts.

Software compatibility and requirements

The procedures in this guide are designed for use with Genexus™ Software 6.2 or later. Version-specific information is provided in the software release notes for your version of the software. An administrator-level user can view the software version in the ⚙ **(Settings) / Software Updates** screen.

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

The operating system of the sequencer is Ubuntu™ 18.041.

Network and password security requirements

Network configuration and security

The network configuration and security settings (for example, firewalls, antivirus software, network passwords) of your laboratory or facility are the sole responsibility of your facility administrators and IT and security personnel. Genexus™ Software does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the sequencer, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss.

Note: If a LIMS system is configured to retrieve analysis files from the sequencer, manual FTP setup or drive mapping is required. This configuration is not provided as part of sequencer installation, and must be set up by your LIMS system integration or IT group.

Password security

Best practice is to maintain unique passwords for all accounts in use in Genexus™ Software. All passwords must be reset upon first sign in to the software. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

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

Installation and verification run

The Genexus™ Integrated Sequencer and Genexus™ Software are locked until a verification run has been performed and the report is signed by a Field Service Engineer.

IMPORTANT! The Genexus™ Integrated Sequencer and the Genexus™ Software are locked until a Field Service Engineer has installed and performed a performance qualification verification run and provided a basic system overview.

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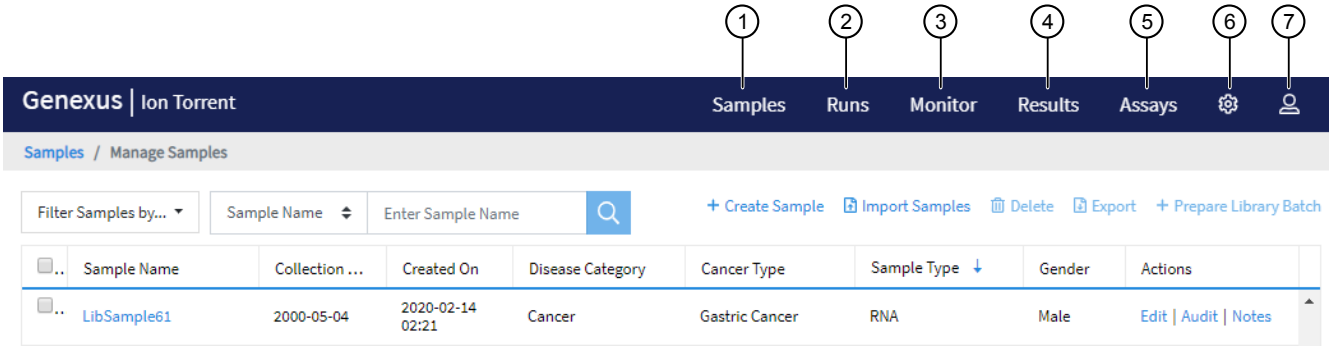
2 Get started with Genexus™ Software

About the Genexus™ Software user interface

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



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

Access End User License Agreement (EULA)

A link to the End User License Agreement (EULA) is located at the bottom of the screen.

1. Scroll to the bottom of the screen.
2. Click the **EULA** link to open a PDF file of the EULA.

Contact Support

You can access Genexus™ Software technical support from the software interface.

1. Scroll to the bottom of the screen.
2. Click **Support** to open **https://www.thermofisher.com/contactus**, where you can request help and technical support.

User-access levels

Users at this level...	Can...
Operator	• Add, import, and export sample files • Prepare library batches • Plan and save runs • Monitor runs • View results and reports • Upload sample results files to Ion Reporter™ Software
Manager	Operator functions, plus: • Create, edit, and delete sample attributes • Create and import assays • Create presets such as annotation sets, filter chains, copy number baselines, and report templates • Edit and delete sample files • Audit and delete runs • Restart a stalled or failed analysis • Reanalyze runs and run plugins • Approve reports and amend a signed-off report • Manage reference sequences and panel, hotspot, and other sequence files • Access services information

Users at this level...	Can...
Administrator	Operator and manager functions, plus: • View, export, and print audit records • Configure network settings • View and manage software updates • Install and manage plugins • Configure data archive and storage settings • Manage sequencer and software log files • Add and manage user accounts

System tracking

The system tracks and checks auditable user activities for samples, assays, QC metrics and information related to reagents.

If the software detects an error at any step—for example, a scanned barcode is inconsistent with the information given for the run—the software alerts the user and does not proceed with the run.

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

Reagent management

Users should put in place a reagent management system to inventory and track reagent use. Proper use of a reagent management system promotes regulatory compliance and ensures optimal use of kits, chips, and reagents.

Request and sign in to a new user account

Operator-level users must request a new user account from the manager- or administrator-level user before they can sign in to Genexus™ Software.

Administrator-level users receive the request and create the user account.

Your user name and password must be unique and not shared with other users.

- To request a new account, contact your administrator-level user.

  After the admin creates a new account, the software sends an email to the new user with a user name and temporary password.

- To sign in to a new account for the first time:
  a. Obtain your user name and temporary password that is supplied by your administrator in an email message.
  b. Browse to the Genexus™ Software home page (for example, ***https://<your server address>***) then sign in using your user name and the temporary password supplied by your administrator.
  c. Press **Enter**, or click **Sign In**.
  d. In the End User License Agreement that appears at first sign on, read the agreement, then click **Accept**.
  e. In the **Change Password** screen, enter your temporary password supplied by your administrator.
  f. Type a new password, confirm the password, then click **Change**.
     - Passwords must be between 6 and 10 characters.
     - Passwords must contain only alphanumeric characters (numbers 0 to 9 and letters A to Z), no spaces or special characters.
     - Passwords must contain at least one alphabetic character (a-z, A-Z).
     - Passwords must contain at least one numeric character (0-9).
     - Passwords are case-sensitive.

Sign in

After registering a new account, sign in to Genexus™ Software in the home page.

1. Go to the Genexus™ Software home page.
2. Enter your user name and password, then press **Enter** or click **Sign In**.

   Note: If you receive a message that indicates that a token is expired, refresh the browser and re-enter the user name and password.

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

Edit a user profile

After your user profile has been established, you can view your user profile information, change your password, or change your email contact information.

1. In the menu bar, click **(Profile)**.
2. In the **Profile** screen, view your profile information.
3. To change your password:
   a. Click **Change Password**.
   b. In the **Change Password** dialog box, enter your current password and a new password. Confirm the password, then click **Change**.
4. To change your email address:
   a. Click **Change Email**.
   b. In the **Change Email** dialog box, enter a new email address, then click **Save**.

Genexus™ Software concepts

The following terms are frequently used in Genexus™ Software. For additional definitions, see "Genexus™ Software definitions" on page 203.

Term or concept	Description	Example
Sample	The genetic material from a single source defined by a set of attributes, such as the date of collection and type of cancer.	DNA from a biopsy or blood draw.
Sample name	A unique identifier for a particular sample.	Sample1
Assay	A collection of settings and parameters for library preparation, templating, sequencing and sequencing data analysis.	Oncomine™ Precision Assay GX
Library	A sample associated with an assay, including barcode information and sample input quantity. Libraries are associated with an assay during library batch creation.	DNA from a non-small cell lung cancer specimen that is sequenced with the Oncomine™ Precision Assay GX.

Term or concept	Description	Example
Library batch	A collection of libraries that are prepped and sequenced in the same run under the same conditions on the Genexus™ Integrated Sequencer.	• A library batch that contains libraries that are different pools from a single sample. • A library batch that consists of one library of sample DNA and another library of sample RNA (from the same sample).
Library name	A unique identifier for a library.	Library1
Library batch ID	A unique identifier for a library batch.	DNA_B1_Lib123456_Samples123

Workflow

Workflow for a sample-to-results sequencing run using the Genexus™ Integrated Sequencer

Create and manage assays (manager/administrator)
(page 94)

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

Enter samples and libraries
(page 18)

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

Plan and manage runs
(page 32)

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

Dilute samples and load the sample plate (see the instrument or assay user guide)

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

Load the sequencer and start a run (see the instrument or assay user guide)

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

Monitor the run
(page 43)

Monitor the run in Genexus™ Software in real time.

Review data and results
(page 44)

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

3 Enter samples and libraries

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

About samples and library batches

Genexus™ Software guides you through the steps of planning a run starting from either nucleic acid samples or libraries.

In Genexus™ Software, the data and attributes that characterize the genomic data from a single source are called samples. You can preselect the attributes that you want to associate with each sample. After adding samples to the software, you can plan a run in five guided steps.

A library batch is a group of sample libraries that will be sequenced in a library run. You can create library batches from samples you have previously added or uploaded to the software. During library batch preparation, you identify the barcode adapters that were used to prepare the libraries. After creating library batches, you can plan and start a library run. The software guides you through four steps (naming and reporting options, selecting assays, selecting library batches, and reviewing) when you plan a library run.

Enter and manage samples

You can add samples to Genexus™ Software in two ways.

- Enter information for each sample into the software
- Create a CSV file of information for a group of samples and import that file.

If you enter samples manually, you can select from system-installed sample attributes that are available for in the software. For more information, see “Create a new sample” on page 19 and “System-installed sample attributes” on page 195.

System-installed sample attributes are also available in the example file that you can use for importing samples. See “Download an example samples file” on page 21 for more information.

Note: LIMS users must create custom attributes before importing sample and run information from LIMS for the attributes to be propagated through to output files. The software ignores all import file content that is not a recognized attribute.

Create a new sample

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

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

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

   For more information, see “Sample attributes (manager/administrator)” on page 21.
4. Click **Save**.

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

Import samples

Sample data files can be used to capture, manage, and edit sample data. You can import sample data files in the following formats: TXT, XLS, XLSX, or CSV. For a list of the sample attributes that are included in the import file, see “System-installed sample attributes” on page 195. For ease of use, you can download a Microsoft™ Excel™ example file to create an import file. For more information, see “Download an example samples file” on page 21.

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

   A progress bar followed by an import report appears. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For troubleshooting, see “Batch sample import fails” on page 198.

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

View sample summary

You can view a summary of a sample in the **Samples / Details** screen. The **Details** screen lists sample attributes as well as the **Extraction Kit Barcode** and the date the sample was created in the software. For more information about sample attributes, see “System-installed sample attributes” on page 195. For more information about the Extraction Kit Barcode, see “Extract samples” on page 23.

To view the sample summary:

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Sample Name** column, click the sample name of interest.
3. When you are finished viewing sample details, click ‹ **Back** to return to the **Manage Samples** screen.

Download an example samples file

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

1. In the menu bar, click **Samples** ▸ **Manage Samples**, then click **Import Samples**.
2. In the **Import Samples** dialog box, click **Click here** to download the Microsoft™ Excel™ example file.

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

   You can now import the file in the **Import Samples** dialog box. For more information, see “Import samples” on page 20.

Sample attributes (manager/administrator)

Manager- and administrator-level users manage sample attributes and create custom sample attributes using the **Samples / Manage Attributes** screen.

- “System-installed sample attributes” on page 195
- “Create a custom sample attribute (manager/administrator)” on page 21

Create a custom sample attribute (manager/administrator)

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

Custom samples attributes cannot be edited or deleted after the samples that use them have been added to a library. To remove a custom sample attribute from use, see “Remove a sample attribute (administrator)” on page 26.

1. In the menu bar, click **Samples** ▸ **Manage Attributes**.
2. In the **Manage Attributes** screen, click **Add New Attribute**.
3. Complete the fields in the **Add New Attribute** dialog box.
   a. In **Attribute Name**, enter the name of the attribute.

      Attribute names are limited to ≤20 alphanumeric characters (0–9 and Aa–Zz), full stops/periods (.), underscores (_), or hyphens (-).

   b. In **Data Type**, specify whether the attribute is text or a number.

   c. To require users to select the new attribute when adding or importing samples, click the toggle button to designate the attribute as **Required**.

   d. Click **Submit**.

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

The new sample attribute is available in the **Create Sample** dialog box and in the **Edit Sample** dialog box, even for samples that were created before the new sample attribute was created. If the new sample attribute is a required attribute, it must be specified in the **Edit Sample** dialog box in order to save the changes. For more information, see "Edit a sample (manager/administrator)" on page 24.

Manage samples

You can find tools for creating, searching, sorting, editing, deleting, and exporting samples, and for viewing the sample history in the **Samples / Manage Samples** screen.

Sort, search, and filter samples

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

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

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

Export samples

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

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

Extract samples

You can designate the samples that have been extracted and assign an extraction kit barcode using the **Extract** feature of the software in the **Samples / Manage Samples** screen.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, in the **Filter Samples by** dropdown list, select **To Be Extracted**.

   Note: The **Extract** option only appears on the screen after samples are filtered by the **To Be Extracted** filter.

   Only samples that are not associated with a barcode are displayed.
3. Click **Extract**.
4. In the **Extract** dialog box, type the **Extraction Kit Barcode** for the sample.

Extracted samples are excluded from the **Manage Samples** screen when **To Be Extracted** is selected in the **Filter Samples by...** dropdown menu.

View notes or add a note to a sample

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

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

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

Edit a sample (manager/administrator)

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

Samples can be edited at any point before a sample run that uses that sample is complete using the **Edit** link. After the sample is used in a run, the **Edit** link is no longer available. To edit a sample after a run is complete, use the **Edit Sample & Amend Report** link instead. For more information, see “Edit a sample and amend a report after a run (manager/administrator)” on page 25.

To add a custom sample attribute to a sample, see “Create a custom sample attribute (manager/administrator)” on page 21.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, in the **Actions** column, click **Edit** in the row of the sample of interest.
3. In the **Edit Sample** dialog box, edit the sample attributes.

   See “Sample attributes (manager/administrator)” on page 21 for more information about the system-installed sample attributes.
4. Click **Save**.

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

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

1. In the menu bar, click **Samples ▸ Manage Samples**.
2. In the **Manage Samples** screen, in the **Actions** column, click **Edit** in the row of the sample of interest.
3. In the **Edit Sample & Amend Report** dialog box, edit sample attributes as necessary.

   See “System-installed sample attributes” on page 195 for more information about system-installed sample attributes.
4. Click **Save**.
   The **Edit Sample and Amend Report** confirmation dialog box opens.
5. Click **Yes** to confirm the changes and continue.
   The sample information is edited, and all runs and reports associated with that sample are updated. Any test report, lab report, tab files and info.csv file that is associated with the sample will be updated with the changes.

Review sample edit history

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

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

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

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

Remove a sample attribute (administrator)

An administrator-level user can use the **obsolete** command to remove user-created sample attributes from use in the software. Sample attributes can be reactivated, and a record of their use is maintained in the audit trail of samples that are created using that attribute. You cannot designate as obsolete system-installed sample attributes.

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

Delete samples

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

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

Enter and manage libraries

You can prepare a library batch manually or import library batch information from a file. To change sample or library batch information, you can edit information in the samples, update library batches, and delete samples and library batches.

Prepare or import a library batch

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

- “Create a new sample” on page 19
- “Import samples” on page 20

Select the library batch when you plan the run. If you are planning a run starting from nucleic acid samples, skip this step and proceed to “Plan a sample run” on page 33.

Note:

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

No-template controls

You can add a no-template control (NTC) to a library when you create a library batch in Genexus™ Software. No-template controls do not contain nucleic acid but are processed in a library run. NTCs are used to monitor contamination or primer-dimer formations that could produce false-positive results. When you plan a library run using a library batch that contains an NTC, data generated from the NTC is shown in the QC results. For more information, see “QC results” on page 55.

Prepare a library batch

A library batch is a group of prepared libraries that are sequenced in the same library run. If you are planning a run starting from libraries that you have already prepared manually, you must first create a library batch in Genexus™ Software from samples that you have added. If you are planning a run starting from nucleic acid samples, skip this step and proceed to “Plan a sample run” on page 33.

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

   See “Sort, search, and filter samples” on page 22 for more information on the **Filter samples** feature.
3. Select samples in the list by clicking the checkbox to the left of each sample, then click + **Prepare Library Batch**.

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

   The assay determines specific parameters of the run, including any required controls and post-run data analysis settings. Only locked assays appear in the **Select Assay** dropdown menu. For instructions to lock an assay, see “Lock a draft assay (manager/administrator)” on page 124.

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

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

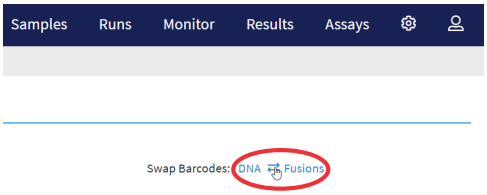
   Library Batch IDs can contain only alphanumeric characters (0–9, Aa–Zz), full stop/period (.), underscore (_), and hyphen (-). Required fields are indicated with a red asterisk (*).

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

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

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

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



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

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

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

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

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

Import a library batch

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

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

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

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

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

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

Template item	Description
Reagents tab	
Assay Name	Auto-populated when assay is selected in step 4 (required)
Library Batch ID	Must be alphanumeric (0–9, Aa–Zz), full stop/period (.), underscore (_), and hyphen (-) (required)
Library kit barcode	For example, Genexus™ Library Strips 1 and 2-AS barcode (optional)
Panel kit barcode	For example, Oncomine™ Comprehensive Assay v3 DNA GX barcode (optional)
Libraries tab	
Sample Name	Same character requirement as Library Batch ID (required)
Library Name	Same character requirement as Library Batch ID (required)
Barcode	Barcodes used for each sample and control library preparation (required)
Nucleic Acid Type	DNA, RNA, or TNA (required)

Template item	Description
Input Quantity	Library input quantity (optional)
No Template Control	To Include a no template control, add a row with **Sample Name** as NTC, **Library Name** as NA, **Barcode** and **Nucleic Acid Type** similar to sample rows (optional)

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

6. Save the file.

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

8. Click **Upload**.
   A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting support, see “Library batch import fails” on page 199.

Manage libraries and library batches

You can find tools for searching, sorting, editing, deleting, and auditing libraries and library batches in the **Samples / Manage Libraries** screen.

1. In the menu bar, click **Samples** ▸ **Manage Libraries**.
2. In the **Manage Libraries** screen, use the tools to perform specific actions.

To...	Do the following...
View more library information	Click a library name in the library list.
View all libraries	In the **Filter Libraries by...** dropdown menu, select **All**.
View libraries that are not in a run	In the **Filter Libraries by...** dropdown menu, select **To Be Planned**.
Search the list	In the search box, select search by **Library Name** or **Library Batch ID**, type a full or partial Library Name or Library Batch ID in the search field above the list, then click **(Search)**. Click ✕ to clear the search criteria and display the complete list.
Sort the list	Click the **Library Batch ID**, **Assay Name**, or **Sample Name** column heading to sort the list by the information in that column. Click the column heading again to sort in reverse order.
View multiple pages of libraries	Click the scroll buttons below the list.
Delete libraries	Select the checkbox next to the library batch ID or related libraries (DNA and RNA), then click **Delete**. You can delete a library only if none of the libraries in the same library batch have been assigned to a run.
Edit a library batch	In the **Actions** column, click **Edit** for the library batch, then modify the information in the **Edit Library Batch** screen. Click **Submit**.
View or export the audit trail of a library batch	1. In the **Actions** column, click **Audit** for the library batch. The **Audit Details** screen opens, displaying details of the most recent auditable action. All audit records for the library batch are listed on the left. 2. To view audit details for other actions, click on the time stamp and action listed in the left pane of the screen. 3. To download the record as a PDF file, in the upper right corner of the screen, click **Download**.
Add or modify notes for a library	In the **Actions** column, click **Notes**.

4 Plan and manage runs

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

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

Before you plan a run

Before you plan a run in the Genexus™ Software, check that:

- Sample information is correctly entered and the assigned sample names are unique.
- For a library run, library batches have been prepared and assigned unique library batch IDs.
- For a library run, each sample library in a library batch has been prepared with and assigned a unique barcode or barcode pair.

The software returns an error message when any of these conditions are not met when planning a run.

Plan a sample run

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

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

Before planning a run for a sample run, you must enter sample information into Genexus™ Software. For more information, see "Create a new sample" on page 19, or "Import samples" on page 20.

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

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

2. In the **Setup** step, enter or make the following selections.
   a. In the **Plan** section, enter a unique name.

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

Reporting option	Description
Generate Report	Select this option to generate a Lab Report using a report template that you specify in the list. To create a report template, click **Assays** ▸ **Manage Presets**, then in the **Report Templates** tab, click + **Add New**.
Upload BAM files to Ion Reporter™ Software	Select this option to automatically upload data for further analysis with Ion Reporter™ Software. You can also upload BAM files after a run if you leave this option unselected. Select your Ion Reporter™ Software account and release version. To configure an Ion Reporter™ Server account, see "Configure an Ion Reporter™ Server account (administrator)" on page 129. To configure an Ion Reporter™ Software on Connect account, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 127.

   c. Click **Next**.

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

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

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

Options	Description
List assays by research application	In **Filter Assays by**, select **Research Application**. You can filter by: • RNA • DNA and Fusions • DNA • Fusions
List assays according to chip type	In **Filter Assays by**, select **Chip Type**.
List assays according to library chemistry	In **Filter Assays by**, select **Library Chemistry**. You can filter by: • Ion AmpliSeq™ • Ion AmpliSeq™ HD
List selected assays only	In **Filter Assays by**, select **Selected**.
Sort the assay list	1. Click a column heading to sort the list by the entries in that column. 2. Click the column heading again to reverse the order.
Search for assays of interest	1. In the **Assay Name** search box, enter the full or partial assay name. 2. Click **(Search)** or press **Enter**. The assay or assays that match the assay name search entry are listed. 3. Click **(Remove)** to return to the complete list of assays.

Note:

- After selecting an assay, the list is filtered to show compatible assays that can be selected and run at the same time.
- To create a new assay, see Chapter 7, “Create and manage assays (manager/administrator)”.

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

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

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

  Note: In order for the Ion Reporter™ analysis workflow to appear in the list, you must tag the analysis workflow for use with the IonReporterUploader plugin. For more information, see "Tag an Ion Reporter™ Software analysis workflow for use with the IonReporterUploader plugin" on page 92.

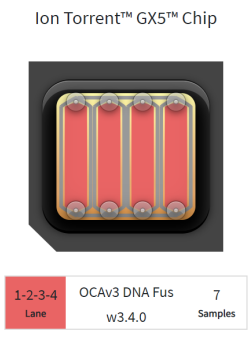
4. In the **Include NTC** column for each assay that you select, click the **Include NTC** checkbox to include a no template control for the assay.

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

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

   The **Chip View** updates to show the lanes to be used in the run. Lane usage is calculated based on the number of samples (including a no template control, if selected), assay type, primer pools used, and minimum reads per sample entered at assay setup. Green denotes a chip lane that will be used in the run containing assigned samples within lane capacity. If the minimum reads per sample × the number of samples exceeds the chip or lane well capacity, a dialog box appears after you click **Assign** asking you to confirm that you want to continue. After confirmation, the **Chip View** updates and shows the lane color as red instead of green. The run is allowed, but you may not achieve the required reads per sample to pass QC metrics.

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



Red lane color denotes sample assignment that exceeds lane capacity.

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

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

9. In the **Sample Plate** step, review sample positions in the sample plate. Drag and drop samples and no template controls to edit the location of samples and controls, if desired.

10. Modify the concentration of samples, if needed.

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

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

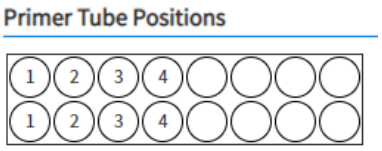
    Note: The sample volume that is required for library preparation is not adjustable. The volume depends on the number of primer pools in the assay, sample type, and library chemistry.

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

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

    The run plan summary lists the following details:
    - the consumables that are required for this run
    - how much sample volume to load
    - where to load samples and primer pool tubes
    - the sample concentration.

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



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

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

Plan a library run

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

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

For more information, see "Create an assay (manager/administrator)" on page 97, "Copy an assay (manager/administrator)" on page 98, and "Prepare a library batch" on page 27.

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

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

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

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

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

Reporting option	Description
Generate Report	Select this option to generate a Lab Report using a report template that you specify in the list. To create a report template, click **Assays** ▸ **Manage Presets**, then click + **Add New**.
Upload BAM files to Ion Reporter™ Software	Select this option to upload data for further analysis with Ion Reporter™ Software. You can also upload BAM files after a run if you leave this option unselected. Select your Ion Reporter™ Software account and release version. To configure an Ion Reporter™ Server account, see "Configure an Ion Reporter™ Server account (administrator)" on page 129. To configure an Ion Reporter™ Software on Connect account, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 127.

c. Click **Next**.

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

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

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

Options	Description
List assays by research application	In **Filter Assays by**, select **Research Application**. You can filter by: • RNA • DNA and Fusions • DNA • Fusions
List assays according to chip type	In **Filter Assays by**, select **Chip Type**.
List assays according to library chemistry	In **Filter Assays by**, select **Library Chemistry**. You can filter by: • Ion AmpliSeq™ • Ion AmpliSeq™ HD
List selected assays only	In **Filter Assays by**, select **Selected**.
Sort the assays list	1. Click a column heading to sort the list by the entries in that column. 2. Click the column heading again to reverse the order.
Search for assays of interest	1. In the **Assay Name** search box, enter the full or partial assay name. 2. Click **(Search)** or press **Enter**. The assay or assays that match the assay name search entry are listed. 3. Click **(Remove)** to return to the complete list of assays.

Note: For the assay to be selectable at this step, you must have prepared a library batch that assigns the assay to the batch. For more information on preparing a library batch, see “Prepare a library batch” on page 27. The assay specifies the barcode set that was used to prepare the sample libraries. To create a new assay, or copy an existing assay, see “Create an assay (manager/

administrator)" on page 97, or "Copy an assay (manager/administrator)" on page 98.

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

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

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

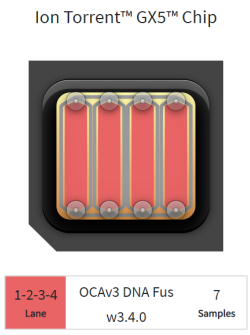
  Note: In order for the Ion Reporter™ analysis workflow to appear in the list, you must tag the analysis workflow for use with the IonReporterUploader plugin. For more information, see "Tag an Ion Reporter™ Software analysis workflow for use with the IonReporterUploader plugin" on page 92.

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

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

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

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



Red lane color denotes sample assignment that exceeds lane capacity.

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

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

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

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

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

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

Manage runs

Runs are listed in the **Runs / Manage Runs** screen. The most recently created run is on the top of the list.

In this screen, you can:

- Sort and search the list of runs.
- Review and print a run.
- View the audit trail of a run.
- Edit a run.
- Delete a run (manager/administrator-level users only).

Search and sort runs

Runs are listed in the **Runs / Manage Runs** screen. The most recently created run is on the top of the list.

- In the menu bar, click **Runs ▸ Manage Runs**.

- To search the run list:

  a. In the **Enter Run Name** search box, enter the full or partial run name, then click **(Search)**.

     The runs matching the search parameters are listed.

  b. Click ✕ to return to the complete list of runs.

- To sort the run list:
  a. Click the column heading of interest.
     The list reorders alphabetically or numerically based on the column name selected.
  b. Click the column heading a second time to reverse the sort order.

Review and print a run

You can review and print run information.

1. In the menu bar, click **Runs** ▸ **Manage Runs**.
2. In the **Manage Runs** screen, click a run name in the list.
3. In the **View Sample Run** screen, review the information that was entered or generated when you created the run.
4. (Optional) Click **Print** to print the information about the run.

Review and download a run history

The entire history of a run is available to review, export, or print using the audit feature. The history shows the original run values and any new values or activities.

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

1. In the menu bar, click **Runs** ▸ **Manage Runs**.
2. In the **Manage Runs** screen, in the row of the run, in the **Actions** column, click **Audit** .
   The **Audit Record Details** screen box opens. Each modifying event for the selected run is listed.
3. In the **Audit Record Details** screen, review the details of changes that have been made.
4. (Optional) Click **Download** to download a print-ready PDF file of the audit record.
5. Click **Back** to return to the **Manage Runs** screen.

Edit a run

You can edit runs that have not been used in a run and have a run status of **Planned**. Parameters that can be edited include reporting options, assays used in the run, samples included in the run, chip assignment, and sample concentration.

You cannot change the run name.

1. In the menu bar, click **Runs** ▸ **Manage Runs**.
2. In the **Manage Runs** screen, in the **Actions** column, click **Edit** for the run of interest.
3. In the **Edit Sample Run** workflow, change the desired settings by navigating through the steps clicking **Next**, then click **Save**.

Delete a run (manager/ administrator)

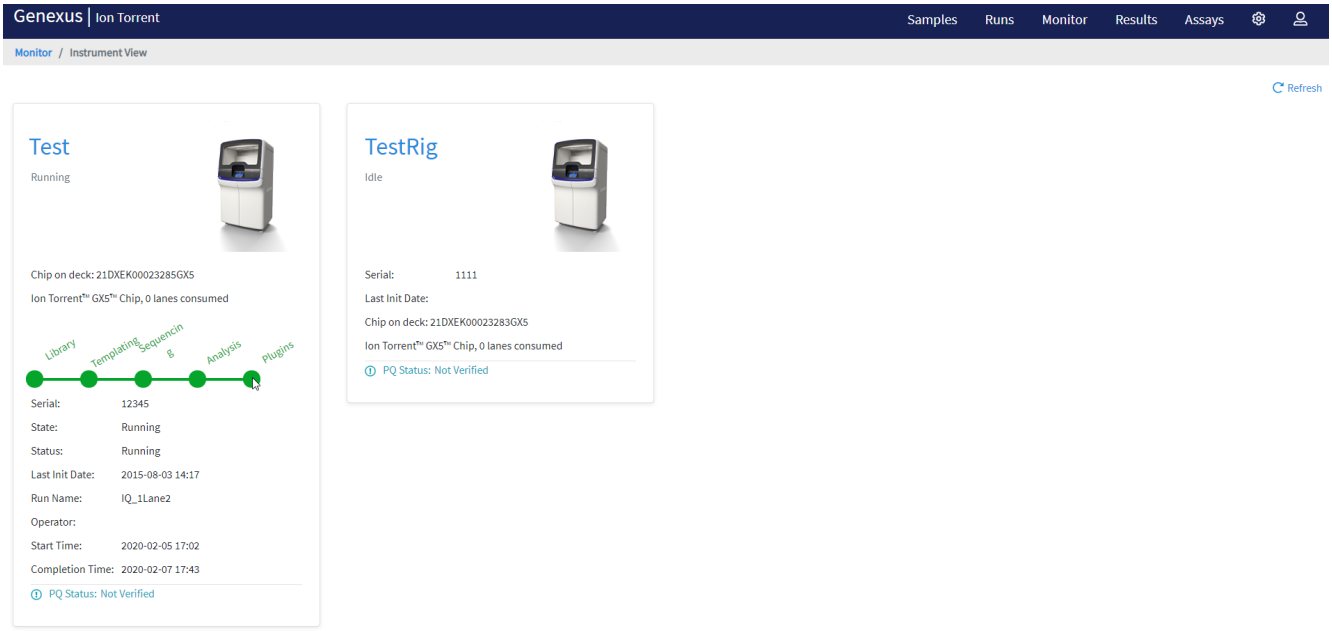
Manager- and administrator-level users can delete runs from the software.

1. In the menu bar, click **Runs** ▸ **Manage Runs**.
2. In the **Manage Runs** screen, select one or more runs to delete by selecting the checkbox next to the run name.

   Select all of the runs in the screen by selecting the checkbox above the column.
3. Click **Delete**.
4. In the **Delete Plan** message box, click **Yes** to delete the selected run.

5 Monitor the run

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



View run progress on the instrument

1. In the menu bar, click **Monitor** ▸ **Instrument View**.
2. In the **Instrument View** screen, view the status of a run in progress, or the status of an instrument.

   The following information is provided:

   - Sequencer, operator, and run names
   - PQ status of the instrument
   - Instrument status at various stages of the run and post-run
   - Status of the results analysis
   - Run start and completion times
3. Click **Refresh** to update the information.

6 Review data and results

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

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

Review sample results

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

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

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

Column	Description
Sample Name	The unique identifier created when the sample was entered into the software. Click the **Sample Name** to open the **Sample Details** screen for the sample. Use the tabs above the Sample Details to view the run summary, assay metrics, quality control, detailed variant results, and results for plugins that are associated with the selected assay, if any.
Sample Name followed by ***(Signed Off)***	Manager- and administrator-level users can provide their electronic signature on sample results for completed runs. A sample name followed by ***(Signed Off)*** indicates that a manager- or administrator-level user has approved the sample results. The signature information appears in the Lab Report PDF file or a user-created report, if selected. For more information, see "Sign off on the run results (manager/administrator)" on page 151.
Assay Short Name	A shortened version of the assay name you imported or created.
Run Name	The unique name of the run given when it was created in the software.
Sample Status	The status of the run or sample (for example: Completed, Running, Failed, Terminated, Pending, Stalled).
QC Status	The QC status of a completed run. **Note:** • ✔ (Passed) indicates the sample passed all QC metrics. • ✘ (Failed) indicates the sample failed a QC metric. • — (Not Calculated) indicates a sample did not undergo QC analysis.
Started On	The date and time when the run analysis was started.
Last Updated On	The date and time when the last action was completed on the run.
Actions	Click the appropriate link. To see more actions, click ⋮ **(More Options)**. • **Lab Report**—Download the **Lab Report** (available only for samples with a sample status of completed. For more information, see "Lab Report" on page 85. • **Audit**—View the audit trail for the run. For more information, see "Review and download a run history" on page 41. • **Notes**—View or add notes to a run. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting. For more information, see "Download a customer support archive" on page 198.

Review run results

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

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

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

Column	Description
Run Name	The unique name of the run given when it was created in the software. Click a run name to open the **Run Summary**. For more information, see "The run summary" on page 47.
Assay Short Name	The shortened unique identifier of an Assay name. You can view the complete list of **Assays** and **Assay Short Names** in the **Assays ▸ Manage Assays** screen.
Total Samples	The total number of samples in a run.
Run Status	The status of the run (for example: Not Started, Pending, Analysis Running, Executing Plugin, Completed, Terminated, Archival: In Progress).
PCR Plate Number	A unique identifier for the 96-well plate used for library preparation and templating. For more information, see "Assign PCR Plate" on page 47.
Started On	The date and time when the run analysis was started.
Last Updated On	The date and time when the last action was completed on the run.
Actions	Click the appropriate link. To see more actions, click **(More Options)**. • **Audit**—View the audit trail for the run. For more information, see "Review and download a run history" on page 41. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting. For more information, see "Download a customer support archive" on page 198. • **Reanalyze**—Reanalyze the results of a completed run, starting from alignment or basecalling. Click **Reanalyze**, then select a new reanalysis assay that you have created with modified settings. For more information, see "Reanalyze a run" on page 87. • **Upload Samples to IR**—Upload sample results files to Ion Reporter™ Software for secondary analysis. For more information, see "Upload sample results files to Ion Reporter™ Software" on page 90. • **Assign PCR Plate**—Enter a unique identifier for the 96-well plate used for library preparation and templating. For more information, see "Assign PCR Plate" on page 47.

Assign PCR Plate

Genexus™ Software allows you to track and associate a run with the PCR plate used in the run. The PCR plate is the 96-well plate that is used for library preparation and templating. You can assign a unique identifier (**PCR Plate Number**) to completed runs and runs in progress. The PCR plate number that you enter is generated on the Lab Report and if needed, can help you track libraries and troubleshoot.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, in the **Actions** column, click ⋮ **(More Options)** ▸ **Assign PCR Plate number** in the row of the run of interest.
3. In the **Assign PCR Plate** dialog box, confirm, edit, or enter the **PCR Plate Number**.

   The PCR plate number must be between 1 and 10 characters. Only alphanumeric characters (numbers 0 to 9 and letters A to Z), periods, underscores, and hyphens are allowed. Spaces are not permitted.
4. Click **Submit** to associate the PCR plate with the run.

Locate unused libraries

You can save and sequence unused libraries from runs planned from samples.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, in the **Run Name** column, click the run name of interest.
3. In the **Run Summary** tab, in the **Sample Locations** table, view the **Library Position** and the **Barcode** for the sample of interest.

Store the unused libraries, if needed. Sequence unused libraries by planning and initiating a library run. For more information, see “Plan a library run” on page 37.

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.

The run information includes the following items:

- **Run Status**
- **Run Start Date**
- **Run Completion Date**
- **Report Template**
- **Ion Reporter™ Software**

Assays table

Item	Description
Assay	The assay or assays used in the run. Click the assay name to display the details of the assay.
Analysis Version	The version of the assay used for analysis.

Item	Description
Research Application	The research application for the assay, such as **DNA** or **DNA and Fusions**.
Chip Type	The semiconductor sequencing chip, such as the Ion Torrent™ GX5™ chip.
Library Chemistry	The type of library chemistry used, such as Ion AmpliSeq™ HD.
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 may correlate with multiple wells on the plate for some assays when multiple nucleic acid types are contained within the sample.

Sample Locations table

Item	Description
Well Pos.	The well position indicates the location of the sample on the plate.
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.
Barcode	The barcode or barcodes associated with the sample.
Assay Short Name	A shortened version of the assay name for 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.

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 the locations of the primer pool tubes.

The instrument information includes the following items:

- **Instrument**
- **Status**
- **Start Date**
- **Completion Date**
- **Operator**

View sequencing results and assay metrics

For every run, you can view assay-specific results and sample-specific results. Assay-specific results include assay metrics, such as final read data, and assay-level plugin information, such as execution of the customer support archive. For more information, see “Assay metrics” on page 53 and Chapter 9, “Plugins for data analysis”.

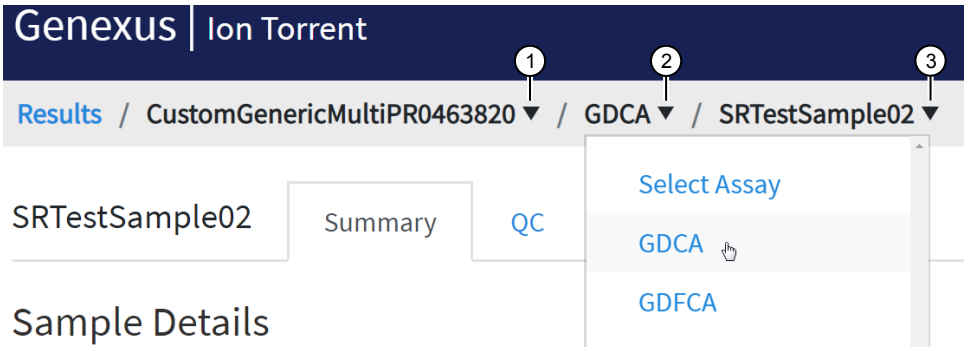
The following sample-specific result information is available.

Tab	Description
Summary	An overview of the results for the sample, including **Sample Details**, **Key Metrics**, and the **Variant Summary**. For more information, see “Summary of the Sample Results” on page 51.
QC	The quality metrics for the sample sequenced in the run. For more information, see “QC results” on page 55.
Variants	Detailed variant results for **SNVs/Indels**, **Fusions**, and **CNVs**. For more information, see “View variant results” on page 59.
Plugins	Results generated from the plugins associated with the assay used to analyze the sequenced sample. For more information, see Chapter 9, “Plugins for data analysis”.

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

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

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



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

Options	Description
Run Results dropdown menu	The run name is listed. Multiple runs are listed only if the run has been reanalyzed. For more information, see “Reanalyze a run” on page 87.
Assays dropdown menu	Toggle between different assays used in the run. If only one assay is associated with the run, only one assay name is listed.
Select Sample dropdown menu	Toggle between different sample results for the selected run and assay.

All of the samples that were run with the same assay share the same assay metrics. All other results are sample-specific. You can view the assay-level results if no sample name is selected from the **Select Sample** dropdown list.

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

Options	Description
Reanalyze	Reanalyze a run with a new assay. For more information, see "Reanalyze a run" on page 87.
Run Plugin	Run plugins on your sequencing data after a sequencing run is complete. For more information, see "Run plugins after a sequencing run is complete" on page 86.
Run Report	The run report includes assay metrics and the record of reagents that were used in a run. For more information, see "View or download a Run Report" on page 55.
Download Files	Download all or selected variant, results, audit and logs, and lab report files. For more information, see "Results files" on page 89.
Generate Report	Generate a Lab Report each sample in a sequencing run. For more information, see "Lab Report" on page 85.
Sign Off	Manager- and administrator-level users can provide their electronic signature on sample results for completed runs. For more information, see "Sign off on the run results (manager/administrator)" on page 151.
CSA	Customer support archive (CSA) log files for the run to help with troubleshooting. For more information, see "Customer Support Archive (CSA)" on page 198.
Upload to IR	Upload results to Ion Reporter™ Software for further analysis. For more information, see "Upload sample results files to Ion Reporter™ Software" on page 90.

Summary of the Sample Results

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

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

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

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

Section	Description
Sample Details	
Sample Name	A unique identifier representing the sample. Click the **Sample Name** to open the **Sample Details** screen for the sample. Use the tabs above the Sample Details to view the run summary, assay metrics, quality control, detailed variant results, and results for plugins that are associated with the selected assay, if any.
Collection Date	The date that the sample was collected.
Gender	The biological sex of the sample: **Female**, **Male**, or **Unknown**.
Sample Type	A term that describes the sample, for example, FFPE, DNA, DNA & RNA
Disease Category	The disease type of the sample.
Cancer Type	The type of cancer that is represented by the sample.
Cancer Stage	The stage of the cancer from which the sample was collected.
% Cellularity	The percentage of tumor cellularity in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is applicable to FFPE samples only.
Key Metrics	
Average Base Coverage Depth	The average number of reads of all targeted reference bases. This is the total number of base reads on target divided by the number of targeted bases, and therefore includes any bases that had no coverage.
Uniformity Of Base Coverage	The percentage of bases in all targeted regions (or whole genome) that are covered by at least 20% of the average base coverage depth reads. Cumulative coverage is linearly interpolated between the nearest integer base read depths.
% Base Reads On Target	The percentage of filtered reads that are mapped to any targeted region relative to all reads mapped to the reference. A read is considered on target if at least one aligned base overlaps at least one target region. If no target regions (file) was specified, this value will be the percentage of reads passing uniquely mapped and/or non-duplicate filters, or 100% if no filters were specified.
Variant Summary	
SNVs/Indels	Lists and describes the SNV, MNV, and INDEL variants that are detected in the sample.
Fusions	Lists and describes the fusions that are detected in the sample.
CNVs	Lists and describes the copy number variants (CNVs) that are detected in the sample.

Assay metrics

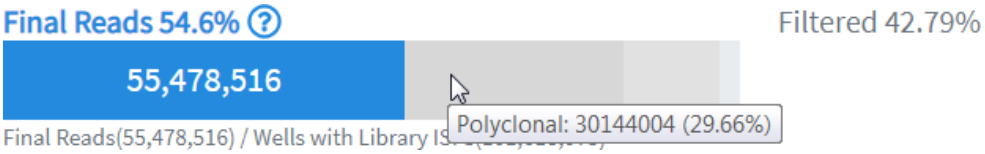
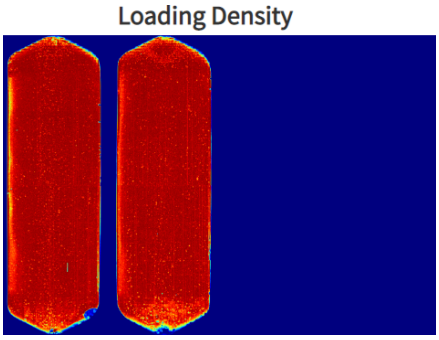
Assay metrics include various chip metrics for the run, such as well and Ion Sphere™ Particles (ISPs) statistics. For runs with multiple assays, metrics are provided for each assay in the run. Summary metrics are displayed at the top of the screen, followed by sample-specific metrics in the **Run Samples**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

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

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

Assay Metrics

Metric	Description
Loading	The number and percentage of total addressable wells on the chip that contain an ISP.
Enrichment	The number and percentage of wells ISPs that contain live ISPs.
Library	The number and percentage of wells with live ISPs that contain Library ISPs.
Final Reads	Library reads passing all filters that are recorded in the output BAM files. This value can be different from the total number of reads due to technicalities associated with read trimming beyond a minimal requirement.
Total Bases	The number of filtered and trimmed base pairs that are reported in the output BAM file.
Mean Raw Accuracy 1x	The mean raw accuracy across each individual base position in a read calculated as, (1–[total errors in the sequenced reads]/[total bases sequenced]) × 100. Raw read accuracy is measured at each base across the length of the read and is based on 1x sequencing coverage; raw read accuracy is *not* based on consensus accuracy across multiple reads for the same base position.
Wells with ISPs	The number of wells that contain an ISP.
Total Addressable Wells	Wells on the chip that can be physically reached by a library.
Empty Wells	The percentage of total addressable wells on the chip that do not contain an ISP.
Wells with Live ISPs	Loaded wells with ISPs with a signal of sufficient strength and composition to be associated with the library or control fragment key.
Wells with Library ISPs	Loaded wells with live ISPs with a key signal that is identical to the library key signal.
Control ISPs	Loaded wells with live ISPs with a key signal that is identical to the control fragment key signal.

Metric	Description
Polyclonal	Wells with a live ISP that carries clones from two or more templates. To view polyclonal metrics, mouse over the first low quality portion (gray) of the **Final Reads** bar plot. 
Low Quality	Loaded wells with a low or unrecognizable signal. To view polyclonal metrics, mouse over the second low quality portion (gray) of the **Final Reads** bar plot. 
Filtered	The total percentage of filtered reads, or the sum of the percentages of polyclonal, low quality, and adapter dimer reads.
Adapter Dimer	Loaded wells with a library template of an insert size less than 8 bases.
Loading Density	A visual representation of chip loading. Red color indicates areas of higher density of loading. Blue color indicates areas of lower density of loading. The following example illustrates a sequencing experiment where two lanes on the chip are uniformly loaded with ISPs. 

Run Samples

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

Column	Description
Sample Name	The unique identifier created when the sample was entered in the software.
Nucleic Acid Type	The sample nucleic acid type, such as DNA, RNA, or TNA.
Barcode	The unique identifiers of the dual barcode pair assigned to the DNA and/or RNA library for a sample.

Column	Description
Total Reads	The total number of filtered and trimmed reads with the listed dual barcodes assigned to the sample. The reads are independent of length reported in the output BAM file.
Mean Read Length	The average length, in base pairs, of usable library reads for each sample.
≥Q20 Bases	The total number of called bases that have ≥99% accuracy (or less than 1% error rate) aligned to the reference for the sample.
Uniformity	The percentage of bases in all targeted regions (or whole genome) with a depth of coverage ≥20% of the mean read coverage.
Read Length Histogram	A histogram that presents all filtered and trimmed library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences. 

View or download a Run Report

You can view a run report or download a run report summary in PDF format. The run report includes assay metrics and the record of reagents that were used in a run.

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

QC results

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

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

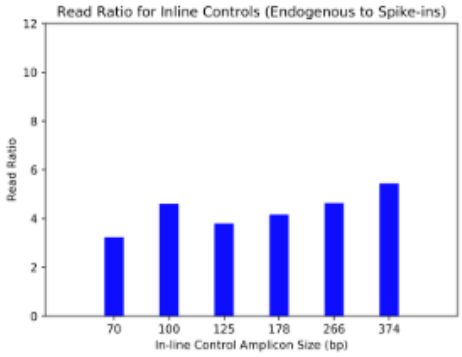
If a sample fails a single test metric, the sample fails that QC test. A sample must meet all QC parameter thresholds of a particular QC test in order to pass. The QC status is broken down into the following categories.

- ✔ (Passed) indicates the sample passed all QC metrics.
- ✘ (Failed) indicates the sample failed a QC metric.
- — (Not Calculated) indicates a sample did not undergo QC analysis.

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

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

Metric	Description
Run QC	**General run quality control information.**
Key Signal	The average signal after software processing for library ISPs that identically match the library key (TCAG).
Percent Loading	The number of wells with ISPs divided by the number of the total addressable wells in a run.
Raw Read Accuracy	The average raw accuracy across each individual base position in a read, where raw read accuracy is calculated as 100 * (1 - (sum(per base error)/sum(per base depth))).
Templating QC—CF-1 Control	**Sequencing quality metrics of the control fragment. These metrics indicate templating success.**
Average Reads Per Lane	The number of CF-1 reads divided by the number of chip lanes used in the run.
Base Call Accuracy	The probability that a given base is called correctly. 1 – (total number of errors for all positions in CF-1) / (total number of CF-1 base reads).
Mean AQ20 Read Length (bp)	Average length, in base pairs, at which the accuracy rate is ≥99% for CF-1 reads.
Sample QC—DNA	**Sequencing quality metrics of the sample DNA library.**
MAPD	MAPD (Median of the Absolute values of all Pairwise Differences) is a quality metric that estimates coverage variability between adjacent amplicons in CNV analyses. A MAPD value of 0.4 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 do not get called. The MAPD metric does not affect SNVs/INDEL calls.
Mapped Reads	The number of reads that are mapped to the reference file.
Mean AQ20 Read Length (bp)	The average length, in base pairs, at which the accuracy rate is ≥99% for all aligned reads of a library.
Mean Read Length (bp)	The average length, in base pairs, of final library reads for the sample.
Uniformity of Amplicon Coverage	The percentage of amplicons that had at least 20% of the average number of reads per amplicon. Cumulative coverage is linearly interpolated between nearest integer read depth counts.
Median Mol Cov	The median number of functional molecule reads per amplicon calculated over all amplicons in the assay. This metric is applicable to Ion AmpliSeq™ HD library chemistry only.

Metric	Description
Uniformity of Base Coverage	The percentage of reads with a depth of coverage ≥20% of the mean read coverage at each position. This metric is applicable to Ion AmpliSeq™ HD library chemistry only.
Read Length Histogram	The histogram presents all filtered and trimmed DNA library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences.
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of genomic sample reads to control reads for each inline control amplicon.  **Note:** The **Read Ratio** for each inline control amplicon is expected to be 3 with 10 ng DNA input for both Ion AmpliSeq™ and Ion AmpliSeq™ HD chemistries.
Sample QC – RNA	**Sequencing quality metrics of the sample RNA library.**
Mapped Reads	The total number of final library reads that are aligned to the reference file for the sample.
Mean Read Length (bp)	The average length, in base pairs, of the final library reads for the sample.
RNA Expression Ctrls Detected	The number of expression control genes detected for the sample. This metric measures the RNA input integrity, input amount, and the fidelity of the reverse transcriptase that was used in library preparation. Fusion panels include primer pairs that cover seven control housekeeping genes.
Mean AQ20 Read Length	The average length, in base pairs, of the final library reads with no more than 1% error rate for the sample. The average length, in base pairs, at which the accuracy rate is ≥99% for all aligned reads of a library.
Read Length Histogram	The histogram presents all filtered and trimmed library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences.

Metric	Description
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of genomic sample reads to control reads for each inline control amplicon. You can use this metric to measure the level of contaminating genomic DNA in RNA libraries when you select the RNA checkbox for **Include Inline Controls** in assay set up. **Note:** The **Read Ratio** for each inline control amplicon is expected to be 3 with 10 ng DNA input for both Ion AmpliSeq™ and Ion AmpliSeq™ HD chemistries.
NTC QC – DNA	**DNA Sequencing quality metrics of the no template control.**
Average Base Coverage Depth	The average number of DNA reads of all targeted reference bases.
Mean Read Length (bp)	The average length, in base pairs, of final DNA library reads for the no template control.
Read Length Histogram	The histogram presents all filtered and trimmed reads for the no template control that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis).
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of no template control sample reads to control reads for each DNA inline control amplicon.
NTC QC – RNA	**RNA Sequencing quality metrics of the no template control.**
Mapped Reads	The average number of RNA reads of all targeted reference bases.
Mean Read Length (bp)	The average length, in base pairs, of final RNA library reads for the no template control.
RNA Expression Ctrls Detected	The number of expression control genes detected for the no template control.
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of no template control sample reads to control reads for each RNA inline control amplicon.

MAPD copy number QC metric

The Median of the Absolute values of all Pairwise Differences (MAPD) score is reported on Aneuploidy analyses and other analyses that detect CNVs.

MAPD is one of the metrics that we define to assess whether the panel data is useful for copy number variation (CNV) analysis.

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 CN calls.

Note: MAPD is not shown on the QC Report.

View variant results

You can view detailed variant results and toggle between different variant results, including SNVs/Indels, Fusions, and CNVs, in the **Results** screen.

Variant Tables

Variant type	Description
SNVs/Indels	• **SNVs**: missense and nonsense single nucleotide variants. Multi-nucleotide variants are also included. • **Indels**: insertion and deletion variants. For more information, see “View SNV/INDEL results” on page 59.
Fusions	Translocations of genetic material. For more information, see “View Fusion results” on page 62.
CNVs	Copy number variations (CNVs) are variations of the number of copies of a given gene. For more information, see “View CNV results” on page 67.

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.
5. *(Optional)* Click **Export** in the upper right corner of the screen to export the results data in tabular format.

SNVs/Indels table

The data displayed in the table depend on the assay that was used in the run. You can adjust the number of columns that are visible using the **Columns** dropdown menu. Deselect the checkbox next to a column heading to remove the column from the table. Select the checkbox to add the column back to the table. Not all of the columns are visible on the screen; use the scrollbar under the table to view additional columns. You can adjust column widths in the column headers to expand or reduce the number of columns visible on the screen.

Filter the results list in the table using the **Filter** dropdown menu. For more information, see "Filter results using a filter chain" on page 71. To sort and filter the results in each column, click **(Filter)**. Options for the column are displayed in the **Filter** dialog box. Check the checkbox next to an option in the Filter dialog box, then click **Filter** to display the option in the column. Deselect the checkbox next to an option to hide the option. Click **Clear** to display all results in the column.

Column	Description
User Classifications	User-defined classification to selected from the list. For more information, see "Create and assign variant classifications" on page 70.
Locus	The chromosome and position of the detected variant.
Oncomine Variant Class	The type of SNV or INDEL at the locus based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function — the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • Loss-of-function — the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Gene	The gene name. Click the link to open the **View Annotation Sources** window to view additional information. For more information, see "View annotation sources" on page 69.
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.

Column	Description
Type	The type of variant that is detected. • snp (single nucleotide polymorphism) • mnp (multi-nucleotide polymorphism) • ins (insertion) • del (deletion) • complex
Call	Indicates the presence or absence of an SNV/Indel variant. When the default filter chain is applied, only the variant calls that are designated with **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants (.vcf)** file (see "Results files" on page 89). • **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** – indicates a high confidence call that passes all filter thresholds at a given variant position. **Note:** ◦ When the default filter chain is applied, **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** indicates the presence of the ALT (alternative) allele. ◦ When the **No Filter** option is applied or when viewing the **Variants (.vcf)** file, **Present** does *not* imply the presence of the ALT (alternative) allele. To infer the presence of the ALT allele, refer to the **Alt** column. • **NO CALL** – while some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call. • **ABSENT** – indicates the presence of a variant that differs from the reference allele at a given position, however, this nucleotide is not an ALT allele that is targeted by this assay.
No Call Reason	The reason why a variant is not reported.
Phred QUAL Score	The relative probability of either the "reference" hypothesis interval [0,cutoff], or the "variant" hypothesis interval [cutoff,1], Phred-scaled (-10*log10). A higher score means more evidence for the variant call.
Raw Read Depth	Total read coverage across amplicon containing SNV/INDEL hotspot locations. Count of chip-level reads aligned at this locus that participate in variant calling.
Effective Read Depth	The number of reads covering the position.
Alt Allele Read Counts	The number of reads containing the alternate allele.
Variant ID	The name of the hotspot as defined in the Browser Extensible Data (BED) file.
Nuc Change	The position and identity of the nucleic acid change.
Allele Fraction	The number of variant read counts divided by the total number of read counts for the sample.

Column	Description
Mol Depth[1]	Reports number of interrogated DNA molecules containing target. It defines limit of detection at hotspot position in a particular run and sample. For instance, if molecular depth is ≥1,500, you can have high confidence that no variant is present at 0.2% LOD. If molecular depth is ≥2,500, you can have high confidence that no variant is present down to 0.1% LOD. For reference calls, Molecular Depth provides measurable metric that serves as confirmation for variant absence among a large number of interrogated molecules.
WT Mol Counts[1]	Number of detected molecules containing the wildtype allele.
Alt Allele Mol Counts[1]	Number of detected molecules containing the alternate allele.
Mol Freq %[1]	Molecular frequency percentage. The percentage of mutant reads over total reads at the locus.
% LOD[1]	Limit of detection (LOD) of a variant allele expressed as a percentage of the WT allele. LOD is the lowest possible variant frequency in the sample that can be detected by the system with a true positive rate greater than 98% for FFPE samples or 95% for cfTNA samples. LOD is dependent on the molecular read depth at the locus. %LOD is reported when there are no variant calls for the gene.

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

View Fusion results

The **Fusions** table lists the calls and other information for the fusions analyzed in each sample in a run.

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.
5. *(Optional)* Click **Export** in the upper right corner of the screen to export the results data in tabular format.

Fusions table

The data displayed in the table depend on the assay that was used in the run. Filter the results list in the table using the filtering tools. For more information, see “Filter results using a filter chain” on page 71.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see “Create and assign variant classifications” on page 70.
Locus	The chromosome positions in the reference genome that define the fusion junction.

Column	Description
Oncomine Variant Class	Oncomine variant class annotation that indicates fusion type based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function — the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • Loss-of-function — the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Genes (Exon)	The name of fusion target and representative acceptor and donor exons.
Read Counts	The frequency that the fusion was detected in the sample.
Type	Assay type (for example, Fusion, RNA exon variant (exon skipping), RNAExon Tile, Proc Control).
Call	Indicates the presence or absence of a fusion or RNA exon variant. When the default filter chain is applied, only the fusion/RNA exon variant calls that are designated with **PRESENT** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants (.vcf)** file (see "Results files" on page 89). • **PRESENT** – indicates a high confidence call that passes all filter thresholds at a given variant position. • **ABSENT** – indicates the absence of a fusion due to the variant call failing internal quality control. • **NO CALL** – while some evidence for the presence of a fusion exists, the call does not pass one or more filters that are required for a high confidence fusion call.
No Call Reason	The reason for reporting a fusion as **NOCALL**.
Failed Reason	The reason for reporting a fusion as **Absent**.
Variant ID	The name of the fusion target as defined in the BED file.
Read Counts Per Million	The number of fusion read counts detected per number of total reads (in millions).
Oncomine Driver Gene	The gene believed to be associated with increased oncogenic properties. The gene is inappropriately activated by the fusion.
Mol Cov. Mutant[1]	The median molecular coverage across a fusion amplicon.

Column	Description
Imbalance Score[2]	Each fusion gene exhibits a characteristic **Imbalance Score** threshold. Scores that exceed this threshold value indicate a high likelihood of the presence of the fusion in the test sample. • **Observed** = (sum of read count coverage of amplicons downstream (3') of a predicted breakpoint in a target gene) / (sum of read count coverage of all amplicons of the gene) [Read counts from test sample] • **Expected** = (sum of baseline value for amplicons downstream of the breakpoint/sum of baseline values) / (sum of baseline values of all amplicons of the gene) [Baseline values computed from normal samples]
Imbalance P-Value[2]	The statistical significance of measure of imbalance relative to a control gene.
Predicted Break-point Range[2]	The exonic range for predicted fusion break point in exon tiling assays.
Ratio To Wild Type[1]	The molecular ratio for exon skipping assays relative to wild type control amplicons.
Norm Count Within Gene[1]	*(Lung panel only)* Exon skipping assay coverage normalized to molecular coverage of wild type (WT) MET control amplicons.

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

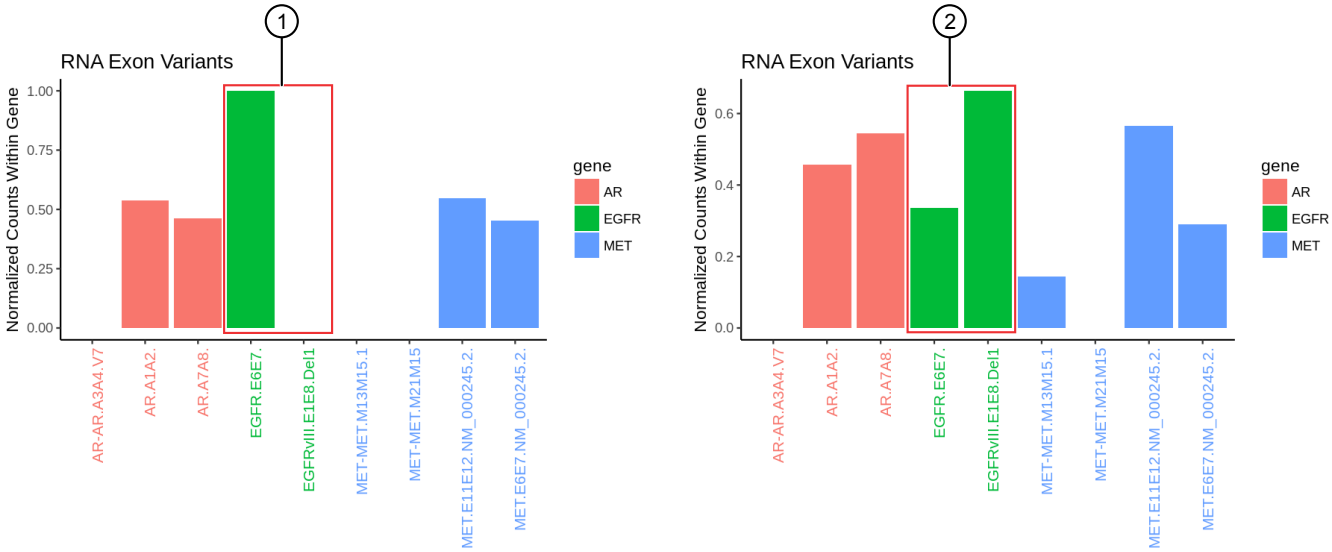
[2] Column appears in analyses that use the exon tiling fusion detection method.

View RNA Exon Variant

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

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample name** column, click the name of the sample of interest.
3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results.
4. In the top right corner of the screen, click **Visualization** ▸ **RNA Exon Variant**, then review the **RNA Exon Variants** plot.

Representative RNA Exon Variant plots



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

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

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

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

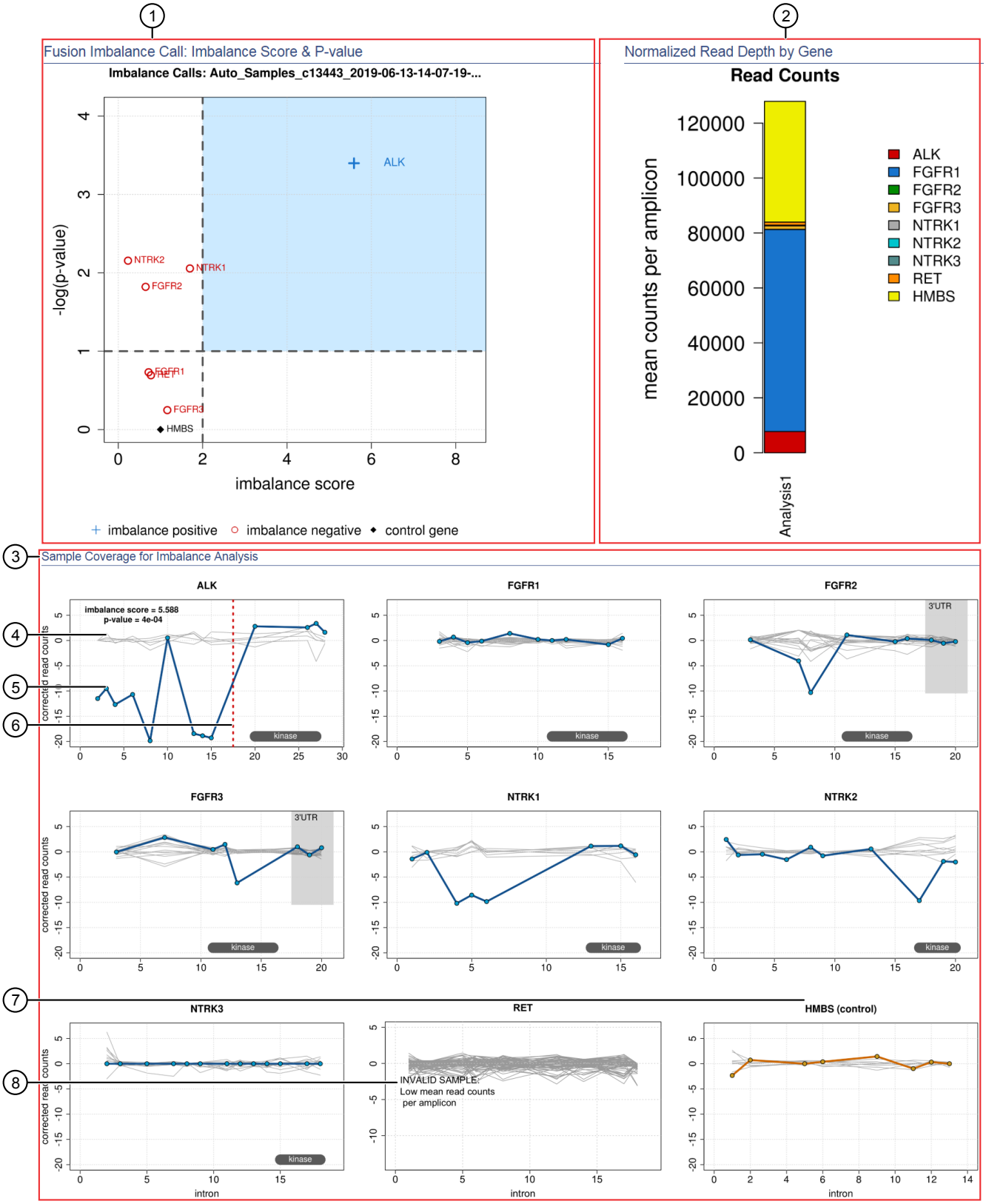
View RNA Exon Tile Fusion Imbalance

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

Note: The exon tiling method for fusion detection and the RNA Exon Tile Fusion Imbalance data view are specific to the Oncomine™ Precision Assay GX. For more information, see the *Oncomine™ Precision Assay GX User Guide* (Pub. No. MAN0018508).

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample name** column, click the name of the sample of interest.
3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results.
4. In the top right corner of the screen, click **Visualization** ▸ **RNA Exon Variant**, then review the RNA Exon Tile Fusion Imbalance plots.

Representative RNA Exon Tile Fusion Imbalance plots



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

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

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

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

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

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

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

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

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

View CNV results

The **CNVs** table lists the calls and other information for the copy number variants (CNVs) analyzed 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 **CNVs** to view the **CNVs** table.
5. *(Optional)* Click **Export** in the upper right corner of the screen to export the results data in tabular format.

CNVs table

The data displayed in the table depend on the assay that was used in the run. Results in the table can be filtered using the filtering tools. For more information, see “Filter results using a filter chain” on page 71.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see “Create and assign variant classifications” on page 70.
Locus	The starting position of the first amplicon covering the CNV gene.
Oncomine Variant Class	Annotation that indicates whether CNV is an amplification or deletion. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.

Column	Description
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function — the altered gene product has a new molecular function or pattern of gene expression, compared to the wild-type gene • Loss-of-function — the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Gene	The gene name, which provides a link to the **View Annotation Sources** dialog box with additional information about the **HotSpot ID**. For more information, see “View annotation sources” on page 69.
Copy Number	The copy number of a CNV gene locus per genome. This column is available when a positive call is made.
Call	Indicates the presence or absence of a CNV. When the default filter chain is applied, only the CNV-positive calls that are designated with **PRESENT** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants (.vcf)** file (see “Results files” on page 89). • **PRESENT** – indicates a high confidence call that passes all filter thresholds. • **PRESENT (GAIN)** – a CNV-positive call that indicates gene amplification; a high confidence variant call that passes all filter thresholds. • **PRESENT (LOSS)** – a CNV-positive call that indicates gene deletion; a high confidence variant call that passes all filter thresholds. • **ABSENT** – the absence of a variant; result is below detection threshold for a CNV call. • **NO CALL**– while some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call.
P-Value	The statistical significance of the CNV ratio measurement.
No Call Reason	The reason for reporting a CNV as No Call.
CNV Confidence	The CNV confidence interval associated with the call. The 5% lower confidence bound value is the ploidy estimate, where there is a 5% chance that the true ploidy is below that value. The 95% upper confidence bound is the ploidy estimate, where it is 95% certain that the true ploidy is below that value.
Variant ID	The name of the hotspot as defined in the BED file.
CNV Ratio	The ratio of measured CNV gene locus coverage relative to coverage of non-CNV loci.
Med Mol Cov Gene [1]	The median molecular coverage of targeted CNV gene.
Med Mol Cov Ref [1]	The median molecular coverage of non-CNV reference loci.
Med Read Cov Gene	The median read coverage of targeted CNV gene.

Column	Description
Med Read Cov Ref	The median read coverage of non-CNV reference loci.
Valid CNV Amplicons	The number of amplicons spanning the CNV call.

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

View annotation sources

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

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

1. In the **SNVs/Indels**, **Fusions**, or **CNVs** table, in the **Gene** column, click the gene symbol.
   The **View Annotation Sources** window opens, which displays information for the particular variant.

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

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

Visualize variants with IGV

You can visualize data from Genexus™ Software with the Broad Institute Integrative Genomics Viewer (IGV). The viewer is available at the Broad Institute website: **http://software.broadinstitute.org/software/igv/home**.

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

2. Click a sample name to open the **Results** screen.

3. Click the **Variants** tab.

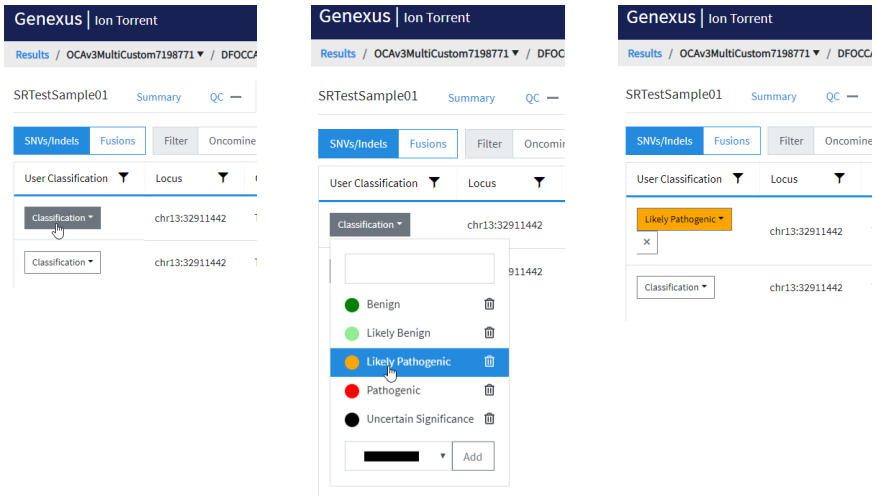
4. In the SNVs/Indels, Fusions, or CNV table, in the **Gene** column, click the gene symbol.

5. In the **View Annotation Sources** window, click **View IGV** to download a JNPL file that can be opened in the Integrative Genomics Viewer (IGV) from the Broad Institute (see **software.broadinstitute.org/software/igv**).

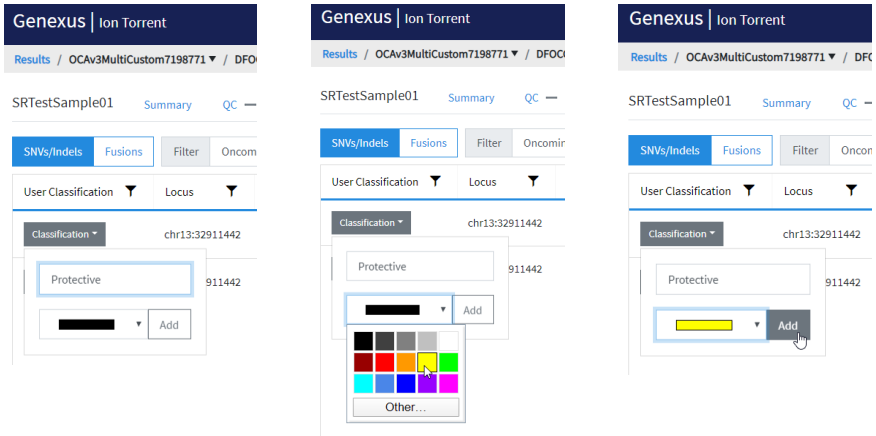
Create and assign variant classifications

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

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



   - 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.

Filter the view of results in the Results screen

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

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

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

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

Filter results using a filter chain

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

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

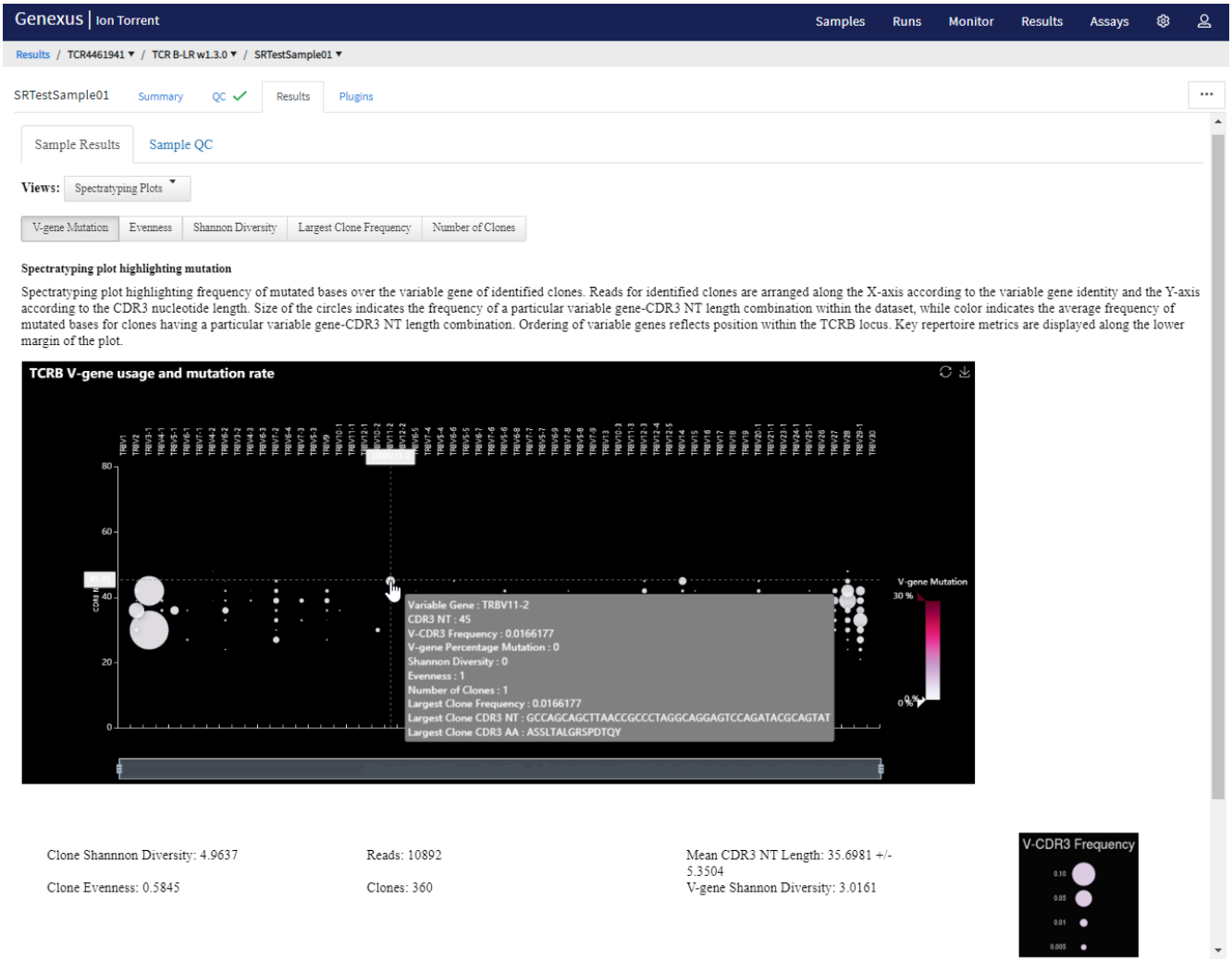
If you save the filter chain to a result, the variants that are included reflect the filtered results when the results are later opened.

For information about system-installed filter chains, see “System-installed filter chains” on page 178. Manager- and administrator-level users can create filter chains. For more information see, “Create a custom filter chain (manager/administrator)” on page 106

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

View Oncomine™ TCR Beta-LR Assay GX run results

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



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

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

Compare sample results

You can compare variant results across samples with Genexus™ Software. Comparisons between a variety of results can be made. However, results generated using the system-installed Oncomine™ TCR Beta-LR Assay GX (or any other immune repertoire assay) can only be compared to samples within the same assay.

- For information about the comparisons of variant results of immune repertoire workflows, see “Compare immune repertoire results” on page 80.
- For more information about the comparisons of variant results across all other workflows, see “Compare variant results” on page 74.

Compare variant results

You can compare variant results and create comparison plots from multiple sample results with Genexus™ Software. A description of the comparison of results from immune repertoire assays is not included here. For more information, see “Compare sample results” on page 74. Comparisons across samples or runs can be used to analyze results for a hotspot of interest. You can also visualize the trends of a reported variant over time.

The following comparisons can be made in Genexus™ Software:

- Comparisons of multiple samples, sequenced and analyzed with the same or different assays
- Comparisons of multiple samples over time, sequenced and analyzed with the same or different assays
- Comparisons of the results from one sample, sequenced and analyzed with different assays

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

View variant result comparisons

You can view variant comparisons in tabular format in **Fusions**, **SNVs/Indels**, and **CNV** comparison tables in the **Compare** screen.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click the checkboxes next to the sample names of interest.

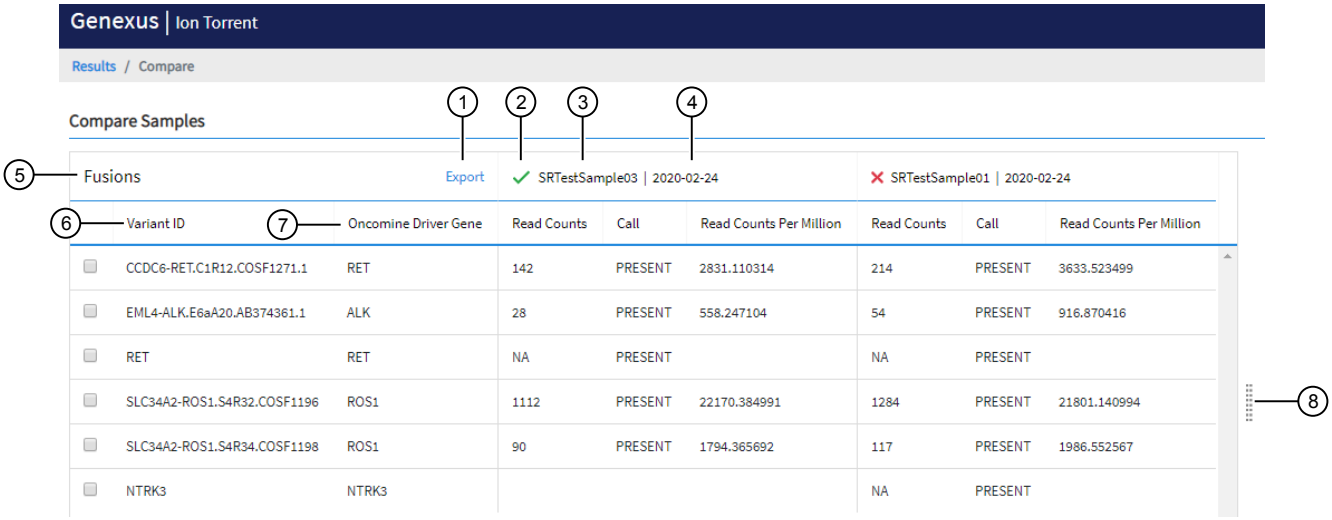
   Note: Select **Filter Runs by** ▸ **Sample Status** ▸ **Completed** to limit the entries that are displayed on the **Sample Results** screen.
3. In the upper right corner of the screen, click **Compare**.

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

The **Compare** screen opens to the **Compare Samples** tables. The **Oncomine™ Extended (5.14)** filter is the default filter for the data displayed in the tables.

4. *(Optional)* In the **Compare** screen, in the upper right corner, click **Filter** to select and apply a different chain to the variants displayed in the tables. For more information, see “Filter results using a filter chain” on page 71.

5. *(Optional)* Click **Export** to download an xlsx spreadsheet file of the variant comparison table.



1. **Export**: Download a Microsoft™ Excel™ file of the sample comparison **Fusions** table.
2. The QC status of the sample.
3. The sample name.
4. The date the sample was analyzed.
5. The variant type. In this example: **Fusions**.
6. **Variant ID**: The name of the hotspot as defined in the Browser Extensible Data (BED) file.
7. **Oncomine Driver Gene**: For fusion class variants, the 5’ gene that drives proliferation of expression.
8. Table slider. Use to extend table to view more samples.

The following table lists and describes the default information shown in the comparison tables.

Column	Description
Fusions comparison tables	
Variant ID	The name of the hotspot as defined in the BED file.
Oncomine Driver Gene	For fusion class variants, the 5' gene that drives proliferation of expression.
Read Counts	The frequency that the fusion was detected in the sample.
Call	The presence or absence of a fusion for the specific hotspot.
Read counts per million	The number of fusion read counts detected per number of total reads (in millions).
SNV/Indels comparison tables	
Variant ID	The name of the hotspot as defined in the BED file.
Gene	The gene name.
AA Change	Identification of the amino acid change using HGVS nomenclature.
Allele Fraction	The number of variant read counts divided by the total number of read counts for the sample.
Call	Indicates the presence or absence of an SNV/Indel variant. See SNV/Indel section for more information.
CNVs comparison tables	
Variant ID	The name of the hotspot as defined in the BED file.
Gene	The gene name.
Copy Number	The frequency that the fusion was detected in the sample. **Note:** Copy Number is not displayed for TNA CNV result comparisons.
Call	Indicates the presence or absence of a CNV.
CNV Ratio	Ratio of CNV amplicons after outlier removal.

Create time series graphs

You can create line graphs to visualize changes and trends in variant results over time.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click the checkboxes next to the sample names of interest.

   Note: Select **Filter Runs by** ▸ **Sample Status** ▸ **Completed** to limit the entries that are displayed on the **Sample Results** screen.
3. In the upper right corner of the screen, click **Compare**.

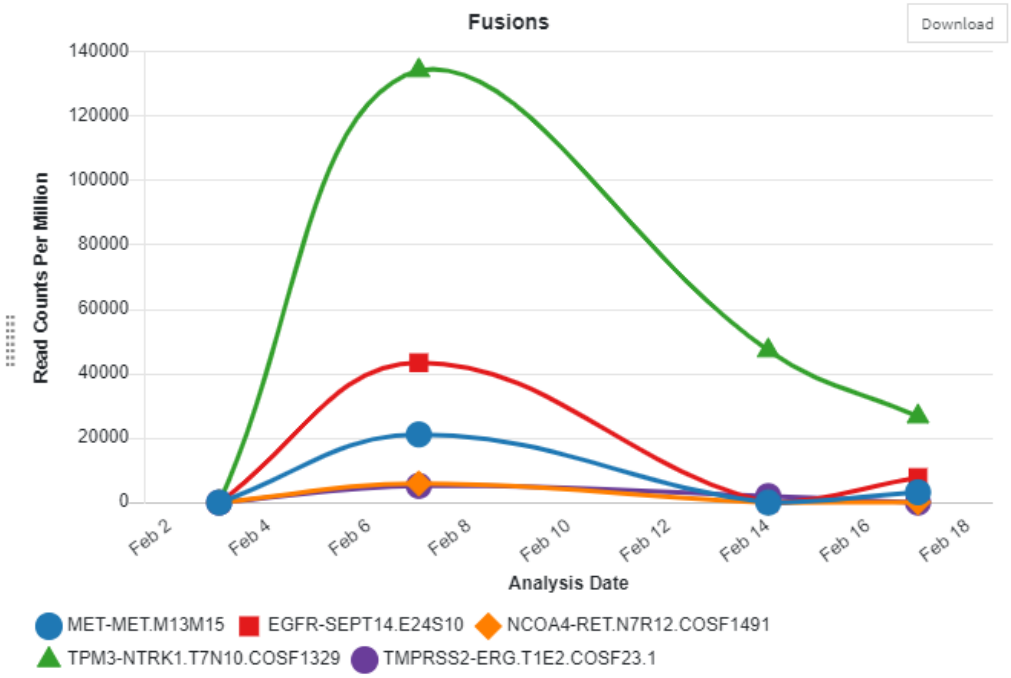
   Note: **Compare** is not enabled until at least two samples are selected

The **Compare** screen opens to the **Compare Samples** tables. **Oncomine™ Extended** is the default filter for the data displayed in the tables.

4. *(Optional)* In the **Compare** screen, in the upper right corner, click **Filter** to select and apply a different chain to the variants displayed in the tables. For more information, see "Filter results using a filter chain" on page 71.

5. In the **Fusions**, **SNVs/Indels**, and **CNV** table, select the checkboxes next to the **variant IDs** you want to visualize in a plot.
The datapoints are added to the line graph with live preview.

6. *(Optional)* Click **Download** to download a PNG file of the time series line graph.



Create and customize comparison plots

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

2. Click the checkboxes next to the sample names of interest.

   Note: Select **Filter Runs by** ▸ **Sample Status** ▸ **Completed** to limit the entries that are displayed on the **Sample Results** screen.

3. In the upper right corner of the screen, click **Compare**.

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

   The **Compare** screen opens to the **Compare Samples** tables. **Oncomine™ Extended** is the default filter for the data displayed in the tables.

4. *(Optional)* In the **Compare** screen, in the upper right corner, click **Filter** to select and apply a different chain to the variants displayed in the tables. For more information, see "Filter results using a filter chain" on page 71.

5. In the **Fusions**, **SNVs/Indels**, and **CNV** table, select the checkboxes next to the **variant IDs** you want to visualize in a plot.
   The datapoints are added to the line graph with live preview.

6. *(Optional)* To customize the chart, click **Chart Options** in the upper right corner of the screen.

7. In the **Preferences** dialog box, change the parameters to customize the plot.

   - X-Axis Options

Option	Description
Sample Name	The unique identifier for a sample.
Analysis Date	The date of the sequencing run. The analysis date or run completion date is typically included in the Sequencing Run Retails of the Lab Report.
Collection Date	The date that the sample was collected.

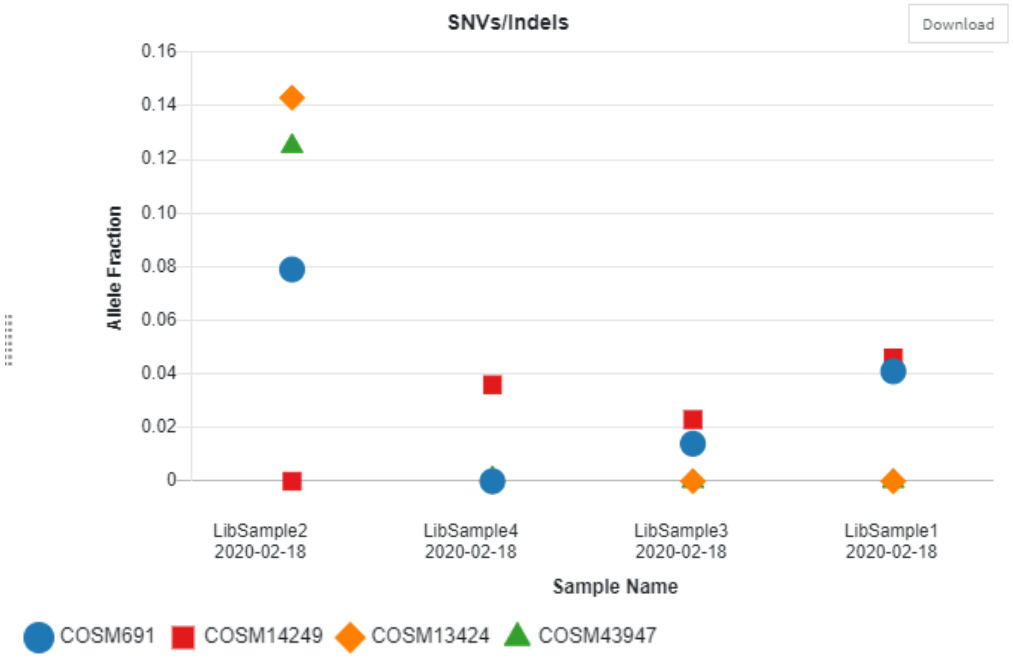
   - Y-Axis Options

Option	Description
SNVs/Indels	See "View SNV/INDEL results" on page 59.
Fusions	See "View Fusion results" on page 62.
CNVs	See "View CNV results" on page 67.

   Note: Y-Axis options depend on the assay or assays used to analyze the samples.

8. Click **Apply** to update the chart with your selections.

9. *(Optional)* Click **Download** to download a png file of the comparison plot or time series line graph.



View and customize comparison tables

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click the checkboxes next to the sample names of interest.

   Note: Select **Filter Runs by** ▸ **Sample Status** ▸ **Completed** to limit the entries that are displayed on the **Sample Results** screen.
3. In the upper right corner of the screen, click **Compare**.

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

   The **Compare** screen opens to the **Compare Samples** tables. **Oncomine™ Extended** is the default filter for the data displayed in the tables.
4. *(Optional)* In the **Compare** screen, in the upper right corner, click **Filter** to select and apply a different chain to the variants displayed in the tables. For more information, see “Filter results using a filter chain” on page 71.
5. *(Optional)* To customize the table or tables, click **Table Columns** in the upper right corner of the screen.

6. Use the checkboxes next to a table column option to add (checked) or remove (unchecked) the column to the table.

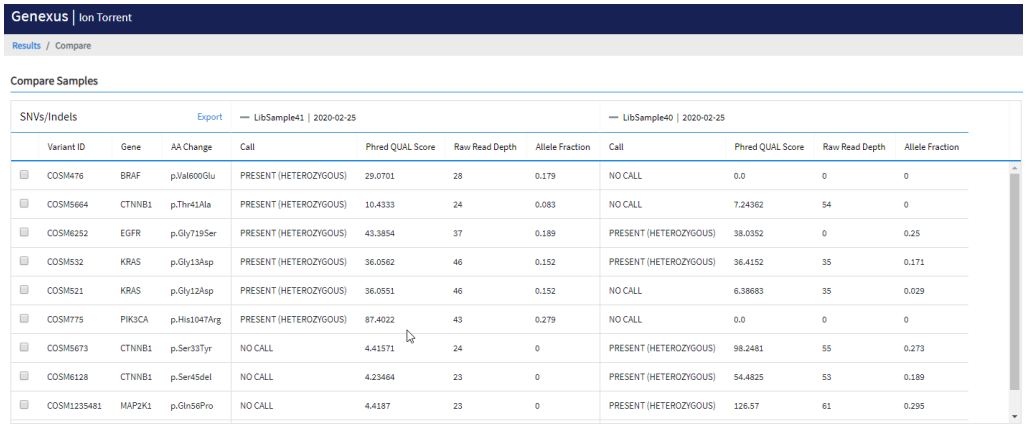
   The available options depend on the assay or assays used to analyze the samples. For descriptions of options, see the following sections.

Option	Description
SNVs/Indels	See “View SNV/INDEL results” on page 59.
Fusions	See “View Fusion results” on page 62.
CNVs	See “View CNV results” on page 67.

   Checked options are added as column headings to the **Fusions**, **SNVs/Indels**, and **CNV** comparison table with live preview.

7. Click **Table Columns** again to close the dialog window.

8. *(Optional)* Click **Export** to download an xlsx spreadsheet file of the variant comparison table.



Compare immune repertoire results

You can compare immune repertoire variant results with Genexus™ Software. A description of the comparison of results generated from all other assays is not included here. For more information, see “Compare sample results” on page 74.

Compare the immune repertoire between samples

Genexus™ Software can perform multi-sample (or cross-sample) analyses to compare the immune repertoire between samples.

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

2. In the **Sample Results** screen, click the checkboxes next to the sample names of interest.

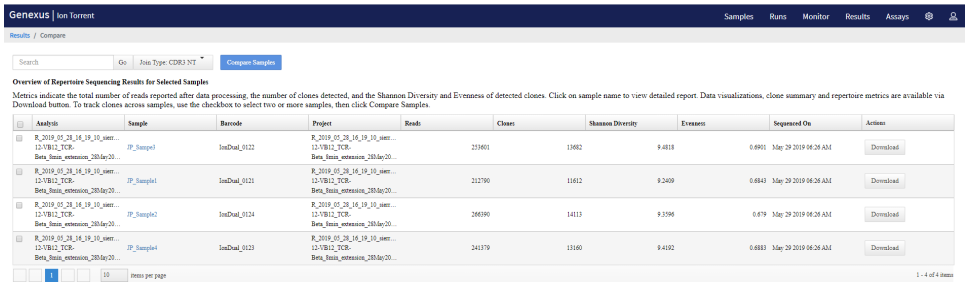
   Select **Filter Runs by** ▸ **Sample Status** ▸ **Completed** to limit the entries that are displayed on the **Sample Results** screen.

   The immune repertoire comparison is only applicable to samples sequenced with the system-installed Oncomine™ TCR Beta LR RNA Only assay (or any other custom or system immuno-oncology workflow).

3. In the upper right corner of the screen, click **Compare**.

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

   The **Overview of Repertoire Sequencing Results for Selected Samples** table is displayed.



4. *(Optional)* Click the hyperlinked Sample name to open the Immune Repertoire Results for that sample. See “View Oncomine™ TCR Beta-LR Assay GX run results” on page 72 for more information.

5. Click the checkboxes next to the analyses you would like to compare.

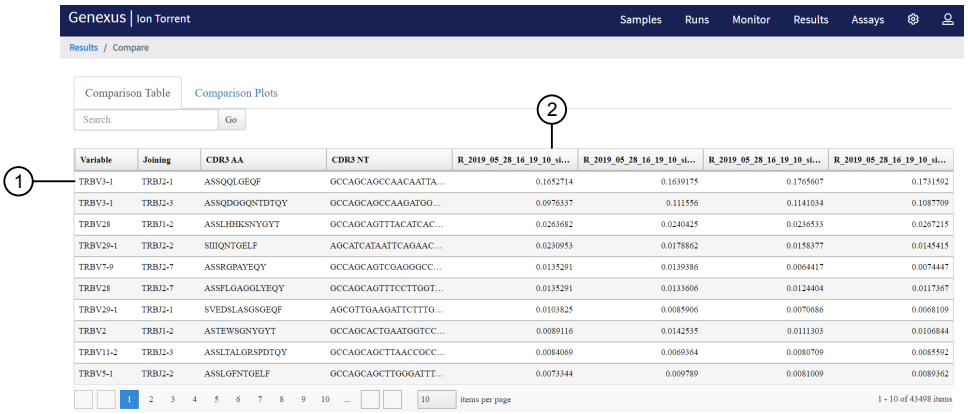
6. In the dropdown menu at the top of the screen, select how you would like to compare clones across the sample.

Options	Description
CDR3 NT	Compare the clones based on the CDR3 nucleotide sequence. If the nucleotide sequence is shared across the samples, those are considered identical clones. This is the least stringent comparison option.
Variable Gene + CDR3 NT	Compare the clones based on the rearrangement of the variable gene plus the CDR3 nucleotide sequence.

7. Click **Compare Samples** to generate a comparison table and plots.

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

8. View variable comparison results in the **Comparison Table** tab.



① Each row represents a clone.
② The frequency of the clone for the listed sample.

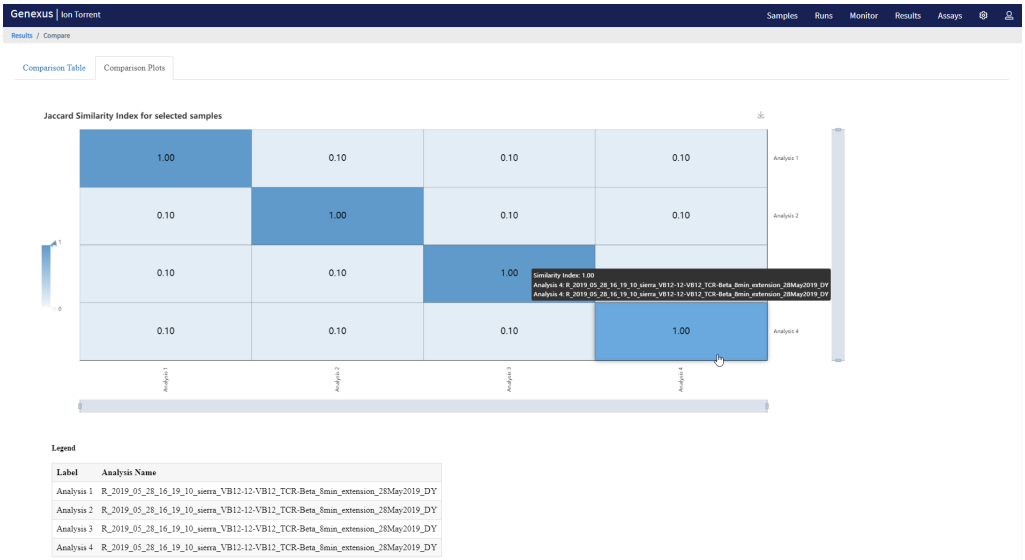
9. View comparison plots in the **Comparison Plots** tab.

Options	Description
Comparisons between 2 analyses.	An interactive scatter plot for the selected analyses is shown in the **Comparison Plots** tab.
Comparisons between more than 2 analyses.	A Jaccard Similarity Index for the selected analyses is shown in the **Comparison Plots** tab.

- The interactive scatter plot indicates the frequency of clones across two analyses. For more information, see “Example correlation and proportion of shared clones” on page 83.
- The Jaccard similarity index is determined for each pairwise comparison and is displayed in heat map format. For more information, see “Example Jaccard Similarity Index for selected samples” on page 83.

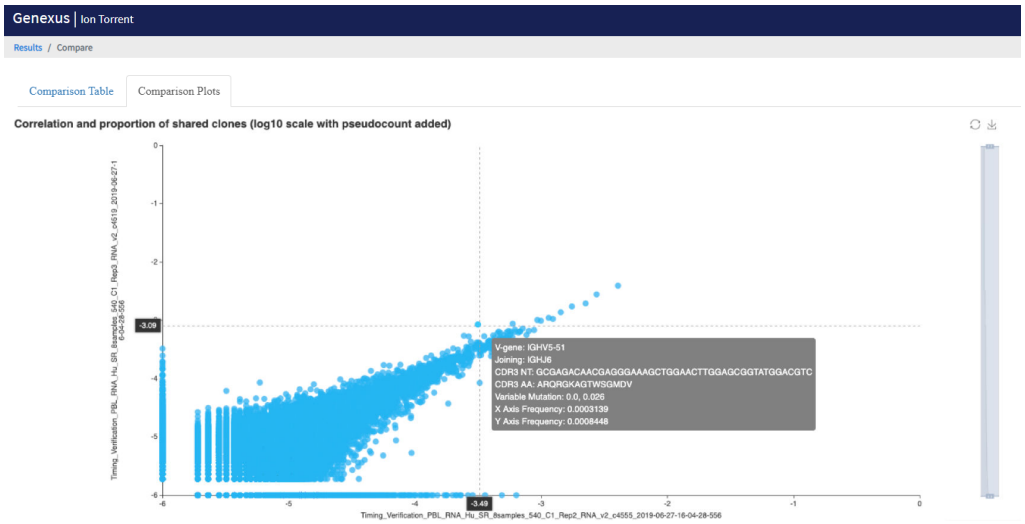
Example Jaccard Similarity Index for selected samples

This is an example of a **Comparison Plots** tab showing the Jaccard Similarity Index for selected samples. The Jaccard similarity index is determined for each pairwise comparison and displayed in heatmap form. The Jaccard Similarity Index ranges from 0 to 1 and is calculated as the total number of shared clones divided by the total number of unique clones across two samples.



Example correlation and proportion of shared clones

This is an example of a **Comparison Plots** tab scatterplot. The illustration indicates the frequency of clones across two samples. Frequency values are log10 transformed with a pseudocount frequency of 1E-6 added to each value. Hovering over a point reveals the CDR3NT and AA sequence of a clone, the variable mutation, and the frequency in either sample.



View completed verification runs

Manager- and administrator-level users can view verification run details, including the run summary, QC summary, and the reagents used in the run. Verification runs are sequencing runs performed during Genexus™ Integrated Sequencer installation to validate the performance of the sequencer.

Note: Field Service Engineers use system-installed verification templates to validate the installation of the Genexus™ Integrated Sequencer. Field Service Engineers can view the template information but cannot modify it.

1. In the menu bar, click **Results** ▸ **Verification Results**.
2. In the **Verification Results** screen, in the **Run Name** column, click the verification run name of interest.
   The **Verification Results Details** screen opens.
3. *(Optional)* Click **Download as PDF** to download the Verification Report file.

Verification runs

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

Column	Description
Run Name	The name of the run, created when the run was planned. Click the name to open the **View Sample Run** dialog box.
Assay Name	The name of the assay used in the run.
Field Engineer Name	The name of the support specialist who performed the run.
Instrument Name	The name of the sequencer that was verified.
Run Status	The current status of the full sequencing run, including analysis.
QC Status	Indicates whether a sequencing run passed or failed, based on the sequencing QC metrics selected for the assay.
Last Updated on	The date and time that the sequencing run was last updated.
Actions	• **Audit**—Click this link to display the list of users who created/edited the run. You can export and print information from the list from the **Audit Trail** dialog box. • **CSA**—Customer Support Archive. Click this link to download all of the sequencer log files. Log files contained within the CSA may be useful when troubleshooting issues with the sequencer. For more information, see “CustomerSupportArchive plugin” on page 170.

Lab Report

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

To automatically generate a Lab Report for each sample during data analysis of a run, select the **Generate Report** checkbox in the **Setup** step when you plan the run (for more information, see Chapter 4, "Plan and manage runs"). To generate a Lab Report for each sample after a run is complete, see "Generate customized reports" on page 88.

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

Lab reports can be electronically signed by manager- and administrator-level users. Electronically signed reports have ***(Signed Off)*** after the sample name in the **Sample Results** screen. The electronic signature is included in the footer of the report. For more information, see "Sign off on the run results (manager/ administrator)" on page 151.

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

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

Download the Lab Report

You can download the Lab Report for a sample of interest.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Actions** column, click **Lab Report** in the row of the sample of interest.
   A ZIP file that contains the PDF report downloads automatically.
3. Extract the downloaded files, then open the PDF file in an appropriate viewer.

Run plugins after a sequencing run is complete

You can run plugins on your sequencing data after a sequencing run is complete using the **Run Plugin** function in the **Results** screen for the sample. Use this command to run one or more plugins on the data for the first time, or to rerun any plugin that has been run before (for example, if a plugin has been updated or failed during analysis).

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name in the **Sample Results** screen.
3. Click ··· **(More Options)**.
4. Click **Run Plugin**.
5. In the **Run Plugin** dialog box, select one or more assays in the run, then select the plugins to run on those assays.
6. Click **Run**.
   While plugins are running, the **Run Plugin** link is unavailable for that run.
7. When analysis is complete, you can review the results. For more information, see Chapter 9, “Plugins for data analysis”.

Reanalyze a run

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

Note:

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

Before you begin, create a new assay or copy the original assay that was used in a run and modify assay parameters as needed. For more information, see Chapter 7, “Create and manage assays (manager/administrator)”.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest.
3. In the upper right corner of the screen, click ··· **(More Options)** ▸ **Reanalyze**.
4. In the **Reanalyze** dialog box, enter or select the following information.
   a. In **Reanalysis Run Name** field, enter a reanalysis run name.
   b. In **Start Reanalysis from** dropdown list, select **Alignment** or **Basecalling**.
   c. In the **Current Assay in Run** column, select the checkbox in the row of each assay that you want to reanalyze, then in the **Choose Assay to Reanalyze** column, select an assay that you want to use for each reanalysis from the dropdown list.
5. Click **Reanalyze**.

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

Generate customized reports

When generating a customized report, you can update any report template selections. For more information on results files, see “Results files” on page 89.

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

Results files

The following files can be downloaded from the **View Results** screen for each sample. The files that are available for download vary depending on the assay used. For a list and descriptions of plugin output files, see "Output files generated by the coverageAnalysis plugin" on page 161 and "Output files generated by the molecular CoverageAnalysis plugin" on page 168.

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

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

Selection	File name	Description
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.
RNA Basecaller FASTAQ File (.fastq)	`<barcode>_rawlib.basecaller.fastq`	FASTQ file generated from the unmapped BAM file of the RNA barcodes used.
Test Fragment Basecaller FASTAQ File (.fastq)	`rawtf.basecaller.fastq`	FASTQ file for the test fragment.
Audit and Log		
Analysis Log File	`analysis.log`	Analysis log file.
Run Summary	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.
Run Audit	`PlannedRun-AuditTrail.pdf`	Contains all audit records pertaining to the run.
Reports		
Lab Report	`<language>_<sample name>_AD_Lab_Report_<assay name>_<date>.pdf`	A PDF report that contains sample-specific results. For more information, see “Lab Report” on page 85.

Upload sample results files to Ion Reporter™ Software

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

You can also upload the samples to Ion Reporter™ Software, then use a custom Ion Reporter™ Software analysis workflow to view analysis results. Or, you can upload sample results files if you want to run assays that are not yet available on the Genexus™ Software Integrated Sequencer.

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

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

- To configure an Ion Reporter™ Server account, see "Configure an Ion Reporter™ Server account (administrator)" on page 129.
- To configure an Ion Reporter™ Software on Connect account, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 127.

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

The sample results are uploaded to Ion Reporter™ Software.

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

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

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

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

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

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

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

Data files and flow

During a Genexus™ Integrated Sequencer run, raw data (DAT) files from the first flows are available. The Genexus™ Software starts processing the data, producing a 1.wells file. Basecalling is performed on the 1.wells file data, producing an unmapped BAM (uBAM) file. Subsequent analysis produces mapped reads (BAM) and variant calls (VCF) files.

The following table shows the flow of data and typical file size generated as the Genexus™ Software processes data.

IMPORTANT! Data from approximately 200 sequencing runs can be accommodated on the sequencer before disk space becomes a limiting factor and data archiving is required. You can perform approximately 45–50 whole chip runs without deleting any wells files on the system. For more information about data archival of the analysis runs, see "Data management (administrator)" on page 141. Note that the 1.wells file is not archived during the archival process.

Process	File type	File size
Sequencing[1]	DAT	2 TB
	▼	
Signal Processing [2]	Wells	250 GB
	▼	
Base Calls (reads)	uBAM	15 GB
	▼	
Mapped Reads	BAM	15–20 GB
	▼	
Variant Calls	VCF	120 Kb

[1] The sequencing raw data (DAT) files are deleted from the sequencer after run completion.

[2] A disk space cleanup process is executed nightly to free up space for future runs and to ensure a 2TB hard disk space is maintained. If the system memory available for storing run analysis data reaches the 2TB threshold, the system auto-deletes 1.wells files for the oldest completed runs. If the system approaches the 2TB threshold, an alert notifies you when you sign in. If desired, you can back up 1.wells files to an external drive.

7 Create and manage assays (manager/administrator)

About assays in Genexus™ Software

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

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

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

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

Find assays

You can sort, search and filter information to narrow the list of assays shown in the **Assays / Manage Assays** screen, then find a specific assay in Genexus™ Software.

Search for assays

You can search for assays Genexus™ Software with keywords or text that are included in an assays name.

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 sample names returns a list of all samples with a name that includes ***demo***. For example, demo1, demo2, and so on.
- Spaces are removed during searches, and are therefore not recommended. For example, a search for ***demo 1*** would return results only for data that includes the string ***demo1***.

1. Click **Assays ▸ Manage Assays** to open the **Manage Assays** screen.
2. In the **Assay Name** field, enter a full or partial assay name, then click **(Search)**.

The list of assays is updated according to the search terms.

Sort the list of assays

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

1. Click **Assays ▸ Manage Assays** to open the **Manage Assays** screen.
2. Click a column heading in the list of assays.
   If the column is sortable, the contents of the column is sorted alphabetically, or chronologically.
3. Click the column heading again to reverse the order.

Filter the list of assays

You can apply filters to the list of assays to narrow search results, or shorten the list of assays to make easier to find assays in Genexus™ Software.

1. Click **Assays ▸ Manage Assays** to open the **Manage Assays** screen.
2. Click the **Filter Assays By** dropdown menu, then apply the filters.
   - Select **Status** to expand the list of options for status, then select a status:
     - Locked & Draft
     - Obsolete
     - Locked
     - Draft

- Select **Research Application** to expand the list of options for research application, then select the research application that is associated with the assay:
  - DNA
  - Fusions
  - RNA
  - DNA and Fusions
- Select **All** to return to the complete list of assays.

The list of assays is filtered according to the selections.

Remove filters

You can remove filters from the list of assays to go back to previous search results or to return to the complete list of assays.

1. Click **Assays ▸ Manage Assays** to open the **Manage Assays** screen.
2. Remove one or both of the filters applied to the list of assays.
   - In **Assay Name** search field, click ✕ **(Remove)** to remove the contents of the field.
   - Select **All** in the field that is used to filter assays by **Status** or **Research Application**.

The list of assays is filtered according to the selections.

Assay creation overview

Manager- and administrator-level users can create custom assays if system-installed assays do not meet needs.

To create a custom assay in Genexus™ Software:

- Copy an existing system-installed assay or a custom assay, then edit its settings to create a new custom assay. For more information, see “Copy an assay (manager/administrator)” on page 98.
- Use a template to create a custom assay that contains unique settings. The template includes settings for common genetic research analysis applications. You can copy an existing custom assay only if it is locked. For more information, see “Create an assay (manager/administrator)” on page 97 and “Lock a draft assay (manager/administrator)” on page 124.

Create an assay (manager/administrator)

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

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

IMPORTANT! For an assay to be used in run planning, it must first be locked.

1. In the menu bar, click **Assays** ▸ **Create Assay**.
2. In the **Create Assay** screen, click the assay type for the template that provides the appropriate kits, chips, primer panels, and adjustable QC thresholds and read mapping parameters.

Assay type	Description
Generic Sequencing Application	Use this template to create a generic sequencing assay with no application-specific post-run analysis. The assay generates BAM files.
DNA Germline	Use this template to create an assay that detects and annotates germline variants in DNA samples.
DNA and Fusions	Use this template to create an assay that detects and annotates somatic DNA variants and RNA fusion targets in a sample.
DNA Somatic	Use this template to create an assay that detects and annotates somatic variants in DNA samples.
Fusions	Use this template to create an assay that detects and analyzes variants in fusion samples.

3. In the **Panel** step, select a panel and other options for the assay. For descriptions of the panel information appropriate for your assay, see “Panel step assay options” on page 99.

   IMPORTANT! You must add an integer value greater than zero for **Minimum Read Count Per Sample**. The remaining options are appropriately populated based on the selected panel.

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 primer panel that you are using, then click **Next**.

   For descriptions of the reagent information appropriate for your assay, see “Reagent step assay options” on page 101.

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

   For descriptions of the QC information appropriate for your assay, see "QC step assay options" on page 102.

7. In the **Parameters** step, accept the recommended default values, or adjust the values within the allowed ranges, then click **Next**.

   Optionally, you can set these parameters by uploading an advanced parameters configuration file that overrides the default settings. To create an advanced parameters configuration file, at the top of the **Parameters** step, click **Download** to download a ZIP file of the JSON template that is appropriate for the assay, edit the file with your custom settings, then save the file to your drive. Then, at the top of the **Parameters** step, click **Upload**, click **Select file** to navigate to the file on your drive, then click **Open** to upload the file. The parameters uploaded in this file override the default values in the template.

   For more information about parameters, see "Parameters step assay options" on page 105.

8. In the **Plugins** step, select one or more plugins to run with this assay, then click **Next**.

   For more information, see Chapter 9, "Plugins for data analysis".

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

   The new assay is listed in the **Manage Assays** screen. The **Status** column indicates that the assay is in **Draft** status. The new assay is available to lock, edit, or delete, and audit. You can also download parameters from assays in the either the **Draft** or **Locked** status.

Copy an assay (manager/administrator)

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

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

2. In the **Manage Assays** screen, in the **Actions** column for the assay that you want to copy, click **Copy**.

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

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

4. When finished, enter a new name and short name for the copied assay in the **Assay Name** field, then click **Save**.

   The newly created assay is added to the list of assays in the **Assays** / **Manage Assays** screen. The assay name is followed by (Draft) in the Assay column. The assay remains in draft status until it is locked.

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

Assay options

Panel step assay options

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

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

Following is an alphabetical list of options that may be available in the **Panel** step, depending on the type of assay.

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

Option	Description
Adjust Sample Copy Number by Cellularity[1] only. It is available when a **Copy Number Baseline for CNV Calling** is selected.	The % cellularity that is the minimum value that the assay workflow requires of a sample. The assay analysis adjusts all copy numbers based on the % cellularity entered. Select **Do Not Adjust CN and Do Not Reject Samples** if you choose to not make the adjustment.
Annotation Sets	The annotation set to use in the assay. Some annotation sets are system-installed. Custom annotation sets can be created if the system-installed sets do not meet needs.
Apply Oncomine Variant Annotations	When 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 “Annotation criteria for Oncomine™ Variant Annotator” on page 172 or contact your local support representative, Field Bioinformatics Specialist (FBS), or Clinical Account Consultant (CAC). This option is available only for assays that use Ion AmpliSeq™ HD and Oncomine™ panels.
Copy Number Baseline for CNV Calling	The software analyzes the CNV results based the selected baseline file. The copy number baseline is a set of control samples that are used to create a baseline for the detection of copy number variants (CNVs).You can use system-installed baselines, import or create a baseline, or copy and edit an existing baseline. You can view the settings that are used for the baseline if you click on the baseline name in the **Assays/Manage Presets/Copy Number Baselines** tab. For more information, see “Copy number variant baselines (manager/administrator)” on page 115.
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.

Option	Description
Fusion Panel	The tab-delimited BED file to use in the assay that defines the coordinates of the regions of interest to be amplified with the selected primer set.
Fusion Reference	The tab-delimited BED file to use in the assay that indicates the positions where an RNA fusion target is expected to occur. It also annotates these positions.
Genome Reference	The genome reference file to use in the assay. The hg19 genome reference is installed with the system.
Hotspots	The hotspots file to use in the assay. The hotspots file is a 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.
Library Chemistry	The type of library chemistry to use in the assay.
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 on the lanes that are available on a chip. The system uses this value to calculate how many lanes a run uses and assigns samples accordingly. 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.
Panel	The panel to use in the assay. The remaining options are appropriately populated based on the selected panel. The panel is a tab-delimited BED file that defines the coordinates of the regions of interest to be amplified with the selected primer set.
Primer Pools	The number of primer pools used in the assay.
Target Regions	The target regions BED file that is appropriate for your sample. Depending on your application, you may need to select a separate DNA and Fusions Target Regions file. Check with your local Field Service Engineer for updates to ensure that the most current files are being used.

[1] This option is available for assays that use **DNA and Fusions Panel**

Reagent step assay options

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

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

Following is an alphabetical list of options that may be available in the **Reagent** step. The available options depend on the type of assay.

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

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	Select **RNA** or **DNA**, or both **RNA** and **DNA**, depending on the assay template that is used. For example, you might want to ensure that both **RNA** and **DNA** are selected if you use the RNA/DNA template. By default, only **DNA** is enabled and **RNA** is disabled. RNA is available for selection only if the assay template includes it.
Sequencing Chip	The sequencing chip to use in the assay.
Sequencing Flows	The number of flows performed by the sequencer during the sequencing stage of the run.
Sequencing Kit	The sequencing kit to use in the assay.
Templating Kit	The templating kit to use in the assay.
Template Size (bp)	The template size, measured in base pairs, to use in the assay. • **200** • **400**

QC step assay options

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

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

Following is an alphabetical list of options that may be available in the **QC** step. The available options depend on the type of assay.

NTC QC - DNA

Option	Description
Average Base Coverage Depth	The average number of reads of all targeted reference bases.
Mean Read Length 9bp)	The average length, in base pairs, of called reads.

NTC QC - RNA

Option	Description
Mapped Reads	**Mapped Reads** and **Mean Read Length** can be configured in the **QC** step or in the **Parameters** step in the **Fusions** section as **Minimum Total Valid Mapped Reads** and **Minimum Mean Read Length for Valid sample QC**. The values configured in the **Parameters** step overwrite those configured in the **QC** step.
Mean Read Length (bp)	The average length, in base pairs, of called reads.

CF-1 Control

Option	Description
Average Reads Per Lane	The average number of reads per chip lane.
Base Call Accuracy	The percentage of the total number of errors for all positions in CF-1/ total number of CF-1 base reads.
Mean AQ20 Read Length (bp)	The average length, in base pairs, at which the accuracy rate is ≥99% for reads of a library.

Run QC

Option	Description
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.
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.
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))).

Sample QC - RNA

Option	Description
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 do not get called. The MAPD metric does not affect SNVs/INDEL calls.
Mapped Reads	**Mapped Reads** and **Mean Read Length** can be configured in the **QC** step or in the **Parameters** step in the **Fusions** section as **Minimum Total Valid Mapped Reads** and **Minimum Mean Read Length for Valid sample QC**. The values configured in the **Parameters** step overwrite those configured in the **QC** step. Best practice to achieve consistent results is to use the same values in both the **QC** step and the **Parameters** step.

Option	Description
Mean AQ20 Read Length (bp)	The average length, in base pairs, at which the accuracy rate is ≥99% for reads of a library.
Mean Read Length (bp)	The average length, in base pairs, of called reads.
RNA Expression Ctrls Detected	The number of expression control genes detected for the sample. This metric measures the RNA input integrity, input amount, and the fidelity of the reverse transcriptase that was used in library preparation. Fusion panels include primer pairs that cover seven control housekeeping genes. For Oncomine™ Precision Assay GX to pass QC, cfTNA samples require 2 out of 7 control genes to be detected and FFPE RNA samples require 5 out of 7 control genes to be detected.

Sample QC - DNA

Options	Description
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.
Mapped Reads	The number of reads aligned to the reference.
Mean AQ20 Read Length (bp)	The average length, in base pairs, at which the accuracy rate is ≥99% for reads of a library.
Mean Read Length (bp)	The average length, in base pairs, of called reads.
Mean Red Cov	Mean molecular coverage of targeted CNV gene.
Median Mol Cov	Median molecular coverage of non-CNV reference loci.

Options	Description
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.
Uniformity of Base Coverage	The percentage of reads showing a depth of coverage ≥20% of the mean base coverage.

Parameters step assay options

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

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

The available options depend on the type of assay. You can view the complete of parameters when the **All** is selected. Or, you can select an option to filter the list:

- **Library Prep & Templating Parameters**
- **Primary Analysis Parameters**
- **Read Mapping**
- **Annotation**
  - CNV Finding
  - Fusions
- **Variant Finding**

You can see a description of most parameters if you hover over ⓘ **(Learn More)** next to a parameter.

In the **Parameters** step, you can accept the default settings for the assay parameters, or adjust the settings if needed.

Filters and filter chains

A filter chain is a set of filters that Genexus™ Software uses to narrow the list of variants that are included in results. There are two ways to use filter chains. You can apply the filter chain to results to dynamically change the view of the variants, then optionally save the filter chain to the results. See “Filter results using a filter chain” on page 71.

Genexus™ Software includes predefined filter chains that you can apply to run results. Most system-installed assays include default filter chains. For information about system-installed filter chains, see “System-installed filter chains” on page 178.

Manager- and administrator-level users can create custom filter chains that are built from system-installed filter chains, or from one or more system-installed filters. For more information, see “Create a custom filter chain (manager/administrator)” on page 106. The system-installed filters are based on public and proprietary annotation

sources and data types that are included in the software. For more information, see “System-installed filters” on page 178.

Review filter chain details

A filter chain is a set of filters that Genexus™ Software uses to narrow the list of variants that are included in results. Administrator-level users create custom filter chains from system-installed filters to meet specific variant filtering needs.

After a run, you can review the details of the filter chains that were applied to the run. If the filter chain does not meet your needs, you can adjust the settings or apply a different filter chain.

1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Manage Presets** screen, click the **Filter Chains** tab.
3. Click a filter chain.

Review the following details:

- Version of the analysis pipeline used for the filter chain
- Whether the filter chain is system-installed
- The date that the filter chain was created
- The filters that are included in the filter chain
- The order in which the filters and/or filter chains contained within are applied

Create a custom filter chain (manager/administrator)

Manager- and administrator-level users can create and manage filter chains. A filter chain is a set of filters that you apply to the variants identified in your results. Filter chains are used to narrow the analysis results to only those variants of interest.

1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Manage Presets** screen, in the **Filter Chains** tab, click + **Add New**.
3. In the **Create Filter Chains** dialog box, enter a name for the new filter chain.
4. (Optional) Add a description for the filter chain.
5. From the **Choose Filter** list, select the filter that you want to use, then click **Set**.
   The selected filters are shown on the right side of the window. If the list does not populate with the filters available in the software, you may need to reboot the sequencer.
6. Click **Save**.
   A confirmation message appears, and the new filter chain is listed in the **Filter Chains** tab and is available for selection when you view results. For more information, see “Filter results using a filter chain” on page 71.

Custom filter chain examples (manager/administrator)

Manager- and administrator-level users can create custom filter chains for use in Genexus™ Software.

Create a filter chain query (manager/administrator)

Manager- and administrator-level users can create custom filter chains that combine filters and specify the order in which the filters are applied. The modifiers are AND and OR, and brackets ( ) are used to set the order. The modifiers AND and OR must be in all caps.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

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1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Manage Presets** screen, click the **Filter Chains** tab.
3. Click + **Add New**.
4. In the **Create Filter Chains** dialog box, enter a name and optional description, then in the **Choose Filter** list, add the filters to create a chain.

   To include unannotated variants in the results, select **Include Unannotated Variants** for each filter selection.

   a. Select **Deamination**, set its value to **In**, then click **Set**.
   The filter name appears on the right side of the **Create Filter Chain** dialog box.

   b. Select a **dbsnp** filter, set its value to **In**, then click **Set**.
   The second filter name appears on the right side of the **Selected Filter Chains** list.

   c. Select **COSMIC(85)**, set its value to **In**, then click **Set**.
   The third filter name appears on the right side of the **Create Filter Chain** dialog box.

5. (Optional) If you want change the order in which your filters are applied, click 🗑 **Delete** to remove a filter, then reselect the filter and click **Set** to reinsert the filter in the **Selected Filter Chains** list.
6. Click **Save**.

A confirmation message appears, and the new filter chain is listed in the **Filter Chains** tab and is available for selection when you view results. For more information, see “Filter results using a filter chain” on page 71.

Create a COSMIC ClinVar and Variant Impact filter chain (manager/administrator)

Manager- and administrator-level users can create a custom filter chain that includes both annotation source filters and variant data type filters. This procedure shows how to create a COSMIC, ClinVar, Allele Fraction, and Variant Effect filter chain.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

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1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Manage Presets** screen, click the **Filter Chains** tab.
3. Click + **Add New**.
4. In the **Create Filter Chain** dialog box, enter a name and optional description.
5. Select the filters to add to the filter chain.

   To include unannotated variants in the results, select **Include Unannotated Variants** for each filter selection.

   a. In the **Choose Filter** list, choose a version of the **COSMIC** filter.

   b. Select the annotations that you want to include in the filter.

   c. Click **Set**.

   d. In the **Choose Filter** list, select a version of **clinvar**.

   e. Select the annotations that you want to include in the filter.

   f. Click **Set**.

   g. In the **Choose Filter** list, select **Allele Fraction**.

   h. Enter a range value, then click **Set**.

   i. In the **Choose Filter** list, select **Variant Effect**.

   j. Select the value that you want to include in the filter to detect the effect of variants on coding sequences.

   k. Click **Set**.
6. Click **Save**.

Your filter chain can now be applied to results. For more information, see “Filter results using a filter chain” on page 71.

Create a PValue/dbSNP/Variant Type filter chain (manager/administrator)

Manager- and administrator-level users can create a custom filter chain with multiple filters. This example shows how to create a filter chain with PValue, dbSNP, and Variant Effect filters.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

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1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Manage Presets** screen, click the **Filter Chains** tab.
3. Click + **Add New**.
4. In the **Create Filter Chain** dialog box, enter a name and optional description.

   To include unannotated variants in the results, select **Include Unannotated Variants** for each filter selection.
   a. Select the **PValue** filter.
   b. Enter a range
   c. Click **Set**.
5. In the **Choose Filter** list, select a version of **dbSNP**.
   a. Set **Filter value** to **In**.
   b. Click **Set**.
6. In the **Choose Filter** list, select **Variant Effect**.
   a. Select the value that you want to include in the filter to detect the effect of variants on coding sequences.
   b. Click **Set**.
7. Click **Save**.

Your filter chain can now be applied to results. For more information, see “Filter results using a filter chain” on page 71.

Delete a custom filter chain

Manager- and administrator-level users can make filter chains unavailable in the software. You cannot designate as obsolete system-installed filter chains.

1. Click **Assays** ▸ **Manage Presets** ▸ .
2. In the **Filter Chains** tab, click **Obsolete** for the filter chain that you want to make unavailable.

The filter chain status is Obsolete. The filter chain is removed from the **Filter Chains** table.

Create a gene symbol filter (manager/administrator)

Manager- and administrator-level users can create a custom filter that is based on gene symbols. This filter can be added to a filter chain that narrows analysis results to only specific genes. If you have numerous gene symbols to filter, you can use a text file to create the gene symbol filter chain. For more information, see “Gene Symbol filter” on page 182.

IMPORTANT! If using a single filter with a NOT operator, do not use a parenthesis. If parenthesis is used, the filter chain can be saved but an error occurs when applying the filter chain in analyses results. For example, use ***NOT Filtered Coverage*** instead of ***NOT (Filtered Coverage)***.

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1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Manage Presets** screen, click the **Filter Chains** tab.
3. Click + **Add New**.
4. In the **Create Filter Chain** dialog box, enter a name and optional description, then in the **Choose Filter** list, choose the **Gene Symbol** filter.
5. Add genes of interest to the filter chain. Select a checkbox for the gene symbol of the gene in the list that you want to add.

   To find a gene symbol in the list, enter the gene symbol into the search field, then click 🔍 **(Search)**.
6. After you select all of the gene symbols that you want to include, click **Set**.

   Your genes appear in the **Selected Filter Chains** list.
7. Click **Save**.

Your filter chain can now be added to an assay, or applied to results. For more information, see “Filter results using a filter chain” on page 71.

Annotation sets (manager/ administrator)

Manager- and administrator-level users use the tools for creating and managing annotation sets that are available in the **Assays / Manage Presets** screen in the **Annotation Sets** tab.

View annotation set details

You can view details about an annotation set in Genexus™ Software.

1. Click **Assays ▸ Manage Presets**.
2. In the **Assays / Manage Presets** screen, in the **Annotation Sets** tab, click the annotation set name.

The following details for the selected annotation set are shown.

Annotation Set Details

Item	Description
Application Version	The analysis pipeline version that is used by the annotation set.
Last Modified By	For custom annotation sets, the sign-in name of the person who created or modified a custom annotation set. Annotation sets that are included with the software are labeled as **System Installed**.
Last Modified On	Date on which a custom annotation set was last modified.
Created By	Sign-in name of the person who created a custom annotation set.
Created On	Date on which a custom annotation set was created.
Status	Statuses of the assay, including: • Locked • Draft • Obsolete
Reference	hg19
Annotations	A list of the annotations included in the annotation, and the version of each annotation.

Create a new annotation set (manager/administrator)

Manager- and administrator-level users can create custom annotation sets in Genexus™ Software. The custom annotation sets are available as an option when you create or copy an assay.

1. Click **Assays** ▸ **Manage Presets**.
2. In the **Manage Presets** screen, in the **Annotation Sets** tab, click + **Add New**.
3. In the **Create Annotation Set** screen, enter a name for the set in **Name**, and an optional description in **Description**.
4. Click the value in **Application Version** to accept the default version of Ion Reporter™ Software, or select the software version that is installed in your lab. The **Application Version** field is highlighted and a checkmark is shown for the selected software version.
5. In **Genome Reference**, the default setting for the sample is **hg19**.

6. In the **Available Annotation Sources** dropdown list, select the annotation source.

Source	Description
5000 Exomes	Population frequency information from the 5000 Exomes project. Source: **https://evs.gs.washington.edu/EVS/**
ClinVar	An assessment of impact of the variants observed from NCBI ClinVar database. Source: **http://www.ncbi.nlm.nih.gov/clinvar**
COSMIC	A catalog of known somatic mutations in cancer. Source: **https://cancer.sanger.ac.uk/cosmic**
dbSNP	A database of genomic variants. Source: **http://www.ncbi.nlm.nih.gov/projects/SNP**
DGV	A curated database of human genomic structural variation. Source: **http://dgv.tcag.ca/dgv/app/home**
DRA	Disease Research Area; a hierarchically organized database of disease categories.
DrugBank	A database from which names of therapies that are used for researching variants are collated. Source: **https://www.drugbank.ca**
ExAC	Exome Aggregation Consortium — A database catalog of variant frequencies. Source: **https://gnomad.broadinstitute.org**
Gene Model	A set of genes that the variant overlaps. Sources: **https://www.ncbi.nlm.nih.gov/refseq** and **http://www.ensembl.org**
Gene Ontology	Standardized ontology for gene and gene projects (for example, functional role or localization). Source: **http://geneontology.org**
Gene Set (Custom)	Select this option to import a TXT file that contains a custom set of variants in a specific set of genes.
Genomic Regions (Custom)	Select this option to import TXT file that contains a custom set of variants in specific gene regions.
OMIM	Online Mendelian Inheritance of Man. Source: **https://www.ncbi.nlm.nih.gov/omim**
Pfam	A database of protein families that includes their annotations and multiple sequence alignments using hidden Markov models. Source: **http://pfam.xfam.org**
PhyloP Scores	A measure of conservation of the protein across a wide range of organisms. Source: **http://compgen.cshl.edu/phast**

Source	Description
SIFT / PolyPhen	A prediction of the functional effect of a variant on a protein. Sources: **https://sift.bii.a-star.edu.sg** and **http://genetics.bwh.harvard.edu/pph2** and
Transcript Set (Custom)	Select this option to import a TXT file that contains a custom set of transcript annotations.
Variant DB (Custom)	Select this option to import a TXT file that contains custom set of variant annotations.

The annotation sources that you select are listed in the **Available Annotation Sources** list.

7. Add annotation sources to the annotation set.

Options	Description
Use an existing source	Select the checkbox next to each annotation source in **Available Annotation Sources** list that you want to include in the annotation set.
Import a new custom source file in a TXT file [1]	Click + **Add New Source** below the list, enter a name and version for the source, browse to and select the source file, then click **Upload**. The new sources are added to **Available Annotation Sources** list.

[1] You must have selected one of the following **Available Annotation Sources**:**Gene Set (Custom)**, **Genomic Regions (Custom)**, **Transcript Set (Custom)**, **VariantDB (Custom)**.

8. After all of the annotation sources that you want to add to the annotation set are selected in the **Available Annotation Sources** list, click **Add** › to move the annotation sources to the **Selected Annotation Sources** list.

9. Review the list of selected annotation sources, then add or remove sources, if needed. To remove a source from the **Selected Annotation Sources** list, select the checkbox, then click **Remove** ✕.

10. Click **Save** to save the annotation set.

The annotation set is listed in the **Annotation Sets** tab and is available when you create an assay.

Make custom annotation sets unavailable

Manager- and administrator-level users can make custom annotation sets unavailable in the software.

You cannot designate as obsolete system-installed annotation sets.

1. In the menu bar, click **Assays ▸ Manage Presets**.
2. In the **Annotation Sets** tab, in the **Actions** column, click **Obsolete** for the custom annotation set that you want to make unavailable.

The annotation set status is **Obsolete** in the list of annotation sets

Copy number variant baselines (manager/ administrator)

A copy number baseline is a set of control samples that are used to create a baseline for the detection of copy number variants (CNVs). A copy number baseline is associated with a panel file in the software. CNV analysis is based on reads counts for each amplicon. Read counts for the sample in question are compared to the expected read counts for the amplicons in a normal sample, which are designated in the copy number baseline. The calculations used to determine the CNV call are based on the parameters set in the copy number baseline.

Known sources of variability include pool imbalance (when an assay has more than one pool of amplicons), total number of reads, per-amplicon attributes of GC proportion, and length of the amplicon insert. In practice, we observe other variability that does not associate with known attributes yet is systematic. The software makes adjustments to account for specific types of variability in the analysis that generates a copy number estimate. The method that is used by the software to identify copy number variations is based on many diverse samples, captures systematic effects, and encodes the samples into a file that comprises the baseline for the panel.

Copy number estimates are made using a proprietary algorithm. The algorithm uses an informatics baseline, which is created using at least 48 diverse samples, to allow assessment of corrected log2ratios of amplicons of identified CNV regions (usually genes) in input sample data. This algorithm is followed by a correction algorithm for the percent tumor cellularity recorded for the sample to give copy number and confidence interval data for the identified CNV regions. The algorithms used to calculate the corrected log2 ratios and the correction for tumor fraction are proprietary.

Manager- and administrator-level users can perform the following functions related to copy number baselines.

Option	See the following section for more information
View details for system-installed, or custom, copy number baselines.	"View copy number baseline details (manager/administrator)" on page 116
View the panel associated with a copy number baseline.	"Panel files (manager/administrator)" on page 145
Create a copy number baseline.	"Create a copy number baseline (manager/administrator)" on page 117
Edit a copy number baseline.	"Edit a copy number baseline (manager/administrator)" on page 118
Import a copy number baseline.	"Import a copy number baseline (manager/administrator)" on page 120
Remove copy number baselines that are not system-installed.	"Remove a copy number baseline (manager/administrator)" on page 120

View copy number baseline details (manager/administrator)

Manager- and administrator-lever users can view details for the system-installed copy number baselines, or for custom copy number baselines.

1. In the menu bar, click **Assays ▸ Manage Presets**.
2. In the **Assays / Manage Presets** screen, click the **Copy Number Baselines** tab.
   The **Copy Number Baselines** table lists both system-installed and custom copy number baselines.
3. Click the name of a copy number baseline.

Review the details in the **Copy Number Baseline Details** dialog box.

Copy Number Baseline Details

Item	Description
Created By	Sign-in name of the person who created the baseline. System-installed baselines are included with the software.
Created On	Date on which the baseline was created.
Number of Normal Samples	The number of normal samples that are included in the baseline.
Gender	The biological sex of the sample: Female, Male, or Unknown.

Item	Description
Amplification Type	Library amplification technology that is used in the baseline (Ampliseq or Ampliseq HD).
Panel Name	Name of the panel that is included in the baseline.
Reference File Name	The name of the genome reference file that is used in the baseline.
Number Amplicons per Bin for GC normalization	A threshold set for the baseline. This threshold should not be more than about 10% of the number of amplicons in the target regions file.
Number PC for correction	The number of principal components to use for correction
Similarity Threshold	Maximum similarity threshold allowed for any two samples used for creating the copy number baseline.
Number Amplicons per Bin for Amplicon Length normalization	A threshold set for the baseline. This threshold should not be more than about 10% of the number of amplicons in the target regions file.

Create a copy number baseline (manager/administrator)

Manager- and administrator-level users can create a copy number baseline.

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

1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Assays / Manage Presets** screen, click the **Copy Number Baselines** tab.
3. Click + **Add New**.
   The baseline creation tool opens in the **Panel** step.
   Use the **Next** and **Previous** buttons to navigate the screens to configure the baseline.
4. Complete the **Panel** step.
   a. Enter a name in the **Copy Number Baseline Name** field.
      The name is limited to 50 characters and no spaces are allowed.
   b. Select a **Panel** to use.

c. Enter information about the baseline into the **Notes** field.

d. Click **Next**.

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

6. In the **Select Samples** step, select samples for the baseline.

   All of the samples that have been run with the selected panel are listed.

   If you do not select the minimum number of samples that are required for the baseline, an error message opens and you cannot proceed. You can adjust the **Minimum number of samples required to add to an existing baseline** parameter in the **CNV Baseline Creation** section of the **Parameters** step.

   a. Select the checkbox next to each sample to use for the baseline.

   b. Click in the **Normal Sample** column to designate a selected sample as "Normal" in the baseline.

      The tube icon changes to green. A **Normal Sample** is designated with .

   c. Click **Next**.

7. In the **Review Selected Samples** step, review the selections, then click **Save**. The new copy number baseline with a status of **Draft** is added to the **Copy Number Baselines** tab.

8. Click **Lock** in the **Actions** column to lock the copy number baseline.

New copy number baselines that are locked are available for selection when an assay is created or copied and edited. Copy number baselines can only be added to **DNA and Fusions**, **DNA Somatic** and **DNA Germline** assays.

Note: If the status of a copy number baseline is **Failed**, click **View Log** in the **Actions** column of the table of copy number baselines.

Edit a copy number baseline (manager/administrator)

Manager and administrator users edit an existing copy number baseline by adding or removing samples in the baseline or by changing the parameters used for analysis. Edited copy number baselines are saved with a new name and are listed as a new copy number baseline; the original copy number baseline used as a base remains unchanged in the software.

When a baseline is augmented with the addition of samples, new samples are run, the size of each systematic affect encoded in the baseline is estimated, and a correction is applied to remove the effect. Any added samples can not be designated

as a **Normal Sample** and should be diverse so as to capture likely systematic variation.

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

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1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Assays / Manage Presets** screen, click the **Copy Number Baselines** tab.
3. Click + **Add New**.

   The baseline creation tool opens in the **Panel** step.

   Use the **Next** and **Previous** buttons to navigate the screens to configure the baseline.
4. Complete the **Panel** step.
   a. Enter a name in the **Copy Number Baseline Name** field.

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

   b. Select a **Panel** to use.

   c. Select the **Use an Existing Copy Number Baseline as Base** checkbox, then select the baseline you would like to edit or augment.

   d. Enter information about the baseline into the **Notes** field.

   e. Click **Next**.
5. In the **Set Parameters** step, accept the default settings or adjust the appropriate parameters, then click **Next**.
6. In the **Select Samples** step, select samples for the baseline.

   All of the samples that have been run with the selected panel are listed.

   If you do not select the minimum number of samples that are required for the baseline, an error message is displayed and you cannot proceed. You can adjust the **Minimum number of samples required to add to an existing baseline** parameter in the **CNV Baseline Creation** section of the **Parameters** step.
   a. Select the checkbox next to each sample to use for the baseline.

   b. Click **Next**.
7. In the **Review Selected Samples** step, review the selections, then click **Save**. The new, edited copy number baseline with a status of **Draft** is added to the **Copy Number Baselines** tab.
8. Click **Lock** in the **Actions** column to lock the copy number baseline.

Copy number baselines that are locked are available for selection when an assay is created or copied and edited. Copy number baselines can only be added to **DNA and Fusions**, **DNA Somatic** and **DNA Germline** assays.

Note: If the status of a copy number baseline is **Failed**, click **View Log** in the **Actions** column of the table of copy number baselines.

Import a copy number baseline (manager/administrator)

1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Assays / Manage Presets** screen, click the **Copy Number Baselines** tab.
3. Click **Import Copy Number Baseline** in the top right of the screen, then browse for and select the file.
   A message confirms that the file import was successful when the file import is complete.
4. Click **Lock** in the **Actions** column to lock the copy number baseline.

Imported copy number baselines that are locked are available for selection when an assay is created or copied and edited. Copy number baselines can only be added to **DNA and Fusions**, **DNA Somatic** and **DNA Germline** assays.

Remove a copy number baseline (manager/administrator)

Manager- and administrator-level users can make copy number baselines unavailable in the software. You cannot designate as obsolete system-installed copy number baselines.

1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Assays / Manage Presets** screen, click the **Copy Number Baselines** tab.
3. In the **Copy Number Baselines** tab, click **Obsolete** for the copy number baseline that you want to make unavailable.

The copy number baseline is **Obsolete** in the list of copy number baselines.

Report templates (manager/administrator)

Manager- and administrator-level users create and manage custom report templates that are used to generate PDF reports after a run. Users select a report template when they plan a run.

Create a report template (manager/administrator)

Manager- and administrator-level users create custom report templates that are used to generate PDF reports after a run.

IMPORTANT! For a report template to be used in a run, it must first be locked.

1. In the menu bar, click **Assays** ▸ **Manage Presets**.
2. In the **Manage Presets** screen, click the **Report Templates** tab.
   Existing report templates are listed in this tab, including any system-installed templates.
3. Click + **Add New**.
4. In the **Create Report Template** screen, enter a unique report name, then complete the remaining sections.
   a. In the **Lab Information** section, enter the laboratory information to display on reports.
   b. In the **Sample Details** section, drag and drop the sample attributes that you want to appear in the report into one of the three column placeholders. You can also drag and drop the additional attributes below the columns into the columns. Select up to two checkboxes next to an attribute to include the attribute in the report header on each page.
   c. Complete the remaining sections, then click **Save**.
      The new report template appears in the **Report Templates** list.
5. To preview the report layout, in the **Actions** column, click ⋮ **(More Options)**, then click **Preview** in the row of the new template. Click **Edit** to make changes.
6. When you are finished, click **Lock**.

Manage report templates (manager/administrator)

Manager- and administrator-level users use the tools in the **Assays / Manage Presets** screen under the **Report Templates** tab to manage report templates.

1. In the menu bar, click **Assays** ▸ **Manage Presets**
2. In the **Manage Presets** screen, click the **Report Templates** tab.
   - To lock a report template, click **Lock**.
   - To preview the template layout, in the **Actions** column, click ⋮ **(More Options)**, then click **Preview**.
   - To make a template unavailable in the software, in the **Actions** column, click **Obsolete**.
   - To add or view a note attached to a report template, in the **Actions** column, click ⋮ **(More Options)**, then click **Notes**.

Manage assays (manager/administrator)

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

Import an assay (manager/administrator)

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

1. In the menu bar, click **Assays** ▸ **Manage Assays**.
2. In the **Manage Assays** screen, click **Import Assay**.
3. In the **Import Assay** dialog box, click **Select Assay file**, navigate to the exported assay ZIP folder on your drive, then select it.
4. Navigate to the exported assay ZIP folder on your drive, select it, then click **Upload**.

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

Export a locked assay (manager/administrator)

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.

1. In the menu bar, click **Assays** ▸ **Manage Assays**.
2. In the **Assays** ▸ **Manage Assays** screen, in the row of a locked assay, in the **Actions** column, click **Export**.

The assay parameter files are downloaded to your local drive as a ZIP file and are available for import to another sequencer.

Panel reference files are not included in the exported file.

Review and export an assay history (manager/administrator)

You can review, export, or print the entire history of changes for an assay. The history shows the original values and any new values or activities.

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

1. In the menu bar, click **Assays ▸ Manage Assays**.
2. In the **Manage Assays** screen, in the row of an assay, **Actions** column, click **Audit** .
   The **Audit Details** screen opens with time-stamped audit records that include assay details and changes for each action performed on the assay.
3. *(Optional)* Review the audit record.

Details in the audit record

Item	Description
User name	The name of the person who was signed in when the action was performed.
Action Performed	Actions performed on the assay include: Create, Edit, Delete, and Obsolete.
Assay Name	The name of the assay.
Time Stamp	The time and date of the action.
Data Object Name	The data object that is affected by the action.
ID	An identifier to represent a specific action in the audit record. Each action in the audit record includes a unique identifier.
New Values	A list of all values in the assay. If a value changed as a result of the action, the new value is shown. If no change was made, the value is listed as **NA**. For example, **Plan Executed Status=false** indicates a change; **Template Kit Barcode=NA** indicates that the value did not change.
Old Values	A list of the previous values in the assay. Values are listed for each change. For example, **State = Draft** indicates that a change was made to the state of the assay. The new value is listed in the **New Values** section.

4. *(Optional)* Click **Download** to download a print-ready PDF file of the audit record.

   You can open the PDF file in an appropriate viewer, then print the record from within the open document.

5. Click **Back** to return to the **Manage Assays** screen.

Edit a draft assay (manager/administrator)

When a manager- and administrator-level user first creates an assay, the assay name is followed by **(Draft)** in the **Assay** column. While the assay is in draft status, it can be edited. A locked assay cannot be edited.

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

1. In the row of a draft assay, in the **Actions** column, click **Edit**.
   The **Create Assay** workflow reopens.

2. Edit the options on each assay step as desired, then click **Save**.

The assay remains a draft until you lock the assay. For more information, see “Lock a draft assay (manager/administrator)” on page 124.

Lock a draft assay (manager/administrator)

When a manager or administrator first creates an assay, the status of the assay is **Draft**. To use the assay in a run, you must lock it. After an assay is locked, it cannot be edited or deleted.

1. In the menu bar, click **Assays ▸ Manage Assays** to open the **Manage Assays** screen.
   A draft assay has **(Draft)** next to the name.

2. In the row of a draft assay, in the **Actions** column, for an assay with a status of **Draft**, click **Lock**.

The assay is now locked and can be used in a run. **(Draft)** is removed from the assay name in the **Assays ▸ Manage Assays** screen.

Delete a draft assay (manager/administrator)

When an assay is in draft status, it can be deleted from the software. Only draft assays can be deleted. Draft assays are indicated by **(Draft)** next to the assay name.

1. In the row of a draft assay, in the **Actions** column, click **Delete**.

2. In the **Delete Assay** dialog box, click **Yes** to confirm the deletion.

The draft assay is removed from the software and is no longer available in the **Assays / Manage Assays** screen.

Remove access to an assay (manager/administrator)

A manager-or administrator-level user can remove a locked assay from use in the software by designating it obsolete. The assay is not deleted and a record of it is maintained in the audit trail. The results for any runs already performed with the assay remain on the sequencer.

1. In the menu bar, click **Assays ▸ Manage Assays**.
2. In the **Assays / Manage Assays** screen, in the row of a locked assay, in the **Actions** column, click **Obsolete**.
3. Click **Yes** to confirm the operation.
   The assay is no longer available for use in a sequencing run.

Download assay parameters (manager/administrator)

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.

Panel reference files are not included in the export.

1. In the menu bar, click **Assays ▸ Manage Assays**.
2. In the **Manage Assays** screen, in the row of a locked assay, in the **Actions** column, click ··· **(More Options)**.
3. Click **Download Parameters**.
   The assay parameter files are downloaded to your local drive as a ZIP file of JSON files and are available for import to another sequencer.

8 System administration and management (manager/administrator)

Manager-level users use tools in the **(Settings)** menu to:

- Create and manage user email settings, and audit these settings.
- Create and link user accounts for to get software updates from the Connect App Store, use Ion Reporter™ Software.
- Create and manage account settings, and audit these accounts.
- View system information and settings.

Administrator-level users use tools in the **(Settings)** menu to:

- View and export audit records.
- Configure user email settings, set up laboratory information, and audit these settings.
- Create and link user accounts for to get software updates from the Connect App Store, use Ion Reporter™ Software.
- Create and manage account settings, and audit these accounts.
- Manage software updates.
- Manage data archive settings and disk space usage.
- Manage log files.
- Manage services for servers, services, and active jobs.
- Create and manage user accounts.

Accounts for use with Connect and Ion Reporter Software (administrator)

Administrator-level users can configure Genexus™ Software to link to one or more Connect user accounts and Ion Reporter™ Software accounts.

Active **Thermo Fisher Connect** accounts can perform the following tasks in the Genexus™ Software:

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

Users who have an active Ion Reporter™ Softwareand an account that is linked to Genexus™ Software can upload samples results to Ion Reporter™ Software, and then use the software to further analyze those results, add annotations and create reports. Genexus™ Software can be linked to either accounts on an Ion Reporter™ Server, or Ion Reporter™ Software on Connect accounts.

Configure Thermo Fisher Accounts in Genexus™ Software (administrator)

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

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

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

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

1. Click **(Settings)** ▸ **Thermo Fisher Account**.
2. In the **Thermo Fisher Account Settings** screen, click **Create Account**.
3. In the **Create Thermo Fisher Account** dialog box, enter the information that is required to create the account.

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

Item	Description
User Name	The username is the email that you used to register for the Connect account.
Password	Enter the password for the Ion Reporter™ Software on Connect account.

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

When the **Thermo Fisher Account** is active, software updates from the Connect App Store are automatically uploaded to the Genexus™ Software and administrator-level users can download the updates. For more information, see "Software updates" on page 138.

Configure an Ion Reporter™ Software on Connect account (administrator)

An administrator-level user can configure a link to an Ion Reporter™ Software on Connect account in Genexus™ Software.

Ion Reporter™ Software on Connect, the cloud-based platform, is available at **https://ionreporter.thermofisher.com**.

When the link is configured, you can upload sample results files from Genexus™ Software directly to the Ion Reporter™ Software on Connect and use Ion Reporter™ Software for further data analysis, annotation and reporting. Unmapped BAM files for the sample results are uploaded.

Access to Ion Reporter™ Software on Connect is controlled by your **thermofisher.com** user name and password. Before you configure the Ion Reporter™ Software on Connect account, you must have a valid Connect account on the **thermofisher.com** website. Users who use the account to access Ion Reporter™ Software on Connect must also have valid Thermo Fisher account accounts.

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

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

Option	Description
Account Type	Select **IonReporter Cloud**.
Name	Enter a name to identify the account in the list of **Thermo Fisher Account Settings** in Genexus™ Software.
User Name	This must the user name (email address) for a valid Ion Reporter™ Software on Connect account.
Password	Enter the password for the Ion Reporter™ Software on Connect account.
(Optional) **Set as Default Account**	Click the toggle button if you want to make the account the default account that is used to upload sample results files.

4. Click **Submit**.

The configured Ion Reporter™ Software on Connect account is added to the **Thermo Fisher Account Settings** table. (See the row with the **Account Name** that you used listed as a **Thermo Fisher Cloud** account.) The account can be selected in the **Setup** step of a run plan to automatically upload sample results file to Ion Reporter™ Software. When sample results are added to a run plan, samples samples that are associated with the run can be uploaded.

Completed sample results files can also be uploaded to Ion Reporter™ Software from the **Actions** column in the **Results / Run Results** screen.

Configure an Ion Reporter™ Server account (administrator)

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

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

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

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

4. Click **Submit**.

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

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

User accounts (administrator)

Administrator-level users can perform user management actions in the **(Settings) / User Management** screen.

- Add new user accounts
- Assign user privileges (roles)
- Edit user information
- View user account audit trails
- Manage user account security policies

In addition, administrator-level users can configure an SSH key in the **(Profile) / My Profile** screen.

Add a new user account (administrator)

Administrator-level users add new user accounts to the software, and grant certain users permissions to electronically sign reports.

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

The email settings in the software must be configured before new users can receive email notifications. For more information, see “Configure email notifications (manager/administrator)” on page 137.

1. In the menu bar, click **(Settings)** ▸ **User Management**.
2. In the **User Management** screen, click **Add New**.
3. In the **Create User** dialog box, enter the user account information.
   User names can contain alphanumeric characters and underscores, periods, and hyphens.
4. From the **Role** list, select the appropriate user-access level (**Administrator**, **Manager**, or **Operator**).
5. To allow a user to sign reports (manager- and administrator-level users only), click the **Electronic Signature** toggle button.
6. Click **Save**.
   The software sends an email notification containing a temporary password to the email address of the new user.

Sort the user list (admin)

Administrator-level users can sort the user list by user name, first name, and last name.

1. In the menu bar, click **(Settings)** ▸ **User Management**.
2. In the **User Management** screen, click the column name of interest to sort the column alphabetically.
3. Click the column name a second time to reverse the order.

Edit user details (administrator)

Administrator-level users can edit user details in the **(Settings) / User Management** screen. An administrator can also suspend or disable user accounts. A history of user account changes is maintained by the audit feature.

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

1. In the menu bar, click **(Settings)** ▸ **User Management**.
2. In the **User Management** screen, in the **Actions** column, click **Edit**.
3. In the **Edit User** dialog box, edit the user details.
   - Enter changes in the appropriate account information fields.
   - In the **Role** list, select the user-access level (**Administrator**, **Manager**, or **Operator**).
   - To enable the ability to sign reports (manager- and administrator-level users only), click the **Electronic Signature** toggle button to the enabled position.
   - In the **State** list, select the appropriate state (**Active**, **Suspended**, or **Disable**).
4. Click **Save** to make the changes.

Reset user password

An administrator-level user resets passwords for user accounts. A history of user password changes is maintained by the audit feature.

After a password is reset, the software sends an email notification to a user of a temporary password, which they must personalize when logging in.

Periodically, the software sends a system-generated email notification to a user that their password is about to expire. The operator follows the directions included in the email notification of a pending password expiration. A user can also request that an administrator reset their password.

Passwords can contain only alphanumeric characters and no special characters or spaces.

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

The email settings in the software must be configured before new users can receive email notifications. For more information, see “Configure email notifications (manager/administrator)” on page 137.

1. Click **(Settings)** ▸ **User Management**.
2. In the **Actions** column, click **Reset Password** for the selected user.
3. In the **Change Password** dialog box, you can change the password in one of two ways.
   - Click **Send password in Email** to send a software-generated password in an email message to the user.
   - Enter a new password into the **Password** field to change the password for the user. Reenter the new password into the **Confirm Password** field, then click **Change**.

   The confirmation message "Password reset link sent to your email account" or "Password changed successfully" appears to indicate the password reset.

View user account history (administrator)

An administrator-level user can view the user account history using the audit feature. The user account audit trail is a record of when the user account was created and modified.

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

1. In the menu bar, click **(Settings)** ▸ **User Management**.
2. In the **User Management** screen, in the **Actions** column, click **Audit**.
3. In the **Audit Details** screen, view the audit trail details. For additional information, in the **Audit Record Details** column, click the time stamp of interest.
4. *(Optional)* In the **User Management** screen, click **Download** to export the record to a PDF file.

Set user account security policies (administrator)

An administrator-level user sets security policies for Genexus™ Software user accounts. User account security policies include the permissible number of failed sign-in attempts, password lifetime, and the length of inactivity before users are automatically signed out of the software. These settings apply to all users.

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

1. In the menu bar, click **(Settings)** ▸ **User Management**.
2. In the **User Management** screen, click **Policies**.
3. In the **Policies** dialog box, select the appropriate values to set the user-account suspension period, password, and session timeout policies.
4. Confirm that the **Enabled** toggle button is set to enable the session policy settings.
5. Click **Save**.

Configure an SSH key (administrator)

Administrator-level users can set up an SSH key in their user profile in the **(Profile) / My Profile** screen. SSH, also known as Secure Shell or Secure Socket Shell, is a network protocol that gives administrator-level users a secure way to access a computer over an unsecured network. SSH also refers to the suite of utilities that implement the SSH protocol.

Configuring an SSH key is optional, but best practice is to configure one or more SSH keys. The ionadmin and ionservice users may need to configure SSH keys so that they can access the system at the operating system level to configure some system-level settings, for example mounting an archive directory on the system. A user can have more than one SSH key.

Before configuring an SSH key, you must first generate keys by using the Keygen command on your local system. When the public and private keys are generated, copy the text string from the public key to use in this procedure.

1. In the menu bar, click **(Profile)** ▸ **My Profile**.
2. In the **SSH Keys** section, click **Add new SSH Key**.
   The **SSH Keys** section is available only to ionadmin and ionservice users.
3. Complete the **Add new SSH Key** dialog box.
   a. In **Label**, type a unique name for this SSH key.
   b. In **SSH Key**, paste or type the SSH key string generated by using the Keygen command on your local system.
   c. Click **Save**.

Audit records (administrator)

Administrator-level users can use the tools in the **(Settings) / Audit Records** screen to configure and manage audit records. Information that can be audited includes activities by users on samples, libraries, assays, planned runs, system setup the sequencer, accounts, and software updates.

The **Audit Records** screen lists actions that are performed on any data object, including the user who performed the action, the action performed, the data object name, and the date and time of the action. Additional details concerning the action are available in an accompanying audit record.

The actions can be searched and sorted, and the audit records can be exported and printed.

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

Note: Library batches and runs that are created in a batch with other objects in a single LIMS transaction do not have a "Create" action that is listed in the **(Settings) / Audit Records** screen. However, you can view complete library batch audit records from the **Samples / Manage Libraries** and **Runs / Manage Runs** screens. Any subsequent actions that are performed on library batches are listed in the **Audit Records** screen. A library batch or run that is created individually in a LIMS transaction is listed.

Search audit records (administrator)

An administrator-level user can use the search tools in the **(Settings) / Audit Records** screen to search for an existing audit record.

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

1. In the menu bar, click **(Settings)** ▸ **Audit Records**.
2. In the **Audit Records** screen, select the search parameters.
   a. To select a date range, click **Calendar** next to **Start Date** and **End Date**.
      If an end date is not selected, the search results include all records from the start date through the current date.
   b. To limit the results list to actions that are performed by a specific user, select the user from the **Select User** list.
   c. To limit the results to specific actions, select from the **Select Action** list.
   d. To limit the results to a particular sample, select from the **Sample** list.
3. Click **Search**.
4. Click **Clear** to return to the complete list of records.

Sort audit records (administrator)

An administrator-level user can sort the columns in the **(Settings) / Audit Records** screen to display the list of audit records in different order. The default order is the newest record on top.

To return to the default display, click the **Audit Records** column heading or click **Clear**.

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

1. In the menu bar, click **(Settings) ▸ Audit Records**.
2. In the **Audit Records** screen, click the column heading of interest.
   The list of records is reordered based on the heading selected. User, Action Performed, and Data Object Name sort alphabetically.
3. Click the column heading a second time to reverse the order of records displayed.
4. Click **Clear** to return to the sorted column to the default.
5. Click the **Timestamp** heading to return to the default order, displaying the most recent record on top.

Export audit records (administrator)

An administrator-level user uses the **(Settings) / Audit Records** screen to export a print-ready PDF file of selected audit records.

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

1. In the menu bar, click **(Settings) ▸ Audit Records**.
2. In the **Audit Records** screen, select the record or records to be exported by clicking the checkbox next to the record of interest. Select all of the records in the screen by selecting the checkbox above the column.
3. Click **Download**.
   A PDF file is generated. Depending on your browser settings, the software automatically downloads the file or prompts you to open or save the file.
4. Open the PDF file in an appropriate viewer to print.

Update the audit configuration (administrator)

An administrator-level user configures the audit details by requiring that a reason for a change be included in audit records when a user changes information. When a reason is required, the user must supply a reason for change when editing the information, and the reason is included in the audit record.

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

1. In the menu bar, click **(Settings)** ▸ **Audit Records**.
2. In the **Audit Records** screen, click **Audit Configuration**.
3. In the **Audit Configuration** dialog box, select the **Require Reason** checkbox next to the **Data Object Name** for which a reason for change is required.
4. Click **Save**.

   To remove a reason for change requirement, open the **Audit Configuration** dialog box, deselect the checkbox, then click **Save**.

Configure email settings (manager/administrator)

Manager- and administrator-level users configure email settings. Administrator-level users can configure support contact information, such as the lab contact and the IT contact for the organization.

Configure email notifications (manager/administrator)

Manager- and administrator-level users configure the Genexus™ Software to send email notifications to a specified administrator.

Administrator-level users receive email notifications when any of the following conditions occur:

- The archive directory location has limited free disk space remaining.
- The archive directory location is not accessible when the system tries to archive a run.

1. Obtain the SMTP server URL and port information from your IT department.
2. Click **(Settings)** ▸ **Configuration**.
3. In the **Configuration** screen, in the **Email Settings** section, enter the server and port information supplied by IT, and the Genexus™ Software user name, password, and email address of the administrator.
4. Click **Update**.
5. Click **Send Test Email** to send a test email to the email address provided in the settings.

Lab information

The **Lab Information** tab in the ⚙ **(Settings) / Configuration** screen provides a reference for support personnel, listing points of contact if a problem with the connected Genexus™ Integrated Sequencer occurs.

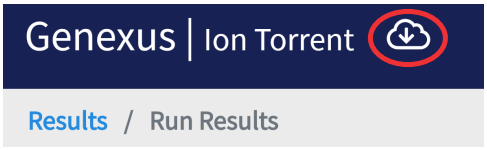
- **Lab Contact**—This is the person in your organization who should be notified during a support request of problems related to the sequencer.
- **IT Contact**—This is the person in your organization who should be notified during a support request of problems related to the sequencer hardware or the network environment.

IMPORTANT! The Administrator should review the contact information periodically to ensure that it is current and accurate.

Software updates

An administrator-level user can select **Software Updates** in the ⚙ **(Settings)** menu to:

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



For users who have set up a Thermo Fisher Connect account, a notification appears in the Genexus™ Software banner on the top of the screen upon login when updated software packages are available for download from the Connect cloud-based platform.

Updated software packages are available from the App Store at **apps.thermofisher.com**. Administrator-level users can download and install software directly from the App Store if a Thermo Fisher Connect account has been configured. Alternatively, a USB drive can be used to install and update the software if the instrument is not connected to the internet.

To...	See this...
Download and install software directly	"Download and install software directly from the App Store (administrator)" on page 139
Download and install software with a USB drive	"Format a USB drive for a software update" on page 140 "Download software from the App Store to a USB drive (administrator)" on page 140

Note: For first-time update of system software from the 5.100.2 version, contact a Thermo Fisher Scientific field service engineer to update the system software.

IMPORTANT! Before updating the software, ensure that the instrument is idle, no analysis jobs are running or are queued to run, and the screen on the instrument is the main menu screen.

Download and install software directly from the App Store (administrator)

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

- Configure a Thermo Fisher Connect account. For instructions, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 127.
- Ensure that the Genexus™ Integrated Sequencer has the latest software version, Genexus™ Software version 6.2*x*.
- Connect your Genexus™ Integrated Sequencer to the internet.

1. In the menu bar, click **(Settings)** ▸ **Software Updates**, then click **Software Updates** in the upper right corner.
   The **Software Updates** screen opens with **App Store** enabled.
2. *(Optional)* Click the desired package name to view the installation details and version information in the **View Package Details** pane.
3. Click **Download** in the **Actions** column in the row of the software package of interest.
   After completion of the download, the **Download** link changes to an **Install** link.
4. Click **Install**. When the confirmation window appears, click **Yes** to confirm that you want to install.
   If you are performing a system software module installation, a command window opens where you can monitor installation progress.

   Note: There may be a delay before the command window opens.
5. When installation of the software package is complete, click **Installed Software** to verify the installation.

Format a USB drive for a software update

Pre-requisites:

- An Ubuntu Linux desktop machine with disk utility software.
- A USB drive with a capacity of 4 GB or more.

1. Insert the USB drive into the Ubuntu Linux machine.
2. With Ubuntu running, open the disk utility by navigating to **System Administration ▸ Disk Utility**.
3. Select the USB Flash Drive (SMI USB DISK) under **Peripheral Devices from Storage Devices** by clicking on it. Highlight the volumes (New Volume FAT ≥4 GB), click **Unmount Volume**, then click **Delete Partition**. Click **Delete** to confirm.
4. Select the free USB volume, then click **Create Partition**.
   The **Create partition on SMI USB DISK** window opens.
5. Deselect the **Take ownership of file system** check box.
6. Select **ext3** from the **Type** dropdown menu, then select the entire size of the partition. Name the partition **usbinstall**.
   The partition name must be completely lowercase.
7. Click **Mount**.
   The mount point of the USB drive is **/media/usbinstall**.

Proceed to "Download software from the App Store to a USB drive (administrator)".

Download software from the App Store to a USB drive (administrator)

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

- Create or access your Thermo Fisher Connect account. For instructions, see "Configure Thermo Fisher Accounts in Genexus™ Software (administrator)" on page 127.
- Format a USB drive for the software update. For more information, see "Format a USB drive for a software update" on page 140.

1. Sign in to the Thermo Fisher App Store on Connect at **apps.thermofisher.com**.
2. On the left side of the App Store screen, click **AppConnect**.
3. On the right side of the screen, under **Resource Libraries**, click **Ion Torrent™ Genexus™**.
4. Click **Software Update**.
5. Select the software package name of interest, such as (name) 6.2*x*.
6. Click **Download** to download the package.
7. Copy the downloaded package (.zip) to the `/media/usbinstall` folder in the USB drive.

8. After completion, unmount the USB drive, then insert the drive into one of the USB ports at the back of the Genexus™ Integrated Sequencer.
9. In the menu bar of the Genexus™ Software, click ⚙ **(Settings)** ▸ **Software Updates**, then click **Software Updates** in the upper right corner.
10. Click the **App Store** toggle button to select **USB**.
11. Click **Install**. When the confirmation window appears, click **Yes** to confirm that you want to install.

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

Data management (administrator)

Administrator-level users use the ⚙ **(Settings) / Data Management** screen to:

- Monitor sequencer disk space usage.
- Manage archive settings.
- View an audit trail of any changes made.

Disk space usage and data archiving (administrator)

An administrator-level user monitors disk space usage and data archiving on the sequencer. Sequencer disk space usage information is available from the ⚙ **(Settings) / Data Management** menu.

To maintain sufficient disk space, best practice is to implement a systematic plan to archive older run data, results files, variants information, and signed reports to an external file storage system. A system administrator or Field Service Engineer is responsible for designing and establishing a data archiving system and database backup options. After an archive system has been established, an administrator-level user configures the archive schedule in the ⚙ **(Settings) / Data Management** screen.

The system ensures that free disk space is available on the sequencer for runs.

- If the sequencer has less than 1 terabyte (TB) of free disk space, no new runs can be started. In this case, an alert notifies the user that there is insufficient disk space when setting up a run on the sequencer. The run cannot proceed until data on the sequencer is archived and deleted.
- If the sequencer has less than 2 terabytes (TB) of free disk space, the raw data for the oldest completed runs is automatically deleted to free up space for future runs.

Contact your local system administrator to archive and delete data manually. The administrator can also change the archive schedule.

Archived results and reports can be restored to the sequencer hard drive and downloaded from the **Results / Run Results** screen. For more information, see “Restore archived results (administrator)” on page 144.

Configure archive settings (administrator)

An administrator-level user views and configures sequencer archive settings in the **(Settings) / Data Management** screen.

The archive settings may need to be updated for various reasons.

- The archive location has limited free disk space remaining.
- The archive location is not accessible when the system tries to archive a run.

Consult your local Field Service Engineer to set up a database archive and backup system. For more information, see “Set up an archive server (administrator)” on page 143.

1. In the menu bar, click **(Settings) ▸ Data Management**
2. In the **Data Management** screen, edit the archive settings.

Setting	Procedure
Auto Archive After	Select the number of months for data to remain on the sequencer hard drive before it is archived on to an external server. To free up additional space on the sequencer hard drive, reduce this interval.
Archive Directory	Enter the file path to the external server where data will be archived. The path must be specified before data can be archived. If blank, and if any archive data is found, the software sends an email notification to set up the archive directory. For more information, see “Data output directory” on page 142.
Data Output Directory	The default file path for the data output directory can be viewed, but cannot be changed.

3. Click **Save**.

Data output directory

The data output directory is the primary location on the hard drive where data generated by the Genexus™ Integrated Sequencer is stored. The path to the directory appears in the **(Settings) / Data Management** screen.

The directory location is locked and cannot be changed. Users should configure their laboratory information management systems (LIMS) to access this folder location to receive sequencing output files.

After a sequencing run and data analysis are complete, the software creates a separate results folder in the data output directory for each sample, and uses the following naming conventions:

```
AssayDev_<Sample Name>_<Assay Name>_<RunPlanShortCode>_
<RunPlanShortCode>
```

Archive notifications

Administrator-level users receive email notifications when any of the following conditions occur:

- The archive directory location has limited free disk space remaining.
- The archive directory location is not accessible when the system tries to archive a run.

To receive email notifications, a valid administrator email address must be entered into Genexus™ Software. To configure email notifications, see “Configure email notifications (manager/administrator)” on page 137.

Set up an archive server (administrator)

Administrator-level users set up an external storage device to be an archive server to store system data.

1. Mount your NFS to the sequencer.

   The following example is for users who have an existing—read and write access—Network File System (NFS). Mount your NFS to the sequencer using fstab so that the mount point is persistent (NFS mounts automatically after rebooting).

   Example:
   For NFS share: `cqtsop4:/md1200`
   Mount point: `nfs1`

2. Create a mount point to mount the external device on nfs share.
   a. Create the directory /nfs1 using the following command:

      ```
      sudo mkdir /nfs1
      ```

   b. Append the mount point entry in /etc/fstab.

      Command example:

      ```
      sudo vi /etc/fstab
      cqtsop4:/md1200 /nfs1 nfs defaults 0 0
      ```

   c. Save, then exit by pressing **Esc** on your keyboard, then type ***:wq***

3. Create a permanent or temporary mount on nfs share:
   - Permanent mount command: `sudo mount -a`
   - Temporary mount command (example): `sudo mount cqtsop4:/md1200 /nfs1`

4. Verify that the NFS share is mounted using the df -kh command.

   ```
   ionadmin@eternity1:~$ df -kh
   Filesystem Size Used Avail Use% Mounted on
   /dev/sda9 253G 5.9G 235G 3% /
   none 4.0K 0 4.0K 0% /sys/fs/cgroup
   udev 63G 4.0K 63G 1% /dev
   tmpfs 13G 1.3M 13G 1% /run
   ```

```
none 5.0M 0 5.0M 0% /run/lock
none 63G 0 63G 0% /run/shm
none 100M 0 100M 0% /run/user
/dev/sda5 19G 2.9G 15G 17% /var
/dev/sda1 938M 37M 854M 5% /boot
/dev/sda6 9.1G 1.7G 7.0G 20% /home
/dev/mapper/raid-serverdata 8.4T 4.3T 4.1T 52% /serverdata
/dev/mapper/raid-servershare 1.0T 9.0G 1015G 1% /
servershare
/dev/mapper/raid-scratch 1.0T 838M 1023G 1% /scratch
/dev/sda7 92G 61M 87G 1% /tmp
pluto:/md1200 37T 1.1T 36T 3% /nfs2
penguin-ts1:/local 11T 9.3T 978G 91% /nfs
ddn4.itw:/gs1/ion-data/dx-data 1.1P 1001T 115T 90% /ddn
cqtsop4:/md1200 37T 37T 77G 100% /nfs1
```

For more information, see **help.ubuntu.com/lts/serverguide/network-file-system-configuration**.

Restore archived results (administrator)

Genexus™ Software can be configured to archive automatically older run data, results files, and signed reports from the sequencer to an external server, based on when the results are generated. For more information, see “Disk space usage and data archiving (administrator)” on page 141) and “Set up an archive server (administrator)” on page 143.

The administrator can later restore the archived results, files, and reports to the sequencer from the **Results / Run Results** screen.

Archived results are listed in the **Results / Run Results** screen with the **Restore** link active in the **Actions** column.

1. In the menu bar, click **Results ▸ Run Results**.
2. In the **Run Results** screen, click the run name of interest.
3. In ⋯ **(More Options)**, click **Restore**.
4. Click **OK** in the confirmation dialog box.
   Click the run name in the **Run Results** screen to view run results.

Log files (administrator)

Administrator-level users use the **Settings** tab in the **Logs** screen to view log files generated by the software. Viewing log files is useful for troubleshooting activities.

Administrator-level users can also use the **Logs** screen to manage the storage of log data by setting the retention period for the audit trail function and enabling auto-deletion rules for logs.

Manage log settings (administrator)

An administrator-level user manages the length of time that log files are kept and configures the software to allow log files to be automatically deleted.

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

1. Click **(Settings)** ▸ **Logs**.
2. In the **Logs** screen, click **Manage Logs**.
3. In the **Manage Logs** dialog box, in **Retention Period**, select the number of months that logs are to be retained on the sequencer.
4. Select **Enable Auto Deletion** to delete log files automatically after the designated retention period.
5. Click **Save**.

Panel files (manager/administrator)

Manager- and administrator-level users use the **Assays / Manage Panels** screen to manage panel files and reference files.

Note: Manager- and administrator-level users can create and obsolete panel and reference files using **Add New** and **Obsolete**, except for those defined by system-installed or locked assays.

- Panel files (BED format)
- Hotspots files (BED format)
- Fusion reference sequences (FASTA format)
- Reference genome files (FASTA format)
- DNA barcode sequence files
- Control fragments

Manage panel files (manager/ administrator)

Manager- and administrator-level users add new panel files to Genexus™ Software, export existing panel files, and make old panel files unavailable in the software.

Operators can view the panel files that are available in the software.

1. In the menu bar, click **Assays** ▸ **Manage Panels**.
   The **Panels** tab in the **Manage Panels** screen opens.
2. Use the following options to manage panel files:
   - To add a new panel file, click + **Add New**, enter a name and panel type. Click **Upload** when you have finished.
     Large files take time to upload and may not appear immediately.
   - To export a panel file, click **Export** in the **Actions** column of the panel that you want to export.
   - To make an old panel file unavailable in the software, click **Obsolete** in the **Actions** column of the panel that you want to obsolete.
     You cannot designate as obsolete system-installed panel files.

Manage hotspot files (manager/ administrator)

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

Operators can view the panel files that are available in the software.

1. In the menu bar, click **Assays** ▸ **Manage Panels**.
2. In the **Manage Panels** screen, select the **Hotspots** tab.
   - To add a new hotspot file, click + **Add New**, then enter a name, select a panel, then select the BED file containing the hotspots information. Click **Upload** when you have finished.
     Large files take time to upload and may not appear immediately.
   - To export a hotspot file, in the **Actions** column, click **Export**.
   - To make a hotspot file unavailable in the software, in the **Actions** column, click **Obsolete**.
     You cannot designate obsolete system-installed hotspot files.

Manage fusion reference files (manager/ administrator)

Manager- and administrator-level users add new, view, and manage the fusion reference files that are available in the software.

Operators can view the fusion reference files that are available in the software.

1. In the menu bar, click **Assays** ▸ **Manage Panels**.
2. In the **Manage Panels** screen, select the **Fusion References** tab.
   - To add a new fusion reference file, click + **Add New**, then enter a name, select a genome reference and select the FASTA file containing the fusion reference information. Click **Upload** when you are finished.
     Large files take time to upload and may not appear immediately.
   - To export a fusion reference file, in the **Actions** column, click **Export**.
   - To make a fusion reference file unavailable in the software, in the **Actions** column, click **Obsolete**.
     You cannot designate obsolete system-installed fusions reference files.

Add reference genome files (manager/ administrator)

Manager- and administrator-level users add new DNA reference genome files so that they are available in Genexus™ Software. New reference genomes are uploaded in a FASTA file.

For information about the system-installed references, see “System-installed genome reference files” on page 194.

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 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.

Control fragments (manager/administrator)

Control fragment sequences are system-installed in Genexus™ Software and are included in reagents. These internal controls are predefined and cannot be modified by users.

Manager- and administrator-level uses can view control fragment information in the **Assays / Manage Panels** screen, in the **Control Fragments** tab.

Column	Description
Name	The control fragment name.
Sequence	Single-letter nucleotide sequence of the control fragment.
Control	The process in which the control fragment is used.
Status	Indicates whether the control fragment information is locked and cannot be modified by the user.

Manage barcode sets (manager/ administrator)

Manager- and administrator-level users add new barcode sets to the Genexus™ Software, and view the barcode IDs and sequences for each barcode set that is available in the software.

For information about system-installed barcode sets, see “System-installed barcode sets” on page 177.

1. In the menu bar, click **Assays** ▸ **Manage Panels**.
2. In the **Manage Panels** screen, in the **Barcode Sets** tab, click the name of a barcode set in the list.
   A new window opens showing the name and sequence information about each barcode in the set.
3. Click ‹ **Back** to return to the list.
4. To add a new DNA barcode set, click + **Add New**, enter the required information in the **Add Barcode Set** dialog box, and browse to and select the barcode set file. Click **Upload** when you have finished.

System services (manager/administrator)

Manager- and administrator-level users can view information about system services using the **(Settings) / Services** screen. The information can be useful when troubleshooting error messages that are received during an active job or why an analysis failed.

Service	Function
Jobs Server	Shows a list of server information and active (running) software.
Services	Shows the service name, description, and status. The Services list can be used for troubleshooting. If a service has stopped during a run, an option to start the service will be visible for manager- and administrator-level users.
Active Jobs	• Shows a list of analysis jobs that are active and queued on the sequencer. • When no job is currently active, displays a "No active jobs" message.
ionCrawler Service Details	Provides information about the data transfer process from the Genexus™ Integrated Sequencer.
Smart Monitoring Service Details	Allows remote monitoring of the Genexus™ Integrated Sequencer.
Download Instrument Diagnostics	Contains the initialization logs of the Genexus™ Integrated Sequencer, and allows you to download the diagnostic archive file (in ZIP format).

Terminate an active job (manager/administrator)

If an active job is not going to be useful or if a different job is urgent, you can opt to terminate an active job. A manager- or administrator-level user can stop an actively running job in the **(Settings) / Services** screen.

1. In the menu bar, click **(Settings)** ▸ **Services**.
2. In the **Services** screen, in the **Active Jobs** section, find the active job that you want to stop, then click **Terminate**.
   A confirmation message opens, **Are you sure you want to terminate <job name> ?**.
3. Click the **Terminate** to terminate the job.

Start a stopped service (manager/ administrator)

A manager- or administrator-level user can use the **(Settings) / Services** screen to restart a stopped service if a service has stopped running for some reason. The two database services, **postgresql** and **mongod** are the only services that can be restarted.

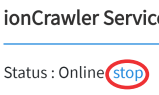
1. In the menu bar, click **(Settings)** ▸ **Services**.
2. In the **Services** screen, in the **Services** section, find the service that you want to reactivate, then click **Start**.

   A confirmation message opens, **Are you sure you want to Start<service name>?**.
3. Click **Yes** to restart the service.

Disable or enable the ionCrawler service (manager/ administrator)

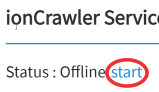
The ionCrawler service typically remains enabled at all times, but can be disabled by manager- or administrator-level users if required for remote service troubleshooting.

- Disable the service.
  a. In the menu bar, click **(Settings)** ▸ **Services**.
  b. In the **Services** screen, in the **ionCrawler Service Details** section, click **stop**.



  c. In the confirmation message, click **Yes** to disable the service.

     The **Stop** option changes to **Start**.



- Enable the service.
  a. In the menu bar, click **(Settings)** ▸ **Services**.
  b. In the **Services** screen, in the **ionCrawler Service Details** section, click **Start**.
  c. In the confirmation message, click **Yes** to enable the service.

     The **Start** option changes to **Stop**.

Disable or enable the Smart Monitoring service (manager/administrator)

The Smart Monitoring service allows Thermo Fisher Scientific personnel to monitor the status of the Genexus™ Integrated Sequencer remotely through an internet connection. Smart Monitoring employs multiple layers of security, including a Secure Sockets Layer (SSL) and Lightweight Directory Access Protocol (LDAP) authentication, to provide real-time troubleshooting and problem resolution for the Genexus™ Integrated Sequencer.

The Smart Monitoring service is active by default and can be disabled by manager- or administrator-level users, if required.

- Disable the service.
  a. In the menu bar, click ⚙ **(Settings)** ▸ **Services**.
  b. In the **Services** screen, in the **Smart Monitoring Service Details** section, click **Stop**.
  c. In the confirmation message, click **Yes** to disable the service.
     The **Stop** option changes to **Start**.
- Enable the service.
  a. In the menu bar, click ⚙ **(Settings)** ▸ **Services**.
  b. In the **Services** screen, in the **Smart Monitoring Service Details** section, click **Start**.
  c. In the confirmation message, click **Yes** to enable the service.
     The **Start** option changes to **Stop**.

Sign off on the run results (manager/administrator)

Manager- and administrator-level users can provide their electronic signature on sample results for completed runs. In the **Results / Sample Results** screen, a sample name followed by ***(Signed Off)*** indicates that a manager- or administrator-level user has approved the sample results. The signature information appears in the Lab Report PDF file or a user-created report, if selected. For more information, see “Lab Report” on page 85, and “Create a report template (manager/administrator)” on page 121.

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

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

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

   Items identified with a red asterisk (*) are required fields.
5. In the **Meaning of Signature** dropdown list, select **Approval**.
6. In the **Report Template** dropdown list, select the report template that you want to use.
7. In the **Report Customizations** section, in the **Lab Report** pane, select the types of variant calls that you want to include in each report. For assays that use Reporting Gene Lists, you can customize the variants by reporting category.
8. In **Footer Field**, enter any text that you want to appear in the footer of the PDF report pages.

   If you entered footer information in the **Footer Field** when you created a report template, the same footer information appears in the **Electronic Signature** dialog box. You can enter new footer information to override the report template.
9. Click **Sign Off** to confirm your electronic signature.
10. In the menu bar, click **Results** ▸ **Sample Results** to return to the **Results / Sample Results** screen.
11. In the row of the sample of interest, in the **Actions** column, click **Lab Report** to download the report.

Amend a signed-off report (manager/administrator)

Manager- and administrator-level users can amend a report within 30 days of signing off. A sample that has a signed-off report is listed with the `sample name` followed by ***(Signed Off)*** in the **Sample Name** column in the **Run Results** screen. You can change sign-off or laboratory comments, the type of calls that are reported, or the text that appears in the report footer.

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

1. In the menu bar, click **Results ▸ Sample Results**.
2. In the **Sample Results** screen, click the signed off sample name of the sample of interest.
3. In the **Results** screen, click ··· **(More Options)**, then click **Amend Report**.
4. In the **Electronic Signature** dialog box, make your changes in the appropriate fields, including changes in the types of variant calls you want to include in the Lab Report, then click **Amend Report**.
5. Return to the **Results / Sample Results** screen. Then, in the **Actions** column for the sample, click **Lab Report** to download the amended report.

9 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 variety of purposes.

An administrator-level user can download and install additional plugins from the Connect cloud-based platform.

coverageAnalysis plugin in Genexus™ Software

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

Review coverageAnalysis plugin results

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

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

You can download coverageAnalysis plugin output files from the **Results** screen for a sample. For more information, see “Results files” on page 89 and “Output files generated by the coverageAnalysis plugin” on page 161.

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

3. Click the **Plugins** tab.
   A summary table of the coverage analysis, by barcode, is included in the **coverageAnalysis** summary pane.

4. *(Optional)* From the **Executed At** dropdown list, select an alternate timestamp, if available, to view additional reports.

5. *(Optional)* Click **View Log** to view the coverageAnalysis log.

6. *(Optional)* Click **Delete** to delete the coverageAnalysis plugin output for the selected timestamp.

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

7. In the **coverageAnalysis** summary pane, in the **Barcode Name** column, click the link in the row of the barcode of interest.
   The detailed **Coverage Analysis Report** for the barcode opens in a separate window.

Reads statistics

The detailed **Coverage Analysis Report** that is available when you select a barcode in the coverageAnalysis plugin summary for a run includes a variety of reads 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 tooltips with definitions. Hover over a heading or description in the report to view the tooltip.

General statistics

Statistic	Description
Number of mapped reads	The total number of reads mapped to the reference genome.
Percent reads on target	The percentage of filtered reads mapped to any targeted region relative to all reads mapped to the reference. If no target regions file is specified, this value will be the percentage of reads passing uniquely mapped and/or non-duplicate filters, or 100% if no filters were specified. A read is considered on target if at least one aligned base overlaps at least one target region. A read that overlaps a targeted region but where only flanking sequence is aligned, for example, due to poor matching of 5' bases of the read, is not counted.

Amplicon read coverage statistics

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

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

Target base coverage statistics

The following statistics describe the targeted base reads of the reference. A base covered by multiple target regions is counted only once per sequencing read.

Statistic	Description
Bases in target regions	The total number of bases in all specified target regions of the reference.
Percent base reads on target	The percent of all bases covered by reads aligned to the reference that covered bases in target regions. Clipped bases, deletions, and insertions (relative to the reference) are not included in this percentage. If no specific target regions was specified, the targeted regions is the whole genome.

Statistic	Description
Average base coverage depth	The average number of reads of all targeted reference bases. This is the total number of base reads on target divided by the number of targeted bases, and therefore includes any bases that had no coverage.
Uniformity of base coverage	The percentage of bases in all targeted regions (or whole genome) that is covered by at least 20% of the average base coverage depth reads. Cumulative coverage is linearly interpolated between nearest integer base read depths.
Target base coverage at *N*x	The percentage of target bases covered by at least *N* reads.
Target bases with no strand bias	The percentage of all target bases that did not show a bias toward forward or reverse strand read alignments. An individual target base is considered to have read bias if it has ≥10 reads and the percentage of forward or reverse reads to total reads is greater than 70%. Target bases with <10 reads are considered to have no strand bias.
Percent end-to-end reads	The percentage of on-target reads that fully cover their assigned amplicon (insert) from 'end-to-end'. To allow for error, the effective ends of the amplicon region for read alignment are within 2 bases of the actual ends of the region.
Bases in targeted reference[1]	The total number of bases in all target regions of the reference.
Bases covered (at least 1x)[1]	The total number of target bases that had at least one read aligned over the proximal sequence. Only the aligned parts of each read are considered. For example, unaligned (soft-cut) bases at the 5' ends of mapped reads are not considered. Covered target reference bases can include sample DNA read base mismatches, but does not include read base deletions in the read, nor insertions between reference bases.
Average base read depth[1]	The average number of reads of all targeted reference bases that were read at least one time.
Genome Base Coverage[1]	Summary statistics for base reads of the reference genome.
Genome base coverage at *N*x[1]	The percentage of reference genome bases covered by at least *N* reads.

[1] Applicable to whole genome library analyses only.

Example charts generated by the coverageAnalysis plugin

Many of the charts in the detailed **Coverage Analysis Report** include a **Plot** menu that allows you to change characteristics of the chart. For example, you can show both strands (see callout 3 in Figure 5).

The 🔍 button (in the top right corner of a chart) opens the chart **Viewing Options** panel. The ? button opens a description of the chart.

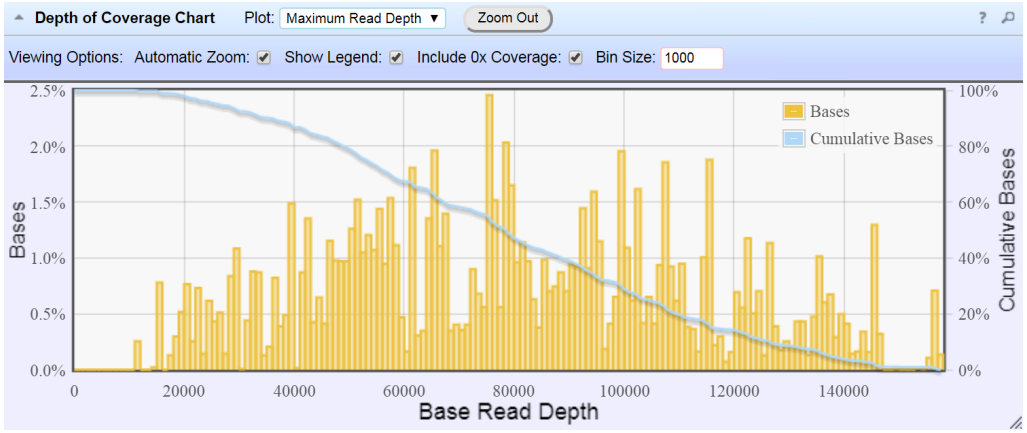


Figure 1 Representative Depth of Coverage Chart

The Depth of Coverage Chart shows the distribution of targeted base coverage. The X-axis represents the base read depth. The left Y-axis represents the number of reads at a given base read depth or a range (bin) of base read depths, as a percentage of the total number of base reads. The right Y-axis represents the cumulative count of the number of reads at a given read depth or greater, as a percentage of the total number of reads. The individual orange bars represent the percentage of reads in the specific range of base read depths. The blue curve measures the cumulative reads at a given base read depth or greater. If your analysis includes a regions of interest file, this chart reflects only target regions (reads that fall within a region of interest).

In most charts, you can click a data point to open a detail pane for that data. For example, in the Depth of Coverage Chart in Figure 1, click an individual orange bar to open the detail pane for bases within a specific range of base read depths. Click a point on the blue curve to open the detail pane for cumulative bases at that base read depth or greater. For example detail panes, see Figure 2. Similarly, you can click any point or bar within the Amplicon Coverage chart to view details for each amplicon coverage bin (see Figure 4).

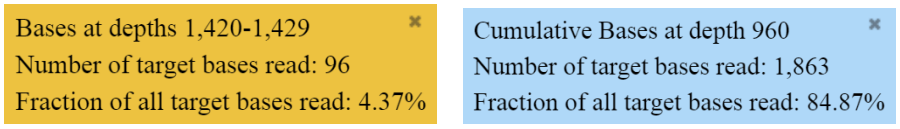


Figure 2 Example detail panes

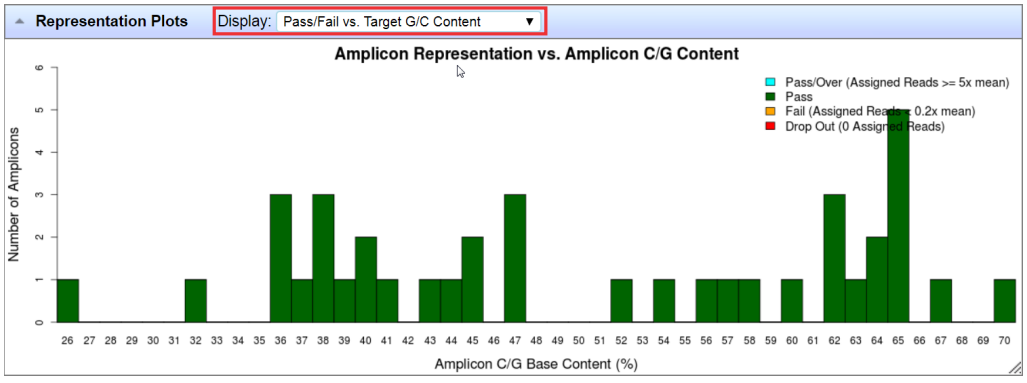


Figure 3 Representation Plots

Use the **Display** list to view different amplicon representation plots. This figure shows an example Pass/Fail vs. Target G/C Content plot.

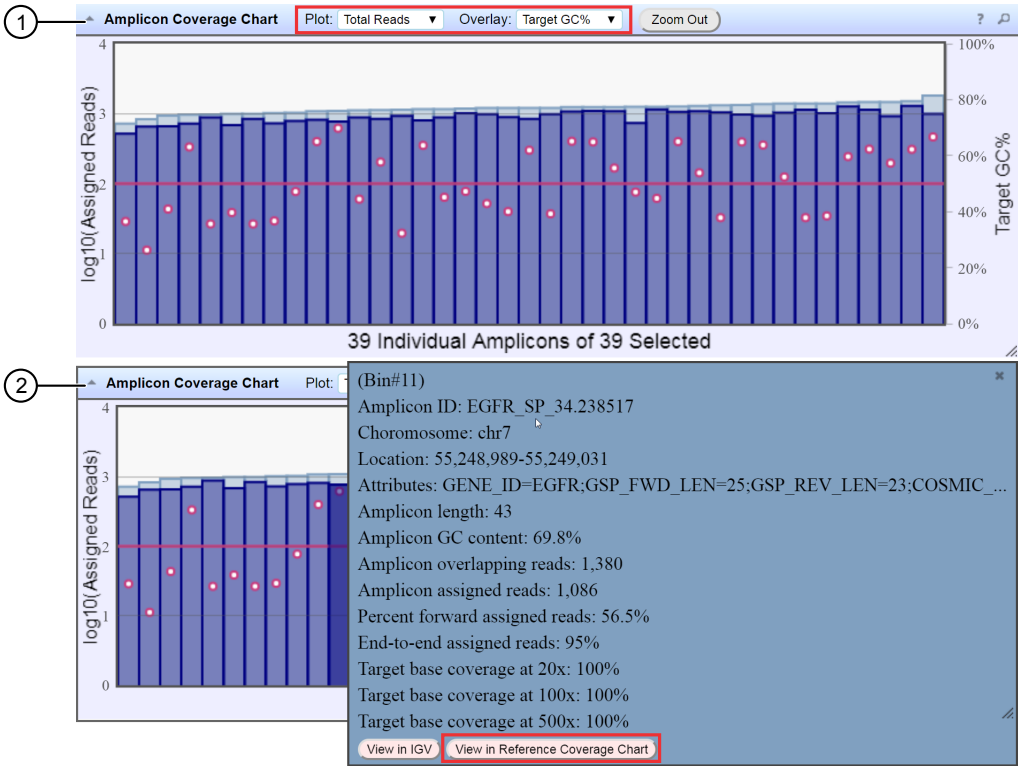


Figure 4 Representative Amplicon Coverage Chart

The Amplicon Coverage charts summarize the amplicon coverage results. Several plots views are available. The X-axis in all plots represents individual amplicons. The left Y-axis in all plots represents assigned reads (on a base 10 log scale). Use the **Plot** and **Overlay** lists to customize the chart view. The right Y-axis varies depending on the plot view that is selected from the **Overlay** dropdown list. Click on any bar or any point on the chart to view the detail pane for that data.

1. Example Total Reads plot with a Target GC percentage overlay
2. Click on the bar to open the detail pane for the specific amplicon. Click **View in Reference Coverage Chart** to zoom in on the amplicon in the **Reference Coverage Chart** (see callout 3 in Figure 5).

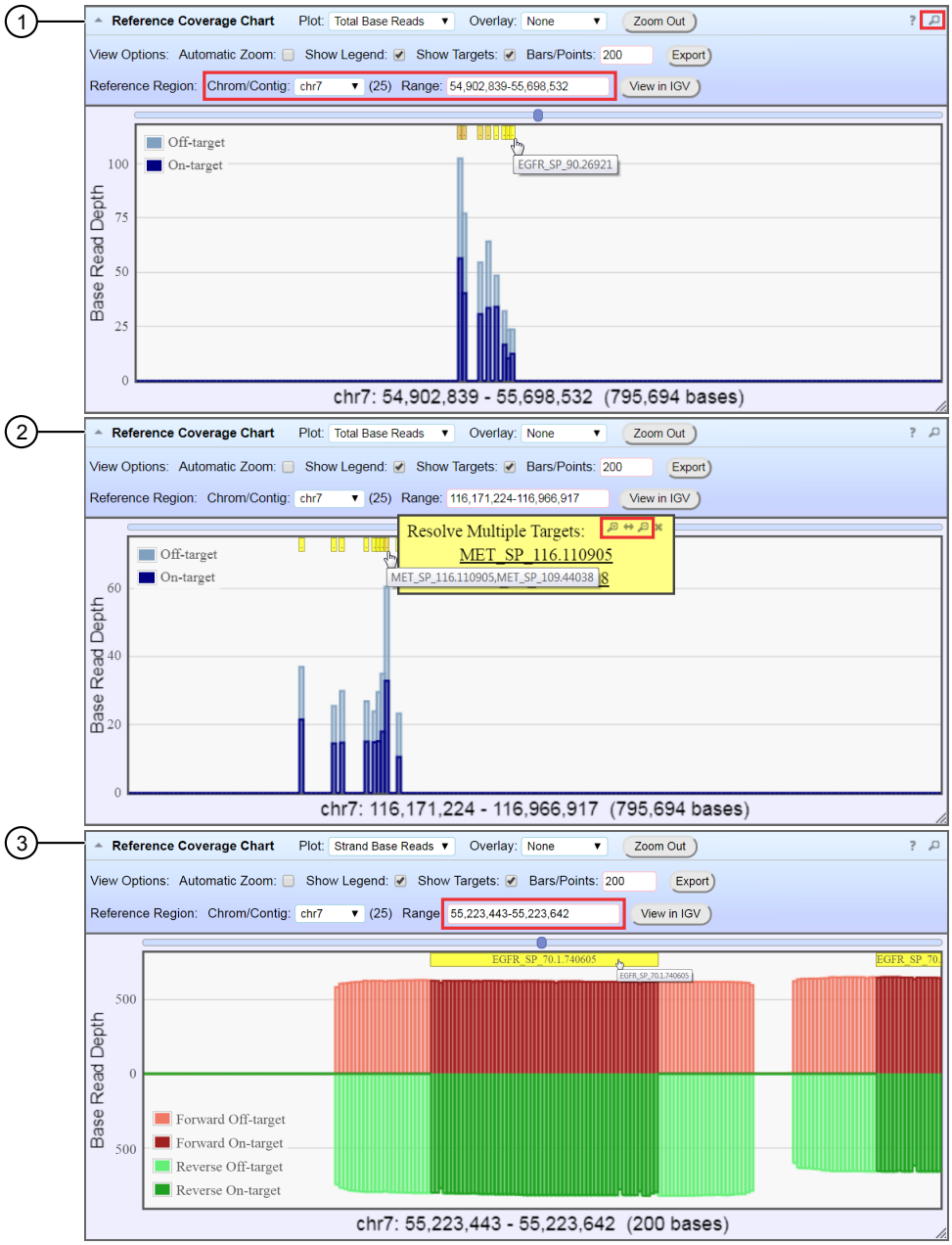


Figure 5 Representative Reference Coverage Chart

The Reference Coverage Chart is an overlay of where target regions are defined and overlap on the reference. The X-axis represents the target region chromosomal position. The Y-axis represents the Base Read Depth.

① Example Total Base Reads plot with Viewing Options panel expanded. The plot is zoomed in on a specific chromosomal region. Click a yellow bar (top of the chart) to open the detail pane for a specific amplicon in the Amplicon Coverage Chart (see callout 2 in Figure 4).

② Example Total Base Reads plot where a chromosomal region has multiple targets. In the Resolve Multiple Targets pane, click to zoom in on the region, or click one of the links to open the detail pane for the specific amplicon in the Amplicon Coverage Chart.

③ Example Strand Base Reads plot that is zoomed in on one specific amplicon. Click the yellow box to open the detail pane for the specific amplicon in the Amplicon Coverage Chart.

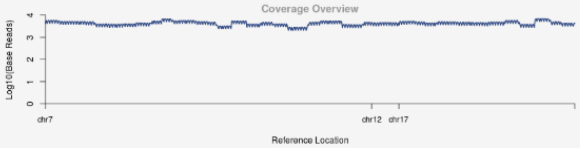
Example Coverage Analysis Report

The detailed Coverage Analysis Report that is available when you select a barcode in the coverageAnalysis plugin summary for a run includes a variety of reads statistics.

Coverage Analysis Report

Sample Name:

Library type: AmpliSeq HD - DNA and Fusions (Single Library)
Reference: hg19 (DNA)
Target regions:



Number of mapped reads	48,721
Percent reads on target	96.16%
Average base coverage depth	1,177
Uniformity of base coverage	100.00%

Amplicon Read Coverage	
Number of amplicons	39
Percent assigned amplicon reads	96.16%
Average reads per amplicon	1,201
Uniformity of amplicon coverage	100.00%
Amplicons with at least 1 read	100.00%
Amplicons with at least 20 reads	100.00%
Amplicons with at least 100 reads	100.00%
Amplicons with at least 500 reads	100.00%
Amplicons with no strand bias	100.00%
Amplicons reading end-to-end	100.00%
Amplicon base composition bias	0.000

Target Base Coverage	
Bases in target regions	2,195
Percent base reads on target	53.78%
Average base coverage depth	1,177
Uniformity of base coverage	100.00%
Target base coverage at 1x	100.00%
Target base coverage at 20x	100.00%
Target base coverage at 100x	100.00%
Target base coverage at 500x	100.00%
Target bases with no strand bias	100.00%
Percent end-to-end reads	96.21%

Output files generated by the coverageAnalysis plugin

You can download coverageAnalysis plugin results files from the **Results** screen for a sample. For more information, see “Results files” on page 89.

Note: Sometimes the file name can be too long to open in applications such as Microsoft™ Excel™. To resolve this problem, right-click the file and click **Save As** to rename the downloaded file.

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

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`

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

File contents

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

File	Description
Amplicon coverage summary	Coverage summary data used to create the Amplicon Coverage Chart. This file contains the following fields: • `contig_id`: the name of the chromosome or contig of the reference for this amplicon. • `contig_srt`: the start location of the amplicon target region. **Note:** This coordinate is 1-based, unlike the corresponding 0-based coordinate in the original targets BED file. • `contig_end`: the last base coordinate of this amplicon target region. **Note:** The length of the amplicon target is given as tlen = (contig_end - contig_srt + 1). • `region_id`: the ID for this amplicon as given as the 4th column of the targets BED file. • `gene_id` or `attributes`: the gene symbol or attributes field as provided in the targets BED file. • `gc_count`: the number of G and C bases in the target region. The %GC that is uses this count divided by the amplicon (insert) length. • `overlaps`: the number of times this target was overlapped by any read by at least one base. **Note:** Individual reads might overlap multiple amplicons where the amplicon regions themselves overlap. • `fwd_e2e`: the number of assigned forward strand reads that read from one end of the amplicon region to the other end. • `rev_e2e`: the number of assigned reverse strand reads that read from one end of the amplicon region to the other end. • `total_reads`: the total number of reads assigned to this amplicon. This value is the sum of fwd_reads and rev_reads and is the field that rows of this file are ordered by (then by contig id, srt, and end). • `fwd_reads`: the number of forward strand reads that are assigned to this amplicon. • `rev_reads`: the number of reverse strand reads that are assigned to this amplicon. • `covNx`: the number of bases of the amplicon target that had at least N reads. There are 3 such columns for the specified coverage tiers, which by default are `cov20x`, `cov100x`, and `cov500x`.
Coverage Analysis Summary (.pdf)	A PDF file that contains the Coverage Analysis Report, including read statistics and charts that are generated by the coverageAnalysis plugin.

molecularCoverageAnalysis plugin in Genexus™ Software

Use the molecularCoverageAnalysis plugin to view statistics and graphs that describe the level of sequence molecular coverage produced for targeted genomic regions. The plugin generates statistics, downloadable data files, and interactive visualization of molecular coverage over targeted regions of the reference genome.

The plugin is only compatible with libraries prepared using Ion AmpliSeq™ HD or Taq Sequencing chemistries.

Review molecular Coverage Analysis plugin results

The molecularCoverageAnalysis plugin generates a Molecular Coverage Analysis Report. This report includes molecule statistics and several charts. The report summary lists the barcodes, the samples, the median molecular coverage, the molecular uniformity, and 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 a list of gene annotations for each sequenced region.

You can download statistics files from the file links at the bottom of the results screen when the **Plugins** tab is selected. After the sequencing run completes, review the plugin results in the report summary.

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 molecular coverage analysis, by barcode, is included in the **molecularCoverageAnalysis** summary pane, located beneath the **coverageAnalysis** summary pane, if present.
4. In the **molecularCoverageAnalysis** summary pane, in the **Barcode Name** column, click a link to open a detailed **Molecular Coverage Analysis Report** for that barcoded sample.
   Alternatively, click the **molecularCoverageAnalysis.html** link to open the summary table for all barcodes in a new window.
5. In the **Molecular Coverage Analysis Report**, review the plugin results.
6. Click the links at the bottom of the **Molecular Coverage Analysis Report** to download associated statistics and summary files for each barcoded sample in the run.

Molecule statistics

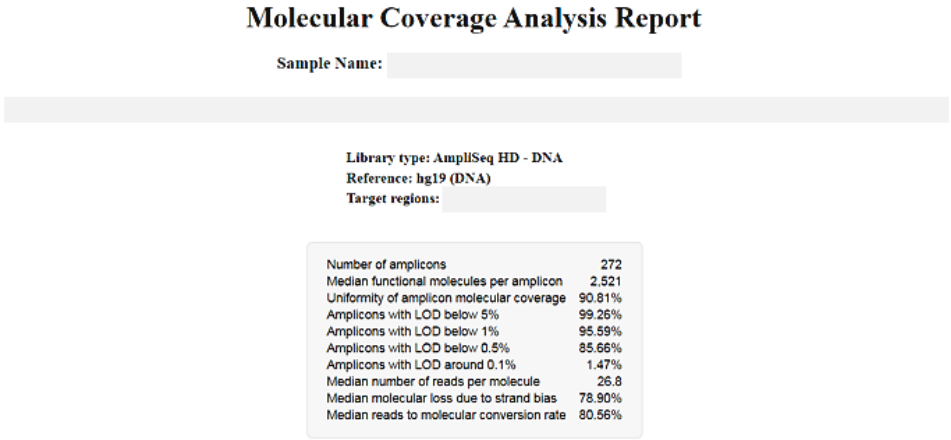
The detailed **Molecular Coverage Analysis Report** that is available when you select a barcode in the coverageAnalysis plugin summary for a run includes a variety of reads statistics. The statistics that are displayed in your report depend on the type of library that is used in your sequencing experiment. Definitions are in tooltips. Almost every statistic, plot, link, and functional widget in the report provides tooltips with definitions. Hover over a heading or description in the report to view the tooltip.

Statistic	Description
Median Functional Molecular Coverage per Amplicon	A functional molecule is a collection of reads that covers the amplicon and satisfies all criteria associated with the parameters (for example, `min_tag_fam_size` and `min_fam_per_strand_cov`) in the parameter file. Molecular coverage is the number of functional molecules. The median is calculated across all amplicons.
Uniformity of Molecular Coverage for all Amplicons	Percentage of amplicons having molecular coverage between 0.5x and 2x of the median molecular coverage.

Statistic	Description
Percentage of Amplicons larger than 0.8x Median Functional Molecular Coverage	Percentage of amplicons having molecular coverage more than 0.8x of the median functional molecular coverage
Median Total Molecular Coverage per Amplicon	The number of molecules that satisfies size criteria in the parameters file. The median is calculated across all amplicons.
Percentage of Reads with Perfect Molecular Tags	Percentage of reads whose molecular tags are exactly the same with design.
Median Functional Molecular Loss due to Strand Bias per Amplicon	The metric is calculated by 1- (`Functional Molecular Coverage`) / (`Molecular Coverage without Strand Constraint`), which measures the loss of functional molecules due to strand constraint for molecular functionality. The median is calculated across all amplicons. This statistic is available in Ion AmpliSeq™ HD runs only.
Median Percentage of Functional Molecules out of Total Molecules per Amplicon	The percentage of functional molecules out of the number of molecules that satisfies size criteria. The median is calculated across all amplicons.
Median Reads per Functional Molecule	For each amplicon, the number of reads supporting each functional molecule is averaged across all functional molecules for that amplicon to determine the number of reads per functional molecule at the amplicon level. The median is calculated across all amplicons.
Median Reads Contributed to Functional Molecules per Amplicon	For each amplicon, the percentage of reads supporting functional molecules is (`Number of Reads Supporting Functional Molecules`) / (`Number of Reads`). The median is calculated across all amplicons.
Percentage of Amplicons below (around) n% LOD	LOD is calculated based on the number of functional molecules for each amplicon and variant calling parameters in the parameter file. Around x n% means in the range from 0.5x n% to 2x n%.

Example Molecular Coverage Analysis Report

The following is an example of a **Molecular Coverage Analysis Report** is available when you select a barcode in the molecularCoverageAnalysis plugin summary.



Molecular Coverage Analysis Report

Sample Name:

Library type: AmpliSeq HD - DNA
Reference: hg19 (DNA)
Target regions:

Number of amplicons	272
Median functional molecules per amplicon	2.521
Uniformity of amplicon molecular coverage	90.81%
Amplicons with LOD below 5%	99.26%
Amplicons with LOD below 1%	95.59%
Amplicons with LOD below 0.5%	85.66%
Amplicons with LOD around 0.1%	1.47%
Median number of reads per molecule	26.8
Median molecular loss due to strand bias	78.90%
Median reads to molecular conversion rate	80.56%

Example charts generated by the molecular Coverage Analysis plugin

The charts in the detailed **Molecular Coverage Analysis Report** include **Plot** and **Overlay** menus that allow you to customize the data that is displayed in each chart.

Click **(Search)** (in the top right corner of a chart) to open the chart **Viewing Options** panel. Click **(Help)** to open a description of the chart.

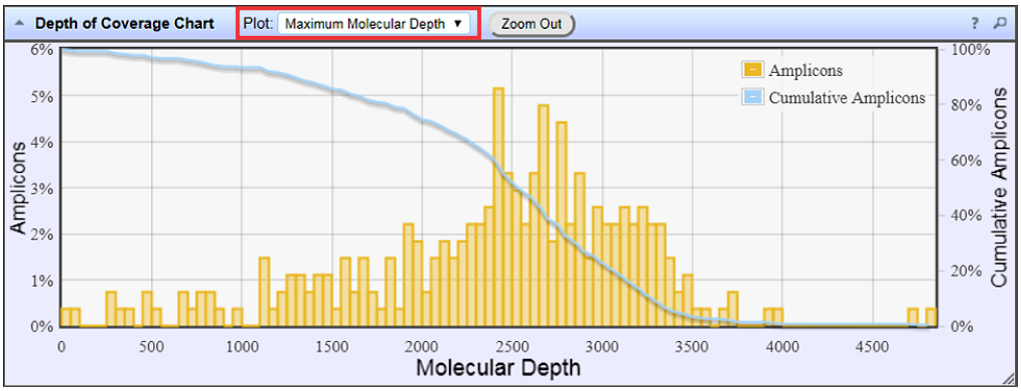


Figure 6 Representative Depth of Coverage Chart

The Depth of Coverage Chart summarizes the amplicon depth of coverage results. The X-axis represents the amplicon molecular depth. The left Y-axis represents the number of amplicons at a given molecular depth or a range (bin) of molecular depths, as a percentage of the total number of amplicons. The right Y-axis represents the cumulative count of the number of amplicons, at a given molecular depth or greater, as a percentage of the total number of amplicons. The individual orange bars represent the percentage of amplicons in the specific range of molecular depths. The blue curve measures the cumulative amplicons at a given molecular depth or greater. Use the **Plot** dropdown list to switch between **Maximum Read Depth**, **99.9% of All Reads**, and **Normalized Coverage** plots.

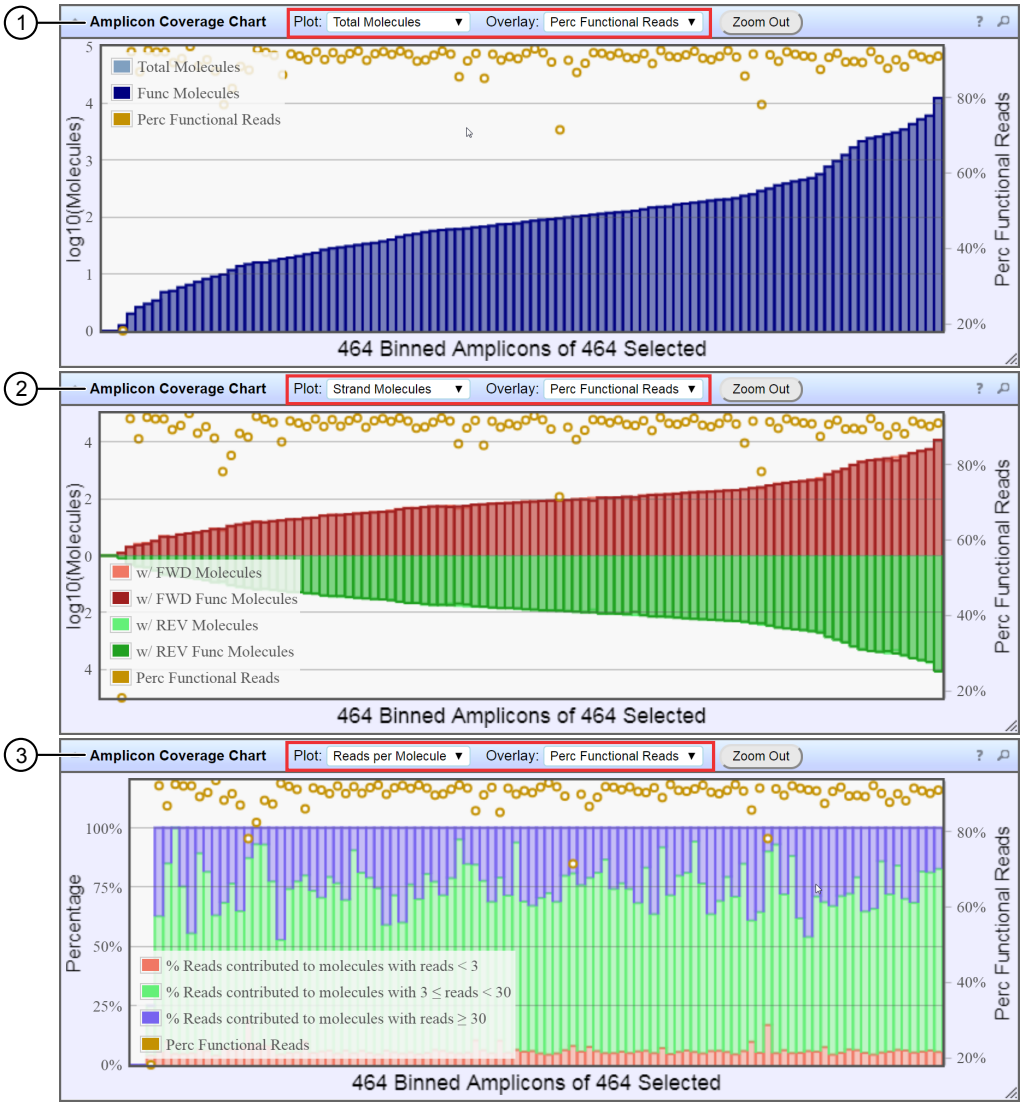


Figure 7 Representative Amplicon Coverage charts

The Amplicon Coverage charts summarize the amplicon molecular coverage results. The X-axis in all plots represents amplicons that are grouped into bins, where each bin contains amplicons that have the same molecular coverage. Depending on the plot selection, the left Y-axis represents either the average number of molecules (on a base 10 log scale) or the percentage of functional molecules in a given bin. The right Y-axis represents the percentage of functional reads in a given bin. Use the **Plot** and **Overlay** dropdown lists to customize the chart view. You can zoom in on a subset of amplicons by using your mouse to draw a rectangle that contains the region of interest. Use **Zoom Out** to revert to the initial view.

① Representative **Total Molecules** plot with an **Perc Functional Reads** overlay
② Representative **Strand Molecules** plot with an **Perc Functional Reads** overlay
③ Representative **Reads per Molecule** plot with an **Perc Functional Reads** overlay

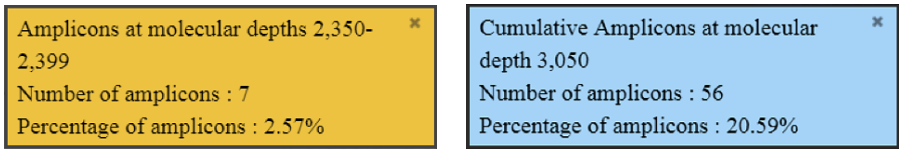


Figure 8 Example detail panes

In most plots, you can click a data point to open a detail pane for that data. For example, in the Depth of Coverage Chart in Figure 6, click an individual orange bar to open the detail pane for amplicons in a specific range of molecular depths or click a point on the blue curve to open the detail pane for cumulative amplicons at that molecular depth or greater. Similarly, you can click any point or bar within each Amplicon Coverage chart to view details for each amplicon coverage bin. For example detail panes, see Figure 8.

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 “Results files” on page 89.

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 CoverageAnalysis plugin. The list of files depends on the application type that was selected during assay creation.

File download selections in the Genexus™ Software

Selection	File name
DNA Molecular Coverage Statistics	`<DNA Barcode><ExpName>.cov.stats.txt`
DNA Molecular depth of coverage	`<DNA Barcode><ExpName>.mol.cov.xls`
DNA Molecular Coverage Analysis Summary	`<DNA Barcode><ExpName>.summary.pdf`
DNA Molecular Amplicon coverage summary	`<DNA Barcode><ExpName>.amplicon.cov.xls`

File contents

File	Description
Molecular Coverage Statistics	This file contains a summary of the statistics presented in the tables at the top of the **Molecular Coverage Analysis Report** 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.
Molecular depth of coverage	Molecular coverage summary data used to create the Depth of Coverage Chart. This file contains the following fields: • `mol_depth`: the depth at which a (targeted) reference amplicon has been read. • `amp_cov`: the number of times any amplicon was read (covered) at this depth. • `amp_cum_cov`: the cumulative number of reads (coverage) at this read depth or greater. • `norm_mol_depth`: the normalized read depth (depth divided by average molecular read depth). • `pc_amb_cum_cov`: same as amp_cum_cov but represented as a percentage of the total reads.
Molecular Coverage Analysis Summary	A PDF file that contains the Molecular Coverage Analysis Report, including molecule statistics and charts that are generated by the molecularCoverageAnalysis plugin.

File	Description
Molecular Amplicon coverage summary	Amplicon molecular coverage summary data used to create the Amplicon Coverage Chart. This file contains the following fields: • `contig_id`: the name of the chromosome or contig (from contiguous) of the reference for this amplicon. • `contig_srt`: the start location of the amplicon target region. Note that this coordinate is 1-based, unlike the corresponding 0-based coordinate in the original targets BED file. • `contig_end`: the last base coordinate of this amplicon target region. Note that the length of the amplicon target is given as (contig_end - contig_srt + 1). • `region_id`: the ID for this amplicon as given as the 4th column of the targets BED file. • `gene_id or attributes`: the gene symbol or attributes field as provided in the targets BED file. • `func_mol_cov`: the number of molecules (functional molecules) which are available for the variantCaller plugin. • `lod`: LOD (limitation of detection) calculated from the number of functional molecules. • `strict_func_umt_rate`: the percentage of functional molecules used with strict molecular tags. • `func_mol_cov_loss_due_to_strand`: the percentage of functional molecules loss due to strand bias. • `fwd_only_mol_cov`: the number of molecules containing forward strand only. • `rev_only_mol_cov`: the number of molecules containing reverse strand only. • `both_strands_mol_cov`: the number of molecules containing both forward strand and reverse strand. • `r2m_conv_rate_all`: the percentage of reads contributed to functional molecules. • `reads_per_func_mol`: the average reads per functional molecules. • `perc_to_mol_(<3_reads)`: the percentage of reads contributed to small size molecules(size<3). • `perc_to_mol_(>=3&<30_reads)`: the percentage of reads contributed to median size molecules (size ≥3 && size <30). • `perc_to_mol_(>=30_reads)`: the percentage of reads contributed to large size molecules (size ≥30).

CustomerSupportArchive plugin

The CustomerSupportArchive plugin generates 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” on page 198.

sampleID plugin

Use the sampleID plugin to track samples or possibly identify misassignment or mix up between samples and barcodes in a sequencing run. The sampleID plugin produces a unique identification code (**SampleID**) for each barcode in a sample.

The plugin can be used with the Ion AmpliSeq™ Sample ID Panel, which is a human SNP genotyping panel, to ensure that the accuracy of samples increase confidence in sample data management. The Ion AmpliSeq™ Sample ID Panel is composed of the identified human sample gender and IUPAC base letters for eight high-frequency noncoding SNPs. Ion AmpliSeq™ Sample ID Panel contains nine primer pairs that can be combined with any Ion AmpliSeq™ Ready-to-Use Panel or Custom Panel.

For the samples to work with this plugin, the Ion AmpliSeq™ library must have been prepared with Ion AmpliSeq™ sample tracking amplicons.

The sampleID plugin is preconfigured and does not require input.

Review sampleID plugin results

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** is shown.
4. Click a barcode name to open the **Sample ID Report**.
5. To return to Genexus™ Software, click back in the browser.

A Reference information

Annotation criteria for Oncomine™ Variant Annotator

When you apply Oncomine™ Variant Annotations to 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.

This section summarizes the requirements that must be met in order for the Oncomine™ Variant Annotator to annotate variants in results for each Oncomine™ Research Assay and Ion AmpliSeq™ HD Panel.

For each variant type in these tables, Oncomine™ Variant Annotator annotates a variant only if all conditions in the corresponding Annotation Criteria column are satisfied. The annotations are shown in results in the Oncomine™ Gene class and Oncomine™ Variant class columns.

Note: You can find all relevant annotation criteria in VCF files.

Oncomine™ Precision Assay GX

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	Gain-of-Function	Amplification	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number deletion	Loss-of-Function	Deletion	• SVTYPE = "CNV" • FILTER = "LOSS" • Occurs in a designated copy-loss gene
Gene fusion	Gain-of-Function	Fusion	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
RNA exon variant	Gain-of-Function	RNAExonVariant	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Expression imbalance	Gain-of-Function	ExpressionImbalance	• SVTYPE = "RNAExonTiles" • FILTER = "PASS"
Missense hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Functional impact is missense • Transcript and coding syntax occur in predefined missense hotspot list
In-frame hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Gain-of-function truncating hotspot mutation	Gain-of-Function	Hotspot	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	Loss-of-Function	Truncating	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
Splice site hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined intronic hotspot list
MNV hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
EGFR exon 19 deletion	Gain-of-Function	EGFRExon19Deletion	• Positive Hotspot mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744-761 of EGFR
EGFR exon 20 insertion	Gain-of-Function	EGFRExon20Insertion	• Positive Hotspot mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762-775 of EGFR or variant is COSM26720
ERBB2 exon 20 insertion	Gain-of-Function	ERBB2Exon20Insertion	• Positive Hotspot mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770-783 of ERBB2
MET exon 14 skipping	Gain-of-Function	METExon14Skipping	• Positive Hotspot mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET Exon 14 Skipping confirmed list

Oncomine™ Comprehensive Assay v3

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Copy number amplification	Gain-of-Function	Amplification	• SVTYPE = "CNV" • FILTER = "GAIN" • Occurs in a designated copy-gain gene
Gene fusion	Gain-of-Function	Fusion	• SVTYPE = "Fusion" • FILTER = "PASS" • Is a targeted fusion isoform
RNA exon variant	Gain-of-Function	RNAExonVariant	• SVTYPE = "RNAExonVariant" or "Fusion" • FILTER = "PASS" • Is a targeted RNA exon variant
Loss-of-function truncating de novo mutation	Loss-of-Function	Truncating	• Positive mutation call • Functional impact is frameshift block substitution, frameshift deletion, frameshift insertion, or nonsense • Occurs in a loss-of-function gene

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
Missense hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Functional impact is missense • Transcript and codon position occur in predefined missense hotspot list
In-frame hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Function, transcript, and coding syntax occur in predefined in-frame hotspot list
Splice site hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript, location, and exon occur in predefined splice site hotspot list
Intronic hotspot mutation	Gain-of-Function Loss-of-Function	Hostpot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined intronic hotspot list
Promoter hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript, location, and coding syntax occur in predefined promoter hotspot list
Gain-of-function truncating hotspot mutation	Gain-of-Function	Hotspot	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a gain-of-function gene
Loss-of-function truncating hotspot mutation	Loss-of-Function	Truncating	• Positive mutation call • Function, transcript, and coding syntax occur in predefined truncating hotspot list • Occurs in a loss-of-function gene
MNV hotspot mutation	Gain-of-Function Loss-of-Function	Hotspot	• Positive mutation call • Transcript and coding syntax occur in MNV hotspot list
EGFR exon 19 deletion	Gain-of-Function	EGFRExon19Deletion	• Positive mutation call • Functional impact is nonframeshift deletion, nonframeshift block substitution • Deletion impacts codons 744-761 of EGFR
EGFR exon 20 insertion	Gain-of-Function	EGFRExon20Insertion	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 762-775 of EGFR or variant is COSM26720

Variant type	Oncomine™ Gene class	Oncomine™ Variant class	Annotation criteria
ERBB2 exon 20 insertion	Gain-of-Function	ERBB2Exon20Insertion	• Positive mutation call • Functional impact is nonframeshift insertion, nonframeshift block substitution • Insertion impacts codons 770-783 of ERBB2
MET exon 14 skipping	Gain-of-Function	METExon14Skipping	• Positive mutation call • Location is splice site in MET exon 14, is intronic >= 4bp deletion in 30 nucleotides preceding MET exon 14, or variant is in MET Exon 14 Skipping confirmed list

System-installed annotation sets

Genexus™ Software includes system-installed annotation sets as presets to use in assays. System-installed annotation sets are locked and cannot be changed.

System-installed annotation sets	Description
All_hg19_514	A system-installed annotation set for use with hg19 assays.
Oncomine™ Comprehensive v3 514 Annotations	A system-installed annotation set for use with Oncomine™ Comprehensive assays.
Oncomine™ Myeloid Assay Annotations v1	A system-installed annotation set for use with Oncomine™ Myeloid assays.
Performance Qualification Annotations V7-1.6	A system-installed annotation set for use with Performance Qualification assays.

System-installed barcode sets

The Genexus™ Software includes system-installed barcode sets as presets to use in assays. System-installed barcode sets are locked and cannot be changed.

System-installed barcode set	Description
Ion AmpliSeq™ HD Dual Barcode Kit 1–24	A set of 24 unique dual-barcode primers designed and validated for optimal performance with Ion GeneStudio™ S5 Series sequencers. For use with Ion AmpliSeq™ HD assays.
IonCode™ Barcodes 1-32	A unique set of 32 barcode adapters designed for optimal performance with Ion GeneStudio™ S5, Ion PGM™, and Ion Proton™ sequencers.
IonCode™ Barcodes 1-384	A unique set of 384 barcode adapters designed for optimal performance with Ion GeneStudio™ S5, Ion PGM™, and Ion Proton™ sequencers.
Ion Select BC Set-1	A unique set of 16 barcode adaptors supplied with the Ion PGM™ Select Library Kit.
Ion SingleSeq™ Barcodes	A unique set of 96 barcode adaptors optimized for use with Ion ReproSeq™ libraries.
Ion Torrent™ Dual Barcode Kit 1–96	A set of 96 unique dual-matched barcode adapters designed to enable optimal performance of amplicon libraries and Ion semiconductor sequencers For use with Ion AmpliSeq™ HD and Oncomine™ assays.
Ion Torrent™ Genexus™ Barcodes 1–32 HD	A unique set of 32 barcode adaptors designed and validated for optimal performance with the Genexus™ Integrated Sequencer For use with Ion AmpliSeq™ HD assays.
Ion Torrent™ Genexus™ Barcodes 1–96 AS	A unique set of 96 barcode adaptors designed and validated for optimal performance with the Genexus™ Integrated Sequencer
Ion Xpress™ Barcodes	A unique set of 96 barcode adaptors for use with Ion AmpliSeq™ libraries and Ion Xpress™ Plus fragment libraries.

System-installed filter chains

Genexus™ Software includes system-installed filter chains as that are included as presets in assays to filter in or our variants of interest in run results. The filter chains can also be used to dynamically change the view of the variants as you view results. System-installed filter chains are locked and cannot be changed. You cannot edit system-installed filter chains, but you can create custom filter chains that are built from the system-installed filter chains.

System-installed filter chain	Description
Oncomine™ Extended (5.14)	This filter chain includes all Oncomine™-annotated variants and variants that may be relevant to cancer due to their inclusion in one or more of the following classes: • Non-targeted fusions. • CNV variants with FILTER value of GAIN or LOSS. • Likely somatic mutations based upon 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. • Variants with ClinVar annotations of pathogenic or likely pathogenic.
Oncomine™ Variants (5.14)	This filter chain includes all Oncomine™-annotated variants.
Variant Matrix Summary (5.14)	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.

System-installed filters

Genexus™ Software includes system-installed filters that you can add to custom filter chains. A filter chain is a set of filters that the software uses to narrow the list of variants that are included in results. When you create a filter chain, you can dynamically change the view of variants in results, or you can filter in or out variants from results by adding the filter chain to an assay that is used for a sequencing run.

The system-installed filters are based on public and proprietary annotation sources and data types that are included in the software.

Annotation source filters

You can create filter chains that identify variants based on the annotations associated with those variants. The software uses annotation sources from public and proprietary genomic databases to apply these annotations to variants during analysis.

Filters that are made from annotation sources are available in the software and you can use these filters to build filter chains that narrow the list of variants that appear in the analysis results.

5000Exomes filters

The 5000Exomes filters can be added to filter chains to detect minor allele frequencies (MAF) for specific population groups in the software. The filters are derived from the 5000Exomes annotation database. The specific filters are:

- 5000Exomes AfricanAmerican MAF(1)
- 5000Exomes AfricanAmerican MAF(20161108)
- 5000Exomes EuropeanAmerican MAF(1)
- 5000Exomes EuropeanAmerican MAF(20161108)
- 5000Exomes Global MAF(1)
- 5000Exomes Global MAF(20161108)

Filter conditions include the ability to set minor allele frequency ranges from 0.0 to 0.5. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns minor allele frequency results in samples that align with the 5000Exomes minor allele frequency database.

Clinvar filter

This filter can be added to filter chains in the software to evaluate the impact of variants observed in samples that match those in the National Center for Biotechnology Information (NCBI) ClinVar database. The specific databases are:

- Clinvar(20180725)
- Clinvar(20190909)

The impact values that can be included in the filter chain are: Pathogenic, Likely benign, Benign, other, Likely pathogenic, Uncertain significance, not provided, and drug response.

The filter returns all variants with the selected impact values that match those in the NCBI ClinVar database.

COSMIC filter

This filter can be added to filter chains in the software to compare variants to the catalog of somatic mutations in tumor tissue as compiled by the COSMIC database. The specific databases are:

- COSMIC(85)
- COSMIC(89)

Filter conditions include the ability to select all COSMIC values, to select specific annotation values, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants that match those in the COSMIC database.

dbSNP filter

This filter can be added to filter chains in the software to compare single nucleotide polymorphism variants in samples against the dbSNP database. The specific filters are:

- dbSNP(151)
- dbSNP(153)

Filter conditions include the ability to select all dbSNP values (In), or to exclude all dbSNP values (Not In).

The filter detects SNP variants that match those in the dbSNP database, which can be included in or excluded from filtered analysis results.

DGV filter

This filter can be added to filter chains in the software to detect human genomic structural variants that match those in the Database of Genomic Variants (DGV). The specific filters are:

- DGV filter(20130723)
- DGV filter(20160515)

Filter conditions include the ability to include all DGV variants (In), or to exclude all DGV variants (Not In) in filtered analysis results.

The filter detects human genomic structural variants as defined by DGV, which can be included in or excluded from filtered analysis results.

DrugBank filter

This filter can be added to filter chains in the software to detect variants that are correlated with drugs and drug targets listed DrugBank database. The specific filters are:

- DrugBank (20180731)
- DrugBank (20190723)

Filter conditions include the ability to select specific values, to include all DrugBank values (In), or to exclude all DrugBank values (Not In). In addition, unannotated variants can be included or excluded in filtered analysis results.

This filter detects variants that are correlated with drugs and drug targets in DrugBank, which can be included in or excluded from filtered analysis results.

ExAC filters

These filters can be added to filter chains to detect rare gene variants for specific population groups in the software. The filters are derived from the Exome Aggregation Consortium (ExAC) database. The specific filters are:

- ExAC AAF(01)
- ExAC EAAF(01)
- ExAC EFAF(01)
- ExAC ENFAF(01)
- ExAC GAF(01)
- ExAC LAF(01)
- ExAC OAF(01)
- ExAC SAAF(01)

Filter conditions include the ability to set a range from 0.0 to 1.0. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter detects rare gene variant results that align with the ExAC database, which can be included or excluded from filtered analysis results.

FATHMM Scores filter

This filter can be added to filter chains in the software to calculate Functional Analysis through Hidden Markov Models (FATHMM) Scores for coding variants, nonsynonymous single-nucleotide variants (nsSNVs), and noncoding variants. The specific filters are:

FATHMM Scores(85)

FATHMM Scores(89)

Filter conditions include the ability to set a range from 0.0 to 1.0 for the score. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter calculates FATHMM scores for coding variants, nsSNVs, and noncoding variants.

Gene Ontology (GO) filter

This filter can be added to filter chains in the software to detect various gene ontologies (functions of specific genes). The specific filters are:

Gene Ontology (20171101)

Gene Ontology (20190930)

Filter conditions include the ability to select all (In), none (Not In), or to select specific annotations, and to include or exclude unannotated variants in filtered analysis results.

This filter returns gene ontologies of samples based on the selections made.

Gene Symbol filter

This filter can be added to filter chains in the software to report on specific genes.

Filter conditions include the ability to select all (In), none (Not In), or specific gene symbols from the list, and to include or exclude unannotated variants in filtered analysis results.

This filter returns the gene symbols of genes that are found in your samples.

Minor Allele Frequency filter

This filter can be added to filter chains in the software to detect variants with minor allele frequencies that match those in the dbSNP database from the 1000 genomes project.

Filter conditions include the ability to set a range from 0.0 to 0.5 for minor allele frequencies. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns variants in samples that match those in the dbSNP database from the 1000 genomes project.

OMIM filter

This filter can be added to filter chains in the software to detect variants that match those in the Online Mendelian Inheritance in Man® (OMIM) database. The specific filters are:

OMIM(20180823)

OMIM(20191001)

Filter conditions include the ability to search for all (In), none (Not In), or specific annotations, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants in samples that match those in the OMIM database.

Pfam filter

This filter can be added to filter chains in the software to detect protein domain families in the coded proteins as defined by the Pfam database. The specific filters are:

Pfam(31)

Pfam(32)

Filter conditions include the ability to include all (In), none (Not In), or specific annotations, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants in the samples that match the variants in the Pfam database.

PhyloP Scores filter

This filter can be added to filter chains in the software to measure the conservation of protein across a wide range of organisms in 16S metagenomics analyses. The specific filters are:

PhyloP Scores(20160919)

Filter conditions include the ability to set a score range from –14.0 to 3.0. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

This filter returns PyloP scores for 16S metagenomics samples.

UCSC Common SNPs filter

This filter can be added to filter chains in the software to detect variants that match those in the UCSC Genome Browser Common SNPs database.

Filter conditions include the ability to include (In) or exclude (Not In) variants that match the UCSC Common SNPs in filtered results.

The filter returns variants in samples that match the UCSC Common SNPs database.

Data type filters

You can create filter chains that are based on variant data types in the software, such as allele frequency and allele ratio. Many data types that are included in the results tables are available as filters.

Allele fraction filter

The Allele fraction filter can be added to filter chains in the software to report the frequency of alleles observed in raw data.

Filter conditions include the ability to set allele frequency ranges from 0.0 to 1.0. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns allele frequencies of alleles observed in raw data.

Allele ratio filter

This filter can be added to filter chains in the software to narrow analysis results to nonreference allele frequencies.

Filter conditions include the ability to set allele ratio ranges from 0.0 to 1.0. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns all variants that have at least one nonreference allele reported with a frequency in the selected filter range.

Allele read-count filter

This filter can be added to filter chains in the software to set the minimum count for genotype alleles.

Filter conditions include the ability to set allele read-count ranges from 0 to 100,000. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter removes variant candidates that do not have the required numbers of supporting reads from analysis results.

Alternate allele count filter

This filter can be added to filter chains in the software to set the minimum count for alternate alleles.

Filter conditions include the ability to set alternate allele read-count ranges from 0 to 100,000. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter removes variant candidates that do not have the required numbers of reads from analysis results.

CNV Confidence Range filter

This filter can be added to filter chains in the software to return copy number variants (CNV) with confidence levels between 10 and 10,000,000 and other variants.

Filter conditions include the ability to set the CNV confidence range from 10 to 1.0E7. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns copy number variants with high confidence levels and other variants.

CNV Confidence Range—CNVs Only filter

This filter can be added to filter chains in the software to return copy number variants (CNV) with confidence levels between 10 and 10,000,000.

Filter conditions include the ability to set the CNV confidence range from 10 to 1.0E7. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns only copy number variants with high confidence levels.

CNV Somatic Confidence Range filter

This filter can be added to filter chains in the software to make ploidy estimates with lower and upper confidence values on somatic samples. The filter detects extra copies over the expected normal ploidy value (2 for autosomes, 2 for X chromosomes in females, and 1 for X chromosomes in males). The 5% lower confidence bound value is the ploidy estimate where there is a 5% chance that the true ploidy is below that value. The 95% upper confidence bound is the ploidy estimate where it is 95% certain that the true ploidy is below that value. The lower bound is the most important for gains. The upper bound is most important for losses.

By default, the filter is set to detect gains and losses using the confidence interval values of 5% confidence interval for Minimum Ploidy Gain over the expected value and 95% confidence interval for Minimum Ploidy Loss under the expected value. The default boundary values are set to 0.0.

If you set the **Minimum Ploidy Gain (5% CI) over expected** to **2.0** (2+2=4) and the **Minimum Ploidy Loss (95% CI) under expected** to **0.0**, you can expect the following example CNV call CI data:

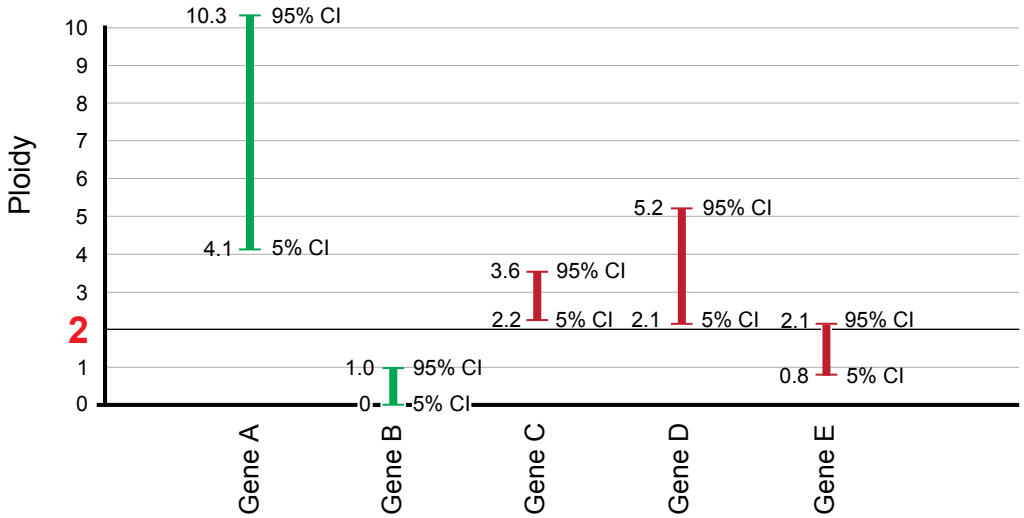
Gene A—A gene with suspected gain of 5% CI = 4.1 and 95% CI = 10.3 filtered in.

Gene B—A gene with suspected loss of 5% CI = 0 and 95% CI = 1.0 filtered in.

Gene C—A gene with suspected gain of 5% CI = 2.2 and 95% CI = 3.6 filtered out (2.2 is less than 4).

Gene D—A gene with suspected gain of 5% CI = 2.1 and 95% CI = 5.2 filtered out (2.1 is less than 4).

Gene E—A gene with 5% CI = 0.8 and 95% CI = 2.1 filtered out (0.8 is less than 4 and 2.1 is greater than 2).



CNV Somatic Confidence—CNVs Only filter

This filter can be added to filter chains in the software to make ploidy estimates with lower and upper confidence levels.

Filter conditions include the ability to enable and set the range for Minimum Ploidy Gain (5% CI) over expected, to enable and set the Minimum Ploidy Loss (95% CI) under expected ploidy values. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns only somatic copy number variants within the lower and upper limits of the set confidence ranges.

Confident Somatic Variants filter

This filter detects confident somatic variants in tumor-normal analyses. The filter compares tumor samples to matched normal samples. It does not apply to single sample analyses.

Filter conditions include the ability to include the confident somatic variants (In), or to exclude the confident somatic variants (Not In) in filtered analysis results.

Deamination filter

Default DNA and Fusions View filter

This filter can be added to filter chains in the software to detect all of the fusion variants, expression control markers, assay 5′/3′ markers, and all DNA variants that the predefined Default DNA and Fusions View filter chain detects.

Filter conditions include the ability to select specific annotations (Present or Absent), to include all default DNA and Fusions view variants (In), or to exclude all Default DNA and Fusions view variants (Not In) in filtered analysis results. In addition, unannotated variants can be included or excluded in filtered analysis results.

The filter detects the fusion variants, expression control markers, assay 5′/3′ markers, and all DNA variants, which can be included or excluded in filtered analysis results.

Default Fusions View filter

This filter can be added to filter chains in the software to detect all of the fusion variants, expression control markers, and assay 5′/3′ markers that the predefined Default Fusions View filter chain detects.

Filter conditions include the ability to select specific annotations (Present or Absent), to include all Default Fusions View variants (In), or to exclude all Default Fusions View variants (Not In) in filter analysis results. In addition, unannotated variants can be included or excluded in filtered analysis results.

The filter detects fusion variants, expression control markers, and assays 5′/3′ markers, which can be included or excluded in filtered analysis results.

Disease Research Area (DRA) filter

This filter can be added to filter chains in the software to annotate disease research categories as defined by the Disease Research Area (DRA) annotation source database. The specific filters are:

- Disease Research Area (DRA)(20170112)
- Disease Research Area (DRA)(20170914)

Filter conditions include the ability to select specific annotations, to include all Disease Research Area values (In), or to exclude all Disease Research Area values (Not In) in filtered analysis results. In addition, unannotated variants can be included or excluded in filtered analysis results.

This filter annotates disease research areas that match the DRA database, and can be included or excluded.

Expected normal ploidy buffer filter

The Expected normal ploidy buffer filter can be added to filter chains in the software to detect mosaic chromosomal aneuploidies in research samples and to set a buffer for normal ploidy values (2 for autosomes, 2 for X chromosomes in females, and 1 for X and Y chromosomes in males) so as to identify the most likely aneuploidy samples. Ploidy calls do not have to be integers because mosaicism detection allows decimal-level Copy Number Gain or Loss calls.

By default, the filter is set to filter out all copy number variant segments of gain or loss within 0.2 ploidy value of expected normal. Gains must be above ploidy 2.2 and below ploidy 1.8 to remain filtered in on autosome and female X, and ploidy 1.2 and ploidy 0.8 on male Y. Filter conditions include the ability to change the amount of buffer by adjusting the From and To values. This Expected Normal Ploidy Buffer overrides any Confidence filtering that may be in effect within the buffer zone.

If default settings are applied, ploidy values of 1.8 to 2.2 are filtered out as normal for autosomes and X chromosomes in females, and 0.8 to 1.2 are filtered out as normal for X and Y chromosomes in males. Values outside this range are detected as chromosomal ploidy samples.

Filtered Coverage filter

This filter can be added to filter chains in the software to review results that meet a specific coverage threshold. By default, the filter reports all results with a coverage threshold greater than 100. Or, you can use the filter settings to change the threshold.

Filter conditions include the ability to set a minimum and a maximum value for minor allele frequency for the coverage threshold. Ranges from 0.0 to 0.0045 can be selected for the minimum in the **From** field and in the **To** field for the maximum. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

Functional Scores filter

This filter can be added to filter chains in the software to provide functional scores based on SIFT, PolyPhen, and Grantham scores. SIFT and PolyPhen scores are predictions of the functional effect of a variant on a protein. A Grantham score attempts to predict the distance between two amino acids, in an evolutionary sense (lower is less distance, higher is greater distance).

Filter conditions include the ability to include or exclude SIFT, PolyPhen, and Gratham scores, and to set ranges for those scores. SIFT and PolyPhen ranges can be set from 0.0 to 1.0, and Grantham can be set from 0.0 to 215.0. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns a functional score based on the three (or fewer) scores.

Fusion Read Counts filter

This filter can be added to filter chains in the software to produce counts for fusion reads.

Filter conditions include the ability to set a minimum count of 0 or higher, to include the boundary value for the count, and to include or exclude unannotated variants in filtered analysis results.

This filter returns results that meet the fusion read minimum count value.

Genomic Coordinates filter

This filter can be added to filter chains in the software to narrow analysis results to specific genomic regions. The filter can also be used to identify regions that are targeted by an amplicon or amplicons in a user-defined assay.

The filter value is one chromosome region, or multiple regions that are separated by OR operators or commas.

The Genomic Coordinates filter returns variants in samples that are in the range for the genomic coordinates that you set.

IMPORTANT! Do not use AND, OR, or NOT when adding values to the Genomic Coordinates filter.

Homopolymer Length filter

This filter can be used in a filter chain in the software to detect INDELs of specific homopolymer lengths.

Filter conditions include the ability to set a range from 0 to 1,000 for homopolymer length. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

This filter returns INDEL variants in samples that meet the specified homopolymer length.

Hotspot filter

This filter can be added to filter chains in the software to detect variants that overlap specific hotspots.

Filter conditions include the ability to include (In) or exclude (Not In) hotspots as defined by a hotspots file in filtered analysis results.

The filter returns variants that overlap hotspots included in an uploaded hotspots file.

Location filter

This filter can be added to filter chains in the software to detect variants in specific positions, such as exonic.

Filter conditions include the ability to select all locations or specific locations in filtered analysis results. Locations include:

- unknown
- intergenic
- intronic
- exonic
- utr_5
- utr_3
- splicesite_5
- splicesite_3
- upstream
- downstream
- exonic_nc
- intronic_nc
- ncRNA
- nonCoding

The filter returns variants in samples that match the selected filter locations.

Named Variants filter

This filter can be added to filter chains in the software to detect known variants in the Ion AmpliSeq™ Community Panel for the cystic fibrosis transmembrane regulator (CFTR) gene, for example: c.2054delA.

Filter conditions include the ability to select all named variants, to select specific named variants, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants in samples that match the CFTR variants that are specified in the filter.

Minimum Limit of Detection at Genomic Location filter

The Minimum Limit of Detection at Genomic Location filter can be added to filter chains to filter for a minimum limit of detection (LOD) at chromosomal locations within a selected range.

This LOD calculation depends on the depth of coverage at a given chromosomal location and the probability that is defined by **min_callable_prob** parameter.

This filter is for use with Ion AmpliSeq analysis results and analysis workflows only. For more information, see “Parameters step assay options” on page 105.

Filter conditions include the ability to set a minimum and a maximum value for minor allele frequency. Ranges from 0.0 to 0.0045 can be selected for the minimum in the **From** field and in the **To** field for the maximum. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

Oncomine™ filter

This filter is the default filter for most Oncomine™ assays. It presents the user with Oncomine™ driver variants that are based on their Oncomine™ Gene Class and Oncomine™ Variant Class annotations. It can be added to filter chains in the software to detect Oncomine™-annotated variants in specific Oncomine™ panels.

Analysis workflows for Oncomine™ assays and analysis workflow templates for Ion AmpliSeq™ HD panels in Ion Reporter™ Software include the Oncomine™ Variant Annotator plugin. The plugin integrates into analysis results data from more than 24,000 exomes across solid tumor and hematological cancer types, and annotates variants relevant to cancer with Oncomine™ Gene class and Oncomine™ Variant class information. For more information and a full list of annotation rules for each Oncomine™ assay, see “Annotation criteria for Oncomine™ Variant Annotator” on page 172, or contact your local support representative, Field Bioinformatics Specialist (FBS), or Clinical Account Consultant (CAC).

The Oncomine™ filter (Oncomine = In) returns variants in the samples that have Oncomine™ Gene class and Oncomine™ Variant class annotation.

This filter can be used only if an Oncomine™ analysis workflow was used for the analysis. You can apply filter chains that include the Oncomine™ filter only if the analysis workflow that was used for the analysis includes the Oncomine™ Variant Annotator plugin. Examples of filter chains that include this filter are the Oncomine™ Variants and Oncomine™ Extended filter chains.

Possible Polyploidy Alleles filter

The Possible Polyploidy Alleles filter can be added to filter chains to detect searches alleles which have passed all variant calling filters used by the Torrent Variant Caller (TVC) module but missed by genotype.

The Possible Polyploidy Alleles filter is useful for identification of variants beyond diploid genomes, that is, cancer cells or polyploidy species.

Filter conditions include the ability to select **YES** to find alleles that have passed all filters, **NO**, to find alleles that have *not* passed all filters in addition to genotype, or both **YES** and **NO**.

PValue filter

This filter can be added to filter chains in the software to determine the *p*-value of variants. The *p*-value is a statistical method for the detection of variant calls from next-generation sequencers.

Filter conditions include the ability to set a range from 0.0 to 1.0. The option to include or exclude unannotated variants in filtered analysis results is also available. The **Inclusive** option, to include or exclude boundary values in the range, is selected by default and cannot be changed.

The filter returns *p*-values for variants calls in the samples.

Variant Classification filter

This filter can be added to filter chains in the software to narrow results to the following user-set variant classifications:

- Unclassified
- Unknown
- Benign
- Suspected Benign
- Likely Benign
- Suspected Deleterious
- Deleterious
- Pathogenic
- Likely Pathogenic
- VUS
- Uncertain Significance
- Technical Artifact

Filter conditions include the ability to include all (Filter Option set to In), none (Filter Option set to Not In), or to select specific classifications in filtered analysis results.

The filter returns variants that are classified by software users.

Variant Effect filter

This filter can be added to filter chains in the software to detect the effect of variants on coding sequences. Specific values include:

- refAllele
- unknown
- synonymous
- missense
- nonframeshiftInsertion
- nonframeshiftDeletion
- nonframshiftBlockSubstitution
- nonsense
- stoploss
- frameshiftInsertion
- frameshiftDeletion
- frameshiftBlockSubstitution

Filter conditions include the ability to select all (Filter Option set to In), none (Filter Option set to Not In), or to select specific values, and to include or exclude unannotated variants in filtered analysis results.

The filter returns variants in samples that match the selected variant effects.

Variant Subtype filter

This filter can be added to filter chains in the software to detect variant subtypes, including:

- BigDel
- BigDup
- GeneCNV
- REF
- NOCALL

Filter conditions include the ability to select all or specific variant subtypes in filtered analysis results.

The filter returns variants that match the selected specific variant subtypes.

Variant Type filter

This filter can be added to filter chains in the software to detect variants that match the following variant types:

- SNV
- INDEL
- MNV
- REF
- NOCALL
- CNV
- LONGDEL
- FUSION
- EXPR_CONTROL
- ASSAYS_5P_3P
- RNA_HOTSPOT
- GENE_EXPRESSION
- RNAExonVariant
- ProcControl
- FLT3ITD

  Note: The FLT3ITD variant type is detected only in Oncomine™ Myeloid Assay analysis workflows.

Filter conditions include the ability to select all variant types or specific variant types in filtered analysis results.

The filter returns variants in analyses that match the selected specific variant types.

Zygosity filter

This filter can be added to filter chains in the software to detect variants that are homozygous or heterozygous.

Filter conditions include the ability to search for both homozygous and heterozygous variants, or either of them, in filtered analysis results.

The filter returns variants in analyses that are homozygous or heterozygous.

System-installed plugins

The Genexus™ Software includes system-installed plugins to use in runs. System-installed plugins are locked and cannot be changed.

System-installed plugin	Description
coverageAnalysis	This plugin provides statistics and graphs that describe the level of sequence coverage that is produced for targeted genomic regions.
CustomerSupportArchive	This plugin generates 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” on page 198.
molecularCoverageAnalysis	This plugin provides statics and graphs that describe the level of sequence molecular coverage that is produced for targeted genomic regions.

System-installed genome reference files

The Genexus™ Software includes system-installed genome reference files as presets to use in assays. System-installed genome reference files are locked and cannot be changed.

System-installed genome reference file	Description
hg19	Human genome reference.

System-installed sample attributes

The following table lists and describes system-installed sample attributes. System-installed sample attributes cannot be edited. Custom sample attributes are not listed in this table.

Sample attribute	Description
Sample Name[1]	A unique identifier representing the sample. The sample name can contain only alphanumeric characters (0–9, Aa–Zz), full stops/periods (.), underscores (_), or hyphens (-), cannot contain spaces, and is limited to a maximum of 20 characters. **IMPORTANT!** To prevent erroneous sample selection during run planning, make sure that you assign a unique and distinguishable sample name for each sample. **Note:** • Samples that have been used in a run cannot be deleted. • To prevent duplication, the software checks all sample names and returns an error message if a non-unique sample name is detected.
Collection Date	The date that the sample was collected. Click **Calendar** to select the date in the correct format.
Gender	The biological sex of the sample: **Female**, **Male**, or **Unknown**. **IMPORTANT!** **Male** or **Female** must be selected for proper measurement of AR CNV.
Sample Type	A term that describes the sample, for example, RNA. You can also select **Other**, then enter a custom sample type.
Disease Category	The disease type of the sample. **Note:** If you select **Cancer** in this list, the **Cancer Stage**, **Cancer Type**, **% Cellularity**, and **% Necrosis** attributes listed below become available in the **Add New Sample** dialog box.
Cancer Stage	The stage of the cancer from which the sample was collected. Select **Stage 0–IV**, or **Primary**, **Unknown**, or **Other**.
Cancer Type	The type of cancer that is represented by the sample. Select the type of solid or hematologic cancer. If cancer type is unknown, select **Unknown Primary Origin**.
% Cellularity	The percentage of tumor cells over normal cells in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is applicable to FFPE samples only. **IMPORTANT!** • If not set, **% Cellularity** is assumed to be 100% in calculations that use this attribute. • **% Cellularity** is a required attribute for CNV analyses. Do not leave the field blank.

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

[1] Required attribute

System-installed verification templates

Field Service Engineers use the system-installed verification templates during Genexus™ Integrated Sequencer installation.

IMPORTANT! The **Assays / Verification Templates** screen lists the verification templates that are used only by service engineers during Genexus™ Integrated Sequencer installation and performance qualification testing. These templates should not be used or modified in any way.

Details about each verification template is available when you click the name of the template in the software.

- Performance Qualification Assay
- System Install Qualification Assay

B

Troubleshooting

Download Instrument Diagnostics

You can download diagnostic files for troubleshooting purposes. Instrument diagnostic files include:

- Diagnostic archive file (ZIP format)
- Init.log file (a text file)
- Installation Acceptance Report (PDF format)

1. In the menu bar, click ⚙ **(Settings)** ▸ **Services**.
2. Scroll to the bottom of the screen.
3. In the **Download Instrument Diagnostics** section, in the **Select Instrument** dropdown menu, select the instrument.
4. Click **Download**.

Customer Support Archive (CSA)

You can download an archive that a Technical Support representative can use to diagnose Genexus™ Software issues. The Customer Support Archive (CSA) contains log files and other technical data about your Genexus™ Software and other files from sequencing runs on a Genexus™ Integrated Sequencer.

You can access customer support archive files from the **Results / Sample Results** and the **Results / Run Results** screens.

Download a customer support archive

Customer support archives are available to download from the **Results / Run Results**, **Results / Sample Results**, and from the ··· **(More Options)** dropdown list in the Sample Summary **Results** screens.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Name** row of the run result for which you want to download the customer support archive, in the **Actions** column, click **CSA**.

An XZ compressed TAR archive (TZV) file is downloaded to the directory location that you specified to download files from the browser. This location depends on your browser settings. You can attach this archive to an email to send to Customer Support.

Troubleshoot Genexus™ Software

Observation	Possible cause	Recommended action
Cannot sign in to the Genexus™ Software	You have either forgotten your password or are signed out due to several failed login attempts.	Contact the Genexus™ Software system administrator.
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique sample name.	Every sample name in the software must be unique. Ensure that the spreadsheet does not contain any duplicate sample names, and repeat the import. Note that the system check is not case-sensitive, so a sample name of ABC1 conflicts with abc1.

Observation	Possible cause	Recommended action
Batch sample import fails *(continued)*	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors.
Library batch import fails	One or more entries in the library batch import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the library batch import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean library batch import template file, and be careful to copy only those rows that contain actual data.
	The library batch import spreadsheet contains a nonunique Library Batch ID.	Every Library Batch ID in the software must be unique. Ensure that the spreadsheet does not contain any duplicate IDs, and repeat the import. Note that the system check is not case-sensitive, so a Library Batch ID of ABC1 conflicts with abc1.
	A sample name entered in the library batch import spreadsheet does not match a sample name listed in the **Manage Samples** screen.	Ensure that the sample names entered into the spreadsheet are correct and match an existing sample name added to the software.
	The Barcode ID name format does not exactly match the format that is used in the **Prepare Library Batch** dialog box.	Use the name format following the Barcode ID name format found in the Barcode Set reference lists (**Settings** ▸ **References** ▸ **Barcode Set**), for example: IonDual_0101 through IonDual_0196, or IonHDdual_0101 to IonHDdual_0132.
	An invalid library, control, or panel kit barcode has been entered in the spreadsheet.	Ensure that you have correctly entered a valid kit barcode in the appropriate cell of the spreadsheet.
The assay I created does not appear in the menu when I plan a run	Forgot to lock your assay.	Go to **Assay tab** ▸ **Manage Assays** and make sure that the assay is locked.
Cannot upload my panel or hotspots	Issues with BED file format or files do not end in (.bed).	Ensure your file is in the correct BED format and has a (.bed) extension.

Observation	Possible cause	Recommended action
Variants tab is missing hotspot entries. The remaining entries are present.	Hotspot BED file contains entries that are incorrectly formatted.	Check that BED file entry is correctly formatted. See the following examples: SNP entry: chr1 2337276 2337277 SVA_322 0 + REF=C;OBS=T;ANCHOR=G AMPL Deletion entry: chr1 201341175 201341180 SVA_497 0 + REF=AGAAG;OBS=;ANCHOR=C AMPL Insertion entry: chr1 236978992 236978992 SVA_621 0 + REF=;OBS=TCTG;ANCHOR=T AMPL
		Confirm the REF values match the actual reference coordinate of hg19.
No information for my loci of interest in the results	The wrong hotspot or BED file is associated with the assay.	1. Check the hotspot and BED files associated with the assay. If either is incorrect, create a new assay. 2. Plan a new run for the sample or sample library with the correct assay. 3. Repeat sequencing of the sample or sample library.
Cannot download run result files	The run failed. Links to run files are not available for runs that fail QC.	Create a reanalysis assay with reduced QC parameters, then reanalyze your sample.
		Repeat the run.

C

Documentation and support

Related documentation

cs_Ion Reporter_pub

Document	Publication number
Genexus™ Integrated Sequencer User Guide	MAN0017910
Genexus™ Integrated Sequencer Quick Reference	MAN0017912
Genexus™ Integrated Sequencer Site Preparation Guide	MAN0017918
Ion Reporter™ Software 5.12 User Guide	MAN0018031
Oncomine™ Precision Assay GX User Guide	MAN0018508
Oncomine™ Comprehensive Assay v3 GX User Guide	MAN0018512
Oncomine™ TCR Beta-LR Assay GX User Guide	MAN0018513

Customer and technical support

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

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

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

Limited product warranty

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

Glossary

Genexus™ Software definitions

adapter dimer (ISPs)	ISPs that carry DNA insert length of less than 8 bp.
aligned read length	The aligned length of a read at a given accuracy threshold is defined as the greatest position in the read at which the accuracy in the bases up to and including the position meets the accuracy threshold. For example, the AQ17 length of a read is the greatest length at which the read error rate is 2% or less. The "perfect" length is the longest perfectly aligned segment. For all of these calculations, the alignment is constrained to start from position 1 in the read—that is, no 5′ clipping is allowed.
aligned reads	The number of bases covered by reads aligned to the reference sequence.
AQ score	An alignment quality (AQ) score defines read accuracy when compared to the reference sequence. The discrepancy can be biological (real variant) or technical (sequencing error). For example, an AQ score of 17 represents 2% aligned read error rate, while an AQ score of 20 represents 1% aligned read error rate when compared to the reference sequence.
average base coverage depth	The average number of reads of all targeted reference bases.
average base read depth	The average number of reads of all targeted reference bases that were read at least once.
BAM file	A BAM (binary alignment map) file (. bam) is the binary version of a SAM (sequence alignment map) file. A SAM file (.sam) is a tab-delimited text file that contains sequence alignment data. A BAM file contains aligned reads sorted by reference location.
barcode	A barcode is a machine-readable code in the form of numbers and a pattern of parallel lines of varying widths, printed on and identifying a product. There are several applications for barcodes. Libraries can be molecularly barcoded with unique nucleic acid sequence identifiers. Library barcodes are used during data analysis to sort the sequencing results from sequencing reactions that contain combined libraries. Chips and sample tubes also contain unique numeric barcodes that aid in the setup of the experimental analysis workflow.

base calling	Base calling is the process by which raw data from the sequencing instrument is converted to nucleotide sequences. This is performed by base calling software that is usually run from the instrument itself.
basecalling input file	Signal processing input files are converted to a single condensed basecalling input file that represents the processed signal. Basecalling input files are required files for basecalling.
bead loading	The percentage of chip wells that contain live ISPs. The percentage value considers only potentially addressable wells. Bead loading is calculated as number of loaded ISPs divided by the number of potentially addressable wells.
bp	Abbreviation for "base pair(s)".
cellularity (%)	The percentage of tumor cells in a given sample.
BED file	Browser Extensible Data file—BED file—defines chromosome positions or regions.
CNV	Copy number variation (CNV) is the variation in copy number of any given gene between two samples. CNV is a phenomenon in which sections of the genome are repeated and the number of repeats in the genome varies between individuals in the human population.
contig	A contig (from contiguous) is a set of overlapping DNA segments that together represent a consensus region of DNA.
control sequence	Control nucleic acid sequences can be added to DNA or RNA samples to facilitate post-sequencing data analysis. Two types of control sequences can be used during sample preparation. ERCC RNA Spike-In Mix is used with RNA samples to achieve a standard measure for data comparison across gene expression experiments. Ion AmpliSeq™ Sample ID Panel, comprised of nine specially designed primers, can be added prior to template amplification to generate a unique ID for each sample during post-sequencing analysis.
CSV file	A comma-separated values (CSV) file is a delimited text file in which each line represents a data record with information fields separated by a comma. A CSV file stores tabular data (numbers and text) in plain text. Each line of the file is a data record. CSV files are easily opened using spreadsheet software, such as Microsoft™ Excel™ or Apache® OpenOffice ™ Calc, where each comma-separated field is listed in a separate column.
DAT file	A DAT file is a generic data file. It contains data that may be opened or referenced by a specific application. While some DAT files can be opened directly, most contain program data that is referenced the program when it is running. Therefore, most DAT files are not meant to be opened manually.

empty wells	Wells on a chip that do not contain an ISP.
enrichment (%)	Predicted number of live ISPs that have a key signal identical to the library key signal. The percent enrichment value reported is the number of loaded ISPs that are library ISPs, after taking out the test fragment ISPs.
FASTA file	A FASTA file is a text-based format for representing either nucleotide sequences or peptide sequences, in which base pairs or amino acids are represented using single-letter codes. A sequence in FASTA format begins with a single-line description, followed by lines of sequence data.
FASTQ file	A FASTQ file is a text-based format for storing both a biological sequence (usually nucleotide sequence) and its corresponding quality scores. Both the sequence letter and quality score are each encoded with a single ASCII character for brevity.
FD (flow disruptiveness)	A data filtering parameter that is used instead of INDEL, SNP, and MNP.
final library reads	Number (and percentage) of reads, passing all filters that are recorded in the unmapped BAM file. This value may be different from the total number of reads located in the Library Summary Section due to specifications associated with read trimming beyond a minimal requirement resulting in total number of reads being slightly less than Final Library Reads.
flow	A flow is the event of exposing a chip to one particular dNTP (deoxyribonucleotide triphosphate), followed by a washing step.
flow order	The order in which a chip is exposed to each particular dNTP. The default Samba flow order consists of a 32-base sequence, repeated. This flow order resists phase errors by providing opportunities for out-of phase molecules to catch up and is designed to sample all dimer (nucleotide pair) sequences efficiently. Samba is the default flow order because it improves sequencing accuracy for longer reads by resisting phase errors.
flow transfer	Progress of the sequencing run expressed as number of total flows completed. For example, a sequencing run set to 500 flows shows 250/500 flows in the status column of the **Runs in Progress** table when the sequencing run is half completed.
fusions	A target technique used for detection and annotation of gene fusions (or translocation of genetic material) in samples.

hotspots file	A BED or a VCF file that defines regions in the gene that typically contain variants. Specifying a hotspots file to use in a run enables Torrent Variant Caller to identify if a specific variant is present or absent. A hotspot file instructs the Torrent Variant Caller to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualified a variant candidate. A hotspot file affects only the variantCaller plugin, not other parts of the analysis pipeline. If you don't specify a hotspots file, the software tells only the difference between your sequence and the reference genome.
IA	Isothermal application (IA) is a template preparation technique that uses nonemulsion isothermal reaction to clonally amplify DNA onto an ISP surface.
INDEL	INDEL is an abbreviation used to designate an insertion or deletion of bases in the genome of an organism.
intermediate files	Intermediate files contain information used for debugging runs.
ISPs	Ion Sphere™ Particles (ISPs) are particles that contain bound copies of a single (ideally) DNA fragment amplified during template preparation.
key signal	Average 1-mer signal in the library key.
library ISPs	Live ISPs that have a key signal identical to the library key signal.
LIMS metadata	Laboratory Information Management System (LIMS) software is used for recording sequencing metadata. Entered text is associated with the Planned Run and can be extracted using APIs for LIMS consumption.
live ISP	An ISP with a signal of sufficient strength and composition to be associated with the library or test fragment key.
low quality ISP	An ISP with a low or unrecognizable signal.
library key	A short known sequence of bases used to distinguish a library fragment from a test fragment (for example, "TCAG").
MAPD	Acronym for median absolute pairwise difference. Assuming that adjacent amplicons in the genome most likely have the same underlying copy number in a sample, the difference between the log2(read count ratio) values against the reference baseline for all adjacent amplicons contains information for the noise level of the data. The median of the absolute values of all such difference in log2(read count ratio) is the measure for how informative the results fare for copy number estimates.
mapped reads	Sequencing reads that have been mapped to the reference sequence.

mean raw accuracy	Average raw accuracy of 1-mers at a specific position in the read.
MNP	Multiple nucleotide polymorphism (MNP) is a genetic mutation in an allele that differs from the reference allele of the same length by >1 nucleotide.
on target reads	Sequencing reads mapped to any target region of the reference. A read is considered to be on target if at least one aligned base overlaps a target region. A read that overlaps a targeted region but where only flanking sequence is aligned, for example, due to poor matching of 5′ bases of the read, is not counted.
Planned Run	A Planned Run is a file that contains executable instructions for individual runs. The file contains all the specifications, settings, and parameters for template preparation and chip loading on the Ion Chef™ Instrument, and sequencing on an Ion S5™/Ion S5™ XL or Ion GeneStudio™ S5 Series system.
Planned Run template	A reusable experimental design (digital protocol) for the sequencing instrument that holds specifications for sample preparation, sequencing, data export, and post-sequencing data analysis.
polyclonal ISP	An ISP that carries clones from two or more library sequences.
primer dimer	A primer dimer (PD) is a potential by-product in PCR, a common biotechnological method. As its name implies, a PD consists of primer molecules that have attached (hybridized) to each other because of strings of complementary bases in the primers
primer dimer ISP	An ISP that carries an insert length of less than 8 base pairs.
read length	The length of called reads measured in base pairs.
read	The sequence of a section of a unique fragment obtained after the end of the sequencing process.
reference library	A consensus nucleotide sequence that represents the genome of a particular species. The results from a sequencing run are compared to the reference library to identify sequence variants.
relationship group	Defines related samples within a Sample Set. Related samples are designated by the same relationship group number.
SAM file	A SAM (Sequence Alignment Map) file is a tab-delimited text file that contains sequence alignment data. It stores biological sequences aligned to a reference sequence.
sample	Genetic material from one source (for example, DNA from one individual).

signal processing input files	Signal processing input files consist of the raw voltage measurement data collected during the sequencing run. These files are required to reanalyze the run from signal processing.
SNP	Single nucleotide polymorphism (SNP) is a genetic mutation in an allele that differs from the reference allele of the same length by one nucleotide.
SSH	SSH, also known as Secure Shell or Secure Socket Shell, is a network protocol that gives users, particularly system administrators, a secure way to access a computer over an unsecured network. SSH also refers to the suite of utilities that implement the SSH protocol.
structural variants	Genetic mutations that cause a change in the organism's chromosome structure, such as insertions, deletions, copy number variations, duplications, inversions, and translocations.
system SNR	System signal-to-noise ratio.
target base coverage	Summary statistics for targeted base reads of the reference. A base covered by multiple target regions is counted only once per sequencing read.
target regions file	A BED file that specifies all of the regions that a panel represents such as the amplified regions that are used with target sequencing. The complete software analysis pipeline, including plugins, is restricted to only these specified regions instead of analyzing the entire reference library.
test fragment	A test fragment (TF) is a known nucleotide sequence that is used to monitor system characteristics.
test fragment ISPs	Live ISPs with a key signal that is identical to the test fragment key signal.
test fragment key	A test fragment key (TF key) is the nucleotide sequence that is used to identify test fragment reads.
TF key peak counts	Signal strength of the first three bases of the TF key.
total reads	Total number of filtered and trimmed reads independent of length reported in the output BAM file.
TSV file	A tab-separated values (TSV) file is a tab-delimited file that is used with spreadsheet software. TSV files are essentially text files, and the raw data can be viewed by text editors, though they are often used when moving raw data between spreadsheets. See also *VCF file*

uBAM file	A uBAM file is a variant form of the BAM file format in which the read data does not contain mapping information. This is basically an "off-label" use of the BAM format (which was specifically designed to contain mapping information) that is used for data management reasons. It allows you to attach metadata to the reads from as early on in the analysis process as possible.
unaligned reads	Nucleotide bases covered by reads that are not aligned to the reference.
uniformity of base coverage	The percentage of bases in all targeted regions (or whole genome) covered by at least 0.2X the average base coverage depth.
usable sequence / usable reads	Usable reads consist of library ISPs that pass the polyclonal, low quality, and primer dimer filter.
VCF file	A variant call format (VCF) file specifies a variant of interest and its location. This file stores only the differences between the BAM file and the reference file.
wells with ISPs	Number of wells that were determined to be positive for the presence of an ISP within the well. Wells containing ISPs have a delayed pH change due to the presence of an ISP slowing the detection of the pH change from the solution.
XLS file	Microsoft™ Excel™ format of a VCF file.