iontorrent

Ion Reporter™ Software 5.6

HELP

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Revision A.0

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ThermoFisher SCIENTIFIC

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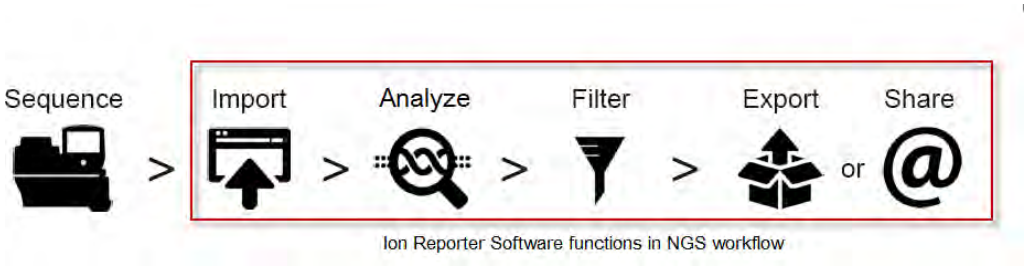
Revision history: Pub. No. MAN0017204

Revision	Date	Description
A.0	Sept. 20, 2017	Updates for Ion Reporter™ Software 5.6 new features: • Immune Repertoire workflow • New workflow parameter that supports single-letter or 3-letter amino acid code annotation • Table preferences improvements • Updates to QC report sections • Shut down button for the Ion Reporter™ Server • New Copy to DataConnect procedure • Updates to Set IRGV as default viewer section • Whole Genome View png call for API • Updates to sections for Ion Reporter Uploader • Removed references to Ion Reporter™ Software 4.4

Contents

About Ion Reporter™ Software



Ion Reporter Software functions in NGS workflow

Ion Reporter™ Software comprises a suite of bioinformatics tools that streamline and simplify the data analysis, annotation, and reporting of Ion Torrent™ semiconductor sequencing data. Designed for both exploratory and research production use in performing sequencing assays, Ion Reporter™ Software helps you overcome the bioinformatics barriers to interpret DNA variants faster and more consistently. For researchers needing a simple, reproducible bioinformatics tool that helps ensure consistency in their results, Ion Reporter™ Software is an ideal solution.

Integrate your variants with comprehensive public and curated annotations, along with your own lab-specific content. Ion Reporter™ Software is designed to reduce the time-to-results. Configurable analysis modules and parameters allow customization of workflows to fit your research needs.

All the steps, from data import to annotating variants, are automated in the Ion Reporter™ Software workflow. Key features include the following:

- Detection of SNPs, indels, and CNVs in one easy to use workflow
- Automated analysis workflows for 1, 2, or 3 samples
- Hosted and local deployment options to suit your laboratory's needs
- Simple interface that users can learn and use intuitively
- Role-based logins and the ability to fully audit user activity so that you can determine what was done, when it was done and by whom
- Extensive annotations to guide understanding of your sample's variants
- Flexible batch export for integration into laboratory systems and APIs in support of third-party tools for external analysis

Ion Reporter™ Software on Thermo Fisher Cloud

Ion Reporter™ Software on Thermo Fisher Cloud is available at **https://ionreporter.thermofisher.com**.

Access to the Thermo Fisher Cloud version of the software is controlled by your **thermofisher.com** user name and password. Samples, data, and results in the software are controlled through "organizations". Members of an organization share

data and results in the Cloud software in the same way that users of a particular Ion Reporter™ Server organization share data and results.

To use the Cloud version of the software, you must first create a **thermofisher.com** account with a user name and password, and then either create a new organization on the Cloud or be invited to join an existing organization. Each Ion Reporter™ Software user can only belong to one organization at a time. You can leave an organization at any time to join or create a different organization, but you lose access to the data and results of the organization you left.

Create a new Ion Reporter™ Software organization on Thermo Fisher Cloud

You can create a new Ion Reporter™ Software organization on Thermo Fisher Cloud. Creating a new organization adds you as the first adminstrator-level user of that organization. You can then begin entering or importing data into the software and invite other Cloud users to your organization to share data and results. (To join an existing organization, see "Join an existing Ion Reporter™ Software organization on Thermo Fisher Cloud" on page 13.)

Note: You can only belong to one organization at a time. If you are already a member of an Ion Reporter™ Software organization on the Cloud, you will not be able to create a new organization until you are removed as a user from your current organization.

1. If you have not already done so, create a new account on **thermofisher.com**. Your user name and password are also used to sign in to Ion Reporter™ Software on Thermo Fisher Cloud. Your user name is your email address.
2. Go to **https://ionreporter.thermofisher.com/**. Or, from the Thermo Fisher Cloud dashboard, under **All Apps**, click on **Ion Reporter**.
3. On software sign-in screen, click **Sign In**. If you are not already signed in to thermofisher.com, you will be prompted to enter your user name and password.
4. When you sign in to Ion Reporter™ Software for the first time, you will be prompted to create a new organization. Click **Yes** and enter an organization name.

   Note: Enter a name that describes your organization to other users whom you will invite to share data and results.

   The **User Manager** screen for the Cloud opens, and you are listed as an administrator-level user.
5. To return to the software, click on the Thermo Fisher Cloud **Home** () button, then click **Ion Reporter** under **My Apps**.
6. Accept the license agreement to begin using the software.

Invite a user to an organization on Thermo Fisher Cloud

An administrator-level user of Ion Reporter™ Software can invite other users on Thermo Fisher Cloud to join their software organization.

1. In Ion Reporter™ Software on the Cloud, click the **Admin** tab. In the **Users** screen, follow the link to **User management**.
2. Above the list of users, click **Action ▸ Invite User**.

3. Enter the email of the user you want to invite into the **Invite User** screen, then enable one or more roles for the user:
   - **Import**
   - **Analyze**
   - **Report**
   - **Admin**
4. Click **Invite**.
   The user receives an email inviting them to join the organization. See "Join an existing Ion Reporter™ Software organization on Thermo Fisher Cloud" on page 13.

Join an existing Ion Reporter™ Software organization on Thermo Fisher Cloud

You can join an existing Ion Reporter™ Software organization on Thermo Fisher Cloud by invitation from a software administrator in that organization (see "Invite a user to an organization on Thermo Fisher Cloud" on page 12).

Joining an organization allows you to share data and results with other users in that organization. (To create a new organization, see "Create a new Ion Reporter™ Software organization on Thermo Fisher Cloud" on page 12.)

Note: You can only belong to one organization at a time. If you are already a member of an Ion Reporter™ Software organization on the Cloud, you will not be able to accept an invitation from another organization until you are removed as a user from your current organization.

1. If you have not already done so, create a new account on **thermofisher.com**. Your user name and password are also used to sign in to Ion Reporter™ Software on Thermo Fisher Cloud. Your user name is your email address.
2. When an Ion Reporter™ Software administrator for the organization adds you as a new user, an invitation is sent to your email address. Click on the link in the email to accept.
3. Your browser opens to a page prompting you to accept the invitation for the organization. Select the organization name to accept.
4. If you do not already have an account on **thermofisher.com** with the email address that the invitation was sent to, you are prompted to create one.

   Note: The email address of your account must match the address that the invitation was sent to.
5. Accept the license agreement to begin using Ion Reporter™ Software.

Sign in to Ion Reporter™ Software on Thermo Fisher Cloud

You must have an account on **thermofisher.com** to sign in to Ion Reporter™ Software on Thermo Fisher Cloud. Your user name and password for the website are also used to log in to the software.

You must also be a member of an organization to use Ion Reporter™ Software on the Cloud. See "Create a new Ion Reporter™ Software organization on Thermo Fisher Cloud" on page 12 or "Join an existing Ion Reporter™ Software organization on Thermo Fisher Cloud" on page 13.

To log in to the software:

1. Go to **https://ionreporter.thermofisher.com**. You can also click on **Ion Reporter** on the Thermo Fisher Cloud dashboard. Then click **Sign In**.
2. Enter your user name and password, then click **Sign In**.

Ion Reporter™ Server

Always access Ion Reporter™ Server through HTTPS (*not* HTTP).

The URL depends on your server configuration, and is named `https://ion-reporter-server-ip-address`, where `ion-reporter-server-ip-address` is the IP address or hostname of your Ion Reporter™ Server.

Sign in to Ion Reporter™ Software on Ion Reporter™ Server

Before you can sign in to Ion Reporter™ Software on Ion Reporter™ Server, you must have a user account on the server. An administrator-level user on the server can create an account for you. To sign in to the software on the server:

1. In a browser on the same network as the Ion Reporter™ Server, enter the server URL: `https://ion-reporter-server-ip-address`, where *ion-reporter-server-ip-address* is the IP address or host name of the server.
2. Enter your user name and password, then click **Sign In**.

Create users on Ion Reporter™ Server

If you are an administrator-level user of Ion Reporter™ Software, you can add new user accounts on Ion Reporter™ Server. You can add individual users manually or add users with a batch upload through a spreadsheet that you create.

Note: Use a minimum of two letters for first and last names in user accounts.

- Create a single new user account.
  a. Sign into Ion Reporter™ Software as an administrative user, then click the **Admin** tab.
  b. Click **Create User** ▸ **Manual**.
  c. Enter the user information, then assign one or more roles to the user account. For details about the roles, see "User roles and permissions" on page 16.
  d. *(Optional)* Select **Requires private folder** if you want to give the user the authority to restrict all visibility to their data. For details, see "Enable or disable private folders" on page 313.
  e. Click **Save**.

The new user will receive an email with a link to activate the account and reset the password.

- Create multiple new user accounts as a batch.
  a. Sign in to Ion Reporter™ Software as an administrative user, then click the **Admin** tab.

  b. Click **Create User ▸ Batch**, then click **download examples** on the **Import Users** page.

  c. Open the UserDefinitionTemplate.csv that is downloaded through the browser.

  Note: You will upload this file to create new Ion Reporter™ Software users.

  d. Enter information for each new user that you want to create into the following columns of the spreadsheet:

  Table 1

Column name	Description
First Name	First name of the account user
Last Name	Last name of the account user
EmailId	Email address of the account user. The email address will be used as the user ID when the user signs in. For example, user@institute.com.
Import Role	Enter 1 to assign the Import role
Analyze Role	Enter 1 to assign the Analyze role
Report Role	Enter 1 to assign the Report role
Administrator	Enter 1 to assign the Administrator role

  e. Save the spreadsheet to a file directory on your hard drive or in a location that you can get back to.

The new users will receive an email with a link to activate the user ID and reset the password.

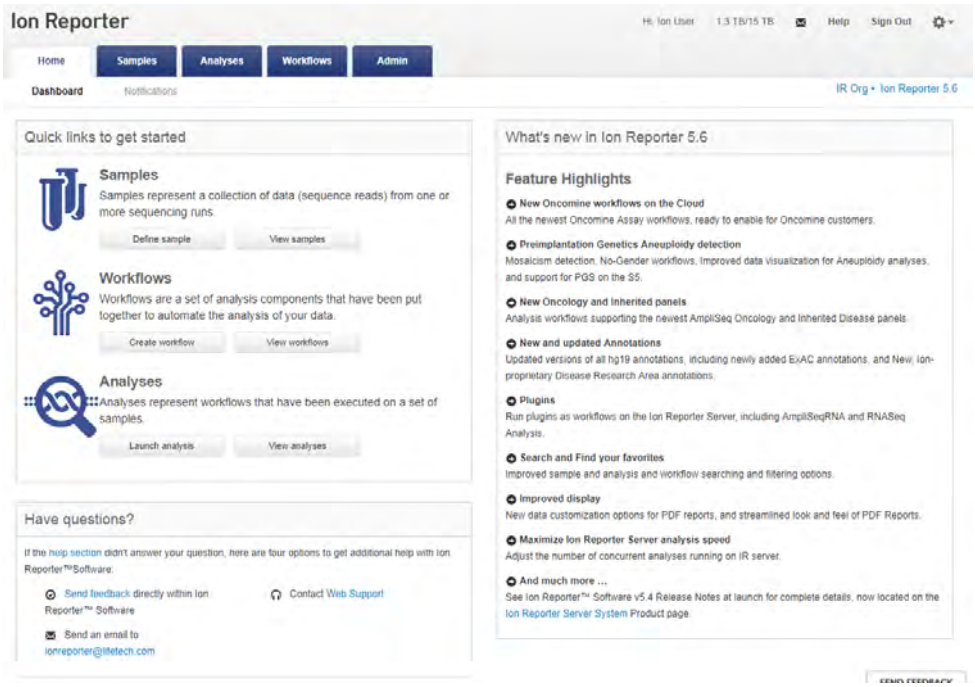
User roles and permissions

Ion Reporter™ Software grants access to specific functions based on roles that are assigned to users. The roles and functions are as follows:

Action	Import User	Analyze User	Report User	Admin User
Upload data	Yes			
Define samples	Yes	Yes		
Create / modify workflows		Yes		
Launch analysis	Yes	Yes		
Review / filter results		Yes		
Select / classify variants			Yes	
Generate report			Yes	
Create / modify users				Yes
Update software (Ion Reporter™ Server System only)				Yes

Ion Reporter™ Software navigation

Use the quick links in the Ion Reporter™ Software dashboard to open the page you want.



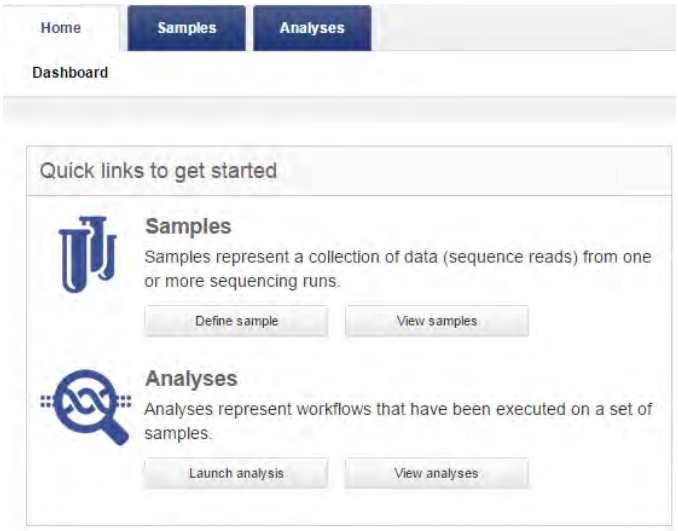
Important notices are displayed in the right pane in the **What's new in Ion Reporter™ 5.6** section.

These links take you to the Samples, Workflows, and Analyses pages.

Click to return to the Thermo Fisher Cloud home screen.

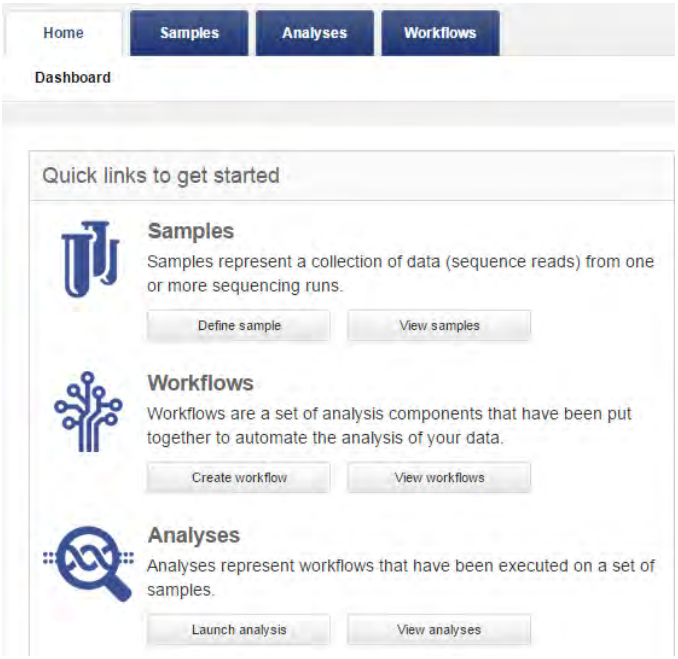
Each role within Ion Reporter™ Software has access to different areas of the software. The dashboard, links, and tabs for the various roles are shown here:

- **Import Role:**



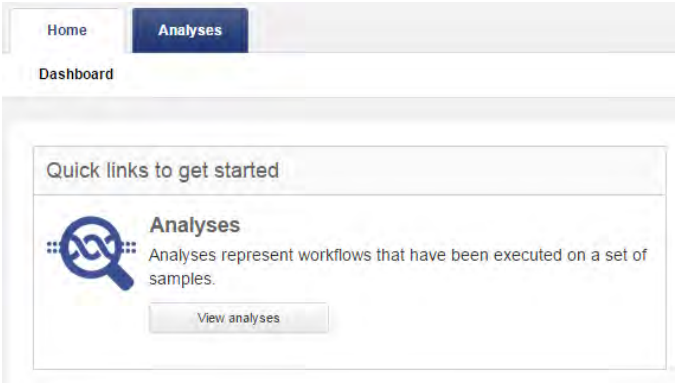
  - View samples
  - Define a sample
  - View analyses list (but not review results)
  - Launch an analysis
  - Access to the Samples and Analyses tabs

- **Analyze Role:**



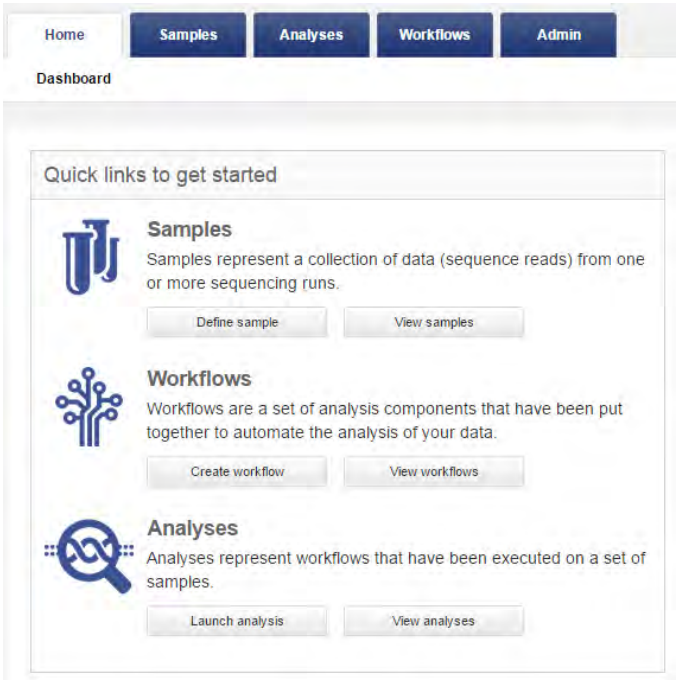
  - View samples
  - Define a sample
  - View workflows
  - Create a workflow
  - View analyses list (and review results)
  - Launch an analysis
  - Access to the Samples, Analyses, and Workflows tabs

- **Report Role:**



  - View analyses list (and view and classify results)
  - Access to the Analyses tab
  - Generate reports

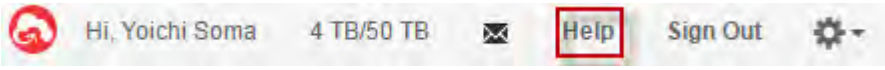
- **Admin Role:**



  - View samples
  - View workflows
  - View analyses list (but not view results)
  - Access to the Samples, Analyses, Workflows, and Admin tabs

Documentation

See help pages for more information

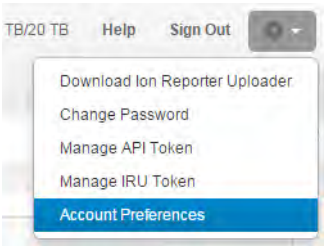


Set IRGV or IGV as default viewer

You can set a preference for your account that determines whether Ion Reporter™ Genomic Viewer (IRGV) or Integrative Genomics Viewer (IGV) opens by default when you visualize analyses. Ion Reporter™ Genomic Viewer is the faster loading genomics viewer that is built into Ion Reporter™ Software. IGV is the Integrative Genomics Viewer made by the Broad Institute.

You can also change these preferences for any Ion Reporter™ Software screen that has a table grid to list reports, results and so on. Account preferences must be changed for each screen that contains a table grid. For details, see "Set default number of rows shown in tables" on page 24.

1. Click **Actions ( )Account Preferences**.



2. In the **Account Preferences** dialog, under **IGV launch preference**, select **IGV** or **IGV**.

   The choice determines which application opens when you click **Locus** for any of the results in the **Analysis Visualization** screen:

   - If you select **IRGV**, Ion Reporter™ Genomic Viewer launches in a separate browser window when you click **Locus** in the **Analysis Visualization** screen. Alternatlively, you can open Ion Reporter™ Genomic Viewer, Ion Reporter™ Genomic Viewer in the same browser window, If you click the Ion Reporter™ Genomic Viewertab to launch the application.
   - If you select IGV, a JNLP file is downloaded when you click **Locus** in the **Analysis Visualization** screen. Double-click on the file to enable IGV to be launched as a standalone application on your computer.

3. *(Optional)* Set number of rows that you want to include in the list of results in the screen.

   The default setting is 20 rows for Ion Reporter™ Genomic Viewer and IGV.

Table preferences

You can configure and save the columns that are displayed in the tables under the **Samples** and **Analyses** tabs in Ion Reporter Software.

Table 2 Table Preference options

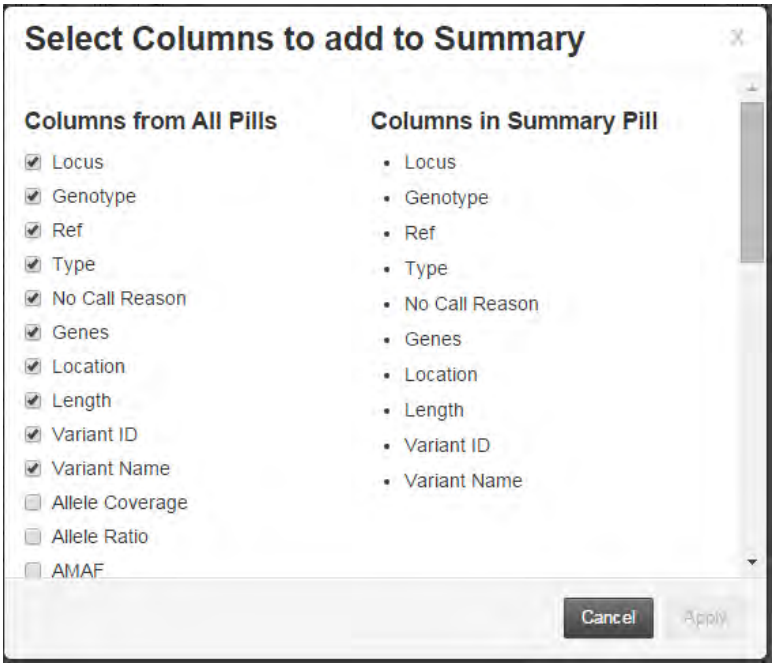
Option	Description
Table Preferences	Select from a list of saved table preferences.
Save Table Preferences	Save the selected column display under the current selected table preference name.
Save Table Preferences As	Save the selected column display under a new user-defined table preferences name.
Select Columns	Select from a list of available columns to display, including any user-defined attributes.
Delete Table Preferences	Delete the user-defined table preference from the list of available columns to display.
Restore Defaults	Restore the default table display.

Set table preferences

You can set **Table Preferences** in Ion Reporter Software to show or hide the columns in any table that lists samples or analyses, or summarizes other sample or analysis information, such as **Analysis Results**. If you save a setting, it applies to all tables. If you save a setting and name it, the preference is added a list of Table Preferences and you can apply that setting at any time to any table. You can delete unused **Table Preferences**.

In the **Samples** tab or **Analyses** tab, click **Overview**, then select a **Analysis** or an **Analysis**.

- To save a Table Preference:
  a. Click **Preferences** ▸ **Select Columns**.
  b. Select and deselect columns that in the **Select Columns to add to Summary** dialog that you want to add or remove from the table. Click **Apply**.



- To create a custom table preference:
  a. Click **Preferences** ▸ **Save Table Preference As**.
  b. Enter a name for the table preference and click **Save**.
  c. Click another analysis.
  d. Click **Preferences** ▸ **Table Preferences**, then select the new **Table Preference**.
  The analysis columns are modified as determined by the applied **Table Preference**.
- To delete an unused **Table Preference**:
  a. Click **Preferences** ▸ **Table Preferences**, then select the **Table Preference** that you want to delete.
  The **Table Preference** is applied.
  b. Click **Preferences** ▸ **Delete Table Preference**.
  c. When you are prompted to delete the **Table Preference**, ensure that the dialog refers to the preference that you want to delete, then click **Yes**.
  The **Table Preference** is removed from the table.

Set default number of rows shown in tables

You can set a preference for your account to set the number of rows that are shown by default when you open any list that appears in a table.

Note: You can also set a preference to run Ion Reporter™ Genomic Viewer or Integrative Genomics Viewer (IGV) by default when you view analyses. For details, see "Set IRGV or IGV as default viewer" on page 21.

1. Click **Settings ( )Account Preferences** in any of the following screens:

Tab	Screen
Home	Notifications
Samples	Overview
	Presets
Analyses	Overview
	Launch
	My Variants
Workflows	Overview
	Presets
Admin	Users
	System Services
	Software Versions
	Plugins

   a. In the **Account Preferences** dialog, select the number of rows that you want to include in the list for the screen that is open.

      The default settings are 20 rows for all screens.

   b. Select the viewer that you will use to launch Ion Reporter™ Genomic Viewer or IGV when you click **Locus** for any result in the **Analysis Visualization** screen.

      If you click **Locus** to launch a visualization application, IGV opens as a standalone application or Ion Reporter™ Genomic Viewer launches in a separate browser window, depending on the account preferences. If you click the tab to launch one of the visualization applications, Ion Reporter™ Genomic Viewer opens in a new tab in the same browser window.

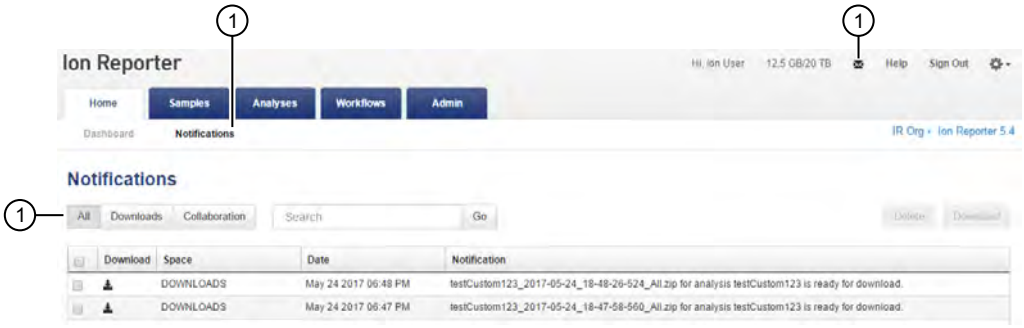
      Note: If you use IGV , you might have to save a JNLP file that is downloaded to your browser before you can open the application.

2. Click **Save**.

3. *(Optional)* Look for the message at the top of the screen to show that your preferences were saved. You can then refresh the screen to show the updated number of rows.

View notifications

From the notifications screen, you can monitor all downloads and collaborations.



① Subtabs for the **Notifications** window
② **Notifications** subtab
③ Envelope icon

Subtabs for Notifications window	Description
All	Shows all downloads and collaboration notifications.
Downloads	Shows all available files for download.
Collaborations	Shows messages from research collaborators and lists files that have been shared.

To view the **Notifications** screen, select one of the following actions.

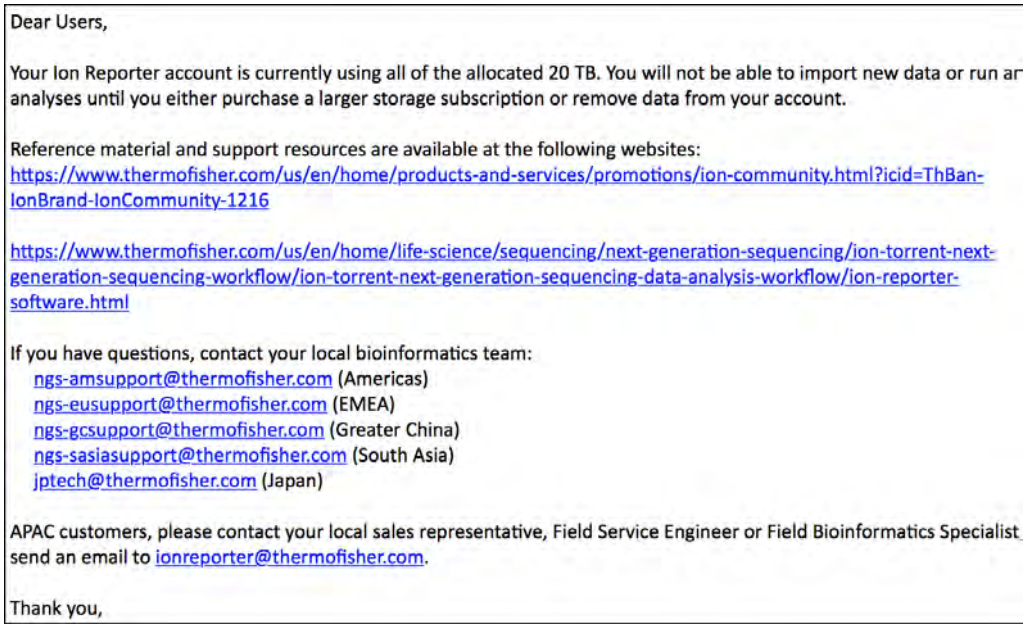
- In the **Home** tab, select the **Notifications** subtab.
- In any tab, click in the Ion Reporter screen menu bar.

Status alert emails

Ion Reporter™ Software sends email status alerts for the following scenarios:

- account creation.
- password reset.
- account deletion.
- sharing of an analysis.
- unsharing of an analysis.
- storage warning.

- creation of a new organization.
- report generation request.



Dear Users,

Your Ion Reporter account is currently using all of the allocated 20 TB. You will not be able to import new data or run ar analyses until you either purchase a larger storage subscription or remove data from your account.

Reference material and support resources are available at the following websites:
https://www.thermofisher.com/us/en/home/products-and-services/promotions/ion-community.html?icid=ThBan-IonBrand-IonCommunity-1216

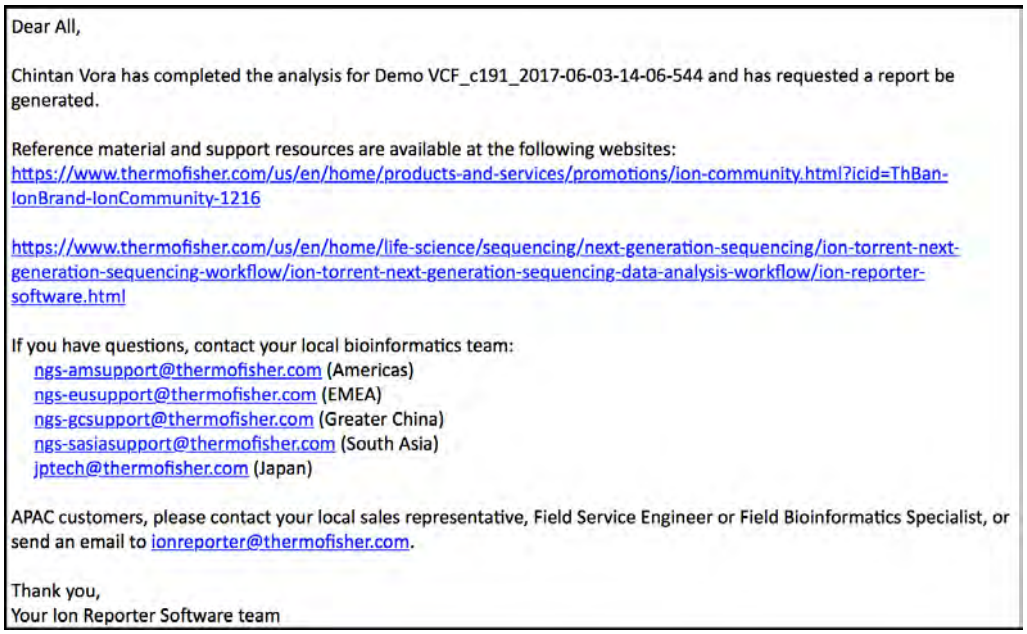
https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-data-analysis-workflow/ion-reporter-software.html

If you have questions, contact your local bioinformatics team:
ngs-amsupport@thermofisher.com (Americas)
ngs-eusupport@thermofisher.com (EMEA)
ngs-gcsupport@thermofisher.com (Greater China)
ngs-sasiasupport@thermofisher.com (South Asia)
jptech@thermofisher.com (Japan)

APAC customers, please contact your local sales representative, Field Service Engineer or Field Bioinformatics Specialist send an email to ionreporter@thermofisher.com.

Thank you,

Example of a storage warning message



Dear All,

Chintan Vora has completed the analysis for Demo VCF_c191_2017-06-03-14-06-544 and has requested a report be generated.

Reference material and support resources are available at the following websites:
https://www.thermofisher.com/us/en/home/products-and-services/promotions/ion-community.html?icid=ThBan-IonBrand-IonCommunity-1216

https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-data-analysis-workflow/ion-reporter-software.html

If you have questions, contact your local bioinformatics team:
ngs-amsupport@thermofisher.com (Americas)
ngs-eusupport@thermofisher.com (EMEA)
ngs-gcsupport@thermofisher.com (Greater China)
ngs-sasiasupport@thermofisher.com (South Asia)
jptech@thermofisher.com (Japan)

APAC customers, please contact your local sales representative, Field Service Engineer or Field Bioinformatics Specialist, or send an email to ionreporter@thermofisher.com.

Thank you,
Your Ion Reporter Software team

Example of a message that is generated when an analysis is complete and a report is generated

GRCh38 human reference

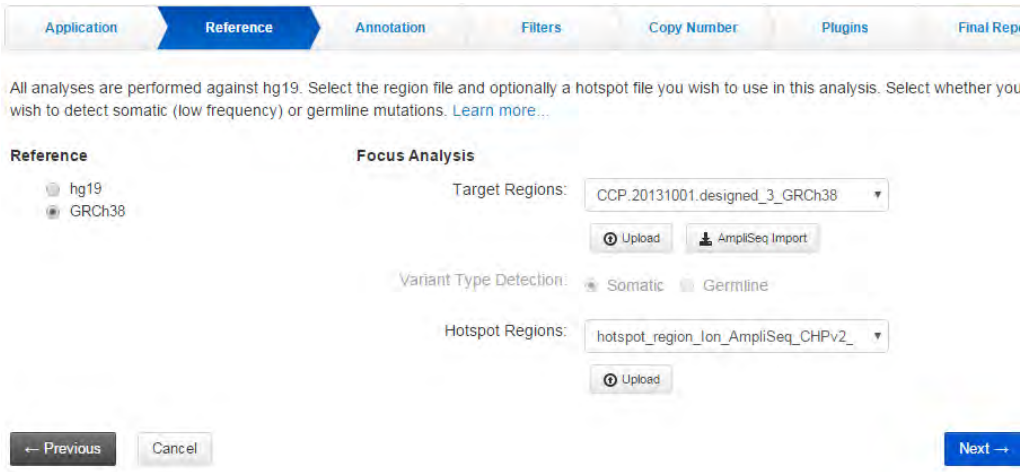
You can use the Ion GRCh38 human reference in custom workflows Ion Reporter™ Software. The Ion GRCh38 Reference Genome is based on the latest GRC human reference assembly and is the first major update since 2009. Highlights include: changes to chromosome coordinates, fixed errors in the former sequence, addition of Mitochondria, and multiple loci for some highly variable genes. In Ion Reporter™ Software, custom workflows can be created using this reference genome.

Note: When editing older version workflows that use the hg19 reference genome, you cannot change the reference genome to Ion GRCh38.

GRCh38 human reference can be used in custom analyses and workflows

AmpliSeq™ Designer currently offers one custom AmpliSeq™ panel and related target and hotspot regions files for GRCh38 experiments. Optionally, you can also convert existing coordinates to GRCh38 by using a publicly available lift-over tool, such as **CrossMap**.

To use the GRCh38 human reference, you must create custom workflows. Use the same approach as with hg19, but select GRCh38 on the Reference screen in the workflow creation wizard. You will also need to upload the AmpliSeq™ GRCh38 Target Regions and Hotspot Regions files or create your own.



See the following topics for custom workflow creation:

- Download the Ion GRCh38 reference from Ion Updates in Torrent Suite™.
- “Create a custom workflow with the Ion Reporter™ wizard“ on page 86
- “Create a Copy Number baseline“ on page 120
- “Launch an analysis“ on page 130

Requirements for GRCh38 custom BED file

If you are creating your own BED file for a custom analysis on the GRCh38 reference, here are some aspects to consider:

Public standard:

- See the BED file specification as described by UCSC.
- Annotation files contain three types of lines: browser lines, track lines and data lines.
- Empty lines and those starting with '#' are ignored.
- A track line begins with the word 'track', followed by one or more key=value pairs.
- There are currently 16 key=value pairs recognized by UCSC, but other key=value pairs are allowed.
- The key db=<UCSC_assembly_name> specifies the reference, but must be a valid UCSC assembly ID (e.g. hg19).

Example:

track name="CHP2_designed" description="Amplicon_Insert_CGP2" visibility=2 db=hg19

track name="CHP2_designed" description="Amplicon_Insert_CGP2" visibility=2 db=hg38

Recommendation:

Include the key=value pair db=<UCSC_assembly_name> as shown in the example.

Define an additional key=value pair in the format reference=hg19 or reference=GRCh38.p2 and include it on the track line.

For hg19, including the key=value pair reference=hg19 is optional.

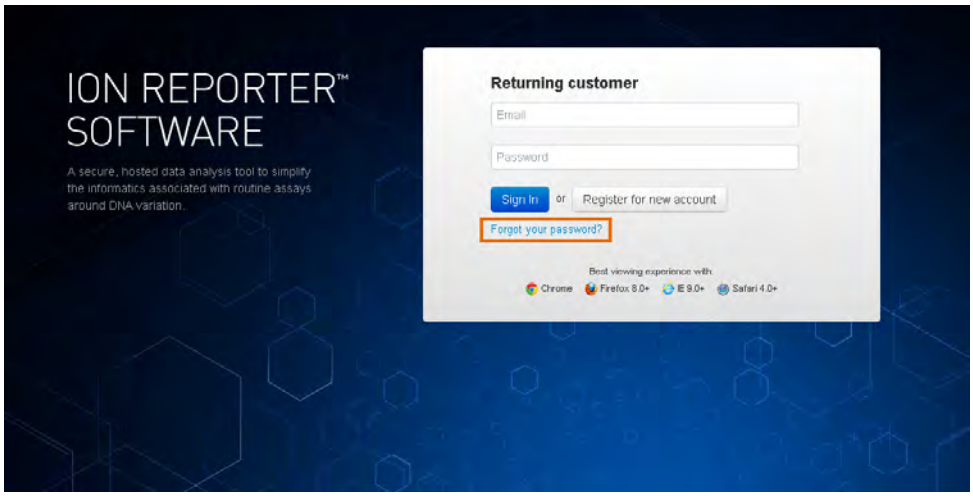
If a URI is desired, define an additional key=value pair in the format referenceURI=http://www.thermofisher.com/GRCh38.p2.fasta .

Troubleshoot sign ins and provide feedback for Ion Reporter™ Software

This section addresses questions that are related to how to troubleshoot sign-ins, provide feedback and get support for the Ion Reporter™ Software.

What can I do if I am locked out of my account?

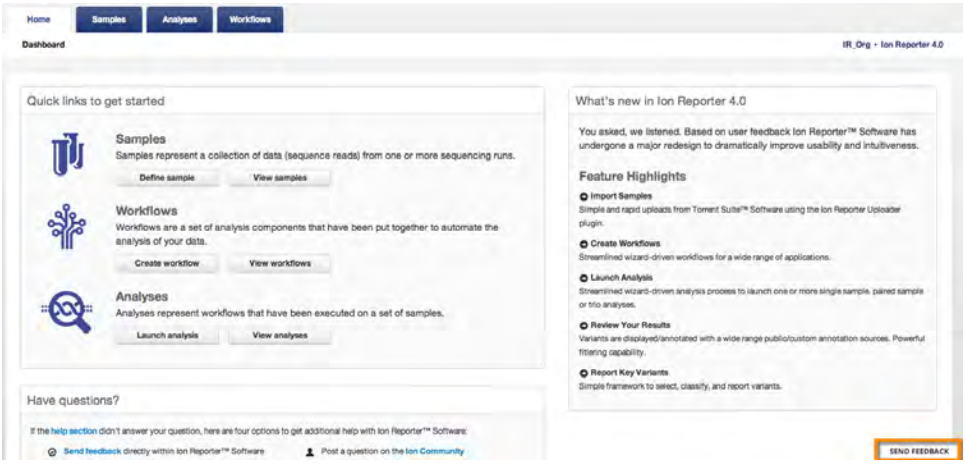
- If you are a server user and have been locked out of your account (system locks you out after five attempts to login in with the incorrect password), you will need to contact your administrator to reset your password. For details on password reset, see the Administration Guide.
- If you are a cloud user, click the **Forgot your password?** link on the sign in form.



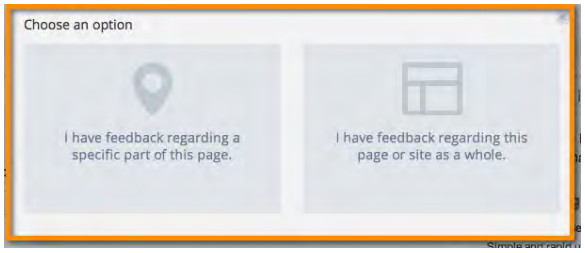
How can I provide feedback?

Feedback on your experience or any recommendations is always welcomed. Providing feedback in Ion Reporter™ Software is very easy:

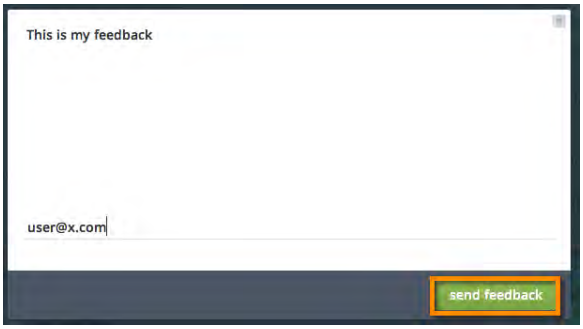
1. Navigate to the page you would like to provide feedback on. In the bottom-right corner of the page you see a **Send Feedback** button.



2. Click on the **Send Feedback** button and then select whether you have general feedback on the page or if your feedback is specific to a certain component.



3. If you select to provide feedback on a specific part of the page, you then are prompted to select that part.
4. Then just enter your feedback and your email address.
5. After you enter your feedback and email, simply click the **Send Feedback** button.



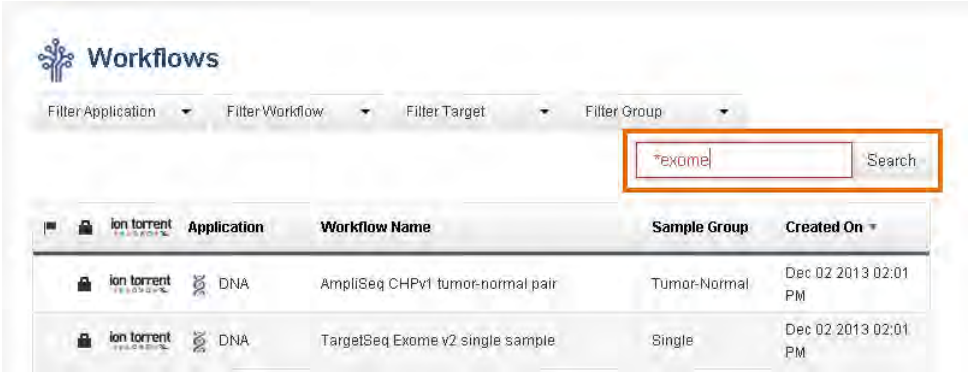
How do I contact support if I have additional questions?

There are three ways that you can get help with Ion Reporter™ Software:

- Send feedback by following the steps outlined above.
- Send an email to **ionreporter@lifetech.com**
- For ordering and commerce issues, please contact your local website support at **http://www.lifetechnologies.com/us/en/home/technical-resources/order-support.html**.

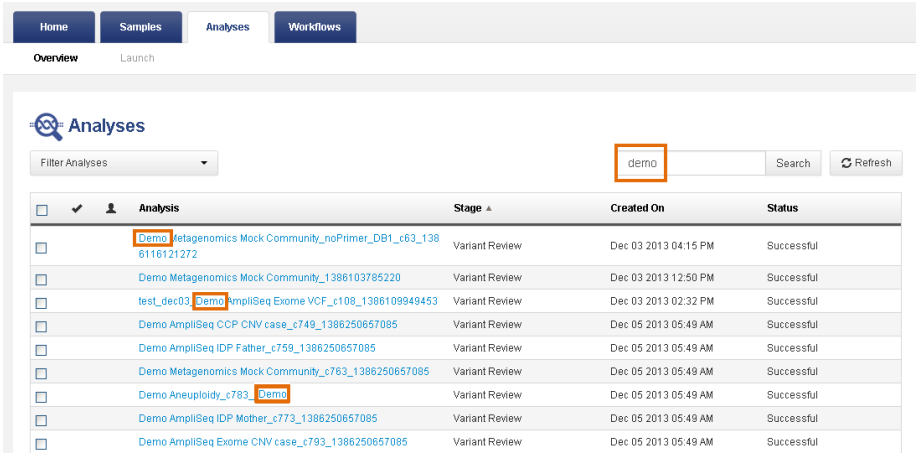
How can I conduct searches?

- At least three characters are required for a search. The search field also appears in red with a bold outline if fewer characters are entered.
- Searches are not case-sensitive.
- If you enter an invalid search character, the search field appears in red with a bold outline.

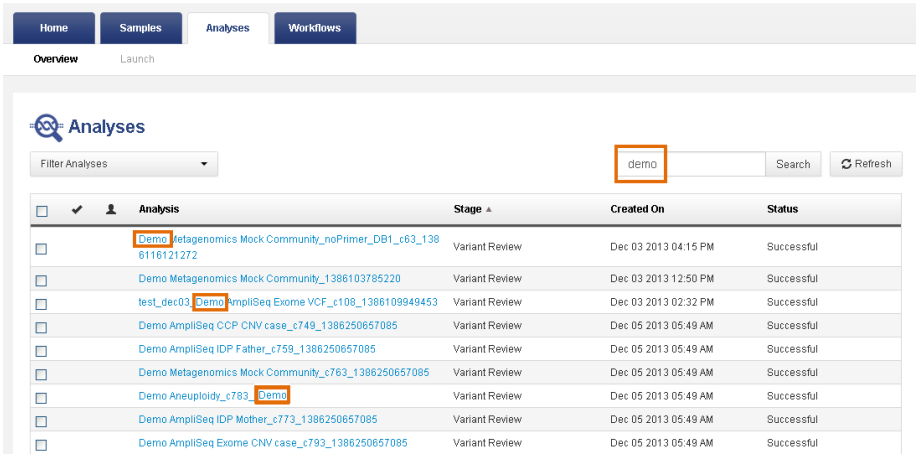


- Search field behavior is different for the variant table search on the Analysis Results page, than for other searches.

- For most searches (except on the variant table on the Analysis Results page):
  - An asterisk (*) is not allowed in a search field.
  - Searches match your search string in any location in the target list. For example, a search on "demo" in workflow names matches workflows with "demo" anywhere in their name (equivalent to *demo* with wildcard searches).



- Only for searches on the variant table on the Analysis Results page:
  - An asterisk (*) is required for some searches here, but is not allowed for other searches. The differences are due to how the different types of information are stored.
  - The asterisk is a search wildcard. Without the asterisk, searches only match the exact string entered.
  - On "exome" in workflow names matches workflows with "exome" anywhere in their name (equivalent to *exome* with wildcard searches).



Are there any naming restrictions?

- Most names (for instance, for analyses, samples, workflows, and users) require at least three characters. The name field appears in red with a bold outline if fewer characters are entered.
- In this release, Ion Reporter™ Software does not support either a first name or last name of only two characters. As a workaround, append an underscore (_) or number to a name that is less than 3 characters.

What special characters are allowed in names?

- Most names (for instance, for analyses, samples, workflows, and users) and other text fields in Ion Reporter™ Software allow these characters:
  - Alphanumeric characters: A-Z a-z 0-9
  - Underscore: _
  - Space: ' '
  - Dash: -
  - Period: .
- If unsupported characters are entered, the field appears in red with a bold outline.

Purchasing and ecommerce

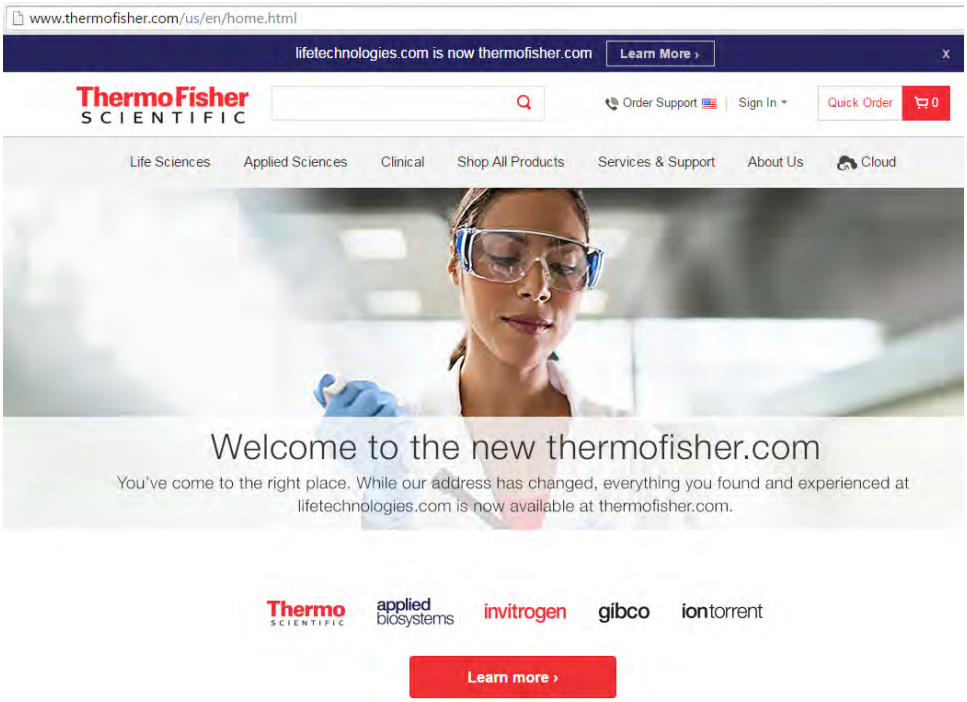
You can use your Thermo Fisher account credentials to manage your Ion Reporter™ Software and related third-party applications.

Note: You do not have to change your lifetechnologies.com user name and password to access . All your legacy account options remain intact.

Create a Thermo Fisher account account

Each Ion Reporter™ Software organization needs at least one user with a full Thermo Fisher account account. To sign up for an account:

1. Go to http://www.thermofisher.com.



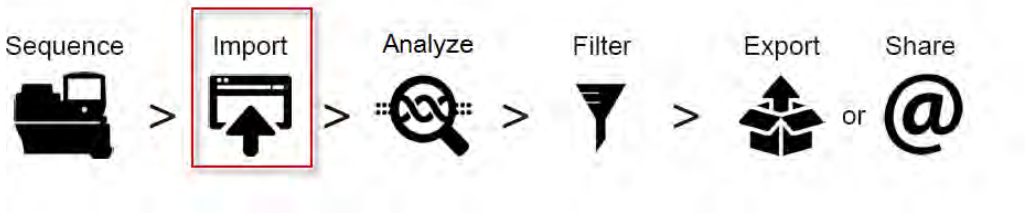
2. Click **Sign In** ▸ **Register**.
3. Fill out the requested information, then click **Create account**.

You will receive a confirmation email after you create the account.

Ion Reporter™ Software storage

To use Ion Reporter™ Software on Thermo Fisher Cloud, subscriptions are not required. Ion Reporter™ customers are given 100GB of storage free on Thermo Fisher Cloud. To obtain larger amounts of space, see the Thermo Fisher Cloud Help for subscription infomation.

Import and manage samples



This section describes three primary methods to transfer data into Ion Reporter™ Software:

- The Ion Reporter™ Software Uploader plugin
- Import a VCF file into Ion Reporter™ Software
- The Ion Reporter™ Software Command-line Uploader

And tools to manage your samples:

- Define a sample manually
- Define a sample via CSV upload
- Edit a sample
- Lock a sample
- Delete a sample
- Create sample presets (attributes)

Import a VCF file into Ion Reporter™ Software

This section describes how to import a VCF file from a local Ion Reporter™ Software Server and define it as a sample so that its variants can be annotated with Ion Reporter™ Software using an annotation-only workflow. This section does not apply to importing a VCF file as a hotspot file.

Note: A sample can only contain one VCF file, not multiple VCF files.

You can use the IonReporterUploader plugin or Ion Reporter™ Command-line Uploader to upload your VCF files to Ion Reporter™ Software, and you can also upload VCF files from your local machine. For details, see "IonReporterUploader plugin" on page 41.

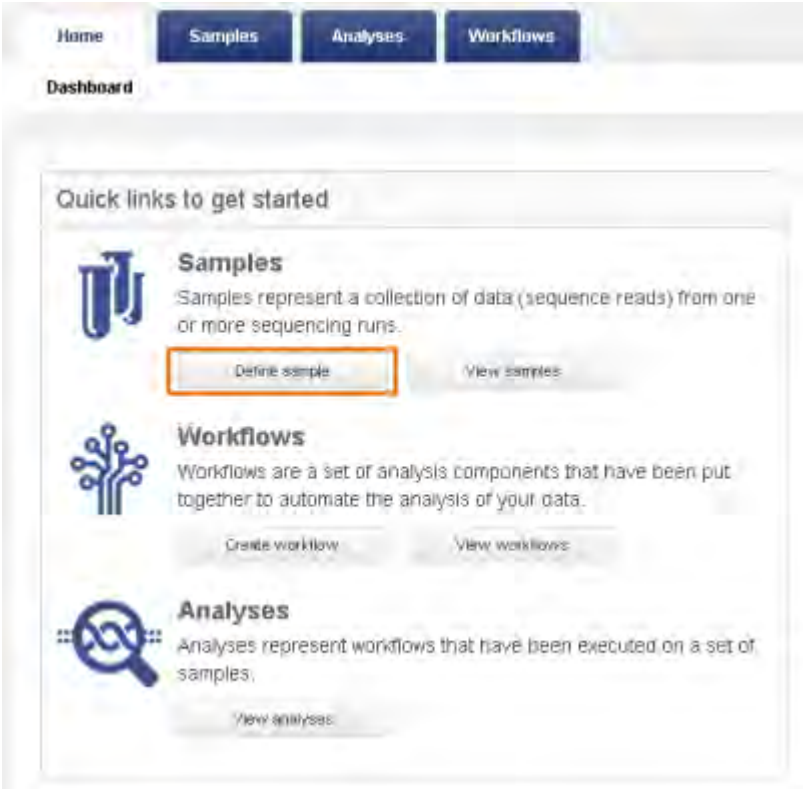
These instructions both import the raw VCF file from your local machine and also define an Ion Reporter™ Software sample from the VCF file.

When you import a VCF file:

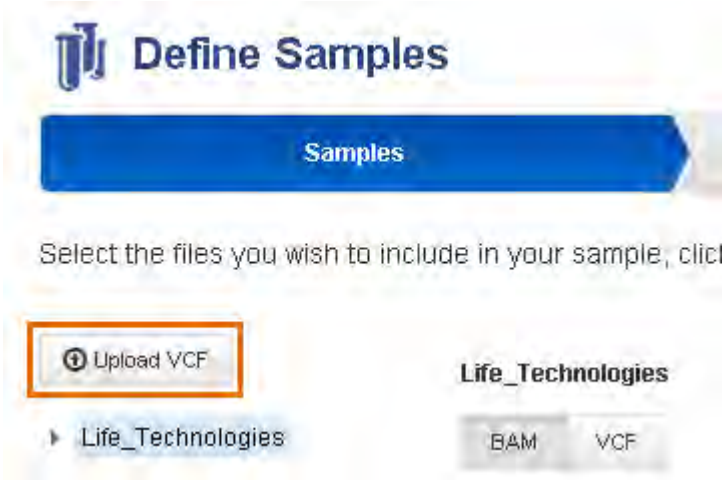
- Annotation-only workflows accept VCF files that conform to the 4.1 VCF standard only.
- Ion Reporter™ Software also accepts a compressed VCF file in .gz format as input for annotation-only workflows. Ion Reporter™ Software unzips the .gz file and works with the resulting VCF file.
- Ion Reporter™ Software can analyze VCF data generated from any platform as long as it conforms to the 4.1 VCF standard.
- Avoid the use of "CHR" instead of "CHROM" as a header column name. See IGV parse header error.

Note: To use an imported VCF with the GRCh38 annotation workflow in Ion Reporter™ Software, the imported VCF file must include the following text in the header line: ##reference=GRCh38

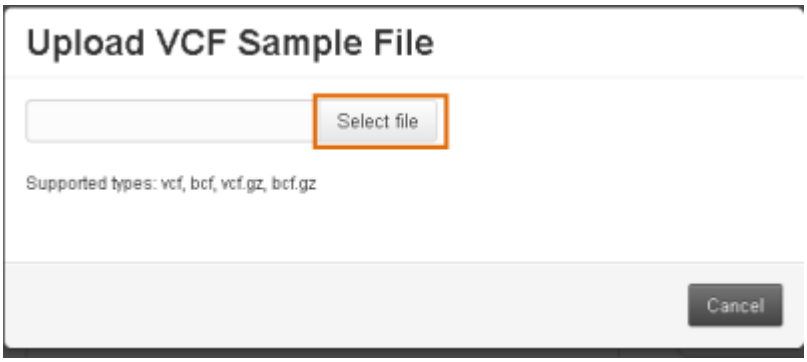
1. Log in to the Ion Reporter™ Software application. In the Home page, click **Define sample**.



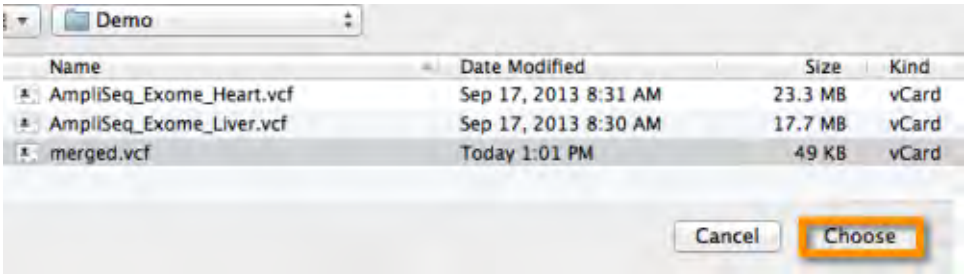
2. Click **Upload VCF**.



3. In the Upload VCF Sample File dialog, click **Select File**.



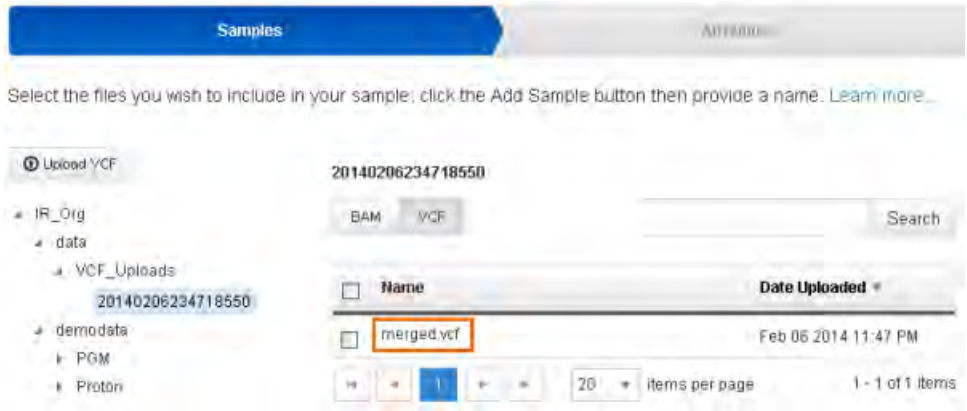
4. Browse to the VCF file on your local machine and click **Open** or **Choose** (depending on your browser).



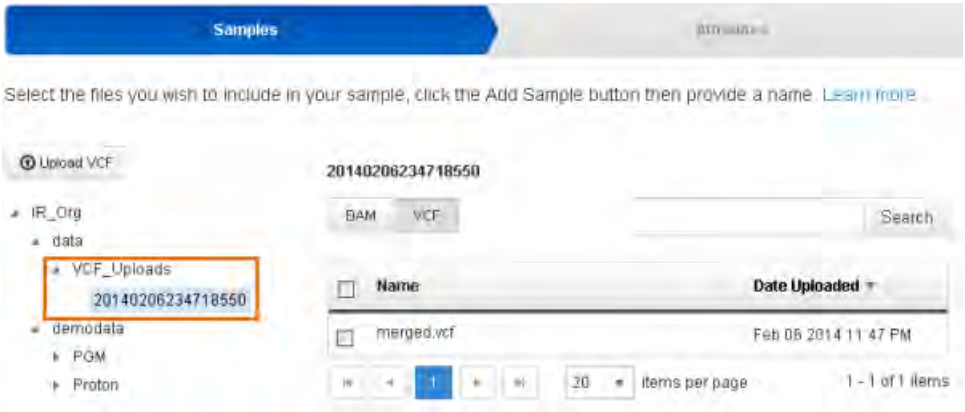
5. Confirm that your file name appears in the Upload VCF Sample File screen (`merged.vcf` in this example). If so, click **Upload VCF**.

6. Wait while your VCF file is verified.
   When verification is complete, click the **Close** button.

   Your file appears in the samples table.



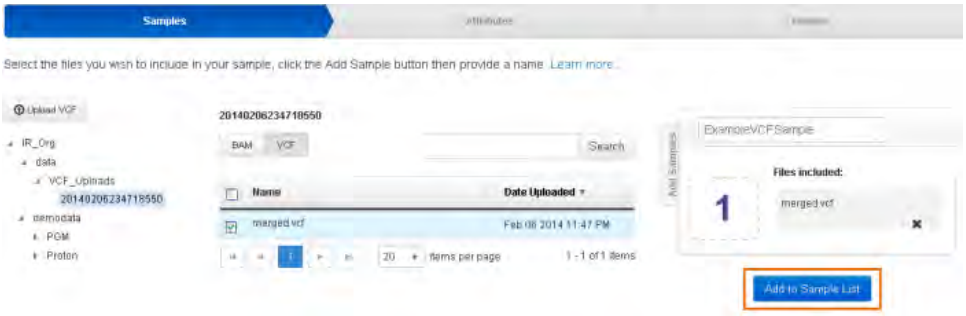
Your new file also appears in the left panel as a time-stamp under VCF_Uploads.



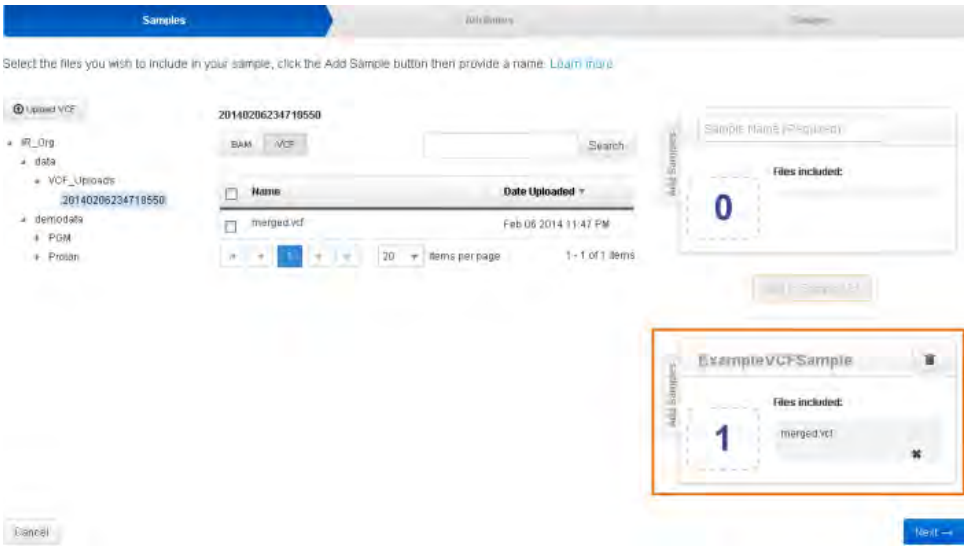
7. In the samples table, enable the checkbox to the left of your VCF file name. This highlights the row and also enables the **Add to Sample** tab in the right panel.

8. Enter a descriptive (and unique) name for the sample in the **Sample Name** field, then click **Add Samples**.
   Your VCF file now appears in the **Files Included** list and the number of data files is shown in the field with dotted lines. In this example, the sample number is 1.

9. Click **Add to Samples List**.



A new box appears below the Add to Sample LisT. This box contains the data file and sample name part of your sample definition.



10. Click **Next**.
    The Attribute chevron opens.

11. If you do not need to set attributes, click **Next**.

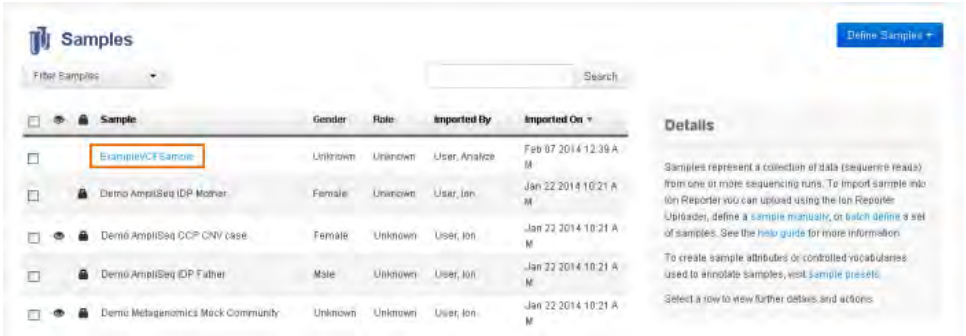
12. To set an attribute, click the area under an attribute heading to set the attribute value.

(In this example, Lab is a user-defined attribute. Click the expansion arrow to open the attribute menu and make your selection.

When you have set the attributes, click **Next**.

13. In the summary chevron, hover over the sample name to see the raw data file. If the sample name and the raw data file are correct, click **Save**.

The Samples Overview tab opens, with your new sample in the sample table. Your new sample is ready to be analyzed.



IonReporterUploader plugin

Analysis files that are generated in the Torrent Suite™ Software can be directly transferred to an organization in Ion Reporter™ Software with the IonReporterUploader plugin.

Ion Reporter™ Software uses the Torrent Suite™ Software output BAM file for analysis. The Ion Reporter™ Software annotation-only workflow also accepts the VCF output file of the variantCaller plugin. Use the IonReporterUploader plugin to transfer these BAM and VCF output files to Ion Reporter™ Software.

There are two ways to run the IonReporterUploader plugin:

- Configure the IonReporterUploader plugin to run by default after every sequencing run. Torrent Suite™ Software results files are transferred to Ion Reporter™ Software and are defined as samples in Ion Reporter™ Software when the plugin is run manually.
- Run the plugin manually. Results files are also defined as samples in Ion Reporter™ Software when the IonReporterUploader plugin is run as part of a Planned Run or Run template. In addition, the workflow of your choice Ion Reporter™ Software is automatically launched on your newly transferred samples Ion Reporter™ Software.

The Ion Reporter™ Software is not included with Torrent Suite™ Software and is available under separate license. Before you run the IonReporterUploader plugin, you must add a valid Ion Reporter™ Software account. For complete details about the IonReporterUploader plugin, see the Torrent Suite™ Software Help. If you do not have access to Torrent Suite™ Software, you can use IonReporterUploader command-line utility. For details, see“Ion Reporter™ Uploader command-line utility“ on page 42.

Note: When the IonReporterUploader plugin defines samples in Torrent Suite™ Software for your newly transferred files, the plugin also defines sample relationships for paired and trio samples and defines sample attributes. For details, see

Transfer limitations

The IonReporterUploader plugin transfers results files for a completed run plan that executed on the Torrent Server where the plugin is configured. The following limitations apply to the IonReporterUploader plugin:

- You cannot add supplemental files to the results files of a run, to have the plugin transfer those files.
- For barcoded runs:
  - For sequencing runs that use barcoded data, the IonReporterUploader plugin only transfers samples if the barcode kit selection is correct. If you correct or add the barcode kit selection on the sequencing instrument, the IonReporterUploader plugin still uses the original run plan information and the results file transfer fails.
  - For manual launches of the IonReporterUploader plugin on barcoded data, the IonReporterUploader plugin uses the barcode kit that you select on the sequencing instrument.

Ion Reporter™ Uploader command-line utility

You can use IRU command-line utility to transfer files from a local machine (that is not an Ion Reporter™ Software server) to the Ion Reporter™ Software server. This utility can be used if you do not have access to Torrent Suite™ Software, and you have files on your local machine that you want to transfer. For example, if you have a BAM or VCF file on your local machine that you want to upload and then analyze the file in Ion Reporter™ Software.

You can also use IRU command-line utility if you have problems using the plugins in Torrent Suite™ Software.

The IonReporterUploader command-line utility is a stand-alone utility that is not part of either Ion Reporter™ Software or the Torrent Browser. This procedure is recommended only for users who are familiar with the command-line utilities.

The Command-line Uploader can be run on any of these systems:

- Your Torrent Server
- A standard Linux™ machine
- A standard Windows™ (XP or later) machine
- A standard Macintosh™ machine

Note: IonReporterUploader command-line utility supports the upload of combined Ion Reporter™ Software analysis results that are output by the **Combine Alignments** option in the Torrent Suite™ Software Projects tab. The IonReporterUploader plugin does not support uploading these files.

Download Ion Reporter Uploader command-line utility

This procedure explains how to download and install the Ion Reporter Uploader command-line utility from Ion Reporter™ Software.

Ideally, download the Ion Reporter Uploader command-line utility onto the machine where you run it. At a minimum, use a machine with the same operating system.

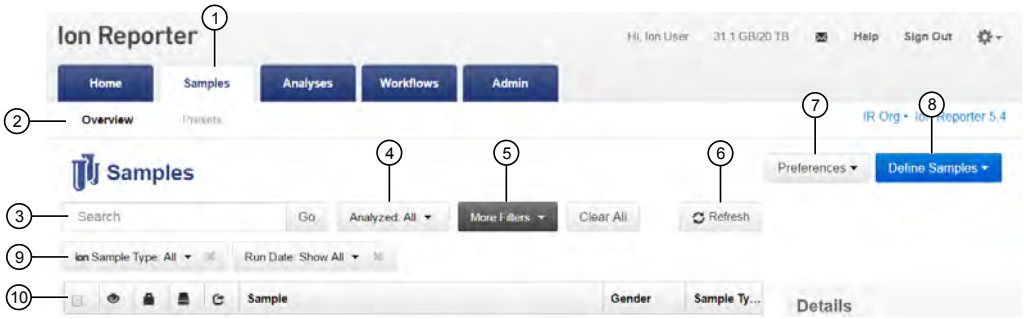
1. Sign in to Ion Reporter™ Software, then click **Settings ( )** ▸ **Download Ion Reporter Uploader**.
2. Click the filename **IonReporterUploader-cli.zip**, then download the file to your target machine.
3. On your target machine, extract the downloaded **IonReporterUploader-cli.zip** file, then copy the **IonReporterUploader-cli** directory to a convenient location.

Run Ion Reporter Uploader command-line utility

The Ion Reporter Uploader command-line utility (irucli) is ready to run after you extract it. Run the Ion Reporter Uploader command-line utility from the IonReporterUploader-cli bin directory (with the **irucli.bat** or **irucli.sh** script). Instructions for using the command-line uploader are downloaded with the utility and with Ion Reporter documentation.

Manage samples

Samples are listed under the **Samples** tab in the **Overview** screen, in a table format. The **Overview** screen has a number of tools for managing samples.



1. **Samples** tab
2. **Overview** screen
3. **Search** field, to search for specific samples in the table
4. Built-in **Analyzed** filter, to filter samples by whether they have been analyzed
5. **More Filters** list, to select additional filters
6. **Refresh** button, to refresh the list of samples
7. **Preferences** button, to configure the columns displayed in the table
8. **Define Samples** button, to add or import samples
9. Additional filter buttons, added using the **More Filters** list
10. Table columns, as defined by **Preferences**

Set table preferences

You can configure and save the columns displayed in the tables under the **Samples** and **Analyses** tabs.

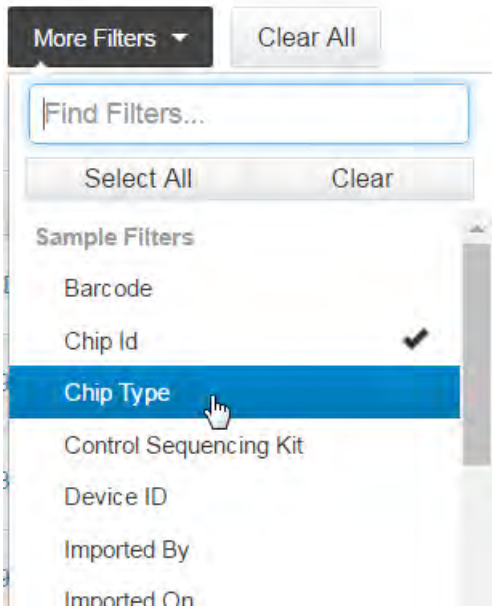
Under the **Samples** or **Analyses** tab, in the **Overview** screen, click **Preferences** and select from the following options:

Select...	To...
Table Preferences	Select from a list of saved table preferences.
Save Table Preference	Save the selected column display under the current selected table preference name.
Save Table Preference As...	Save the selected column display under a new user-defined table preferences name.
Select Columns...	Select from a list of available columns to display, including any user-defined attributes.
Restore Defaults	Restore the default table display.

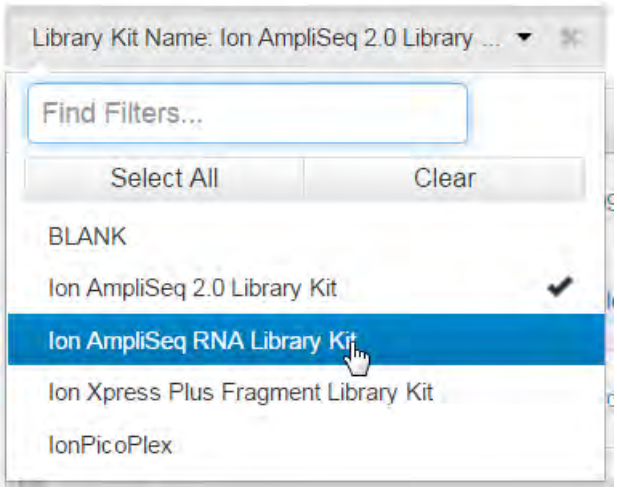
Filter the sample table

Tools for filtering samples based on sample attributes are located under the **Samples** tab in the **Overview** screen.

- The **Analyzed** filter is available by default. Click **Analyzed** above the sample table to filter the list by whether a sample has been analyzed (select **Yes** from the dropdown list) or not (select **No**).
- Click **More Filters** to select additional filters from a dropdown list.



  - Use the **Find Filters** field to search for filters in the list.
  - Select the filter you want from the list. A button for that filter is added above the table, and the filter is listed with a check mark in the **More Filters** list.
  - To remove a filter button, click it again in the **More Filters** list to deselect it.
- Each filter button you add to the table has different options to filter by, based on the attributes of the samples in the table. For example, the **Library Kit Name** filter has a list of the library kit names that are associated with the samples in the list.



- Click each filter button you add to display a dropdown list of options.
  - Select an option in the list to filter by that selection. The table will reconfigure to list only the samples that meet the filter criteria.
  - Click a selected option again to deselect it and no longer filter by that option.
  - Click **Select All** to select all the options for that filter.
  - Click **Clear** to clear the selections.
- Click **Clear All** above the table to clear all selected filters.

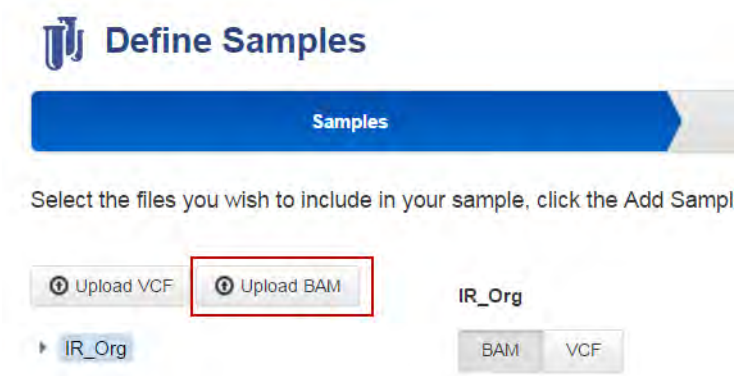
Gender information requirements

Some workflows require that a sample includes a sample attribute for gender information. The trio workflows require accurate sample gender information. You enter gender information when you define your sample manually, import samples, or edit a sample.

Upload a .bam file as a sample

You can upload a .bam file as a single sample.

1. In the Ion Reporter™ Software, click the Samples tab, then click **Define Samples** ▸ **Manual**.
2. In the Define Samples screen, click **Upload BAM**.



3. In the Upload file dialog, click **Select File**, browse and select the .bam file, then click **Upload BAM**.

Define a sample manually

You can define a new sample manually from raw data files for certain protocols. For example, use the procedure if you want to analyze data from multiple combined runs.

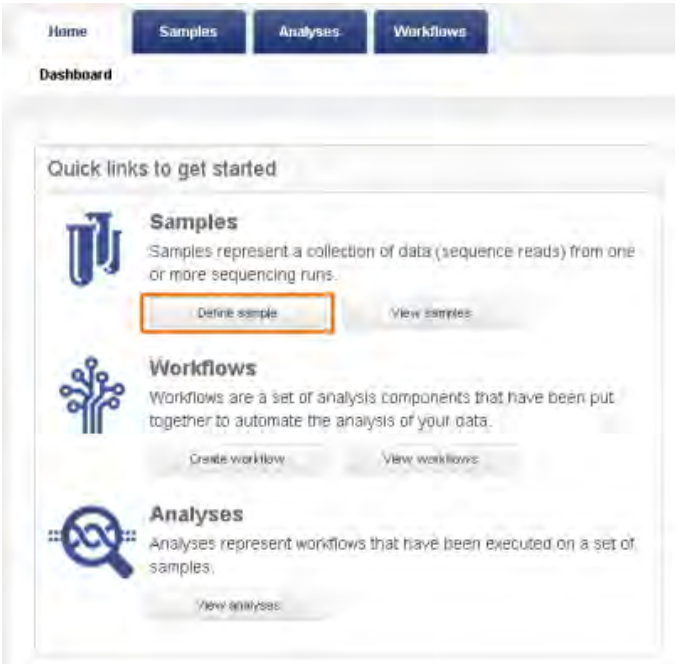
Note: If you used the Ion Reporter™ Uploader plugin or the Ion Reporter™ Command-line Uploader to upload data, the sample definition is done automatically and you do not need to use this procedure.

Before you use this procedure, load raw data files into Ion Reporter™ Software or use the demo data files available in Ion Reporter™ Software .

IMPORTANT! Multiple BAM files can be combined into a sample. Multiple VCF files are not supported and mixed file types are not supported.

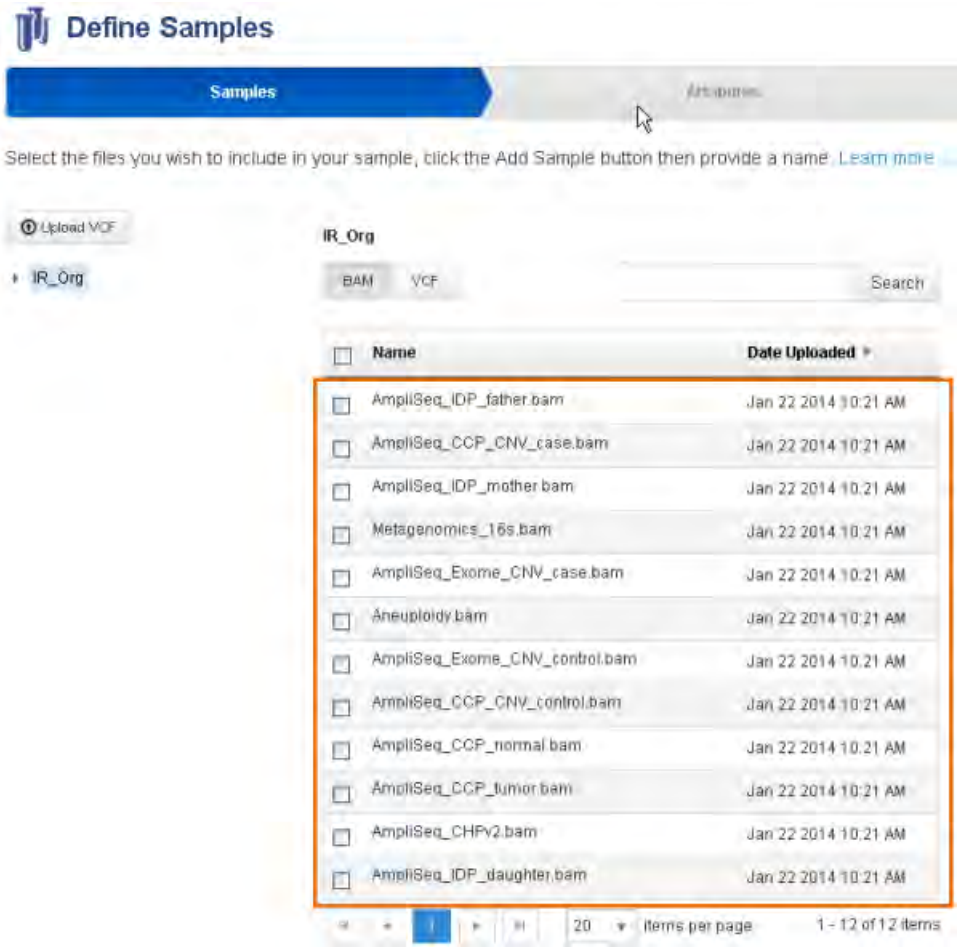
Note: To import a VCF file as a sample (not as a hotspot file) that contains a set of variants from your local machine, see Import a VCF file into Ion Reporter™ Software

1. Log in to the Ion Reporter™ Software, then do one of the following:
   - Click the Home tab, and then select **Define sample**:

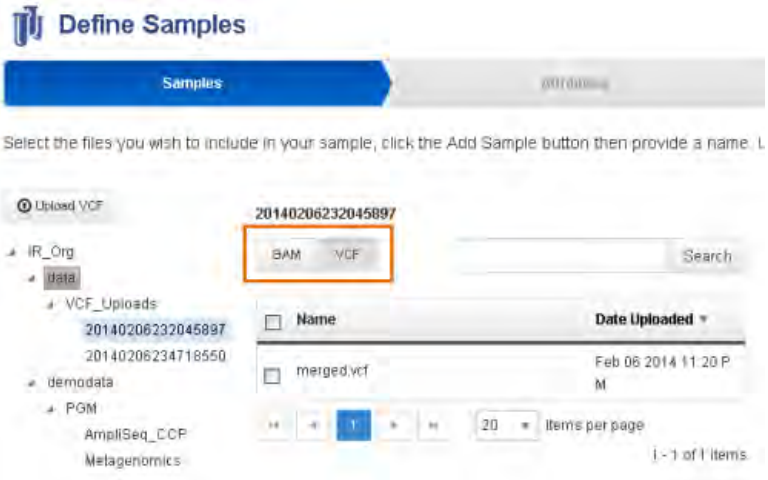


   - Or, click the **Samples** tab, select **Overview** and then click **Define Samples** ▸ **Manual** .

The name of your organization is shown on the left of the screen, and sample files for your organization are listed in the table. In this example, "IR_Org" is the organization name.

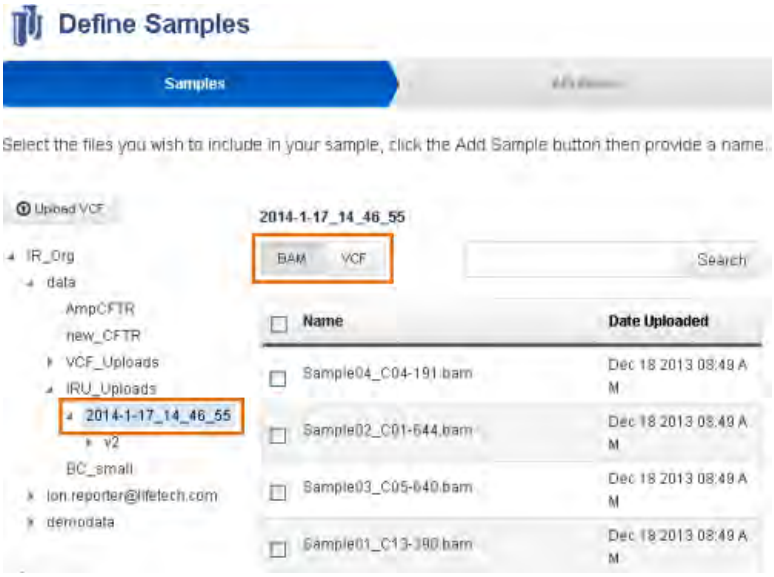


2. Click **BAM** or **VCF** above the list to filter for either .bam or .vcf files. VCF is selected in this example.



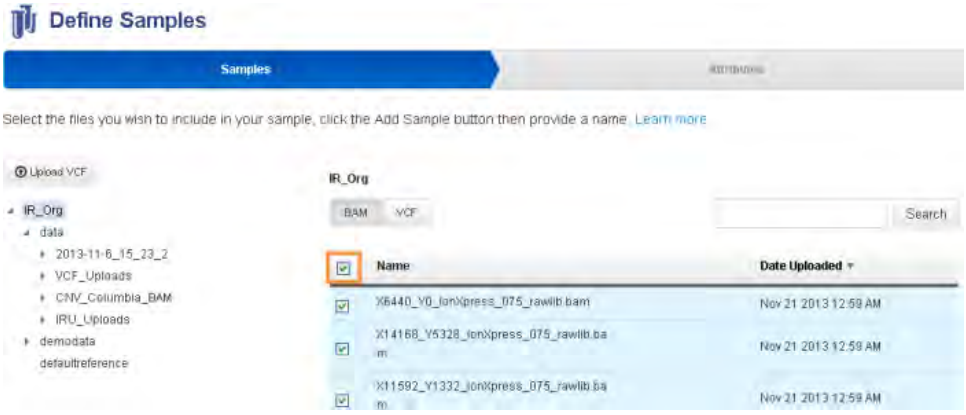
3. To view the files in either your upload directory or the demodata directory, expand the menu on the left that matches your organization name, then expand the data file directory that contains data files of interest.

   When a data file directory is selected, it is highlighted in blue and the contents of the file list change to reflect the files from the directory that you select.

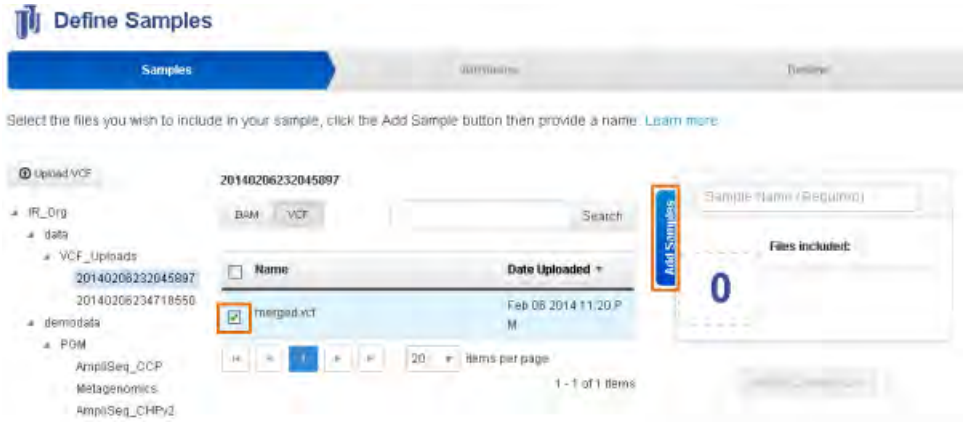


Note: Files transferred by IonReporterUploader appear under data/IRU_Uploads, in time stamp folders. Demo data files appear under the demodata folder.

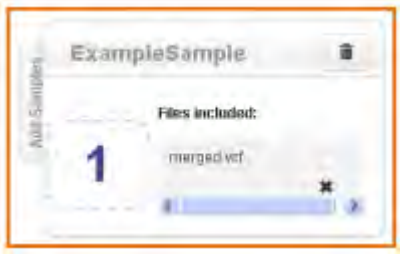
4. Enable the checkbox for each data file that you want to add to your sample, and then click **Add to Sample**. To select all samples in the list, click the checkbox in the header.



Note: The Add to Sample tab on the right is active after you enable at least one data file in the table.



The **Files included:** area lists the files for this sample, and the count of data file is displayed. A new sample box appears and **Next** is enabled.

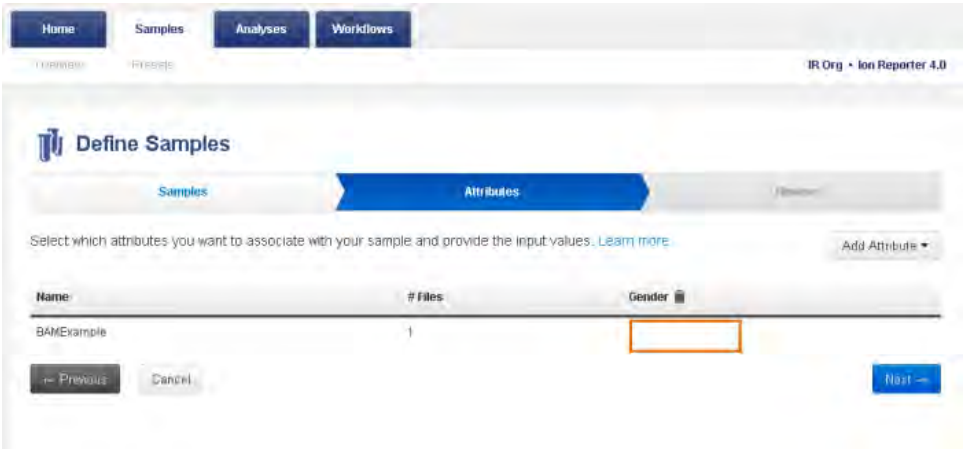


5. Enter a descriptive name for your new sample in the field at the top of the sample list. When the sample box contains the correct data files and the sample name for your sample, click **Add to Sample List**.

6. (*Optional*) Do one of the following:

   - Add more data files to the newly defined sample; Enable the checkbox for each data file that you want to add to your sample, and then click **Add to Sample**.
   - Repeat this procedure to define multiple new samples, each in a separate sample box.

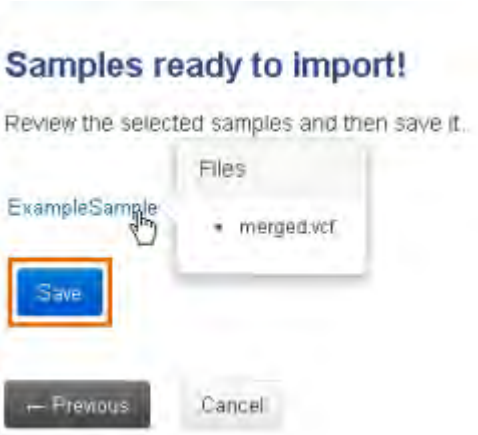
7. Click **Next**.
   - If you have any additional attribute information to associate with the sample, click **Add Attributes**. A new column opens for your attribute.
   - To set an attribute, click the area under an attribute heading to set the attribute value. Gender is selected in this example.



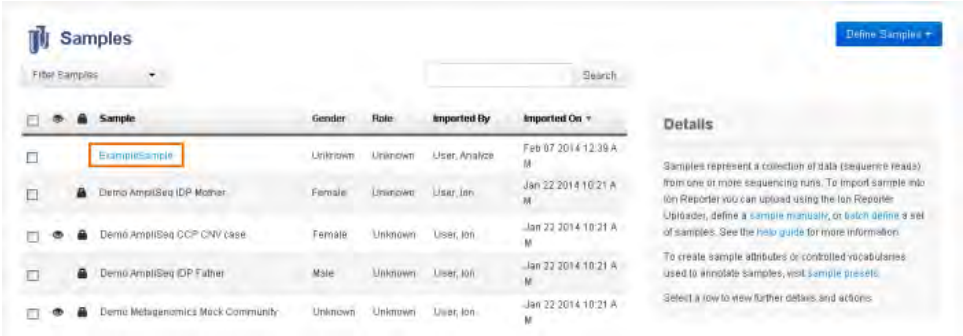
   Note: The range for the cellularity attribute is 0 to 100.

8. Review the sample files that are listed in the Review chevron.
   - If the sample name and the raw data files are correct, click **Save**.
   - To change the sample, click **Previous**.

9. In the Review chevron, hover over the sample name to see the raw data file (or files).



10. The Samples Overview tab opens, with your new sample in the sample table.

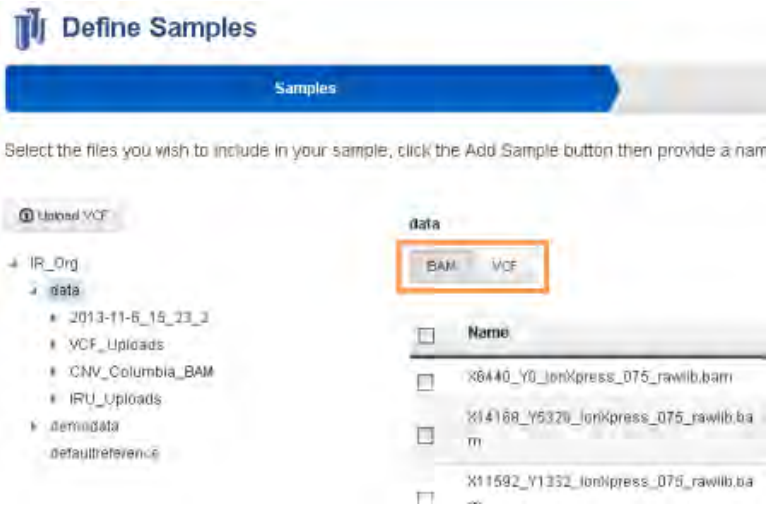


Your new sample is ready to be analyzed.

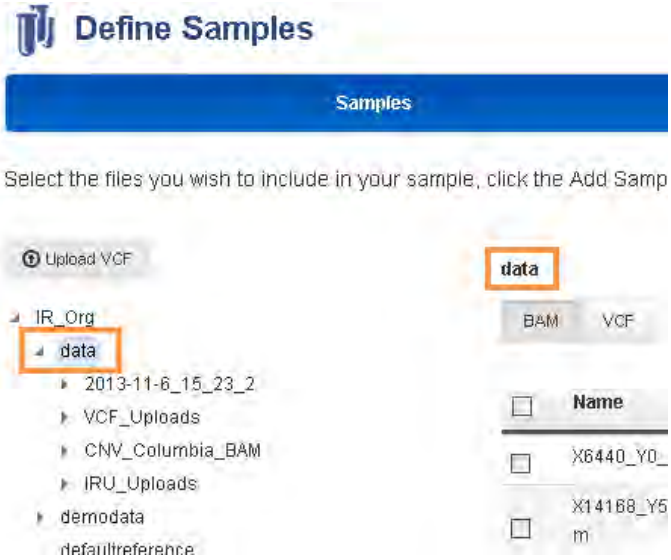
Notes about manual sample definition

Keep the following in mind when defining samples:

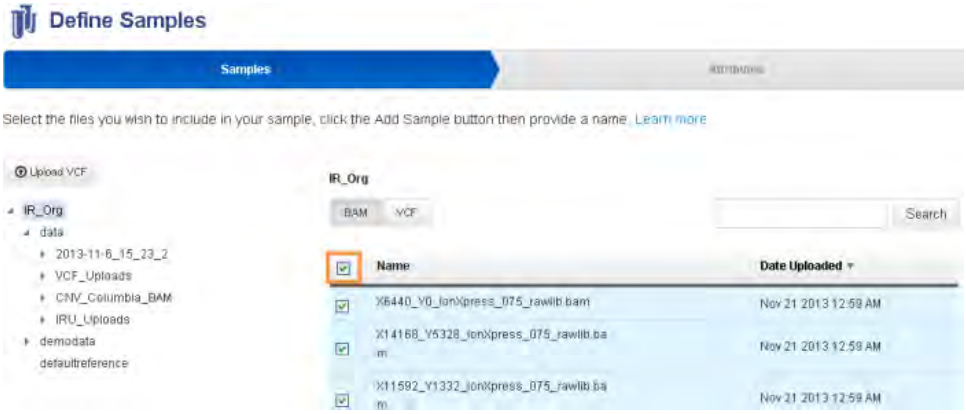
- The table lists either BAM or VCF files (not both at the same time). Click **BAM** or **VCF** to change the list. The file type that is not selected is filtered out and those files do not appear in the table.



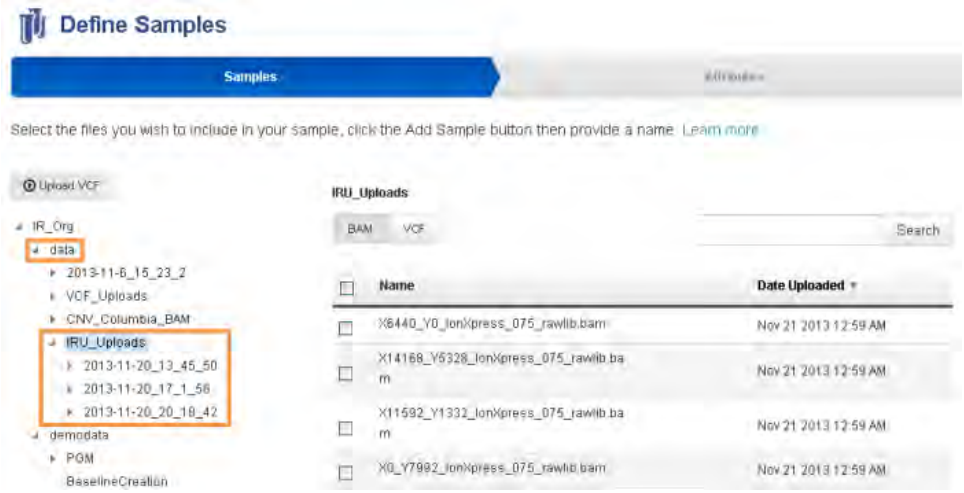
- The data file browser on the left provides a cumulative list of all files at and below the folder that is highlighted in blue. When you click a folder in the data file browser, the list in the table changes to reflect the contents of the selected folder. The selected folder name also appears at the top of the table listing.



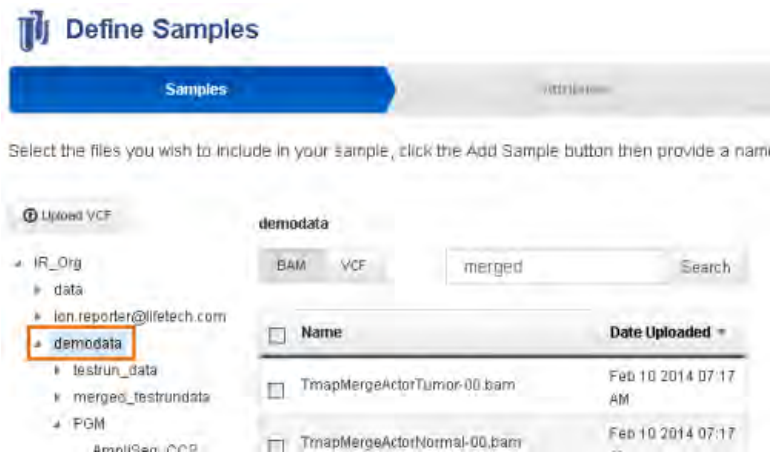
- Click the checkbox in the header to select all samples in the selected directory.



- Files that are transferred by IonReporterUploader appear under data/IRU_Uploads, in folders that include time stamps.



- Demo data files appear under the demodata folder.



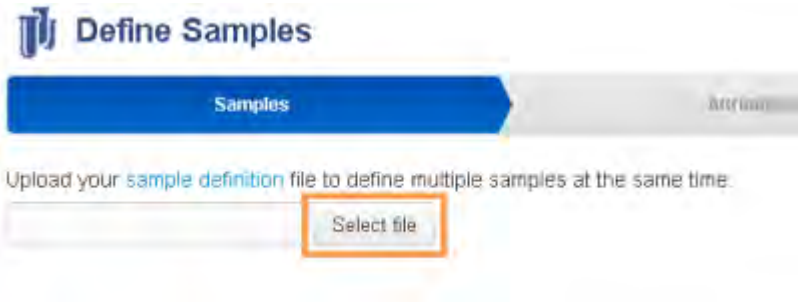
- Multiple BAM files can be combined into a sample. Multiple VCF files are not supported and mixed file types are not supported.
- The range for the cellularity attribute is 0 to 100.

Define a sample via CSV upload

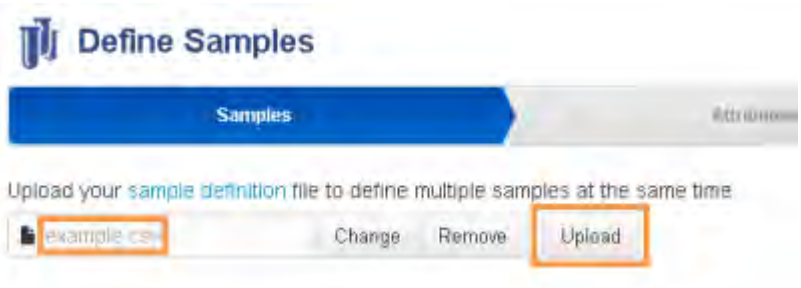
Follow these steps to upload a CSV file and manually define a sample or samples:

1. Log in to the Ion Reporter™ Software and click the **Samples** tab.
2. Click **Define Samples** ▸ **Batch**

3. Click **Select File** and browse to the CSV file you want to upload.



4. Confirm the displayed filename and click **Upload**.



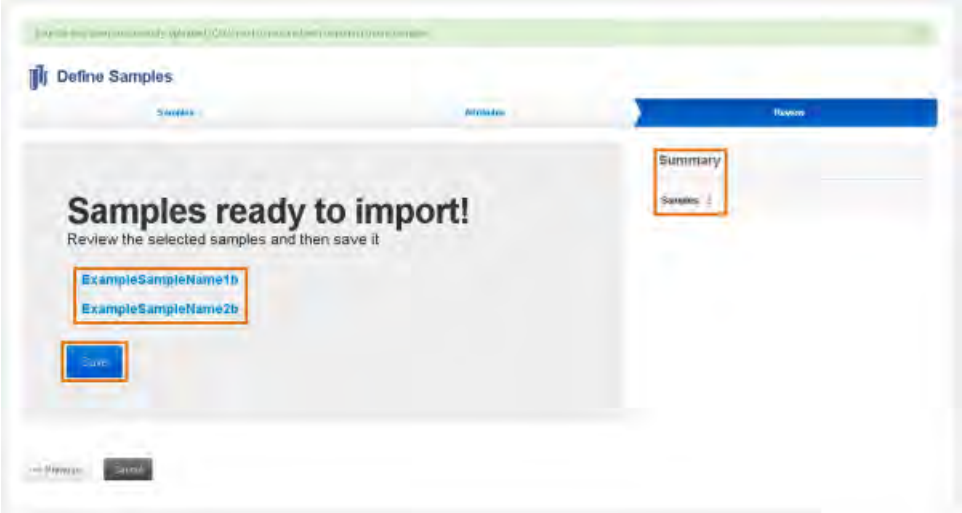
Wait while your file is verified.

5. Click **Next**. In the sample list, verify that your sample information is correct.

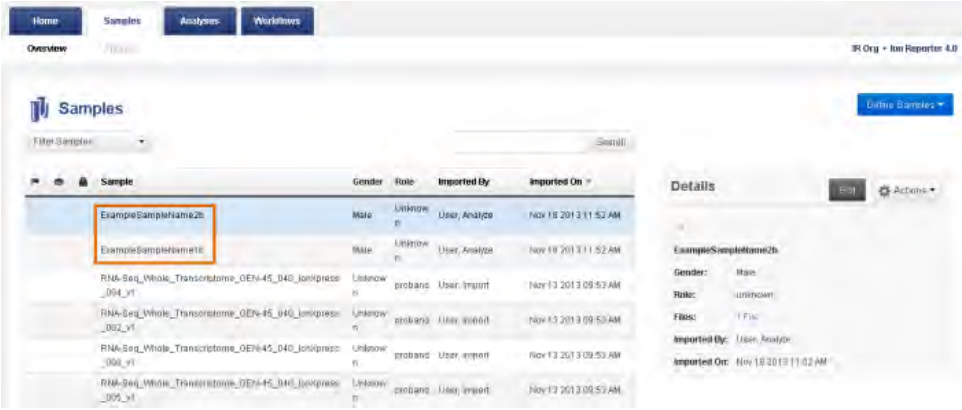
6. (*Optional*) If you have any additional attribute information to associate with the sample, click **Add Attributes**. A new column opens for your attribute.

7. When your sample information is complete, click **Next**.

8. In the "Samples ready to import" screen, check your sample names and number of samples. If the sample information is correct, click **Save**.



9. You are brought back to the sample list where you can see your new sample(s).



Define samples as no-template controls

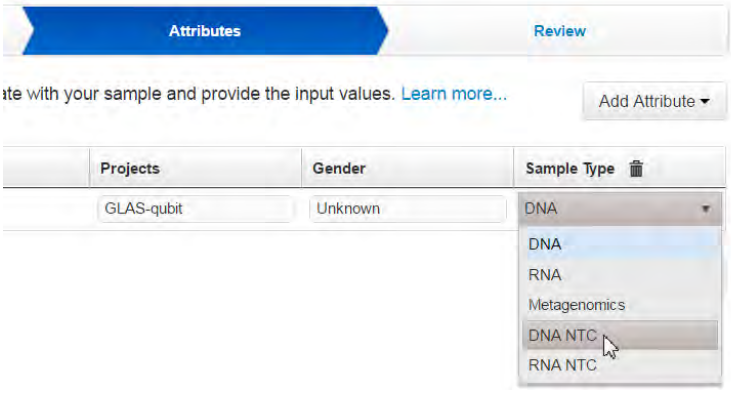
No-template control (NTC) samples can be defined in Torrent Suite™ Software or Ion Reporter™ Software.

If samples are defined as NTCs in Torrent Suite™ Software, the sample definition attributes transfer to Ion Reporter™ Software automatically.

Note: If you define NTCs with an incorrect sample type, Oncomine™ DNA and Fusions analyses and Oncomine™ DNA analyses do not complete successfully in Ion Reporter™ Software due to lack of sufficient reads.

To define a sample as an NTC in Ion Reporter™ Software, perform the following steps.

1. Under the **Samples** tab, in the **Overview** screen, click the sample name that you want to designate as an NTC.
2. In the **Define Samples** workflow bar, click **Attributes**. If the **Sample Type** attribute is not displayed, click **Add Attribute** to add it.
3. Under the **Sample Type** column, click the button to select **DNA NTC** or **RNA NTC**.



4. If you define the sample as **DNA NTC**, you must also add the **Percentage Cellularity** attribute, then set the cellularity to 100%.

   Note: Cellularity is not required for RNA samples.

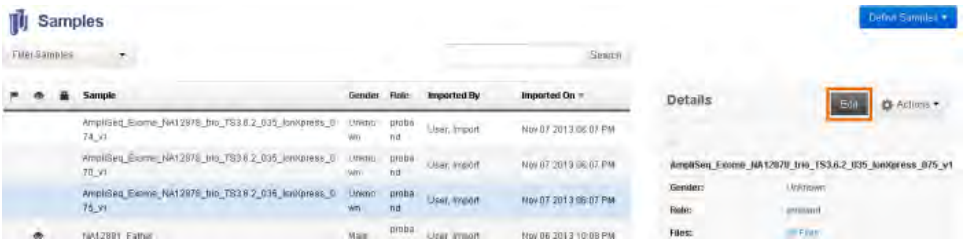
Edit a sample

When you edit an existing Ion Reporter™ Software sample, you can add raw data files to the sample, remove raw data files from the sample, or edit the sample attributes.

Note: You cannot edit demo data samples that are provided with Ion Reporter™ Software. These samples do not include the Edit option when you click **Actions** in the sample Details.

Follow these steps to edit an existing Ion Reporter™ Software sample:

1. Log in to the Ion Reporter™ Software application and click the Samples tab.
2. Select the sample that you want to edit from the table, and then click **Edit** in the sample Details.



3. Add the raw data files from the table to your sample.
4. Click the **Next** to move to Attributes in the workflow bar. You can now edit the attributes associated with your sample. For example, to change the gender attribute of your sample, click on the gender value ("Unknown" in the example"), then in the menu select the correct gender ("Female" in this example).

5. To add an additional attribute to your sample, click **Add Attribute**. (The attribute must already be defined as a Sample Preset. For details, see Create sample presets.
6. After you have made your changes, click **Next**.

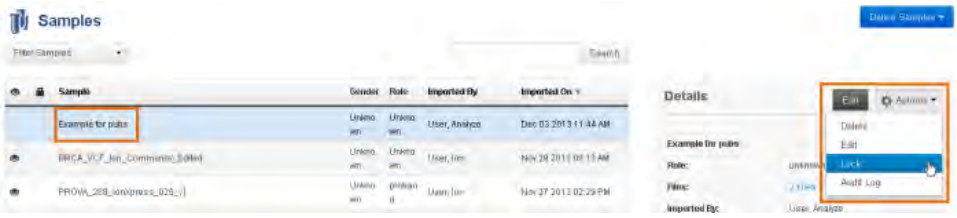
7. In the Review chevron, click **Save** .

Lock a sample

IMPORTANT! After you lock a sample, you are no longer be able to edit the file or attributes associated with it. To edit a sample that has been locked, you must first define a new sample.

Follow these steps to lock a sample:

1. Log in to the Ion Reporter™ Software application and click the **Samples** tab.
2. Select the sample in the table, then click **Actions** ⚙ ▸ **Lock** from the in the sample details section.



3. Click **Yes** to confirm that you want to lock the sample.

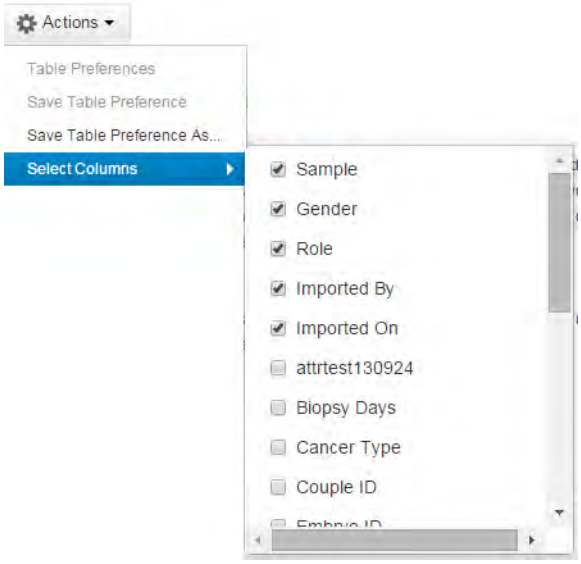
4. You are brought back to the samples page. Your sample is locked and is marked with the locked icon 🔒.

Add sample attributes to Ion Reporter™ Software

When you assign custom sample attributes in a Torrent Suite™ Software run plan, you can access these attributes to access them in Ion Reporter™ Software

Note: Do not create a custom sample attribute that has the same name as a factory-shipped sample attribute.

1. Click **Samples** ▸ **Overview** and select the imported samples that have custom attributes.
2. Click **Actions** ⚙ ▸ **Select Columns**.
3. Select the column types that you want to add your custom attributes to, such as Biopsy Days or Couple ID.

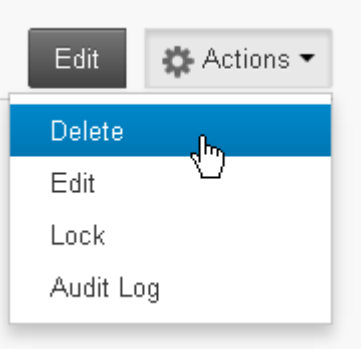


The sample attributes are now added as columns in the Samples table.

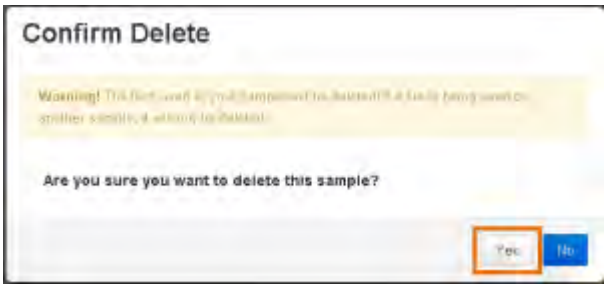
Delete a sample

IMPORTANT! When you delete a sample, this action also erases the raw data associated with the sample. If the raw data is being used with other samples, this action only deletes the data uniquely assigned to the sample that you delete.

1. Log in to the Ion Reporter™ Software and click on the **Samples** tab.
2. With the left column checkboxes, select the sample (or samples), and then select **Delete** in the **Actions** menu in the sample details section.



3. Review the Confirm Delete message, then click **Yes** to delete the sample.

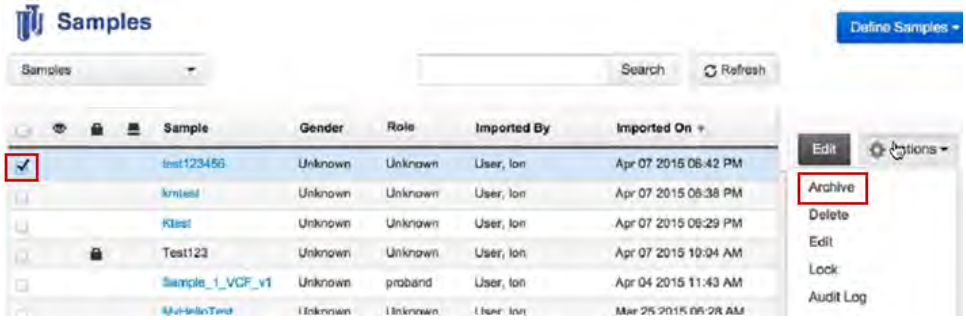


Your sample is deleted and you are brought back to the Samples Overview page.

Archive samples (local server only)

If you have a local Ion Reporter™ server, you can archive samples to your TS Storage device to free up disk space.

1. On the **Samples** page, select samples you wish to archive.
2. Click **Actions** ▸ **Archive**.

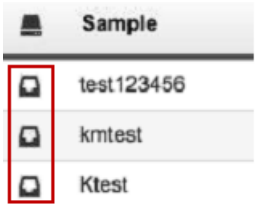


The sample now has an archive icon in its row.

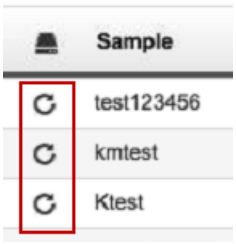
3. You can also select multiple samples and archive them.

4. Click **Yes** to confirm batch archive.

Archive icons now appear in the sample rows.



5. To undo archival, select the archived samples and click **Actions** ▸ **Restore**. A restore icon appears in the sample rows.



Archival location must be specified

New users and users upgrading from a previous version must specify a storage location for archiving samples and analyses in Ion Reporter™ Software 5.4. Change archivalMountPath=/tmp/ to archivalMountPath=/storage IP address/ in the ionreportermanager/server/server.properties file, then restart the Tomcat server.

To check the data that you archived, go to the storage location of the archived data, which is set up in /share/apps/IR/ionreportermanager/server/server.properties file as archivalMountPath.

Create sample presets

You can create custom attributes, which are known as sample presets, to describe samples that are used in your research.

Note: Do not create a custom sample attribute that has the same name as a factory-shipped sample attribute.

Ion Reporter™ Software supports the following types of attributes:

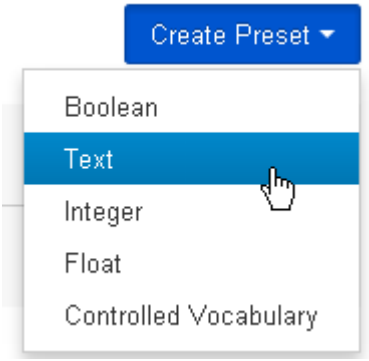
- Boolean -- Creates a list of these two choices: True, False
- Text -- Accepted values: general text
- Integer -- Accepted values: integer numbers (0, 9, -44, and so forth)
- Float -- Accepted values: decimal numbers (0.1, 9.0, -44.3, and so forth)
- Controlled Vocabulary -- Creates a list of these choices that you define with the preset terms.

Note: Ion Reporter™ Software gives you the option to encrypt the internal storage for a sample attribute. In the preset form, click the **Encrypted** checkbox.

This example shows a text sample preset:

1. Log in to the Ion Reporter™ Software application. Click the **Samples** tab, then select **Presets**.

2. Click **Create Presets** , then select the type of preset you want to create.

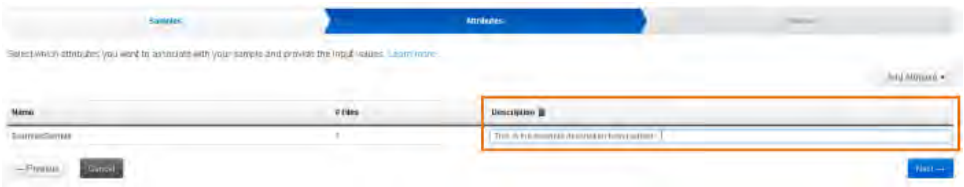


3. Fill out the preset form with the attribute name and your description of the meaning of the attribute. The name that you enter is used for the attribute during sample definition.

4. *(Optional)* If you want the internal storage for the attribute to be encrypted, click the **Encrypted** checkbox.

5. Click the **Save** button. Your new preset is now available in the **Add Attribute** menu during sample definition:

6. To see how the attribute is entered during sample definition, click the image:



Example boolean preset

This is a completed Boolean preset form:

It appears during sample definition.

Example Controlled Vocabulary preset

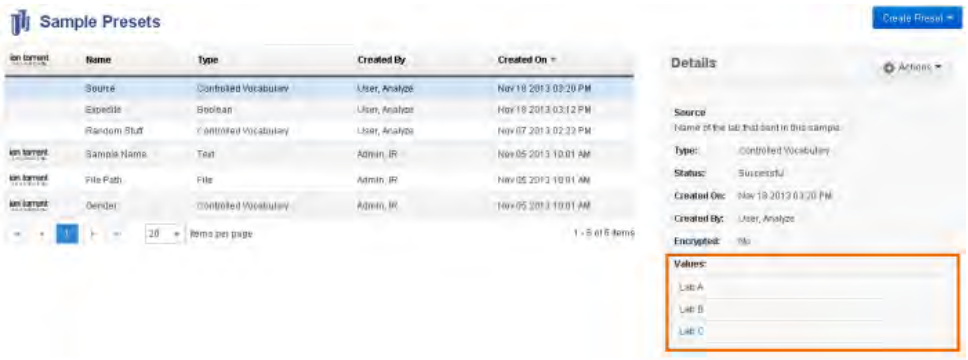
With a Controlled Vocabulary preset, you create a pull-down menu for an attribute. This type of preset gives you control over your sample metadata by preventing misspellings or alternate wordings for common entries.

Click these images to see a Controlled Vocabulary example:

- A completed Controlled Vocabulary preset form:

- How the attribute appears during sample definition:

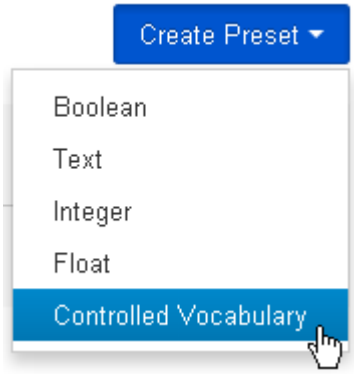
- How the attribute appears in the Samples Presets tab (with the menu options shown in the details Values area):



Create a Controlled Vocabulary preset

1. Log in to the Ion Reporter™ Software application. Click on the **Samples** tab and then on the **Presets** sub-tab.

2. Click the **Create Presets** button and select **Controlled Vocabulary**.



3. In the Controled Vocabulary Preset form, enter the name and description of the new sample attribute. In the Terms field, enter the first entry for the attribute's pull-down menu.

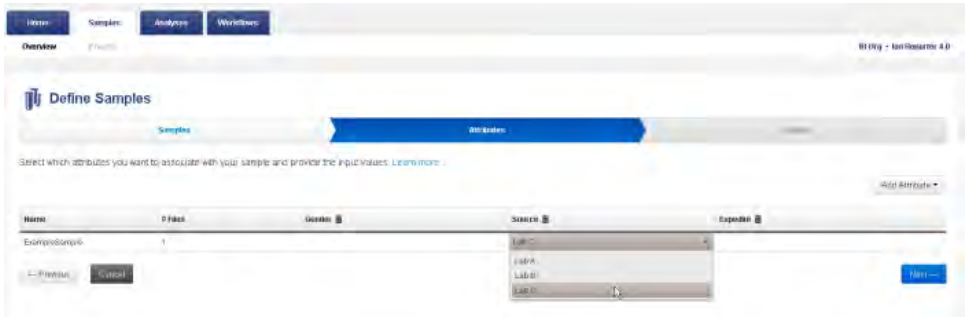
4. Click the **Add** button for an additional Terms field. In the new field, enter the second entry for the attribute's pull-down menu.

5. Again click the **Add** button for an additional Terms field. In the new field, enter the third entry for the attribute's pull-down menu.

6. Click the **Save** button.

7. Your new preset is appears in the Sample Presets table. In the Details area, the Values section lists the menu selections available to the user.

8. During sample definition, to define this attribute, the user selects an entry from the pull-down menu.

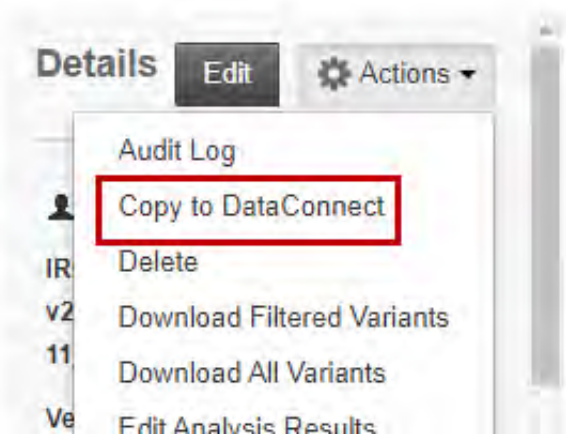


Copy results files to DataConnect

You can copy VCF analysis results from successful analyses to Thermo Fisher Cloud for storage and to share analyses with other users.

Note: If the analysis has an associated filter chain, the exported VCF file in the Thermo Fisher Cloud is the filtered set.

1. Go to the **Analyses** tab, then select a successful analysis by clicking the checkbox.
2. In the **Details** pane, click **Actions** ▸ **Copy to DataConnect**.



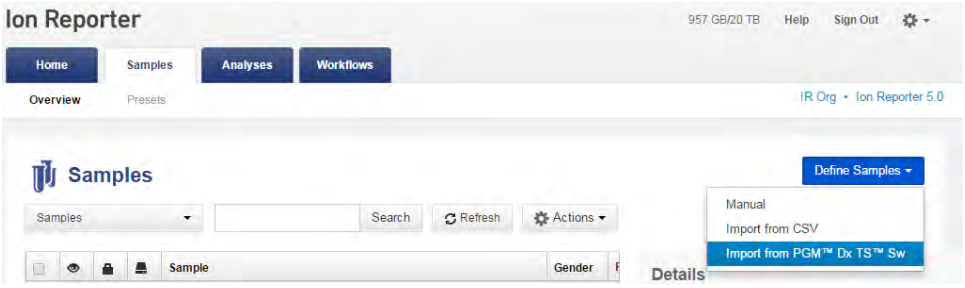
3. In the Export to Data Manager, click **Confirm**.
   A message confirming that the files have been copied to the cloud appears.
4. To view the files, go to Thermo Fisher Cloud. The samples are named the same as the analysis name that was copied.

   Note: Analyses that are run on Ion Proton™ instrument automatically receive 1 TB of storage space. When copied to Thermo Fisher Cloud, VCF files from analyses that are run on Ion Proton™ instruments automatically receive a one-time increment of 1 TB of storage space.

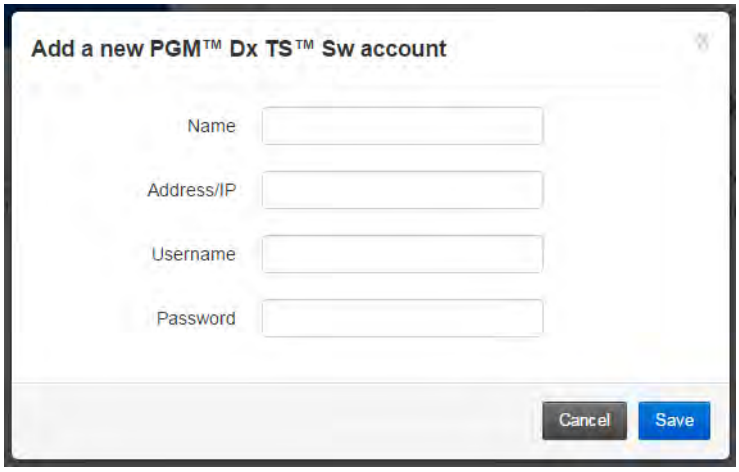
Import samples from Torrent Server running Assay Development Software

Ion Reporter™ local servers can now import samples from an Ion PGM™ Torrent Server running Assay Development Software 5.0, or later.

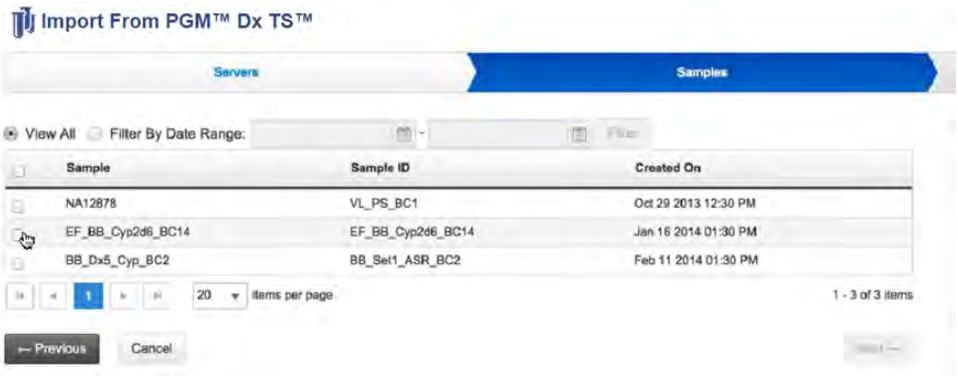
1. In the **Samples** ▸ **Overview** page, click **Define Samples**.
2. Select **Import from PGM™ Dx TS™ Sw**.



3. On the Servers screen, click **Add Server**.
4. Enter name, IP address, username and password. Click **Save**, then click **Next**.

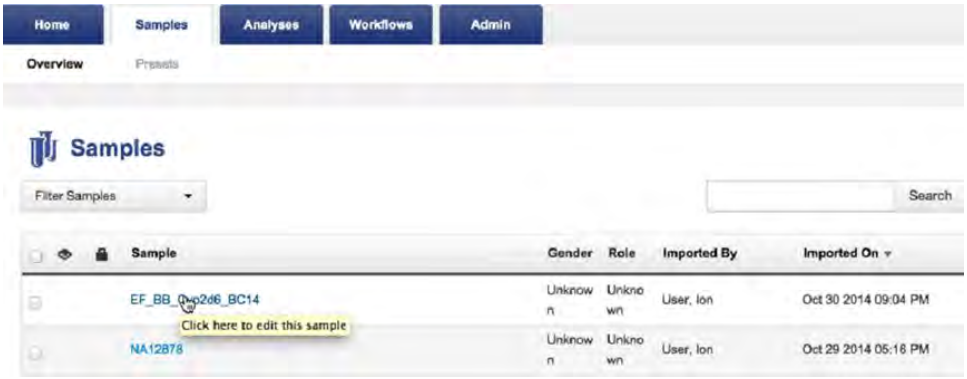


5. Select the samples you want to import and click **Next**.



6. In the Review screen, click **Import**.

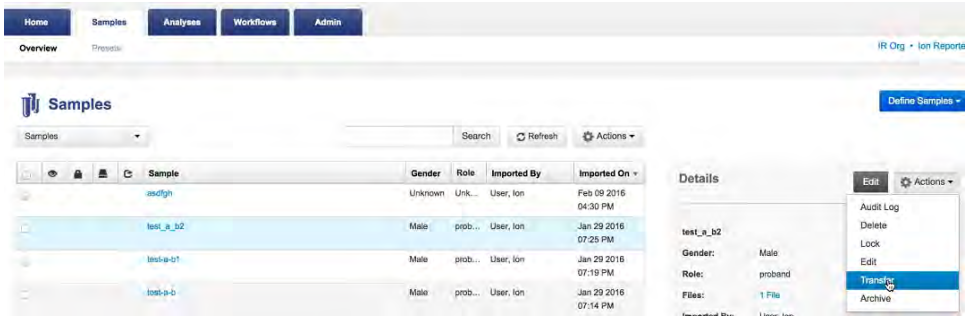
The samples now appear in the Samples list on the Ion Reporter™ local server.



Transfer sample to users in another organization

You can now transfer samples to other users in different organizations. All custom sample attributes are also transferred with the sample. This feature particularly benefits core labs when they complete analysis work for clients.

1. In the **Sample** ▸ **Overview** page, highlight the sample you want to transfer and click the **Actions** drop-down menu and select **Transfer**.



2. In the **Transfer Sample** dialog, enter the address of the intended recipient(s) and click **Transfer**.

A status appears that your sample transfer has been initiated.

Batch transfer samples to user in another organization

You can also transfer multiple samples to user in another organization.

1. In the **Samples** ▸ **Overview** page, select multiple samples and then click **Actions** ▸ **Transfer**.
2. In the Batch Transfer dialog, enter the email address of the intended recipient and click **Transfer**.

A status appears stating your samples transfer has been initiated.

Accept (or reject) a sample from a user from another Org

You can now accept samples transferred from a user in another organization.

1. In the **Home** ▸ **Notifications** page, locate the new sample and click the click the download icon to initiate the transfer.

2. Go to the Samples tab to locate the transferred sample.
3. To reject the sample, go to **Actions** ▸ **Delete**.

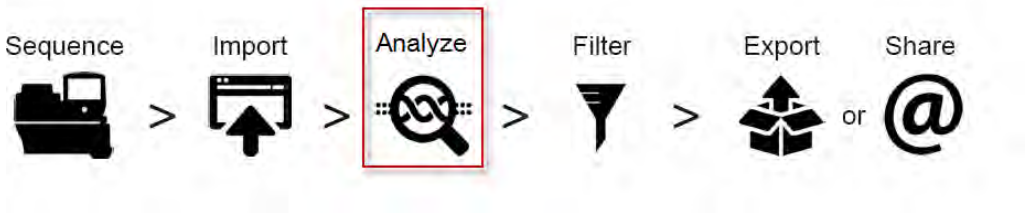
Delete transferred sample after transfer

After you have transferred a sample to a user in a different organization and the recipient has accepted the sample, you can delete it to free up storage space.

1. From the **Home** ▸ **Notifications** page, view transferred samples in the Transferred column.

2. Delete any transferred samples you no longer need.

Workflows in Ion Reporter™ Software



Each workflow is a set of instructions that tell the Ion Reporter™ Software what data to expect, what to do with the data, and what to provide as output.

You can use predefined workflows that are ready to use or can create custom workflows. All of your workflows can be found under the **Workflows** tab.

View workflow details in Ion Reporter™ Software

When you select a single workflow that is in the **Workflows** tab in Ion Reporter™ Software.

1. Sign into the Ion Reporter™ Software and click the **Workflows** tab.
2. In the **Workflows** list, select a workflow.

Details about the workflow open in the **Details** section.

Note: If you select more than one workflow in the list, workflow details are not available.

Pre-installed workflows in the Ion Reporter™ Software

Tumor-Normal pair workflows

These workflows provide more than a calculation of the intersection of variants in the two samples.

These workflows do analysis reads of a tumor sample and reads of the related normal sample. However, these workflows are optimized to find somatic variants, which appear in the tumor sample and *do not appear in the normal sample*. These workflows perform a statistical evaluation of the likelihood that the tumor allele is *not present in the normal sample* and calculates a P-value representing the statistical confidence of that call.

At each position with a variant in the tumor sample, the evidence for that allele in the normal sample is examined. If the tumor allele is seen in the reads of the normal sample above the error rate, we cannot say that this is a tumor-specific (somatic) variant and the variant is rejected (not called).

Tumor variants in which the same position in the normal sample has no coverage or low coverage are designated as non-confident. Non-confident variants are not assigned a P-value, and are instead flagged with NC-LC (non-confident because of low-coverage) in the adjacent field in the output VCF file.

Some non-confident variants receive the NC-LF (non-confident because of low-frequency) flag instead of a P-value. Note that these variants are not assigned a P-value. This happens to variants for which both of these conditions are true:

- The variant's allele frequencies are less than 10% in both the tumor and normal sample.
- The variant has a non-zero allele frequency in the normal sample.

A Non-Confident variant call means that we are not confident that the variant appears *only* in the tumor sample, *i.e.* the variant is also in the normal sample. This could be a germline variant or a systematic error present in both samples.

In the VCF file, "./." means no-call in the normal and "0/0" means homozygous reference call.

The TumorNormal workflow template is designed to be run on a pair of research samples from the same individual. Ideally both research samples are sequenced on the same chip. (With large panels such as the Ion AmpliSeq™ Comprehensive Cancer Panel, this might require an Ion Proton™ instrument.)

Note: When creating a custom workflow for AmpliSeq Exome tumor-normal pair analysis, in order to have the correct parameters that are applied, it is advised to either:

1. Make a copy of the factory AmpliSeq Exome tumor-normal pair workflows and edit any desired parameters.
2. Use the factory-shipped BED file to create a new custom workflow.

Using the **Import from AmpliSeq** feature or manually uploading the AmpliSeq Exome panel BED file is not recommended.

FAO calculation

Key for formula below

AF: Allele frequency

AO: Alternate allele depth at position

RO: Reference allele depth at position

DP: Total depth at position

FAO: Flow space alternate allele depth at position

FRO: Flow space reference allele depth at position

FDP: Flow total depth at position

1. FAO is usually equal to AO; however, due to complex alleles and/or downsampling*, FAO may differ from AO.
2. AF=FAO/(FAO+FRO) and not FAO/FDP. This is because FDP may include reads that don't fit the flow space profile of any hypothesis; in such cases, FDP>=FAO +FRO and this is not used in allele frequency calculation.

Exception: When flow correction is not performed and there are no F tags in the VCF file, then DP=AO+RO and AF=AO/DP.

*FAO along with all the F tags are subject to downsampling but AO/DP/RO/SAF/SAR/SRF/SRR are not. So when total coverage is higher than the downsampling cutoff, FAO tends to be smaller than AO.

Ion AmpliSeq™ RNA Lung Fusion workflow

The Ion AmpliSeq™ RNA Lung Fusion workflow detects gene fusions that are targeted by the Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel.

Reference file

The reference file for this workflow is constructed of all the fusion isoforms in the Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel. The workflow uses a related BED file that describes the breakpoints between the two genes that are associated with each fusion. (Both the reference file and the BED file are included in Ion Reporter™ Software.)

(We loosely refer to this as a reference file. More properly, it is a panel-specific gene fusion mapping file.)

Sensitivity settings

The deployed workflow uses the **MEDIUM** optimized parameters settings, which we recommend for most use. If you want to use higher or lower sensitivity settings in your analyses, create a custom workflow. In the Parameters chevron, click the **Fusions** link in the left panel and select either **LOW** or **HIGH** sensitivity.

Setting	LOW	MEDIUM	HIGH
Min Overlap Percent Fusions	80	70	60
Min Overlap Percent Non-Fusions	70	50	50
Min Exact Match Percent	75	66.7	50
Min Count For Fusions Call	30	20	10
Min Count For Novel Fusions Call	125	100	100
Min Count For Expression Control Genes	20	15	10

Note: You cannot change an individual parameter setting. You can select either the LOW, MEDIUM, or HIGH group of settings.

CNV and aneuploidy research

The Aneuploidy workflow supports chromosome and sub-chromosome-level aneuploidy detection down to multi-megabase resolution. The CNV detection that is used in the single-sample and two-sample Ion AmpliSeq™ workflows calls copy number results down to the gene and sub-gene-level ploidy variants.

In the Aneuploidy workflow and the single- and two-sample Ion AmpliSeq™ workflows, which contain a CNV detection module, read coverage is corrected for GC bias. Corrected coverage is compared to a baseline coverage from control samples of regions with known correct ploidy (ploidy of 2). A statistical model of the likelihood that a genomic region belongs to an alternate ploidy state is developed.

Note: The aneuploidy baseline that is used in Ion Reporter™ Software 5.0 or later is different from the Ion Reporter™ Software 4.4 aneuploidy baseline because new exclusion regions are used. Do not use an Ion Reporter™ Software 5.0 or later aneuploidy baseline with a version 4.4 workflow. Conversely, if you created an aneuploidy baseline in Ion Reporter™ Software 4.4 and want to continue using the baseline, then use it only with Ion Reporter™ Software 4.4 workflows.

The Aneuploidy workflow

The following apply to the Aneuploidy workflow (and to workflows that you create from the Aneuploidy workflow template):

- The input data is only a test research sample. A control research sample is not necessary, since a pre-computed Informatics Baseline Control is used as a copy number reference.
- The input sample is from a whole genome amplified library.
- The volume of the sample can be small.
- The average coverage can be small, in the order of 0.01x.
- This workflow identifies regions of the genome that are duplicated or deleted. The variant length detectable is typically from ~10 Mb up to a whole chromosome.
- This workflow overcomes the variations in coverage that are typical with amplified data.
- With this workflow, the coverage is typically too low to call SNPs or indels.

Mosaicism

Mosaicism detection is enabled by allowing decimal-level Copy Number Gain or Loss calls. This workflow setting allows non-integer ploidy calls. For each tile, the algorithm fits the data to all ploidy with a step of 0.05. This parameter can be turned on or off in aneuploidy workflows that are copied, edited or newly created. Mosaicism is turned on by default in the workflow "ReproSeq Mosaic PGS w1.1", and turned off in other Ion factory-shipped aneuploidy workflows.

Smoothing

Improved visualization of aneuploidy detection is enabled by the default smoothing of the discrete data points in all Ion Reporter™ Software 5.4 aneuploidy workflows. The smoothing shown in the display is based on overlapping neighbor-averaging which smooths the coverage values of the tiles. The copy number calls made by the detection algorithm are not changed if the smoothing parameter is turned on or off. You can control whether or not to smooth the data with an aneuploidy workflow-specific Smoothing parameter.

No Gender

You can disable the recording of the called gender of samples with the **Hide Gender** setting that is available for samples analyzed in aneuploidy workflows. In aneuploidy workflows with **Hide Gender** set to true, sex chromosome data is not shown in the graphical plots or recorded in results files, and the sex chromosomes are given a call of either "SxChrNrml"(if the sample is "normal" XX or XY) or "SxChrAbrml"(if the sample has any ploidy differences from XX or XY). "No Gender" results are turned on by default in the workflow "ReproSeq No Gender PGS w1.1", and turned off in other Ion factory-shipped aneuploidy workflows.

See “Gender calling in the aneuploidy workflow“ on page 78 and “Copy and edit a workflow“ on page 79 for further details.

Gender calling in the aneuploidy workflow

When you want to combine Mosaicism, Smoothing, and NoGender settings in a custom workflow, you can combine the Advanced CNV-finding Aneuploidy parameters in an Aneuploidy workflow that is copied or edited. Because NoGender workflows are not able to be edited to be gender-calling workflows, and vice versa, it is important that you copy the correct type of workflow that you want to customize: either NoGender, or one of the gender-calling workflows. The correct workflow type must be selected if you are to access the desired combination of settings.

Aneuploidy samples with very low total read counts may have analysis results where the gender could not be called. While such samples may have other issues to investigate, samples with low read numbers that are not getting gender calls can be run through a custom Aneuploidy workflow with a lowered value for the parameter `CNV Gender Min Autosomes Count`. The default value is 25000. You can change ths setting under on the Advanced tab of the CNV Finding settings, Gender calling section. See “Copy and edit a workflow“ on page 79 for an example of how to change this setting.

Copy Number detection in the Ion AmpliSeq™ single- and two-sample workflows

The following apply to copy number detection in the single- and two-sample Ion AmpliSeq™ workflows (and in workflows that you create from single- and two-sample Ion AmpliSeq™ workflow templates):

- For input data, both a test sample and a control sample are required for the two-sample workflows. Single-sample workflow CNV detection uses a pre-computed Informatics Baseline Control.
- These workflows detect regions of the genome that are different in the test sample that is compared to the control sample (or from the Informatics Baseline Control).
- Sensitivity is determined by the number of amplicons that cover the variant region. Approximately 200 amplicons is the smallest panel size tested that has shown robust copy number detection.
- Both research samples in the two-sample workflows can, but are not required, to be sequenced on the same chip. (With large panels such as the Ion AmpliSeq™ Comprehensive Cancer Panel, this might require a Ion Proton™ instrument.)

Visualization of CNV coverage data in the IGV genomic browser

You can visualize multiple files that are generated by the CNV detection module in the IGV browser. These files can also be downloaded from the IGV browser using the "Save As" menu option.

Three browser tracks are of particular interest for looking at and downloading tile coverage values:

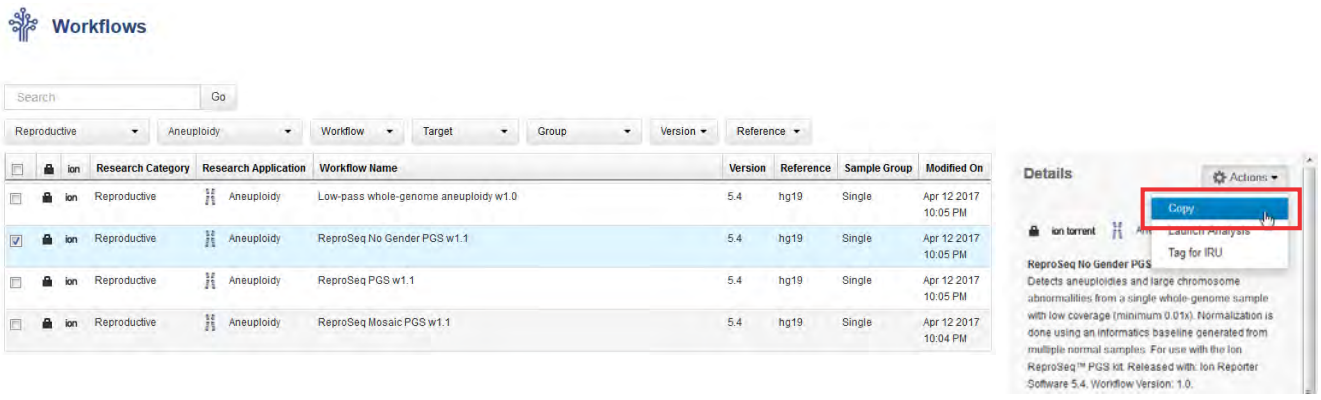
- The "DNA Coverage" track shows the average per-base coverage that is normalized by the median of all tiles (the mode).
- The "DNA Coverage Ratio" track expresses the difference in test and control coverages in a ploidy-centric Y-axis space using the following formula: difference = (test coverage that is divided by the control coverage) x (expected normal ploidy). This tile data is shown in the context of a golden called ploidy line in the Browser. Mosaic Copy number gains can be viewed in this track as contiguous data points consistently slightly above or slightly below, respectively "the gold expected normal ploidy line".
- There is no control coverage track in single sample workflows such as the Aneuploidy workflow and other CNV-detecting single sample workflows, since there is no control sample. The control for single sample CNV-detecting workflows is the informatics baseline that was selected. However, the "Control Coverage" track is present in a Paired sample workflow. This track shows the average per-base coverage that is normalized by the median of all tiles (the mode).

Note: For larger files like AmpliSeq Exome, coverage file loading is disabled.

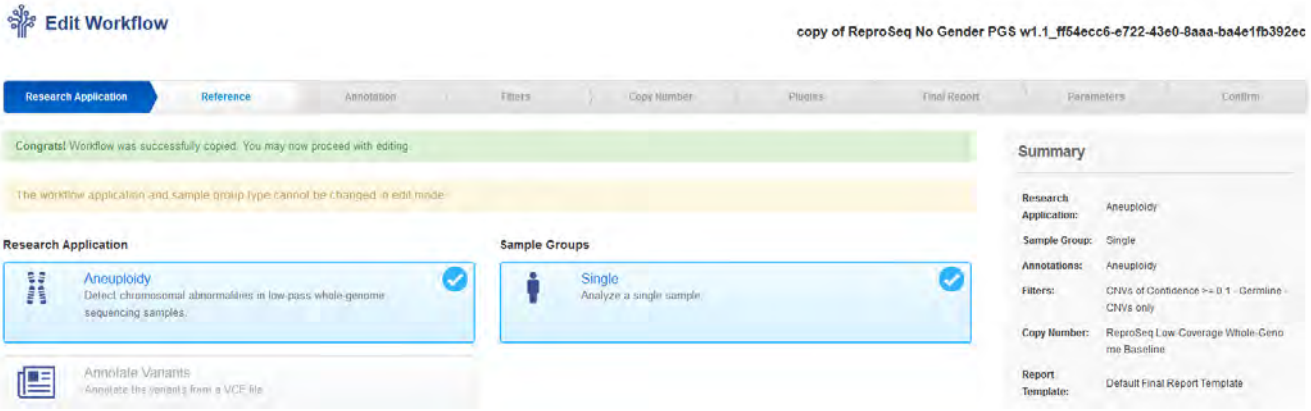
Copy and edit a workflow

You can create a new workflow from an existing Ion Reporter™ aneuploidy workflow by copying and editing it. For example, after copying the ReproSeq No Gender PGS w1.1 workflow, you can enable mosaicism detection (which reports a CNV event as a decimal ploidy value instead of an integer value), change the CNV sensitivity, or disable smoothing, by modifying appropriate workflow settings in the **Parameters** tab, and saving the changes. The following steps describe how to copy and edit an existing workflow to create a new Ion Reporter™ workflow.

1. On the Ion Reporter™ home page, click **View workflows**, select **Reproductive** from the **Research Category** dropdown list, then select **Aneuploidy** from the **Research Application** list. Select the workflow of interest, then click **Copy** from the **Actions** dropdown list.

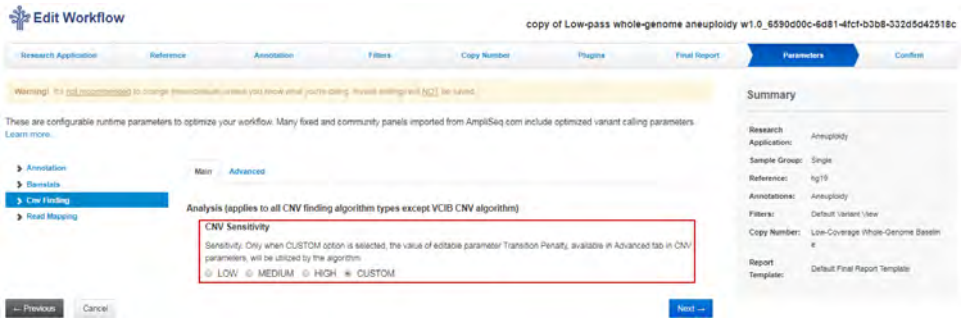


2. Click **Next ->** on each page, making any necessary changes, to proceed to **Parameters**.

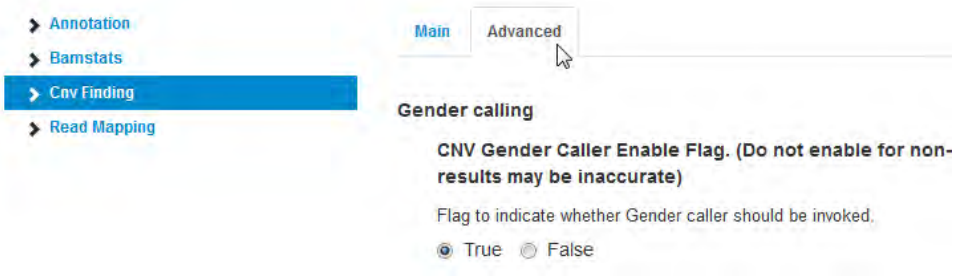


3. Click **Cnv Finding**, then change the **CNV Sensitivity** setting.

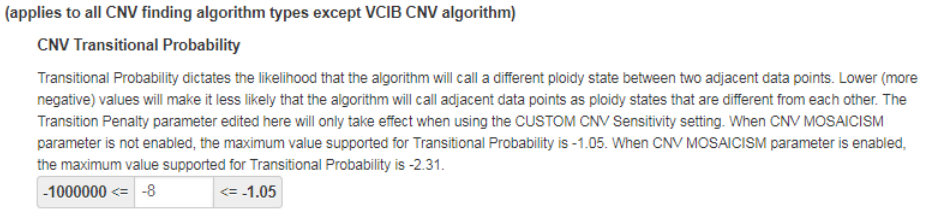
   Note: Low sensitivity results in fewer false positives, but more false negative calls. High sensitivity results in fewer false negatives, but more false positive calls. High sensitivity is required to make segmental aneuploidy calls of ~20 Mbp. Custom sensitivity allows you to make changes to the CNV Transitional Probability that more precisely call the true start and stop of subchromosomal segments.



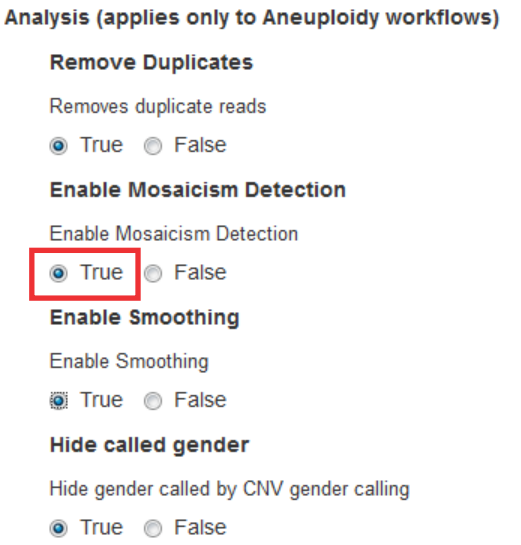
4. Click the **Advanced** tab to access more analysis parameter settings.



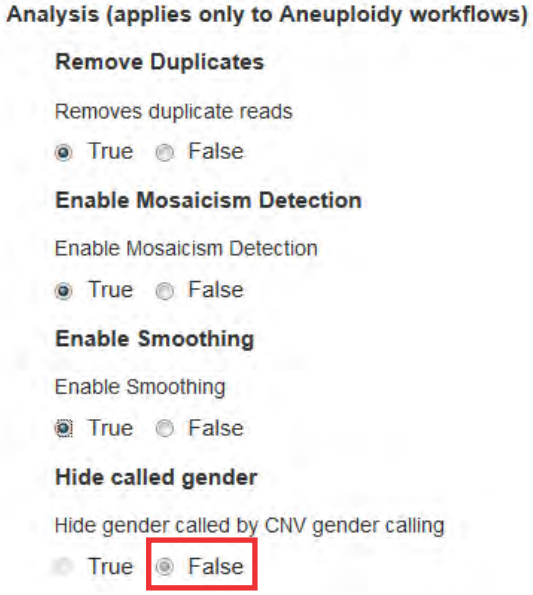
- You can change the CNV Transitional Probability setting if the Custom sensitivity option is selected.



5. Scroll down to the **Analysis (applies only to Aneuploidy workflows)** section, and make desired changes. In this example, select **True** under **Enable Mosacism Detection** to make this change.

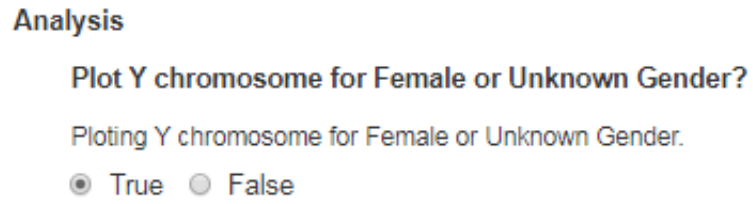


- To enable mosaicism detection and/or smoothing to a No Gender workflow, copy and edit the Reproseq No Gender w1.1 workflow as described. You cannot enable gender hiding in the other ReproSeq workflows because the **Hide called gender** setting is locked as **False** (gender is called), and cannot be set to **True**.



- To always show Y chromosome data for every sample, whether it is female, male or unknown, set **Plot Y chromosome for Female or Unknown Gender** to **True**.

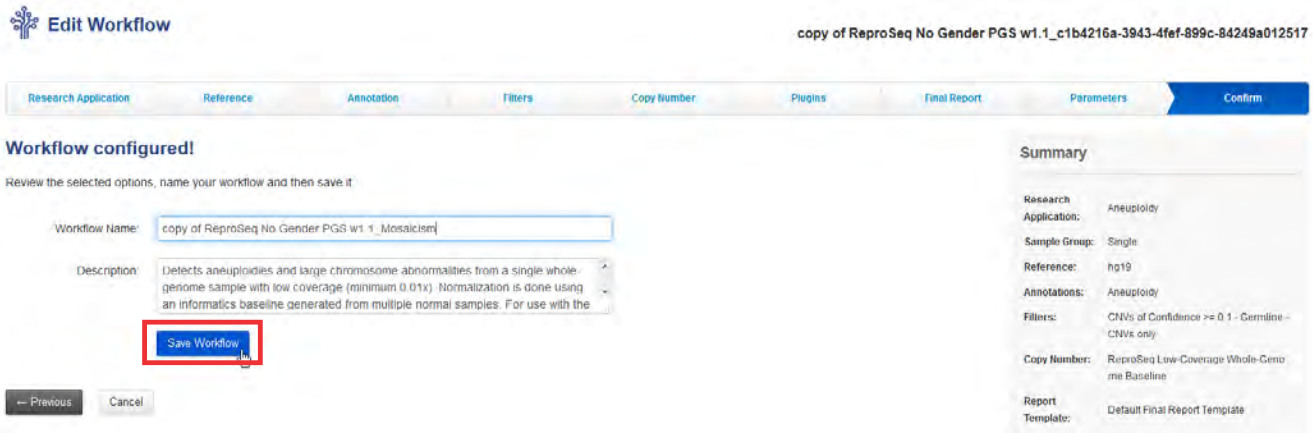
The default value for this setting is **False**, which will show Y chromosome data only if the sample is male.



6. There are additional workflow parameters if you click the Annotation, Bamstats, and Read Mapping links at the left of the page. When you have completed your edits, click **Next ->** at the bottom of the page to proceed to **Confirm**.

   Note: Do not change parameters from the default settings unless you understand how the change can affect your analysis.

7. Rename the workflow, then click **Save Workflow**.



The workflow appears in the list of available Ion Reporter™ workflows.

Variability Correction Informatics Baseline (VCIB)

A Copy Number Variation baseline, called the Variability Correction Informatics Baseline (VCIB), is available for some Oncomine™ Assays.

When creating a new or augmenting an existing workflow that includes CNV detection, you have three choices:

- Create a new VCIB CNV baseline.
- Use an existing VCIB CNV baseline as is.
- Augment an existing VCIB CNV baseline.

VCIB background

Copy number estimates are made using a proprietary algorithm.

The VCIB algorithm uses an informatics baseline 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 is followed by a correction 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 compute the corrected log2 ratios as well as the correction for tumor fraction are proprietary.

Known sources of variability include pool imbalance (when the assay has more than one pool of amplicons), total number of reads and 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 procedure we use trains on a large number of diverse samples, captures systematic effects, and encodes these into a file (the "baseline").

When augmenting a baseline, new samples are run, the size of each systematic effect encoded in the baseline is estimated, and a correction is applied to remove the effect. These added samples need not be normal and should be diverse so as to capture likely systematic variation.

Note: Logs for both successful and failed analyses include the "BaselineCreation.log" file that has the BAM files named that were rejected due to similarity to other files in the baseline, as well as the "map.TmapMergeActor-00.err" file that has the BAM files named that were rejected due to QC failure.

Understanding CNV Somatic Confidence Range

The somatic CNV algorithms in Ion Reporter™ Software deliver not only a ploidy estimate call, but also a pair of 90% confidence values. The 5% lower confidence bound value is the ploidy estimate where there is a 5% chance 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. For calling focal amplification the lower bound is important and not the upper bound.

Default filtering for the Ion AmpliSeq™ Immune Repertoire Assay Plus—TCRβ looks for Gains whose 5% Confidence interval (5% CI) is at least a ploidy of 2 extra copies over the expected normal ploidy value (expected ploidy is 2 for autosomes, and X in females and 1 in X in males). The filter will look for Oncomine™ Annotated variants, and for Oncomine™ Annotated CNVs, in most cases gains of 2 + 2 (gain + expected normal)= 4. Currently the Oncomine™ Variant Annotator plugin annotates somatic CNVs on all chromosomes for the known copy gain genes in the Ion AmpliSeq™ Immune Repertoire Assay Plus—TCRβ results whose 5% CI is ≥4.

How do I look for other gains than the default?

To create a custom somatic CNV filter, choose "CNV Somatic Confidence Range" filter, which has OR selected, and uncheck the "Enabled" checkbox for the "Minimum Ploidy Loss (95% CI) user expected" filter. Double check that the "Minimum Ploidy Gain (5% CI) over expected" Enabled checkbox is selected. The new value should be the ploidy of gain (over expected normal) you wish to threshold on when looking at the 5% CI value. So if looking for gains whose 5% CI value is anything over expected normal of autosomes (2), leave the value set at 0.0.

The default of 0.0 will find all gains whose 5% CI value is of ploidy 2 or greater. Setting the value to 1.0 will cause the filter look for all gains whose 5% CI value is of ploidy 3 or greater, for example.

Note: The OVAT annotation will be added to known copy gain genes on each assay's CNVs by looking at the 5%CI value of ≥4.

How do I set the filter to look for gains and for losses?

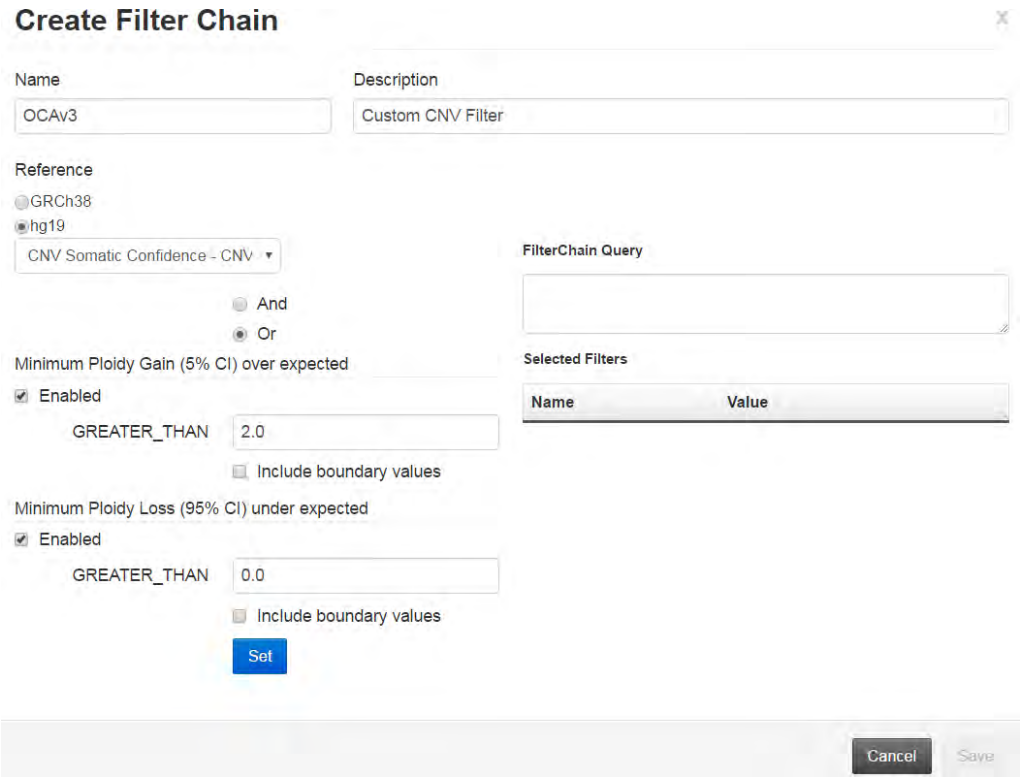
This is relevant especially for the Ion AmpliSeq™ Immune Repertoire Assay Plus—TCRβ CNVs where Tumor Suppressor genes are involved and deletion may have a biological impact.

To set a custom filter to look for gains and losses using the CI values, choose the filter "CNV Somatic Confidence Range", then Enable both the "Minimum Ploidy Gain (5% CI) over expected" and the "Minimum Ploidy Loss (95% CI) under expected" filter.

We are now asking the 5% CI value and filter to threshold for Gains, and the 95% CI value and filter to threshold Losses.

If we set the "Minimum Ploidy Gain (5% CI) over expected greater_than" to 2.0, (2+2=4 ploidy for gains, so anything 4 and above will be filtered in as a gain), and the "Minimum Ploidy Loss (95% CI) under expected to be greater_than" value to 0.0 (2-0=2 ploidy for losses, so nothing exceeding expected normal of 2 will be filtered in as a loss), we can expect the following example CNV call CI data to cause the CNV data to be filtered in or out of the results:

- A gene with suspected gain with 5% CI = 4.1 and 95% CI = 10.3 will be filtered in
- A gene with suspected loss with 5% CI = 0 and 95% CI = 1.0 will be filtered in
- A gene with suspected gain with 5% CI = 2.2 and 95% CI = 3.6 will be filtered out (2.2 is less than 4)
- A gene with suspected gain with 5% CI = 2.1 And 95% CI = 5.2 Will be filtered out (2.1 is less than 4)
- A gene with 5% CI = 0.8 and 95% CI = 2.1 will be filtered out (0.8 is less than 4 and 2.1 is greater than 2)



Custom workflows

There are two ways to create custom workflows for Ion Reporter™ Software.

- You can copy an existing workflow, then edit its settings. See "Copy and edit a workflow" on page 108.
- You can also use the wizard to create a new workflow. See "Create a custom workflow with the Ion Reporter™ wizard" on page 86.

Create a custom workflow with the Ion Reporter™ wizard

Ion Reporter™ Software provides a wizard to guide you through workflow creation.

Note:

- To create a custom workflow that analyzes your Ion AmpliSeq™ custom panel, in the Reference tab, import your custom panel as a target regions file. The wizard provides details when you are asked to set up the Reference step. Details are provided in the Reference step (see the table immediately preceding. (Import a design from AmpliSeq.com).
- Copying and editing from a factory-shipped workflow will always start with the same optimized set of parameters as the original workflow (until edited).
- Creating a custom workflow can have different default parameters based on the workflow and template, and may not contain the most-optimized set of parameters.
- Our recommendation for custom-panel workflows is to copy and edit a factory-shipped workflow most similar to the panel and algorithmic processing you desire, instead of creating a new workflow. If you are creating a new workflow, compare its parameters with those of an existing factory-shipped workflow to optimize performance.
- There may be some types of algorithmic calculation modules that cannot be included in custom workflows created with the Ion Reporter™ Software wizard. Those algorithmic calculation modules also may not be available if the workflow cannot be copied and edited.

1. Log in to the Ion Reporter™ Software, then click the **Workflows** tab.
2. Click the **Create Workflow** button.
3. The workflow wizard opens to guide you through the process of creating a workflow. Follow the instructions that are presented by the wizard. The following sections describe each step in more detail.

Select an application

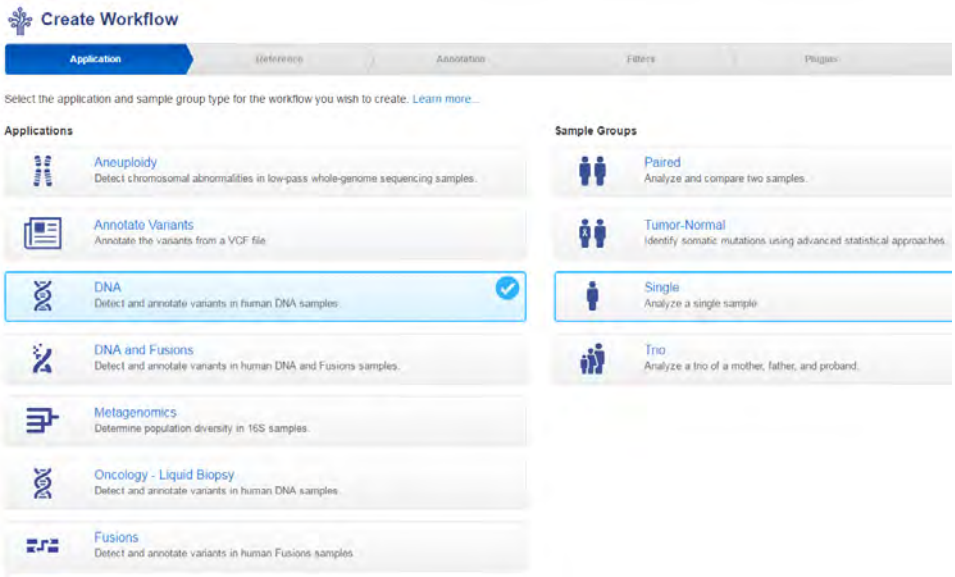
The available applications are:

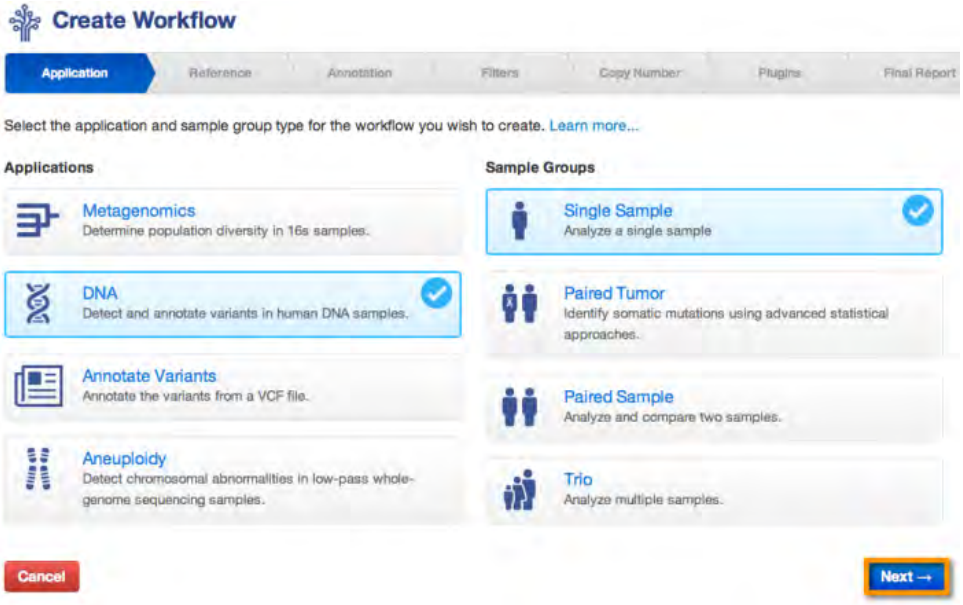
Application	Description
Aneuploidy	Detect human chromosomal large structural abnormalities in low-pass whole-genome sequencing samples
Annotate Variants	Annotate the variants from a VCF file
DNA	Detect and annotate variants in human DNA research samples
DNA and Fusions	Detect and annotate variants in human DNA and Fusions samples
Metagenomics	Determine population diversity in polymicrobial samples using detection of 16S gene variable regions
Oncology-Liquid Biopsy	Detect and annotate variants in human samples
Fusions	Detect and annotate gene fusions in human DNA research samples

The available sample group types are:

Sample group	Description	Application(s)	
Paired	Analyze and compare two samples	DNA	
Tumor—Normal	Identify somatic mutations using advanced statistical approaches	DNA	
Single	Analyze a single sample	Aneuploidy, Annotate Variants, DNA, Fusions, DNA and Fusions, Metagenomics, Oncology-Liquid Biopsy	
Trio	Analyze a trio of a mother, father, and proband.	DNA	
Single Fusions	Analyze a single Fusions sample	Fusions	
Single/Multi	Determine the microbial diversity of a 16S sample in one or more samples	Metagenomics	

1. Select the application type and sample group type for your new workflow.





The **Next** button appears after you select your sample group type.

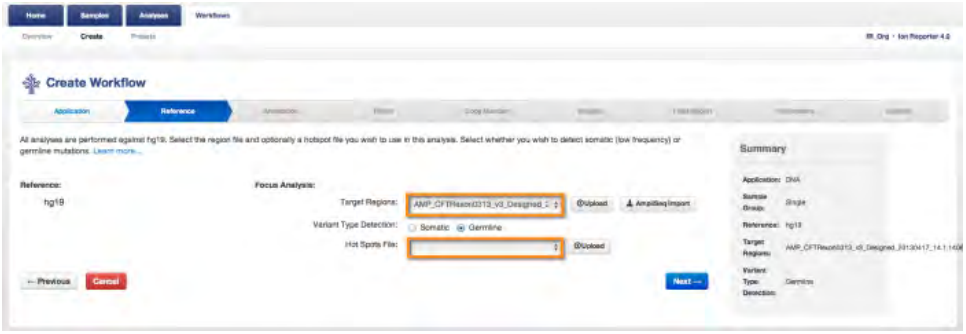
2. Click the **Next** button.

Select a Reference

For Aneuploidy, Annotate Variants, DNA and DNA and Fusions workflows

Ion Reporter™ Software offers hg19 and GRch38 references for human-scale DNA workflows. For DNA applications, you also select files for the target regions and hotspot regions for use in the analysis. For DNA and Fusions workflows, you select a fusions file.

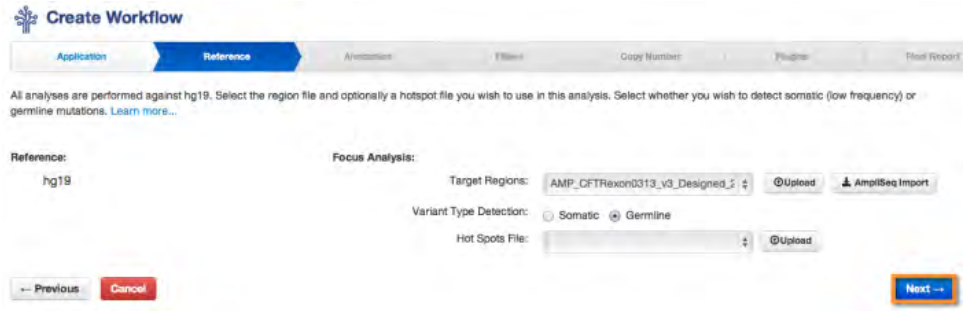
1. Select the files that you wish to use for the regions / hotspot files using the drop-downs.



The names of the files you select or upload are added to the Summary.

2. For single sample DNA workflows, select either **Somatic** or **Germline** variant detection type.

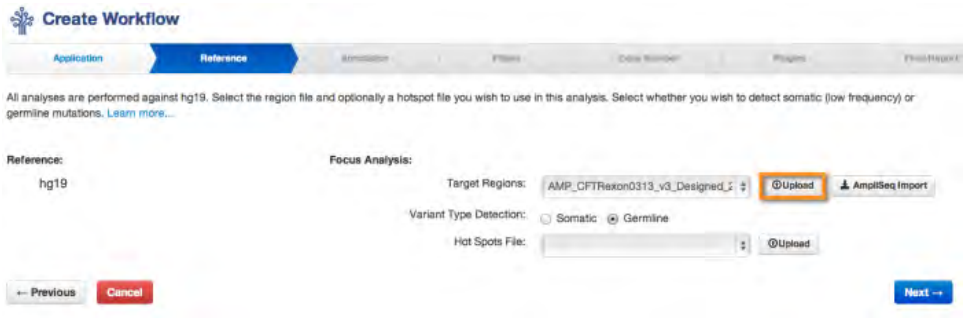
3. Click the **Next** button. (The Next button appears after you make your Variant Type Detection selection, either Somatic or Germline.)



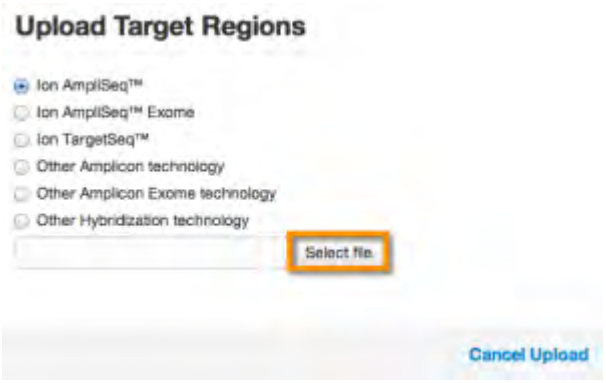
Target regions and hotspot files

If your regions or hotspot file does not appear in the drop-down list, you can upload a new one as follows:

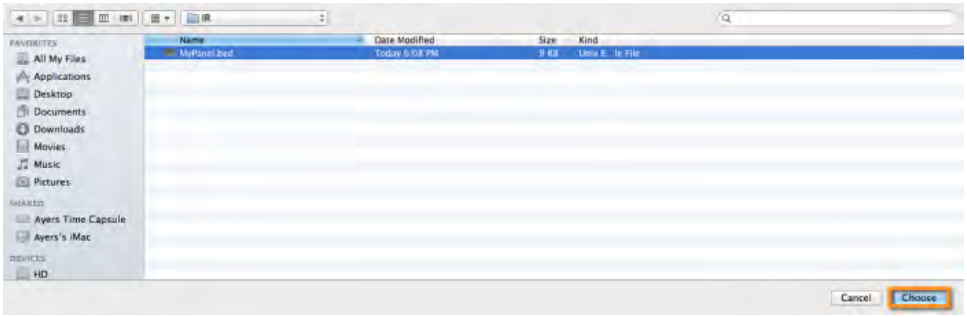
1. Click **Upload**.



2. Select the type of file you are uploading and click **Select file**.



3. Navigate to the folder containing your BED file, select it, and click **Select** or **Choose**.



4. Confirm that the correct filename appears in the display field. Click **Upload**.

5. Your BED file is uploaded and verified. When verification is complete, click **Close**.

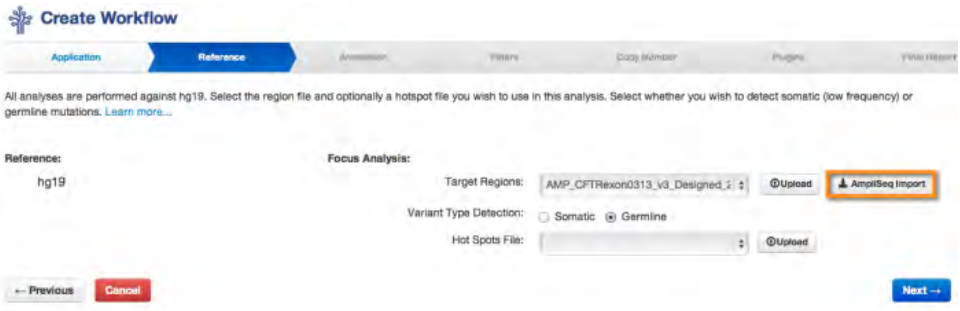
6. Your BED file is added to the list of files selectable in the drop-down for Target Regions or Hotspot Regions, depending on which type of file or files that you uploaded.

Import a design from AmpliSeq.com

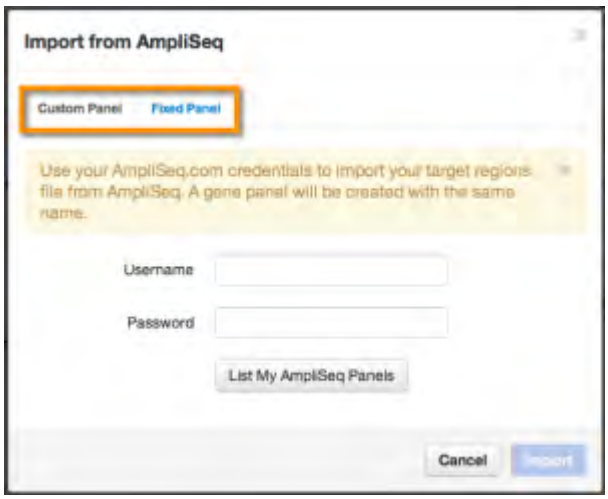
Use this process to import a target regions file that matches your custom design on AmpliSeq.com. You can also import a target regions file for a fixed panel, but target regions files for fixed panels typically are supplied with Ion Reporter™ Software.

To import a design from AmpliSeq.com, follow these steps:

1. Click the **AmpliSeq Import** button.



2. Select an import type, either: **Custom Panel** or **Fixed Panel**.



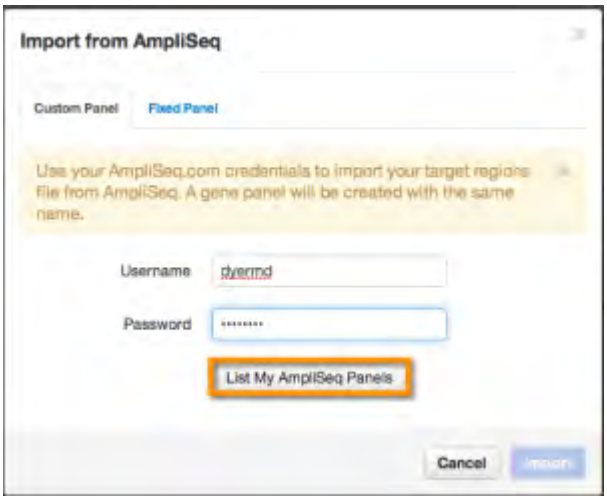
For a fixed panel:

- Select the panel that you want to import from the **Fixed Panels** list and then click **Import** .

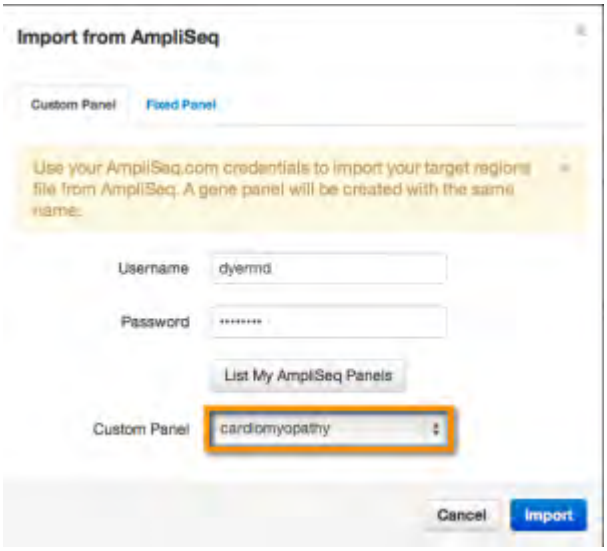
- Your BED file is added to the list of files selectable in the drop-down.

For a custom panel:

- Enter your user name and password and then click**List My AmpliSeq Panels**.



- Select the panel that you want to import from the drop-down box and then click **Import** .



- Your BED file is added to the list of files selectable in the drop-down.

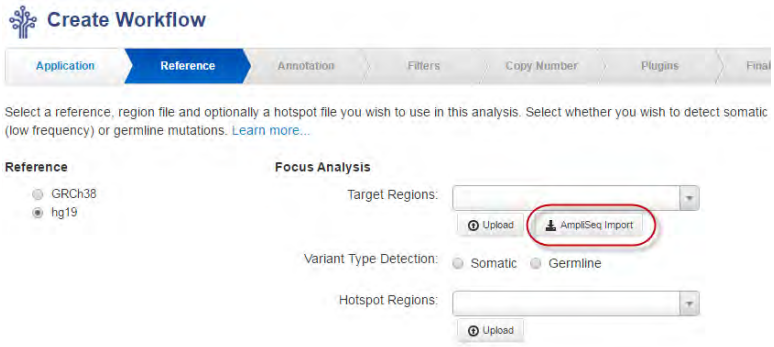
Import panel for Ion 540™ Chip analysis

To analyze data from an Ion 540™ Chip that was prepared using the Ion AmpliSeq™ Exome RDY S5 Kit, you must import the latest version of the ExomePanel_Hi-Q panel into Ion Reporter™ Software. You can download and import this panel from ampliseq.com as part of creating a workflow in the software.

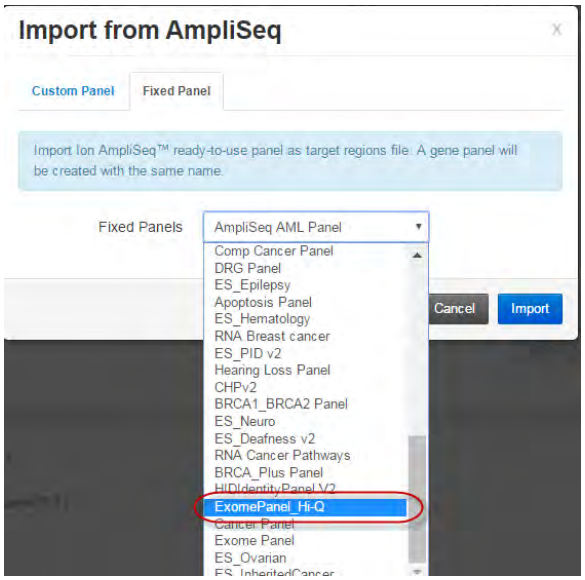
IMPORTANT! If your Ion Reporter™ Softwarehas an older version of the ExomePanel_Hi-Q target regions BED file, you must delete it and any workflows that use it before importing the new version. Older versions do not support Ion 540™ Chip analysis.

To import the ExomePanel_Hi-Q panel:

1. In Ion Reporter™ Software, go to **Workflows ▸ Create**.
2. In the **Application** tab, click **DNA**, click **Single**, then click **Next**.
3. In the **Reference** tab, click **AmpliSeq Import** below the **Target Regions** dropdown list.

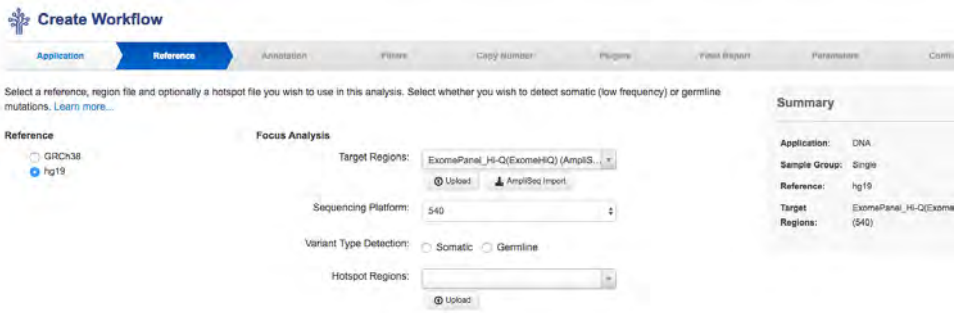


4. In the **Import from AmpliSeq** dialog, select the **Fixed Panel** tab, then select **ExomePanel_Hi-Q** from the list. Click **Import**.



Note: The import takes several seconds.

5. When the import is complete, the panel is listed in the **Target Regions** field. To proceed with workflow creation for Ion 540™ Chip analysis, select **540** for the Sequencing Platform, then select the remaining workflow settings.

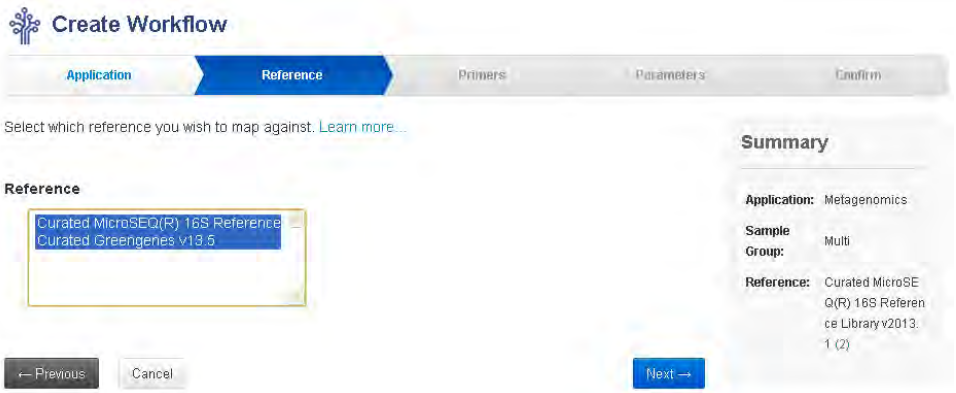


After import, the ExomePanel_Hi-Q panel will be available in the **Target Regions** dropdown list when you create subsequent workflows. The parameters associated with this panel will be listed in the **Parameters** tab, **Variant Finding** subtab.

Select references for metagenomics workflows

The metagenomics workflow provides access to two reference databases for mapping: the curated MicroSEQ™ ID database and curated GreenGenes database. If both databases are selected, data are first mapped against the curated MicroSEQ™ database. Next, reads that were not mapped against the curated MicroSEQ™ database are mapped against the curated **/GreenGenes** database.

1. Select one or more reference databases to map the data against.

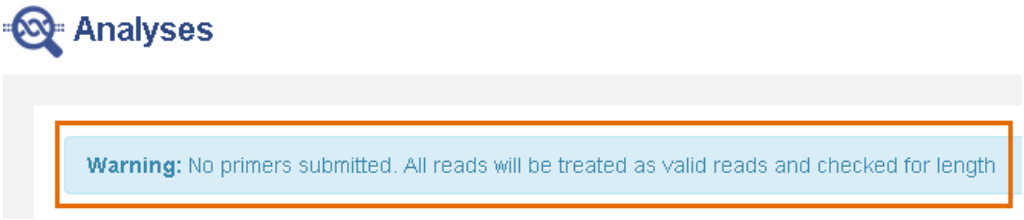


2. Click the **Next** button.

Select Primers for Metagenomics workflows

Note: In this release, the UI allows you to create a workflow that does not contain any primer information. However, we recommend that you always add primer information to your metagenomics workflow. When the primer information is

missing, no trimming is performed on your reads. A warning message appears during analysis review when primer information is missing.

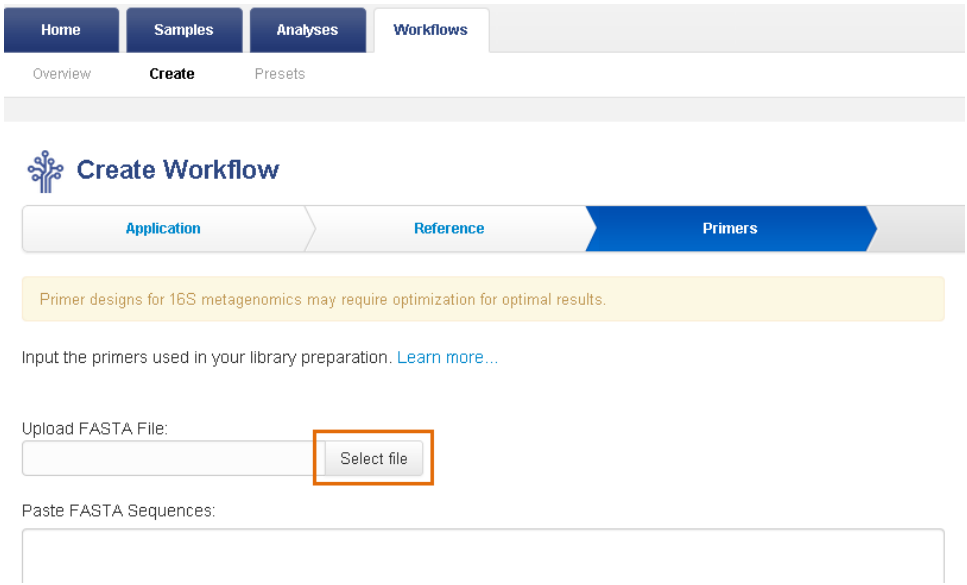


The Primers tab is only used for the metagenomics workflow. Here you either upload or paste in a set of primer sequences that were used to prepare your samples. If provided, these sequences are trimmed from the reads before mapping. The names of forward primers must end in "_f*" and reverse primer names must end in "_r*". Primers in a pair must otherwise have identical names so that the software can match them up during the analysis. An example of a FASTA formatted file is provided below:

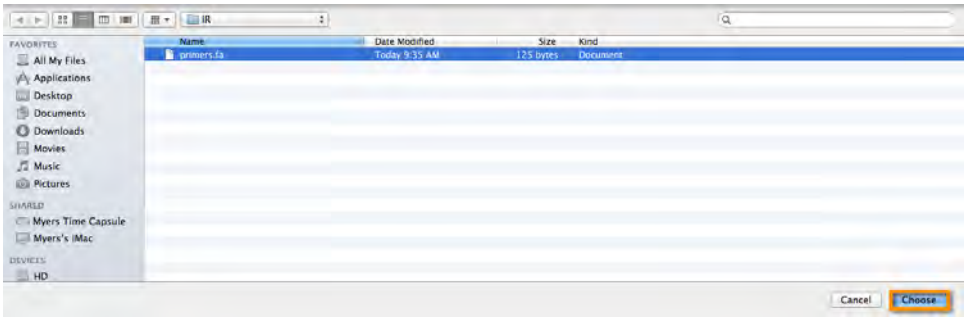
```
>MyFavoriteV5_forward    ACTCGGTCCARACTGAGACT
>MyFavoriteV5_Rev     TTACCGRGGCGTATGCGG
>MyFavoriteV8_Fwd     CCARAACTCGGTCTGSGACT
>MyFavoriteV8_r     RGGCGTATGCSTACCGGG
```

To upload your FASTA file containing your primer sequences:

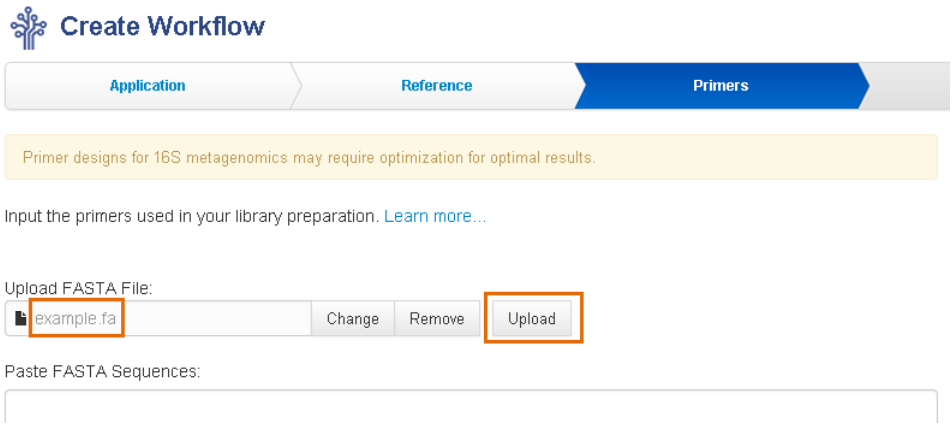
1. Click the **Select file** button.



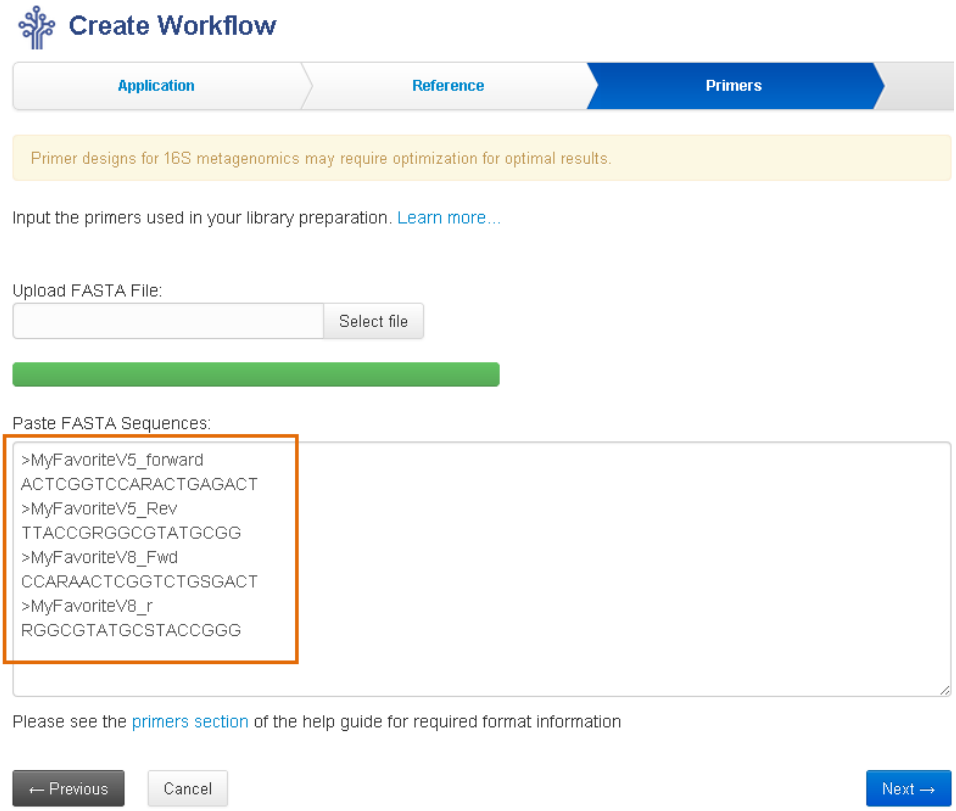
2. Navigate to the folder containing your FASTA file, select it, and click the **Choose**, **Open**, or **Save** button.



3. The Select button is replaced by Change, Remove, and Upload buttons. Ensure that the correct filename appears in the Upload FASTA File field, then click the **Upload** button.



4. Your primer sequences are uploaded and you can edit them if needed in the text window. Alternatively you can just paste in your primer sequences directly into the text box.

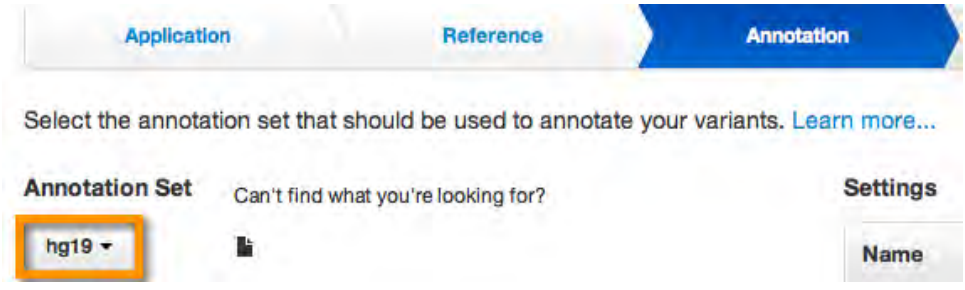


5. Click the **Next** button.

Select an Annotation set

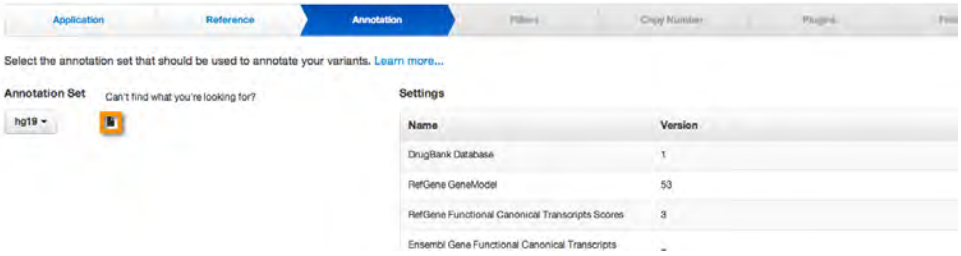
Annotation sets are the group of annotation sources that you want to attach to variants identified in your analysis. To select an annotation set:

1. Select the annotation set you wish to use from the drop-down box.



2. The annotation sources contained in the annotation set are shown.

3. To create a new annotation set, click the **New** icon.

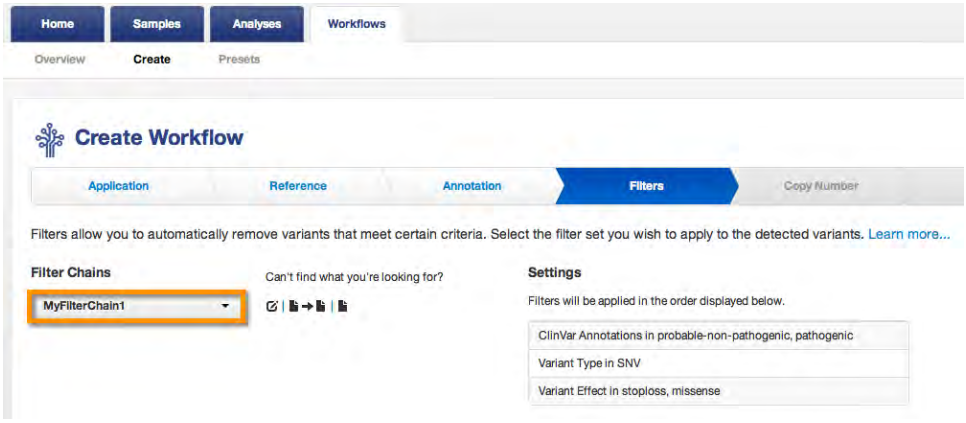


4. Click the **Next** button.

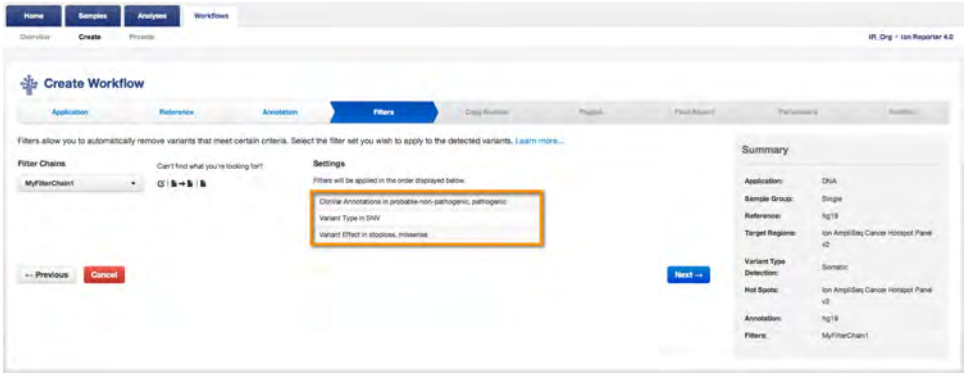
Select a Filter Chain

Filter chains are a set of filters that you want to apply to variants identified in your analysis. To select a filter chain:

1. Select the filter chain of your choice from the **Filter Chains** list.

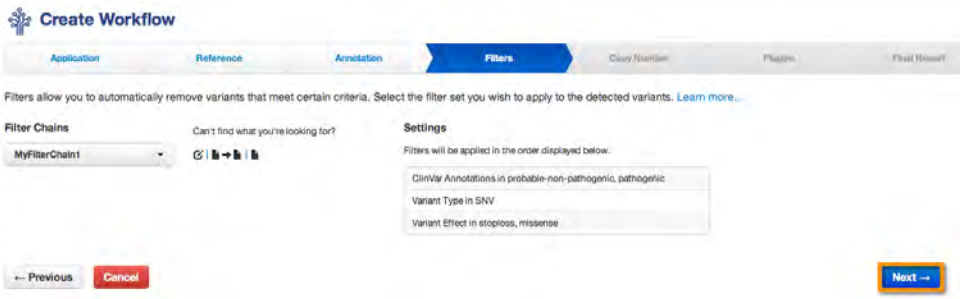


2. The filters contained in the filter chain are shown.



3. To create a new filter chain click the **New** icon.

4. Click the **Next** button.

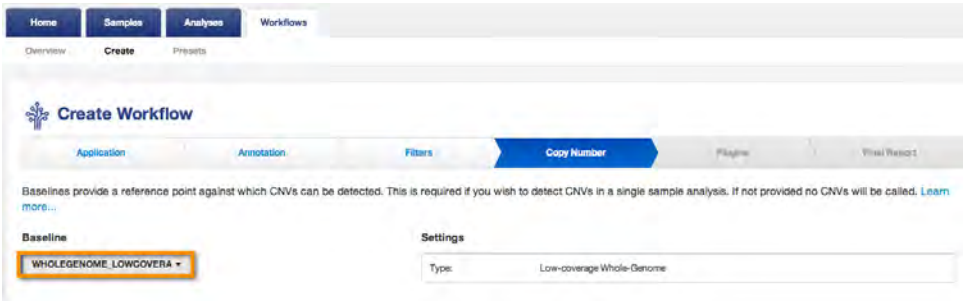


You can change or modify the filter chain applied to your analysis from the variant review screen.

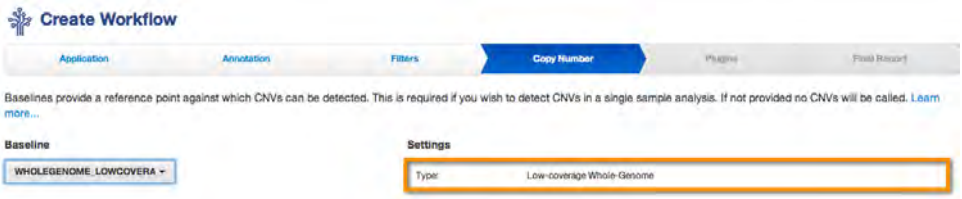
Select a Copy Number baseline

The Copy Number chevron is only present in the aneuploidy and single sample DNA workflows. This is where you can select a copy number baseline to use when detecting CNVs. You are only shown baselines that were created using the same regions file selected in the Reference chevron of the workflow creation process. The selection **Do not call CNVs** causes the CNV calling step of the workflow to be skipped during analyses. To select a baseline:

1. Select the baseline you wish to use from the drop-down box.



2. The settings for this baseline are displayed.



3. Click the **Next** button.

See CNV baselines in the workflow presets section for information on how to create your own CNV baseline.

Allele-specific (Proper) view of variants

When you set workflow parameters, you can select variants per position, **Classic**, or variants per allele, **Proper**.

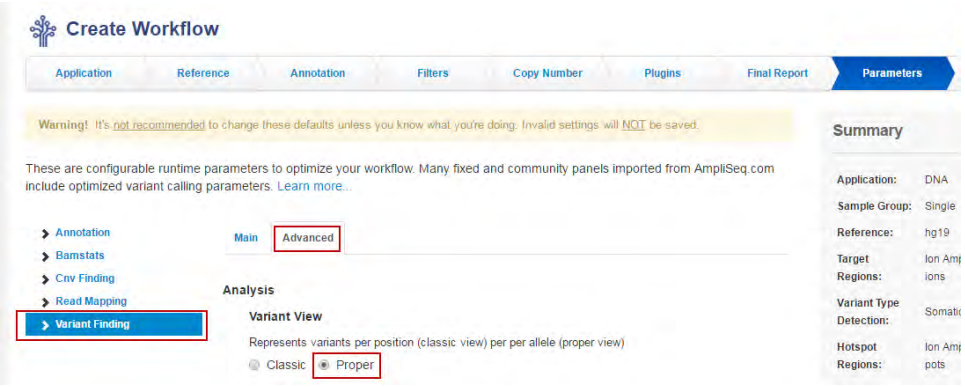
The default variant output in Ion Reporter™ Software is a *Classic* view (locus-centric), with each line of the VCF file corresponding to one line of Ion Reporter™ Software variant output. In **Classic** view, there can be multiple alternate alleles for a given locus. Ion Reporter™ Software also offers a *Proper* view output feature which is "allele-centric" with each allele displayed as a single line of record in the output (Per-allele view). The VCF file that is generated by *Classic* view is called "Classic VCF". The VCF file that is generated by the *Per allele* view is called "proper VCF".

The FDP (Flow Evaluator read depth at the locus) and DP (Total read depth at the locus) are subfields inside the INFO field of the VCF file. The values of these two fields remain the same for both the "classic VCF file" and the "proper VCF file". These two fields, FDP and DP, are based on the TOTAL SUM of ALL the FAO (Flow Evaluator Alternate allele observations) and AO (Alternate allele observations) fields, which are based on the following formulas:

"FDP=SUM(FAO)+FRO or DP=SUM(AO)+RO"

Note: In Ion Reporter™ Software 5.6, the proper VCF file includes values of the FDP and DP fields that remain the same, although each allele is displayed as a single line of record in the proper VCF file. In a future version of Ion Reporter™ Software, since these fields do not really apply to per-allele view, the values of these two fields might be replaced with missing values "." in the proper VCF file.

1. When creating or modifying a workflow, in the Parameters screen, select **Variant Finding ▸ Advanced**.
2. Under Variant View, select **Proper**.



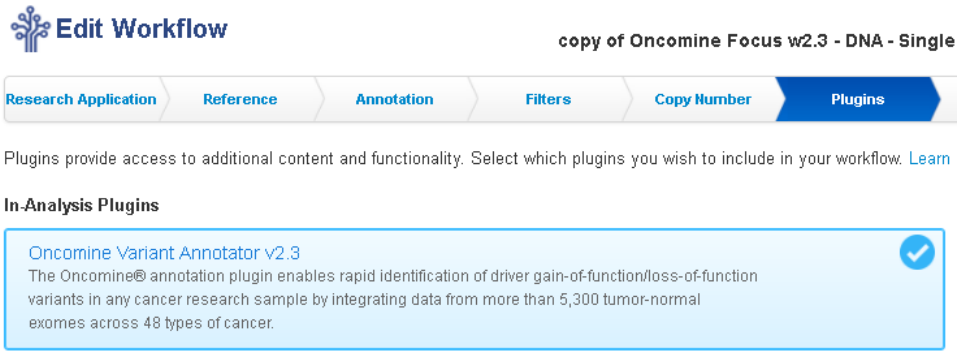
Add plugins to a workflow

Plugins provide additional functionality and content to the workflow. In the Plugins step of the workflow bar, you can optionally select an in-analysis plugin to include in your workflow.

Note: Functionality for the Oncomine Variant Annotator plugin is highly dependent upon the panel used for the assay, so only data from supported panels should be run through workflows that contain this plugin.

1. To select an in-analysis plugin to include in your workflow, click anywhere on its logo, title, or text. The plugin box is highlighted and marked with a checkmark.

   Note: The Oncomine Variant Annotator plugin is selected by default for some Oncomine workflows.



2. Click **Next**.

You can also add in-analysis plugins when you launch an analysis and post-analysis plugins on the variant review page.

Final Report

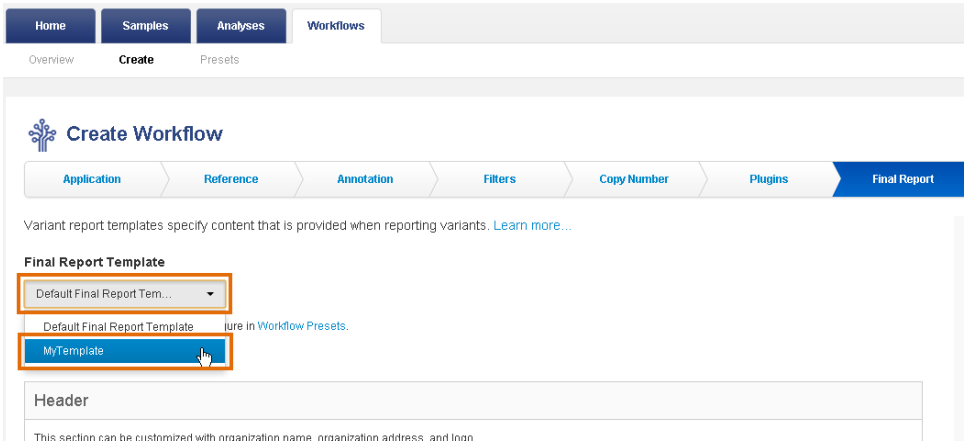
For the annotate, aneuploidy, and DNA workflows, the final report provides a mechanism to summarize the data for a set of variants selected by the user. The report contains a number of sections, which are described in this table.

Section	Description
Header	Contains date and time information and can be customized with a logo.
Background	Contains summary text describing the purpose of the report generated by the workflow.
Analysis information	Provides summary information about the analysis that was run, including the Ion Reporter™ Software version, the user who imported the data, the user who analyzed the data, and more.

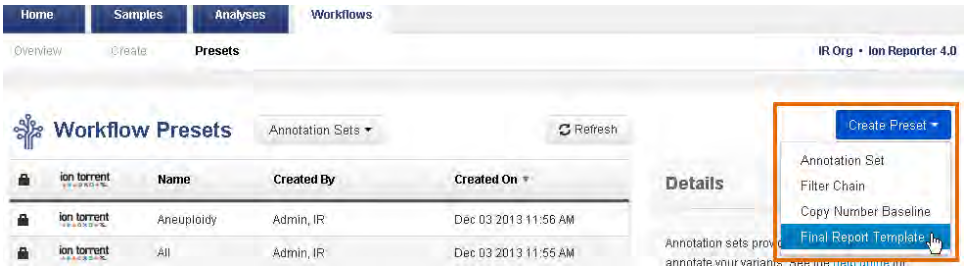
Section	Description
Sample information	Provides detailed information about each sample: the chip type, file path, bar code, sample name, library preparation, gender, chip ID, device ID, sequencing run type, sample ID, and relationship.
Reported variants	Lists the variants that were reported and their classification, gene, and locus information.
Variant details	Provides detailed information about each variant along with annotation and comment information.
Comments	Provides a mechanism for the user to give analysis specific comments to include in the final report
Sign-off	Provides signature lines for a configurable set of persons identified by their name and title.
Disclaimer	Provides a customizable legal disclaimer that is placed at the end of the report.

You can view an example final report. To select a report template:

1. Select the report template you wish to use from the report template drop-down menu.



2. The settings for the report template are displayed under the Sections area.

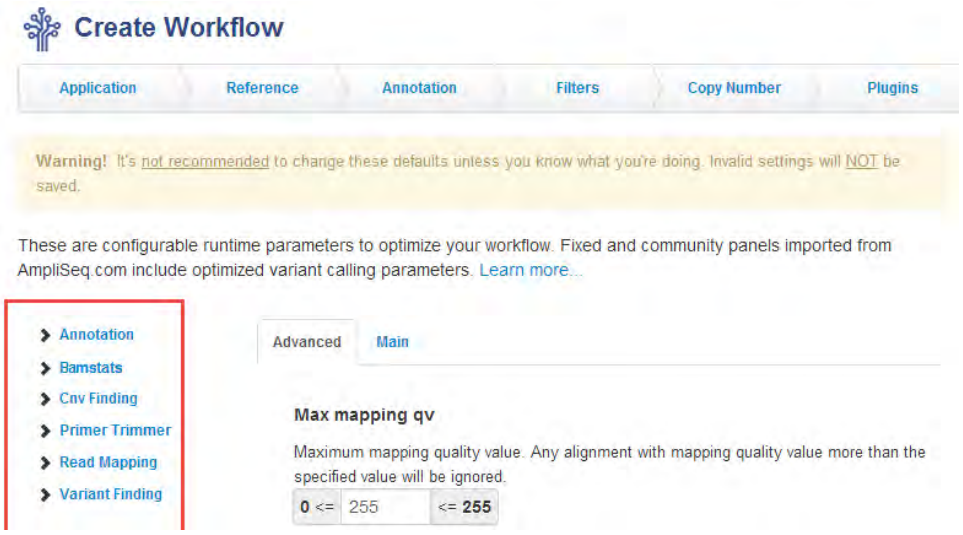


3. To create a new report template click the **New** icon.
4. Click the **Next** button (at the bottom of the page).

Accept default or change Parameters

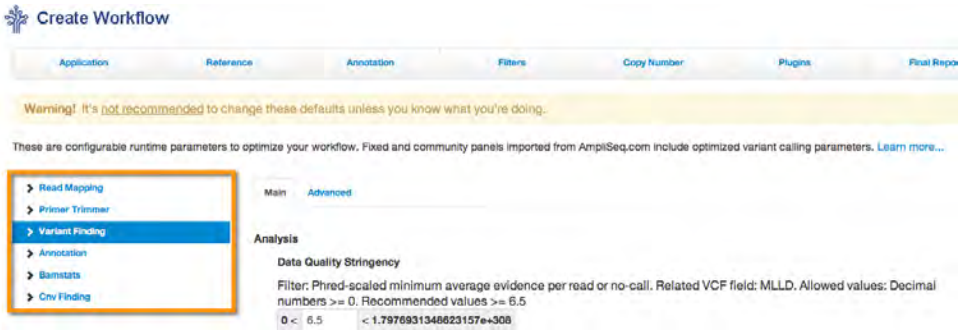
The Parameters chevron is where you can modify any of the run time parameters. It is always recommended to **use the default settings** unless you know what you are doing.

The modules that you can customize vary by workflow. Example:



Follow these steps to change any settings:

1. Select the parameter section on the left and modify the parameters as needed.



2. Click the **Next** button.

QC report metrics and thresholds

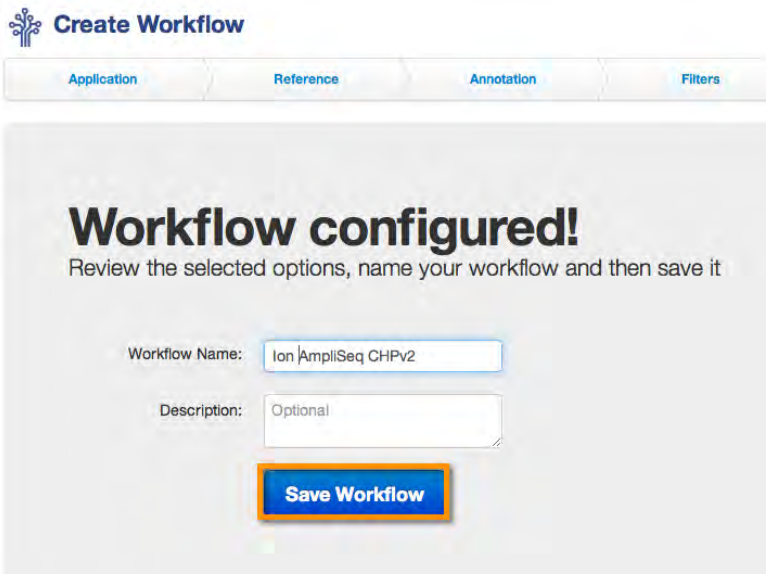
QC report settings can be customized in the Bamstats module. Bamstats parameters are described in the Create Workflow wizard Parameters chevron. Click **Bamstats** in the module list on the left.

There are also QC metrics not present in the QC report: MAPD for Copy Number and Total Mapped Fusion Panel Reads for fusions.

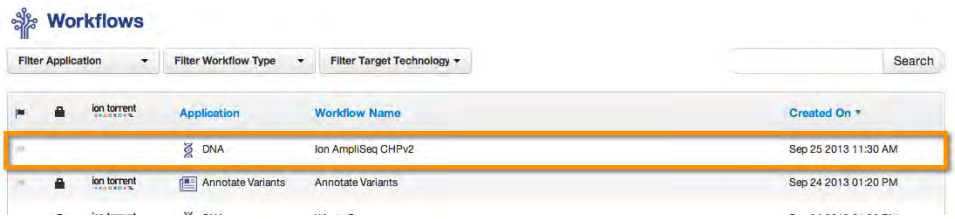
Confirm and create workflow

The Confirm chevron is the last step in the workflow creation process. To confirm and create your workflow:

1. Provide a descriptive name for your workflow.
2. Optionally provide a description for your workflow.
3. Click the **Save Workflow** button.



4. Your workflow is saved and is listed in the workflows table.

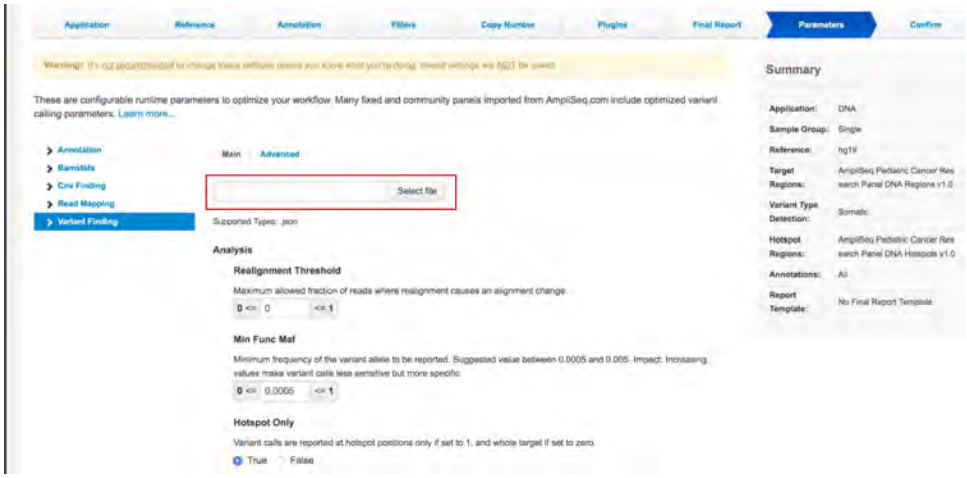


Import custom TVC parameters

If you would like to use custom Torrent Variant Caller variant finding parameters in Ion Reporter™ software, export the parameters JSON file from Torrent Suite™ software. Then, import this file from the Parameters screen when creating a workflow.

1. Export the TVC parameters from Torrent Suite™ software and store the JSON file on your computer or network.

2. Begin building your workflow in Ion Reporter™ software and on the **Parameters** tab, select **Variant Finding**.

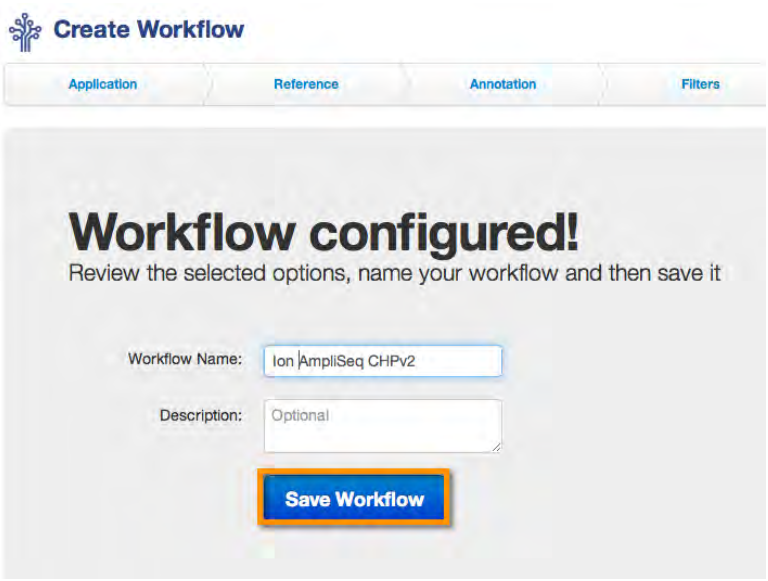


3. Click **Select File** and browse to your TVC parameters JSON file and click **Import**. The TVC parameters replace the default settings. Error messages will appear if any of the imported parameters are out-of-range or if a JSON file with an incompatible format was imported.

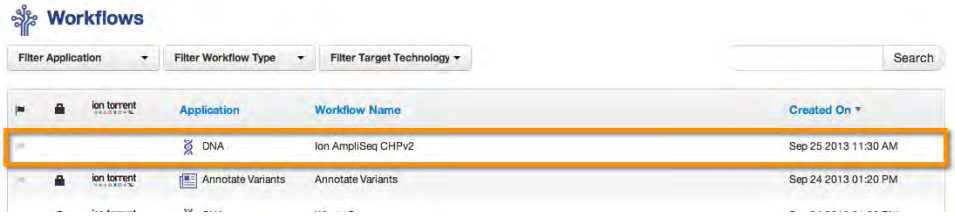
Confirm and create workflow

The Confirm chevron is the last step in the workflow creation process. To confirm and create your workflow:

1. Provide a descriptive name for your workflow.
2. Optionally provide a description for your workflow.
3. Click the **Save Workflow** button.



Your workflow is saved and is listed in the workflows table.

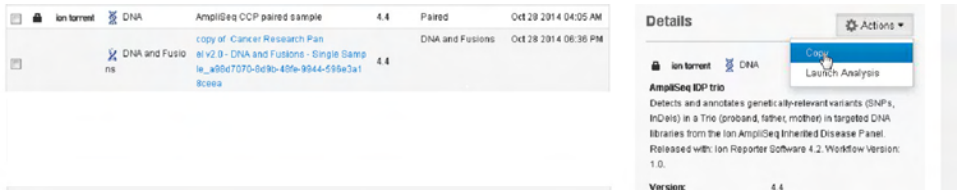


Copy and edit a workflow

To save time, you can copy workflows for reuse.

1. Go to **Workflows ▸ Overview**
2. Search for the workflow you want to copy.

3. Select workflow and click **Actions** ▸ **Copy**. This launches you into the editing mode for the selected workflow.



4. Click **Next** button to advance through Reference, Annotations, Filters, Plugins, Final Report, and Parameters chevrons to accept default settings.

5. On the Confirm page, name the workflow and click **Save Workflow**.

Note: For metagenomics workflows, you cannot view the proprietary primer details of an Ion default metagenomics workflow when you copy the workflow.

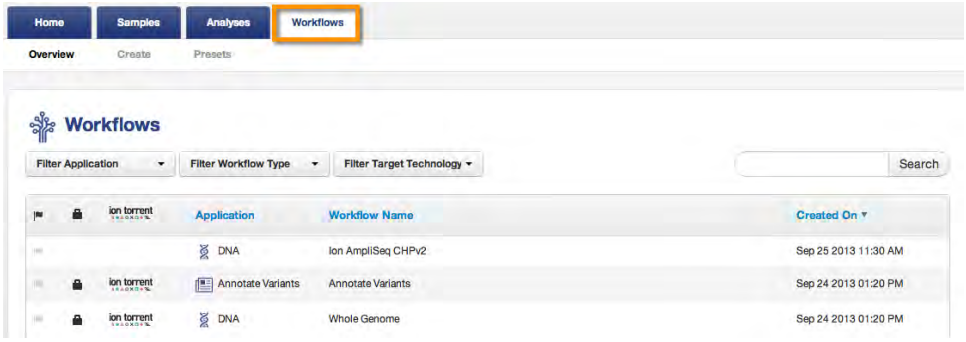
Edit custom workflows

You can edit a workflow to change the settings or parameters associated with the workflow.

Note:

- You cannot edit preinstalled workflows and locked workflows. Once a workflow is locked, that action can not be undone.
- To edit a workflow created in a version of Ion Reporter™ Software earlier than version 5.2, you must select a MyVariants database in the Annotation chevron to proceed.

1. In the **Workflows** tab, select the workflow that you want to edit in the Workflows list, then click **Edit** in the **Details** section.



2. Proceed the workflow wizard as described in "Create a custom workflow with the Ion Reporter™ wizard" on page 86.

Workflow presets

Workflow presets are the components that are used in creating a workflow. The following list describes the types of workflow presets:

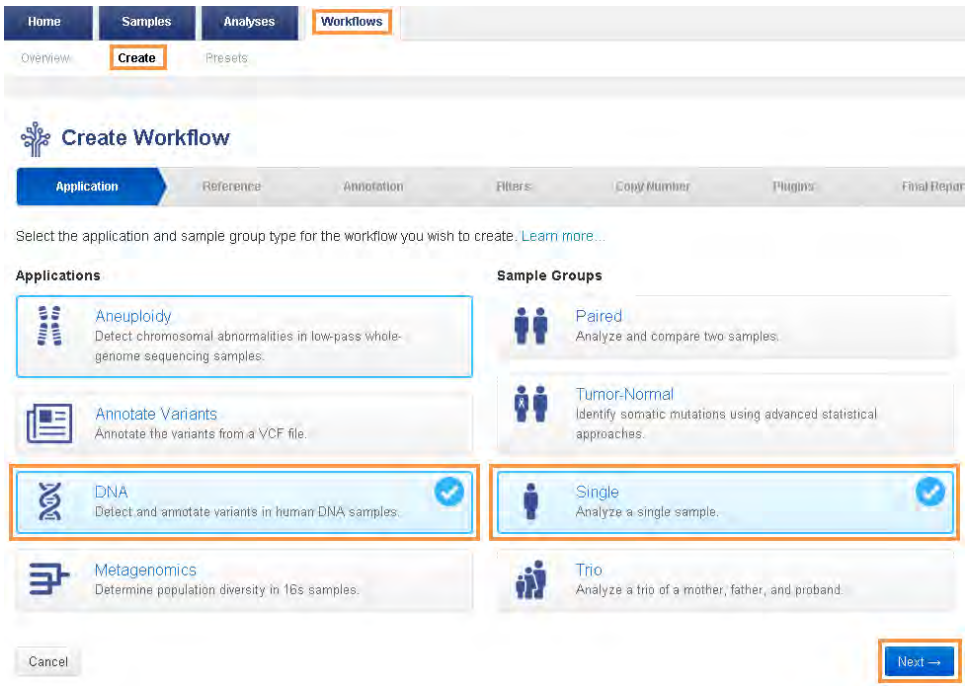
Preset type	Description
BED files	Files that are accessible for selection in the Reference chevron of creating a workflow. BED files are used as the following: • Target regions files, which restrict analysis to only regions specified in the file • Hotspot files, which cause the hotspot positions to be listed in the variants table, even if a variant is not called at those positions
Annotation sets	Set of annotation sources to apply to variants for selection in the Annotation chevron of creating a workflow.

Preset type	Description
Filter chains	Set of filters to apply to variants for selection in the Filter chevron of creating a workflow.
CNV baselines	Set of control samples that are used to create a baseline for detecting CNVs. The baselines are accessible in the Copy Number chevron when you create a workflow.
Report templates	Final report templates that are accessible for selection in the Final Report chevron of creating a workflow.

Create a Target regions BED file workflow preset

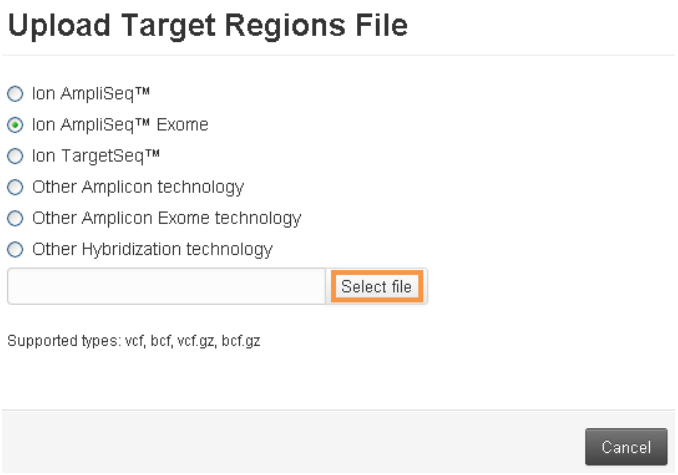
IMPORTANT! Use only BED file names that do not contain spaces. If a file name includes spaces, the analysis fails.

1. In the **Workflows** tab, click **Create**.
2. In the **Research Application** column, click **DNA**. Click any type of sample group.

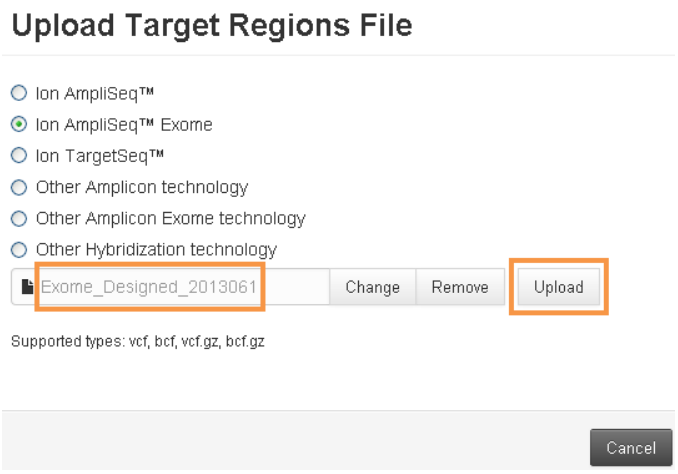


3. In the Reference chevron, in the Target Regions section, click **Upload**.
4. In the **Upload Target Regions File** dialog:
   a. Select your library type or technology.

b. Click the **Select File** button, then browse to your BED file.



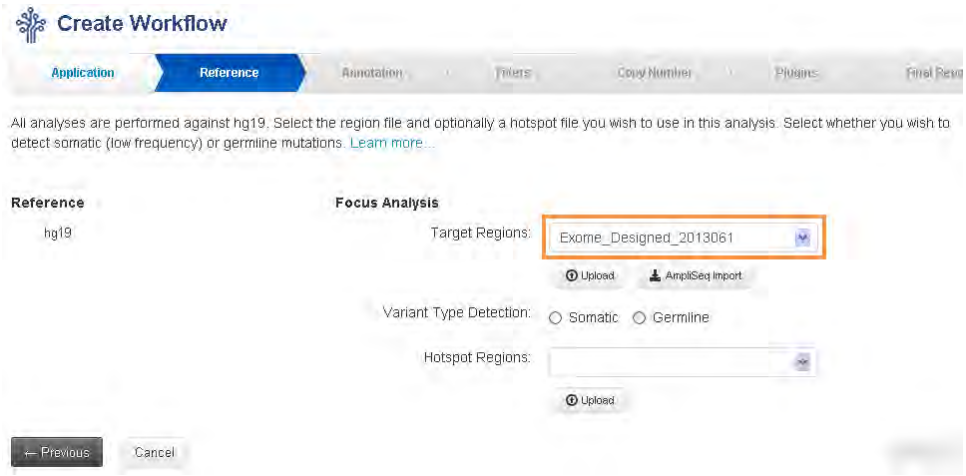
c. Ensure that the correct BED file name appears in the display field and click **Upload**.



Ion Reporter™ Software uploads and verifies your BED file. When verification is complete, the progress bar changes to green and you see the "available for use" message.

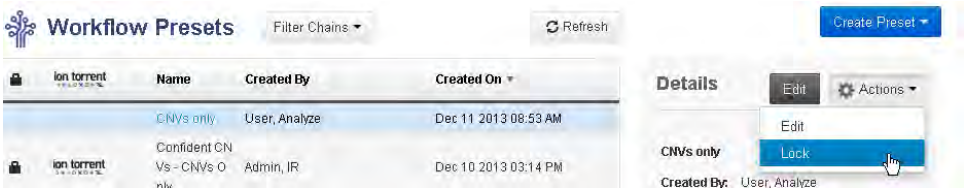
d. Click **Close** .

The new BED file preset appears in the **Target Regions** section of the References chevron.



5. You can now upload additional BED files, continue to create a workflow, or cancel the workflow creation.
   If you go to the **Workflows** tab, then click **Presets**, then select **Annotation Sets ▸ Target Regions Files** you see your new BED file that is listed in the **Workflow Presets** table.

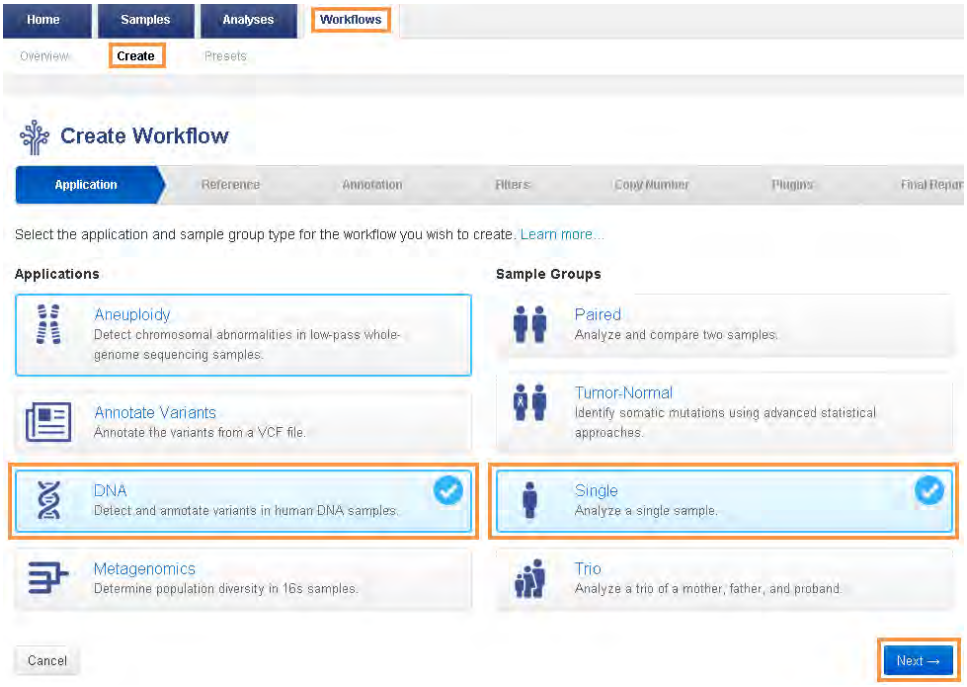
6. *(Optional)*When your preset works as you intend, then is ready for use in production, select the preset in the **Workflow Presets** table, then click **Actions ▸ Lock**to lock the workflow. You cannot undo a lock action.



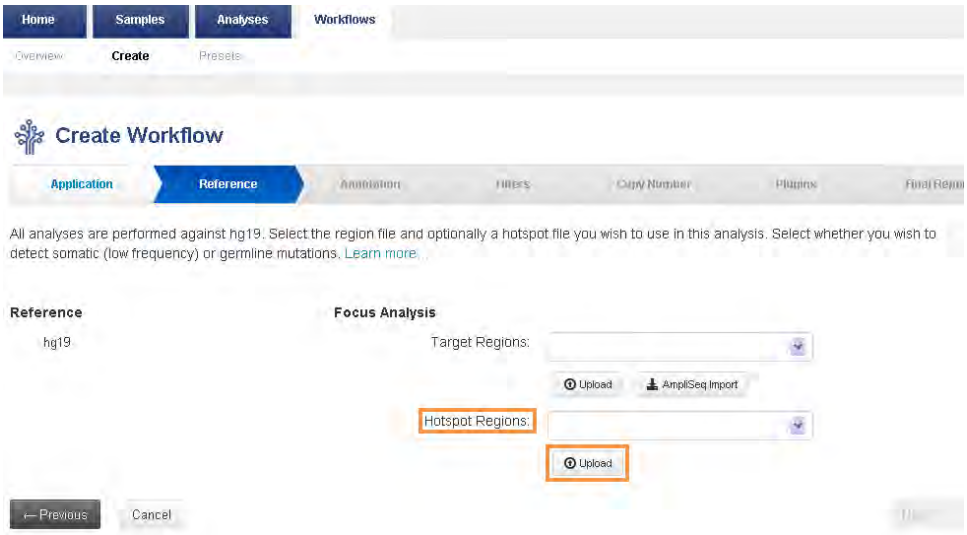
Create a Hotspot regions BED file preset

IMPORTANT! Use only BED file names that do not contain spaces. If a file names include spaces, the analysis will fail.

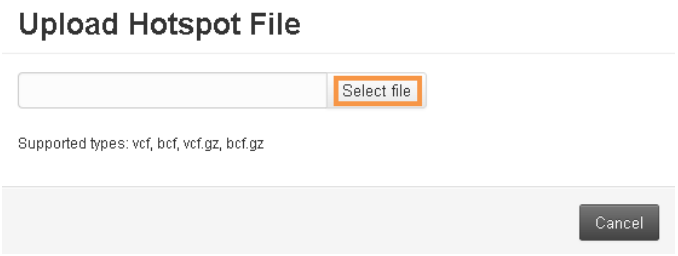
1. In the **Workflows** tab, click **Create**.
2. In the **Research Application** column, click on **DNA**. Click on any type of sample group.



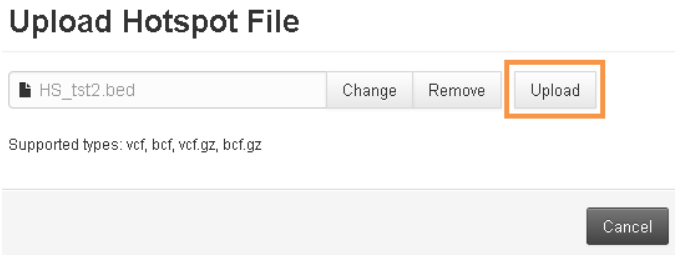
3. In the Reference chevron, in the **Hotspot Regions** section, click **Upload**.



4. In the Upload Hotspot File dialog:
   a. Click **Select File** and browse to your BED file.



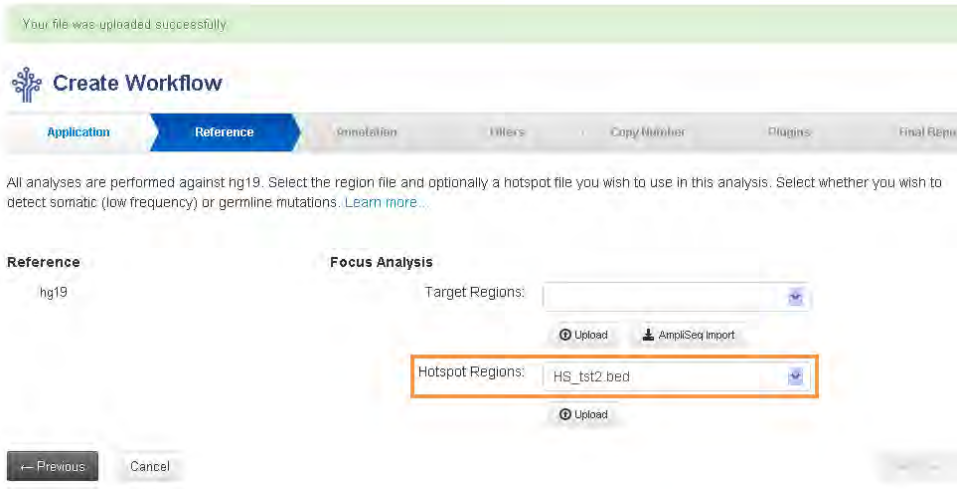
   b. Confirm that the correct BED file name appears in the display field and click **Upload**.



   Ion Reporter™ Software uploads and verifies your BED file. When verification is complete, the progress bar changes to green and you see the "available for use" message.

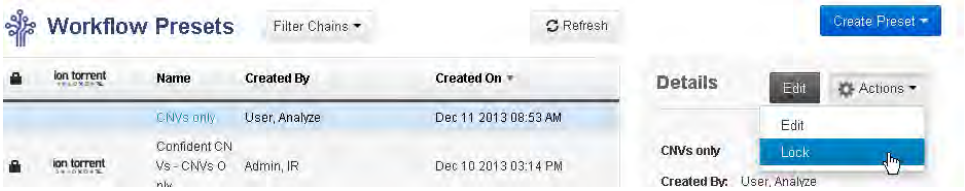
   c. Click **Close**.

The new BED file preset appears in the **Hotspot Regions** section of the References chevron.



5. You can now upload additional BED files, continue to create a workflow, or cancel the workflow creation.
   If you go to the **Workflows** tab, then click **Presets**, then select **Annotation Sets ▸ Hotspot Regions Files** you see the new BED file listed in the **Workflow Presets** table.

6. *(Optional)*When your preset works as you intend and is ready for use in production, select the preset in the **Workflow Presets** table and click **Actions ▸ Lock**to lock the workflow. You cannot undo a lock action.

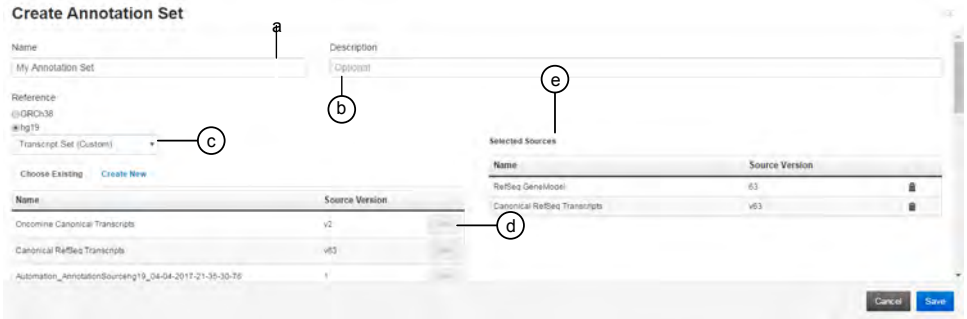


Create annotation set workflow presets

1. In the **Workflows** tab, click **Presets**.

2. Click **Create Preset ▸ Annotation Set**.

3. In the **Create Annotation Set** screen:



   a. Enter a descriptive name.

   b. *(Optional)* Enter a description of the annotation set.

   c. Select the annotation source that you want to add to the preset from the list.

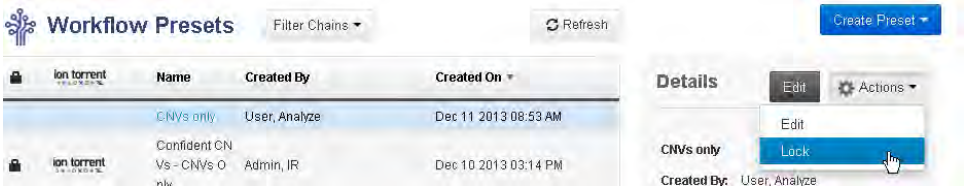
   d. Click **Use** next to the annotation source that you want to use.
   The source is added to the list of **Selected Sources** in your annotation set.

e. Repeat this process for each annotation source you want to add to your annotation set until your list of **Selected Sources** is complete.

Note: Ion Reporter™ Software includes two gene models: RefGene and Ensembl. When you select annotation sources, to use a factory-shipped canonical transcript set, you must use the compatible gene model. For example, use the RefGene gene model with Refseq canonical transcript set.

f. Click **Save** to save the annotation set.
The annotation set now appears in the **Workflow Presets** table and is available in the Annotation chevron when you create a workflow in Ion Reporter™ Software.

4. *(Optional)*When your preset works as you intend, then is ready for use in production, select the preset in the **Workflow Presets** table, then click **Actions** ▸ **Lock**to lock the workflow. You cannot undo a lock action.



View custom annotation sources

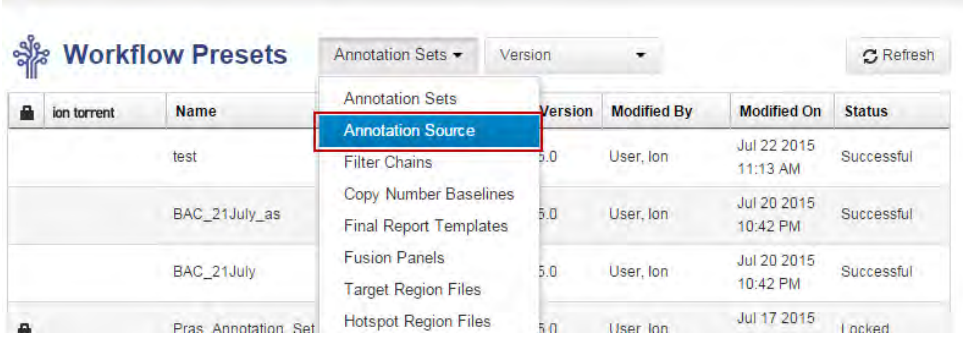
Annotation sets created by users are displayed in the Annotation Source dropdown list. See "Create annotation set workflow presets" on page 116 for details.

Note: For custom annotations that are very long, you can drag the column to the right to resize the content for easier reading.

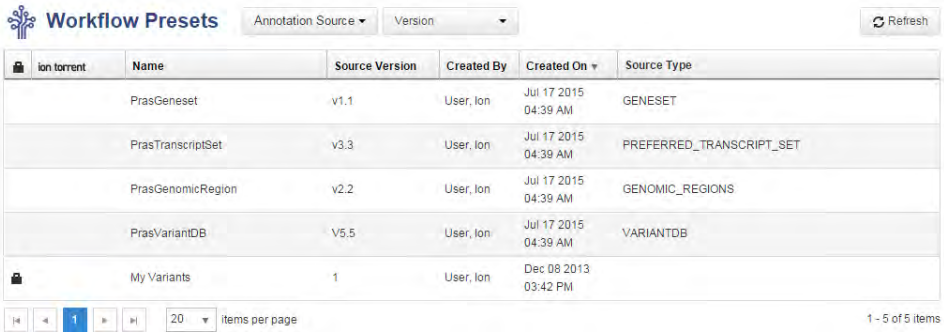
To view current list of custom annotation sets:

1. In the **Workflows** tab, click **Presets**.

2. Click **Annotation Set** ▸ **Annotation Source**.



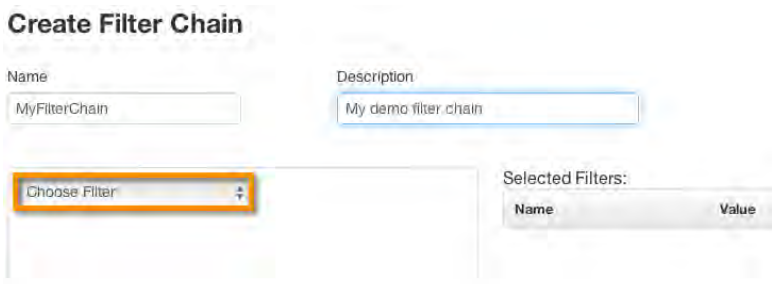
A table listing the custom annotation sources appears. You can use these custom annotation sources to create custom annotation sets.



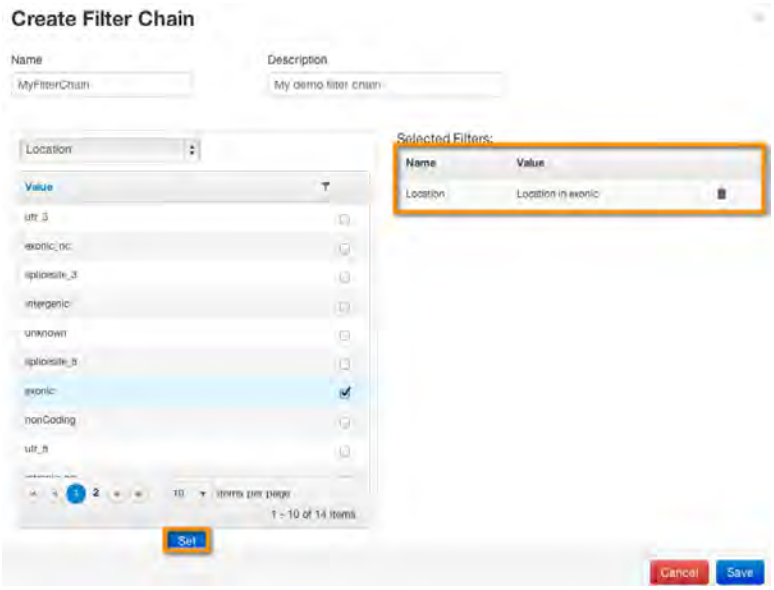
Create filter set workflow presets

1. In the **Workflows** tab, click **Presets**.

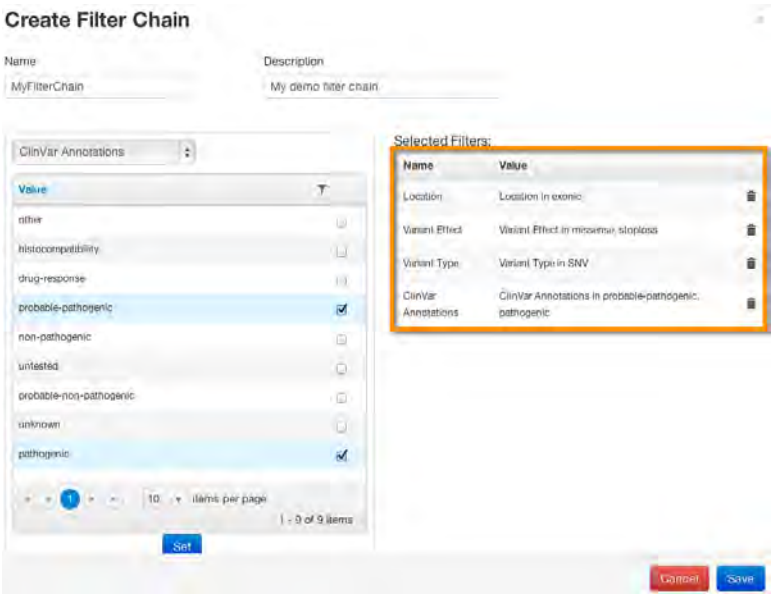
2. Click **Create Preset** ▸ **Filter Chain**.
3. Enter a meaningful name and description of the filter chain.
4. Select the type of filter you want to add from the dropdown list.



5. Click the **Set** button when you have configured it.



6. Repeat this process for each filter you want in your filter chain.



7. Click the **Save** button to save the filter chain.
   The filter chain now appears in the **Workflow Presets** table and is selectable in the Filters chevron of creating a workflow.

Create a Copy Number baseline

This section completes the instructions in Create workflow presets (annotation sets, filters, reports, and so on), for CNV baseline presets. These are used in the Copy Number chevron of creating a workflow.

Notes:

- Workflows that use baseline controls can provide better copy number detection (for both CNV variants and aneuploidy) than a paired sample workflow.
- In this release, use only male samples for creating an informatics baseline control.
- Copy Number estimates are made by counting reads for each amplicon, making adjustments to account for certain types of variability, comparing those read counts to expected counts for those amplicons in a "normal" sample and then making further changes.
- Known sources of variability include pool imbalance (when the assay has more than one pool of amplicons), total number of reads and 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.

To create a Copy Number baseline workflow preset, walk through the wizard and complete each tab (Baseline Type, Samples, and Confirm tabs). See also the notes about baseline samples, in the Samples tab section.

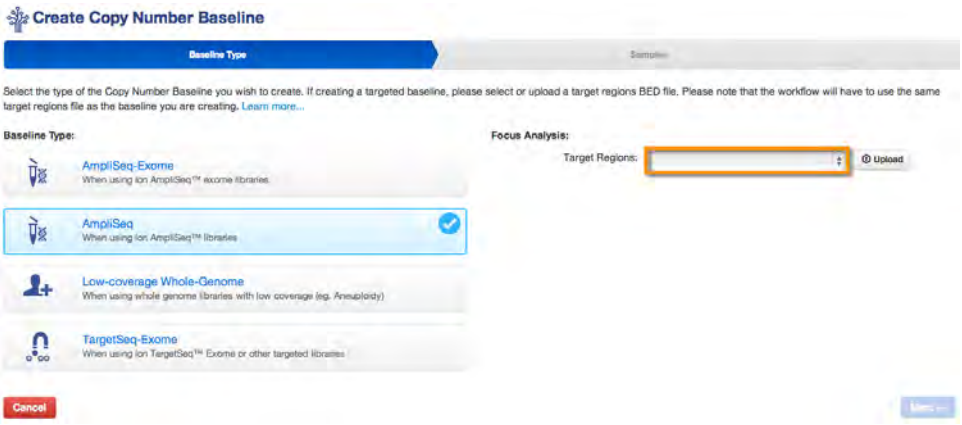
Configure Baseline Type

In the Baseline Type chevron, you select the type of baseline that you want to create and select the regions file that corresponds to panel you are using. You can create the following types of baselines:

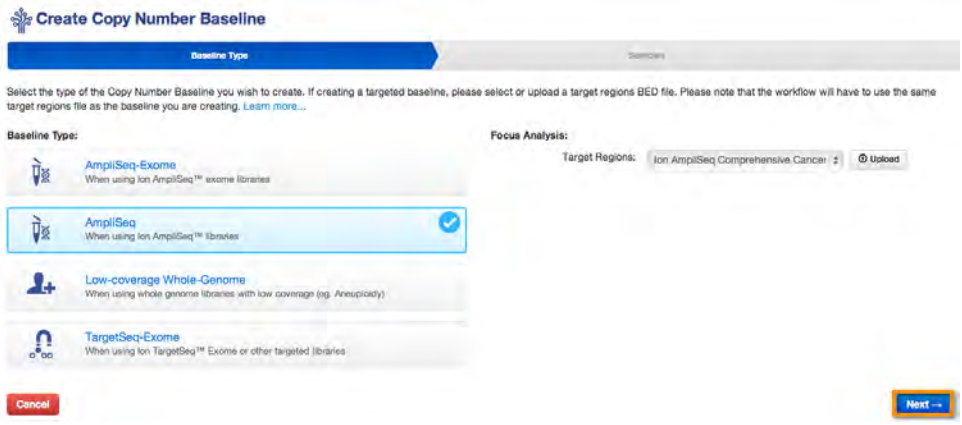
Type	Description	
AmpliSeq	Create CNV baselines when using Ion AmpliSeq™ libraries (not Exome).	
AmpliSeq-Exome	Create CNV baselines when using Ion AmpliSeq™ Exome libraries.	
TargetSeq Exome	Create CNV baselines when using Ion TargetSeq™ Exome or other targeted libraries.	
Low-coverage Whole-Genome	Create CNV baselines when using whole genome libraries with low coverage (e.g. Aneuploidy).	

To configure your baseline type:

1. Select the baseline type, then select the regions file from the dropdown list.



2. Click **Next**.



Select samples

In the samples chevron, select the samples to be used in your baseline creation, based on the following guidelines:

Samples that are used in CNV baselines:

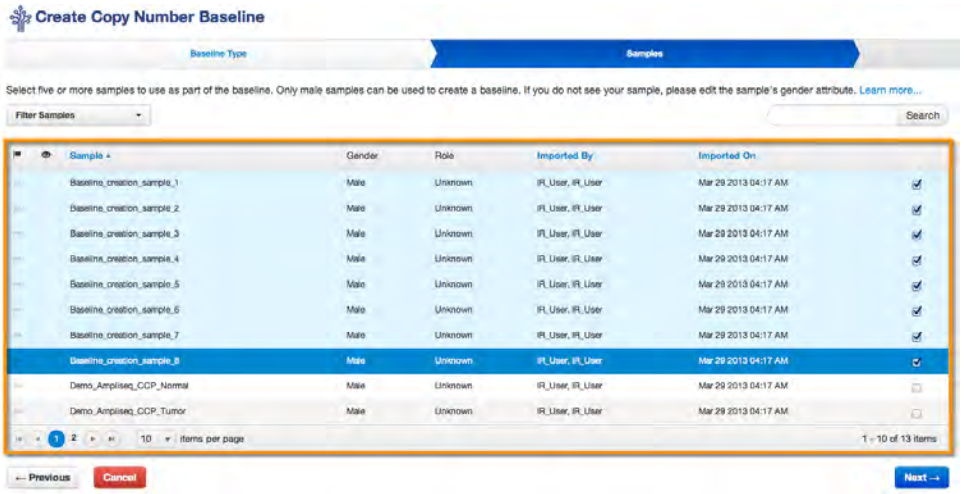
- Use only male samples in the baseline creation process in Ion Reporter™ Software 4.x.x.
- Although at least five samples are required in the baseline creation, ten or more samples are recommended for satisfactory results. (The use of more samples for baseline calculation substantially reduces sample-to-sample variance in coverage and results in fewer false positive calls.)
- Select control samples to add to your baseline which have no known CNVs in any region that is covered by the Ion AmpliSeq™ panel that is used, if possible. The presence of a CNV in your control samples can cause false positive CNVs to be reported for that region in your test samples.

Sample creation and gender assignment:

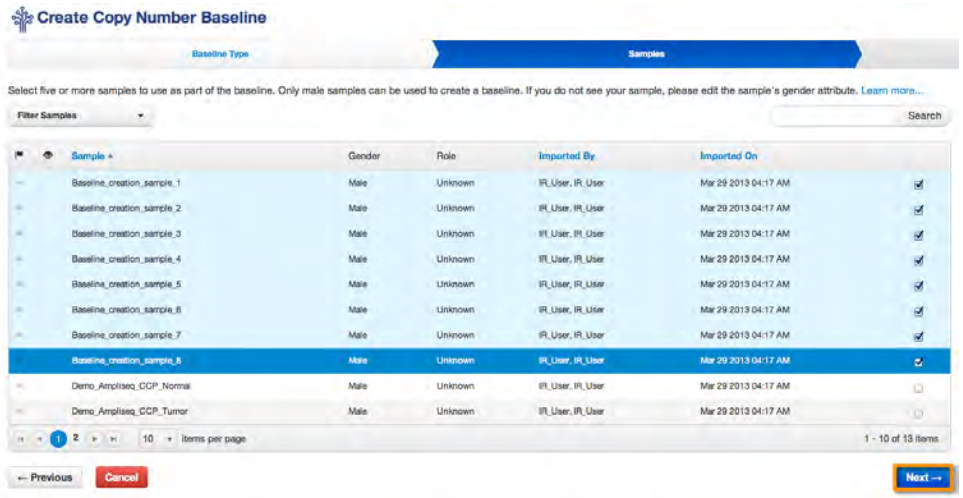
- If you do not see your samples in the table, see “Manage samples“ on page 43 for information on how to upload or define a sample.
- See “Gender information requirements“ on page 45 for details.
- Samples must have "gender" attribute set to "male" to be selectable when creating a baseline.

Follow these steps to select your samples:

1. Select all files for this baseline. The **Next** button is not active until you select at least five samples.



2. Click **Next**.

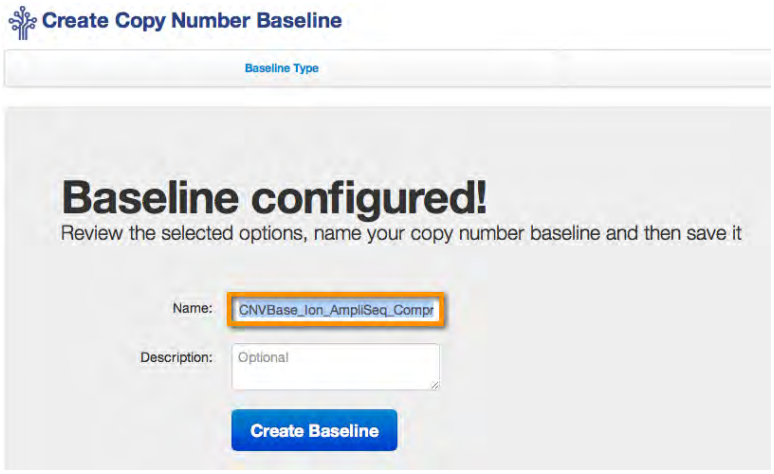


Confirm baseline

Use the Confirm chevron to name your baseline and review everything before you launch the baseline creation.

Follow these steps to finish your baseline and start the baseline creation analysis job :

1. Provide a descriptive name for your baseline.



2. (Optional) Provide a description for your baseline.
3. Click the **Create Baseline** button.
4. The baseline creation job is started and the baseline with its status now appears in your table. When the analysis completes, it is selectable in the Copy Number chevron of creating a workflow.

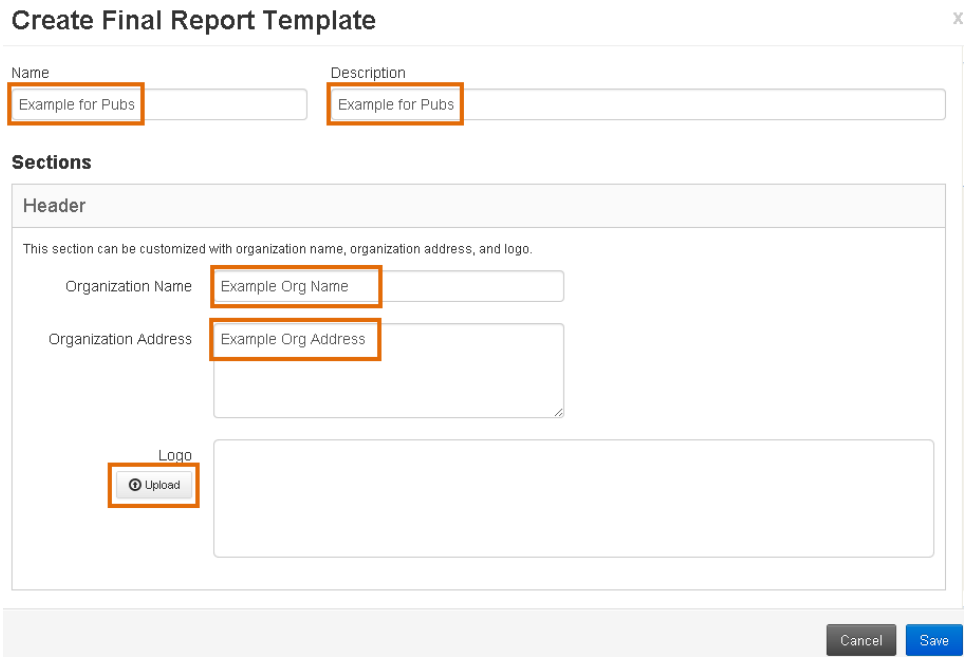
If you click a baseline in the table, the details are provided on the right.

Create a report template

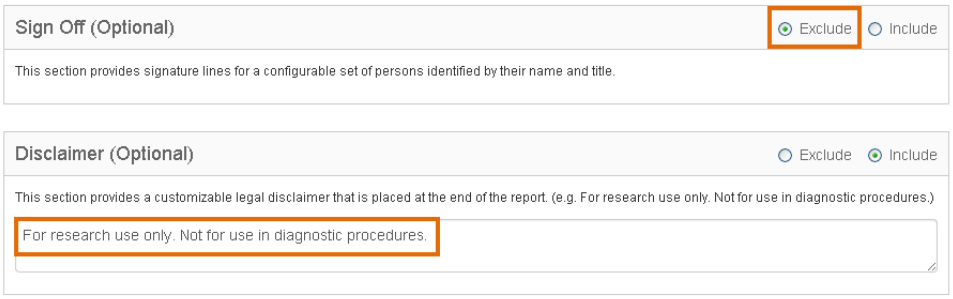
1. In the **Workflows** tab, click **Presets**.

2. Click **Create Preset** ▸ **Final Report Template**.
3. A skeleton report template opens.
4. At the top of the report template, enter a name and description for this template.

5. In the Header section, enter the organization name and address. Click the **Upload** button to add the organization's logo.



6. For each optional section Background, Sign Off, and Disclaimer), either enter the content for the section to contain or click the **Exclude** button for the section.



7. If you want the Sign Off section to list more than one required approver), click the **Add** button to add an additional approver.

8. Click the **Save** to create your report template preset.

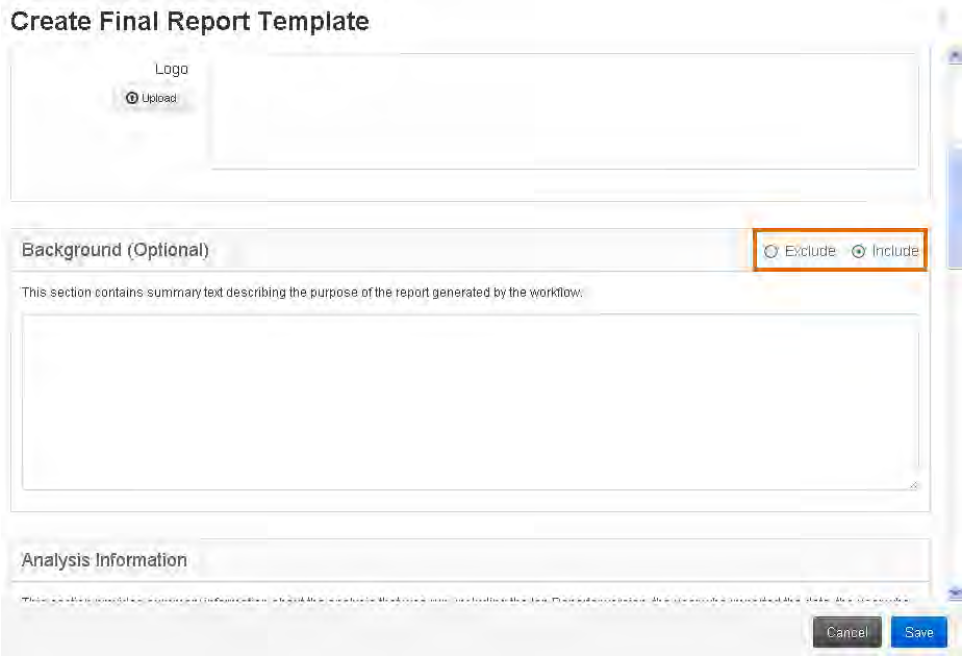
Fixed sections

The following sections always appear in every final report template. These sections are not optional and are not configurable. Ion Reporter™ Software generates the content for these sections for every analysis (in workflows that use this template).

- Analysis Information
- Samples Overview
- Reported Variants
- Variant Details
- Comments

Optional sections

The following sections are optional. You control whether or not each of these sections appears in the final report with Exclude and Include radio buttons.



The information that you enter in these sections is fixed and appears as-is for every analysis (in workflows that use this template).

- Background
- Sign Off
- Disclaimer

The information that you enter in the Header section also is fixed and appears as-is for every analysis (in workflows that use this template). You specify the content for the Header section.

Export and import a custom workflow

Ion Reporter™ Software gives you the ability to export a custom workflow, then import that workflow to another Ion Reporter™ Server or Ion Reporter™ Software on Thermo Fisher Cloud account. Exporting and importing a workflow that has the settings you require saves time, since you can resuse the custom workflow, rather than creating a new workflow for each server or account.

You must use an Ion Reporter™ Software account with the analyze role to export and import a workflow. A workflow can only be exported from, then imported to, a single Ion Reporter™ Software organization. Each compressed file directory can contain only one workflow.

Note: The target regions, hotspot regions, and CNV baseline files, are not included in the compressed directory that is exported. Presets for the workflow, including presets for the annotation set, filter chain, copy number baseline, final report template, fusion panel, target regions file, and hotspot regions file are also not included in the export directory. The files must be uploaded separately, and the presets must be present in the organization into which the workflow is imported.

1. In the **Workflows** tab, click **Overview**, then find the custom workflow that you want to transfer, then select the workflow.
2. Click **Actions** ▸ **Export Workflow**.
3. Browse to the directory where you want to download the compressed directory of the files used for the workflow, then click **Export**.
4. Sign into the Ion Reporter™ Server or Ion Reporter™ Software on Thermo Fisher Cloud account that you want to use to import the workflow. In the **Workflows** tab, click **Overview**, then click **Import Workflow**.
5. Click **Select File**, then browse to and select the compressed directory (ZIP) file. The filename of the workflow directory is *workflow name*.zip.

   The workflow directory contains two files: a file with metadata to set the workflow parameters, and a separate parameters file.
6. Click **Open**, then click **Import**.
7. Refresh the **Workflows** list, or search for the workflow by name, then verify that the workflow is in the list for the Ion Reporter™ Server or Ion Reporter™ Software on Thermo Fisher Cloud account on which you imported the workflow.

You can begin to use the workflow immediately, provided that the target regions, hotspot regions, and CNV baseline files, and presets are available in the Ion Reporter™ Server or Ion Reporter™ Software on Thermo Fisher Cloud account.

Workflow revision autonumbering

Ion Reporter™ Software displays workflow revision numbers and automatically increases workflow revision numbers when you copy or edit a workflow.

To view workflow version numbers:

1. Click **Workflows ▸ Overview**, then select the desired workflow.

   The workflow revision number is displayed on the Details pane. By default, every workflow starts at revision number 0; that number increases if you copy or edit either a factory or custom workflow, then keep the name of the previous version as the new workflow name.

   You can only copy factory and custom workflows that are created in the most recent version of the software. Older version workflows that are unlocked can be edited, but the older versions workflows cannot be copied to create additional workflows.

   The revision numbers appear at the end of the workflow name (r.#). This revision level also appears on the QC Report and Audit log.

2.

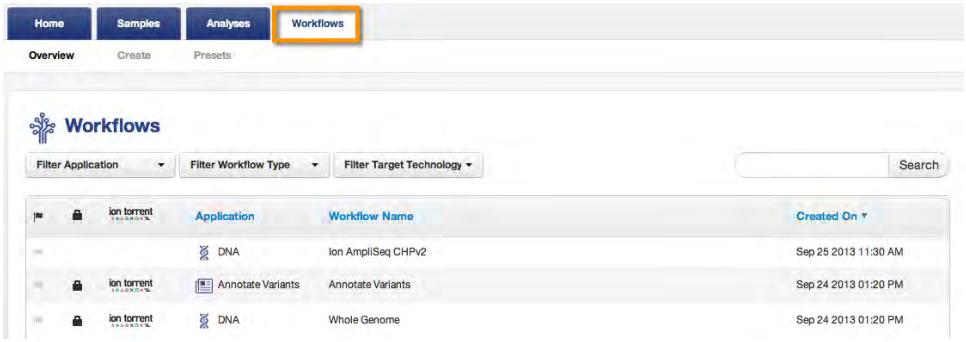
Lock a workflow

Locking a workflow allows you to ensure that no changes can be made to settings or parameters.

Note: Once a workflow has been locked, it can not be unlocked.

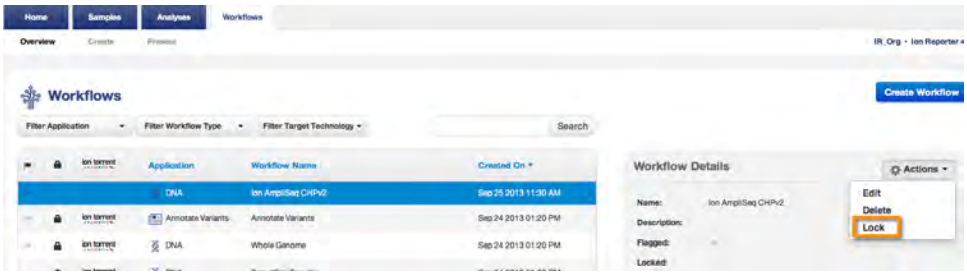
Follow these steps to lock a workflow:

1. Log in to Ion Reporter™ Software and click on the **Workflows** tab.

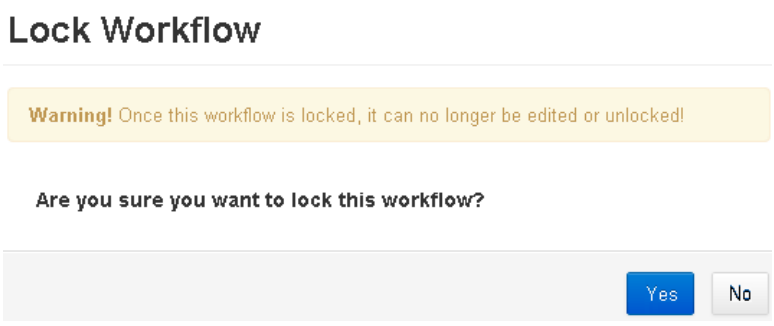


2. Select the workflow you wish to lock from the table and the select **Lock** from the **Actions** drop-down menu in the workflow details section.

   Note: Once a workflow is locked, that action can not be undone.



3. Confirm you want to lock the workflow.



The workflow is now locked and cannot be edited.

Run a plugin as part of a workflow

Use this procedure to run a plugin as part of a workflow.

- Before a plugin can be used as part of a workflow, it must be uploaded into the Ion Reporter™ Software.
- Currently, there are two ready-to-use plugins that are compatible with Ion Reporter™ Software:
  - AmpliSeq RNA (version 5.4.0.1-IR1)
  - RNASeq (version 5.4.0.1-IR1)
- The AmpliSeq RNA and RNASeq plugins can be downloaded from the Thermo Fisher Cloud and uploaded into the Ion Reporter™ Software (see "Download plugins from Themo Fisher Cloud" on page 316).
- See the "Create your own plugins'.

1. In the **Ion Reporter** screen, select the **Workflows** tab.
2. Select a workflow, then click **Actions**, then select **copy** in the dropdown list menu.

   To create a new workflow, select the default plugin workflow (**Run Plugin w1.0**). The default plugin workflow is a template for running plugins as part of a workflow, but does not have any specific plugins uploaded.
3. In the **Edit Workflow** screen, select the **Plugins** subtab.
4. Select a plugin or plugins from the list by clicking each plugin.

   Note: Plugins can also be added when launching an analysis (see "Add plugins" on page 140).
5. (Optional) Click ⚙ to configure plugin.
6. Click **Next** or click the **Confirm** tab.
7. Enter the name for the workflow.
8. *(Optional)* Enter a description.
9. Click **Save Window**.

   The saved workflow now appears in the list of workflows in the **Workflows** screen.

   For plugins that support multi-sample visualizations or comparisons, to show results that compare samples analyzed together for a plugin, all samples that you want to compare must be added to a single analysis and launched as a single analysis.

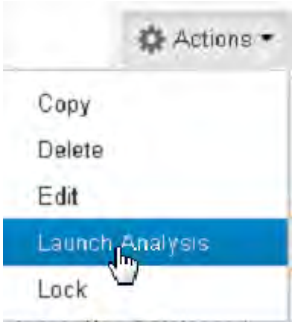
Analyses in Ion Reporter™ Software

Ion Reporter™ Software provides tools that enable you to manage your analyses. In the **Analyses** tab, you can launch new analyses and review results of your completed analyses.

Launch an analysis

Use one of the following methods to launch a single analysis in the analysis wizard:

- Launch an analysis from the Workflows page:
  a. Log in toIon Reporter™ Software and click on the **Workflows** tab.
  b. Select the row for the workflow that you want to launch.
  The row that contains the workflow is highlighted and information about the workflow is shown in the Details pane.
  c. Click **Actions**, then select **Launch Analysis** from the dropdown list.



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Proceed to “Choose samples“ on page 134

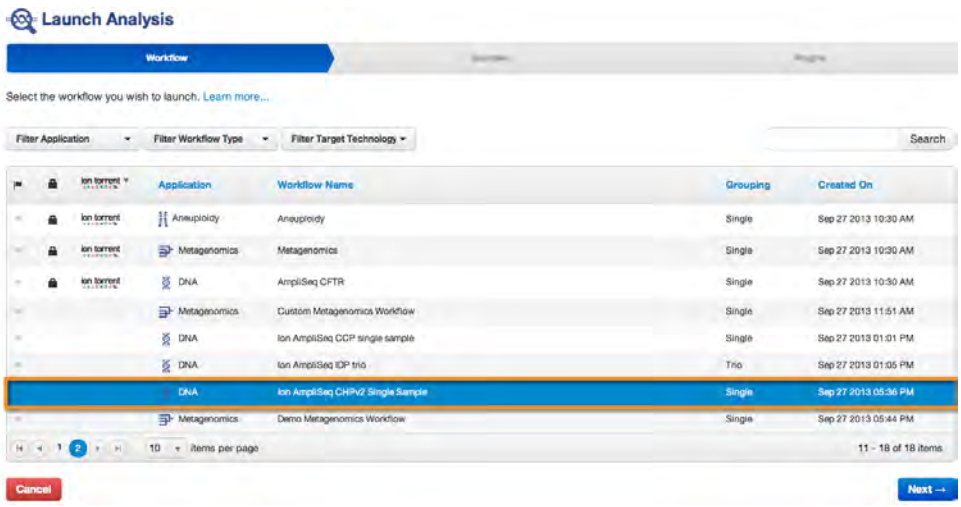
- Launch an analysis from the Analyses Overview page:
  a. Log in to Ion Reporter™ Software, then click on the **Analyses** tab.
  b. Click **Launch Analysis**, then select **Manual**.

Proceed to “Select a workflow“ on page 131.

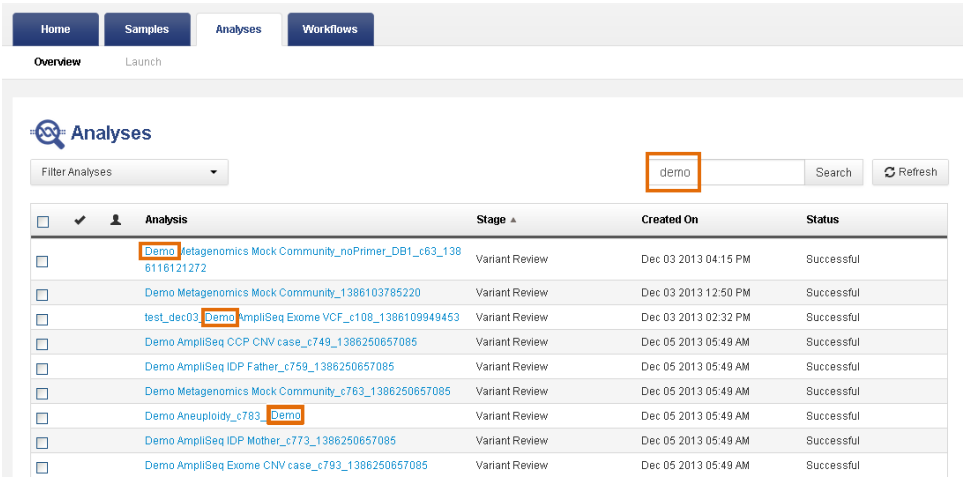
Select a workflow

In the Workflow chevron, you select a workflow to launch. If you have previously selected a workflow, proceed to "Choose samples" on page 134

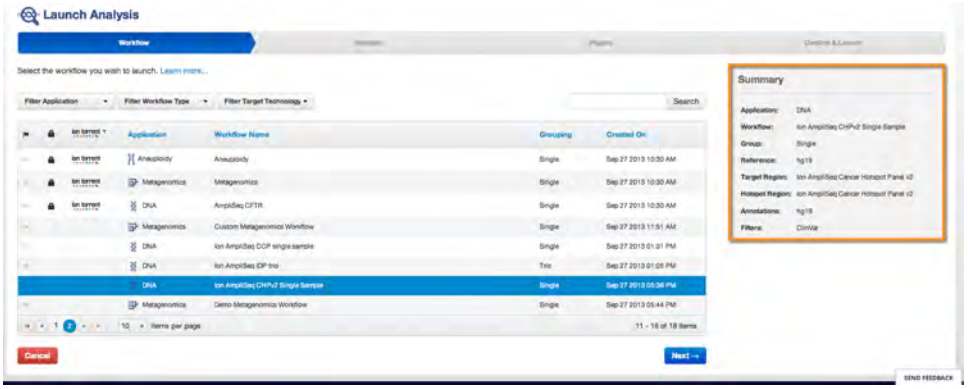
1. In the workflow table, select the workflow that you want to launch. Click a row to highlight a workflow.



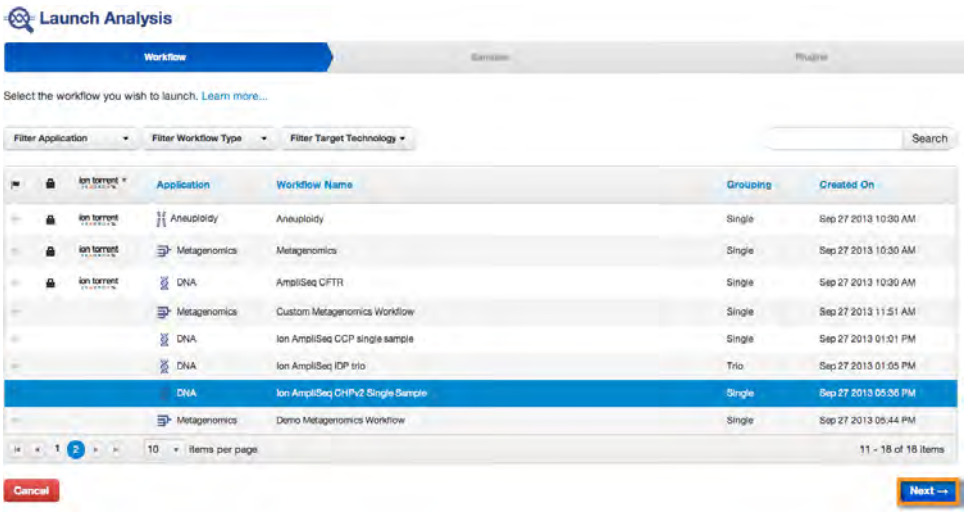
Note: To find a workflow in the table, you can use**Search**. Enter a string such as "CCP", "Exome", "IDP", "tumor", "TargetSeq", or your own custom workflow name and then click **Search** to get a list of results:



2. View details for the workflow in the Summary on the right.



3. Click the **Next** button.



Proceed to “Choose samples“ on page 134.

Demonstration samples

Use the workflows and demonstration sample data listed here to practice working with analyses.

Workflow	Demonstration sample name	Variant detection type
AmpliSeq CCP single sample	Demo AmpliSeq CHPv2 tumor, **or** Demo AmpliSeq CCP tumor	Somatic
AmpliSeq CCP tumor-normal pair	Demo AmpliSeq CCP normal and Demo AmpliSeq CCP tumor	Somatic
AmpliSeq CCP paired sample	Demo AmpliSeq CCP CNV control and Demo AmpliSeq CCP CNV case, **or** Demo AmpliSeq CCP normal and Demo AmpliSeq CCP tumor	Somatic
AmpliSeq Exome paired sample	Demo AmpliSeq Exome CNV control and Demo AmpliSeq Exome CNV case	Germline
AmpliSeq Exome single sample (Germline)	Demo AmpliSeq Exome CNV case	Germline
AmpliSeq Exome single sample (Somatic)	Demo AmpliSeq Exome CNV case	Somatic
AmpliSeq IDP single sample	Demo AmpliSeq IDP Daughter	Germline
AmpliSeq IDP trio	Demo AmpliSeq IDP Daughter, Demo AmpliSeq IDP Father, and Demo AmpliSeq IDP Mother	Germline
AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample	Demo AmpliSeq CHPv2 tumor and Demo AmpliSeq RNA Lung Fusion	Somatic
AmpliSeq RNA Lung Fusion single sample	Demo AmpliSeq RNA Lung Fusion	—
Aneuploidy	Demo Aneuploidy	—
Metagenomics 16S™ beta **Metagenomics 16S™ w1.1**	Demo Metagenomics Mock Community	—
Annotate variants single sample	Demo AmpliSeq Exome VCF	—

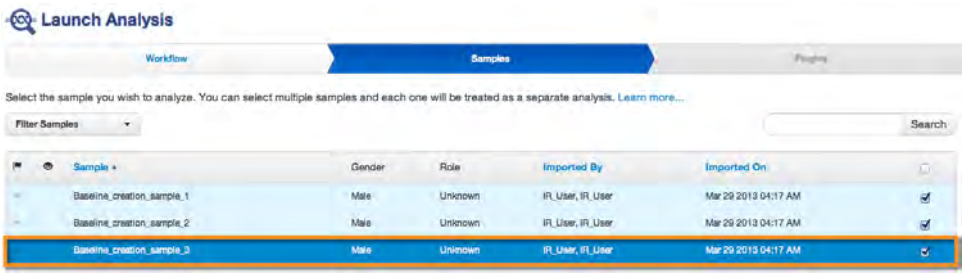
Choose samples

In the Samples chevron, you select the samples to be analyzed.

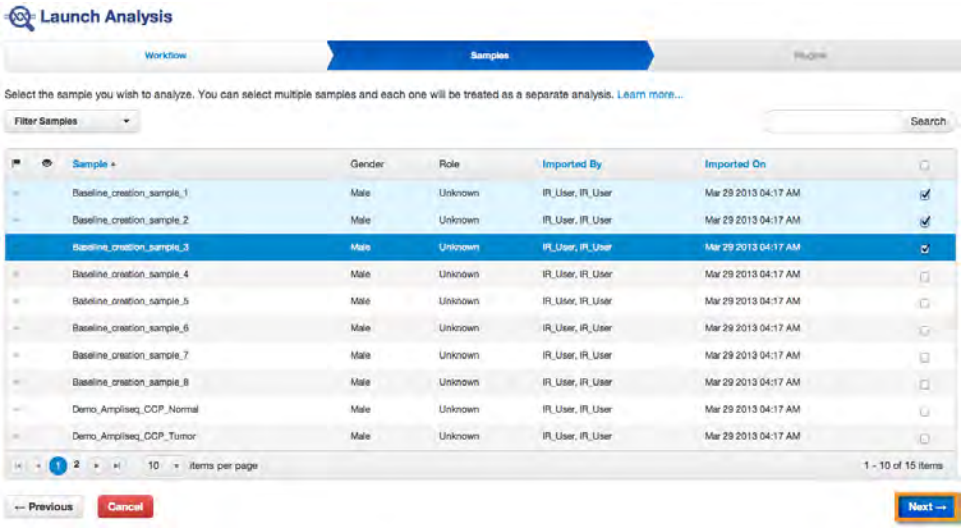
Select a single-sample or multi-sample analyses

Follow these steps to select samples for single sample DNA workflows or multi-sample metagenomics workflows:

1. In the samples table, select the sample or samples to analyze. Click a row to highlight the sample.



2. Click **Next**.



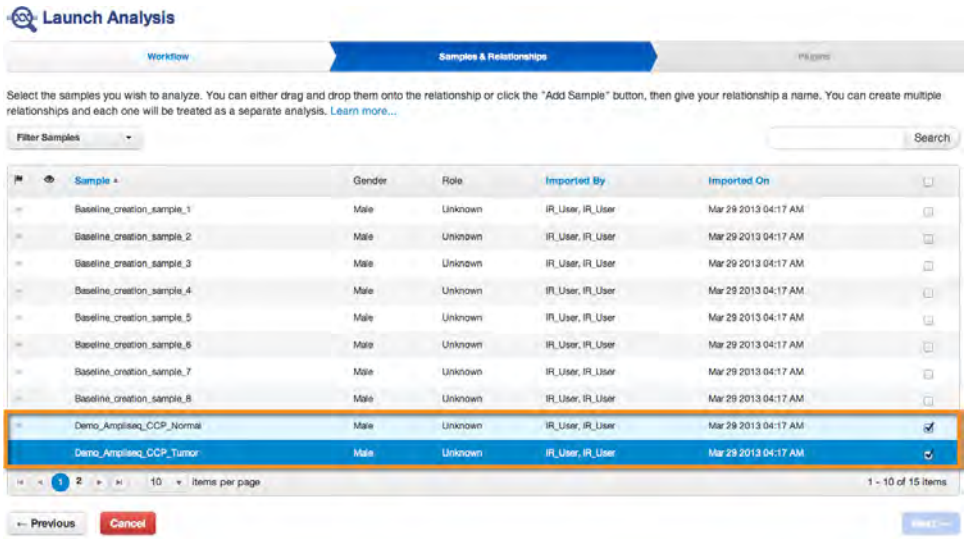
Note: For DNA workflows, if you select multiple samples on this page, Ion Reporter™ Software creates a separate analysis for each sample.

Select paired-sample or tumor-normal analyses

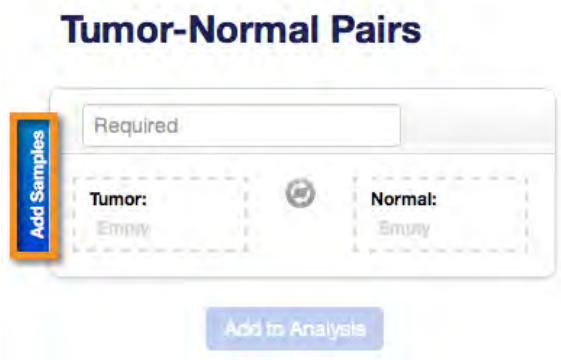
Note: For demo data samples with the tumor-normal workflow, use only the Demo AmpliSeq CCP normal and Demo AmpliSeq CCP tumor data sets.

Follow these steps to select samples for paired-sample or tumor-normal workflows:

1. In the samples table, select the samples to analyze. Click on both samples to highlight those samples rows for the pair.



This activates the **Add Samples** button on the pair window.

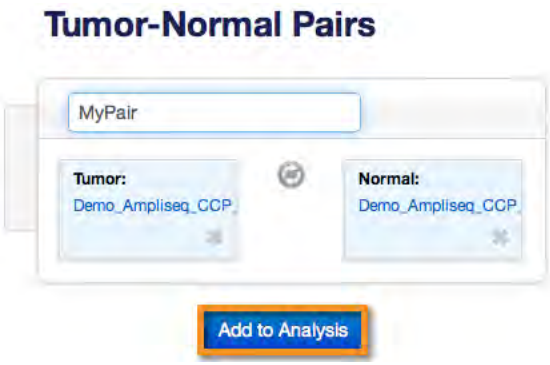


2. Click **Add Samples** to add the samples to the pair.

3. If the samples are placed in the incorrect roles, click the swap icon to switch the placement of the samples.

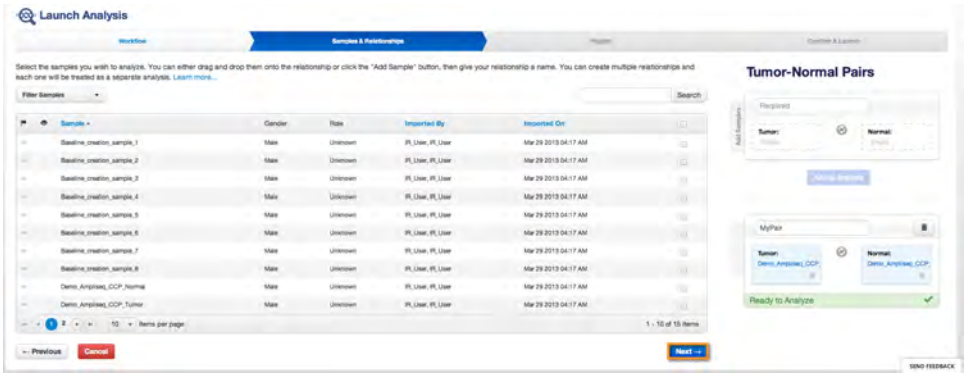
4. Give your pair a descriptive name.

5. Click **Add to Analysis**.



6. Repeat to add multiple pairs. If you select multiple pairs, Ion Reporter™ Software creates a separate analysis for each pair.

7. Click **Next**.

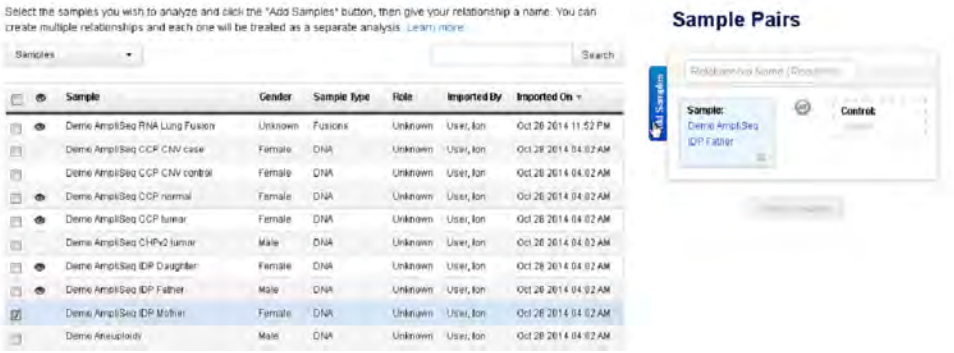


Control sample for CNV workflows

CNVs are reported based on their copy number relative to the control sample used. For best results, choose a control sample with no known CNVs in any region that is covered by the Ion AmpliSeq™ panel used. If most or all test samples are reported as having a CNV in the same region, one possible cause is that the control sample actually has a CNV in that region (leading to unexpected results).

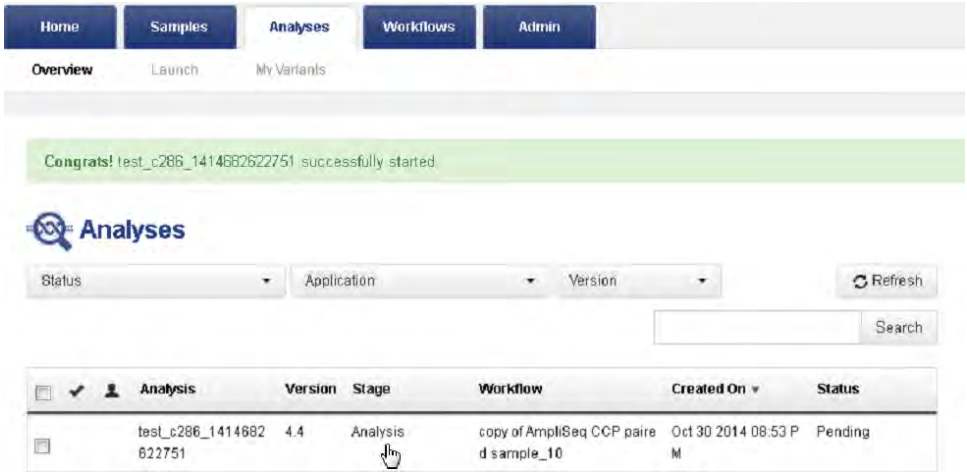
Analyze sample pairs

1. Select one or more samples and click **Add Samples**.



2. Name the sample pair and click **Add to Analysis**.

3. Review the sample that is Ready to Analyze and then click **Next**.
4. Click **Next** on the Launch Analysis page to bypass the option to install plugins.
5. Click **Launch Analysis**.
   You are returned to the Analyses Overview page.
6. Verify there is a message that states the analysis started successfully:



Select Trio analyses

Follow these steps to select samples for trio workflows:

1. In the samples table, select the samples to analyze. Click on each row of the sample trio to highlight the samples for the trio.

   This activates the **Add Samples** button on the trio window.

2. Click **Add Samples** to add the samples to the trio.

3. If the samples were placed in the incorrect roles, click the swap icon to switch the placement of the samples.

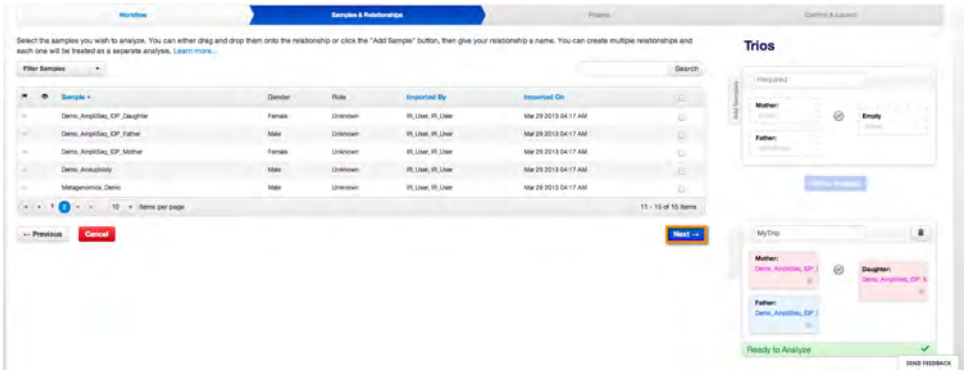
4. Give your trio a descriptive name.

5. Click **Add to Analysis**.

6. Repeat to add multiple trios.

   Note: If you select multiple trios, Ion Reporter™ Software creates a separate analysis for each trio.

7. Click **Next**.



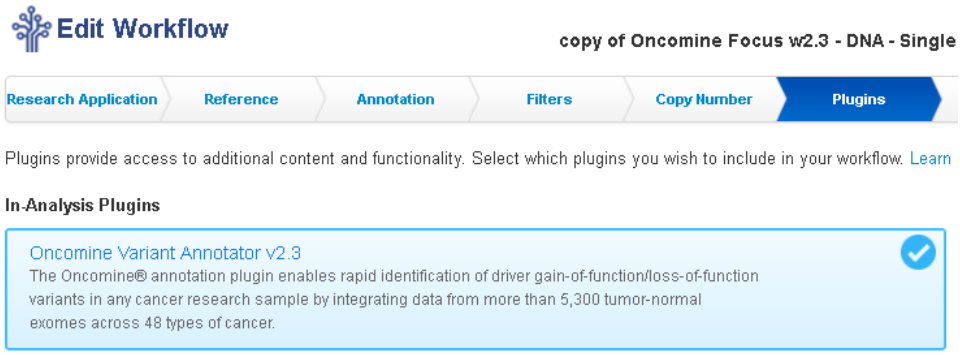
Add plugins

Plugins provide additional content or functionality to an analysis. Follow these steps to select the plugins you wish to include as part of your analysis:

Note: Functionality for the Oncomine Variant Annotator plugin is highly dependent upon the panel used for the assay, so only data from supported panels should be run through workflows that contain this plugin.

1. To select an in-analysis plugin to include in your analysis, click anywhere on its logo, title, or text. The plugin box is highlighted and marked with a checkmark.

   Note: The Oncomine Variant Annotator plugin is selected by default for some Oncomine workflows.



2. Click **Next**.

Confirm and launch an analysis

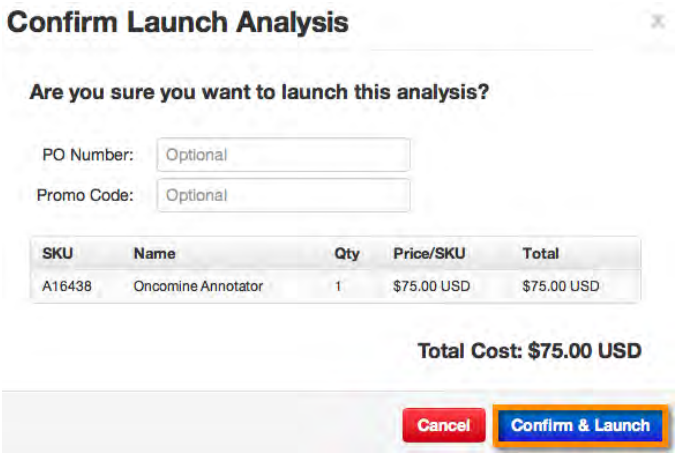
Your analysis is now ready to launch. Review the details of the analysis. When you are ready to start your analysis follow these steps:

1. Provide a name for your analysis or use the default name. Optionally, add a description.

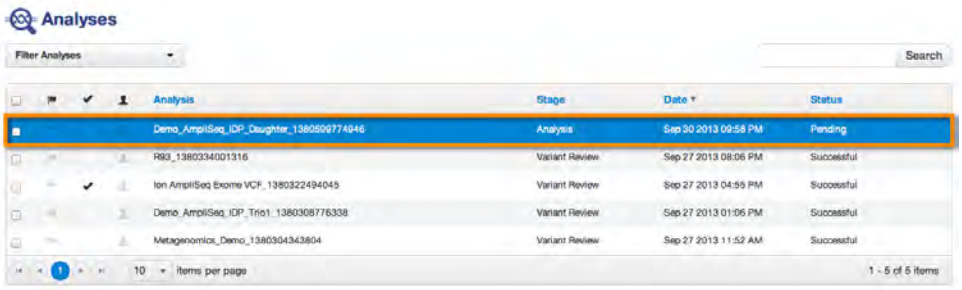
   If you have multiple analyses, repeat this step for each analysis.

2. Click **Launch Analysis**.

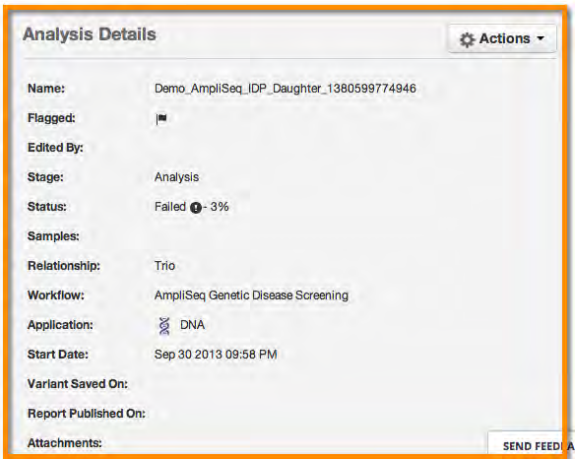
3. If you have included a plugin in your analysis, you are presented with a cost breakdown and confirmation screen. Click **Confirm & Launch**.



4. Your analysis is launched and the analysis table reopens.



5. Click on an analysis to see more information in the Details section on the right.



How to launch multiple analyses with the analysis wizard

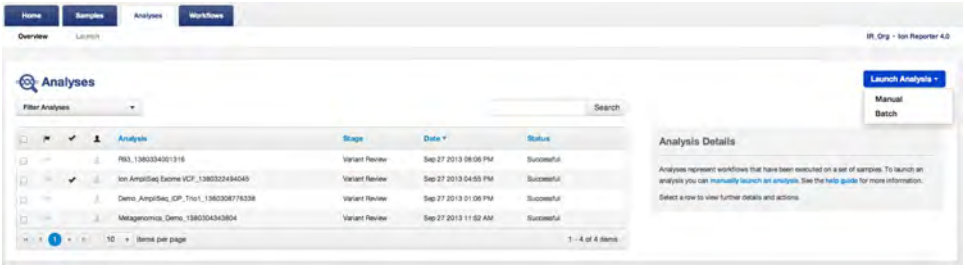
This technique analyses multiple samples, or multiple sets of related samples, using separate analyses with the same workflow. For example, with one use of the wizard, you can launch several trio workflows, or several paired workflows -- and each analysis has its own related samples.

These steps follow the regular analysis launch, except for sample selection.

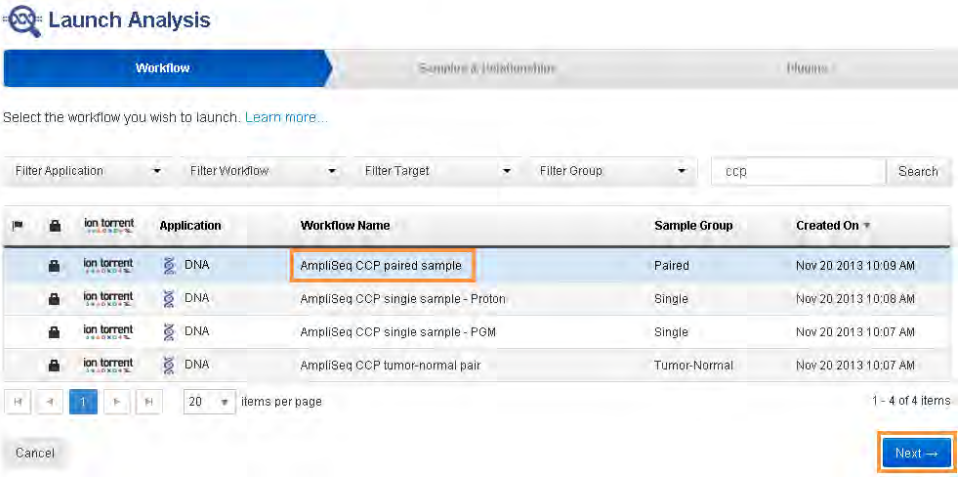
Follow these steps to launch multiple analyses with the analysis wizard:

1. Log in to Ion Reporter™ Software and click on the **Analyses** tab.

2. Click on the **Launch Analysis** button and select **Manual**.

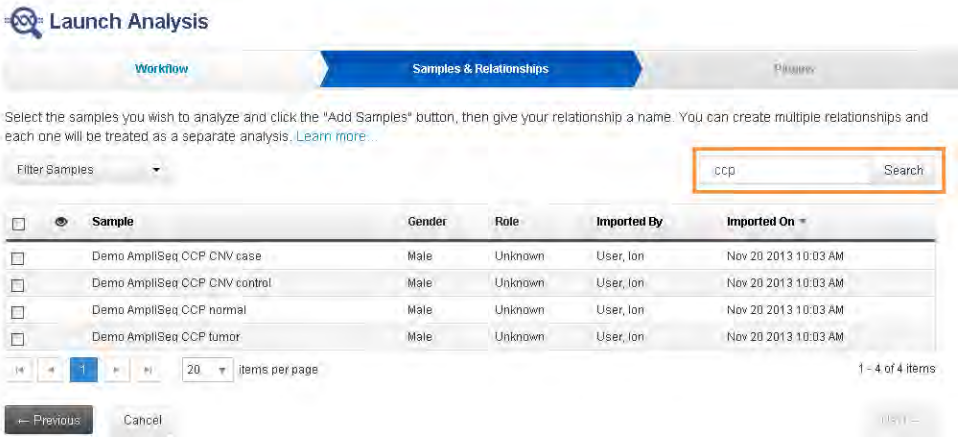


3. Select your workflow.

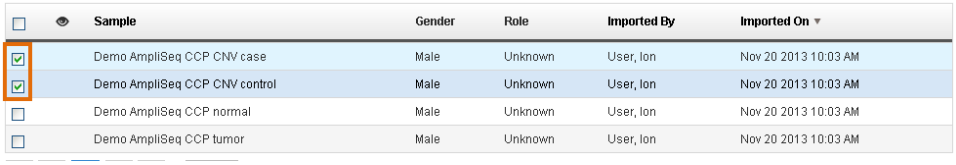


Click the **Next** button.

4. In the Samples & Relationships chevron, for ease in finding and selecting the correct samples, enter a search string that includes all the samples for all your multiple analyses. In this example, only CCP samples are shown.



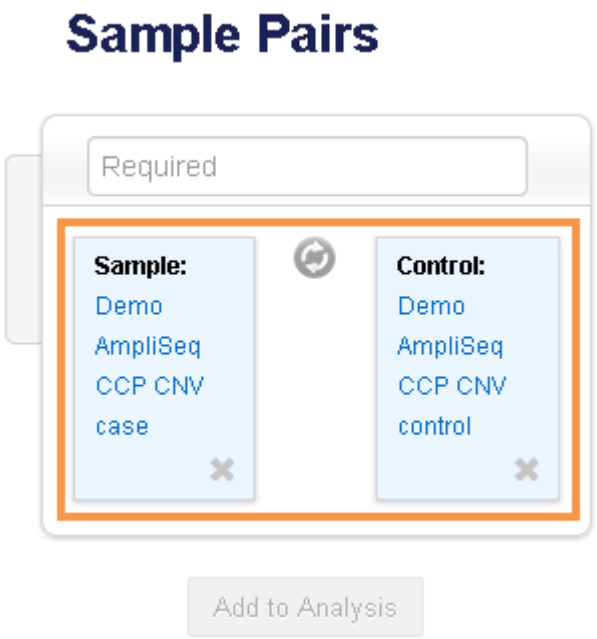
5. Enable the checkboxes for the samples in your first analysis.



Follow the same steps as you would to analyse these samples in a single analysis:

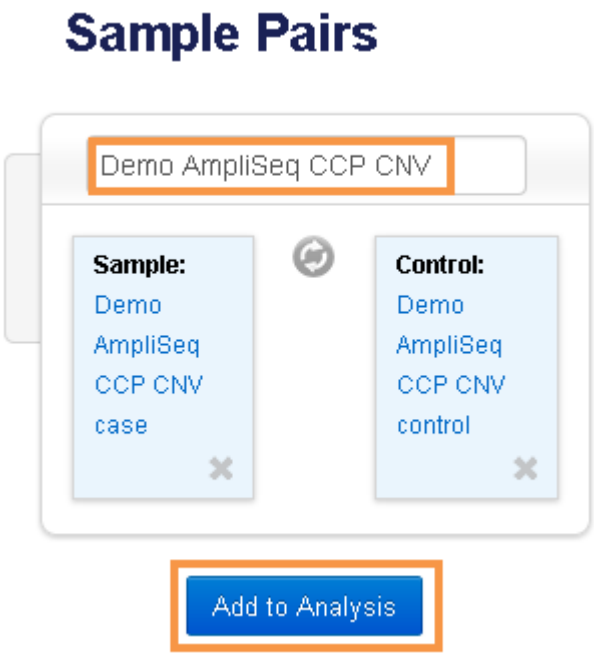
a. Click the **Add Samples** button.

b. Your samples are added to the placeholders in the samples area.



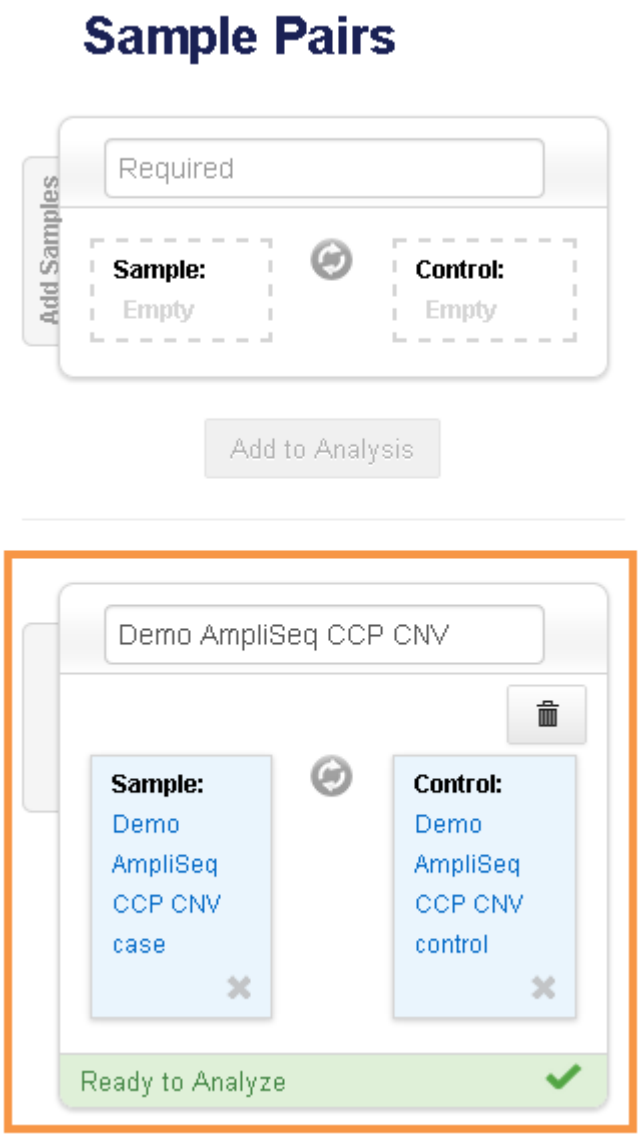
c. If the samples are placed in incorrect roles, swap them by clicking the swap icon .

d. In the text field above your samples, enter a name for this set of samples.



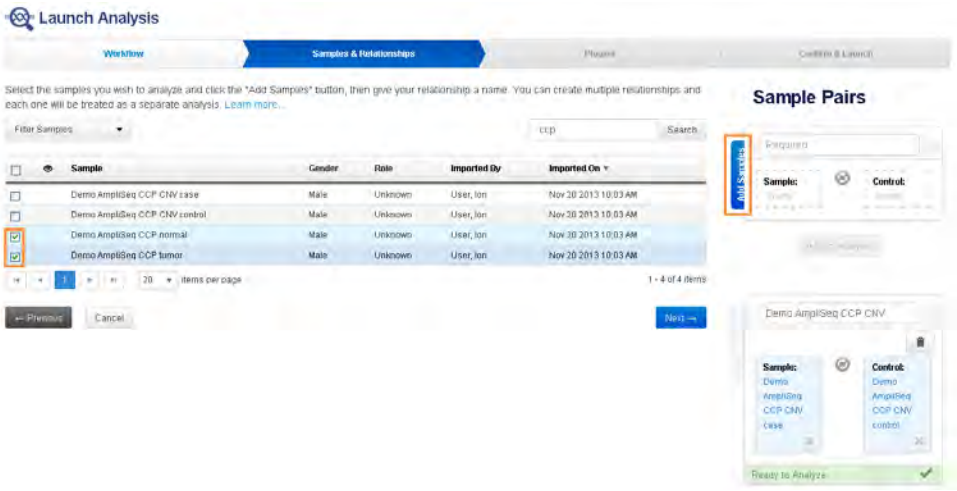
Click the **Add to Analysis** button.

e. Your samples are added to the Ready-to-Analyze area.

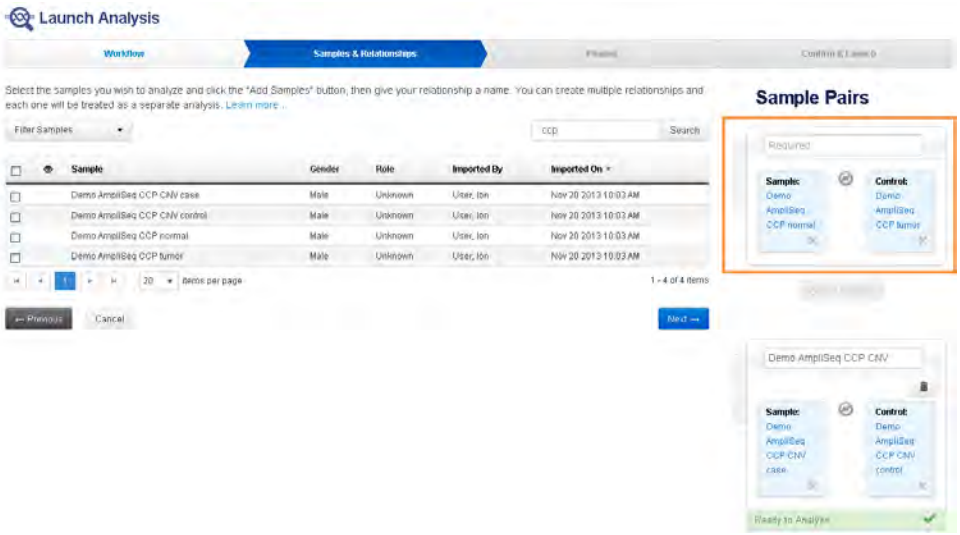


The Ready-to-Analyze area represents one separate analysis.

6. Repeat this process for the set of samples for your second analysis:
   a. Enable the checkboxes for the samples in your second analysis and click the **Add Samples** button.



   b. Your second set of samples are added to the placeholder area.

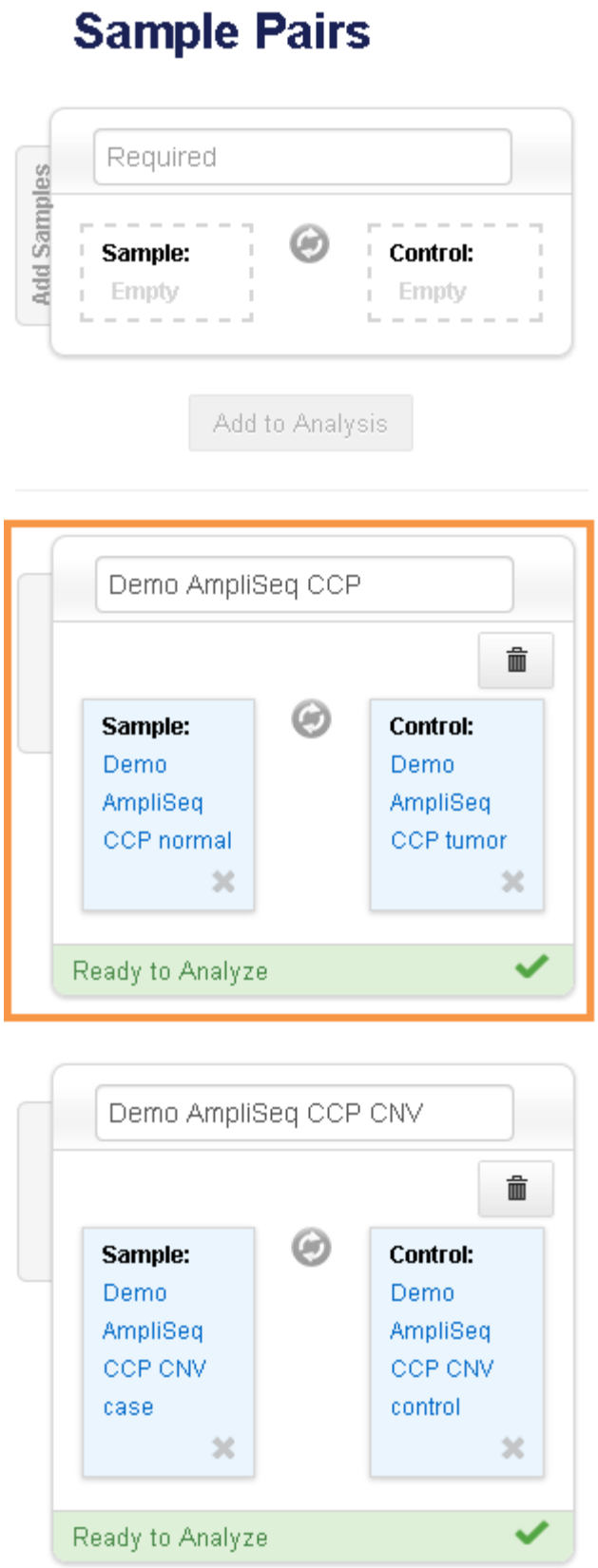


   c. If necessary, swap the sample relationship with the swap icon .

d. In the text field above your samples, enter a name for this set of samples.

Click the **Add to Analysis** button.

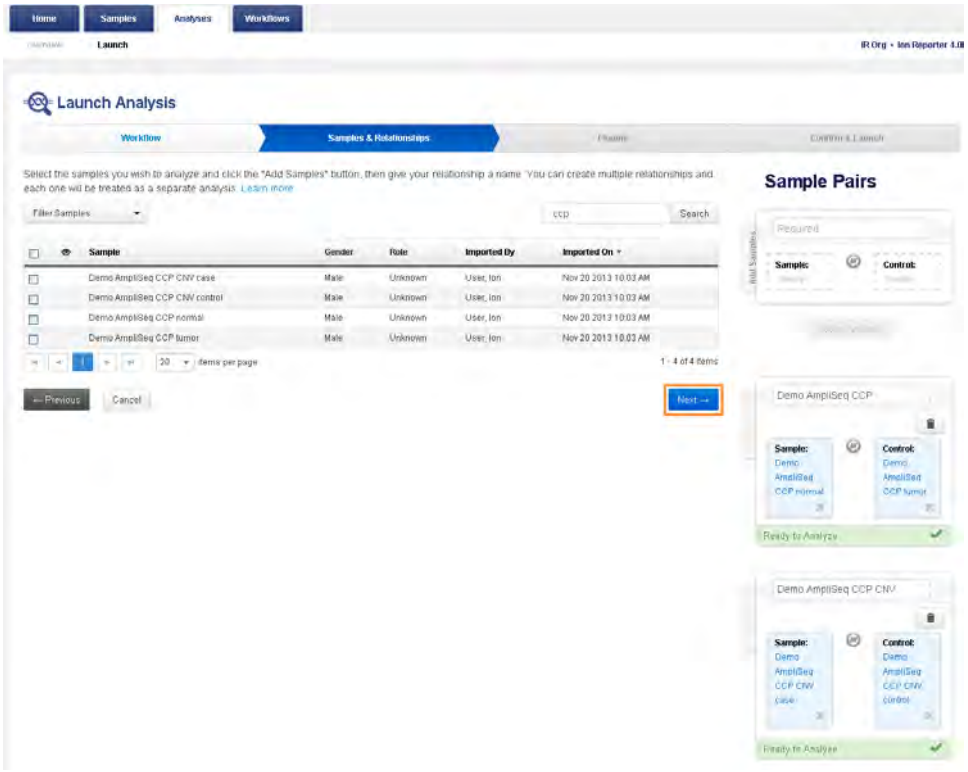
e. Your samples are added to a new Ready-to-Analyze area.



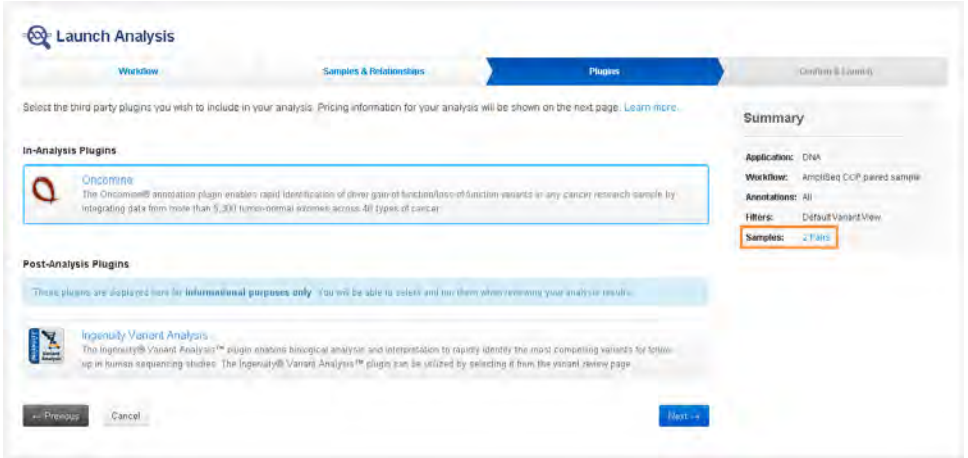
This new Ready-to-Analyze area represents a second separate analysis.

7. Repeat this process for every set of samples to be analyzed. You will have one Ready-to-Analyze area for each separate analysis.

8. Check each Ready-to-Analyze area for the correct sample names and relationships. When you are ready, click the **Next** button.



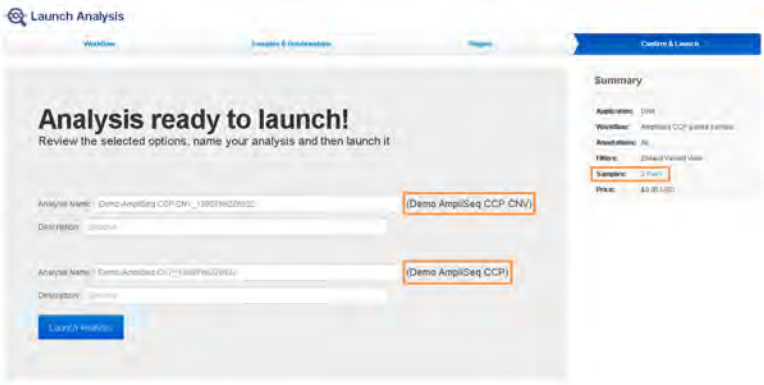
9. In the Plugin chevron, select any in-analysis plugin that you want to be included in these analyses. (If you select a plugin, it is added to all of your analyses being launched now.)



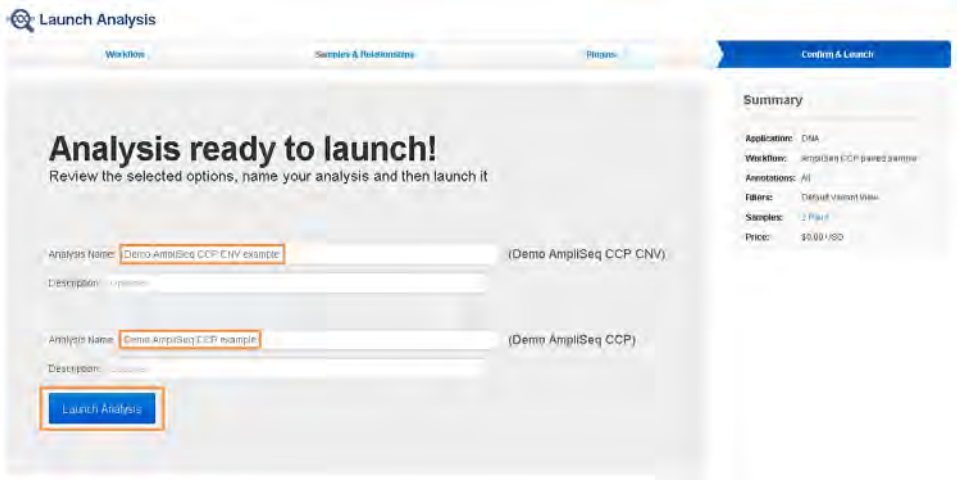
Note: In the Summary panel, "Samples: 2 pairs" refers to the two analyses.

10. Click the **Next** button.

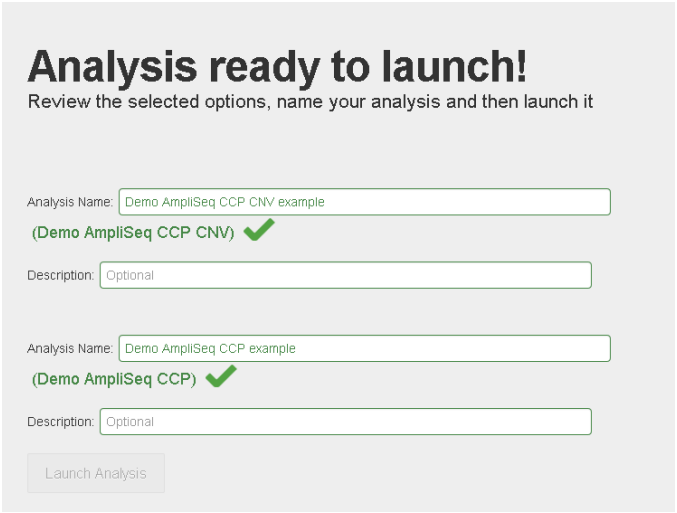
11. In the Confirm & Launch chevron, each analysis is named with the name that you gave to the related samples.



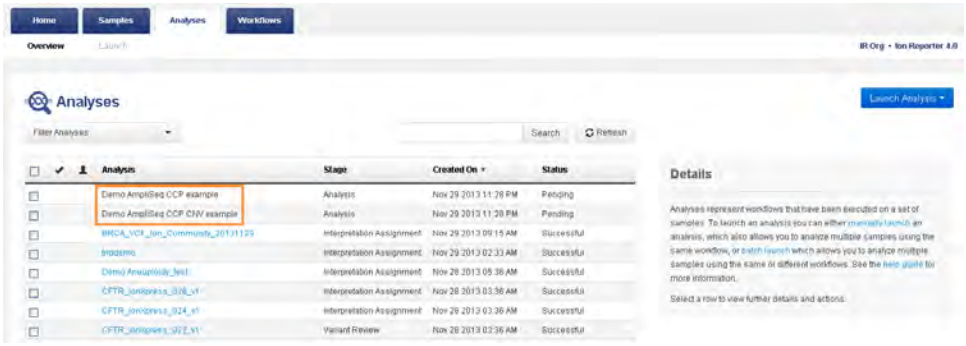
12. You can rename your analyses. Click the **Launch Analysis** button.



13. Each analysis that is successfully launched is shown with a large checkmark.



14. You are return to the analysis table.



Click on an analysis to see more details in the analysis details section on the right.

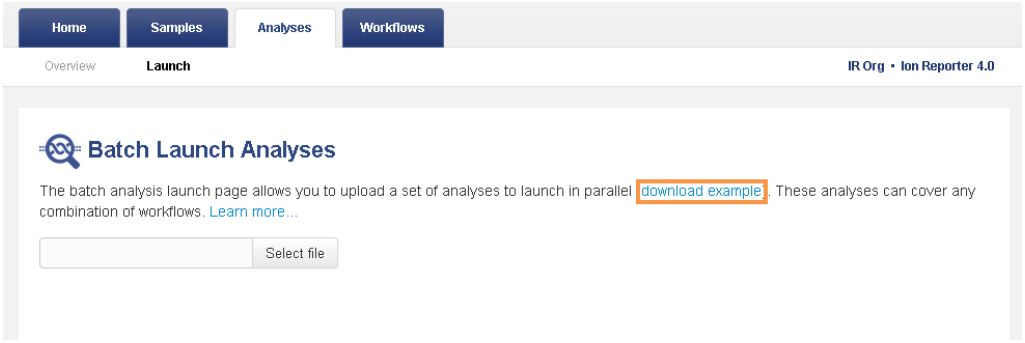
Batch launch analyses

To use batch analysis launch, describe your analyses in a CSV file and upload that file in the Batch Launch Analysis page. Here is an example of the headings required:

	A	B	C	D	E	F
1	#Lines starting with # are ignored					
2	#'|' is used to create a different analysis with a same workflow					
3	#You have to specify whether the workflow or the sample is provided by Ion Torrent.					
4	#Multiple samples can be defined for a workflow using the pattern - sample1:IsIonDefault(yes/no):relation;sample2:IsIonDefault(yes/no):relation					
5	type	workflow name	version	IsIonDefault?	samples	plugins
6	single	AmpliSeq CHPv2 single sample	4.6	yes	Demo AmpliSeq CHPv2 tumor	
7	paired	AmpliSeq CCP paired sample	4.6	yes	Demo AmpliSeq CCP CNV case:yes:sample;Demo AmpliSeq CCP CNV control:yes:control	
8	paired_tumor_normal	AmpliSeq CCP tumor-normal pair	4.6	yes	Demo AmpliSeq CCP tumor:yes:tumor;Demo AmpliSeq CCP normal:yes:normal	
9	trio	AmpliSeq IDP trio	4.6	yes	Demo AmpliSeq IDP Father:yes:father;Demo AmpliSeq IDP Mother:yes:mother;Demo AmpliSeq IDP Daughter:yes:proband	
10	multi	Metagenomics 16S beta	4.6	yes	Demo Metagenomics Mock Community:yes:none	
11	single	AmpliSeq CHPv2 single sample	4.6	yes	Demo AmpliSeq CHPv2 tumor	
12	paired	AmpliSeq CCP paired sample	4.6	yes	Demo AmpliSeq CCP CNV case:yes:sample;Demo AmpliSeq CCP CNV control:yes:control	
13	SINGLE_RNA_FUSION	AmpliSeq RNA Lung Fusion single sample	4.6	yes	Demo AmpliSeq RNA Lung Fusion	
14	DNA_RNA_FUSION	AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample	4.6	yes	RNA_sample_name:RNA_FUSION;DNA_sample_name:DNA_FUSION	

Please read these notes about the use of the batch analysis CSV file:

- Download the example CSV file from the Batch Launch Analysis page **download example** link.



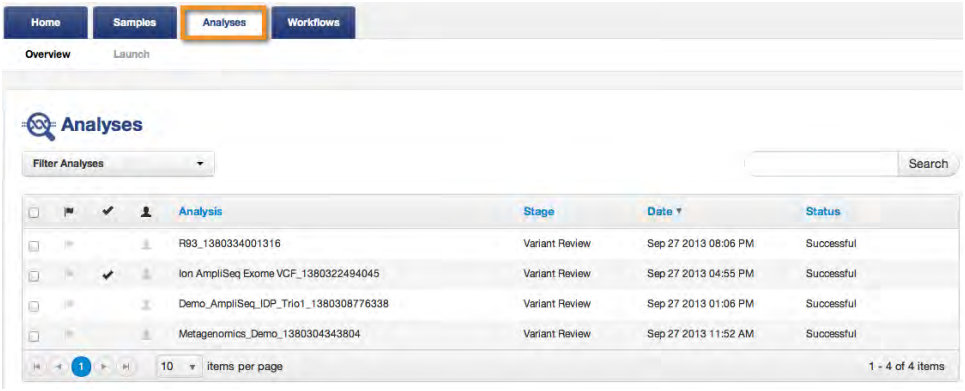
- Each line defines a separate analysis.
- Order is important within an analysis entry.
- The first three of these fields are required:
  `type,workflow name,samples,plugins`

- In the Type column, if you are uploading fusions, please enter "DNA_RNA_FUSION" for DNA and Fusions, and "SINGLE_RNA_Fusion" for RNA only samples.
- The plugin field is optional (but the comma is still required).
- This entry: `single,AmpliSeq CHPv2 single sample,Demo AmpliSeq CHPv2 tumor,` has the following meaning:
  - `single`: This analysis uses a single sample.
  - `AmpliSeq CHPv2 single sample`: The name of the workflow.
  - `Demo AmpliSeq CHPv2 tumor`: The name of the sample.
  - `(blank field after the comma)`: No plugins are used.
- For multiple samples, use a colon to add the role to each sample name. For example:
  Demo AmpliSeq IDP Daughter:proband

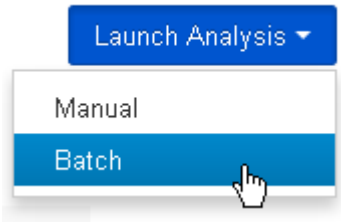
IMPORTANT! Do not remove or edit the line `type,workflow name,samples,plugins`. This line is required.

Follow these steps to batch launch a set of analyses:

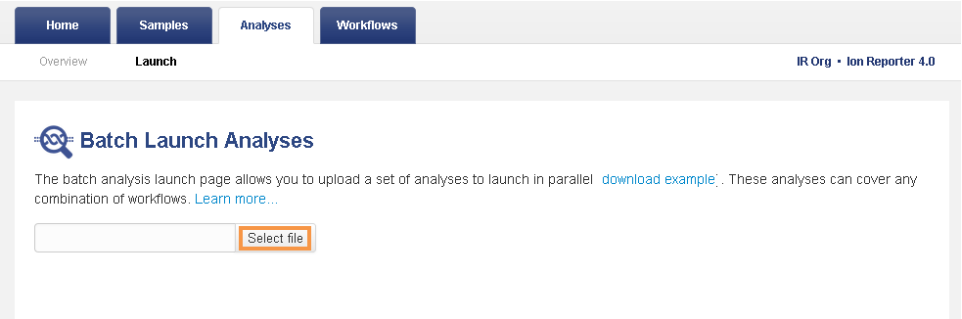
1. Prepare your CSV file with analysis descriptions in the pattern "type,workflow name,samples,plugins" as described above.
2. Log in to Ion Reporter™ Software and click on the **Analyses** tab.



3. Click on the **Launch Analysis** button and select **Batch**.

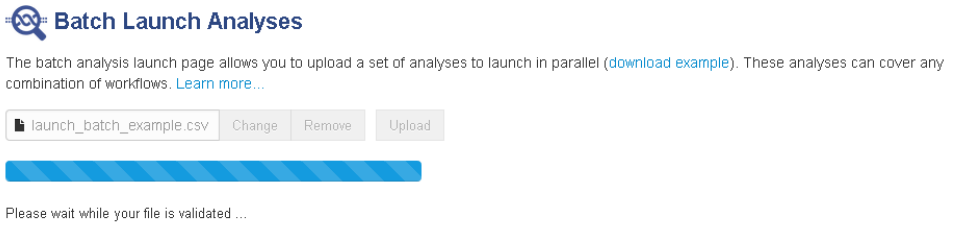


4. Click the **Select file** button and upload your CSV file.



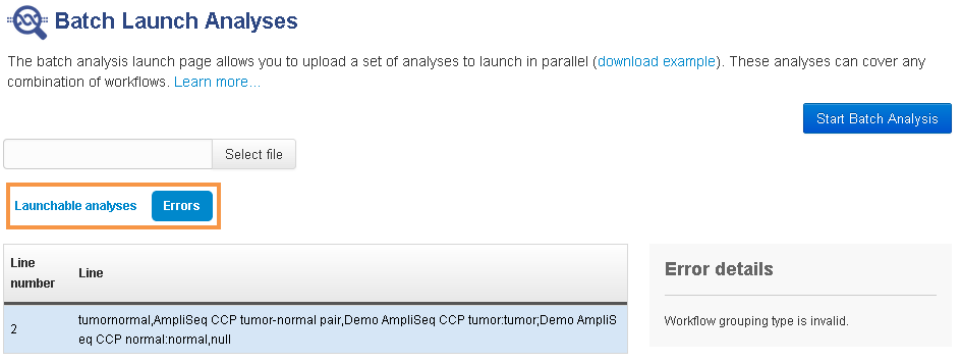
5. Confirm that the correct file name appears in the display field and click the **Upload** button.

6. Please wait while your file is verified and the analyses are created.



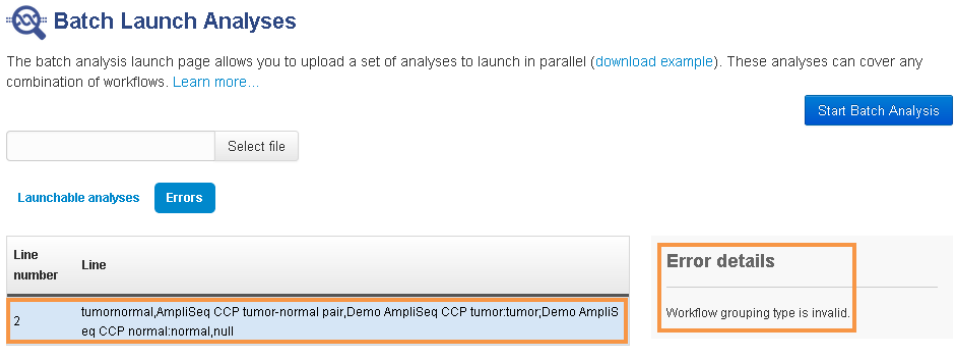
7. If your analyses are created successfully, click the **Start Batch Analyses** button.

If the analysis creation runs into an error, a button appears to switch between analyses that can be launched and analyses that got an error.

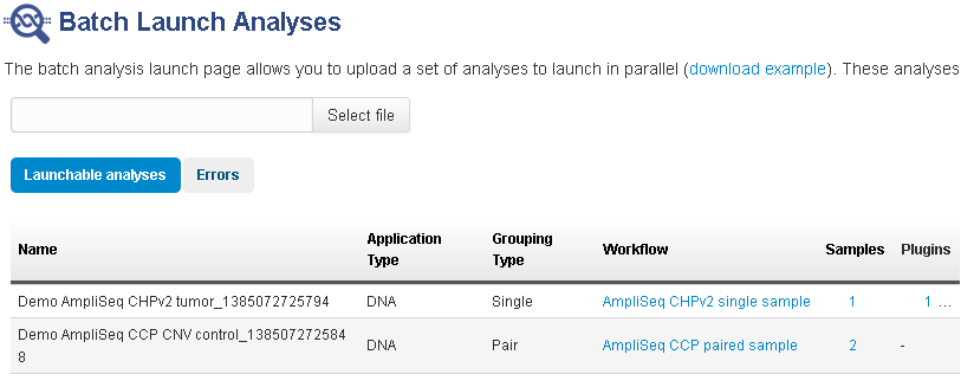


The following describe an example of an analysis creation error:

- The **Errors** button shows the CSV line that contains an error.



- The error message appears in the Errors Details section. In this case the workflow sample group is incorrect. The CSV file contains `tumornormal`, while the correct sample group name is `paired_tumor_normal`.
- Click the **Launchable analyses** button to see analyses that were created correctly.

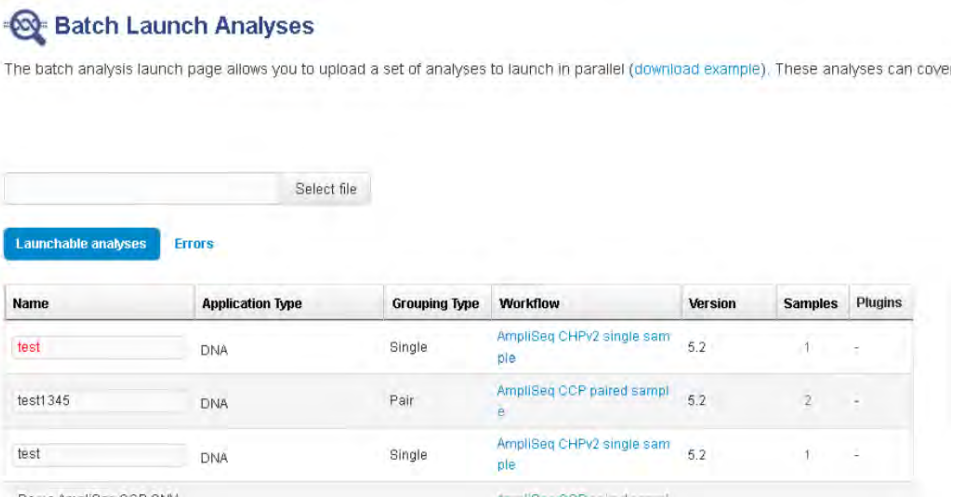


- You can click the **Start Batch Analyses** button to launch the analyses listed under **Launchable analyses**, or you can correct the CSV file and upload it again.

Rename analysis during Batch Upload

If you are uploading multiple analyses with a CSV file that contains duplicate analysis names, the duplicate analyses are listed at the top of the table in red text. You can edit the text to change the name of that analysis and proceed with your batch analyses work.

1. If you find you have a duplicate analysis name during CSV upload, click on the red text and change the name and click **Enter**.



2. You can now proceed with your batch analyses work.

Set table preferences

You can configure and save the columns displayed in the tables under the **Samples** and **Analyses** tabs.

Under the **Samples** or **Analyses** tab, in the **Overview** screen, click **Preferences** and select from the following options:

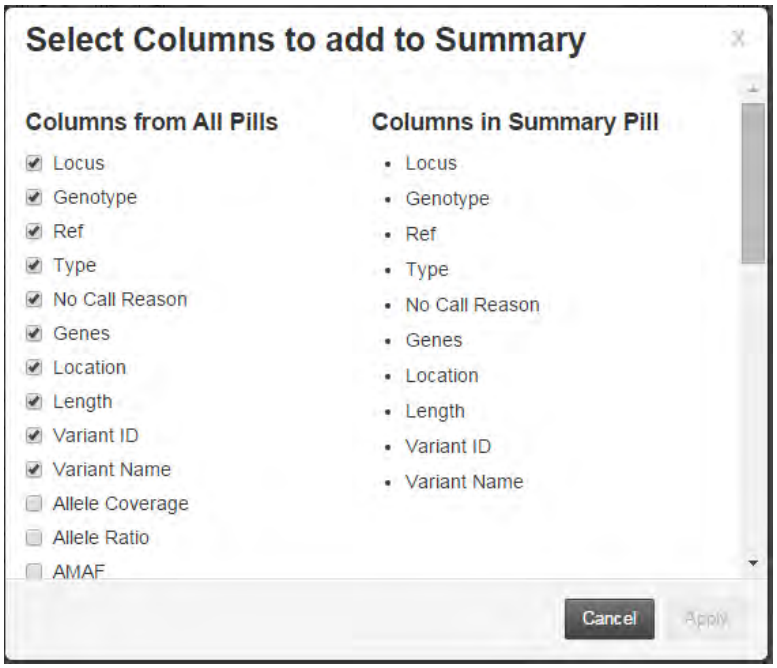
Select...	To...
Table Preferences	Select from a list of saved table preferences.
Save Table Preference	Save the selected column display under the current selected table preference name.
Save Table Preference As...	Save the selected column display under a new user-defined table preferences name.
Select Columns...	Select from a list of available columns to display, including any user-defined attributes.
Restore Defaults	Restore the default table display.

Set table preferences

You can set **Table Preferences** in Ion Reporter Software to show or hide the columns in any table that lists samples or analyses, or summarizes other sample or analysis information, such as **Analysis Results**. If you save a setting, it applies to all tables. If you save a setting and name it, the preference is added a list of Table Preferences and you can apply that setting at any time to any table. You can delete unused **Table Preferences**.

In the **Samples** tab or **Analyses** tab, click **Overview**, then select a **Analysis** or an **Analysis**.

- To save a Table Preference:
  a. Click **Preferences** ▸ **Select Columns**.
  b. Select and deselect columns that in the **Select Columns to add to Summary** dialog that you want to add or remove from the table. Click **Apply**.



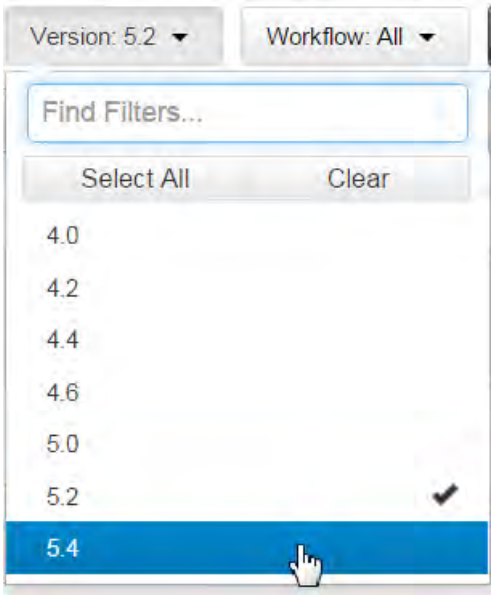
- To create a custom table preference:
  a. Click **Preferences** ▸ **Save Table Preference As**.
  b. Enter a name for the table preference and click **Save**.
  c. Click another analysis.
  d. Click **Preferences** ▸ **Table Preferences**, then select the new **Table Preference**.
  The analysis columns are modified as determined by the applied **Table Preference**.
- To delete an unused **Table Preference**:
  a. Click **Preferences** ▸ **Table Preferences**, then select the **Table Preference** that you want to delete.
  The **Table Preference** is applied.
  b. Click **Preferences** ▸ **Delete Table Preference**.

c. When you are prompted to delete the **Table Preference**, ensure that the dialog refers to the preference that you want to delete, then click **Yes**.
The **Table Preference** is removed from the table.

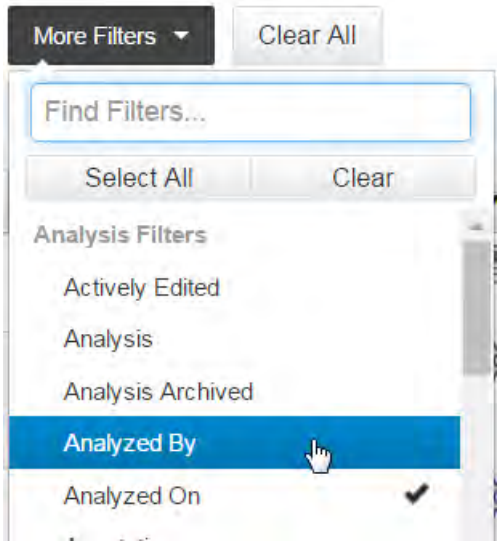
Filter the analyses table

Tools for filtering analyses based on attributes are located under the **Analyses** tab in the **Overview** screen.

- The **Version** and **Workflow** filters are available by default. Click each button above the table to filter the list by analysis version and/or the workflows associated with the analyses.

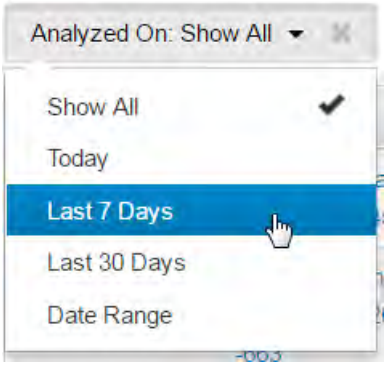


- Click **More Filters** to select additional filters from a dropdown list.



  - Use the **Find Filters** field to search for filters in the list.
  - Select the filter you want from the list. A button for that filter is added above the table, and the filter is listed with a check mark in the **More Filters** list.
  - To remove a filter button, click it again in the **More Filters** list to deselect it.

- Each filter button you add to the table has different options to filter by, based on the attributes of the analyses in the table. For example, the **Analyzed On** filter lets you filter by ranges of dates when analyses were last performed.



- Click each filter button you add to display a dropdown list of options.
  - Select an option in the list to filter by that selection. The table will reconfigure to list only the analyses that meet the filter criteria.
  - Click a selected option again to deselect it and no longer filter by that option.
  - Click **Select All** to select all the options for that filter.
  - Click **Clear** to clear the selections.

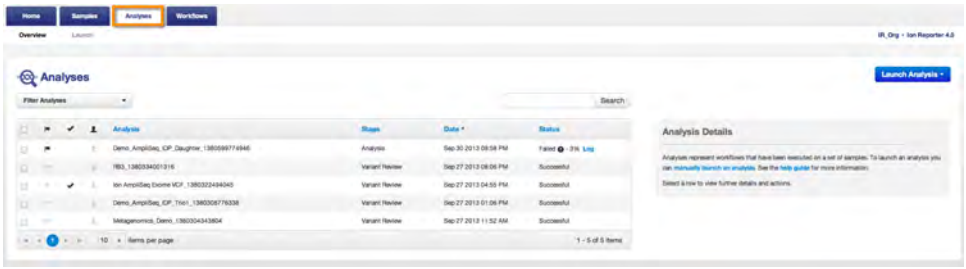
- Click **Clear All** above the table to clear all selected filters.

Delete an analysis

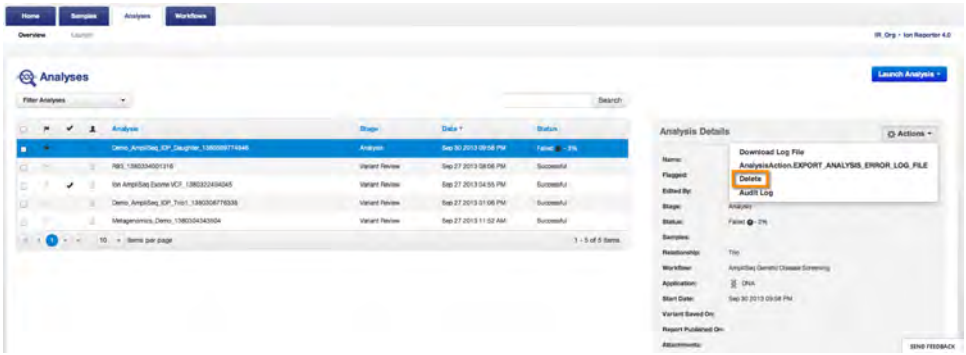
IMPORTANT! When an analysis is deleted, the action cannot be undone.

Follow these steps to delete an analysis:

1. Log in to the Ion Reporter™ Software, then click on the **Analyses** tab.



2. With the left column checkboxes, select the analysis (or analyses) in the table, and then select **Delete** in the **Actions** dropdown list in the Details section.



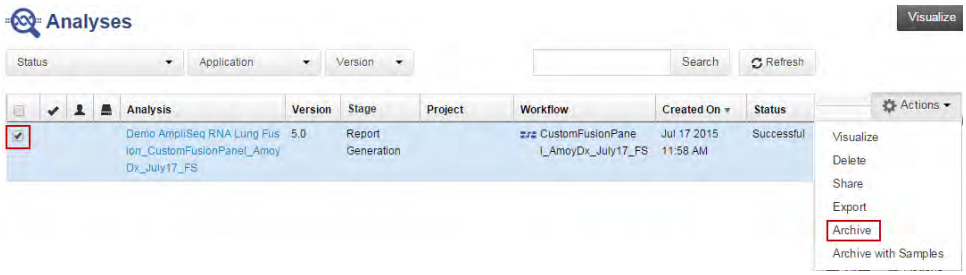
3. Click **Yes** in the pop-up window to confirm that you want to complete the deletion.

4. You are brought back to the analysis page and the analysis or analyses are deleted.

Archive analyses

If you have an Ion Reporter™ local server, you can archive analyses to free up disk space.

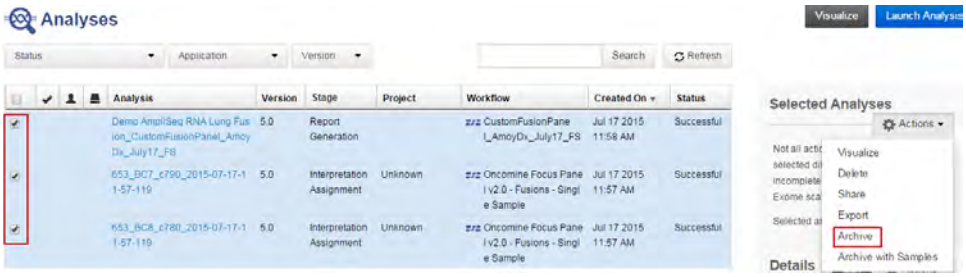
1. On the **Analyses** page, select the analysis you want to archive.
2. Go to **Actions** and click **Archive** or **Archive with Samples**. Note: You can only archive the Analysis and the samples that were used by that analysis, if no other analyses used those samples.



3. Click **Yes** to confirm you want to archive.

An Archive icon appears in the analysis row.

4. To undo an archive, select the archived analysis and go to **Actions** ▸ **Restore**.
5. To archive multiple analyses, select multiple and go to **Actions** button and select **Archive** or **Archive with Samples** from the drop-down menu.



Archival location must be specified

New users and users upgrading from a previous version must specify a storage location for archiving samples and analyses in Ion Reporter™ Software 5.4. Change archivalMountPath=/tmp/ to archivalMountPath=/storage IP address/ in the ionreportermanager/server/server.properties file, then restart the Tomcat server.

To check the data that you archived, go to the storage location of the archived data, which is set up in /share/apps/IR/ionreportermanager/server/server.properties file as archivalMountPath.

Monitor analyses

You can actively or passively monitor your analyses.

1. To actively monitor, go to the **Analyses** ▸ **Overview** page and check the **Status** column.
   Your analysis will be reported as **Pending**, **% complete**, **Successful** or **Failed**.
2. To passively monitor, check your email. Ion Reporter™ Software sends an email when your analysis is complete (success or failure).

Reanalyze an aborted analysis

You can reanalyze the results for an analysis run that has been aborted.

1. In the **Analyses** tab, select an analysis of interest that has a status of **Aborted**.
2. Click **Actions** ▸ **Reanalyze**.

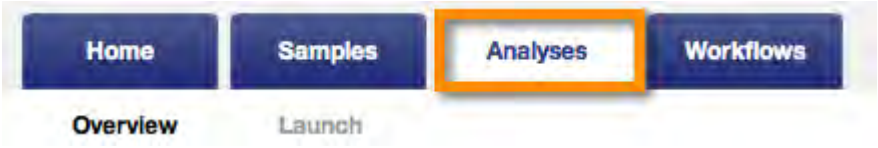
Analysis results

This section describes how to sort and display your analysis results. Analysis results are displayed under the **Analyses** tab in the **Overview** screen. Click on an analysis in this screen to display the results.

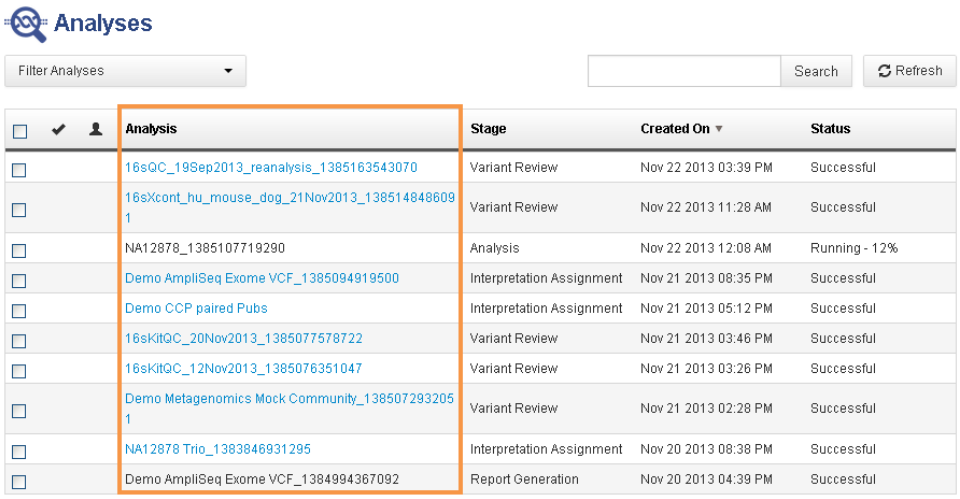
Review and interpret your DNA analysis results

To access the results of your DNA analysis:

1. Sign in to Ion Reporter™ Software, then click **Analyses**.



2. Click on the name of your analysis in the **Analyses** list.

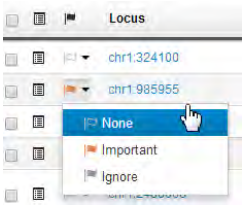
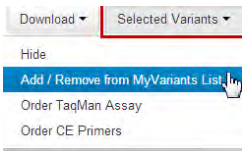


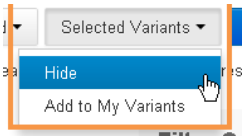
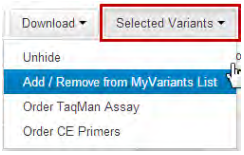
This action opens the analysis in edit mode.

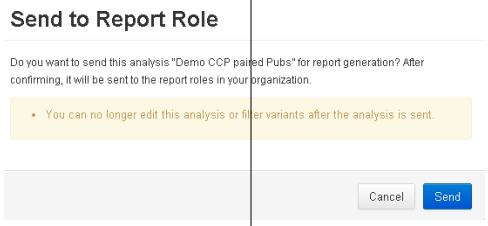
Note: You cannot review analyses that are still running.

To review your DNA analysis results, use any of the following tasks:

Purpose	Your action	
Display different data and annotations	Click one of the following options above the variants list: • **Summary** • **Functional** • **Population** • **Ontologies** • **Pharmacogenomics** • **Somatic** • **QC** The first few columns are the same for each option. If necessary, use the horizontal scroll bar under the table to see all the columns.	
View a variant's detail card	Click the detail icon for the variant.	
View the original source of annotation information	Click on a blue link in the **Genes** column or the **Variant Details** card. The links opens to a public database that contains the variant (such as OMIM or COSMIC).	
Filter variants	See "Filter variants" on page 197.	
View the variant in IGV	Click on the variant position link in the Locus column. See "Visualize other analyses with Ion Reporter™ Software" on page 268.	

Purpose	Your action	
Compare the results of multiple samples	Select multiple analyses in the Analysis Overview tab, and select **Actions** ▸ **Visualize**.	
Add a variant to the My Variants annotation source	Enable the checkbox for the variant, then click ▸ **Important** The selected variants (with flags now in orange) are added to the My Variants annotation source. See also "About the My Variants flags" on page 172.	
Remove one or more variants from the My Variants annotation source	Click the flag menu in the My Variants column and click the **None** option.  Or, enable its checkbox and click **Remove from My Variants** in the Selected Variants menu.  See also "About the My Variants flags" on page 172.	
Search variant information	See "Search on the Analysis Results list" on page 167.	

Purpose	Your action	
Hide variants	Enable the checkboxes for the variants to be hidden, click the **Selected Variants** menu, and click **Hide**.  Hidden variants do not appear in the Analyses list.	
Display hidden variants	In the **Filter Options**, click **Hidden Variants** link. 	
Restore hidden variants	Under **Filter Options**, click **Hidden Variants**. Enable the checkboxes for the variants that you want to restore, then click **Selected Variants ▸ Add / Remove from MyVariants List**, then optionally add or change the flag for the variant, then click **Confirm**. 	

Purpose	Your action	
Send to the Report Role	After you filter out unwanted variants, click **Send To Report Role** for interpretation. Read the consequences in the confirmation box: you cannot edit the analysis or filter its variants if you send it to a **Report Role**.  If you agree, click **Send**	
Leave the Analysis Results page	Click the **Back** in the browser to return to the **Analysis Results**.	

Note: The Allele Coverage column reports counts for all reported alleles, not only genotype alleles. In contrast, the Allele Read Count filter sets the minimum count of the genotype alleles.

Search on the Analysis Results list

Most searching is better done with filters, rather than with the search field. For most searches, use a filter instead.

Searches of analysis results can be performed in the Variant Review page using a controlled vocabulary query language. This allows more advanced searches, including OR and AND searches than is currently possible using filter chains. This feature is available in both a single-analysis variant review table and a multi-sample visualization table. However, the feature has only been tested on the single-analysis variant review table.

Filter terms

Notation	Meaning
key:value1,value2	key=value1 OR key=value2
key:[min,max]	min <=key <=max
key:(min,max)	min < key < max
key:[min,]	min <= key

key:[,max]	key <= max
key:(min,)	min < key
key:(,max)	key < max
key:value*	key contains value
key:*	key exists (key has any value)
-key:value	key != value

The following keys are supported:

Key	**Example**
locus	chr2:123456
function	missense
location	exonic
cosmic, omim, pfam, drugbank, go	glioma
dbsnp	rs12345
gene, transcript	TP53, NM_01010.1
maf	[0.0,0.05]
coverage	[1000']
sift, polyphen, grantham	['0.05]
type	INDEL
comment	something*
vkb	*

Search field behavior is different here on the variant table in the Analysis Results page, than for searches on other pages.

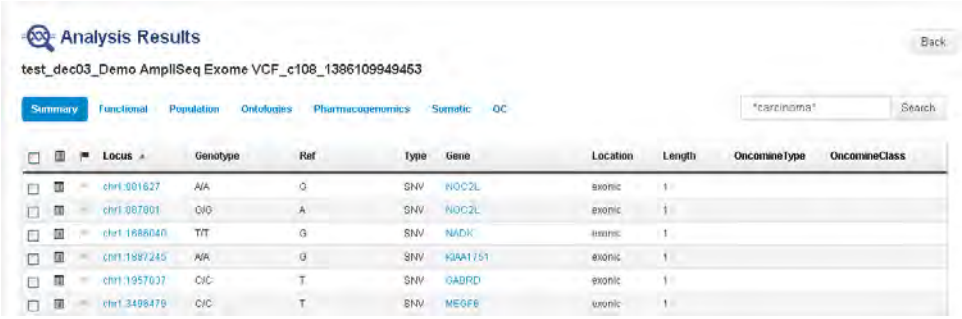
"vbk" in the table above stands for MyVariants.

Asterisks (*) for *some* variant table searches on the Analysis Results page

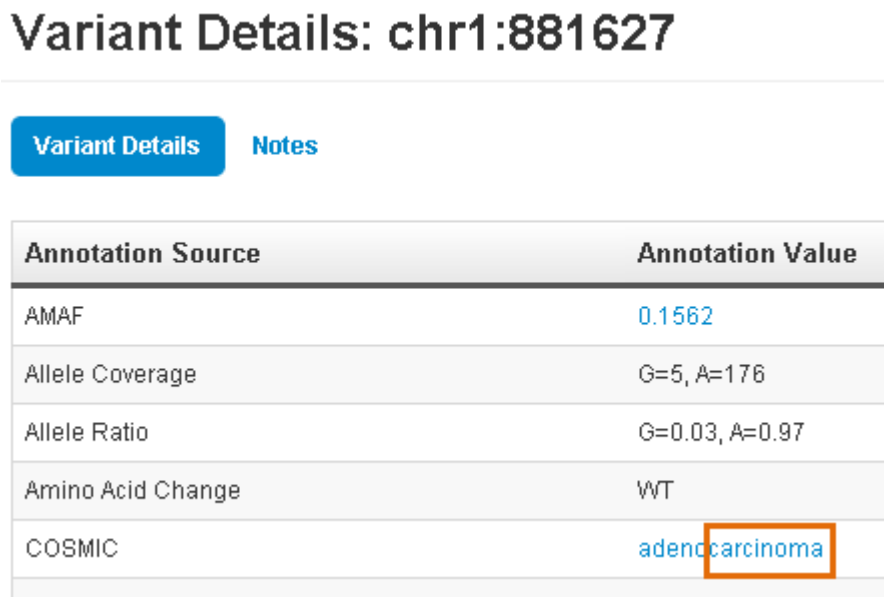
- An asterisk (*) in the search field is only allowed on the Analysis Results page.
- An asterisk (*) is required for some searches here, but is not allowed for other searches. The differences are due to how the different types of information are stored.
- The asterisk is a search wildcard. Without the asterisk, searches only match the exact string entered. With asterisks both before and after your search string, matches at the beginning, middle, and end are all found.

Recommended search with asterisks

- Asterisks are useful to search for matches in any annotation source. (By contrast, a filter search matches only one annotation source.)
- This example shows a search for *carcinoma*.



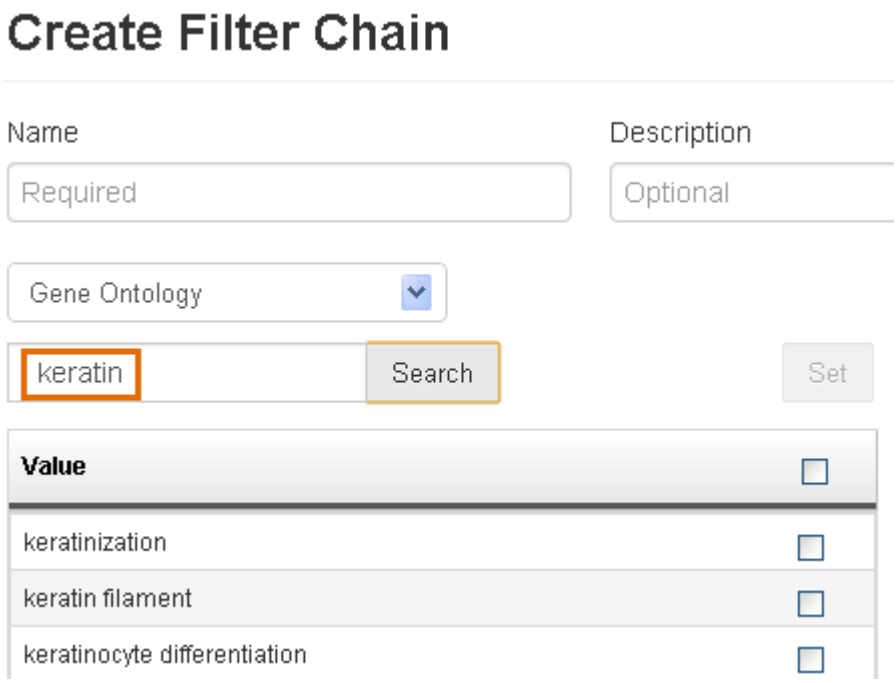
This search lists all variants that have an OMIM, COSMIC, ClinVar, DrugBank, and so on, annotation that contains "carcinoma" anywhere in the annotation. Click the detail icon for one of the matched variants to open its variant detail card. Scroll down to fine the entries that contain "carcinoma".



Searches without asterisks

- To search for a locus, enter the complete locus entry, with chromosome number and full position number.

- Do not use an asterisk (*) for a locus search. Searches on a chromosome number by itself or with a partial position number also are not supported.
- For other information, use a filter.
- Search terms within a filter chain also do not allow an asterisk.



Examples

- `(gene:TP53 OR (function:missense,nonsense AND (maf:[0.0,0.05} OR -dbsnp:*))) OR type:CNV`

  This retrieves all variants that fall in TP53, all CNVs, plus variants that have a functional impact of missense or nonsense AND either have a minor allele frequency less than 0.05 or are noval (not found in dbSNP).

- `cosmic:carcinoma* AND ((type:SNV AND sift:[0.0,0.10]) AND coverage:[300,] OR locus:chrX`

  This retrieves all SNV variants annotated with COSMIC histology terms containing "carcinoma" with a deleterious SIFT score (<0.10) and high coverage(> 300), plus any variant that falls on chromosome X.

Add or edit a note to a variant

1. In the Analysis Results screen, click + or ▪ in the **Variant Details** column (second from the leftmost column).

   Note:
   - The + icon indicates that the variant does not have any notes that are associated with that variant.
   - The ▪ icon indicates that the variant already has a note that is associated with that variant.

   The **Variant Details** screen appears.

2. Click **Notes**.
   A new **Variant Details** screen appears.
3. Enter the note in the **Note text** textbox.
4. Click **Add Note**.
   - You are returned to the **Analysis Results** screen.
   - If the variant previously did not have a note that is attached, the icon changes from + to .

View note attached to a variant

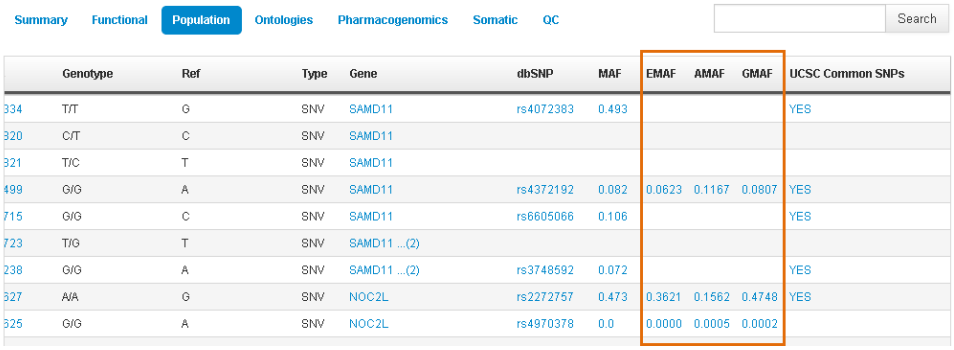
Variants with notes attached, display in the **Variant Details** column (second from the leftmost column).

In the **Analysis Results** screen, click in the **Variant Details** column for the variant of interest.
The **Variant Details** screen opens. Notes can be viewed in the **Variant Details** screen.

Links to external databases

In the following cases, the links to external databases that are included in the **Analysis Results** can lead to database pages that are not specific, contain an error, or are blank.

- The 5000 Exomes database link in Ion Reporter™ Software uses gene information. Because the 5000 Exomes site does not provide a unique identifier for each record, if the website does not have specific information about the gene of a variant, the link in Ion Reporter™ Software leads to a blank page. In this case, try to search for the rsID of the variant on the 5000 Exomes site. (Not all 5000 Exomes records contain rsIDs.) This image shows example 5000 Exome links in the Population tab of the variant table:



	Genotype	Ref	Type	Gene	dbSNP	MAF	EMAF	AMAF	GMAF	UCSC Common SNPs
334	T/T	G	SNV	SAMD11	rs4072383	0.493				YES
320	C/T	C	SNV	SAMD11						
321	T/C	T	SNV	SAMD11						
499	G/G	A	SNV	SAMD11	rs4372192	0.082	0.0623	0.1167	0.0807	YES
715	G/G	C	SNV	SAMD11	rs6605066	0.106				YES
723	T/G	T	SNV	SAMD11 ...(2)						
238	G/G	A	SNV	SAMD11 ...(2)	rs3748592	0.072				YES
327	A/A	G	SNV	NOC2L	rs2272757	0.473	0.3621	0.1562	0.4748	YES
325	G/G	A	SNV	NOC2L	rs4970378	0.0	0.0000	0.0005	0.0002	

- When a ClinVar annotation does not have a CLNACC ID associated with it, the annotation string is "untested" and the link leads to a generic ClinVar page instead of to the specific variant page.

- Some COSMIC records' link-outs may no longer be displayed on the COSMIC website. In such cases, the link-out leads to a generic COSMIC page that displays "no entry found".

About the My Variants flags

The My Variants flags store information about specific variant positions (loci) in the My Variants database (formerly known as VariantKB).

You can view and export My Variants annotations in reports. As you analyze and create reports, you can view the list of variants in the **My Variants** screen under the **Analysis** tab. You can see the flag status, locus, and overlapping gene(s) of each variant in **My Variants**. You can see additional details for each variant such as the list of analyses in which the variant was called. You can also click through the list of My Variants and search on the list of My Variants.

Note: Parameter settings for the Hit Level in the My Variants database are deprecated. For instance, the attribute **gene** does not work.

There are three options for the My Variants flag:

- **None:** Removes current flags and removes the variant from your MyVariant's annotation source.
- **Important:** Adds a variant to the MyVariants annotation source marked as **Important**.
- **Ignore:** Adds a variant to the MyVariants annotation source marked as **Ignore**.

My Variants flags affect analyses in the following ways:

- **Appear in all analyses in your organization:**These flag settings affect all of your analyses, including completed analyses. For example, if you set a variant as Important, then open a previous analysis that includes that variant, the variant is flagged as Important in the Analysis Review page.
- **Appear in other users' analyses:** These flag settings affect all analyses in your organization (not just your own analyses).
- **Supports only one setting:** The last setting of the My Variants flag takes effect for that variant in all your organization's analyses.

  Note: If multiple people in your organization use the **My Variants** flag, it is possible for inadvertent conflicts to happen. For example, one person could set a variant as **Important** and another person, reviewing a different analysis, could set the flag to **Ignore** for the same variant. In this case, only the last flag setting is kept in Ion Reporter™ Software. More importantly, the difference of opinion between the two reviewers is lost. If it is important for your organization to keep

a record of decisions about a particular variant, consider using the **My Variant Notes** to manually record the following information about the flag when a setting is reversed or undone.

- **In a deleted original analysis:** If you delete the analysis in which you originally flagged a variant, its My Variants status is not affected.

Add notes to My Variants

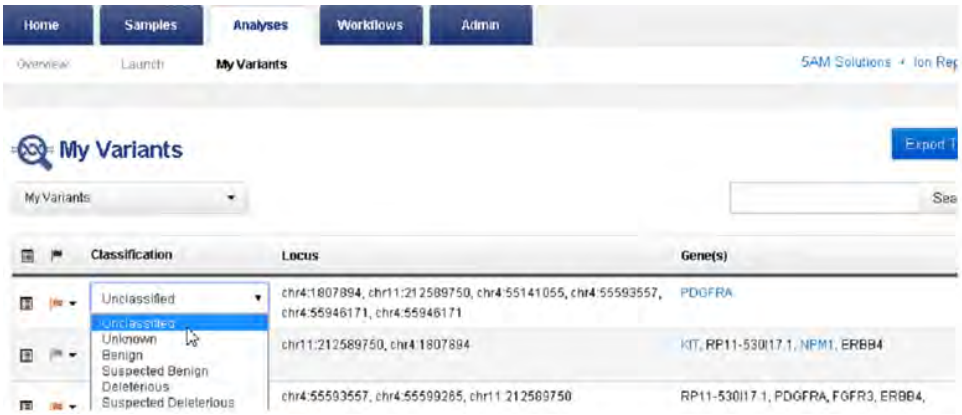
You can add notes to My Variants to explain why a variant is flagged as important, or flagged to be ignored. You can also use the **My Variant Notes** to record information about a flag when a flag setting is reversed or undone, if it is important for your organization to record decisions about why a flag was changed.

Note: These notes are seen only within the analysis in which the notes were originally entered.

1. Click the detail icon for the variant to open the Variant Details card, then click the **Notes** tab.
2. Enter text for the note, then click **Add Note**.
   The **Notes** field automatically records the date and your login ID when you add the Note.

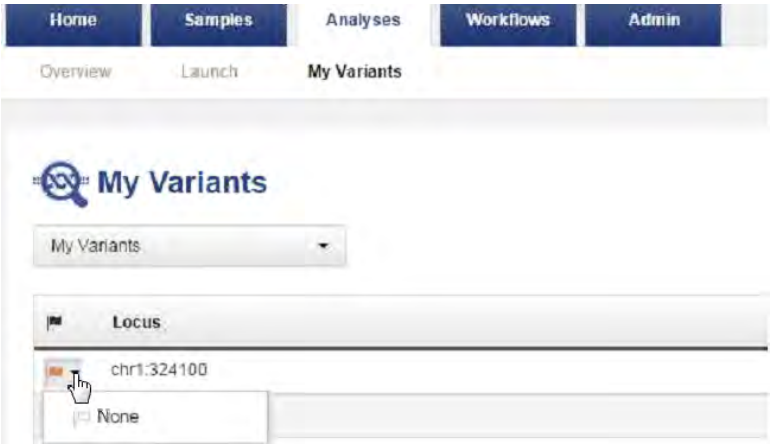
Classification column in My Variants

A Classification column is now visible on the My Variants list. Variant classifications can now be viewed in different analyses. Classifications include: Unclassified, Unknown, Benign, Suspected Benign, Deleterious, and Suspected Deleterious.

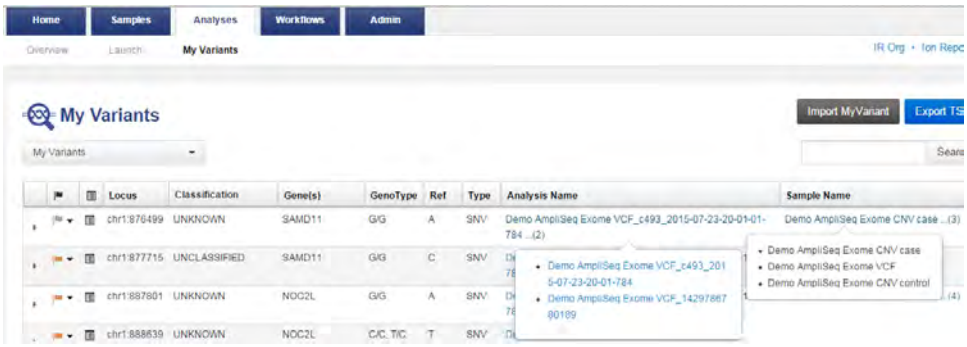


You can view notes about variants in the **My Variants Notes** section. Click on the notes tab of the details card to view notes for the My Variants.

To remove a flag from a variant, right-click on the flag and select **None**. The flag will be removed when you refresh your screen.



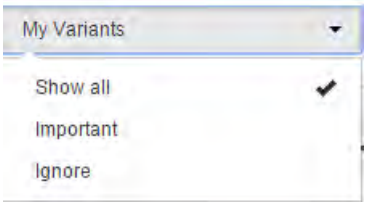
In addition, links in the Analysis Name and Sample Name columns link to analyses and samples that contain the variants.



View and export My Variants annotations

You can now view and export all favorite variants, that is, those flagged **Important** or **Ignore** in reports.

1. Under the **Analyses** tab, click **My Variants** to open the list of variants.
2. Click **My Variants**, then select **Show All**, **Important**, or **Ignore**.



3. Click **Export TSV** .

4. When the download is finished, a myvariants_results.zip file is downloaded in the browser. Open or save the file to your hard drive.

   The TSV file contains the information for each variant such as Locus, Gene, Type, Flag Type, Analysis Name, and so on. You can also view additional details for each variant such as the list of analyses in which the variant was called.

   Note:
   - When flagging a variant from a paired-sample analysis, the variant from the primary sample (not control) is picked and stored in the My Variants database. If there is no explicit call at the location in the primary sample, then nothing gets added to the My Variants database.
   - Sorting on the **Gene(s)** column factors for each gene in a multi-gene result. Therefore the order of the genes in one row is by chromosomal order, not alphabetical order.
   - My Variants from analyses in Ion Reporter™ Software 4.6 and later show all details correctly in the My Variants table (comments, analysis/sample names, and so on). Full details might not appear in Ion Reporter™ Software 4.4 and earlier analyses, due to backward incompatibility.

Variant uniqueness in MyVariants databases

The variant fields that are examined to evaluate whether a variant is already present in a MyVariants database are the following:

- Chromosome number
- position
- end position
- variant id
- ncbi (for GRCh38)
- chr_type (for GRCh38)
- CNV subtype
- observed allele
- genotype
- fusion 3' breakpoint
- fusion 5' breakpoint

Differences between VariantDB and My Variants

VariantDB is an annotation source preset used during workflows to annotate analyses. VariantDB is not designed to be dynamically updated and changes made to its contents will not impact previously-run analyses. My Variants flagging functionality provides the ability to dynamically annotate variants as "Important" or "Ignore" for analyses which have been run previously, and those flags are stored and will be applied in future analyses until removed with the "None" flag.

CNV subtypes in the Analysis Results screen

Some CNV subtypes in the **Analysis Results** screen are specific for Oncomine™ research assays. See your Oncomine™ assay user guide for more information.

Known common CNVs

CNV variants should be interpreted in light of a resource, such as the **Database of Genomic Variants**, that contains known common CNVs.

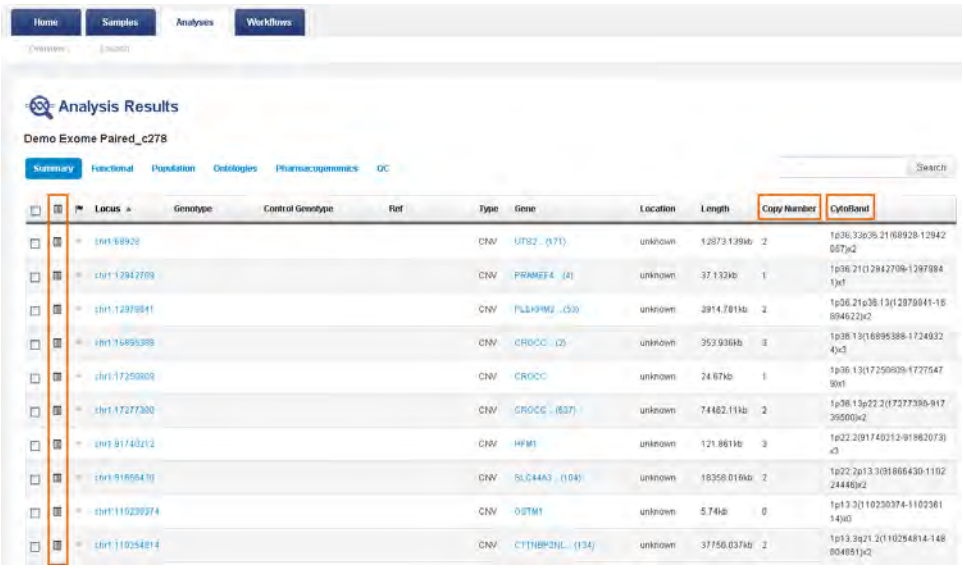
This database shows where common and (usually) non-pathogenic CNVs often occur in various populations. When you design amplicons to detect CNVs, avoid putting amplicons in regions that contain many common variants. Amplicons in these regions tend to indicate common non-pathogenic copy number variants that are based on population differences rather than on pathogenic events.

CNV variant filter

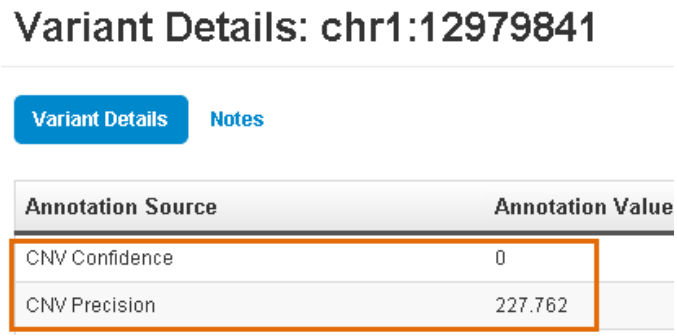
For germline analyses, select the filter "Confident Germline CNVs - CNVs Only". This filter is not automatically applied as a default. Save the filter chain in an Analysis Results page for the filter to be the default filter for that analysis.

CNV variant information

The Copy Number and CytoBand information for a CNV variant appear in the Summary tab of the variant table.



The Confidence and Precision scores appear in the variant detail card.



(Click the detail icon for the variant to open the variant detail card.)

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 (CN) 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.

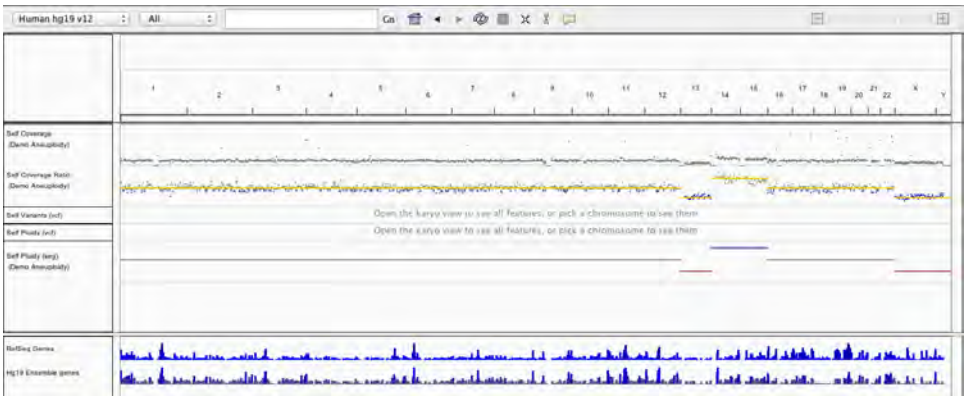
Note: MAPD is not shown on the QC Report.

Mitochondrial Autosomal read count ratio scores

For aneuploidy analyses, the ratio of mitochondrial(chrM) DNA read counts to autosomal (chr1-22) DNA read counts is reported.

CNV variant visualization in IGV

The coverage tracks in the IGV browser visualize mosaic data whether or not a copy number gain or loss is called in the region.



This information serves two purposes:

- Confirms that the data supports the ploidy calls made by Ion Reporter™ Software: The colored tile data appears on or very close to the called ploidy change.
- Helps identify mosaic copy number changes that are not called as a ploidy change:
  Non-integer copy number changes are apparent in the colored tile data as a cluster of tile coverage ratio data that is consistently a little above or a little below the gold ploidy line.

Notes about CNV and ploidy visualization in IGV:

- The red lines represent regions of copy number loss. The blue lines represent copy number gains.
- The gold line in the Coverage Ratio track gives the ploidy call made by Ion Reporter™ Software.
- The colored data points around the gold line are the coverage ratio signal intensities for the individual Amplicon Designer tiles as expressed in ploidy space. These data points are the raw tile data.
- Both the Coverage and Coverage Ratio tracks present mosaic data.
- Notes about the Coverage Ratio track:
  - This track has coverage overlaid on ploidy calls to show the data supporting the ploidy calls.
  - This track's data scale is less compressed than the scale of the Coverage track.
  - This track plots the estimate of the most likely copy number (the raw copy number without rounding) of the test sample at this tile, given only the coverages at this tile.

The IGV Coverage tracks contain coverage data that is not available in either the variant detail or the variant table. This data is available in the workflow output files and can be exported. (This applies to the aneuploidy workflow and to the single sample workflows, paired workflows, and tumor/normal workflows that support CNV calls.)

Note: IGV assumes that a sample's gender is female, if the sample's gender is not specified or is specified as "Unknown".

Gender Calling in the Aneuploidy workflow

Starting with the Ion Reporter™ Software version 4.2, the Aneuploidy workflow will make a call as to the gender of the sample (Male or Female) base on the presence or absence of a Y chromosome. This call does not overwrite an user-entered gender information. The Called Gender is used to determine the expected ploidy of the sex chromosomes in the sample and to report aberrant Copy Number variants. The 4.0 versions of the Aneuploidy workflow will have sex chromosomes called according to the table below.

Copy number calls on X and Y chromosomes in non-Aneuploidy workflows which detect CNVs

This table shows how the user-assigned gender of the control and test samples affects CNV calling restrictions in Ion Reporter™ Software. These rules apply to the single sample workflows, the paired workflows, the tumor/normal workflows, and the 4.0 Aneuploidy workflow.

Test sample	Control sample	CNV calling restrictions
Male	Male	Call CNVs on all chromosomes
Male	Female	Do not call CNVs on the Y chromosome
Female	Female	Do not call CNVs on the Y chromosome
Female	Male	Do not call CNVs on the Y chromosome
Unknown	Female, Male, or Unknown	Do not call CNVs on the X or Y chromosome
Female, Male, or Unknown	Unknown	Do not call CNVs on the X or Y chromosome

Ploidy states and CNV variants in control samples

Copy number state and confidence scores on X and Y chromosomes are determined relative to the gender of the control sample. For example, the expected ploidy of X for a male control sample is 1. If the test sample also has 1 copy of X, the confidence score for a CNV will be 0, since there is no evidence of an unexpected ploidy state.

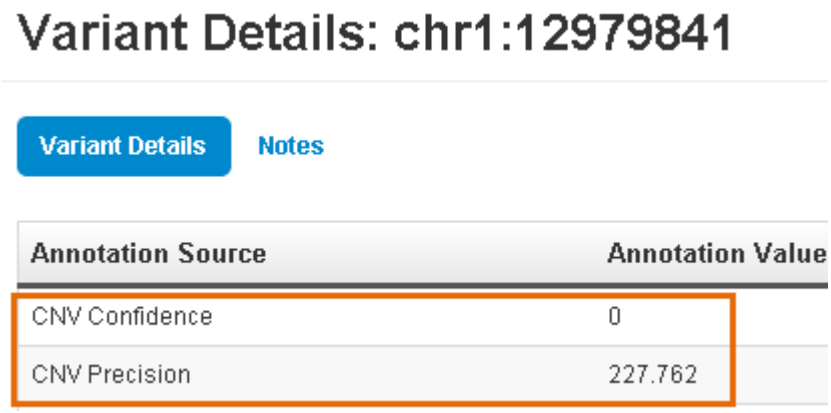
If most or all test samples report a CNV in the same region, one possible cause is that the control sample actually has a CNV in that region (leading to unexpected results).

Copy Number scores

Germline Copy Number variant calls involve the following scores:

- **Copy number** -- The predicted copy number for the region.
- **Confidence** -- The log likelihood that the called copy number state is not normal ploidy, i.e. 2 on autosomes (reflects the likelihood of the region's ploidy number being different than the normal ploidy 2).
- **Precision** -- The log likelihood that the called copy number state is different than next closest copy number state (reflects the likelihood that the precise ploidy number is correct).

Note: Confidence and Precision scores appear in the variant detail card.



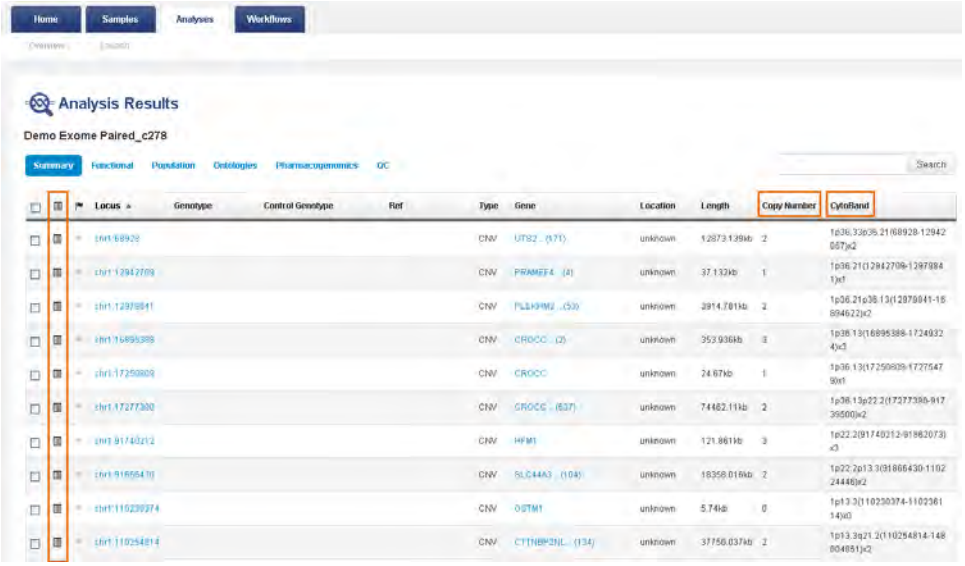
Click the detail icon to open the variant detail card.

Note that the algorithm can call a ploidy variant with good confidence in the variant (a high confidence score) but without confidence in the called ploidy number (a low precision score), especially for high ploidies. For example, a ploidy variant with copy number 5 might have high confidence but low precision. These scores mean that we are confident that the ploidy is not 2, but we also are not confident that the ploidy is exactly 5.

IMPORTANT! Ploidy results depend on the correct gender attribute being assigned to the input samples.

Copy Number

The Copy Number value gives the predicted number of chromosomes for the variant region. The column appears in the variant table (on the Analysis Results page)



Note: The maximum copy number that we report for a variant is 10. A copy number value of 10 is interpreted as copy number 10 or higher.

The length of the affected region is also given (in the Length column).

Precision

The Precision score reflects the likelihood that the stated copy number number is correct. This score is expressed as the log likelihood of the copy number number.

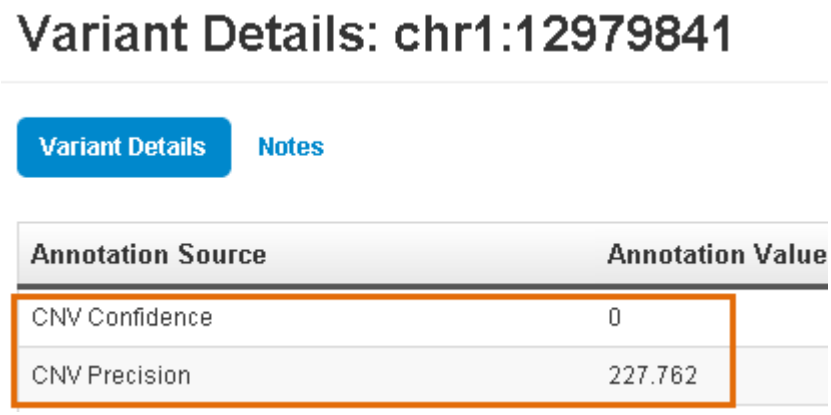
The precision is defined as the likelihood determination of the assigned copy number state and a state that is closest to it. Small precision denotes the uncertainty in the absolute copy number value assignment. Quite often high copy number states have a low precision and high confidence, indicating that the algorithm is uncertain about the absolute copy number value (N or N-1 or N+1), but quite certain about the existence of a copy number state that is different from the expected copy number (N ! = 2 for expected diploid region).

Note: The expected copy number states are set from the customer-specified gender of the sample. Gender defaults to male if unknown. Correct scores depend on the gender of the input samples being correctly specified.

Confidence

The Confidence score describes the likelihood of the region's copy number number being different than the normal copy number 2.

The Confidence Score appears in the variant detail card.



This list describes the confidence score:

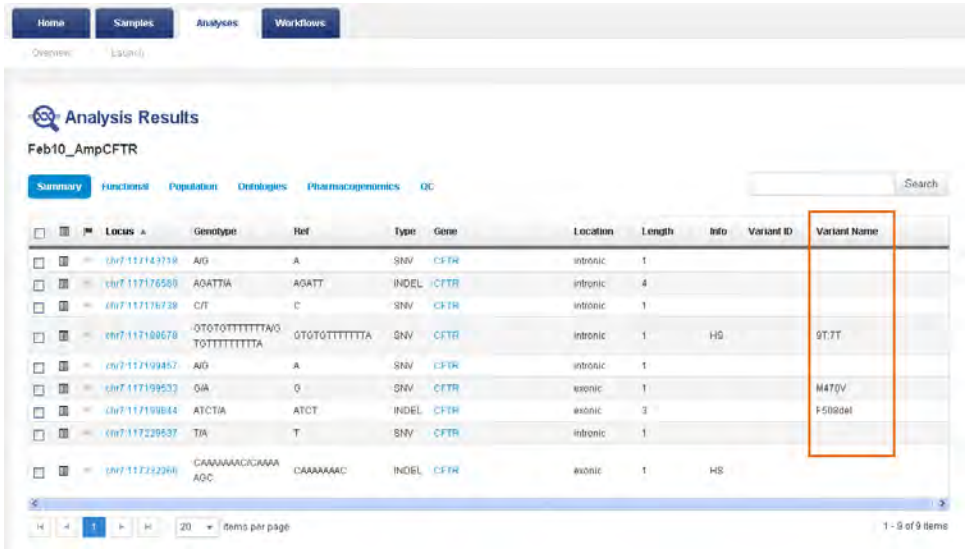
- A confidence score of 0.0 indicates copy number 2.
- Variants with a higher confidence score are more likely to be true positives. Variants with lower scores are less likely to be true variants.
- A confidence score of 10 or 20 or higher indicates a high quality copy number variant.
- True copy number variants might still have a low confidence score if the data is noisy, if the variant region is short, or if coverage is low.
- The confidence score addresses the likelihood of the copy number number being different than normal. The confidence score specifically does not predict that we know the correct copy number number.

Confidence is defined as the likelihood determination between the called copy number state of the segment and the expected copy number state. Confidence is small for segments that have copy number state likelihood close to the expected copy number state likelihood. A high confidence score means that the algorithm is very certain that there is a copy number state that is different from the expected.

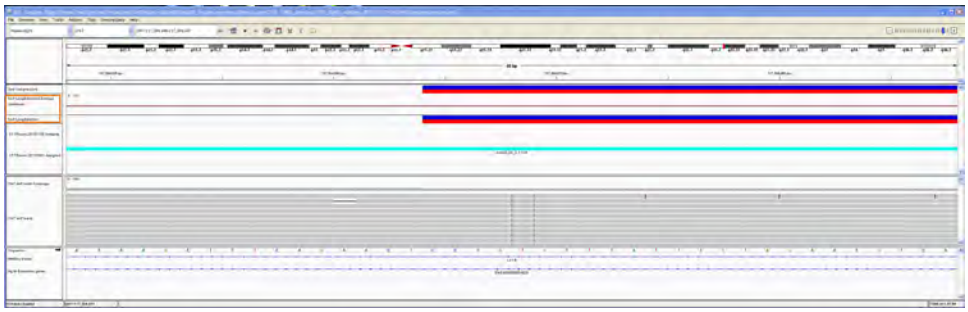
CFTR analysis results

Review the following examples for information about how to review data in the analyses results from CFTR workflows:

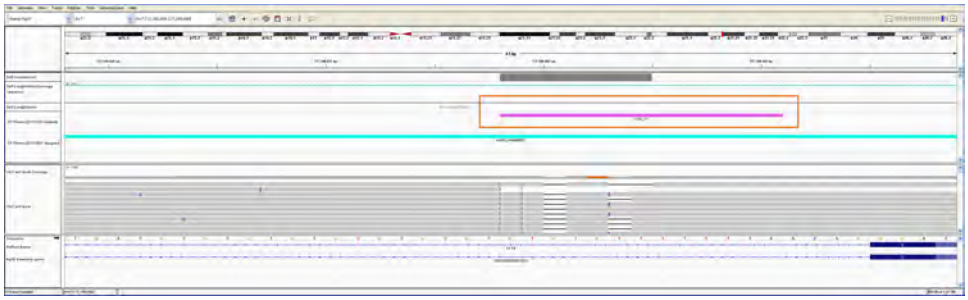
- The **Variant Name** column reports matches with the Named Variants annotation source.



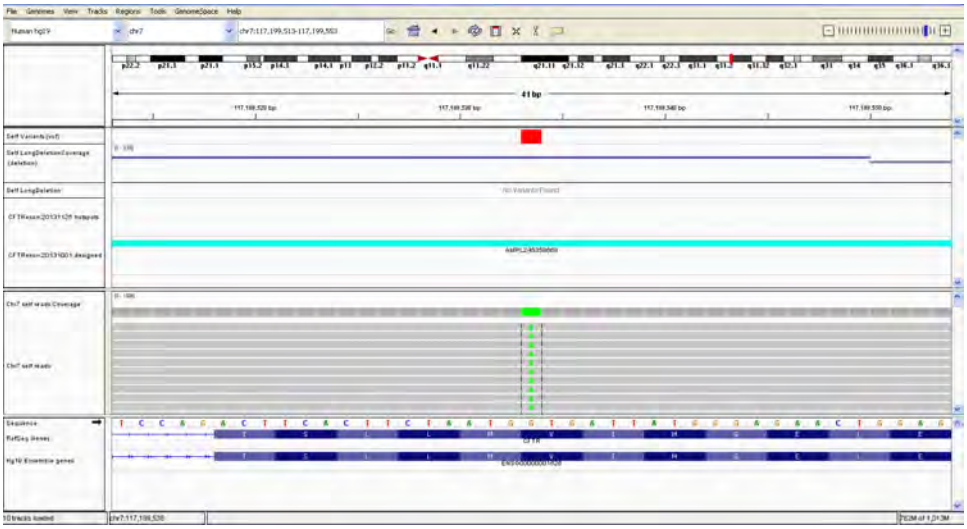
- The following IGV visualization for CFTR analysis results include tracks for long deletions:



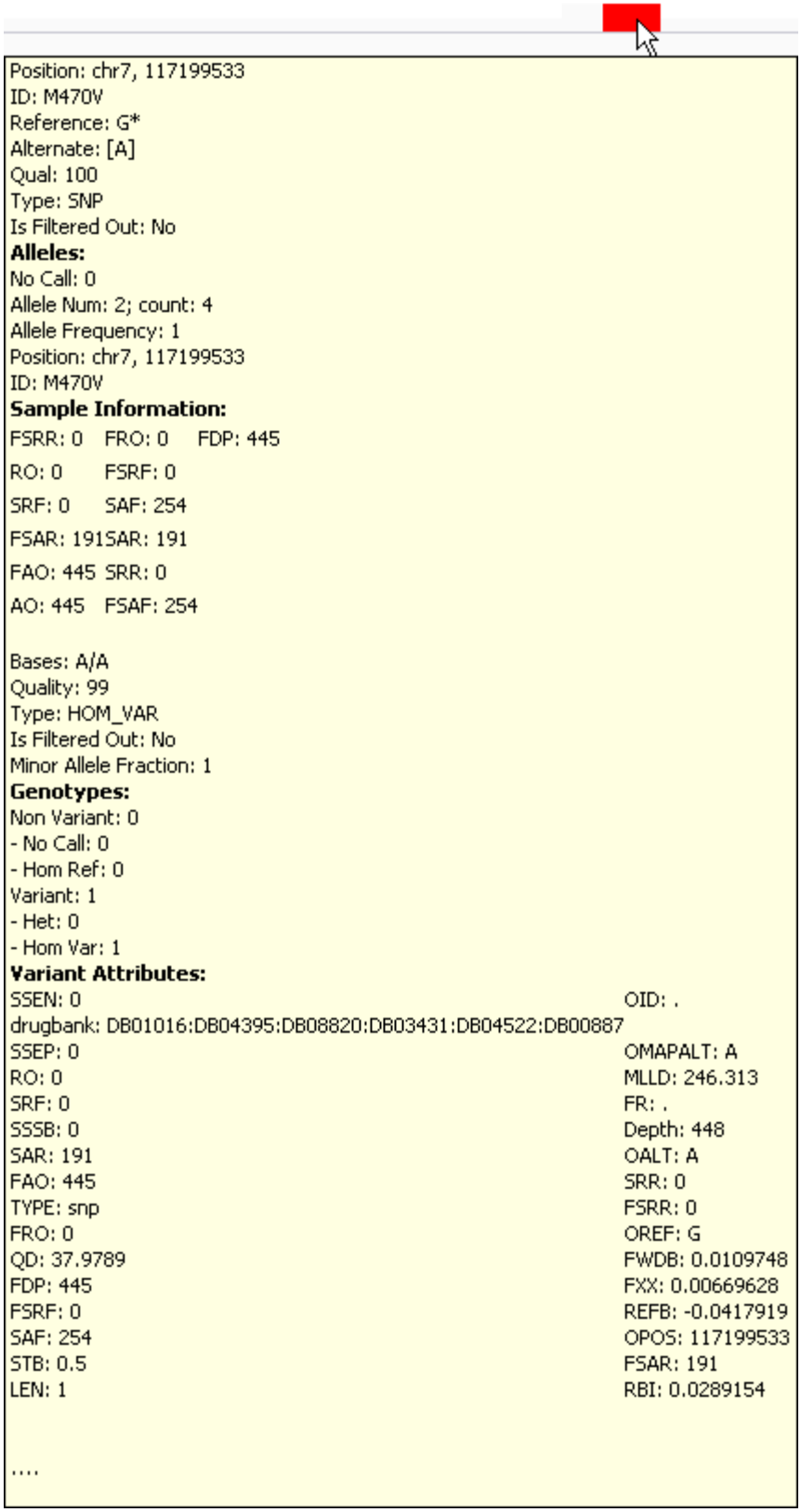
- The following IGV visualization for CFTR analysis results include a named variant:



The following IGV visualization for CFTR analysis results include a hotspot position:



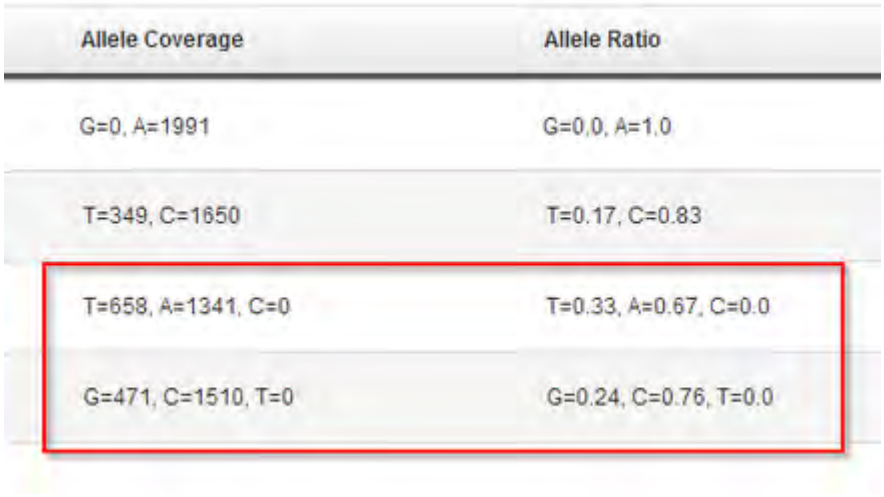
Hover the mouse over a position in the IGV visualization to view details such as the following:



Note: The factory-provided CFTR workflow includes long deletion detection. You can copy/edit the CFTR workflow and preserve long deletion detection. If you create your own CFTR workflow (even with the CFTR regions file), your workflow includes the standard Ion AmpliSeq™ workflow template and does not include the long deletion detection module.

Allele calls for hotspot positions

For hotspot positions, sometimes an allele with zero coverage is reported in the Allele Coverage and Allele Ratio columns of the Analysis Results table.



Allele Coverage	Allele Ratio
G=0, A=1991	G=0.0, A=1.0
T=349, C=1650	T=0.17, C=0.83
T=658, A=1341, C=0	T=0.33, A=0.67, C=0.0
G=471, C=1510, T=0	G=0.24, C=0.76, T=0.0

This is by design and occurs only with hotspot positions. For hotspot positions, the results table reports all alleles that have any evidence in either basespace or in flowspace. Alleles with zero coverage appear in the results table for a hotspot position if the second alternate allele has non-zero reads reported in basespace (even though zero reads are reported in flowspace).

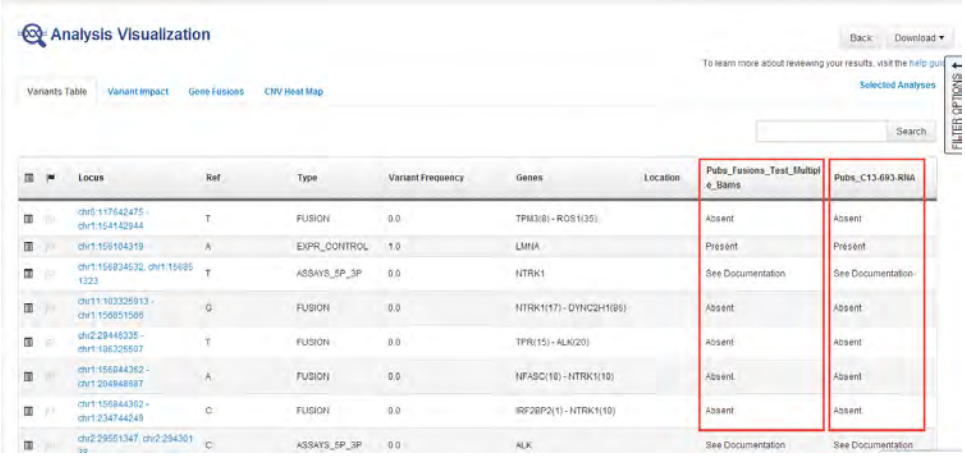
Phred QUAL Score

Phred quality score column is added to the **Analysis Results**.

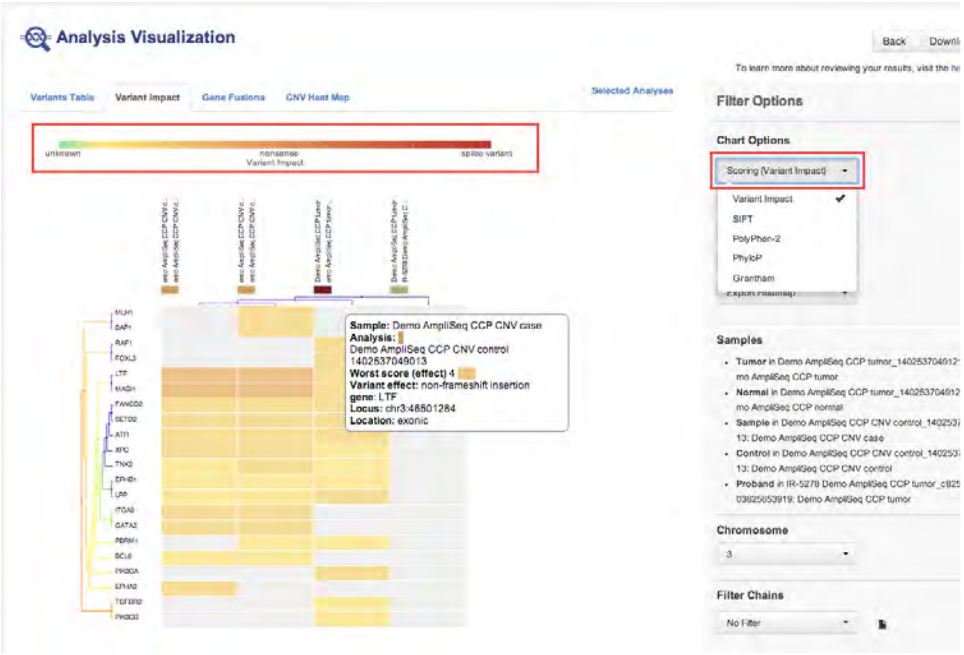
Visualize results of single or multiple analyses

You can visualize results for single analyses, or compare the results of multiple analyses. Multi-analysis visualization supports the following views, depending on workflow type.

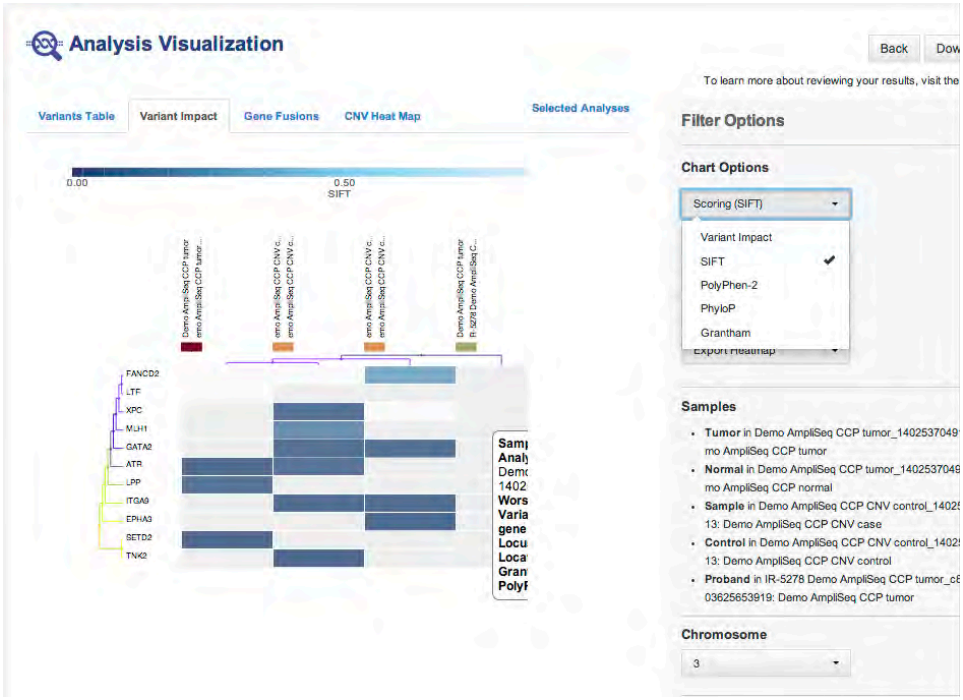
- Table view, with side-by-side columns to compare variant calls in different analyses:



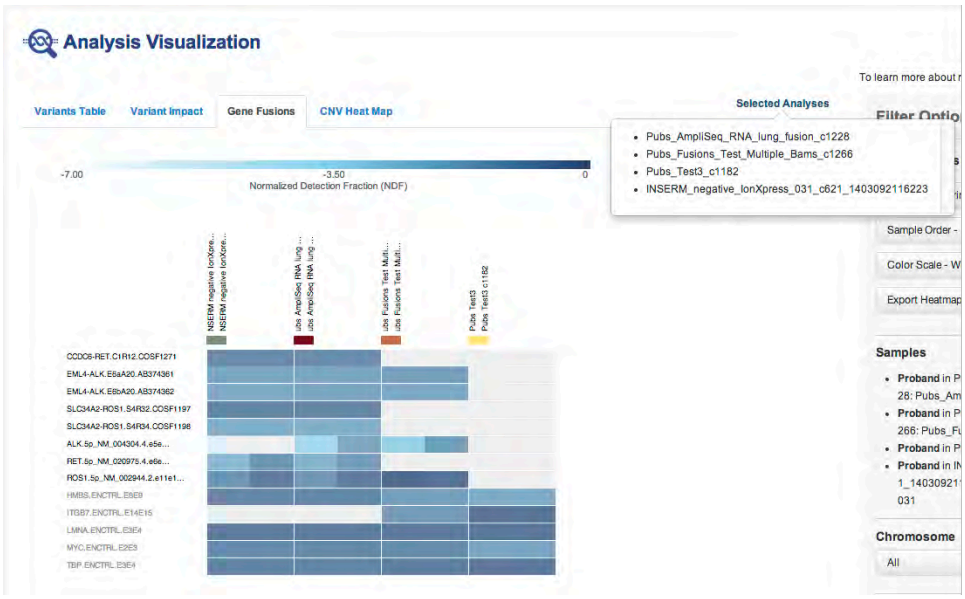
- Variant Impact heat map that is based on the predicted variant impact:



Variant Impact heat map that is based on other scoring:

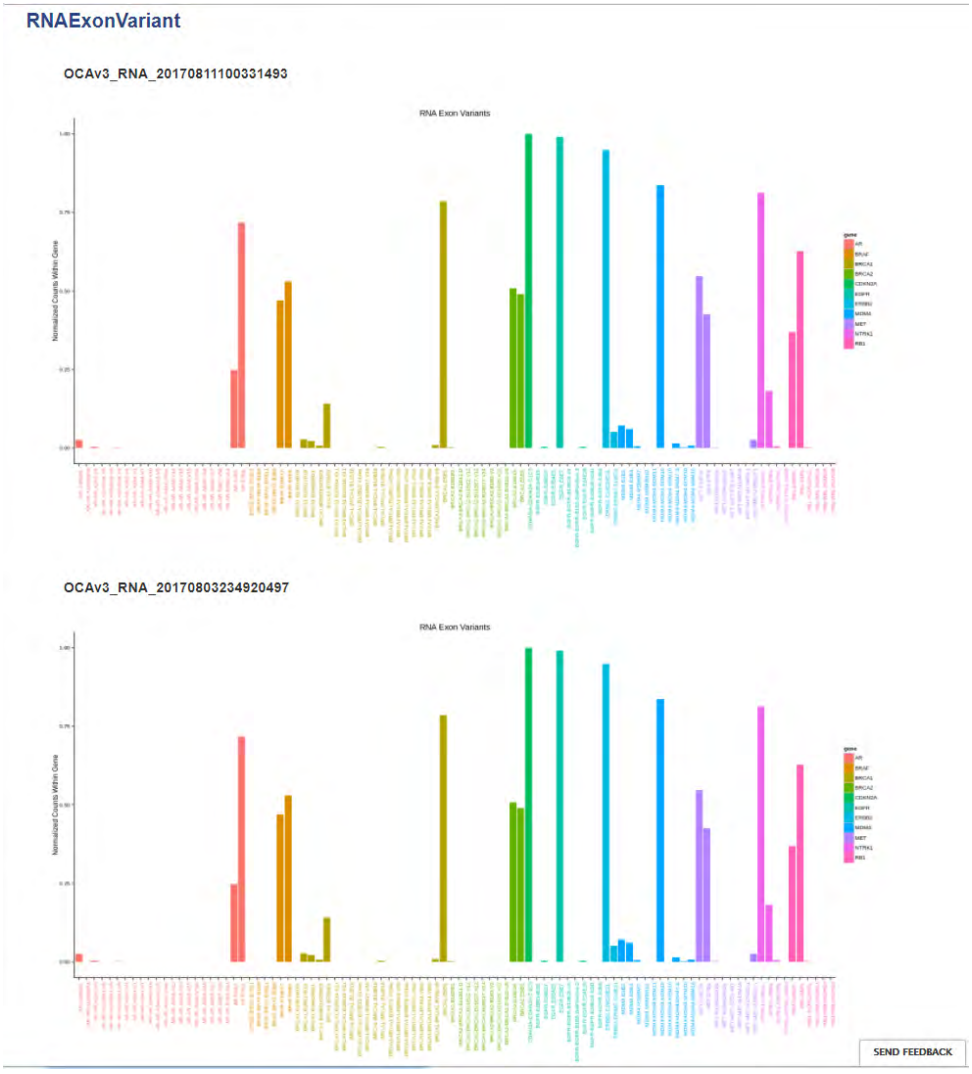


- Gene Fusions heat map:



- CNV heat map:

- RNA Exon Variants:



Note:

- In most cases, you will compare similar analyses. However, Ion Reporter™ Software does not restrict comparisons to only similar analyses.
- You can compare one trio or one paired analysis without selecting multiple analyses. Each trio or paired analysis contains more than one set of variants.
- Hover over the legend of a heat map:

Hover over the legend of a cell to see more information:

Visualize Multiple Analyses with Venn Diagrams

The following attributes are used to compare what variants fall in the intersecting areas versus the non-intersecting areas of the Venn diagrams for the different tabs in the Visualize page. If these attributes of the compared samples' variants are the same, they are considered "common" and add a count of 1 in the corresponding samples' intersecting area. Otherwise, they are considered different and add a count of 1 to each of the sample's non-intersecting areas.

Main Variant Table	variant chromosome + start position + end position + optional fusion ID
Variant Impact Heatmap	gene symbol
CNV Heatmap	gene symbol + CNV chromosome
Fusion Heatmap	fusion 5' breakpoint chromosome + pos + fusion ID

Therefore the counts in the Venn diagram (as well as the counts for the Total, Filtered-In, and Filtered-Out) represent the following:

- Main Variant Table: number of unique variants
- CNV Heatmap: number of unique genes (genes with same name but on different chromosomes are treated as different)
- Fusion Heatmap: number of unique fusions

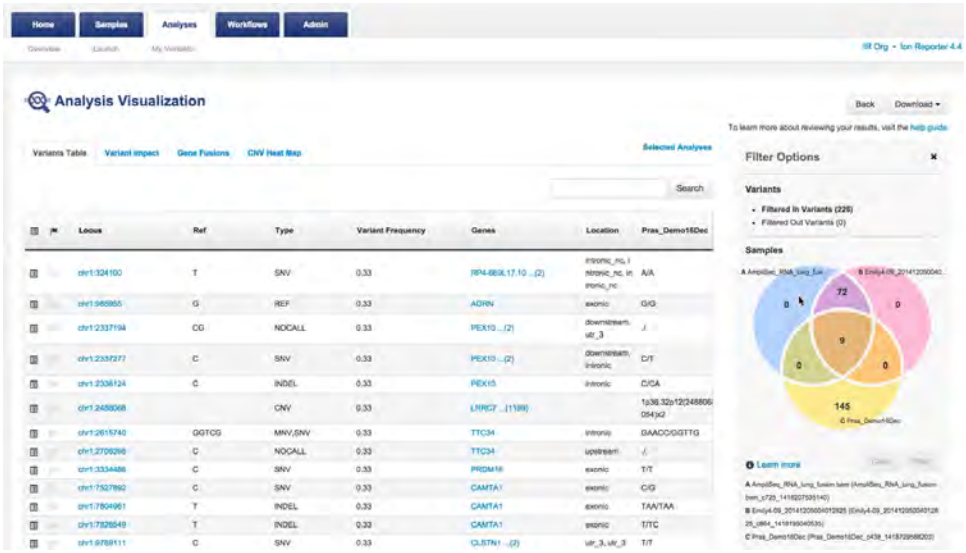
Note: If you edit an analysis and concurrently try to view it on another page, a negative filtered-out count may occur. Perform these tasks consecutively.

Compare multiple analyses and download TSV file

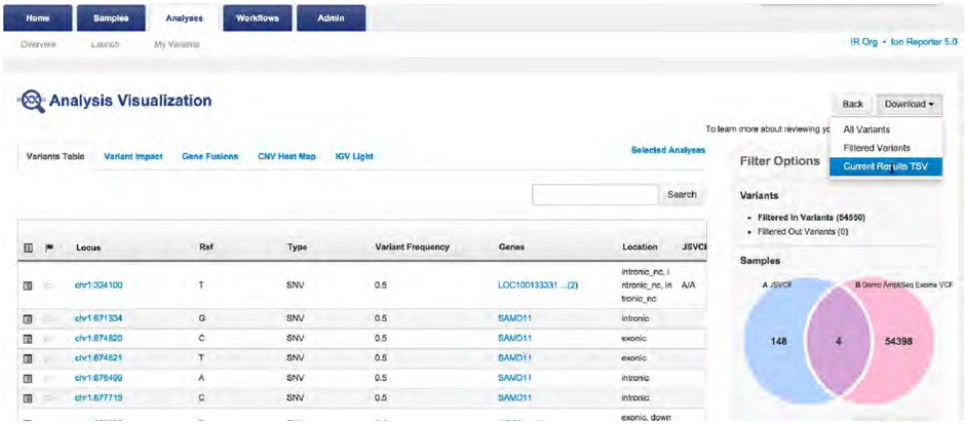
Follow these steps to compare multiple analyses:

1. In the Analysis > Overview tab, select multiple analyses.
2. *(Optional)* Use the Filter Applications menu to view only analyses of interest.
3. In the **Actions** menu on the right, select **Visualize**.

4. All results are shown initially.



5. Click the numbers in the Venn components to toggle for inclusion.
6. Then use the Filter button to update the results to include only toggled components.
7. Use the Clear button to deselect all Venn components.
8. When no components are selected, no Venn filtering is performed.
9. Hover over a sample name to see the analysis to which it belongs.
10. To download the results, go to **Download ▸ Current Results TSV**.



The software outputs a zipped TSV file of all the analyses for comparison.

Clustering in CNV heat map For CNV heat maps, ploidy is used as the score for every gene-sample pair to generate the heat map. Thereafter hierarchical clustering is conducted for clustering genes and samples within the heat map.

Note on the Variants Table view

The contents of the Samples column vary by variant type:

- **SNVs and indels:** Genotype
- **CNVs:** Cytoband
- **Fusions:** Presence or Absence detection call

Restrictions on analysis selection

- Comparison and visualization are not supported for Metagenomics analyses. If one of these is among the analyses selected, then the Visualize option is not available in the Action menu.
- If a Failed or In-Progress analysis is among the analyses selected, then the Visualize option is not available in the Action menu.
- If only one set of variants is selected, the Visualization option is not available:
  - Trio and paired analyses each contain more than one set of variants. These analyses support visualization one a single trio or paired analysis.
  - A tumor-normal analysis has only one set of variants and does not support visualization. (A tumor-normal analysis reports only the set of variants for the tumor sample, not the set of variants for the normal sample.)

Variant Impact tab

The legend at the top of the heat map is color-coded for the following variant impacts using the associated score values:

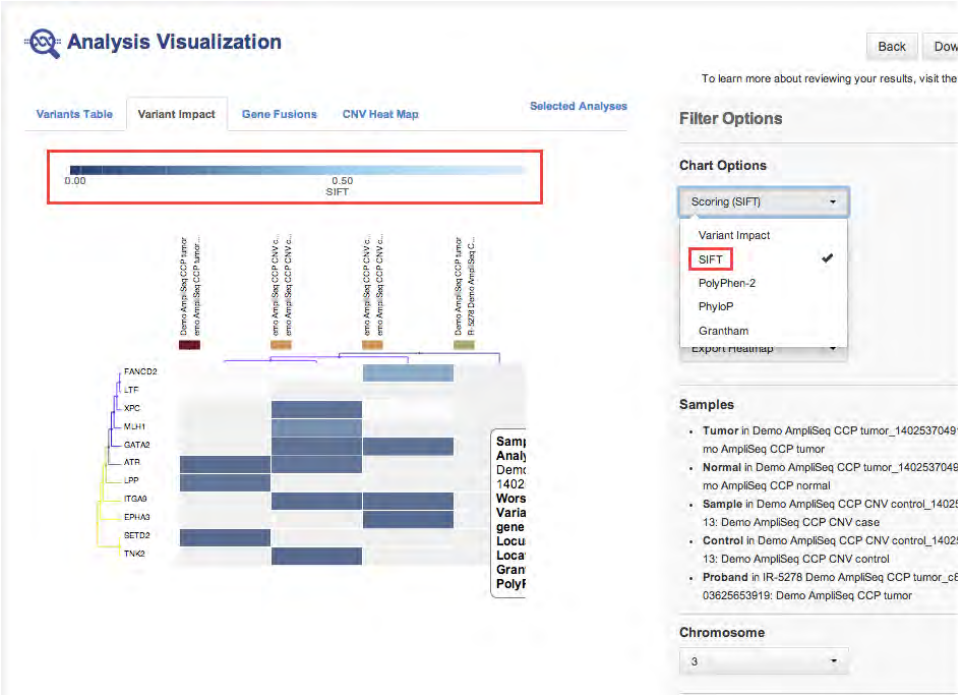
- unknown: 0
- synonymous: 1
- missense: 2
- non-frameshift block substitution: 3
- non-frameshift insertion: 4
- non-frameshift deletion: 4
- nonsense: 5
- stop-loss: 6
- frameshift block substitution: 7
- frameshift insertion: 7
- frameshift deletion: 7
- splice variant: 8

Set the Scoring menu to a different option, such as SIFT or PolyPhen2 , to base the legend and the heat map on those scores.

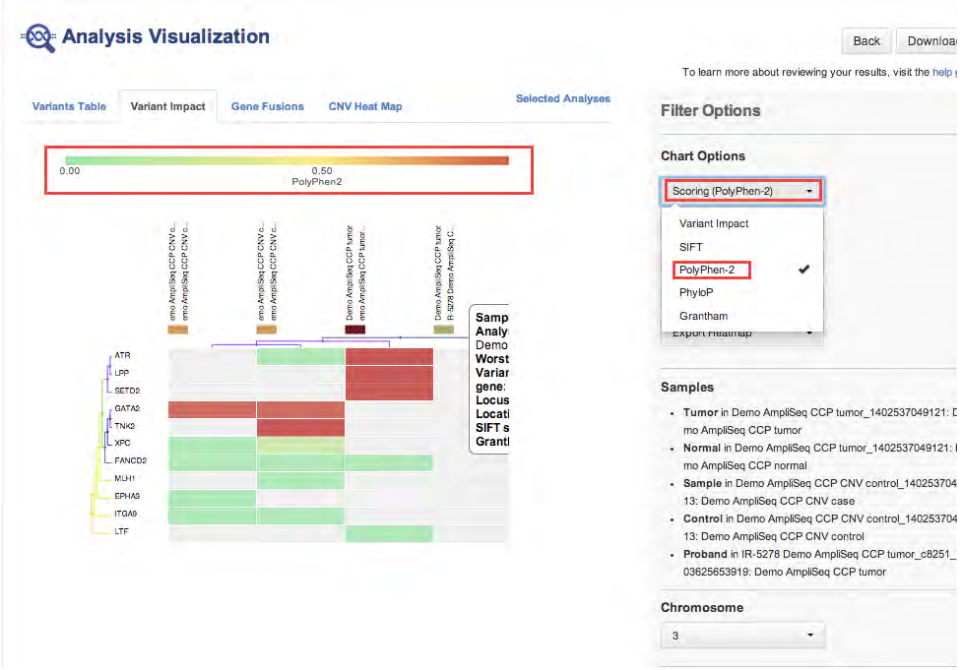
Clustering in Variant Impact heatmap For variant impact heat maps, based on the scoring criteria selected from chart options, most deleterious score is picked for every gene-sample pair to generate the heat map. Thereafter hierarchical clustering is conducted for clustering genes and samples within the heat map.

Chart options

Set the Scoring menu to a different option, such as SIFT

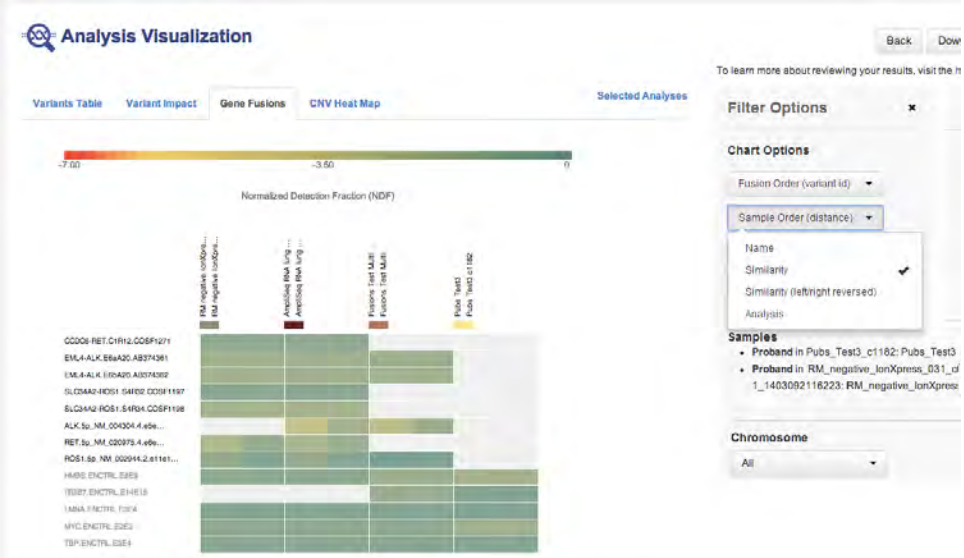


or PolyPhen2



, to base the legend and the heat map on those scores.

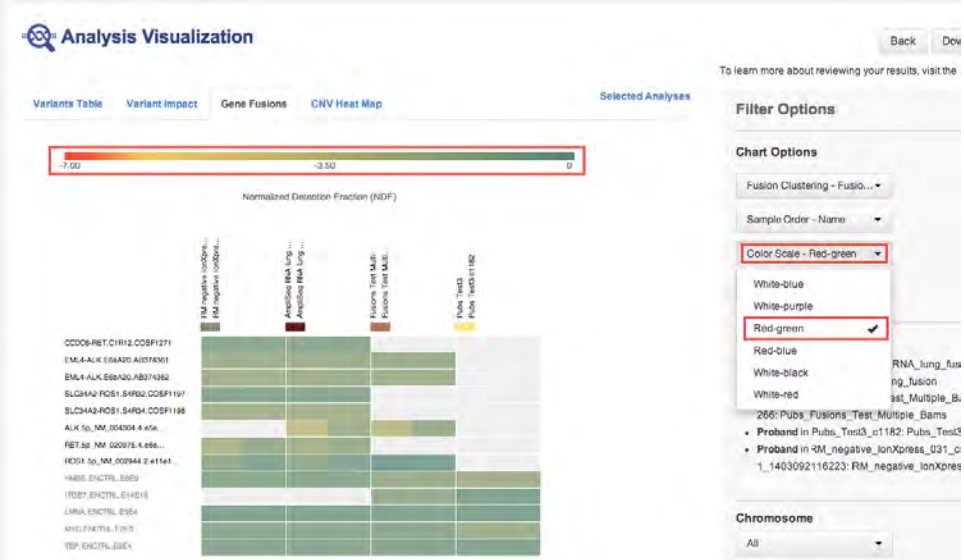
Set the Sample Order menu to base the presentation order of the sample on name or on their similarity in terms of variants found.



The following ordering options are supported:

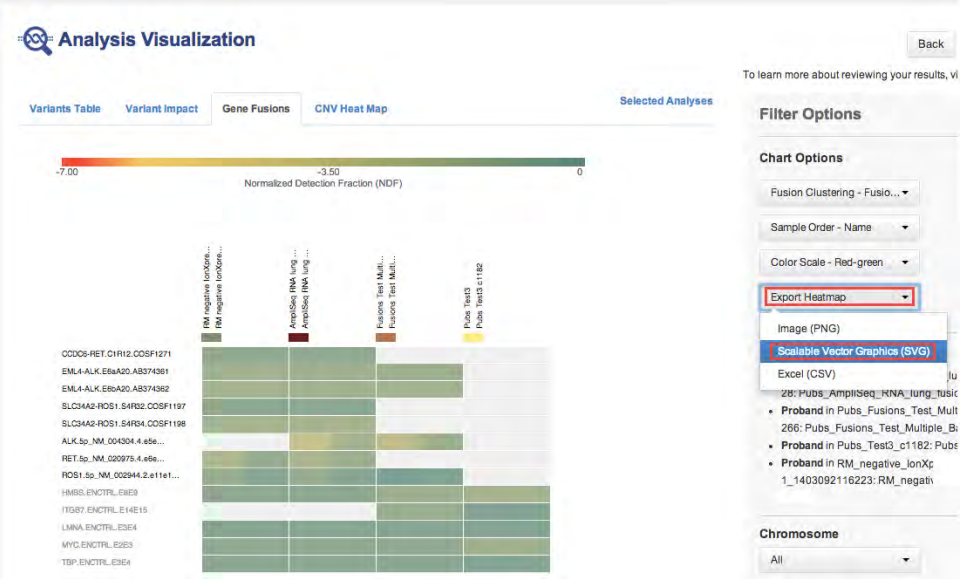
- Name: Alphabetically by sample name
- Similarity: By how similar the samples are in the fusion calls made (presented from fewest fusions to most fusions)
- Similarity (reversed): By how similar the samples are in the fusion calls made (presented from the most fusions to the fewest fusions)
- Analysis: Alphabetically by analysis name

Set the Color Scale menu to a different option, such as SIFT, to base the legend and the heat map on those colors.



How to export heatmaps

Use the Export Heatmap menu to download your heatmap.



The supported download formats include PNG, SVG, and comma-separated file. This works best in Firefox, Chrome and Safari browsers.

Filter impact on the variants displayed in heat maps

If you save your filter chain on the Analysis Results page, then the heat maps and other multiple analysis visualizations show only filtered in variants.

If you do not save your filter on the Analysis Results page, then the heat maps and other multiple analysis visualizations show only the entire result sets (nothing is filtered out).

Filter variants

With filters, you change which variants are displayed in the variant table or remove variants that are not likely to be relevant to your research. You filter out uninteresting variants when you save your filter chain.

Note: The data in the backend can contain more significant digits than the data that is shown in Ion Reporter™ Software. Therefore, to capture all intended data, you can add an extra digit to threshold values when you filter data.

Shipped filters

The following filters are shipped with Ion Reporter™ Software. Several of these filters are automatically applied when an analysis is first opened.

Note: Filters that are automatically applied to a workflow the first time you review the **Analysis Results** screen Ion Reporter™ Software are referred to as *default filters* in this guide.

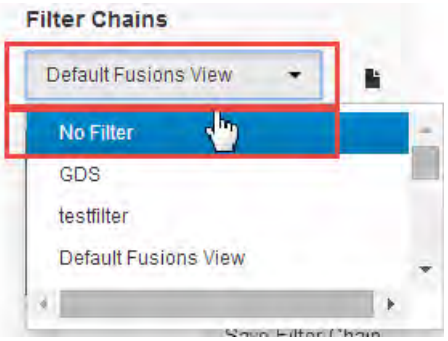
Filter name	Description	
CNV Somatic Confidence - CNV	Includes 5% Confidence Interval Range and 95% Confidence Interval Range.	
Default Variant View	Filters in DNA variants SNVs, indels, MNVs, long deletions, and confident CNVs (which are germline Copy Number variants with a Confidence value >= 10). Default filter for the AmpliSeq Exome single sample (both Germline and Somatic), TargetSeq Exome v2 single sample, AmpliSeq CHPv1 tumor-normal pair, AmpliSeq CHPv2 tumor-normal pair, AmpliSeq CCP tumor-normal pair, AmpliSeq Exome paired sample, AmpliSeq CCP paired sample, and Low-pass whole-genome aneuploidy workflows.	

Default Fusions View	Filters in all of the following: • FUSIONS variants that are detected as Present • All EXPR_CONTROL markers • All ASSAYS_5P_3P markers Default filter for the AmpliSeq RNA Lung Fusion single sample workflow.	
Default DNA and Fusions View	Filters in all of the following (a combination of Default Variant View and Default Fusions View): • FUSIONS variants that are detected as Present • All EXPR_CONTROL markers • All ASSAYS_5P_3P markers • DNA variants of all types Default filter for the Ampliseq Colon Lung v2 with RNA Lung Fusion single sample workflow.	
Genetic Disease Variants	Filters in genetically-relevant variant types such as IsNewlyHomozygous NonRef, HasUnknownX, etc. Default filter for the AmpliSeq Exome trio and AmpliSeq IDP trio workflows.	

Homopolymer Length	A range filter that only applies to indels. Default range is 0 to 1000, including boundaries.	
AmpliSeq Exome Tumor Normal v1	Filters in both of the following: • All CNVs • Confident somatic variants with the following: – Allele Ratio between 0.1 and 1.0 – Allele Read Count between 4 and 100000 – PValue between 0 and 5.0E-6 Default filter for the AmpliSeq Exome tumor-normal Pair workflow.	
Confident Germline CNVs - CNVs Only	For germline analyses, filters in Copy Number variants with a Confidence value >= 10. Not a default filter.	
Variant Classification	Filters on classification options: unknown, benign, suspected benign, suspected deleterious, and deleterious.	

Notes:

- To see the results without a filter applied, set the Filter Chains menu to **No Filter**.

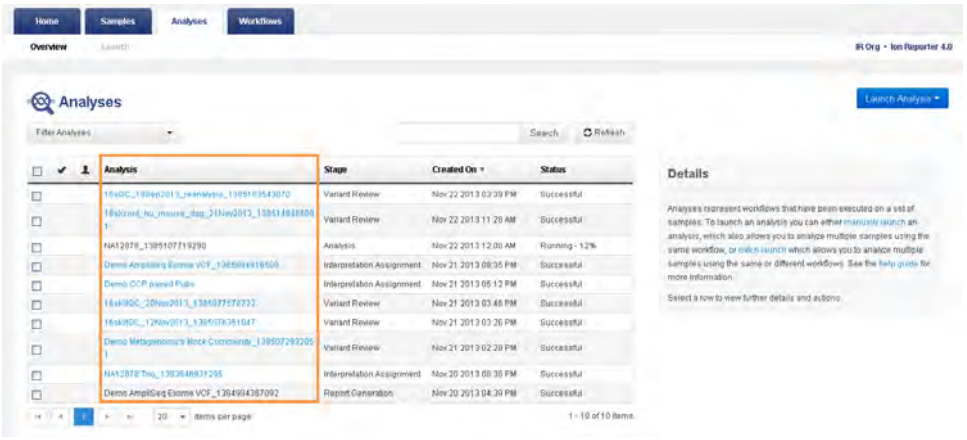


- If you or another user save a filter setting, the next time the Analysis Results page for that analysis is opened, it opens with that filter.

Use filter chains

Open an analysis:

1. Sign in to Ion Reporter™ Software, then click on **Analyses**.
2. Click on the name of your analysis in the **Analysis** list:



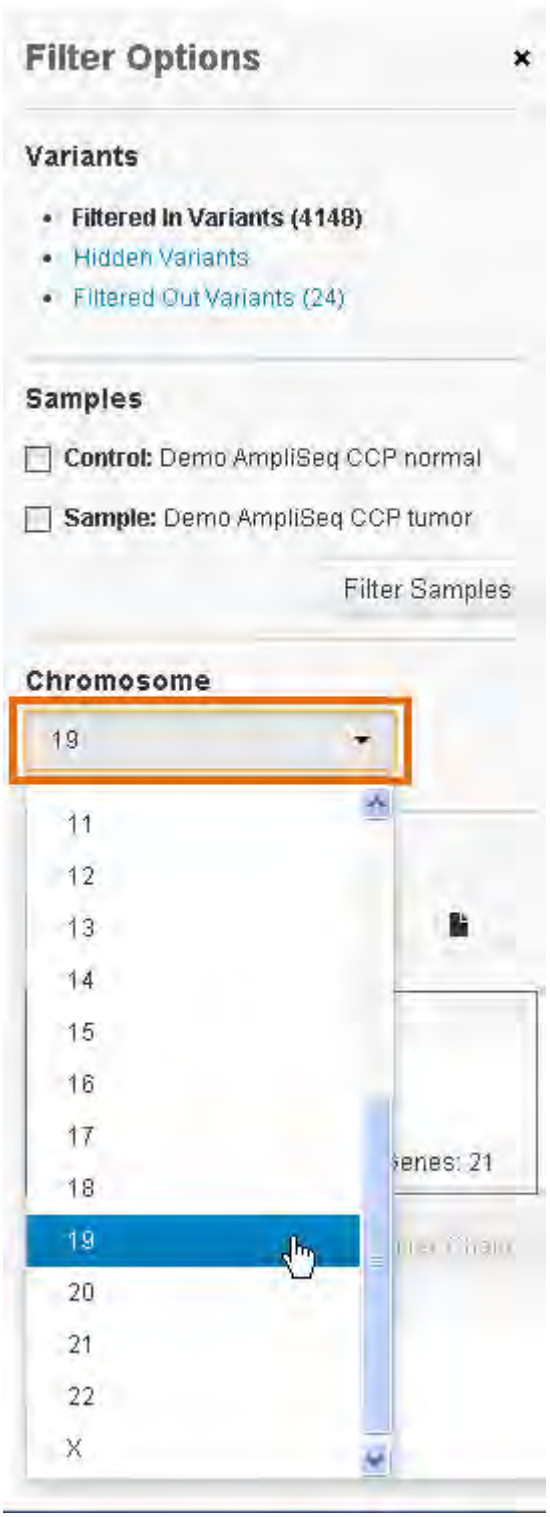
A list of variants opens in the **Analysis Results**.

Use quick filters

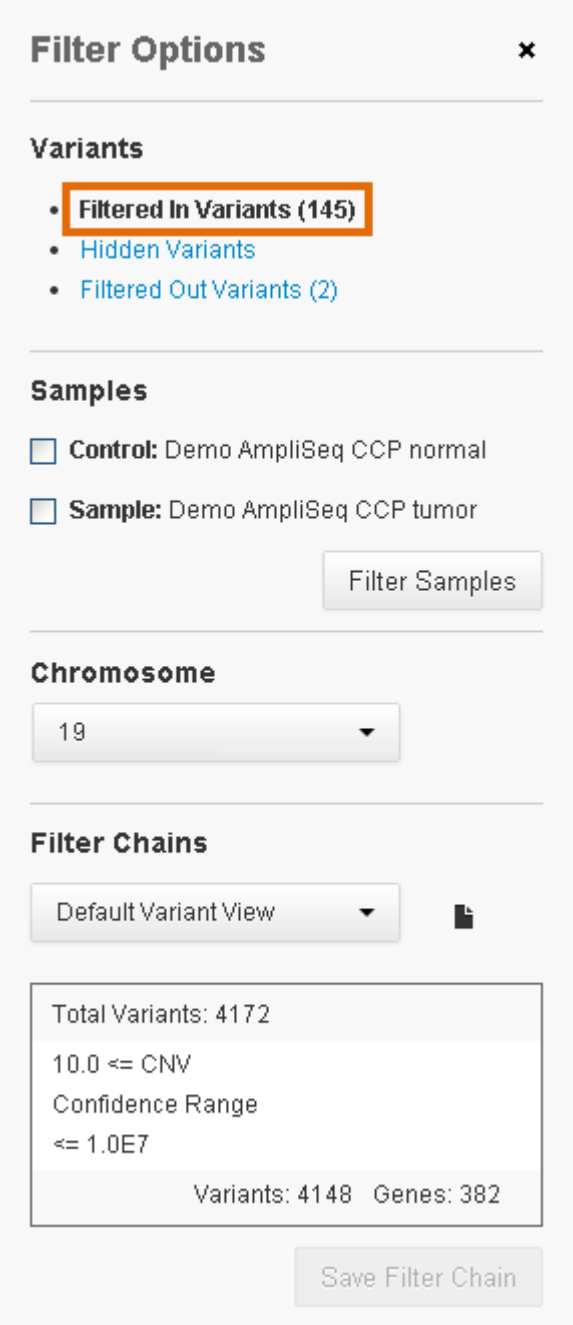
The Chromosome menu

Use the Chromosome menu to restrict the displayed variants to only those called in one chromosome:

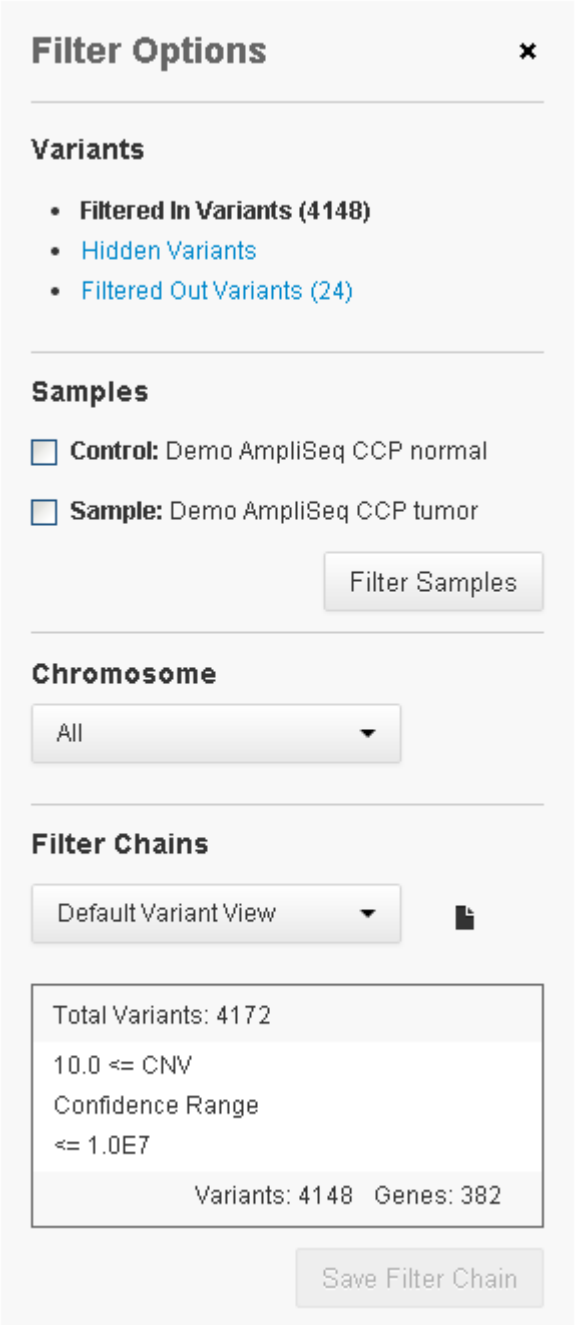
1. Click the **Chromosome** menu and select the chromosome of interest.



2. Notice that the number of Filtered In Variants changes to reflect only those in the selected chromosome. Because variants called in other chromosomes are considered to be "not displayed", as opposed to "filtered out", their variant totals are not added to the Filtered Out Variants.



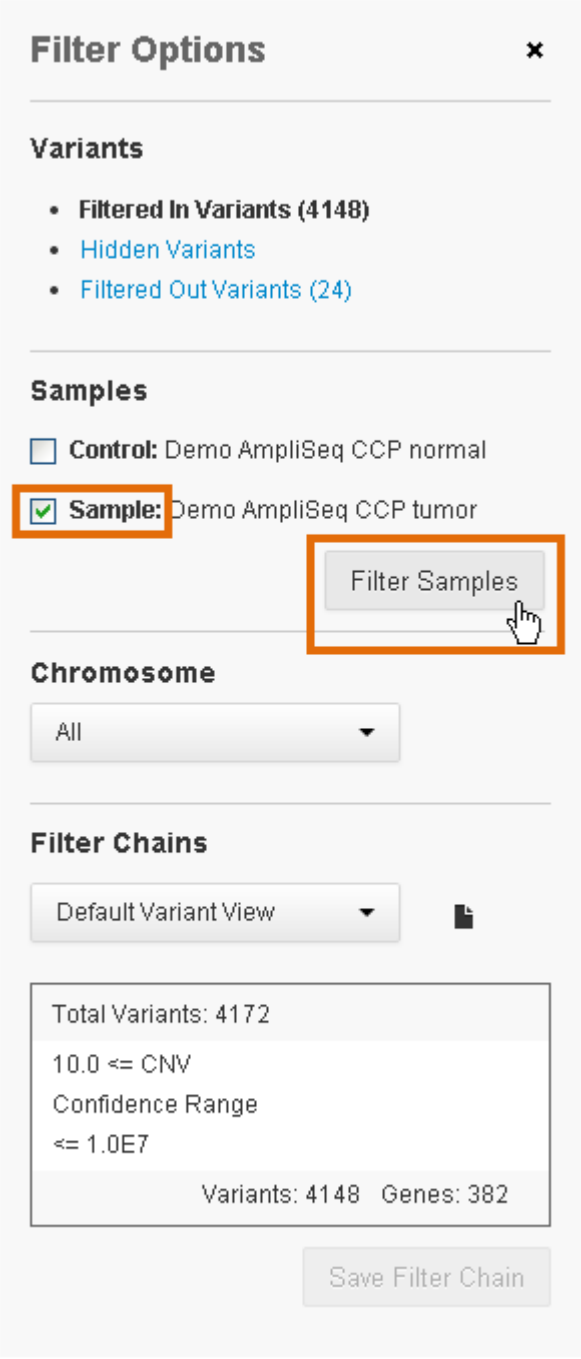
3. Compare with the totals when the Chromosome filter is not used.



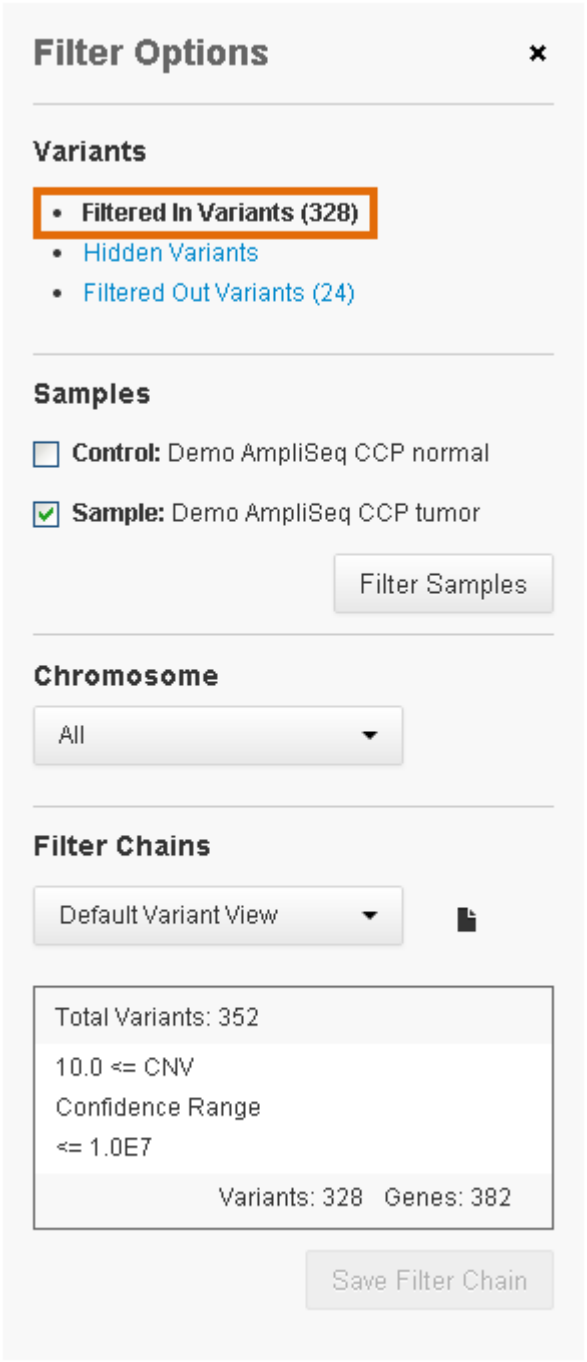
The Sample checkboxes

This filter is available only with the paired samples workflows. Use this filter to display only those variants that are called in the test sample and do not appear in the control sample:

1. Click the **Sample** checkbox to enable it. Leave the **Control** checkbox not enabled.

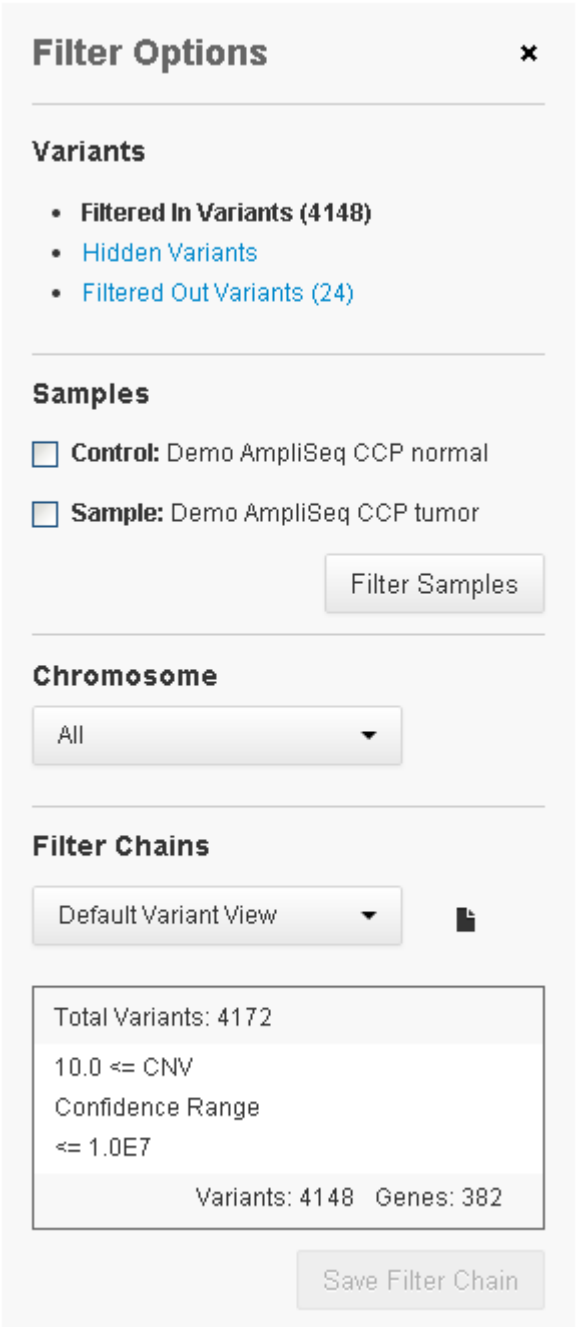


2. The Filtered In Variant total changes to the number of variants that are in the Sample (test) sample and not in the Control sample.



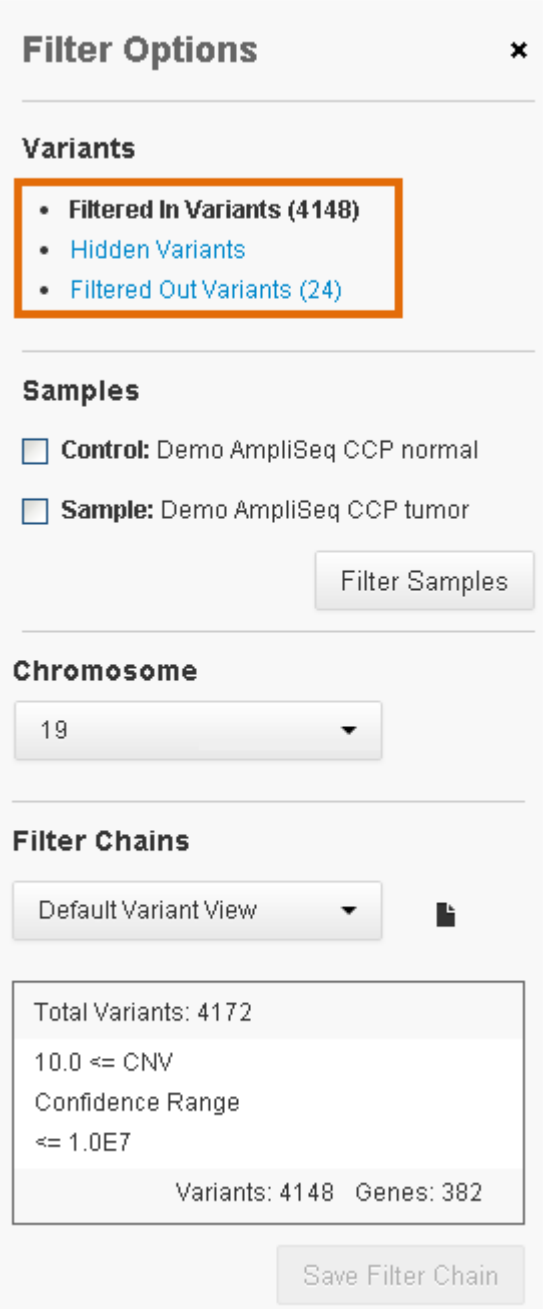
The variant table also shows only this set of variants.

3. Compare with the totals when the Sample checkbox filter is not used.



The Filtered and Hidden links

While not strictly filters, these links change the set of variants that are displayed in the Analysis Results page.

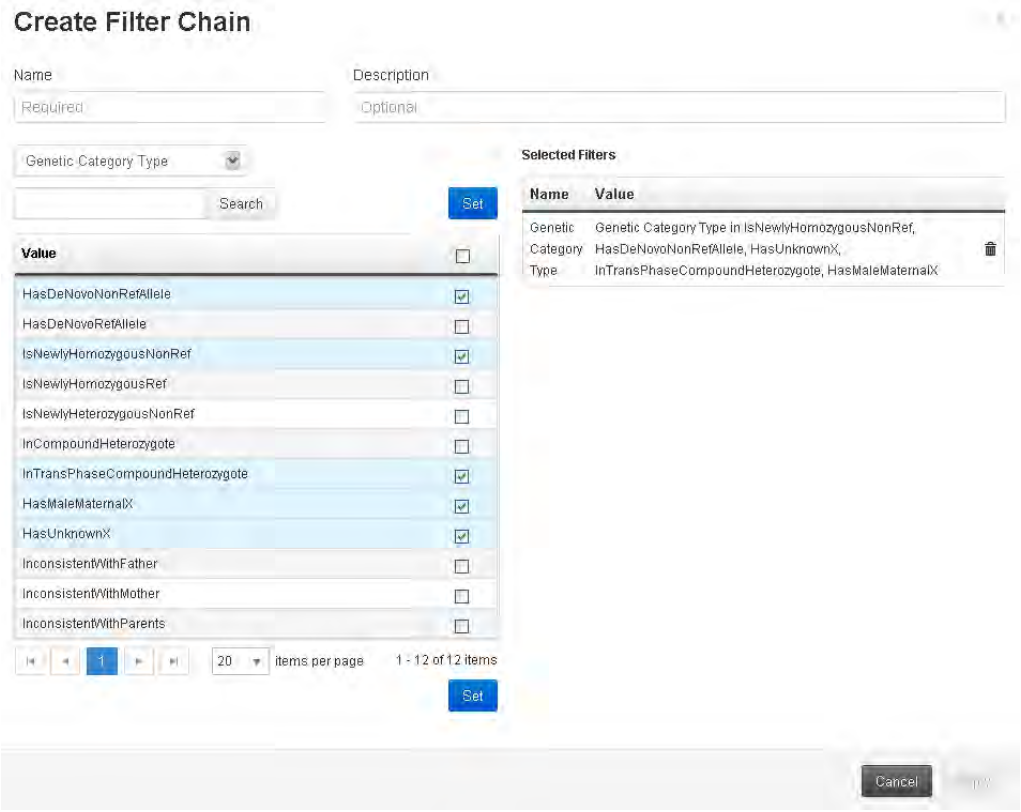


- **Filtered In Variants**: Displays variants that are still being considered for inclusion in the final report or as interesting for your research. This is the default view.
- **Hidden Variants**: Displays variants that have been explicitly hidden with the Selected Variants menu.
- **Filtered Out Variants**: Displays variants that are no longer being considered because of the filter chain setting.

Filter chains

With filter chains, you also restrict the displayed variants according to variant annotations or metrics.

Note: Specific filters only appear in workflows for which they are relevent. For example, the Genetic Category Type filter is available with trio analyses, but not with other analyses.



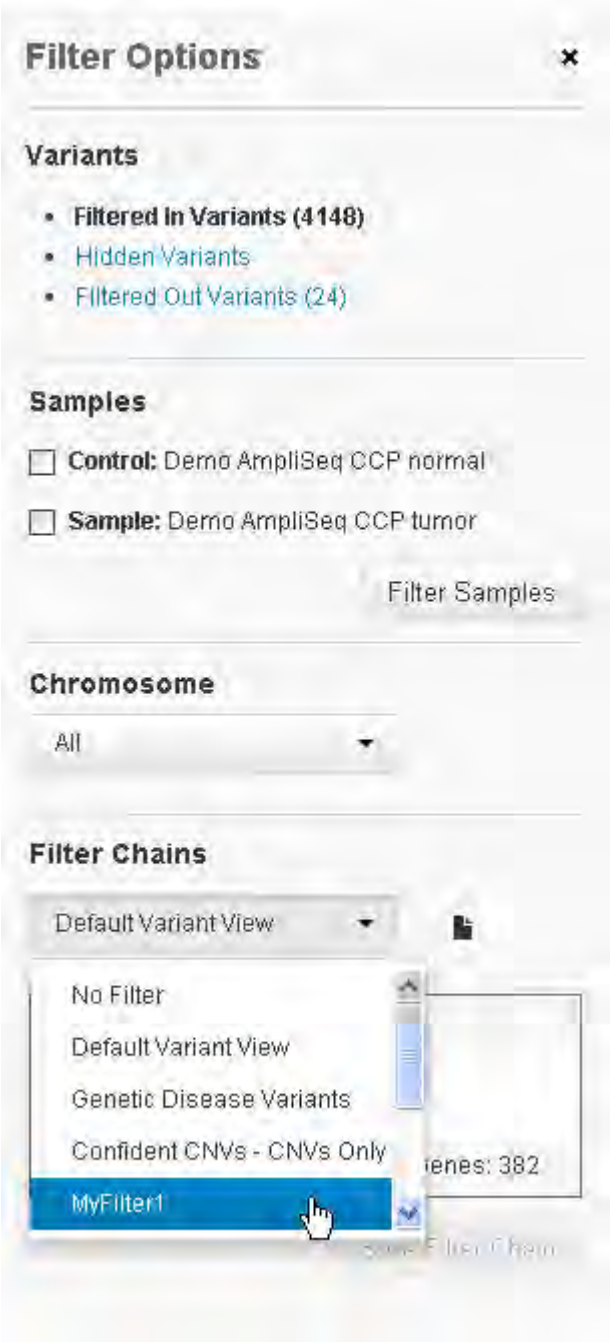
Saved filter chains

When you save a filter chain, you restrict the variants in the analysis' results set.

The saved filter chain also becomes available in the Filter Chain menu for use in other analyses.

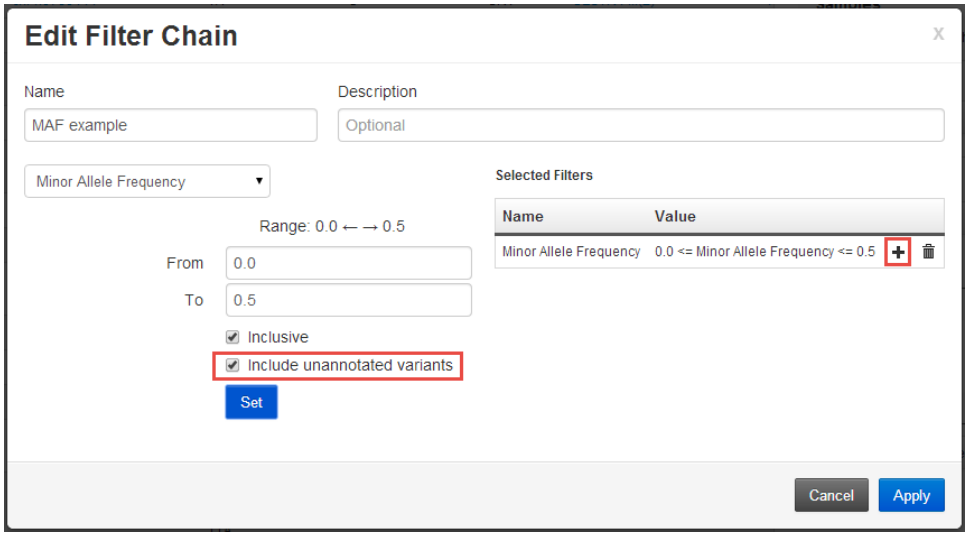
Use an existing filter chain

If a filter chain already exists with the filters you want, click the Filter Chains menu and select that filter chain.



Include unannotated variants option

Enable the Include unannotated variants option to have the filter include variants that have not been annotated with the specific filter key. This behavior is similar to the Ion Reporter™ Software 1.x "Include if missing" option. This example includes variant that with a blank MAF value (as well as variants with MAF values in the specified range).



The plus sign in the Selected Filters area listing indicates that the filter includes variants that are missing the annotation.

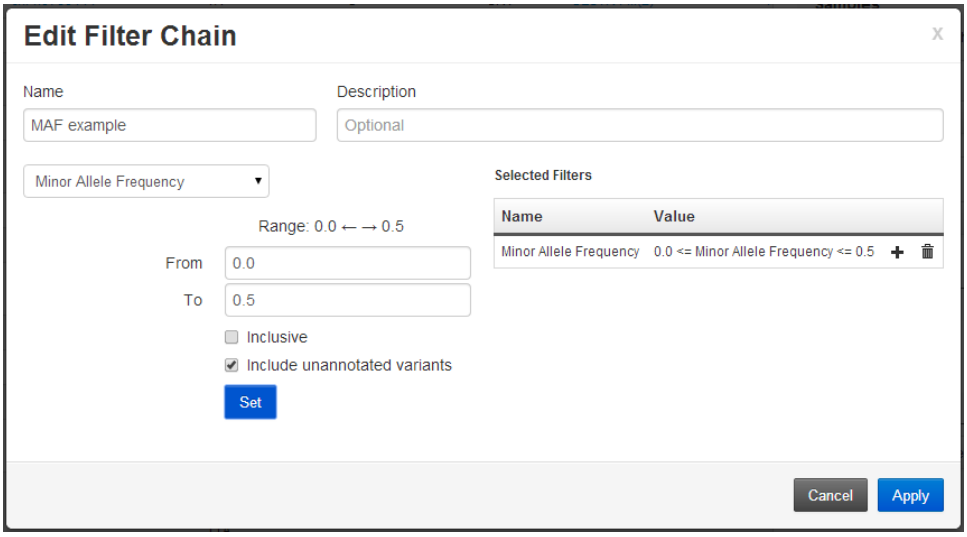
Inclusive checkbox

The **Inclusive** checkbox controls whether a range includes its end-points or not.

When the **Inclusive** checkbox is enabled, variants with the same values as end-points are filtered in.

When the **Inclusive** checkbox is not enabled, variants with the same values as end-points are filtered out. To be filtered in, variants must have values that are in between the range's end-points.

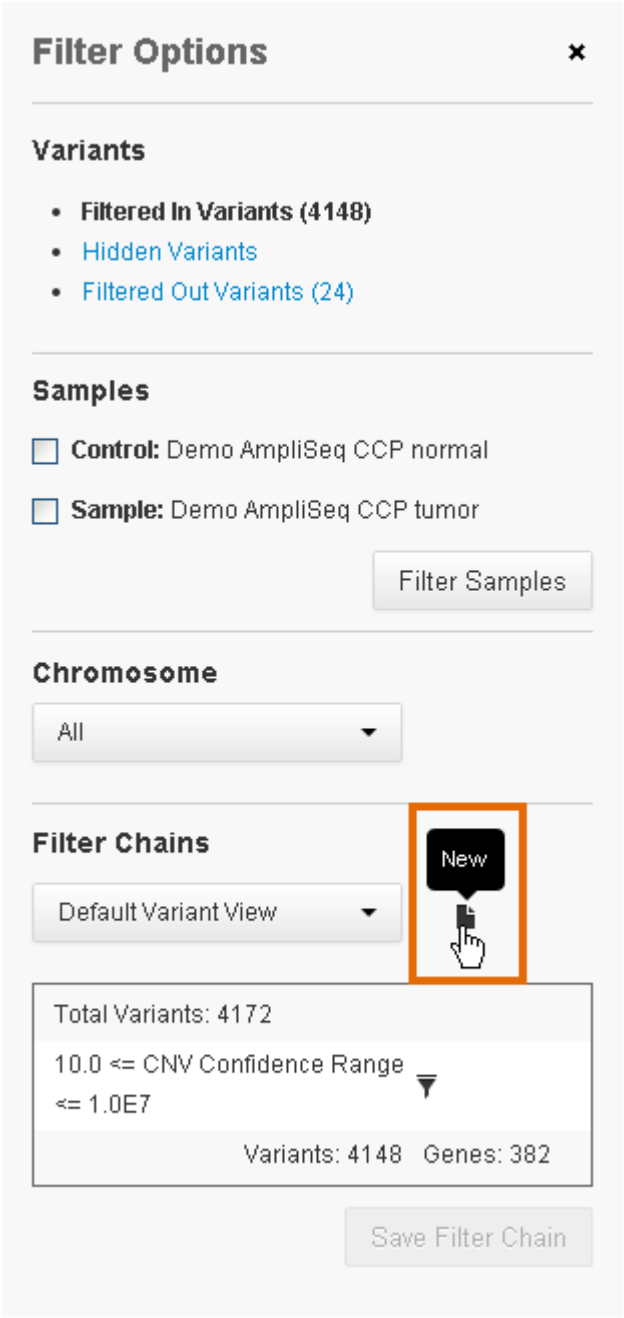
In this example, the **Inclusive** checkbox is not enabled. If a variant has an MAF value of 5.0 (the same as the end-point), the variant is filtered out and not displayed.



Follow these steps in these sections to create a filter chain.

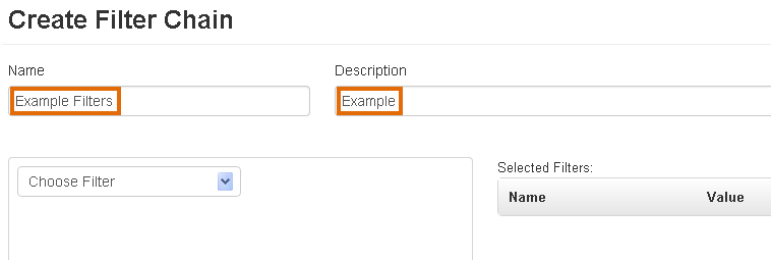
Open the filter chain

Click the **New** icon to the right of the Filter Chain menu.

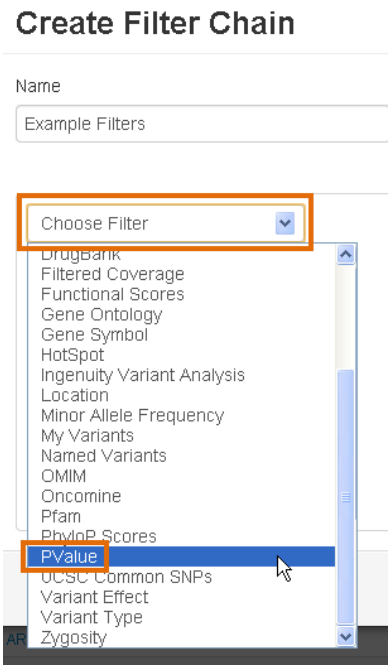


Create the first filter in the chain

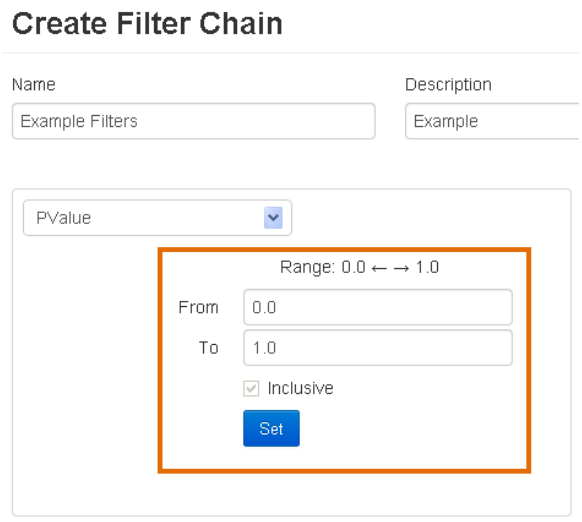
1. Enter a name and description for your new filters. The name is used if you later save your new filters.



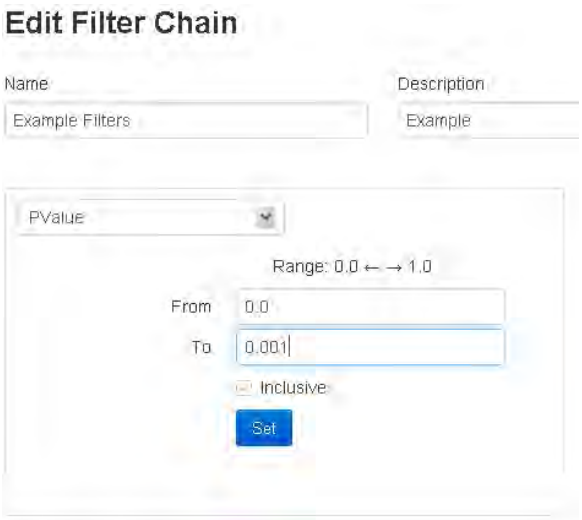
2. In the **Choose Filter** menu, select the type of filter ("PValue" in this example).



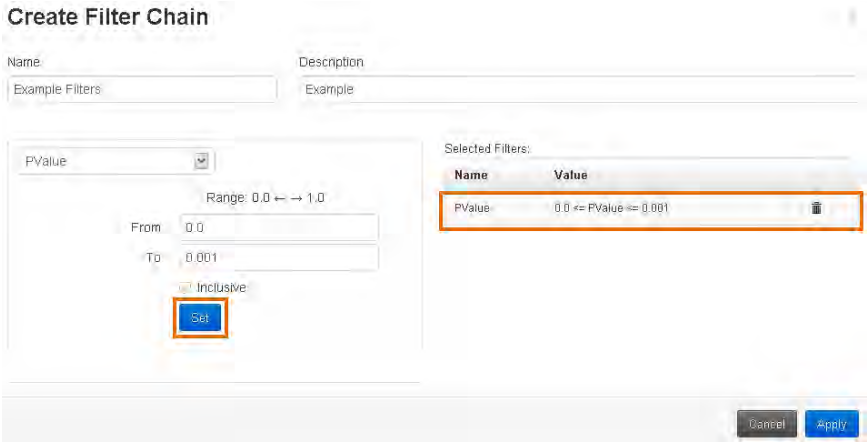
3. The PValue default settings appear next to the Choose Filter menu.



4. Change the PValue settings to your desired value ("0.001" in this example).



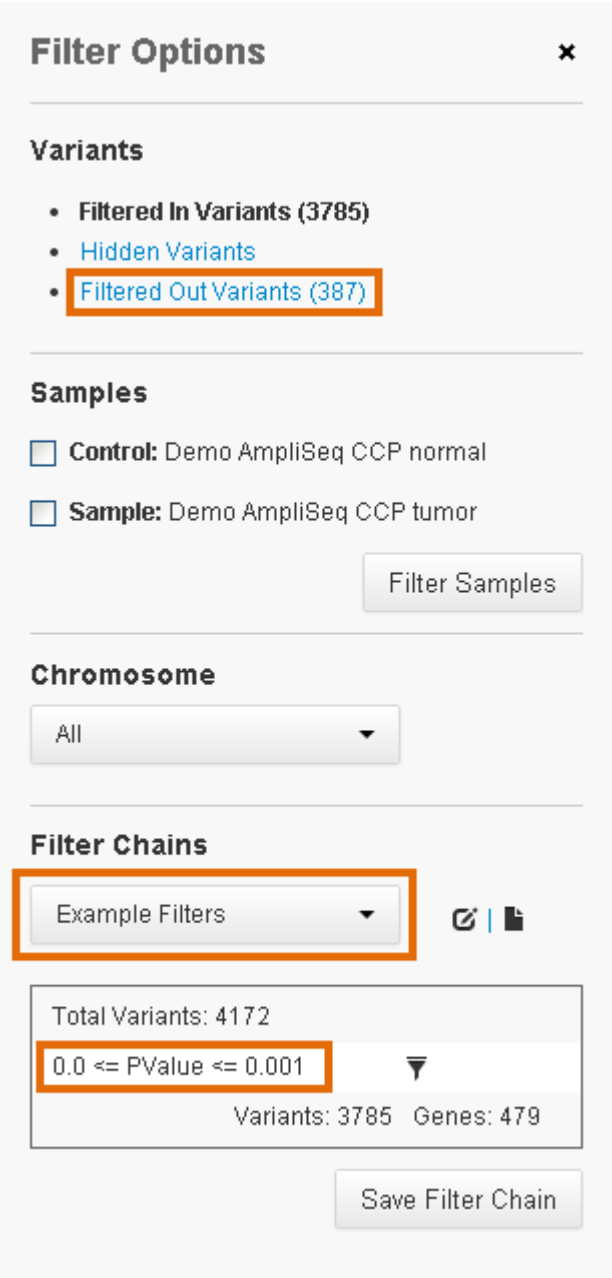
5. Click the **Set** button. Your new PValue range appears in the Selected Filters area.



6. At this point, you can select another filter in the Choose Filter menu. Or, click the **Apply** button to see how many variants are filtered out.

See the effects on the Analysis Results page

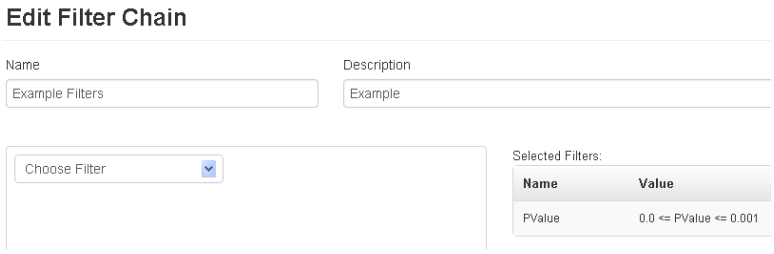
You return to the variant review page. The Filter Options panel has these changes:



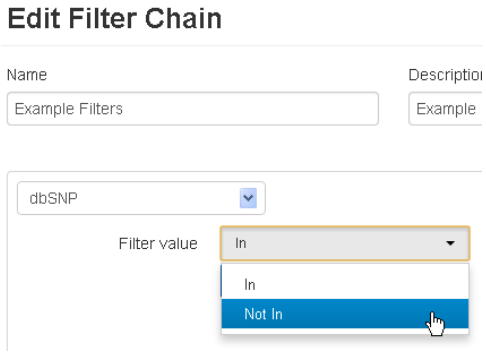
1. The Filtered Out Variants number is increased, due to variants which fail your new PValue threshold. These filtered-out variants are also dropped from the variant table.
2. The Filter Chains menu shows the name you entered in the Create Filter Chain popup.
3. Your new PValue filter is shown in the Filter Chains area.

Create another filter in the chain

1. Click the **Edit** icon to modify your filter chain. The Edit Filter Chain popup opens (with your PValue setting still listed in the Selected Filters area).

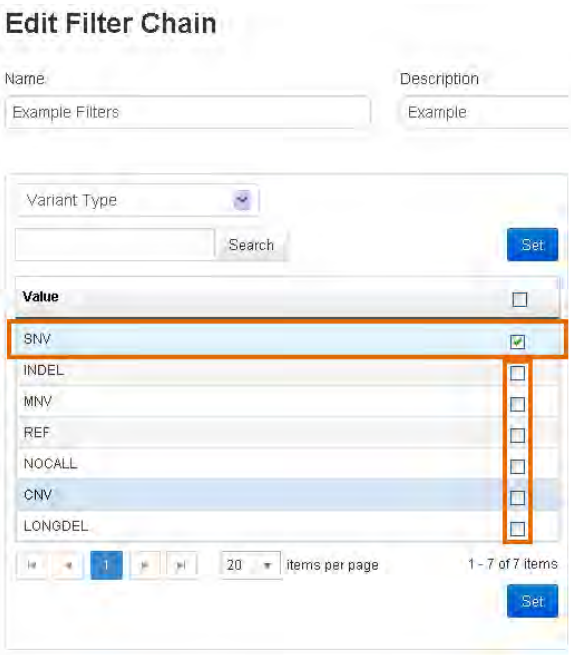


2. Follow these steps in the Edit Filter Chain popup:
   a. In the **Choose Filter** menu, select **dbSNP** as a new filter to add. Change the dbSNP setting default setting ("In") to **Not in**.

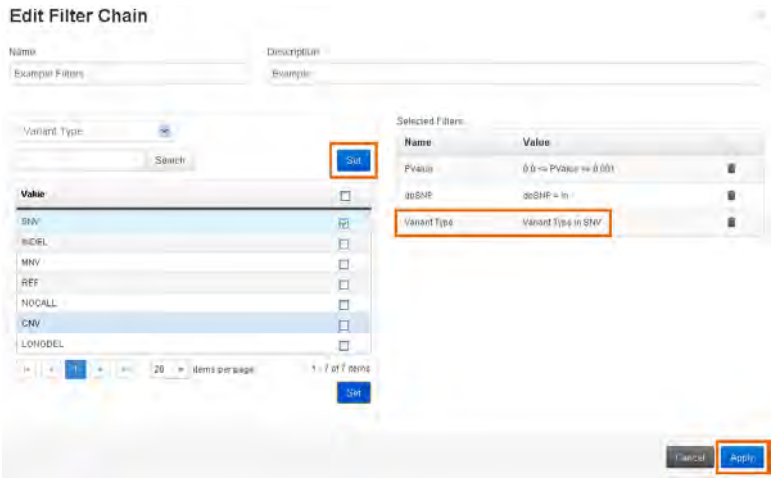


   b. Click the **Set** button. Your new dbSNP setting appears in the Selected Filters area.

c. In the Choose Filter menu, select **Variant Type** as a new filter to add. Change the Variant Type to only **SNV** (Deselect the other checkboxes.).



d. Click the **Set** button. Your new Variant Type setting appears in the Selected Filters area.



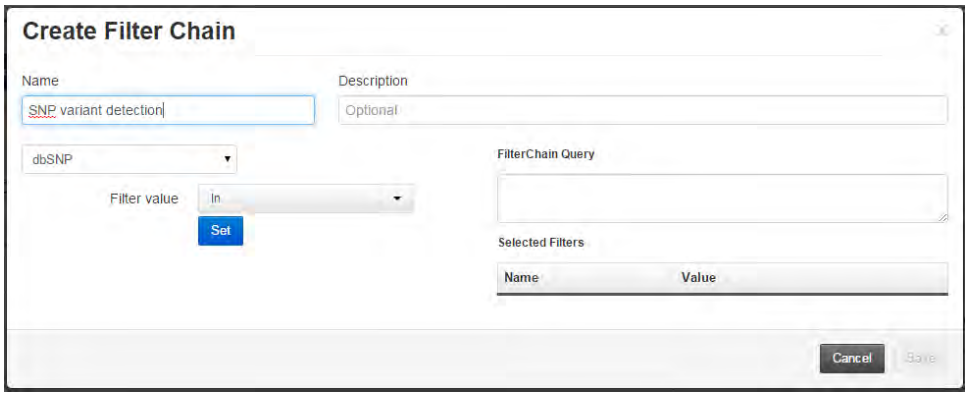
e. Click the **Apply** button.

Create an advanced filter chain query

You can use filter names and AND and OR modifiers and parentheses to construct precise custom queries to filter your variant data.

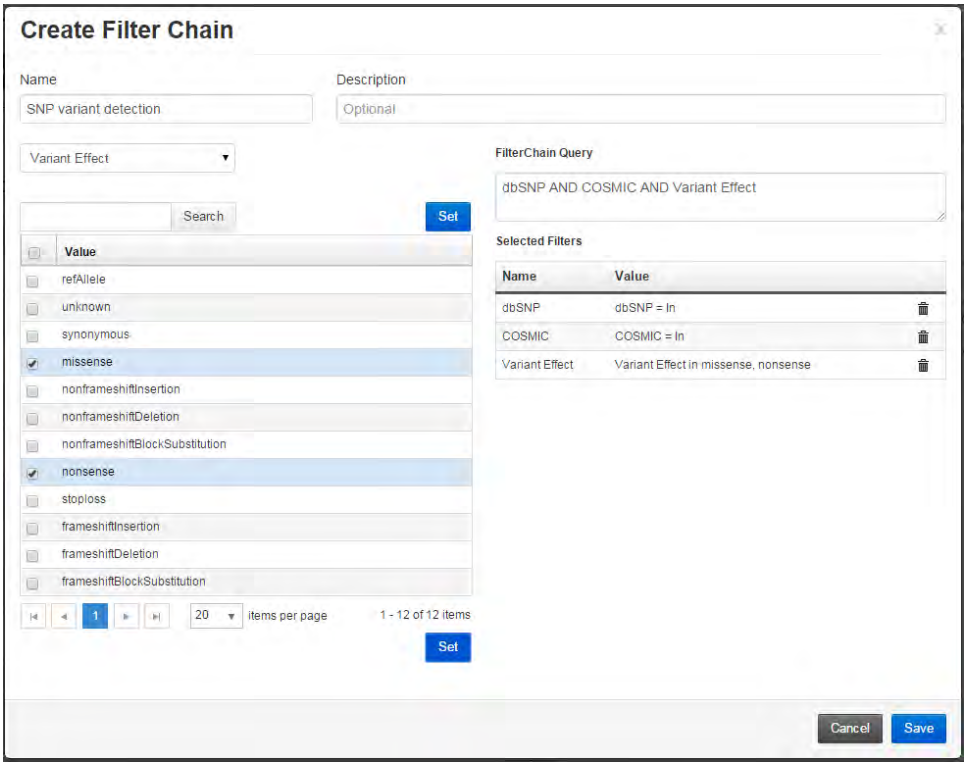
Note: AND and OR must be all caps.

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Filter Chain**.
3. Name your filter chain. In this example, the name is SNP variant detection.
4. Select filters one at a time. In this example, the selections are: **dbSNP** and filter value is **In**. Click **Set**.
   The filter appears on the table on the right.

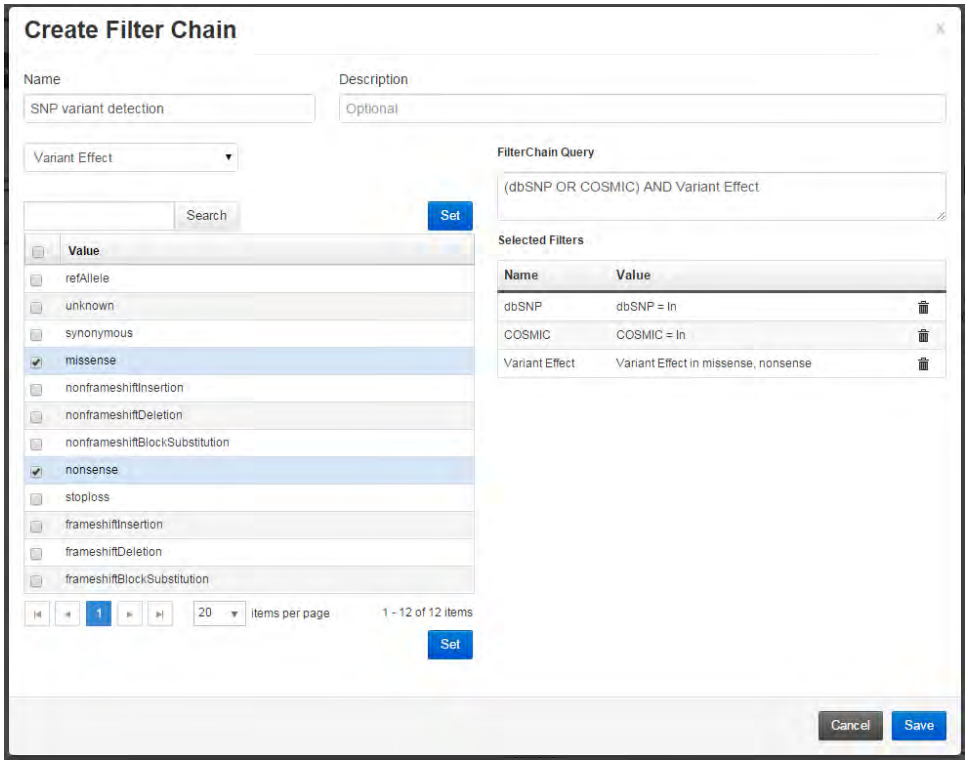


5. Add another filter. For example, add **COSMIC**, then set filter value to **In**. Click **Set** to add the filter to the table on the right.

6. Add a third filter. For example, add **Variant Effect**, then select values **Missense** and **Nonsense**. Click **Set** to add the filter to the table on the right.

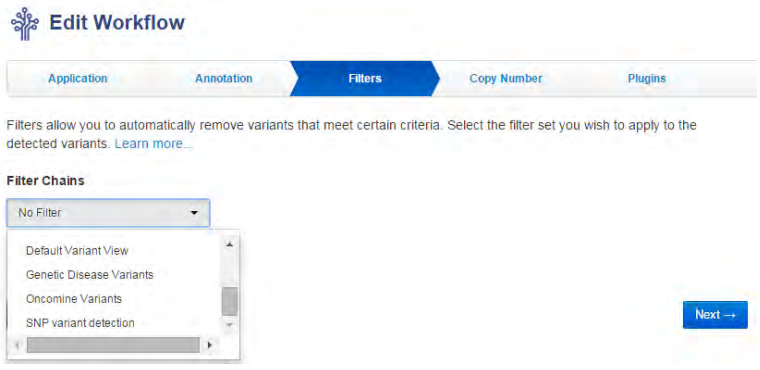


7. To change the order or manner in which these filters are applied, use brackets () to group the filters you want to apply first. You can also change AND to OR to alter the filtering algorithm in the **FilterChain Query** dialog.



Note: When you use a custom grouping different from AND-ing each filter, then the progressive filter counts are not shown on the Analysis Results page.

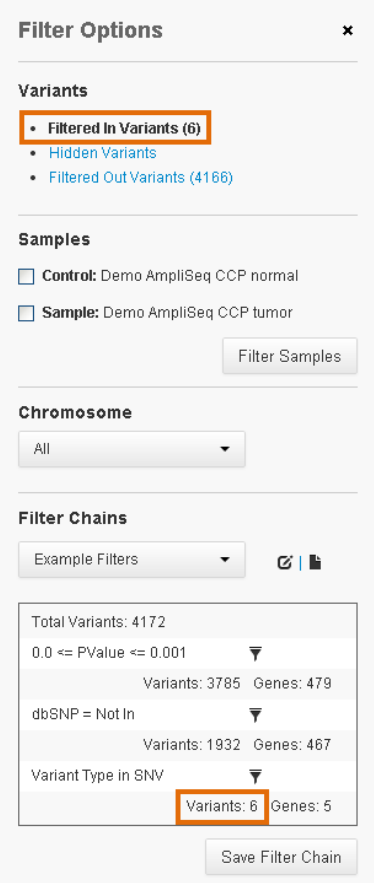
8. Click **Save**. The filter chain is available to use from Filters dropdown list during workflow creation, workflow editing, and on Analysis Variant Review screen. The new filter option is available on the Filter chevron.



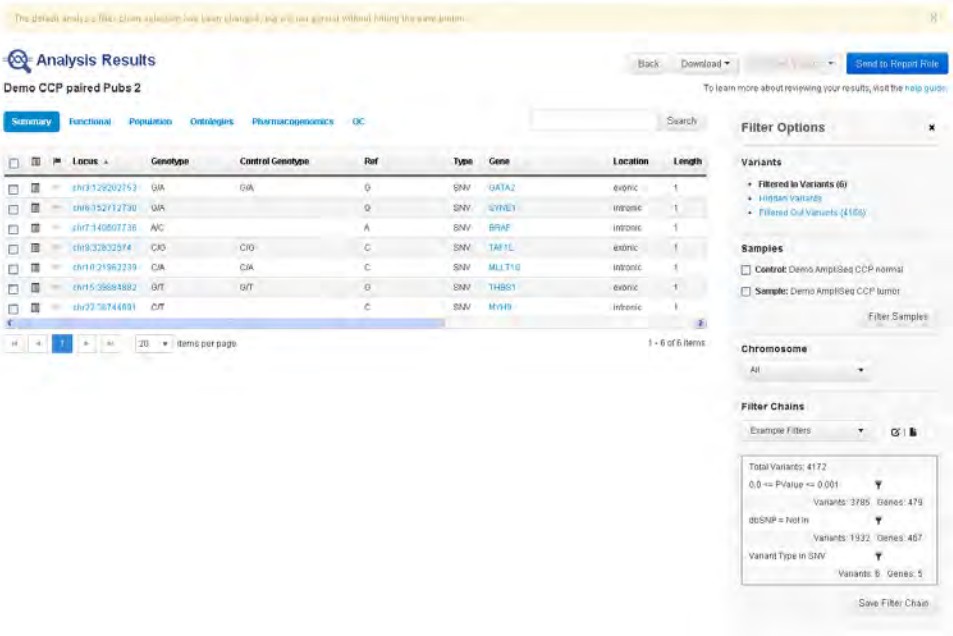
When used, the details of the advanced filter query appear on the Summary Panel under Filter Chains. All filter components are listed.

See the effects on the Analysis Results page

You return to the variant review page. Only 6 variants are still filtered in.

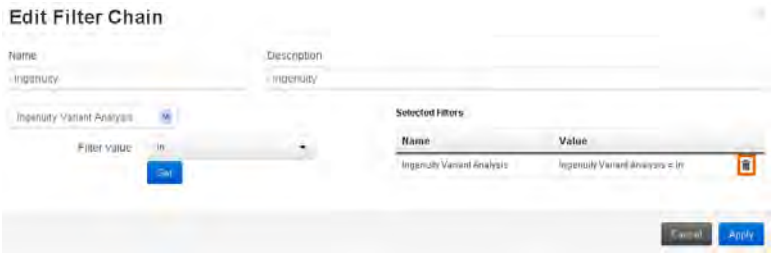


This image shows the entire page.



Additional steps

- You can repeat the filter chain edit to add additional filters to the chain.
- You can edit the filter chain at any time (before you save it) to rename it.
- Click the trash can icon to remove a filter from a filter chain that you are editing.



- Click the **Save Filter Chain** button if you want to reuse this filter in other analyses.

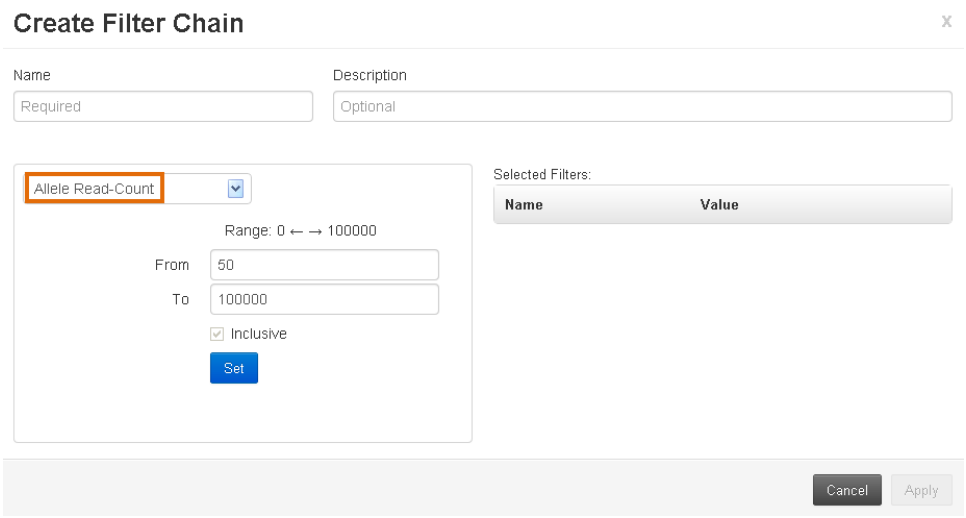
Types of filters and filter chains

Note: If the filter you use includes Annotation Source versions that differ from the version that is used in the analysis, it will not affect filtering. For example, if you apply a gene ontology filter with Gene Ontology version 20160928 and Gene

Ontology version 1.218 is used in the analysis, variants are returned without any change.

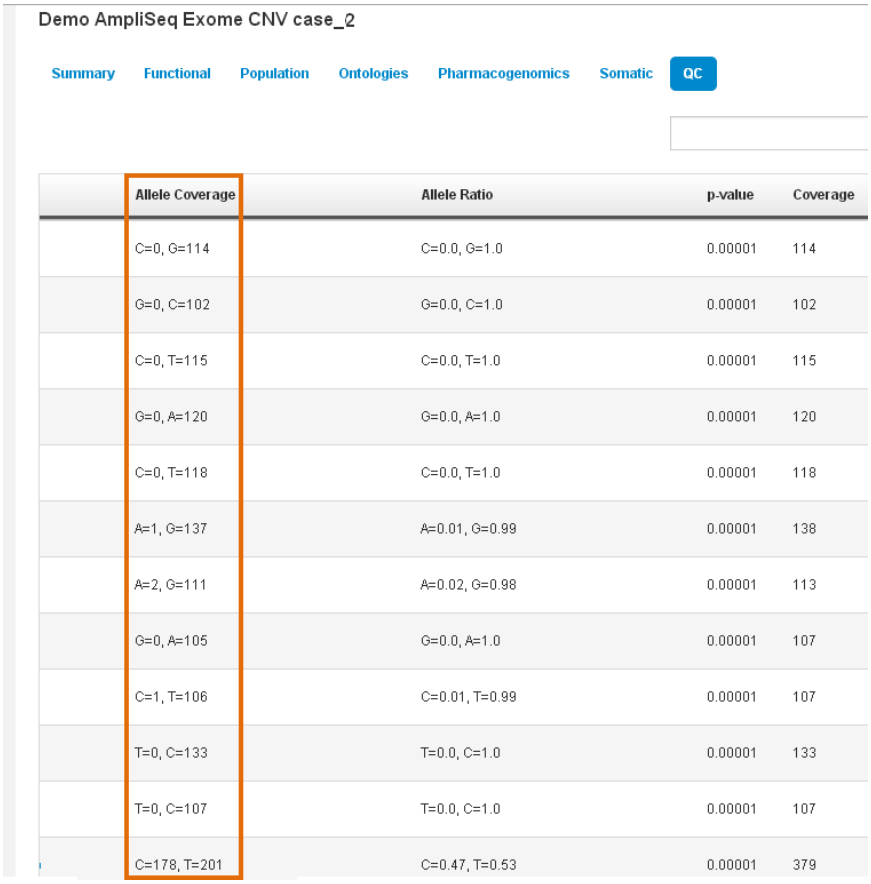
Note: For some annotation sources, multiple versions for each source are available for a given reference. When a filter chain contains a filter that uses a single version of the annotation source is used to filter multiple analyses that might not use the same annotation source version, the filtering uses the original version of the annotation source used in that analysis.
For example, if two analyses (A and B) that use different annotation source versions are visualized: (A): uses dbSNP 138 - filter chain filters in 47331 variants for a generic dbSNP In filter. (B): uses dbSNP 147- filters in 52044 variants for a generic dbSNP In filter.
Multi-visualization of these two analyses A and B: Application of a dbSNP In filter for this multi-visualization shows 47331 variants for analysis A and 52044 variants for analysis B.

- Allele Ratio Filter: The Allele Ratio filter filters on non-reference allele frequencies. All variants that have at least one non-reference allele reported with a frequency in the selected filter range is returned when applying this filter.
- Allele Read Count: The Allele Read Count filter sets the minimum count for genotype alleles. Use this filter to filter out variant candidates that do not have at least this number of supporting reads. More specifically, the Allele Read Count filter uses the minimum value in FAO if it exists, else it falls back to AO if it exists (which can be the case for some indel variants). The Allele Ratio filter uses the same logic. It uses FAO/sum(FRO+FAO) values if they exist, else AO/sum(RO +AO) if it exists.



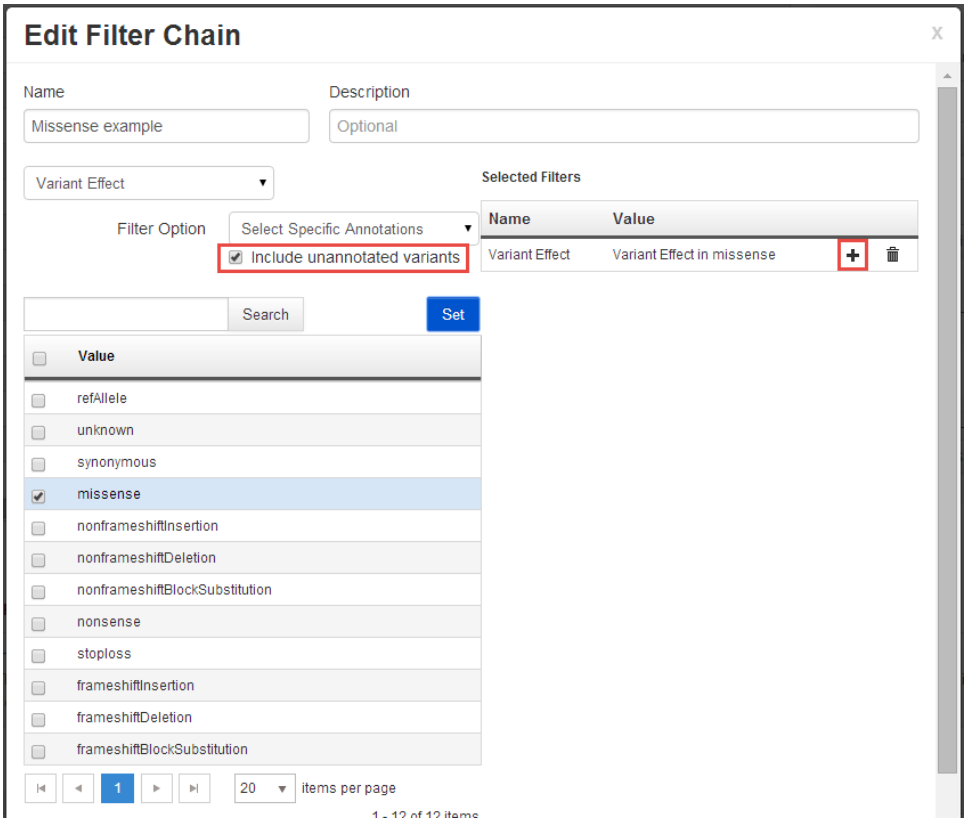
In contrast, the Allele Coverage column in the variant table reports counts for all reported alleles, not only genotype alleles. In Ion Reporter™ Software the allele ratio that is displayed in the graphical user interface is slightly different from the allele ratio used in filtering due to rounding. The filtering happens on a value

stored in the database, which is sometimes different from the value that is displayed in the user interface.



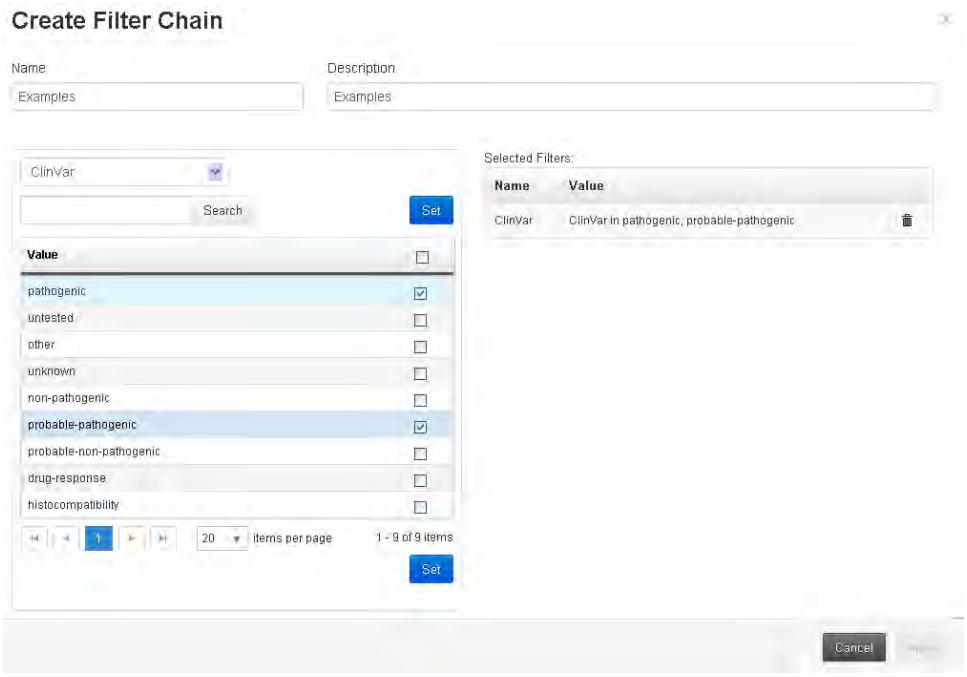
Allele Coverage	Allele Ratio	p-value	Coverage
C=0, G=114	C=0.0, G=1.0	0.00001	114
G=0, C=102	G=0.0, C=1.0	0.00001	102
C=0, T=115	C=0.0, T=1.0	0.00001	115
G=0, A=120	G=0.0, A=1.0	0.00001	120
C=0, T=118	C=0.0, T=1.0	0.00001	118
A=1, G=137	A=0.01, G=0.99	0.00001	138
A=2, G=111	A=0.02, G=0.98	0.00001	113
G=0, A=105	G=0.0, A=1.0	0.00001	107
C=1, T=106	C=0.01, T=0.99	0.00001	107
T=0, C=133	T=0.0, C=1.0	0.00001	133
T=0, C=107	T=0.0, C=1.0	0.00001	107
C=178, T=201	C=0.47, T=0.53	0.00001	379

- CNVs: CNV variant calls can be filtered out by several filters that do not apply to CNVs. For example, CNVs are filtered out by the Variant Effect filter. To instead include CNVs in the filter, select the "Include unannotated variants" checkbox. To exclude CNVs, you must include an explicit Variant-Type filter.

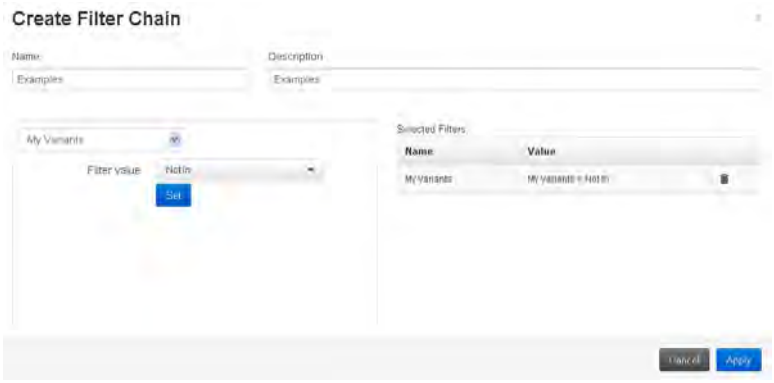


- CNVs: The Ion-supplied filter "Confident CNVs (Germline)- CNVs Only" applies to analyses that employ germline CNV detection. This filter has no effect on analyses that use somatic CNV detection.
- Genomic Coordinates: This filter filters by genomic regions, and can be used to identify regions that are targeted by an amplicon or amplicons in a user-defined assay. The filter value is a chromosome region or multiple regions that are separated by OR operators, such as: chr1:11174371-162724600 OR chr2:24991141-42492092.
- Minor Allele Filter (MAF): The MAF filter filters on 1000-Genomes Global MAF values (as obtained from the dbSNP database). This filter excludes locations/variants where the minor allele happens to be the same as the reference allele. Therefore, the filter can return fewer variants than expected.
- Named Variants Filter is made up only of variants in the CFTR gene panel.
- 5000Exomes MAF filters: If a variant is missing a value for a 5000Exomes MAF field, that variant is filtered out by the 5000Exomes MAF filter (5000Exomes AfricanAmerican MAF, 5000Exomes EuropeanAmerican MAF, and 5000Exomes Global MAF filters).
- Paired Analysis Filtering: In a Paired Sample analysis review page, Filtered Out Counts is only designed to get counts from the filter chain, not from using just the Sample Filter.

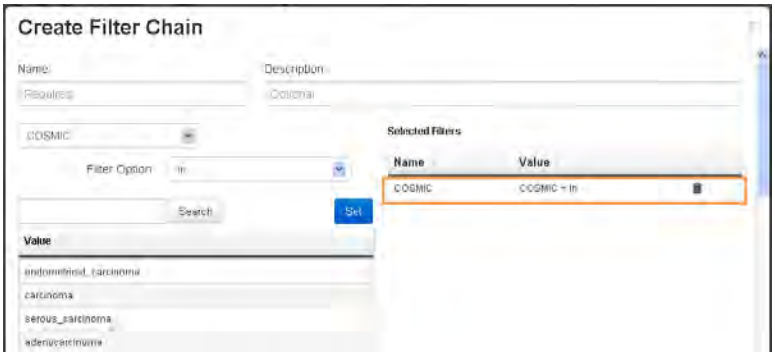
- These images give examples of some filters:
  - Variants that are annotated as either pathogenic or probable-pathogenic in ClinVar:



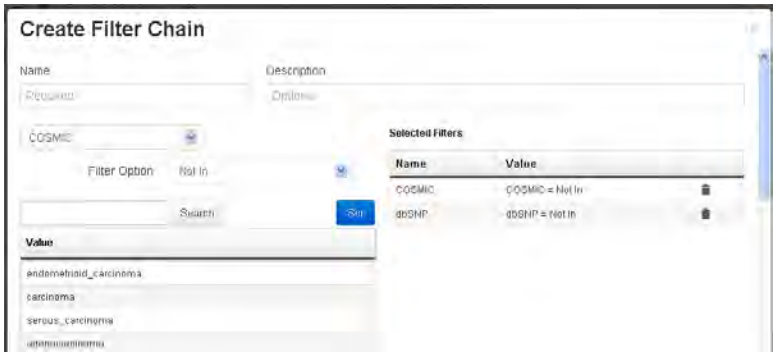
  - Variants that are not in My Variants knowledge base:



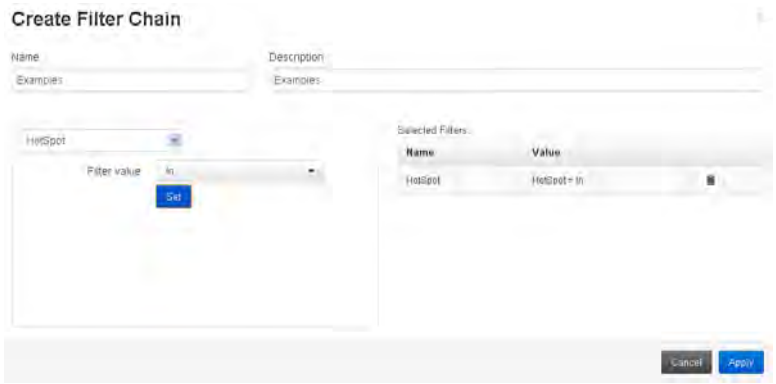
  - Variants that appear in the COSMIC database (no matter what the annotation):



– Variants that do not appear in either the COSMIC or the dbSNP databases:

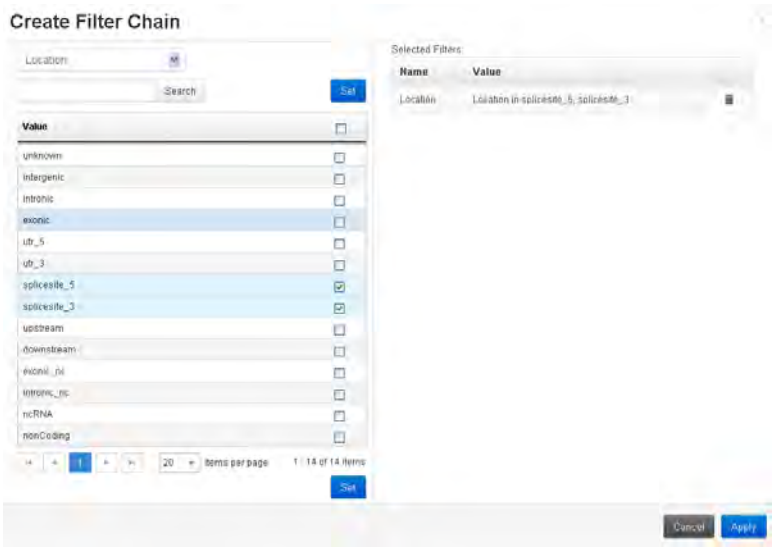


– Variants that match a hotspot position (based on the hotspots file that is used with the analysis):



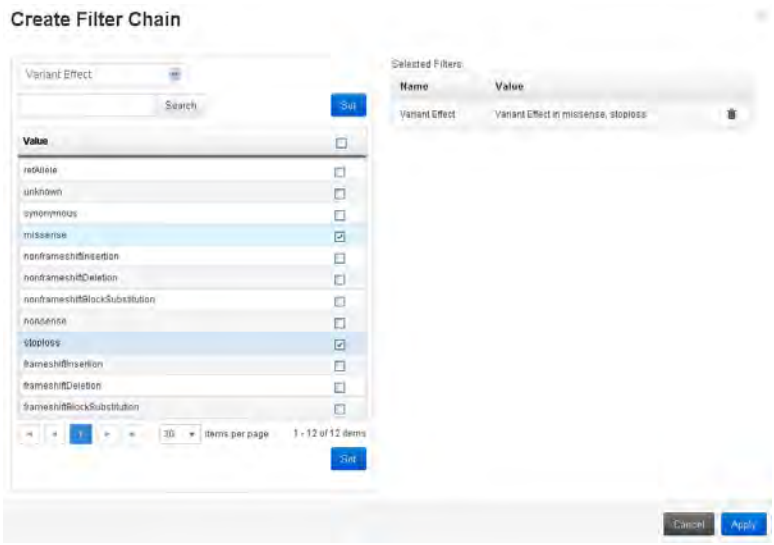
– Variants that do not match a hotspot position (based on the hotspots file that is used with the analysis):

– Variants that are annotated as either splicesite_3 or splicesite_5 (with the Location filter):

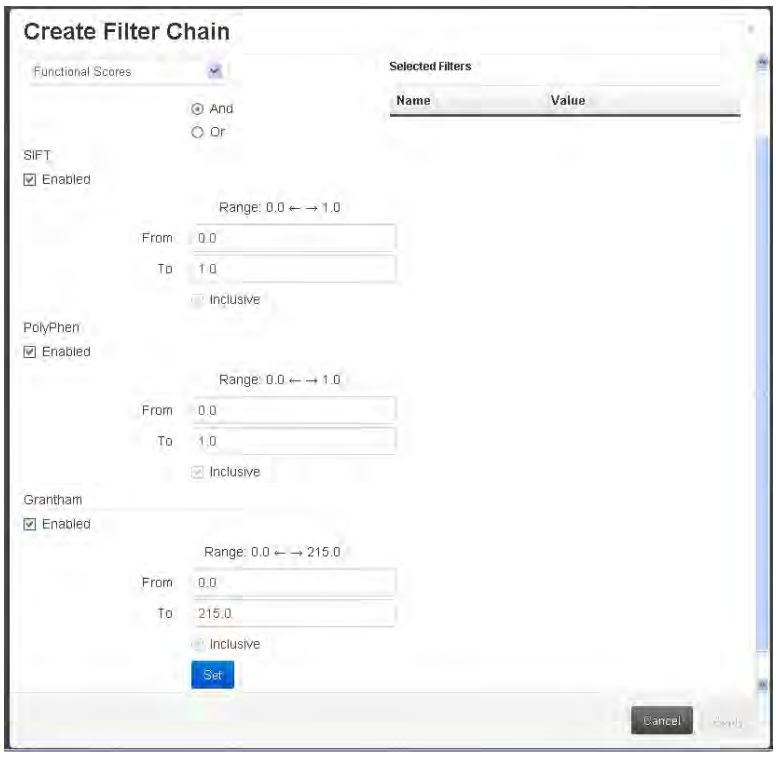


Note: Third-party algorithms that make more precise predictions of splice-site variants can be used independent of Ion Reporter™ Software. These include: SpliceSiteFinder-like, MaxEntScan, GeneSplicer, NNSPLICE, and Human Splicing Finder.

– Variants that are annotated as either missense or stoploss (with the Variant Effect filter):

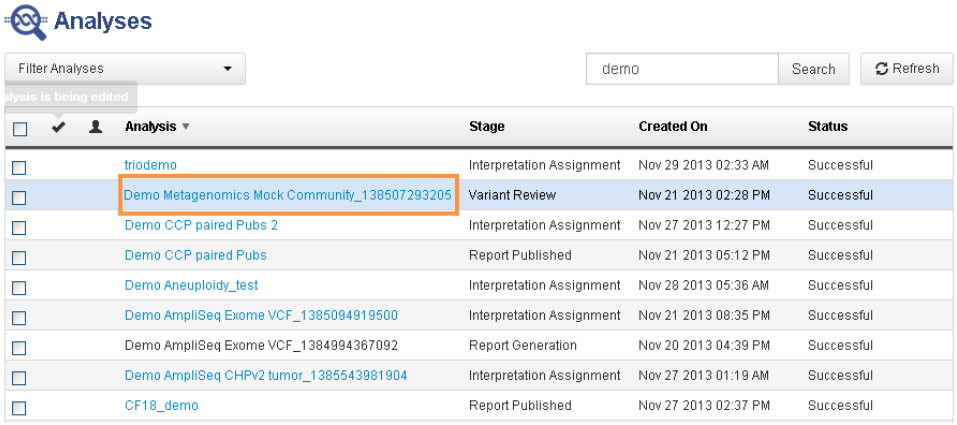


– Grantham, Polyphen, or SIFT scores (with the Functional Scores filter):



Metagenomics 16S analysis results

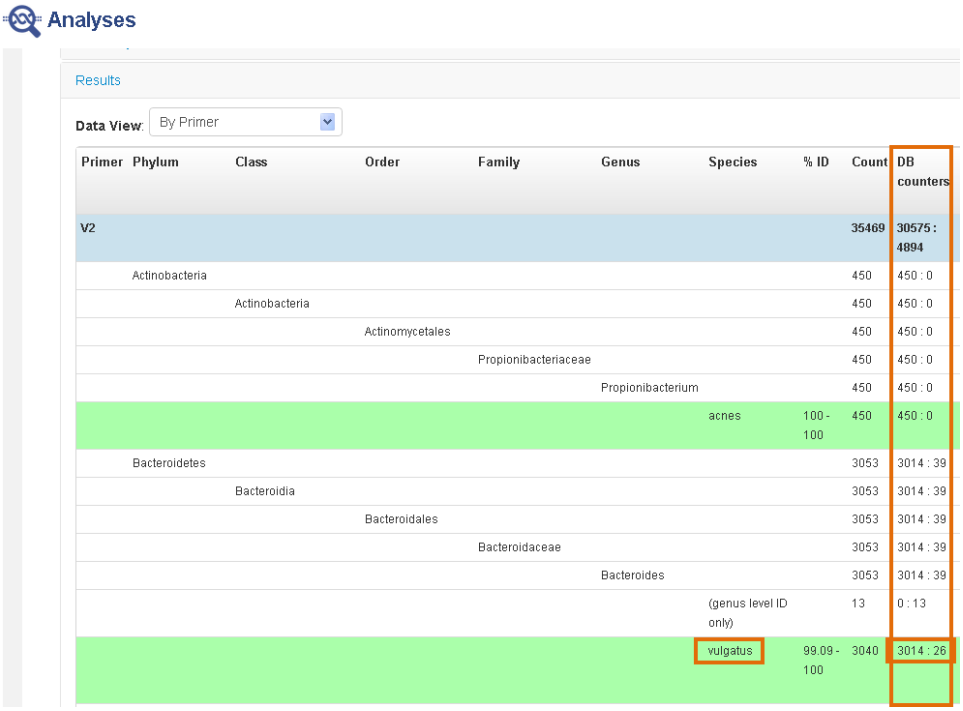
The results page contains the following sections and features:



- Header: States the analysis name.
- View Sample Results menu: For a multi-sample analysis, switches to data for a different sample.
- Visualization / Downloads area: Contains links to download results files and to visualize your results with Krona™.
- Summary: Contains analysis and parameter information and read metrics by primer.
- Results: Contains either consensus data (combined from all primers) or data broken out by primer. Reads that can be identified down to the species level are marked in green.

Note: In the results table, Total Mapped Reads in a sample differs from the sum of the bottom species-level counts. The reason is that the Total Mapped Reads are filtered by genus cutoff, species cutoff, and minimum alignment coverage. The topmost number in the "count" column will differ from the sum of the taxonomy levels below it, since the reads that did not satisfy the filters are also included in the total mapped reads.

Note: When your metagenomics workflow uses two databases, only reads that do not map to the first database are attempted with the second database. In the Results section, the DB counters column shows how many reads matched sequences in each database.



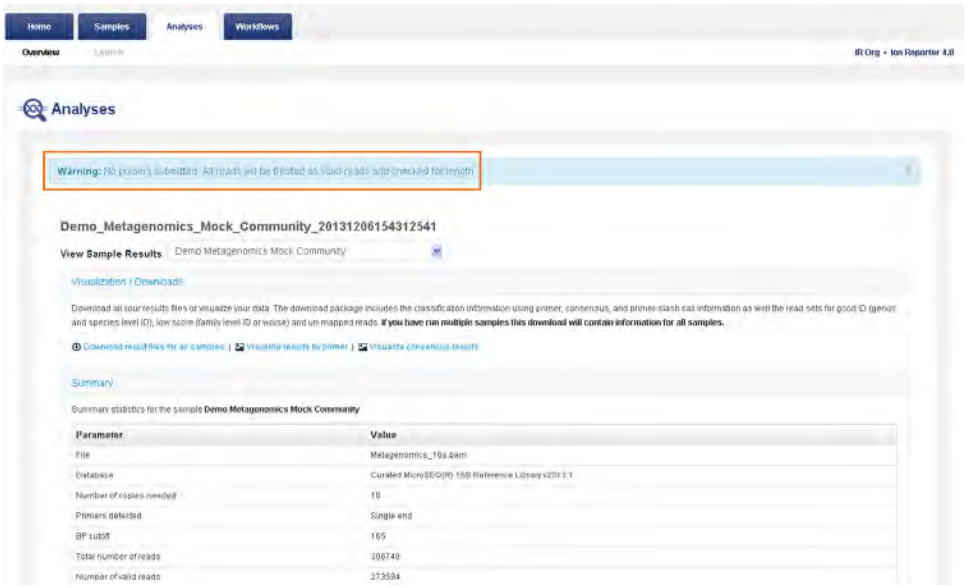
Primer	Phylum	Class	Order	Family	Genus	Species	% ID	Count	DB counters
V2								35469	30575 : 4894
	Actinobacteria							450	450 : 0
		Actinobacteria						450	450 : 0
			Actinomycetales					450	450 : 0
				Propionibacteriaceae				450	450 : 0
					Propionibacterium			450	450 : 0
						acnes	100 - 100	450	450 : 0
	Bacteroidetes							3053	3014 : 39
		Bacteroidia						3053	3014 : 39
			Bacteroidales					3053	3014 : 39
				Bacteroidaceae				3053	3014 : 39
					Bacteroides			3053	3014 : 39
						(genus level ID only)		13	0 : 13
						vulgatus	99.09 - 100	3040	3014 : 26

For example, in the last row, for the species vulgatus, the value

```
3014:26
```

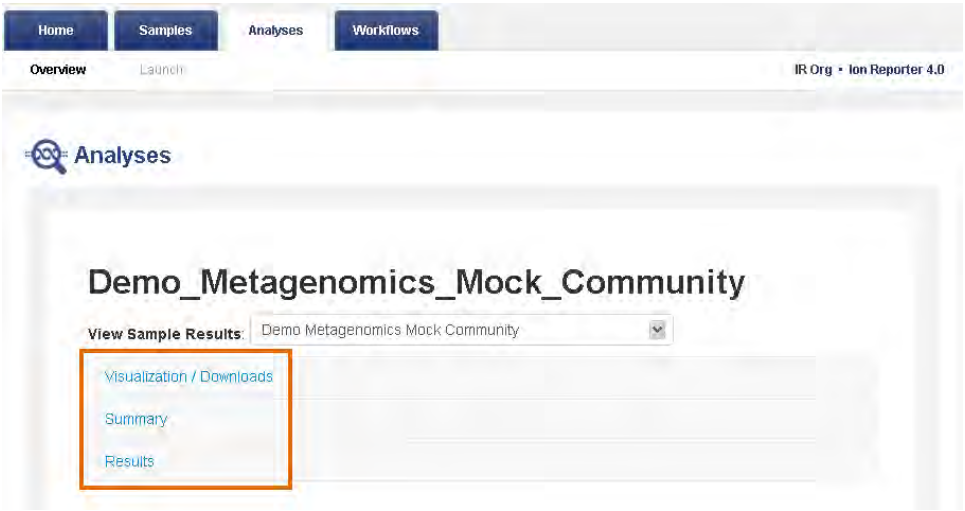
means that 3014 reads matched sequences in the MicroSEQ™ database and 26 matched sequences in the Greengenes database.

Note: When primer information is missing in the metagenomics workflow, a warning message appears during analysis review.

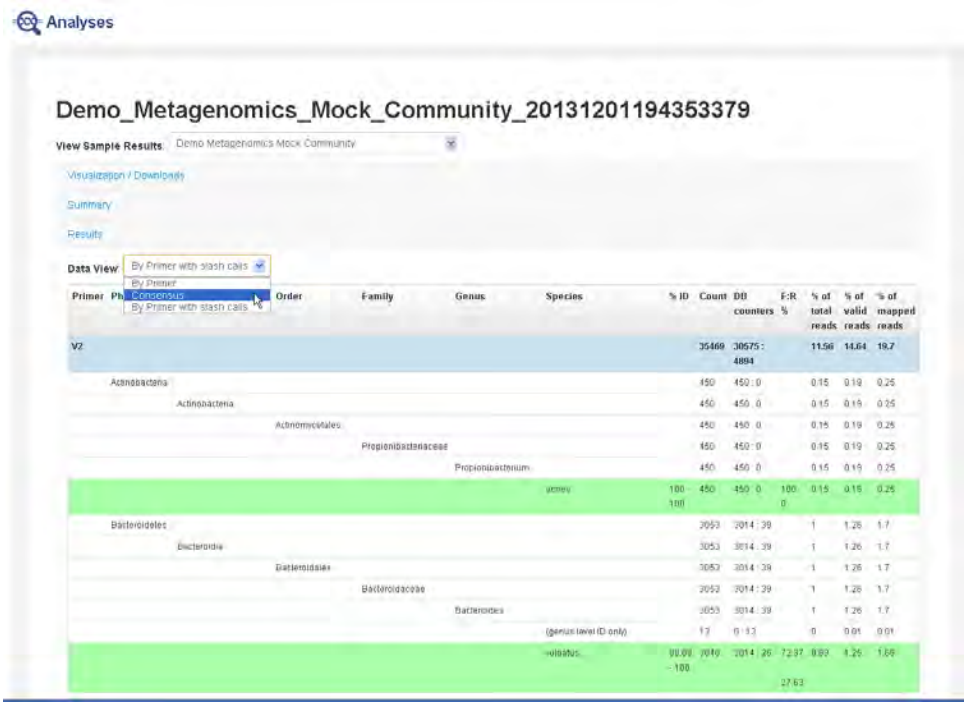


Steps to interpret your metagenomics 16S™ analysis results

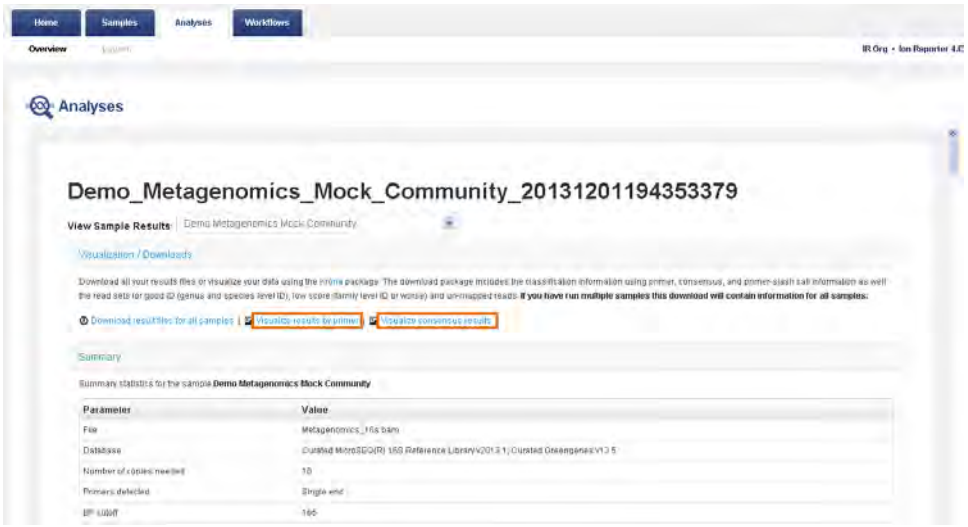
1. Log in to the Ion Reporter™ Software application and click on the **Analysis** tab.
2. Click on the name of your metagenomics analysis in the analysis table.
3. Click on the Visualization / Downloads, Summary, or Results section headers to collapse the section.



4. Click on the **Data View** menu to display either consensus data (combined from all primers) or data broken out by primer, with or without slash calls.



5. Click on one of the visualization links to view your results with Krona™ charts.



Note: Sequences from different 16S databases may use different taxonomic hierarchies. For example, Greengenes may have de novo classified sequences labeled as Archaea>Crenarchaeota>Thaumarchaeota where Thaumarchaeota is a class of Crenarchaeota. Records from other databases may treat both Thaumarchaeota and Crenarchaeota as phyla. Therefore, you may see differences in taxonomies when you compare 16S analysis results from the two databases used in Ion Reporter™ to other, external databases.

Alpha-beta diversity results

The 16S Metagenomics workflow in Ion Reporter™ Software includes alpha diversity calculations and beta diversity calculations.

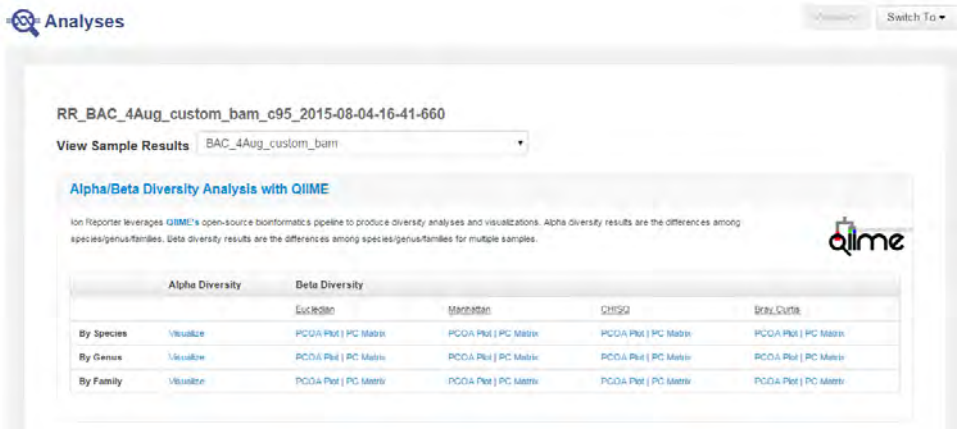
Alpha diversity results describe the diversity in a single sample at the Species, Genus, and Family levels. Beta diversity results describe the diversity between multiple samples at the Species, Genus, and Family levels. For results interpretation guidance, see the QIIME online help at **qiime.org**.

Note: If you only run one sample, Ion Reporter returns only alpha diversity results. If you have two or more samples, you get beta diversity results. However, PCOA plots can only be generated for more than three samples, depending on the quality of data input.

Quantitative Insights Into Microbial Ecology (QIIME) algorithms determine what species, genus, or families are present.

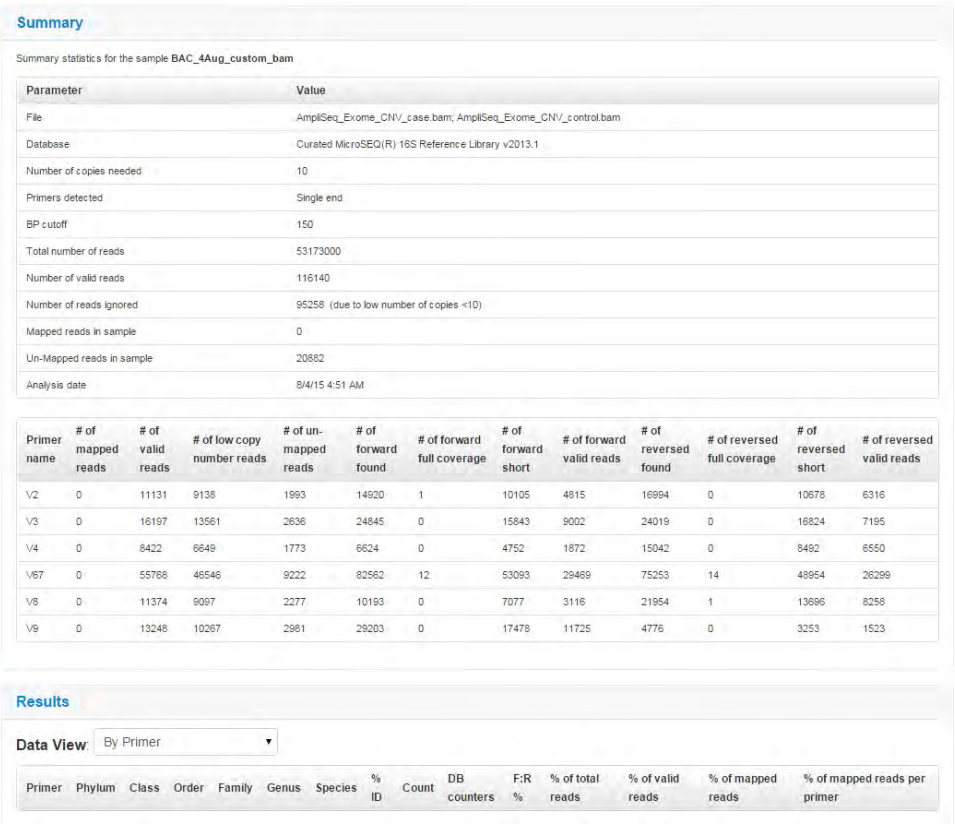
To review results:

1. In the **Analyses**, click **Overview**, then select the analysis of interest.
   An Analyses summary page appears.



2. Review your results by clicking the Visualize links in the **Alpha Diversity** column or the various plots and matrices links in the **Beta Diversity** column.

3. Scroll down to Summary and Results sections to see overall statistics.



Summary

Summary statistics for the sample **BAC_4Aug_custom_bam**

Parameter	Value
File	AmpliSeq_Exome_CNV_case.bam; AmpliSeq_Exome_CNV_control.bam
Database	Curated MicroSEQ(R) 16S Reference Library v2013.1
Number of copies needed	10
Primers detected	Single end
BP cutoff	150
Total number of reads	53173000
Number of valid reads	116140
Number of reads ignored	95258 (due to low number of copies <10)
Mapped reads in sample	0
Un-Mapped reads in sample	20882
Analysis date	8/4/15 4:51 AM

Primer name	# of mapped reads	# of valid reads	# of low copy number reads	# of un-mapped reads	# of forward found	# of forward full coverage	# of forward short	# of forward valid reads	# of reversed found	# of reversed full coverage	# of reversed short	# of reversed valid reads
V2	0	11131	9138	1993	14920	1	10105	4815	16994	0	10678	6316
V3	0	16197	13561	2636	24845	0	15843	9002	24019	0	16824	7195
V4	0	8422	6649	1773	6624	0	4752	1872	15042	0	8492	6550
V67	0	55766	46546	9222	82562	12	53093	29469	75253	14	48954	26299
V8	0	11374	9097	2277	10193	0	7077	3116	21954	1	13696	8258
V9	0	13248	10267	2981	29203	0	17478	11725	4776	0	3253	1523

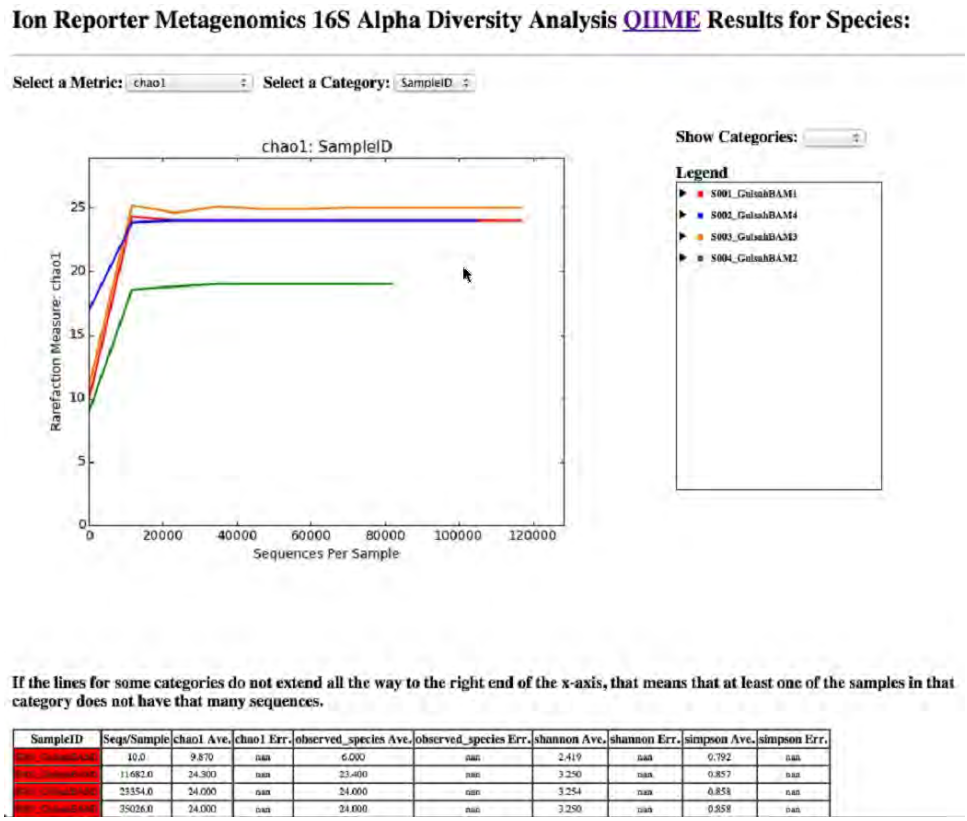
Results

Data View: By Primer

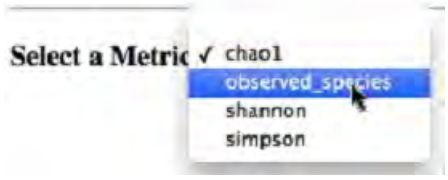
Primer	Phylum	Class	Order	Family	Genus	Species	% ID	Count	DB counters	F:R %	% of total reads	% of valid reads	% of mapped reads	% of mapped reads per primer

Alpha-beta diversity calculations are based on the information that is gathered from the consensus files that are generated by the 16S Metagenomics workflow. Read counts per sample for species, genus, and family are collected. Operational

taxonomic unit (OTU) tables are generated. These tables are used by QIIME to generate alpha-beta diversity results.



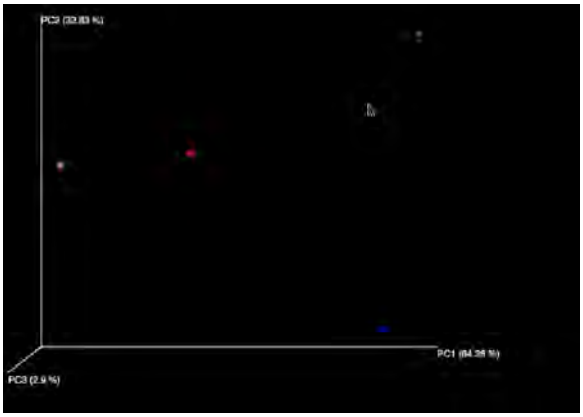
4. Select your metrics.



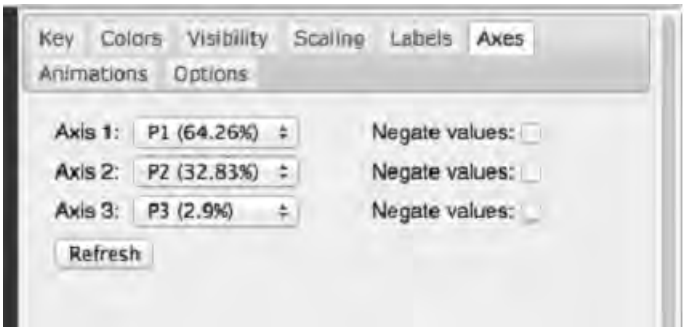
5. Repeat for Genus and Family links under Alpha Diversity Results to see the genus and families that are in the sample.

6. Click Species under Beta Diversity Results.

7. Here you can view several plot types, including: Eucledian, Manhattan, Chi-Square and Bray Curtis PCOA Plots and PC Matrices.



8. Tools on the right side can be used to produce an image.

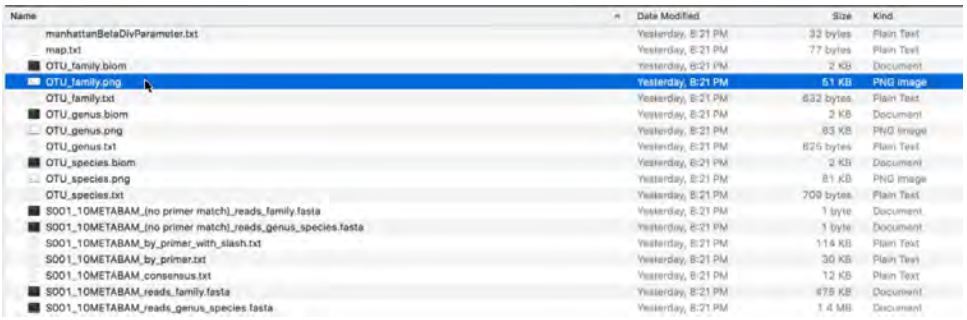


Visualize OTU results with R-scripts graphs

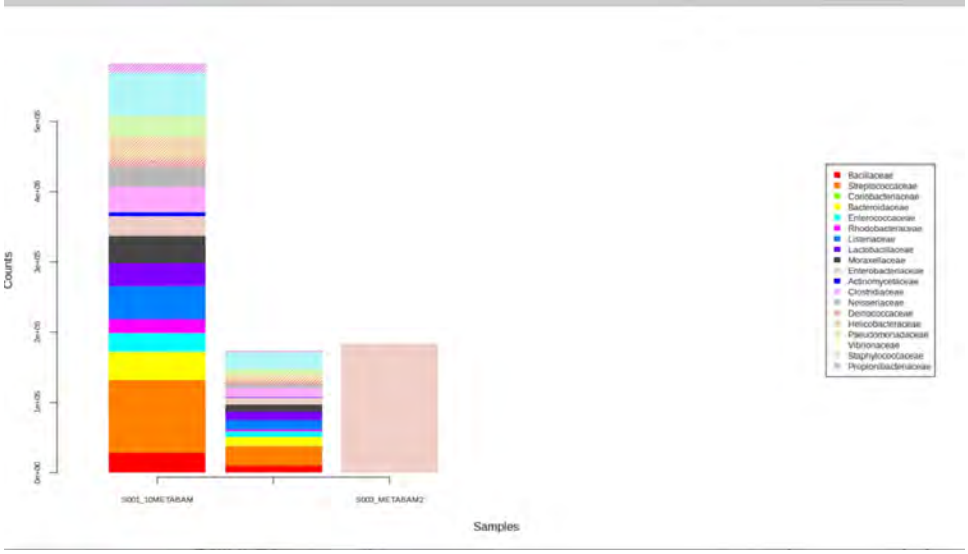
New in Ion Reporter™ software, OTU files are displayed graphically. For instance, in the example below, the OTU_family.txt file is rendered graphically in the OTU_family.png file.

1. Download your results.

2. Click on the PNG file of the results.



The data is displayed visually.

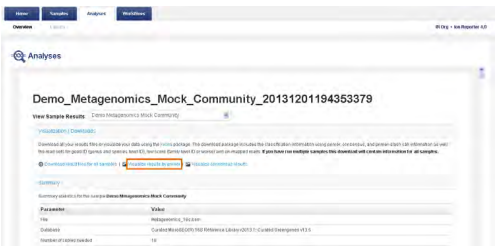


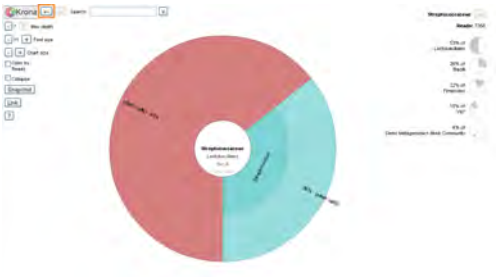
Visualize your metagenomics 16S results with Krona charts

The Krona™ visualization package enables you to browse through your data using interactive zoomable pie charts. Krona™ documentation is available on their **github site**.

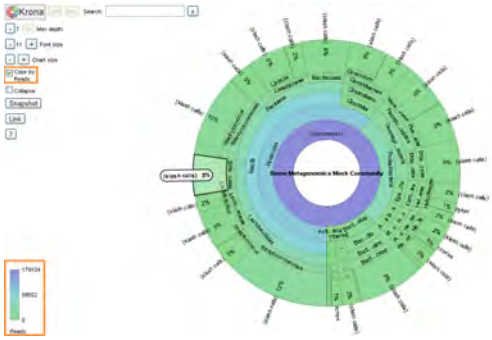
Note: The Snapshot button does not work on the Krona visualization page in the Metagenomics workflow. Krona™ documentation states that their charts are best viewed with the Firefox™ browser.

This table describes your actions to visualize your metagenomics 16S™ results with Krona™ charts:

Purpose	Your action
Open your analysis data in a Krona™ chart	Click on the **Visualize results by primer** link. 

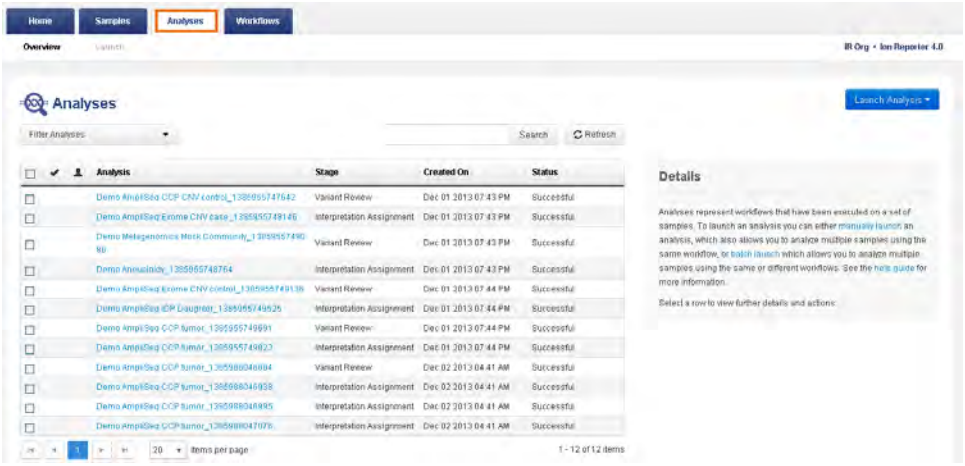
Purpose	Your action
Open Krona™ on your consensus data	Click the **Visualize consensus results** link. 
Display percentages for one area of the chart	Click on that area of the chart. The percentages for that area are displayed in the top right of the page. 
Make that area the new focus of the chart	Click the expand box in the top right of the page. 
Return to the previous chart focus	Click the back arrow ← near the Krona™ logo in the top left of the page. (Only one go-back is supported at a time.) 

Purpose	Your action
Change the number of circles shown in the chart	Click the minus or plus icons [-] [+] near the **Max depth** label. 
Change the font size	Click the minus or plus icons [-] [+] near the **Font size** label. 
Change the size of the chart	Click the minus or plus icons [-] [+] near the **Chart size** label. 

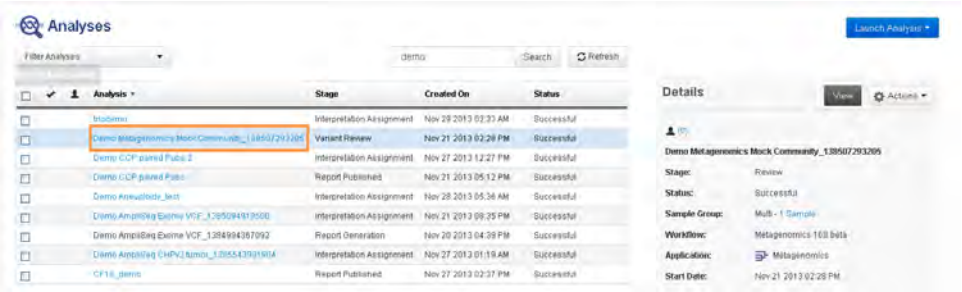
Purpose	Your action
Show read depth by color	Enable the **Color by Reads** checkbox. A color legend appears in the bottom left. (This example shows a consensus chart.) 
Simplify wedges	Enable the **Collapse** checkbox. This selection combines redundant wedges (that contain only another wedge). 
Create an image of the pie chart	Click the **Snapshot** button.  This button creates a SVG (Scalable Vector Graphics) format image file. See Krona™ documentation on their **github site** for browser support. (For example, on Chrome an SVG file is not created directly. You have to save the page as a PDF.)

Purpose	Your action
Create a sharable link to the chart	Click the **Link** button. 
Open Krona™ documentation	Click the question mark button. This link opens the Krona™ sourceforge documentation in a new tab. 

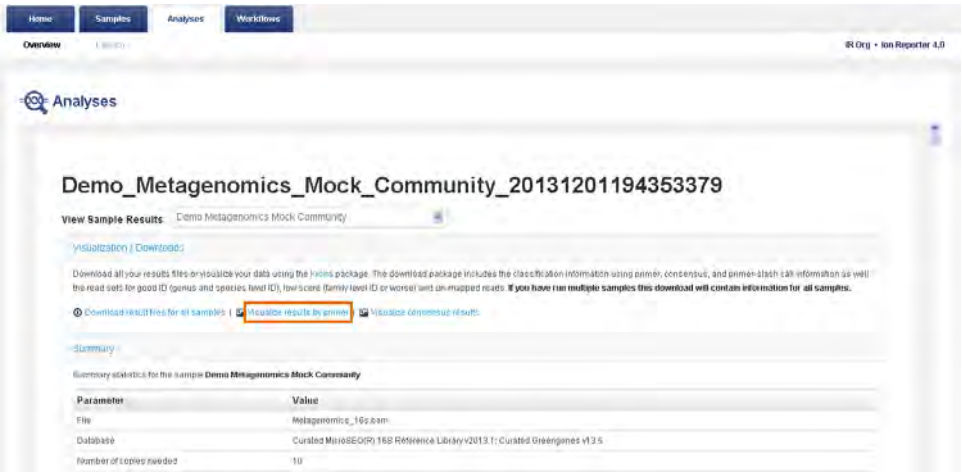
1. Log in to the Ion Reporter™ Software application and click on the **Analysis** tab.



2. Click on the name of your metagenomics analysis in the analysis table.



3. Click on the link next to the **Krona™ piechart** label.



4. See the table above or Krona™ documentation on their **github site** for next steps.

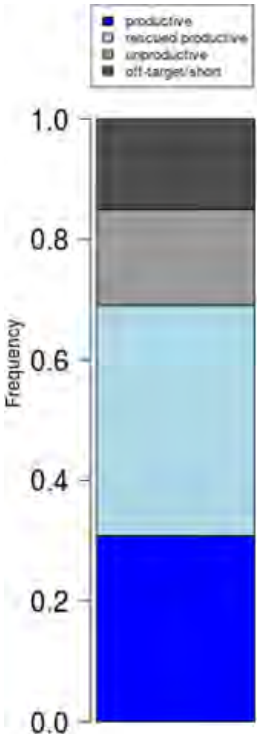
Immune Repertoire analysis results

Sample QC tab

The Ion AmpliSeq™ Immune Repertoire Assay results are represented graphically. Select the QC metric to view from the **Views** dropdown list.

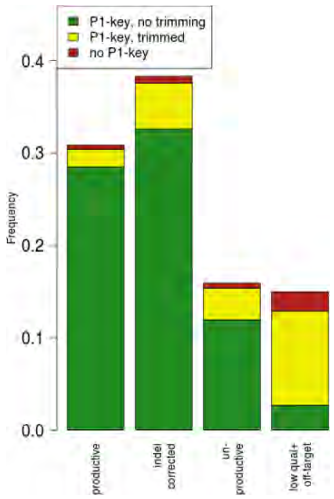
Read classification

After the first stage of data processing raw sequencing reads are classified and proportionally represented in a stacked barplot. Actual read counts for each classification are listed below the figure in the results report.

Read classification	Description	Example
Off-target/low-quality (dark gray)	Reads which are of low quality or represent the product of an off-target amplification.	
Unproductive (gray)	Reads that have uncorrectable sequencing or PCR errors which lead the rearrangement to have out-of-frame variable and joining genes or a premature stop codon.	
Rescued productive (light blue)	Reads having an in-frame variable and joining gene, and no stop codons after indel error correction.	
Productive (blue)	Reads having an in-frame variable and joining gene, and no stop codons.	

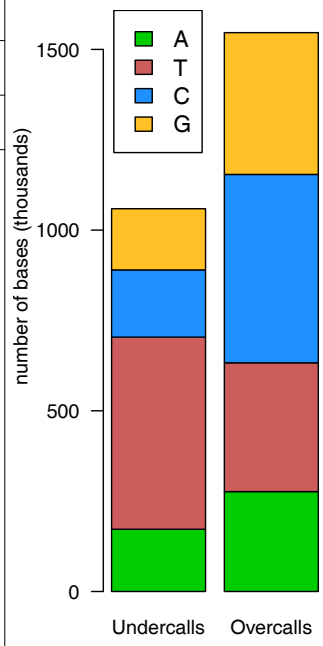
Proportion of full length, quality trimmed and reads lacking P1-key, by read classification

Stacked barplot indicating the frequency of quality trimming for reads classified as productive, rescued productive, unproductive and off-target/low-quality. Full length reads categorized as low quality/off-target are likely to represent off-target amplifications.

Color	Description	Example
Red	Quality trimmed reads lacking an identifiable P1-key.	
Yellow	Quality trimmed reads having an identified P1-key.	
Green	Full length reads having an identified P1-key, and no quality trimming.	

Base composition of overcalled and undercalled homopolymers

Stacked barplot indicating the nucleotide composition of overcalled bases (base insertion sequencing errors) and undercalled bases (base deletion sequencing errors). Highly skewed nucleotide composition may indicate lower quality sequencing or low library diversity.

Color	Description	Example
Orange	Guanidine (G)	
Blue	Cytosine (C)	
Pink	Thymidine (T)	
Green	Adenosine (A)	

QC metrics

The QC metrics include the read classification counts and strand QC metrics.

Category	Description
Read classification	
Total productive reads	Productive + rescued productive reads
Productive reads	Reads having an in-frame variable and joining gene, and no stop codons.
Rescued productive reads	Reads having an in-frame variable and joining gene, and no stop codons after indel error correction.
Unproductive reads	Reads that have uncorrectable sequencing or PCR errors which lead the rearrangement to have out-of-frame variable and joining genes or a premature stop codon.
Off-target/ low-quality	Reads which are of low quality or represent the product of an off-target amplification.

Category	Description
Strand QC metrics	
Plus strand (v-side) read counts	Number of sequence read counts from the plus (+) strand.
Minus strand (c-side) read counts	Number of sequence read counts from the minus (–) strand.
Plus strand CDR3 avg PHRED	Average PHRED score for plus (+) strand reads.
Minus strand CDR3 avg PHRED	Average PHRED score for minus (–) strand reads.

Sample Results tab

The Ion AmpliSeq™ Immune Repertoire Assay results are represented graphically. Select the Sample Results to view from the **Views** dropdown list.

Spectratyping plots

The immune repertoire within a sample is represented in each spectratyping plot by the range of CDR3 lengths and their pattern of distribution. Reads for identified clones are arranged along the X-axis according to the variable gene identity and the Y-axis according to the CDR3 nucleotide length. The variable gene order reflects the gene position within the TCRβ locus. Dots are separated vertically along the Y-axis by 3 nucleotides (one codon), the higher up the Y-axis the longer the CDR3 region. Circle size indicates the frequency of a particular variable gene-CDR3 nucleotide length combination within the dataset. Circle color represents a forth metric specific to each graph (e.g., shannon diversity, evenness, clone frequency). Key repertoire metrics are displayed along the lower margin of the plot.

In the Ion Reporter™ Software the spectratyping plots are interactive, allowing you to adjust the data and access clone details. Drag the ends of the horizontal bar below the X-axis to limit the region (v-genes) to view in the plot. Drag the ends of the color range up or down to limit the clones that are viewed. Hover your cursor over any dot to view the details of an individual clone. Click ↻ to restore the default plot view, click ⬇ to download a static image of the plot (if you have adjusted the plot view, the adjusted plot is downloaded).

Note: T-cell Leukemia (Jurkat) Total RNA is derived from a cell line consisting of a single T-cell clonotype. Running the Ion AmpliSeq™ Immune Repertoire Assay Plus—TCRβ on Jurkat Total RNA should detect a single clonotype (a single spot on spectratyping plot).

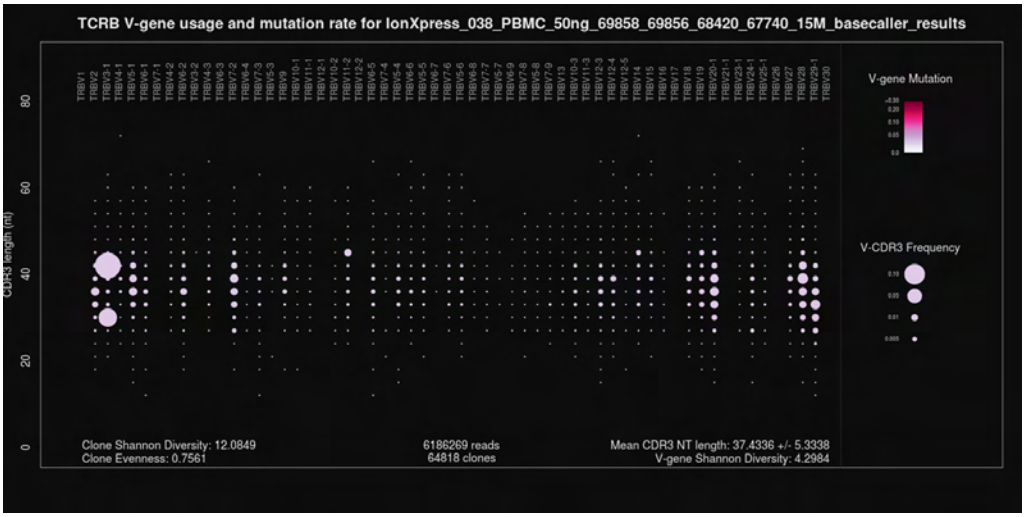


Figure 1 TCRB V-gene usage and mutation rate

Spectratyping plot highlighting frequency of mutated bases over the variable gene of identified clones. Circle color indicates the average frequency of mutated bases for clones having a particular variable gene-CDR3 nucleotide length combination. The two large circles in the TRBV3-1 column indicate the presence of expanded clones having that particular variable gene. The light color of the circles indicates that the variable gene sequences of the sample closely match reference variable gene sequences in the IMGT database. Systematic differences with respect to reference may indicate the presence of polymorphism within the variable gene that is not captured by the IMGT database.

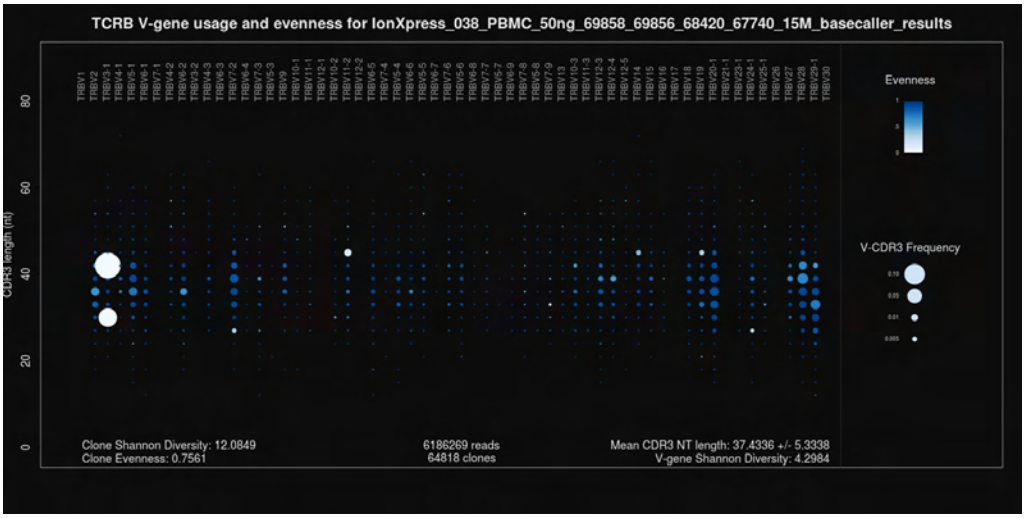


Figure 2 TCRB V-gene usage and evenness

Spectratyping plot highlighting evenness of identified clone sizes (Normalized Shannon Entropy). Circle color indicates the evenness of clone sizes for clones having a particular variable gene-CDR3 nucleotide length combination. Values range from 0 to 1, with 1 indicating most even clone sizes. In this representation, portions of the repertoire containing highly expanded clones appear white.

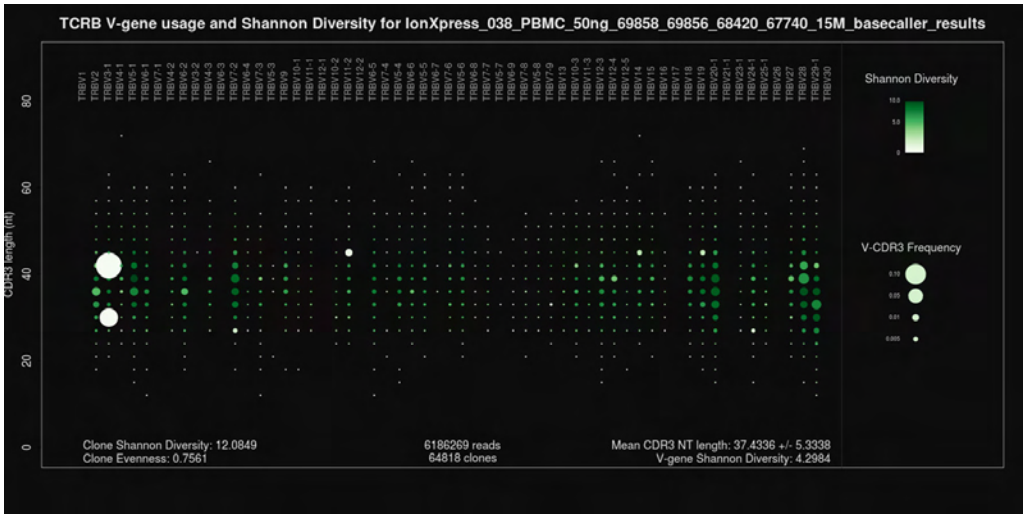


Figure 3 TCRB V-gene usage and shannon diversity

Spectratyping plot highlighting Shannon Diversity (entropy) of identified clones. Circle color indicates the Shannon Diversity of clones having a particular variable gene-CDR3 nucleotide length combination. Portions of the repertoire containing highly expanded clones typically have a corresponding low Shannon Diversity value.

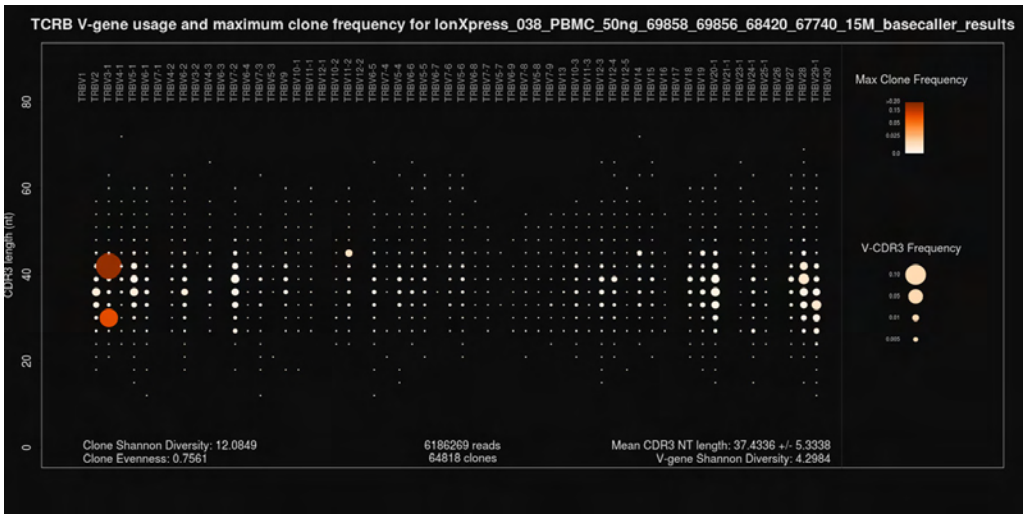


Figure 4 TCRB V-gene usage and largest clone frequency

Spectratyping plot highlighting the frequency of the largest clone for each variable gene-CDR3 nucleotide length combination. Circle color indicates the frequency of the largest clone having a particular variable gene-CDR3 nucleotide length combination. Dark color indicates the presence of particularly expanded clones. These may be indicative of response to antigen but are also more commonly found in aged healthy individuals.

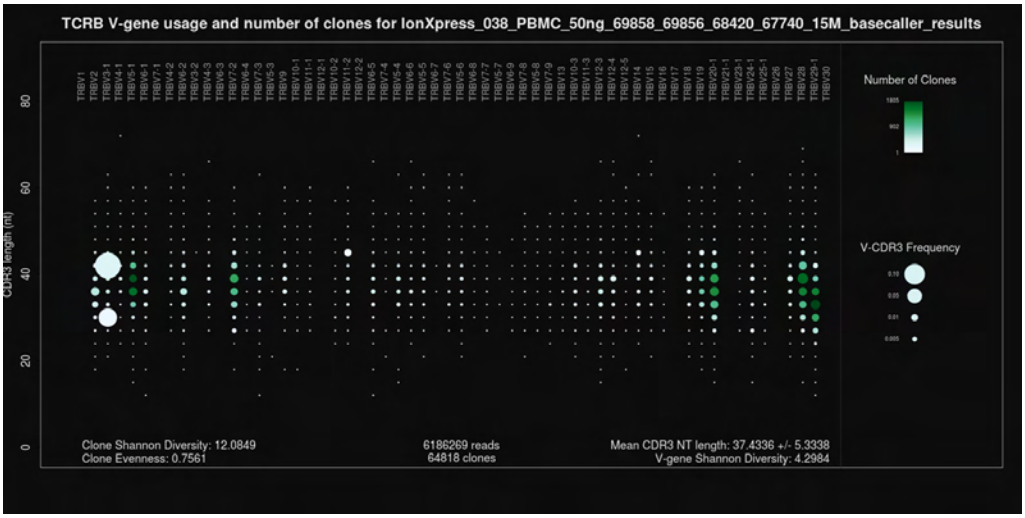


Figure 5 TCRB V-gene usage and number of clones

Spectratyping plot highlighting the number of clones that are identified for each variable gene-CDR3 nucleotide length combination. Circle color indicates the number of clones having a particular variable gene-CDR3 nucleotide length combination. Specific variable genes may more frequently participate in VDJ recombination, leading to an enrichment in distinct clones for those variable genes.

V-gene usage

The Ion AmpliSeq™ Immune Repertoire Assay results are represented graphically. Select **V gene usage** from the **Views** dropdown list. The stacked barplots indicate the representation of variable genes among identified clones. Ordering of variable genes reflects position within the TCRβ locus.

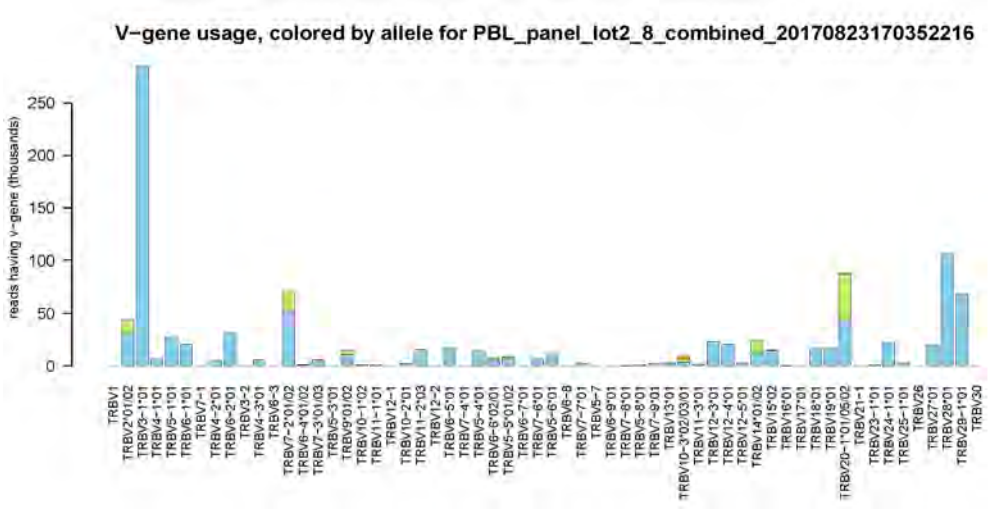


Figure 6 V-gene usage highlighting alleles

Color segments within each bar indicate the frequency of particular variable gene alleles, arranged by frequency from rarest (top) to most common (bottom).

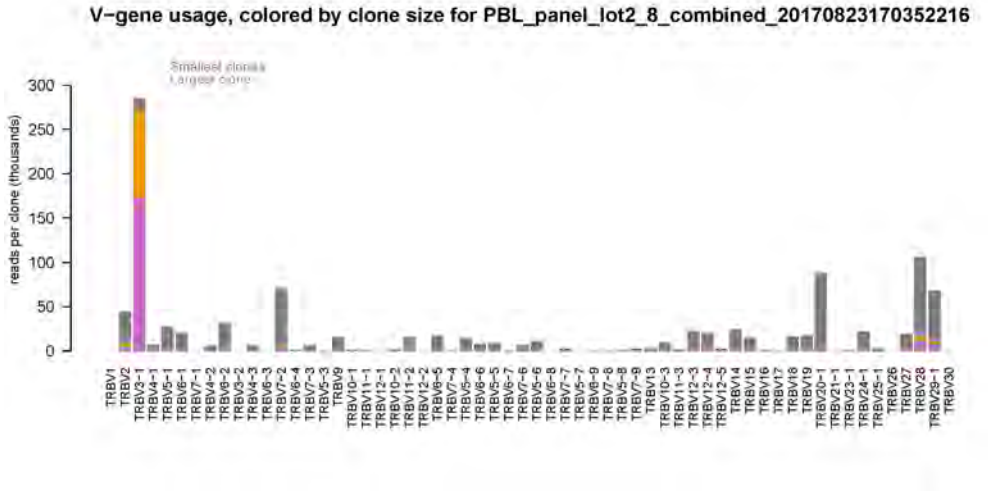


Figure 7 V-gene usage highlighting clone sizes

Color segments within each bar indicate the contribution of individual clones, arranged from smallest clones (top) to largest clones (bottom).

VJ-gene usage heatmap

The Ion AmpliSeq™ Immune Repertoire Assay results are represented graphically. Select **VJ- gene usage heatmap** from the **Views** dropdown list.

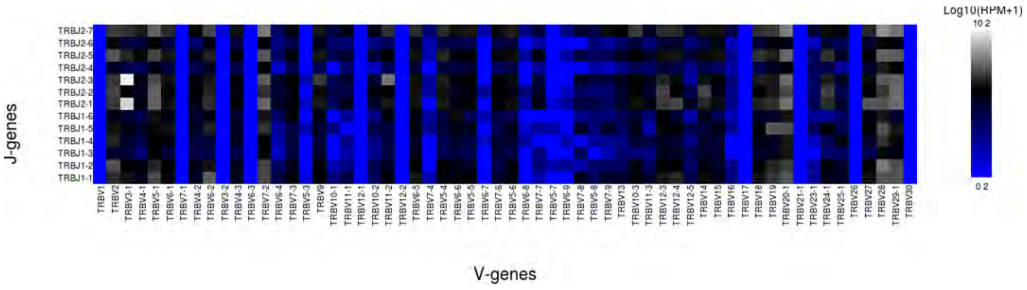
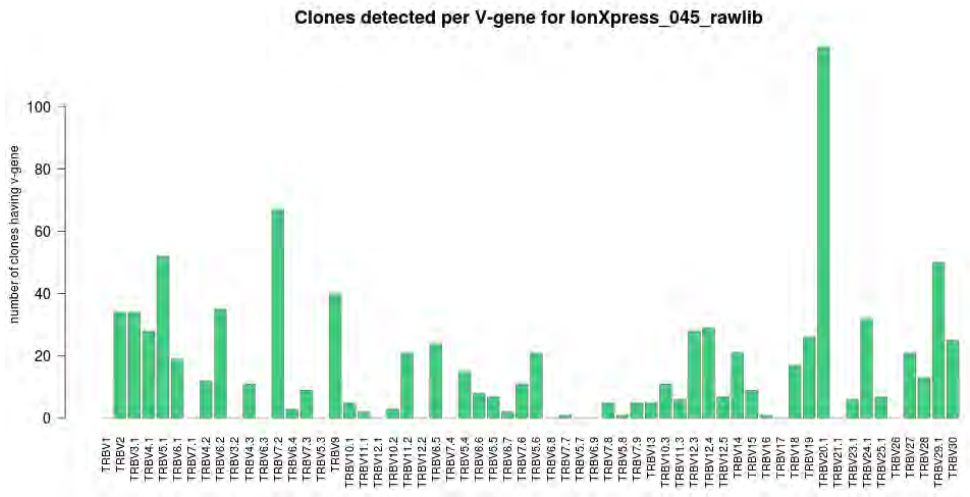


Figure 8 VJ-gene usage heatmap

Heatmap illustrating the frequency of particular Variable gene-Joining gene combinations for identified clones. Frequencies are log transformed with pseudocount added. Over-represented Variable-Joining gene combinations (white) may indicate presence of a highly expanded clone. Ordering of variable genes reflects position within the TCRß locus.

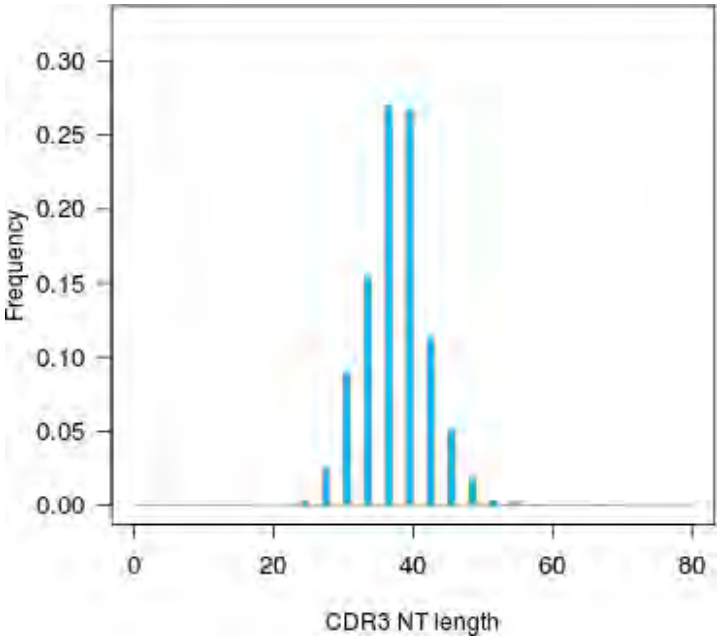
Clones detected per variable gene

The Ion AmpliSeq™ Immune Repertoire Assay results are represented graphically. Select **Clones detected per variable gene** from the **Views** dropdown list. The barplot indicates the number of identifed clones having a particular variable gene. Ordering of variable genes reflects position within the TCRβ locus.



CDR3 histogram

The Ion AmpliSeq™ Immune Repertoire Assay results are represented graphically. Select **CDR3 histogram** from the **Views** dropdown list. The histogram indicates the distribution of identifed clones with a given CDR3 nucleotide length.



Relative frequency (Y-axis) of identifed clones with a given CDR3 nucleotide length (X-axis).

Clone summary table

The **Clone Summary** table lists the identified clones in rank order from the most frequently occurring to least frequent. Each row represents an individual clone. For each clone the identified **Variable** and **Joining** region are listed as well as the amino acid (**CDR3 AA**) and nucleotide (**CDR3 NT**) sequences of the CDR3 region.

For multi-sample analyses the **Clone Summary** table lists the frequency of each clone identified in any of the samples—sorted in descending order based on the frequency of the clones in the leftmost column—for each sample in the analysis.

① Sample 1 ② Sample 2

Table 3 Clone summary information fields

Coloumn header	Description
Variable	The best matching IMGT variable gene of the rearrangement.
Joining	The best matching IMGT joining gene of the rearrangement.
CDR3 AA	The CDR3 amino acid sequence of the rearrangement, denoted using the IMGT definition of the CDR3 region.
CDR3 NT	The CDR3 nucleotide sequence of the rearrangement, denoted using the IMGT definition of the CDR3 region.
Count	The total number of reads mapping to the rearrangement after quality filtering.
Frequency	The frequency of the rearrangement as a proportion of total reads passing quality filtering.
Rank	The frequency rank of the rearrangement.

Note: Additional details are available by downloading the **Clone Summary** table.

Download the clone summary table

1. In the **Immune Repertoire Results** screen, **Sample Results** tab, select **Clone Summary** from the **Views** dropdown list.
2. Click **Download Clone Summary**.
   The clone summary CSV file downloads automatically.
3. Open the clone summary CSV file to view the additional information that is included in the spreadsheet.

Table 4 Additional clone summary information fields

Column header	Description
Diversity	The best matching IMGT diversity gene of the rearrangement. This field is blank if the diversity gene cannot be identified with confidence, often as a consequence of exonucleotide chewback.
Plus Counts	The number of reads mapping to the plus strand (V to J orientation) after quality filtering.
Minus Counts	The number of reads mapping to the minus strand (J to V orientation) after quality filtering.
Variable Mutation	The proportion of bases within the variable gene region that do not match the IMGT variable gene reference sequence. Variable gene alleles that are not found in the IMGT database will have a non-zero value for this metric.
Variable Gene Allele	The allele of the best matching IMGT variable gene.
Diversity Gene Allele	The allele of the best matching IMGT diversity gene. This field is blank if the diversity gene cannot be identified.
Joining Gene Allele	The allele of the best matching IMGT joining gene.
Constant Gene	The constant gene of the rearrangement.
Sequence	The sequence of the read corresponding to the rearrangement, presented in plus strand (V to J) orientation.
Reference Sequence	The combined sequence of the best matching IMGT variable, diversity and joining genes, aligned to the rearrangement sequence. Dashes indicate positions within the reference VDJ sequence that do not match the rearrangement sequence. Often these represent N-additions within the V-D and D-J junctions due to the action of terminal deoxynucleotidyl transferase (TdT).

Manually launch an analysis in Ion Reporter™

1. Sign in to the Ion Reporter™ software.
2. Launch the analysis.

From the...	Directions
Home tab	1. In the **Dashboard** screen, click **Launch analysis**. 2. In the **Launch Analysis** screen, select **Immune Repertoire** from the **Research Application** dropdown list. 3. Click **Next**.
Analyses tab	1. Click **Launch Analysis**, then select **Manual** from the dropdown list. 2. In the **Launch Analysis** screen, select **Immune Repertoire** from the **Research Application** dropdown list. 3. Click in the **AmpliSeq Immune Repertoire Plus TCR beta chain - w1.0 - Single Sample** row, then click **Next**.
Workflows tab	1. Select **Immune Repertoire** from the **Research Application** dropdown list. 2. Click in the **AmpliSeq Immune Repertoire Plus TCR beta chain - w1.0 - Single Sample** row, then select **Launch Analysis** from the **Actions** dropdown list in the **Details** pane.

3. Search by any unique identifier you used to label your samples during setup, select one or more samples to include in the analysis, then click **Next**.
4. *(Optional)* Select plugins to run with your analysis.
   a. Click ⚙ adjacent to the selected plugin.
   b. Configure the plugin parameters as necessary, click **Submit**, then click **Close**.
5. Click **Next**.
6. Enter an **Analysis Name** and **Description** *(Optional)*, then click **Launch Analysis**.

The Ion Reporter™ Software performs the analysis and the results will appear on the **Analyses** page.

Multi-sample analysis

The Ion Reporter™ Software can perform multi-sample (or cross-sample) analyses to compare the immune repertoire between samples. To perform a multi-sample analysis:

1. Under the **Analyses** tab, in the **Overview** screen, select AmpliSeq Immune Repertoire Plus TCR beta chain - w1.0 - Single Sample from the Workflow filter dropdown list.
2. Click the adjacent checkboxes to select 2 or more results files from the available list of analyses, then click **Visualize**.
3. Click the checkboxes to select 2 or more samples for multi-sample analysis, then click **Compare Samples**.

The multi-sample analysis results are reported side-by-side in a clone summary table (see page 254) in rank order from most to least frequent.

Visualize results with Ion Reporter™ Genomic Viewer or IGV

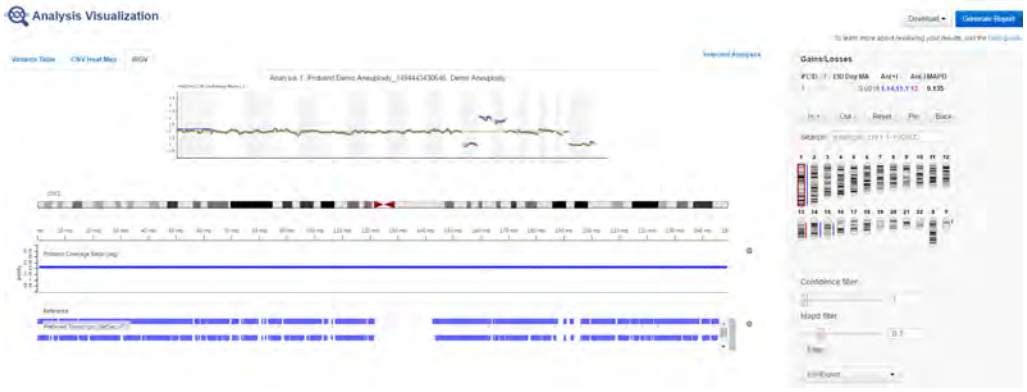
Ion Reporter™ Genomic Viewer overview

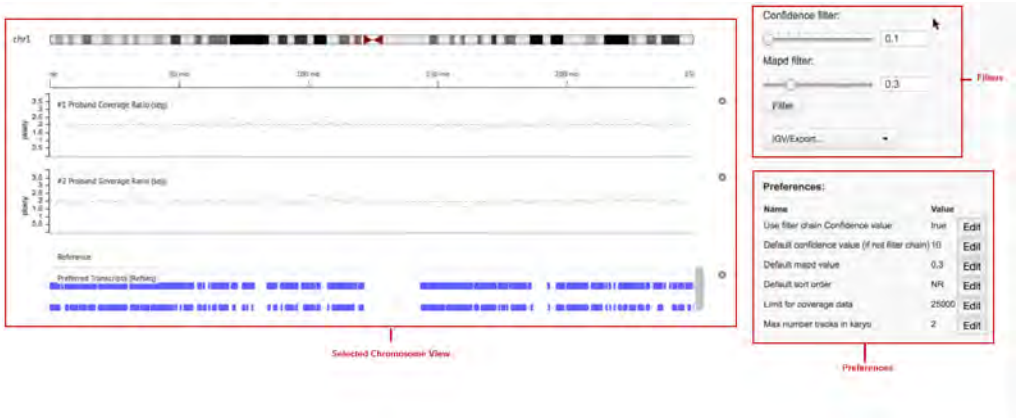
Ion Reporter™ Genomic Viewer, formerly IGV Light, is a streamlined version of the Integrative Genomics Viewer (IGV) that the Broad Institute offers. Ion Reporter™ Genomic Viewer is faster loading, but not as granular as the IGV application. You can set Ion Reporter™ Genomic Viewer preferences in Account Preferences, such as an option to determine how many rows appear in the list of results on the**Analysis Visualization** screen. For more information, see "Set IRGV or IGV as default viewer" on page 21.

Note: The maximum number of analyses you can open in a single visualization depends on the size of the analyses results data.

The following aneuploidy visualization shows the main area of the viewer.

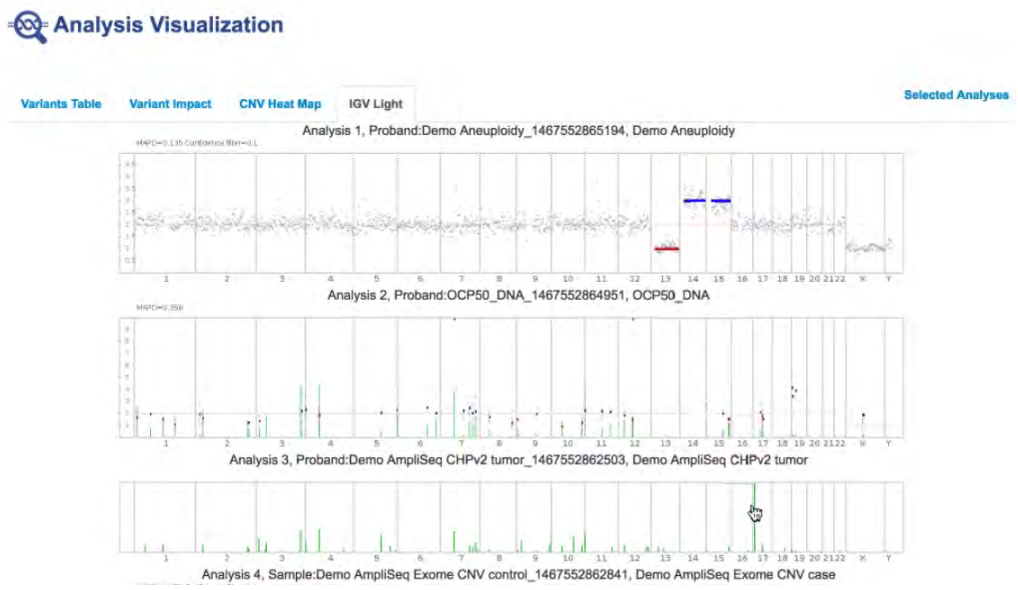
- Whole Genome View
- Selected Chromosome View
- Summary Table
- Filters
- Preferences





You can view multiple analyses at a time and multiple types of analyses. The image below is a Whole Genome View of an aneuploidy, Oncomine™, and cancer hotspot analyses. Notice, that different types of analyses look different in the Whole Genome View and have different contents in the Summary table.

Note: The maximum number of analyses you can open in a single visualization depends on the size of the analyses results data.

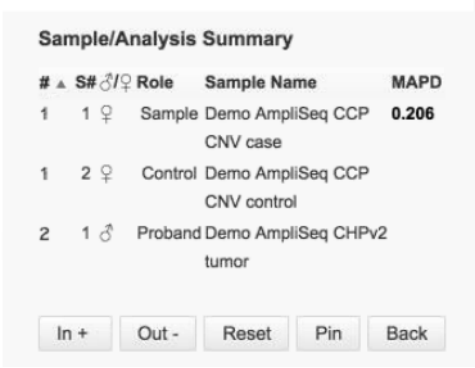


The first Whole Genome View includes CNV calls, a MAPD call, and a Confidence Filter threshold. Blue and red bars in the whole genome view represent CNV calls. Blue bars represent calls above the expected normal ploidy value (Copy Number Gains/Duplications/Insertions) for that chromosome (chr14 and 15 in this aneuploidy example). The Red bars represent calls below the expected ploidy value for the chromosome (Copy Number Losses/Deletions, chr 13 in this aneuploidy example).

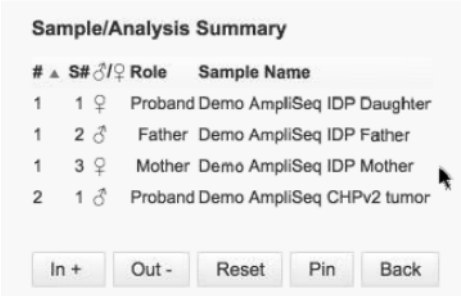
The second Whole Genome View above is an Oncomine analysis where somatic CNV calls have been made. Instead of using a confidence filter, this data uses 5% and 95% Confidence intervals that are not shown in this view. The green dots are a density plot of SNPs, Indels, and other calls. Click on any green density plot and the software zooms and displays the area in detail.

The various types of analyses also produce differing summary tables.

CNV analysis summary table



Trio analysis summary table

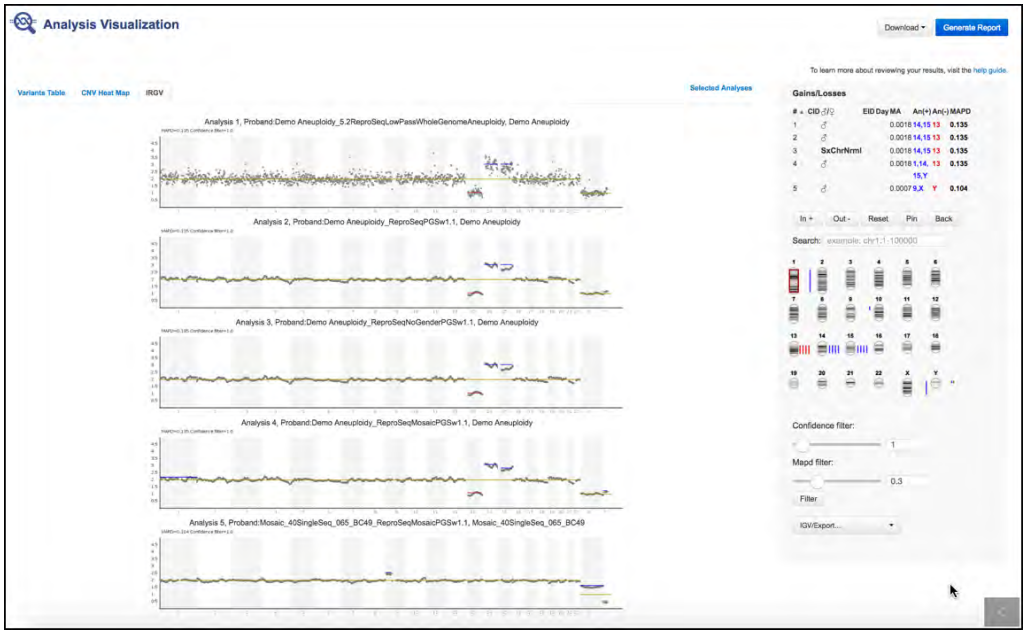


Aneuploidy analysis summary table

RNA View summary table

Smoothing, No Gender and Mosaicism results in IRGV

This image shows five aneuploidy analyses run in various Ion Reporter Software 5.4 aneuploidy workflows that show how smoothing, No Gender and Mosaicism appear in Ion Reporter™ Genomic Viewer visualizations. To make data points easier to see on the screen and in reports, visualization of aneuploidy detection is enhanced by tile-by-tile data points that are rendered as easier-to-see circles in the whole genome views. All results from Ion Reporter™ Genomic Viewer 5.4 aneuploidy workflows use this enhanced view.



Note: Analyses 2 through 5 have smoothing of the discrete tile data turned on by default. Smoothing of the discrete data has no impact on the calls that are made by the aneuploidy pipeline in the software. Smoothing can be turned on or off in any workflow.

Analysis number	Description
1	An analysis performed with the Ion Reporter™ Software 5.2 version of the Low Pass Whole Genome Aneuploidy workflow and the Demo Aneuploidy sample that is available in the software, with a loss of one copy of chr13 and single copy gains of chrs 14 and 15; this analysis was run with a Ion Reporter™ Software 5.2 workflow version which does not include smoothing of the discrete tile data.
2	An analysis of the Demo Aneuploidy sample run through the default ReproSeq workflow in Ion Reporter™ Software 5.4.

Analysis number	Description
3	An analysis of the Demo Aneuploidy sample run through the ReproSeq No Gender workflow. Use of this No Gender workflow generates analysis results that do not record or display the called gender of the sample in display or in data files; instead, a called gender value of SxChrNrml is given when the sample is either a normal XY male or a normal XX female with no sex chromosome aberrations, or a gender call of SxChrAbnrml when the sex chromosomes deviate from normal male or normal female by either whole-chromosome or sub-chromosomal aneuploid events. Aneuploidies on autosomes do not impact the called gender value, and can be present in samples with normal sex chromosomes.
4	An analysis of the Demo Aneuploidy sample run through the ReproSeq Mosaic workflow. Note that some chromosomes can look like they were called aneuploid if the copy number is called as CN loss =ploidy 1.95, or CN Gain = ploidy 2.05 (as shown in this example on chr1), and on the Y chromosome as a slight policy gain. Gain or Loss events with ploidy very close to expected normal ploidy can be a result of slight differences in normalization instead of true biological ploidy changes.
5	Analysis 5 is an analysis of a spiked-in sample that was mixed to have a chr9p event of relative copy number ploidy of 2.5, analyzed through the ReproSeq Mosaic workflow. The two mixed samples consisted of one male and one female, one of which had an integer copy number gain of ploidy=3 for chr 9 while the other sample was normal ploidy=2 for chr 9, and the mixing of these two samples of different gender which can be seen in the observed ploidies of the X and Y chromosomes.

Navigate from variant to variant in IGV and Ion Reporter™ Genomic Viewer

You can use the links from the variant review table and coverage analysis table to move from location to location in the same Ion Reporter™ Genomic Viewer tab or in the same IGV browser tab or window.

1. Click on one locus link to open the Ion Reporter™ Genomic Viewer or IGV browser at the location of the relevant experimental data, panel files, and genomic annotation sources. If opening IRGV, drag the newly opened IRGV's web browser tab out to make it a separate window.

2. Click on another locus link in the Analysis Results table to navigate the browser to the next variant.

Note: In some web browser versions, this functionality may be blocked for IGV. If you click on subsequent variants, the next variant's locus is not selected, and a new instance of IGV opens instead, use Ion Reporter™ Genomic Viewer or use a different web browser for the variant-to-variant navigation.

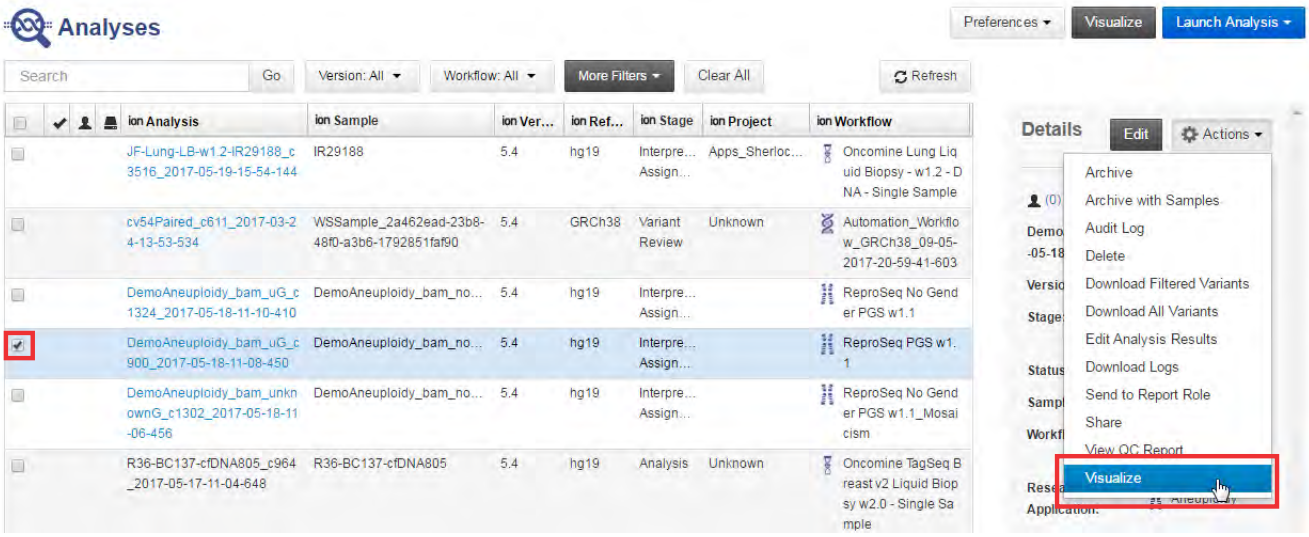
Visualize aneuploidy analyses with Ion Reporter™ Genomic Viewer

You can visualize aneuploidy results and generate reports with the Ion Reporter™ Genomic Viewer (IRGV). With this viewer, you can visualize multiple analyzes at once.

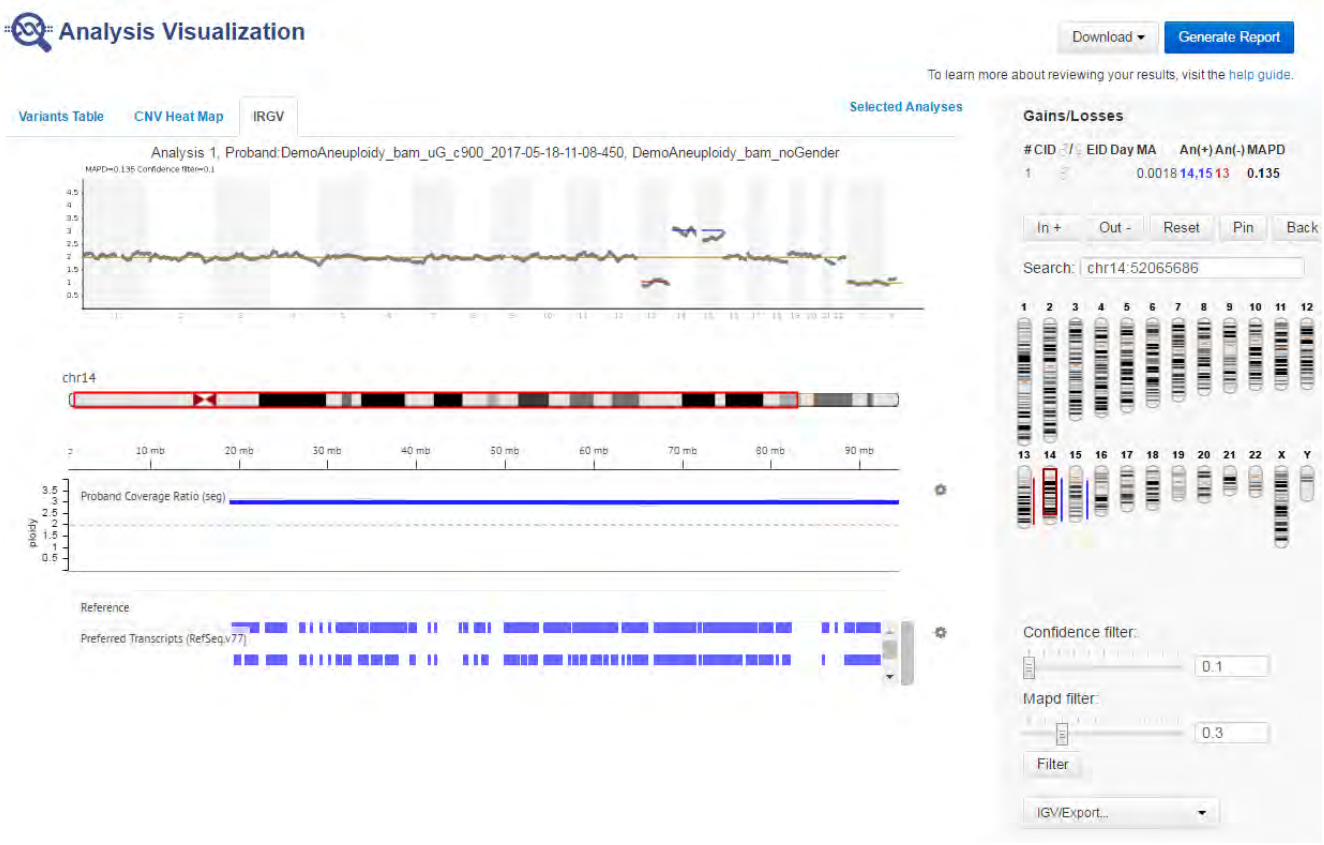
1. Under the **Analyses** tab, click **Overview**.

2. Select one or more analyses then click **Visualize** from the **Actions** dropdown list.

   Note: You can also click **Visualize** to complete this step.

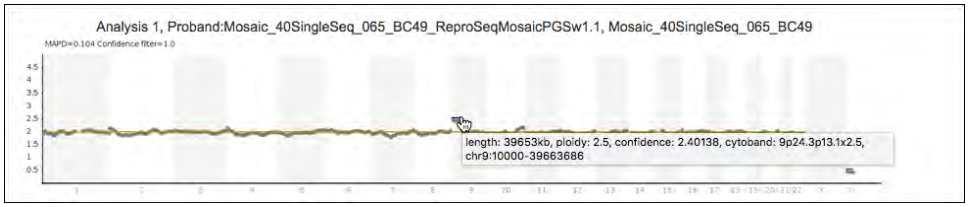


The **Analysis Visualization** page opens to the **IRGV** tab. A copy number histogram for each analysis selected appears, along with ploidy maps for selected chromosomes or chromosome regions, and karyograms showing copy number gains and losses.



Note: If you select multiple analyses generated from workflows with different confidence filter settings, the software will use the lowest confidence filter setting among the analyses and apply it across the group. You can adjust the Confidence and Mapd filters with the slider controls in the lower right corner of the screen.

Example of an IRGV plot showing non-integer ploidy of the short arm of chromosome 9, indicating that the sample was mosaic.



3. To set preferences, click **IRGV/Export ▸ Show Ion Reporter Genomic Viewer Preferences**:

Table 5

Option	Description
Use filter chain Confidence value	If set to **True**, smallest confidence filter chain value would be used as confidence filter. If set to **False**, the value set as default confidence filter below will be used.
Default confidence value (if not filter chain)	If no filter chain is used in the analysis, or **Use filter chain Confidence value** is set to **False**, then this value will be used as confidence filter by default.
Default mapd value	This value will be used as default value in the Mapd filter.
Default sort order	The preferred sort order for Aneuploidy analyses (NR, CID, EID or BIOPSY) for the summary table and tracks. NR sorts by the analysis nr, CID sorts by couple ID, EID sorts by embryo ID and BIOPSY sorts by biopsy days.
Analysis limit for BAM tracks	Maximum number of analysis that will include BAM tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and also a crowded view.
Analysis limit for BED tracks	Maximum number of analysis that will include BED tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and also a crowded view.
Limit for coverage data	By default, the maximum limit of 25000 is used. Reduce this number if you do not want to see the coverage data, or you have problems with the browser, such as freezing.
Max number tracks in karyo	By default, 5 tracks are shown. You can set a value of 1 to 10. When you increase the number of tracks, more space is used in the Karyo view.

4. For each value that you want to change, click **Edit** next to the value that you want to change, then enter a new value into the **Edit Preferences** window, then click **OK**.

5. When your edits are complete, click **Save Preferences**.

   Click **Reset Preferences** to reset all of the Preferences to the original values.

6. Select **Open in IGV (download .jnlp file)** from the **IGV/Export** dropdown list, or click **Download** at the upper right to download a file from which to launch the IGV application, or download the analysis results package.

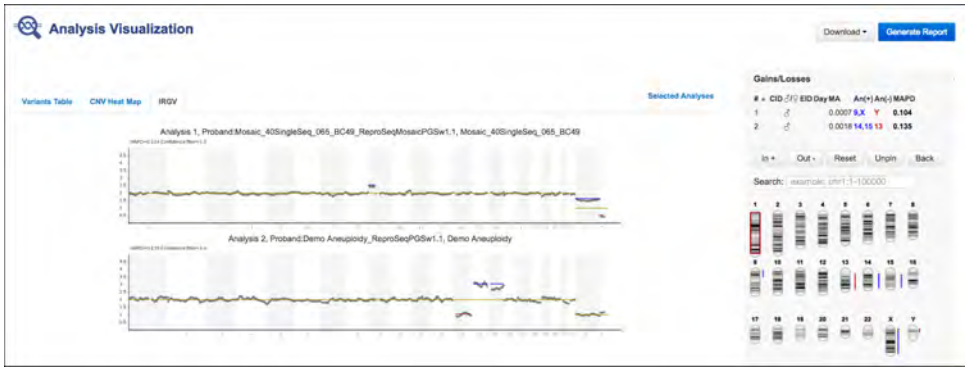
Generate aneuploidy report with Ion Reporter™ Genomic Viewer graphics

When you visualize multiple aneuploidy results in Ion Reporter™ Genomic Viewer, you can generate a single PDF report that contains graphics from each results.

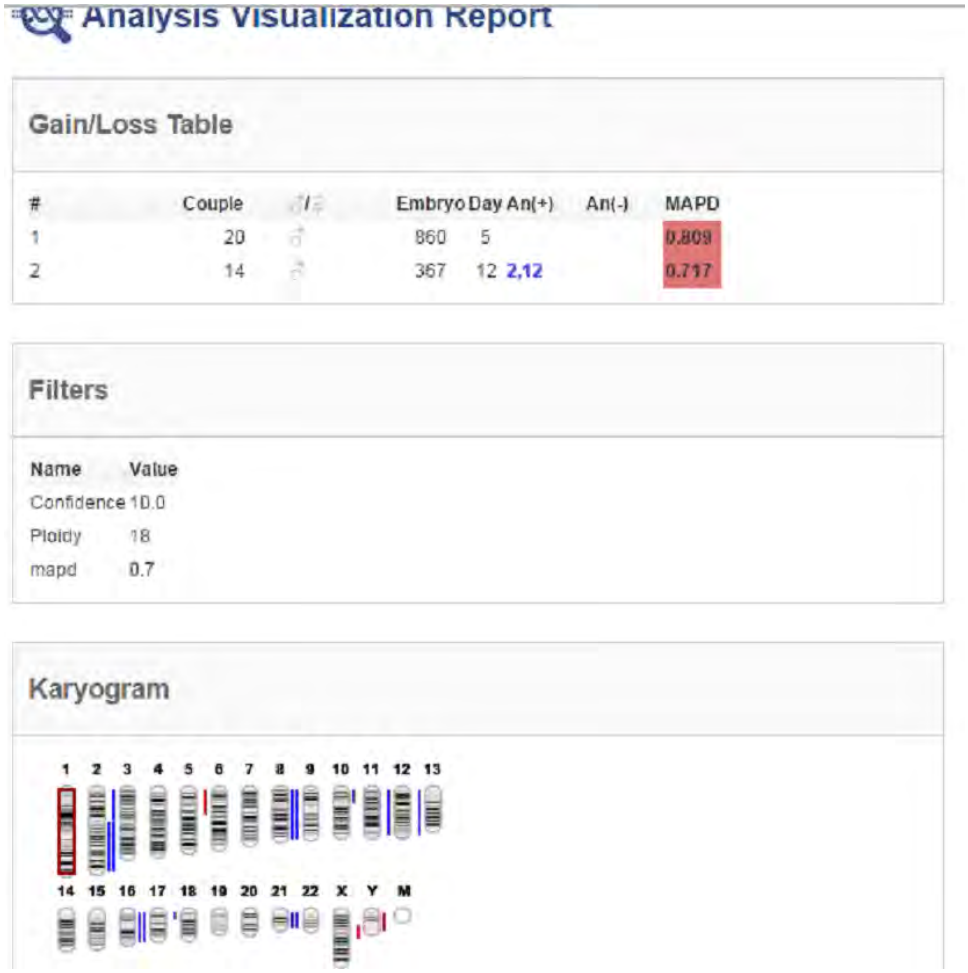
1. Under the **Analyses** tab, click **Overview**, then select the aneuploidy workflows that you want to visualize in Ion Reporter™ Genomic Viewer.

2. Click**Visualize**.

3. Click **Generate Report**.



Note: For six or more aneuploidy analyses, the Whole Genome Karyoview is not able to show Gain and Loss data segments.



A preview of the report is shown. You can customize the report.

4. Make changes to the report if desired. Enter an organization name for the report.

5. Click **Download PDF**.

The report is downloaded to the location used for downloads from the browser. This location depends on your browser settings.

Visualize other analyses with Ion Reporter™ Software

You can visualize any human DNA or RNA analysis type with the Ion Reporter™ Software.

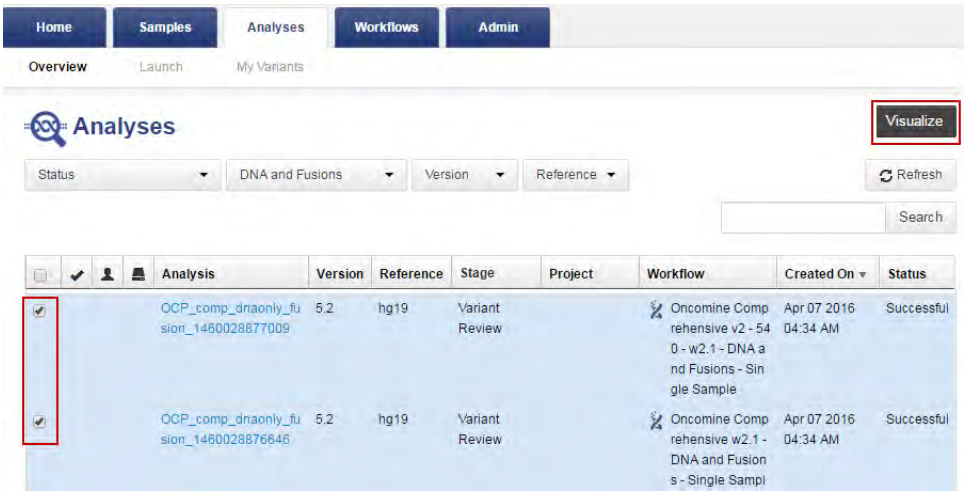
Note: When displaying Hotspots files as a track in IGV Browser, you may see Hotspot annotations displayed in the track which were not used to call bases in the data.

You can also visualize many at a time. In the example below, we look at DNA and Fusions analyses.

1. Under the **Analyses** tab, click **Overview**.

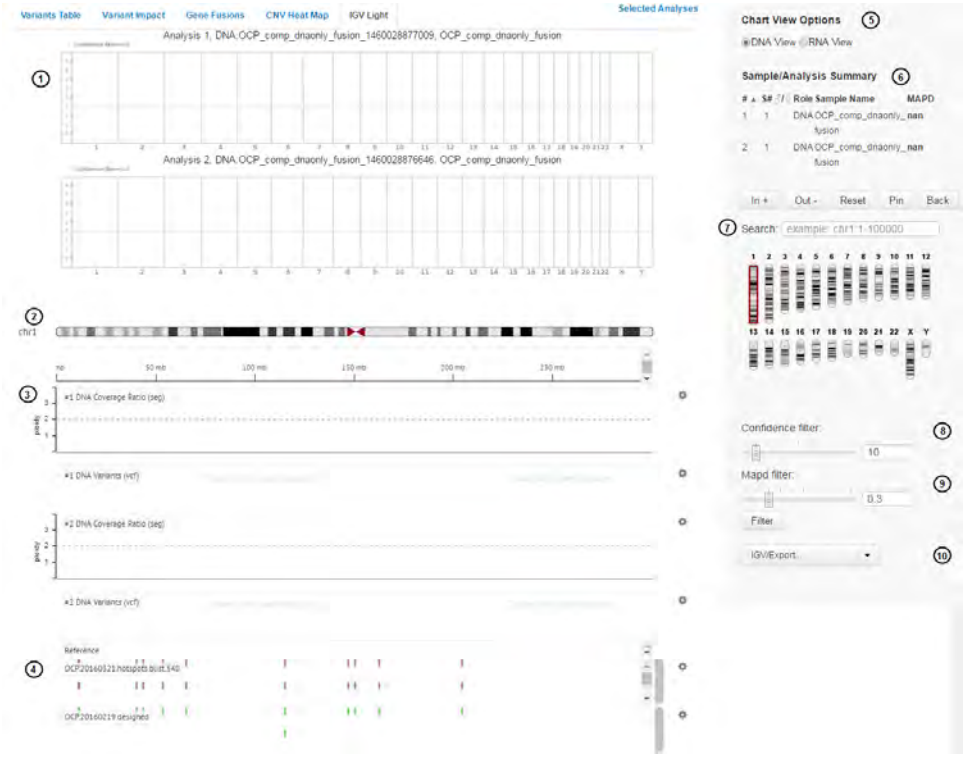
   Note: You can also launch Ion Reporter™ Software from the heatmap views (variant impact, CNV, fusion plots) and from the variant review table and the coverage analysis table. However, the preferences used for IGV Browser track loading will differ depending on where the IGV browser is launched from, either Analysis Results or Visualize > Multianalysis results.

2. Select one or more analyses and click **Visualize**.



In this example, the IRGV tab opens. The tab that opens depends the type of workflows that you selected and the number of analyses.

3. Click on the **IRGV** tab and view your results.



① Analysis Whole Genome View labels – top left, show CNV confidence threshold and MAPD threshold.
② Selected Chromosome bar – shows variant location on selected chromosome.
③ DNA coverage bars – show coverage data for CNV segments.
④ Annotations – at bottom, show annotation source results. You can click the gear button and rearrange the annotation source tracks.
⑤ Chart View Options – top right, allow you to toggle between a DNA or an RNA view.
⑥ Sample/Analysis Summary – allows you to sort by Sample number and rearrange the analysis rows on the left.
⑦ Search – allows you to search by chromosome coordinates or gene names
⑧ Confidence filter – allows you to set a CNV confidence level.
⑨ MAPD filter – allows you to set a threshold for the display of passed and failed MAPD values.
⑩ IGV/Export – allows multiple export options, including export to the full IGV viewer app, and access to preferences.

Use **In+** and **Out -** to zoom in and out on the selected chromosome.

Track order for non-aneuploidy analyses

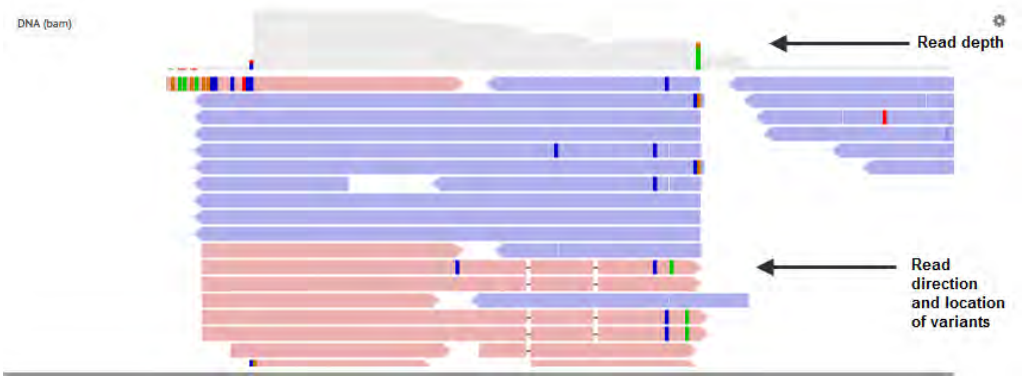
Note: In the GRCh38 genome reference, there exist alternate loci which are not part of the 1-22, X,Y chromosome contigs, and so are not annotated by the software (by design), for example chr22_K1270879v1_alt. Ion Reporter™ Software does, however, annotate the GSTT1 gene which is also located on an alternate loci.

After the Whole Genome View of each non-aneuploidy analysis, the various data tracks are loaded in this order by default:

1. Selected chromosome ideogram
2. Copy Number segment (.seg) file
3. .vcf file
4. .bam track for each sample
5. genome reference track
6. design .bed files and hotspot files
7. annotation tracks (COSMIC, ClinVar, and preferred transcript annotation tracks)

Coverage histogram

When you zoom in on an analysis, you see the coverage histogram and reads from the .bam track. The histogram in gray shows read depth at that location and reads from the .bam track show read direction and location of variants.



Set Ion Reporter™ Genomic Viewer preferences

Before you set preferences, generate an analysis visualization report for Aneuploidy results. For details, see "Generate a report and include Ion Reporter™ Genomic Viewergraphics" on page 272.

You can modify the elements that are included in analysis visualization.

1. Select one or more analyses in the **Analysis Results** screen, then click **Visualize**.
2. In the open Visualization, click **IRGV/Export** ▸ **Show IRGV preferences** .
3. In **Preferences** you can set the following options:

Option	Description
Use filter chain Confidence value	If set to **True**, smallest confidence filter chain value would be used as confidence filter. If set to **False**, the value set as default confidence filter below will be used.
Default confidence value (if not filter chain)	If no filter chain is used in the analysis, or **Use filter chain Confidence value** is set to **False**, then this value will be used as confidence filter by default.
Default mapd value	This value will be used as default value in the Mapd filter.
Default sort order	The preferred sort order for Aneuploidy analyses (NR, CID, EID or BIOPSY) for the summary table and tracks. NR sorts by the analysis nr, CID sorts by couple ID, EID sorts by embryo ID and BIOPSY sorts by biopsy days.
Analysis limit for BAM tracks	Maximum number of analysis that will include BAM tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and also a crowded view.
Analysis limit for BED tracks	Maximum number of analysis that will include BED tracks in IRGV (if available). Loading higher number of tracks would result in longer loading time and also a crowded view.
Limit for coverage data	By default, the maximum limit of 25000 is used. Reduce this number if you do not want to see the coverage data, or you have problems with the browser, such as freezing.

Option	Description
Max number tracks in karyo	By default, 5 tracks are shown. You can set a value of 1 to 10. When you increase the number of tracks, more space is used in the Karyo view.
Max Ploidy in Whole Genome View	Maximum value of the Y-axis (ploidy) in the Whole Genome View graphs.

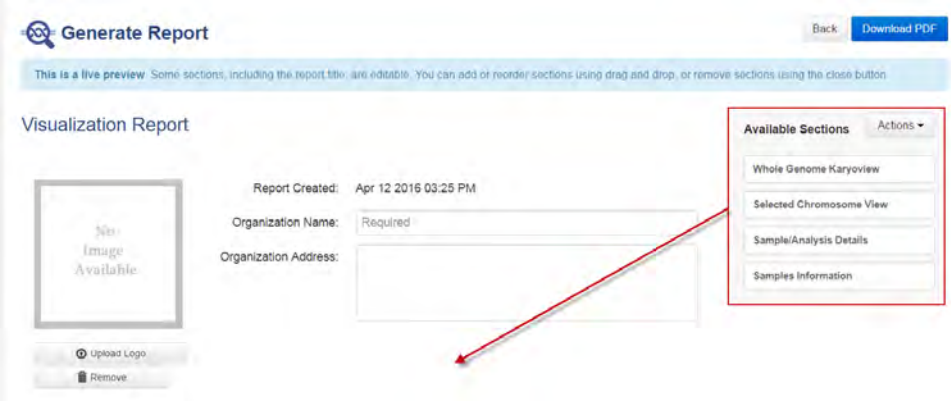
4. Click **Save Preferences** and your selections are used when you visualize other analyses. To restore the preferences to the default settings, , click **Reset Preferences**.

Generate a report and include Ion Reporter™ Genomic Viewergraphics

You can use the Ion Reporter™ Genomic Viewer to create images for reports.

1. When you have a view on the screen that you would like to use in a report, click **Generate Report**.

2. On the Visualization Report template, select the views you want to include by dragging them from Available Sections to the Report.



3. Enter any other relevant information and then click **Download PDF**.

Visualize variants with IGV

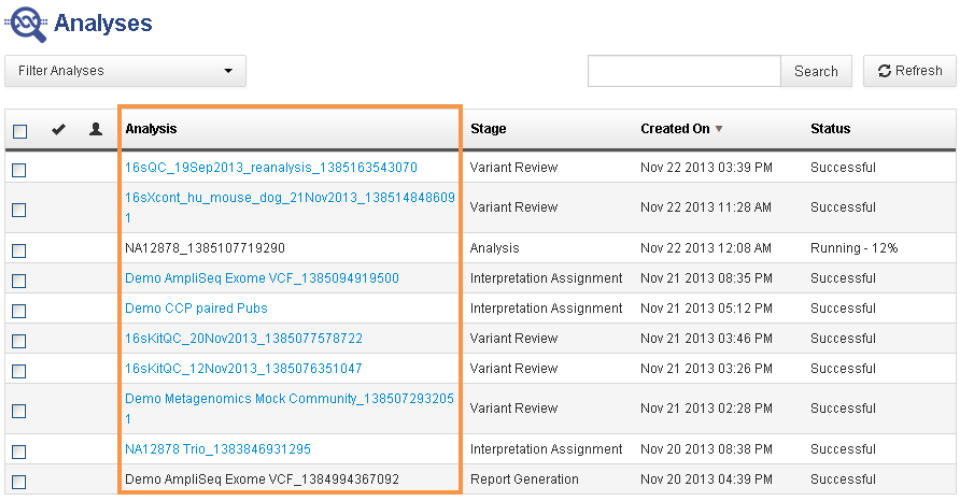
Ion Reporter™ Software leverages the Broad Institute Integrative Genomics Viewer (IGV)**http://software.broadinstitute.org/software/igv/home**) to provide visualization capabilities.

Note: When displaying Hotspots files as a track in IGV browser, you may see Hotspot annotations displayed in the track which were not used to call bases in the data.

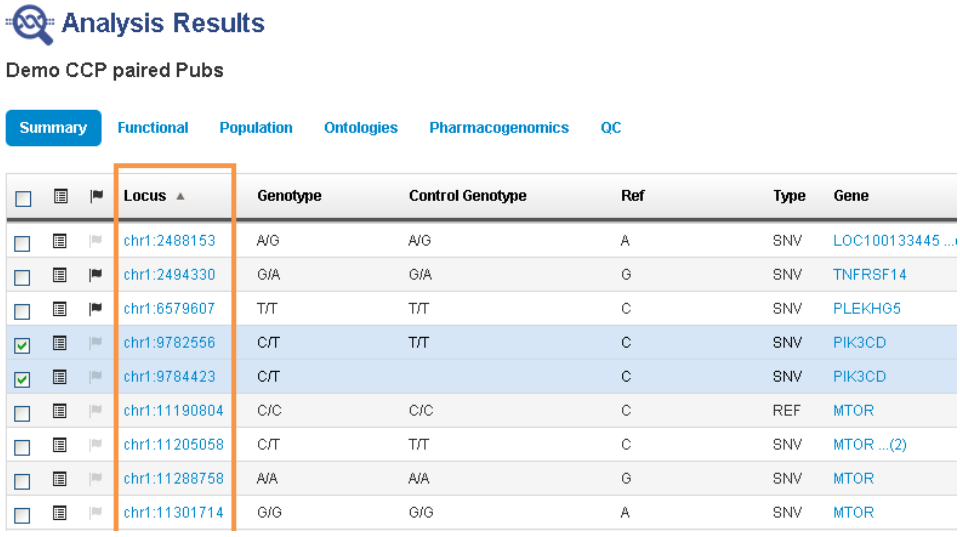
The instructions vary slightly by platform and browser. These steps are for Chrome™ browser on the Windows™ operating system.

Follow these steps to visualize a variant in IGV:

1. Sign in to the Ion Reporter™ Software and click on the **Analysis** tab.
2. Click on the name of your analysis in the analysis table.

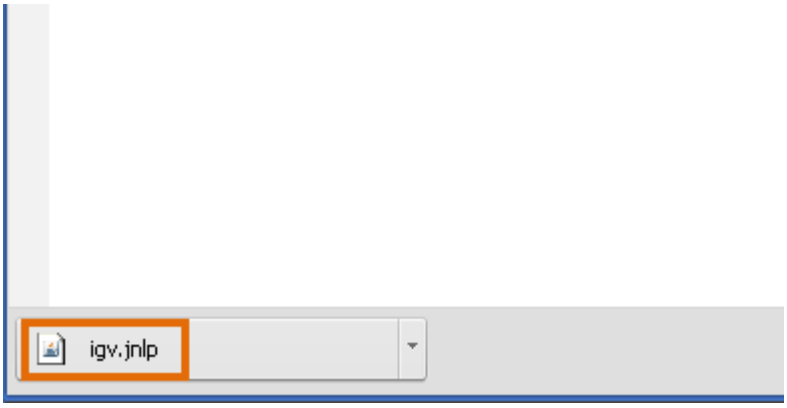


3. In the Analysis Results page, click on the locus of a variant.

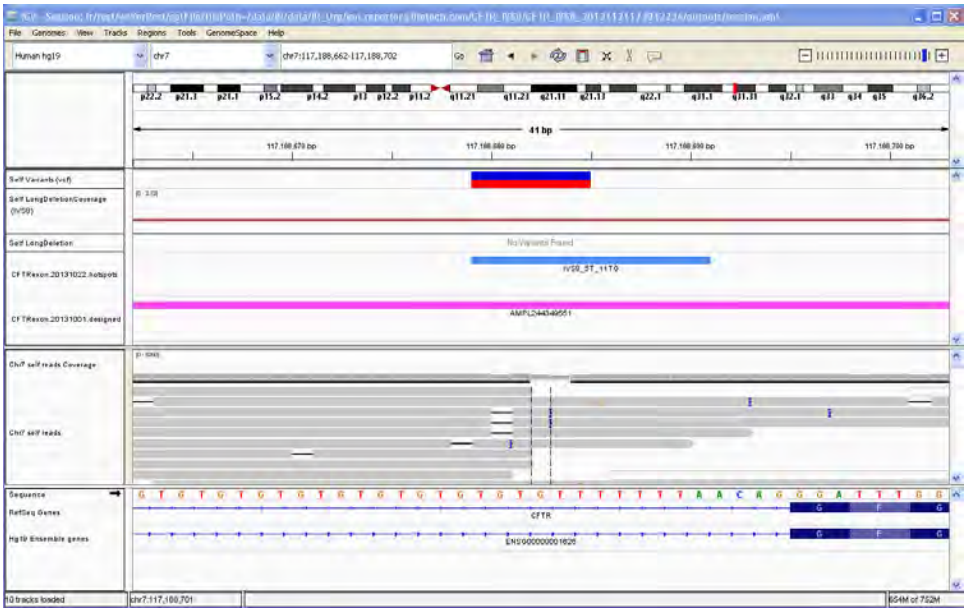


4. A notice appears in the browser download bar. Click the **Keep** button.

5. Double-click the **igv.jnlp** link on the browser download bar (or the **igv.jnlp** filename in the explorer).



This image is an example of the IGV browser.



Note: When comparing different analyses in IGV that use the same samples or the same panel files (but different algorithm versions, for example), the reads, read counts, BED file, and other tracks will load only once in the browser. Only exact duplicate tracks have this behavior of being loaded only once; any differences in results data or input tracks between multiple analyses will trigger any and all unique tracks to be loaded for visualization of the requested analyses.

IGV parse header error

IGV does not load variants and displays this error message when a VCF file uses "CHR" instead of "CHROM" for a column header name:

```
Errors were encountered loading the session: Unable to parse
header with error: Your input file has a malformed header:
```

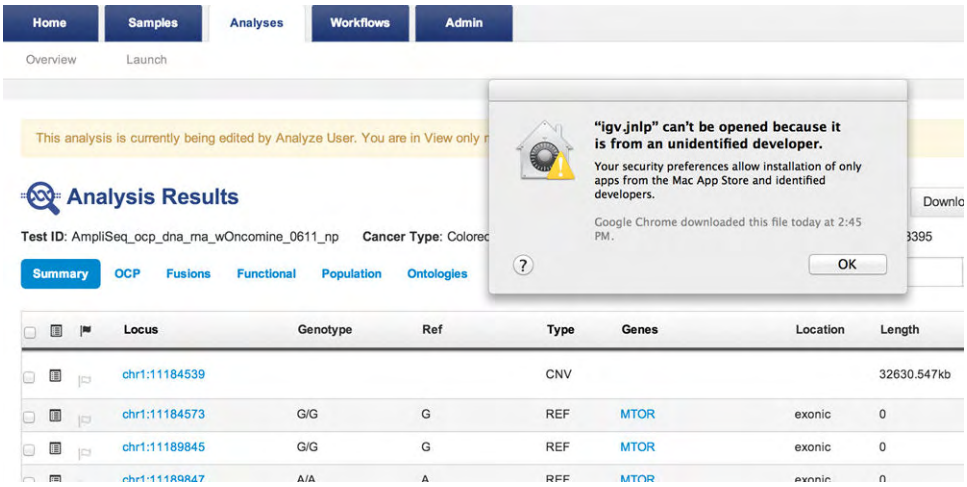
```
unknown column name 'CHR'; it does not match a legal column
header name.
```

The Ion Reporter™ Software annotation-only workflow successfully analyzes a VCF file with a "CHR" column header name. The error is seen when you attempt to view one of the variants in IGV.

To avoid this error, use the "CHROM" column header name in input VCF files (for example: #CHROM POS ID REF).

IGV unidentified developer error on Macs

The error message "igv.jnlp cannot be opened because it is from an unidentified developer" is seen on Macintosh™ computers, depending on security settings.



To open IGV after seeing this error, change either your Macintosh™ security preferences or your browser settings to allow applications from unidentified developers.

Run IGV as an applet

You can run IGV as an applet, even on a Macintosh™ computer.

1. Go to https://[IRServer]/IgvServlet/igvclient.html. Replace IRServer with your Ion Reporter™ server address.

   Note: To enable this on a Macintosh™ computer, you must enable the Java Plugin in Safari and run it in "unsafe" mode. Otherwise it cannot access the local IGV cache folder.

2. Go to Safari/Preferences/Security tab, then select Java plugin on the left.

3. Then select **Run in Unsafe Mode** and **When visiting other websites**.

Export and share results

This section describes how to export and share data analysis results. Topics include:

- Export data from the analysis table for single and multiple samples
- Export data from the Variant Review page
- Export results as a TSV file
- Understanding interpretive reports
- Interpret a VCF file

Share data from an Ion Reporter Software analysis

Ion Reporter™ Software provides a simple mechanism to share data with anyone. They do not have to be users of Ion Reporter™ Software.

By default, all data in your organization is visible only to users within your organization. You can use share data with users from other organizations, even though they do not have access to all of your data. Users whose permissions include the analysis role can also share analysis reports.

When data from analysis is shared:

- The people with whom you share data or analysis reports receive an email with links to download the variant information (filtered and unfiltered) for DNA datasets and species quantification data for metagenomic datasets. Registered users of Ion Reporter™ Software can view the shared analysis in their organization.
- You are notified about the status of the share in your Ion Reporter Software Home page. If you share data with registered Ion Reporter™ Software users, they also receive notifications.
- You are sent an email to confirm that the share is complete. If you shared the data with multiple users at the same time, you will receive just one email for the entire share.
- When the share is complete, this icon → appears beside the report that contains shared data on the Analyses Overview page for you and the recipients of the shared data. When a share is in progress, you see this icon: .

- The statuses in the messages that are shown at the top of the **Analyses** page are as follows:

  Table 6

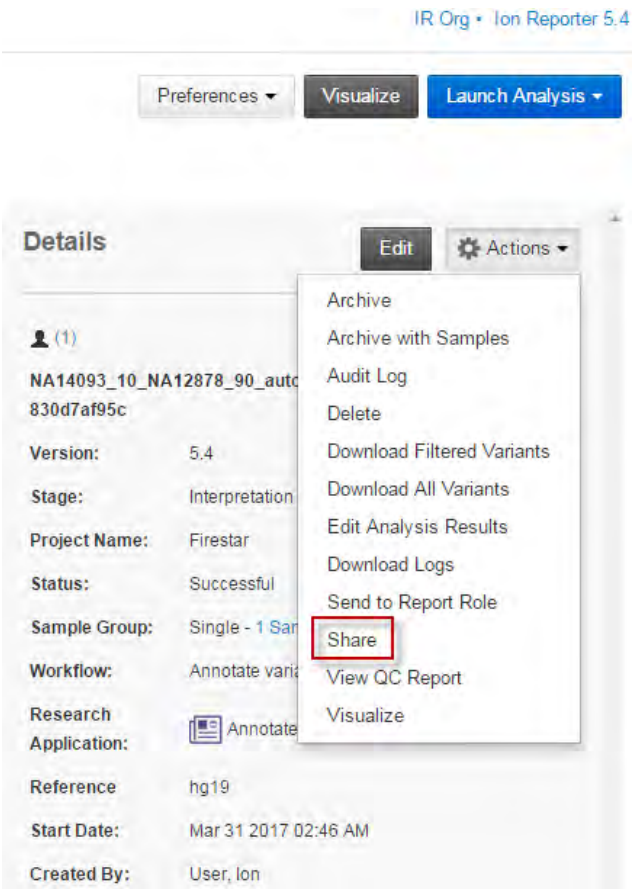
Status	Description
Sharing/Unsharing	A share is in progress.
Shared	The share is successful.
Failed	The share failed. The notification includes the reason for the failed action. **Note:** The Analyses Overview page does not show the latest status. It shows failed status if any shares for that analysis has failed.

Share analyses data

You can share data from Ion Reporter™ Software with anyone, whether or not they are users of the software.

Note: When a share is in progress, the analysis and the sample(s) associated with it can not be edited, archived or deleted.

1. In the Analyses tab, select the analysis that contains the data that you want to share, then click **Actions** ▸ **Share** in the analysis **Details**.



2. Enter the email address of the person with whom you want to share data in the **Share Analysis** dialog, then click **Share**.
   A message about the status of the share opens briefly. When a share is in progress, you see this icon: . You can continue with other tasks inIon Reporter™ Software while the share is in progress. When the sharing is complete, you and the recipients of the shared data see this icon beside the report that contains shared data: .

3. Click the **Home** tab, then click **Notifications** to view notifications about the share.

Note: Variants cannot be flagged when an analysis report has been shared by a user in another organization. The flag column on the on the **Analysis** tab and the option to flag multiple variants are not available for shared analysis reports.

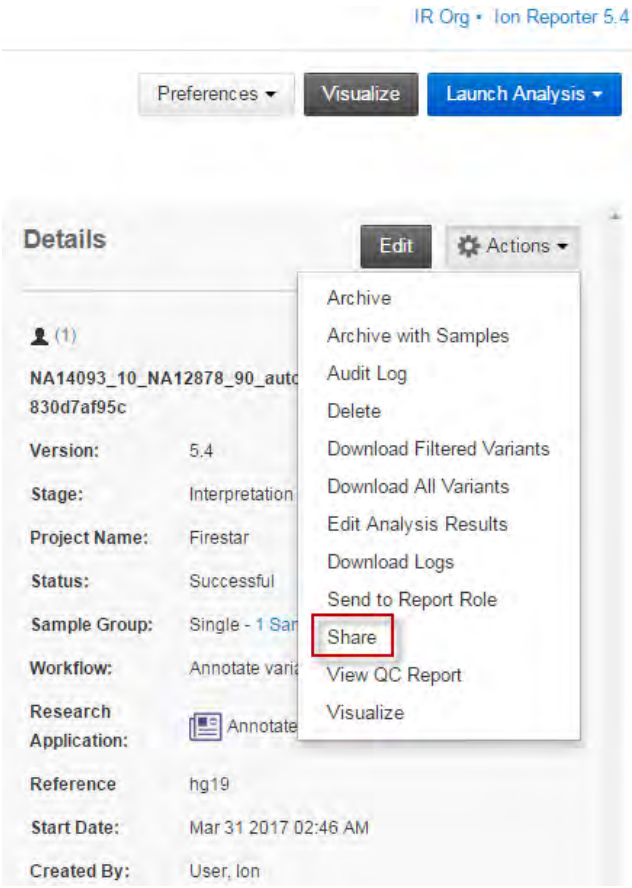
Unshare analyses data

When you unshare an analyses data, the links to the data contained in the notification emails are inactivated. After you unshare the data, subsequent emails with information about the unshare action are sent to both you and your destination users. For Ion Reporter™ Software users, the copied analysis is removed from their organization.

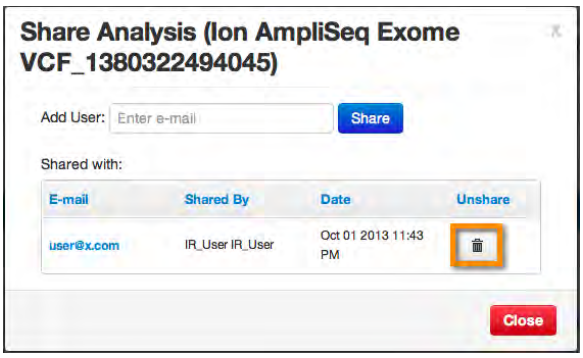
Note: This action can not be undone.

1. Log in to Ion Reporter™ Software, then click the **Analyses** tab.
2. Select the analysis that you want to unshare, then click **Actions** ▸ **Share** in the **Details**.

   Note: This icon appears next to analyses that are shared in the **Analyses** list: ➜.



3. Click the trash can icon next to the user or users to unshare the dataset.



4. Click **Confirm** in the **Unshare** column to complete the unshare.

Search on the Analysis Results list

Most searching is better done with filters, rather than with the search field. For most searches, use a filter instead.

Searches of analysis results can be performed in the Variant Review page using a controlled vocabulary query language. This allows more advanced searches, including OR and AND searches than is currently possible using filter chains. This feature is available in both a single-analysis variant review table and a multi-sample visualization table. However, the feature has only been tested on the single-analysis variant review table.

Filter terms

Notation	Meaning
key:value1,value2	key=value1 OR key=value2
key:[min,max]	min <=key <=max
key:(min,max)	min < key < max
key:[min,]	min <= key
key:[,max]	key <= max
key:(min,)	min < key
key:(,max)	key < max
key:value*	key contains value
key:*	key exists (key has any value)
-key:value	key != value

The following keys are supported:

Key	Example
locus	chr2:123456
function	missense
location	exonic
cosmic, omim, pfam, drugbank, go	glioma
dbsnp	rs12345
gene, transcript	TP53, NM_01010.1
maf	[0.0,0.05]
coverage	[1000']
sift, polyphen, grantham	['0.05]
type	INDEL
comment	something*
vkb	*

Search field behavior is different here on the variant table in the Analysis Results page, than for searches on other pages.

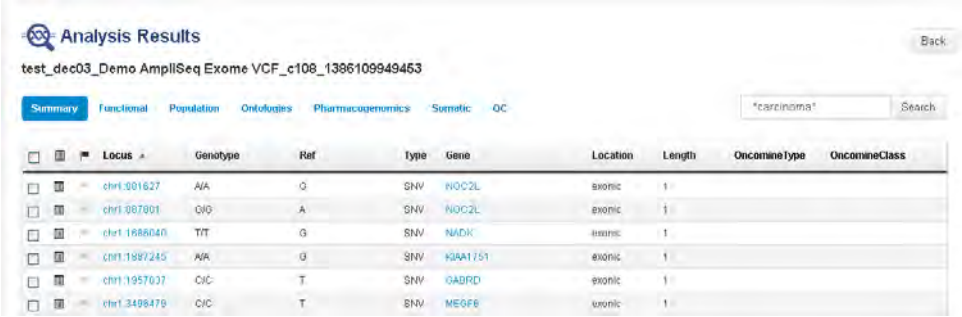
"vbk" in the table above stands for MyVariants.

Asterisks (*) for *some* variant table searches on the Analysis Results page

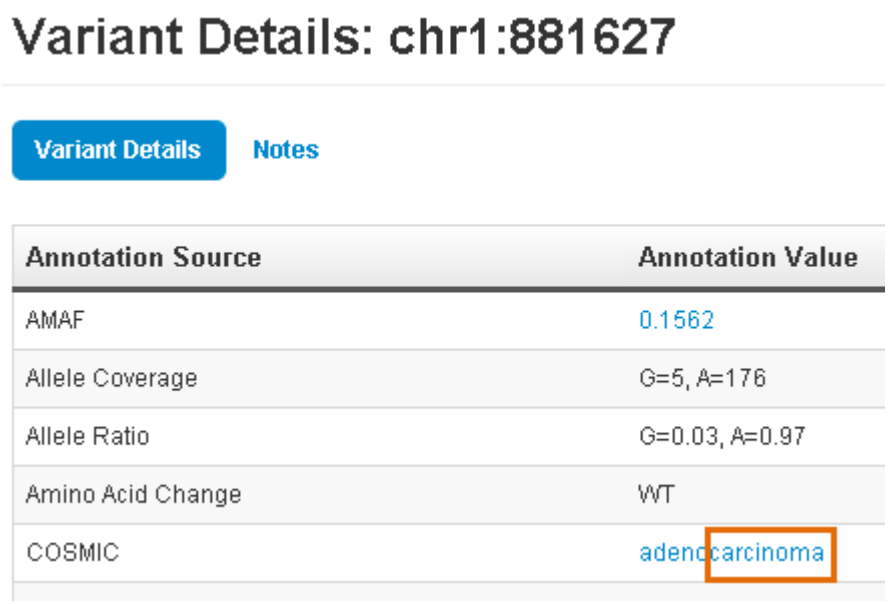
- An asterisk (*) in the search field is only allowed on the Analysis Results page.
- An asterisk (*) is required for some searches here, but is not allowed for other searches. The differences are due to how the different types of information are stored.
- The asterisk is a search wildcard. Without the asterisk, searches only match the exact string entered. With asterisks both before and after your search string, matches at the beginning, middle, and end are all found.

Recommended search with asterisks

- Asterisks are useful to search for matches in any annotation source. (By contrast, a filter search matches only one annotation source.)
- This example shows a search for *carcinoma*.



This search lists all variants that have an OMIM, COSMIC, ClinVar, DrugBank, and so on, annotation that contains "carcinoma" anywhere in the annotation. Click the detail icon for one of the matched variants to open its variant detail card. Scroll down to fine the entries that contain "carcinoma".

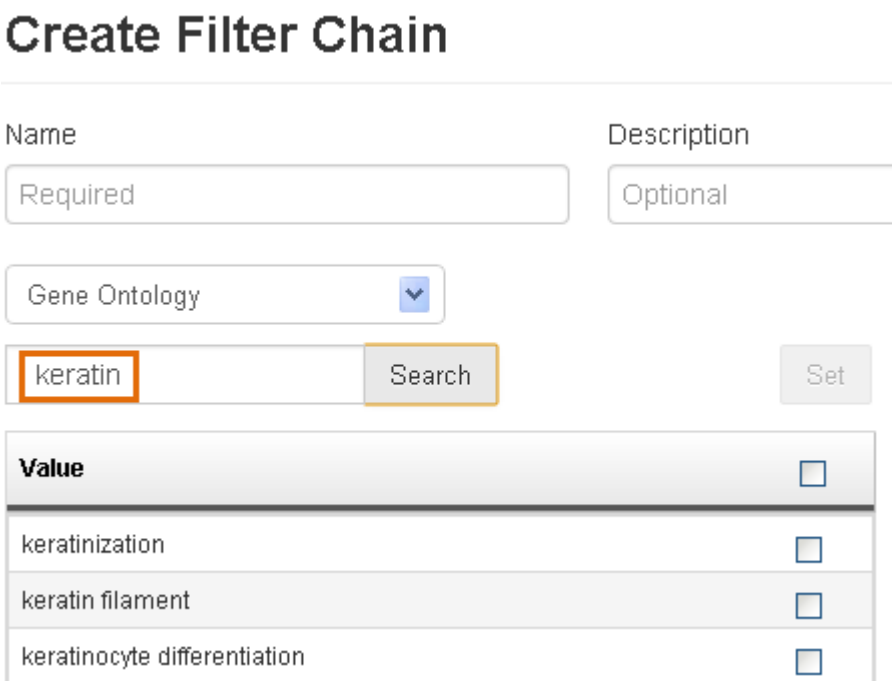


Searches without asterisks

- To search for a locus, enter the complete locus entry, with chromosome number and full position number.

- Do not use an asterisk (*) for a locus search. Searches on a chromosome number by itself or with a partial position number also are not supported.
- For other information, use a filter.

- Search terms within a filter chain also do not allow an asterisk.



Examples

- ```
  (gene:TP53 OR (function:missense,nonsense AND (maf:
  [0.0,0.05} OR -dbsnp:*))) OR type:CNV
  ```

  This retrieves all variants that fall in TP53, all CNVs, plus variants that have a functional impact of missense or nonsense AND either have a minor allele frequency less than 0.05 or are noval (not found in dbSNP).

- ```
  cosmic:carcinoma* AND ((type:SNV AND sift:[0.0,0.10]) AND
  coverage:[300,] OR locus:chrX
  ```

  This retrieves all SNV variants annotated with COSMIC histology terms containing "carcinoma" with a deleterious SIFT score (<0.10) and high coverage(> 300), plus any variant that falls on chromosome X.

Generate reports

This section describes how to generate reports, including:

- Audit log - analysis
- Audit log - sample
- QC report
- Final report

Reports

Ion Reporter™ Software provides a wide range of reports that are generated as part of each analysis.

- Audit log - analysis
- Audit log - sample
- QC report
- Final report

Each of these reports is explained in the following sections.

Note: Each report can be viewed directly in the UI and can be exported as a PDF file. The report layout is different in the UI than in the PDF.

Customize PDF reports

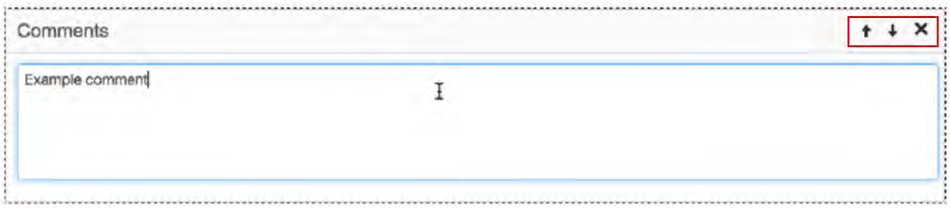
New in Ion Reporter™ Software 5.4, you can customize reports in more ways than in previous versions.

1. In the **Analyses** tab, select the **Overview** subtab.
2. Select the analyses to be included in the report, by clicking the checkbox next to each analysis. See the figure in step 3.

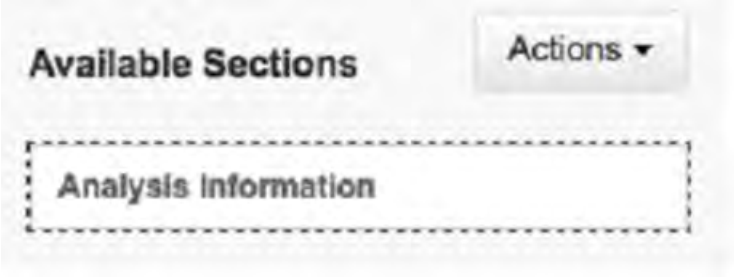
3. Click **Generate Report**.

4. In the **Select Final Report Template** dialog, select a template, then click **OK**, or click **Create Report Template** to create a new report template.

5. Click the section blocks to edit, delete, or move up or down in the report.



Deleted section elements are moved to the right under Available Sections. Click and drag back to the left if you decide you need them for your report.



6. Enter information.

Category	Description
Organizational information	• Date: Date and time when the report was created. • **Required**: Organizational name. • *(Optional)* Upload a logo and/or enter the address
Background	*(Optional)* Enter background information
Comments	*(Optional)* General comments
Sign–Off	Affix an author's name and qualification to the report
Disclaimer	*(Optional)* Enter a custom legal disclaimer.

7. To select annotations, click the appropriate checkbox.

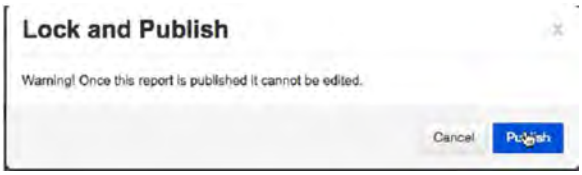
8. Edit Analysis or Sample information by clicking a topic in either section.

9. *(Optional)*To customize the report display, in the **Reported Variants** section, click **Select Columns**.

Option	Description
Add or delete columns.	Select one of the following: • Select a column button in the **Select Reported Columns** dialog to delete that column. • Click **Select Columns** in the **Reported Variants** section to open a menu, then click a column to add, remove, and rearrange columns.
Change column order	Drag a column button in the **Select Reported Columns** box to the desired position.

10. Click **Next**.
    A preview of the PDF report opens. Click **Previous** to go back and change the report.

11. When you are satisfied with the report sections, click **Lock and Publish**. A warning appears. Click **Publish**.

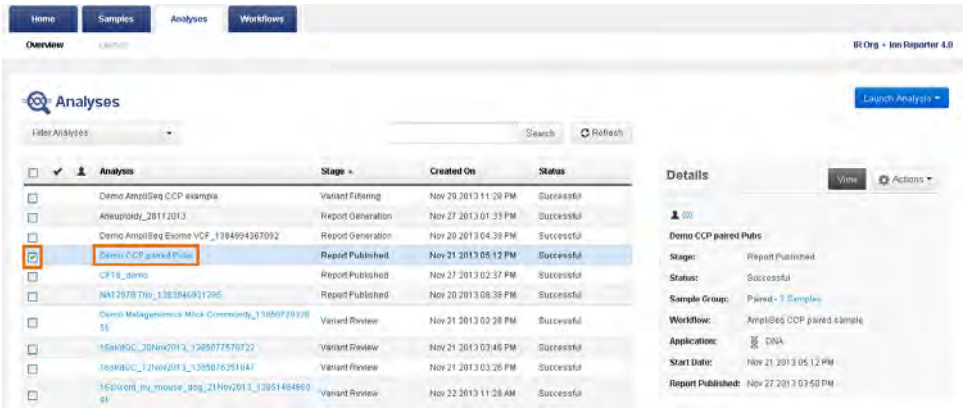


12. Click **Download** to download the publication in PDF format.

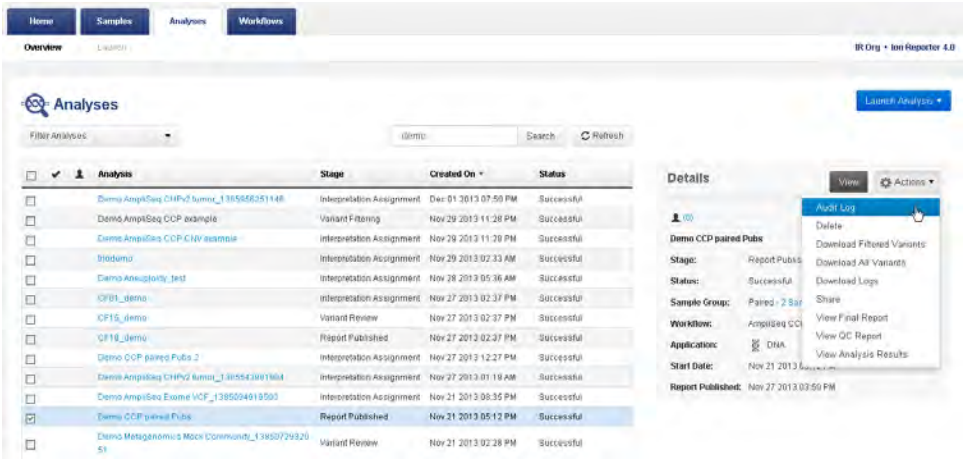
Access Audit log - analysis

The analysis audit log allows you to see which users have accessed a particular analysis and what was done by that user. Follow these steps to access the analysis audit log for a completed analysis:

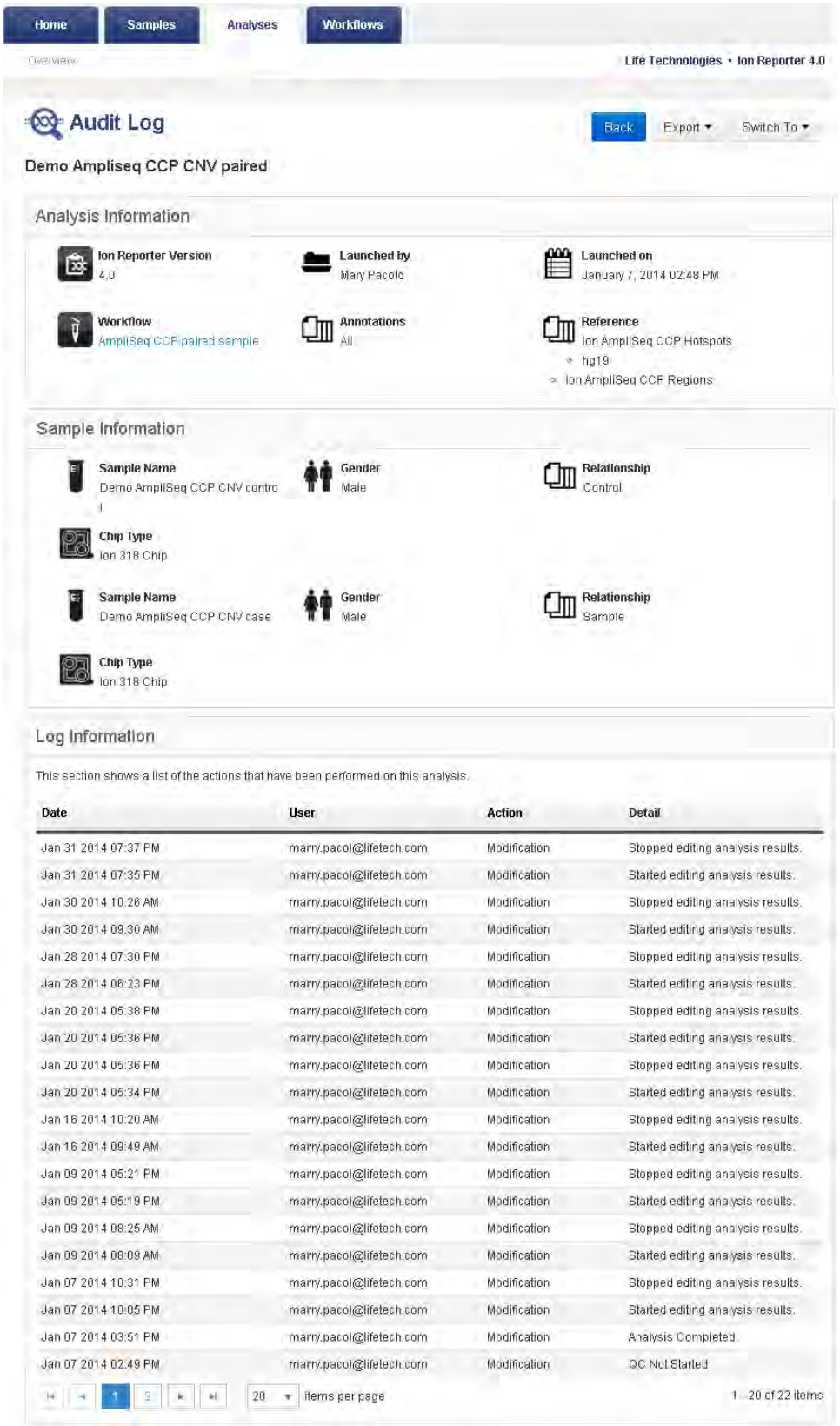
1. Find your analysis in the analysis table and click its row to highlight it (do not enable its checkbox).



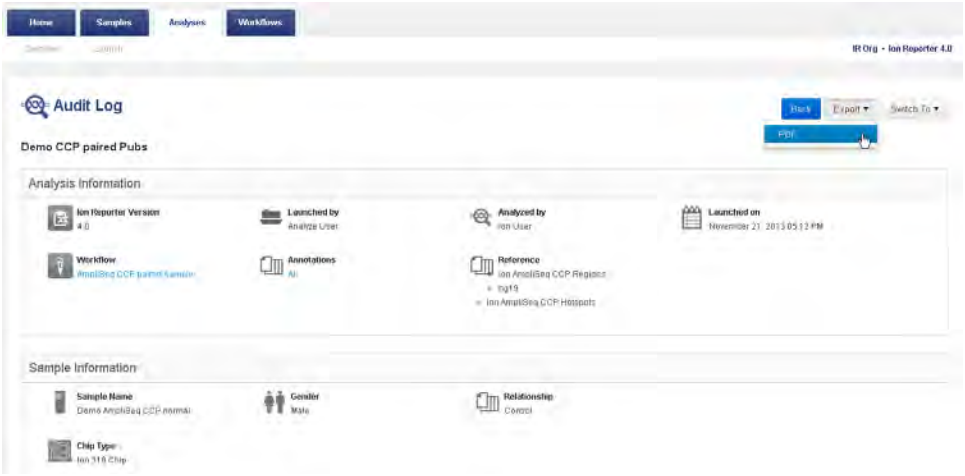
2. In the Actions menu, select **Audit Log**.



3. The audit log opens.



4. (*Optional*) Download a PDF of the audit log from the Export menu. Select **PDF**. (The downloaded filename is analysis_*id*_audit_log.pdf, where *id* is a system-generated analysis id.)



The analysis audit log contains three sections.

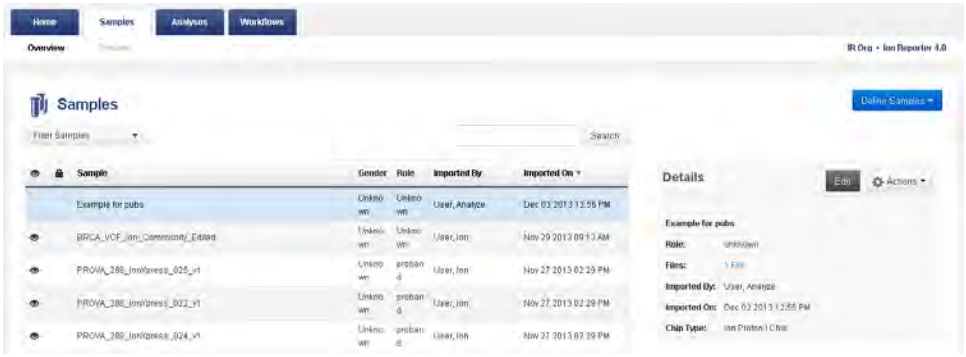
Section	Description
Analysis information	Meta-information on the analysis that was performed
Sample information	Meta-information on the samples that are part of the analysis
Log information	The individual events that have been logged for this analysis including: date / time, user, action, and details on the action performed

Access Audit log - sample

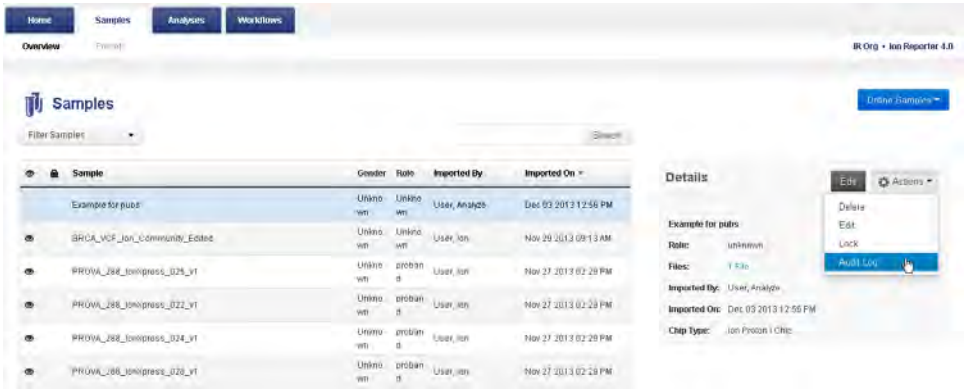
The sample audit log allows you to see which users have accessed a particular sample and what was done by that user. Sample audit logs are not available for the pre-installed demo data samples.

Follow these steps to access the sample audit log:

1. Find your sample in the sample table. Click the sample to highlight it.



2. In the Actions menu, select **Audit Log**.



3. The audit log opens.

4. (*Optional*) Download a PDF of the audit log from the Export menu. Select **PDF**. (The downloaded filename is *sample_name*.pdf, where *sample_name* is the name of the sample.)

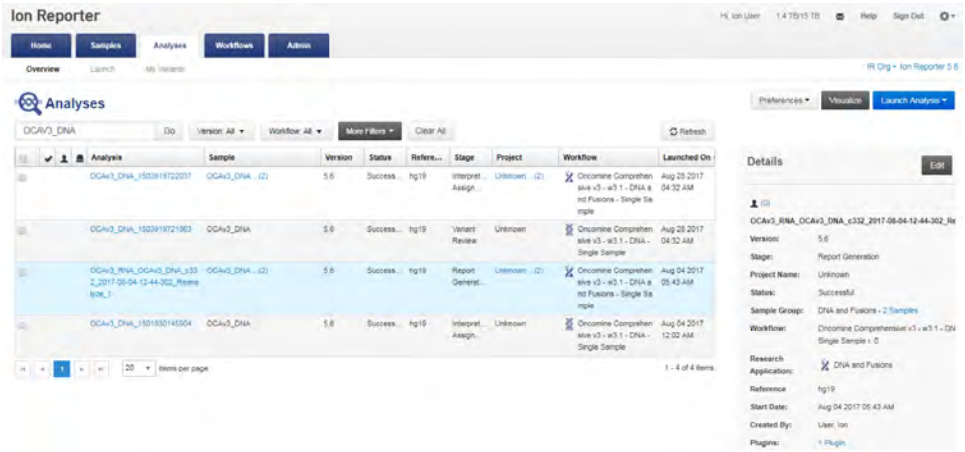
View Quality Control (QC) report

You can access a Quality Control (QC) report for a completed analysis in Ion Reporter™ Software. The QC report provides a high level summary on key metrics for a particular analysis to assess the quality of the data.

1. In the **Analysis** tab, click **Overview**.

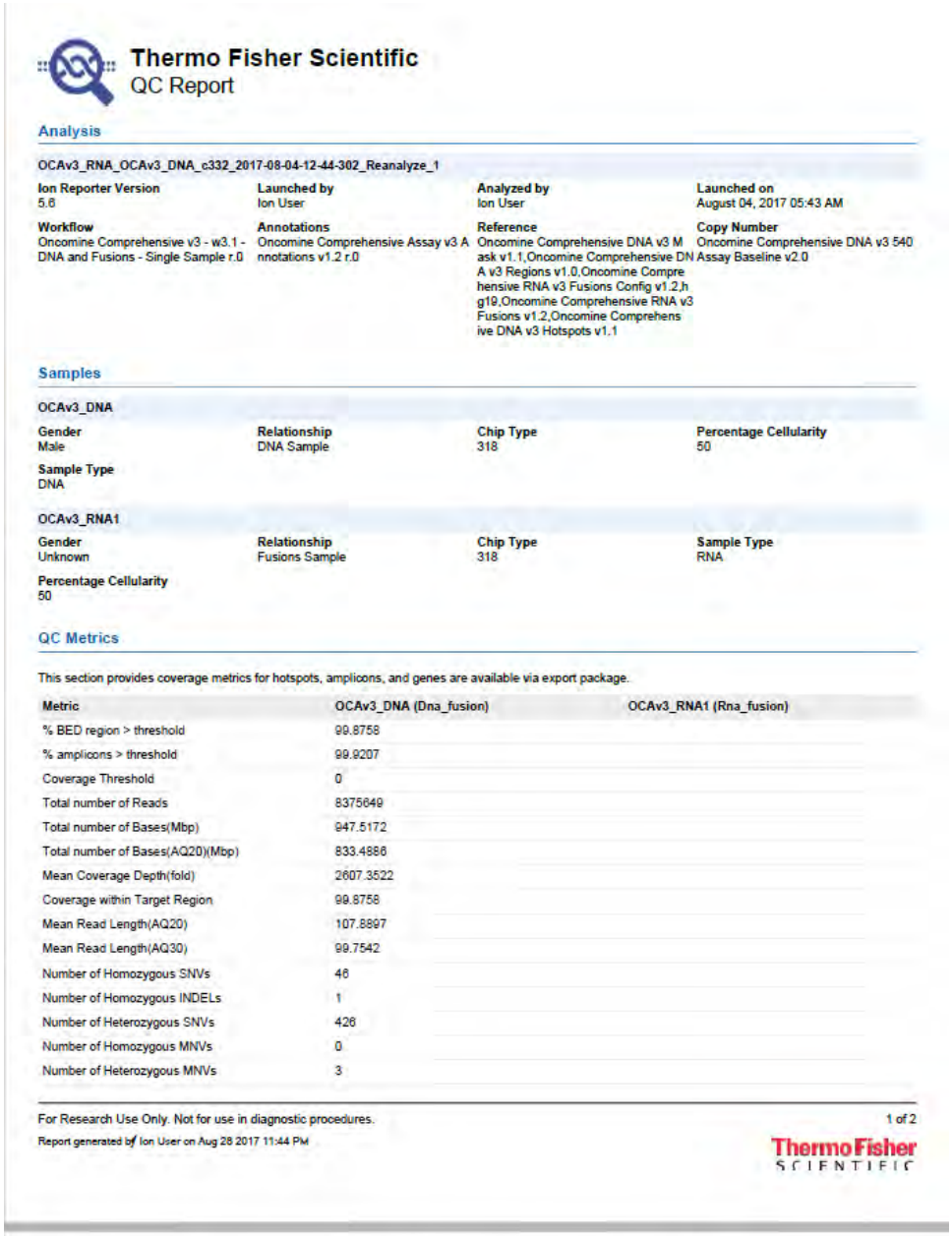
2. Find the analysis of interest, then select the row that lists the analysis to generate a QC report.

   Note: Alternatively, you can select the checkbox in the row to generate the QC report.



3. Click **Actions** ▸ **View QC Report**.

The QC Report opens:



Thermo Fisher Scientific
QC Report

Analysis

OCAv3_RNA_OCAv3_DNA_c332_2017-08-04-12-44-302_Reanalyze_1

Ion Reporter Version	Launched by	Analyzed by	Launched on
5.6	Ion User	Ion User	August 04, 2017 05:43 AM

Workflow	Annotations	Reference	Copy Number
Oncomine Comprehensive v3 - w3.1 - DNA and Fusions - Single Sample r.0	Oncomine Comprehensive Assay v3 Annotations v1.2 r.0	Oncomine Comprehensive DNA v3 Mask v1.1,Oncomine Comprehensive DNA v3 Regions v1.0,Oncomine Comprehensive RNA v3 Fusions Config v1.2,hg19,Oncomine Comprehensive RNA v3 Fusions v1.2,Oncomine Comprehensive DNA v3 Hotspots v1.1	Oncomine Comprehensive DNA v3 540 Assay Baseline v2.0

Samples

OCAv3_DNA

Gender	Relationship	Chip Type	Percentage Cellularity
Male	DNA Sample	318	50

Sample Type
DNA

OCAv3_RNA1

Gender	Relationship	Chip Type	Sample Type
Unknown	Fusions Sample	318	RNA

Percentage Cellularity
50

QC Metrics

This section provides coverage metrics for hotspots, amplicons, and genes are available via export package.

Metric	OCAv3_DNA (Dna_fusion)	OCAv3_RNA1 (Rna_fusion)
% BED region > threshold	99.8758	
% amplicons > threshold	99.9207	
Coverage Threshold	0	
Total number of Reads	8375649	
Total number of Bases(Mbp)	947.5172	
Total number of Bases(AQ20)(Mbp)	833.4886	
Mean Coverage Depth(fold)	2607.3522	
Coverage within Target Region	99.8758	
Mean Read Length(AQ20)	107.8897	
Mean Read Length(AQ30)	99.7542	
Number of Homozygous SNVs	46	
Number of Homozygous INDELs	1	
Number of Heterozygous SNVs	426	
Number of Homozygous MNVs	0	
Number of Heterozygous MNVs	3	

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Report generated by Ion User on Aug 28 2017 11:44 PM

ThermoFisher SCIENTIFIC

Metric	OCAv3_DNA (Dna_fusion)	OCAv3_RNA1 (Rna_fusion)
Number of Heterozygous INDELs	26	
Ti/Tv Ratio (SNPs)	2.301	
dbSNP concordance	0.691	
Heterozygotes/Homozygotes	9.617	
Indels/Total	0.018	
Indels/kb	0.077	
SNPs/kb	1.351	
CNV/Total	0.096	
LongDels/Total	0.000	
Number of CNVs	146	
Number of LongDels	0	
MAPD	0.176910164821	
Fusions/Total		0.013
Number of Fusions		10
Total Mapped Fusion Panel Reads		4338550
Fusion Sample QC		PASS,[TotalMappedFusionPanelReads>500000,MeanReadLength>60]
Fusion Overall Call		POSITIVE,[3pGene=RET,IsoformsDetected=CCDC6-RET.C1R12.COSF1271,CCDC6-RET.C1R11.CCDC6-RET.C1R11.1][3pGene=ALK,IsoformsDetected=EML4-ALK.E6aA20.AB374361,TRMT61B-ALK.T1A9,EML4-ALK.E6bA20.AB374362][3pGene=ROS1,IsoformsDetected=SLC34A2-ROS1.S4R34.COSF1198,SLC34A2-ROS1.S4R32.COSF1196][3pGene=MET,IsoformsDetected=CAPZ

4. (*Optional*) Click **Export ▸ PDF** to download a PDF version of the QC report. Select **PDF**, then download the file through your browser.
   The filename for the PDF is *id*_QC.pdf, where *id* is a system-generated analysis identifier.

Quality Control (QC) report contents

You can access a Quality Control (QC) report for a completed analysis in Ion Reporter™ Software that provides a high level summary on key metrics for a particular analysis to assess the quality of the data. The QC report contains three sections:

Section	Description
Analysis Information	Meta-information on the analysis that was performed
Sample Information	Meta-information on the samples that are part of the analysis
QC Metrics	Quality and variant summary information

QC metrics that are available for QC reports are as follows.

QC Metrics field	Description
Total Number of Reads	Total number of reads
Total Number of Bases (Mbp)	Total number of bases, in million base pairs
Total Number of Bases (AQ20)(Mbp)	Total number of bases at AQ20 (1% error rate) accuracy, in million base pairs
MAPD	Median Absolute Pairwise Difference, a metric for noise in Copy Number data
Mean Coverage Depth (fold)	Mean depth of coverage
Coverage within Target Region	Coverage with target regions (defined by the input regions of interest file)
Mean Read Length (AQ20)	Mean read length at AQ20 (1% error rate) accuracy
Mean Read Length (AQ30)	Mean read length at AQ30 (0.1% error rate) accuracy
Number of Homozygous SNVs	Number of homozygous SNV calls
Number of Homozygous Indels	Number of homozygous indel calls
Number of Heterozygous SNVs	Number of heterozygous SNV calls
Number of Homozygous MNVs	Number of homozygous MNV calls
Number of Heterozygous MNVs	Number of heterozygous MNV calls
Number of Heterozygous Indels	Number of heterozygous indel calls
Ti/Tv Ratio (SNPs)	Ratio of transition to transversion substitutions

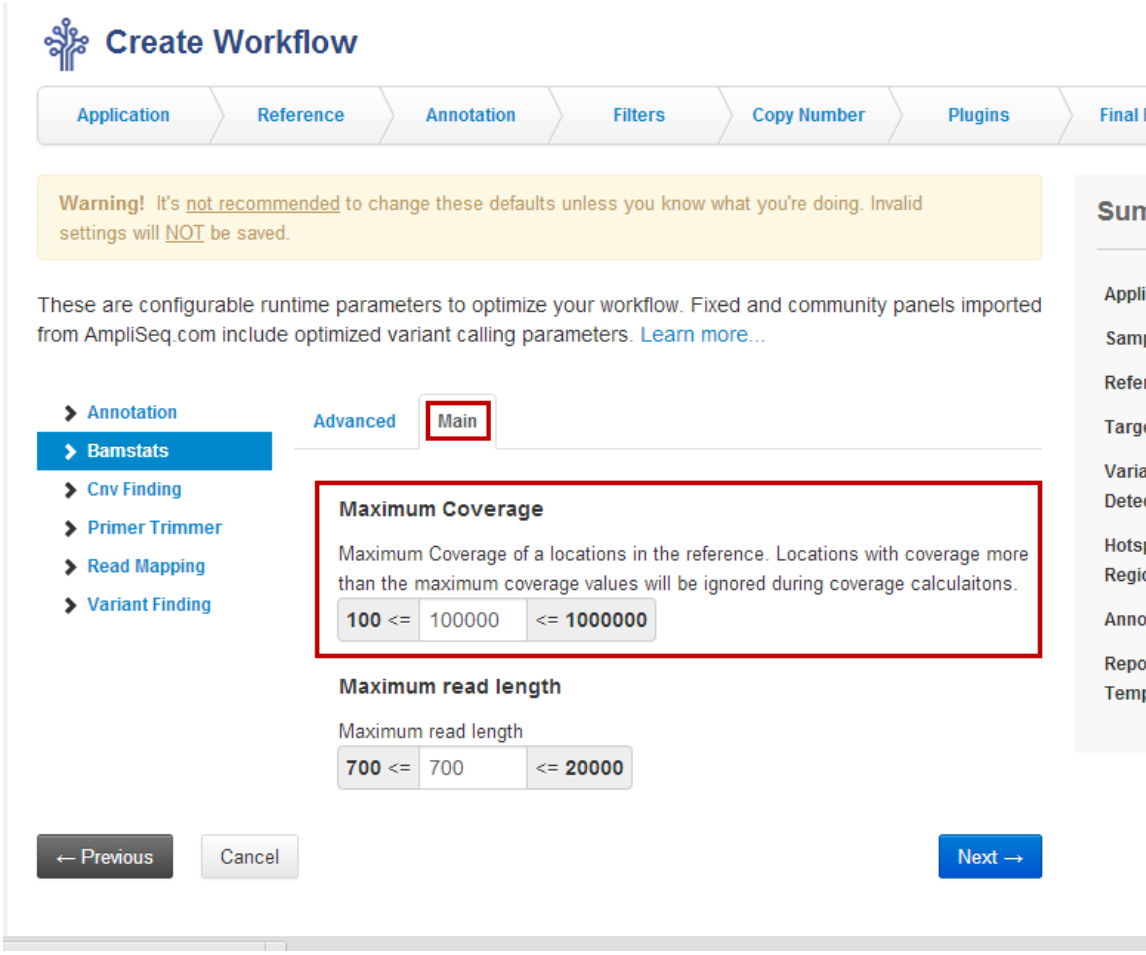
QC Metrics field	Description
dbSNP concordance	Overall dbSNP concordance The ratio of the number of SNP and indel calls that appear in dbSNP with hit level "locus" or "allele", to the total number of SNP and indel calls.
Heterozygotes/Homozygotes	Ratio of heterozygotes to homozygotes This ratio is calculated from SNP and indel variants only. MNVs are not considered.
Indels/Total	Ratio of indel calls to all variant calls
Indels/kb	Number of indel calls per thousand bases For an analysis that uses a regions of interest BED file, the number of bases in those regions is used.
SNPs/kb	Number of SNP calls per thousand bases
Total Mapped Fusion Panel Reads	Sum of all reads for Fusion variants and for ASSAYS_5P_3P, and EXPRESSION_CONTROL markers on a fusion panel
Expression Controls Total Reads	Sum of all reads that are assigned to all of the expression control targets in the sample. If panels contain multiple pools, **ExpressionControlTotalReads** is the aggregate sum from all expression control targets in all the pools.
POOL-*n* Expression Control Total Reads	The sum of expression control reads for individual reads are also reported separately as **POOL-1 ExpressionControlTotalReads** and **POOL-2 ExpressionControlTotalReads**, and so on.

About the QC package coverage columns

The coverage distribution is calculated for each region and reported in the No, Low, Medium, and High columns based on the values of Bamstats input parameters.

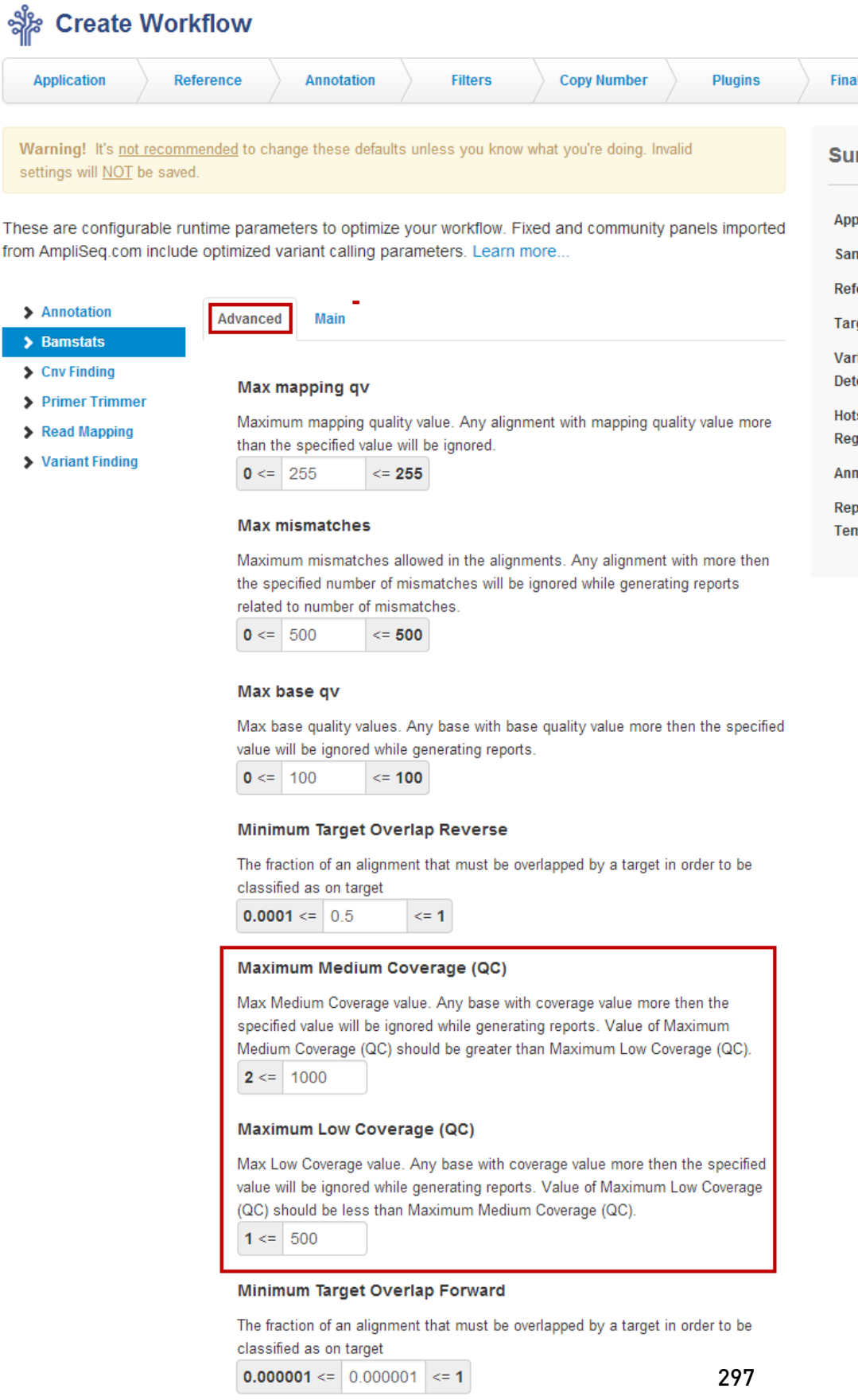
These Bamstats parameters (which are set when the workflow is created) control the reporting of the coverage distribution in the coverage statistics files:

- Maximum Coverage



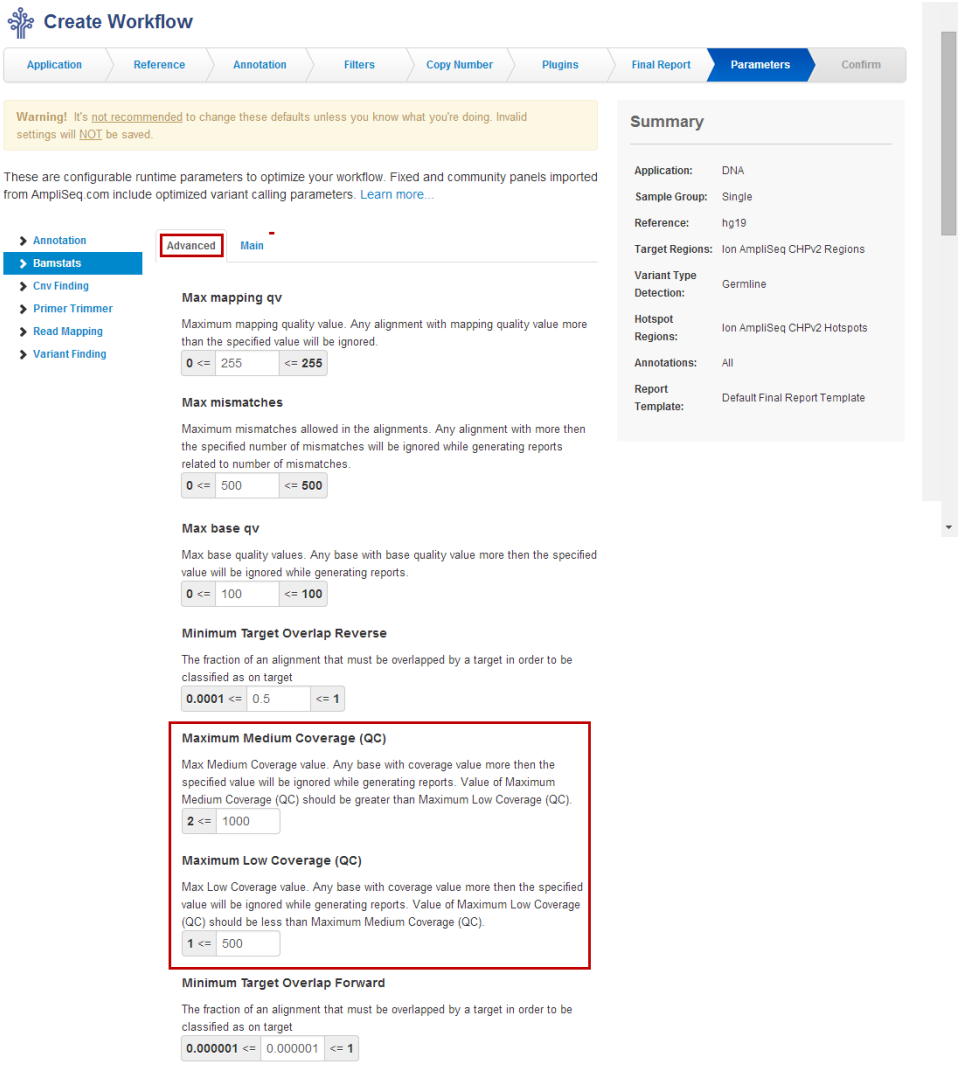
(Main parameter tab)

- Maximum Medium Coverage (QC)



(Advanced parameter tab)

- Maximum Low Coverage (QC)



(Advanced parameter tab)

This table shows how the Bamstats coverage parameters affect the coverage reporting in the coverage statistics files:

Coverage column	Description
No	Zero coverage (not controlled by Bamstats parameters)
Low	Values from 1 to `Maximum Low Coverage (QC)`

Medium	Values from 1 more than
	`Maximum Low Coverage (QC)`
	to
	`Maximum Medium Coverage (QC)`

High	Values from 1 more than
	`Maximum Medium Coverage (QC)`
	to
	`Maximum Coverage (QC)`

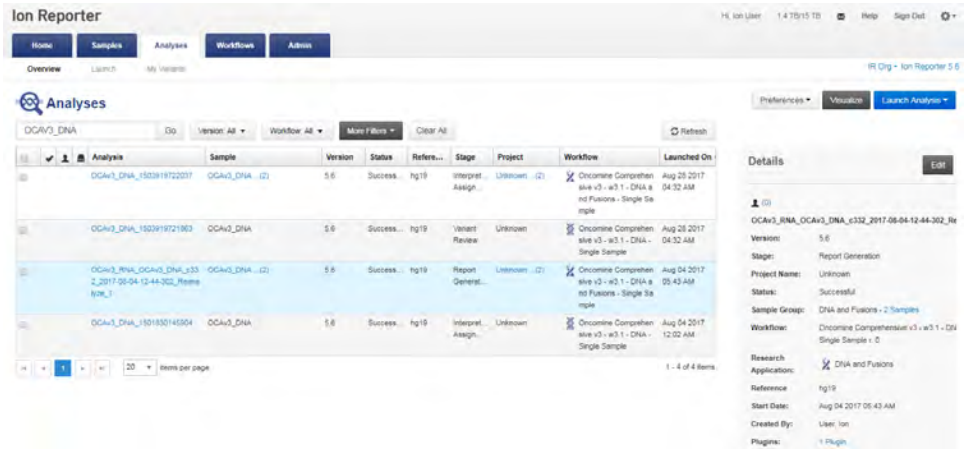
Download Quality Control (QC) files

You can download files from a Quality Control (QC) report that you open in Ion Reporter™ Software. The compressed directory contains the following Quality Control (QC) files:

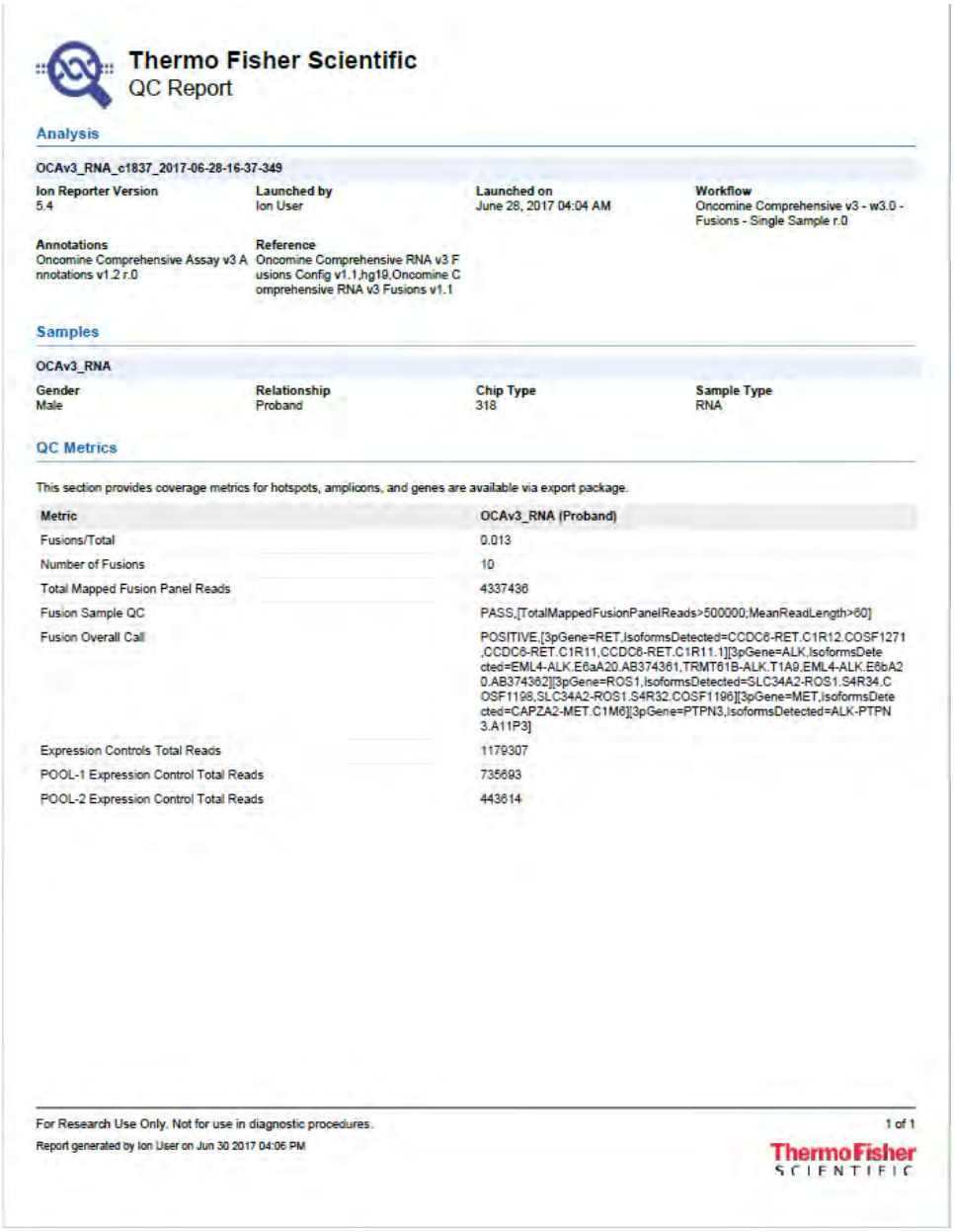
- **QC/*id*.pdf**: A copy of the QC Report PDF file.
- `QC/sample_name/AnnotatorActor/variome-stats.csv`: A tab-separated file of variants statistics for this sample. These statistics are also listed in the QC report PDF file.
- `QC/sample_name/StatsActor/amplicons_low_no_coverage_statistics.txt`: Coverage statistics by amplicon.
- `QC/sample_name/StatsActor/analysis_low_no_coverage_statistics.txt` : Coverage statistics for the analysis.
- `QC/sample_name/StatsActor/genes_low_no_coverage_statistics.txt` : Coverage statistics by gene.

1. In the **Analysis** tab, click **Overview**.
2. Find the analysis of interest, then select the row that lists the analysis to generate a QC report.

   Note: Alternatively, you can select the checkbox in the row to generate the QC report.



3. Click **Actions** ▸ **View QC Report**.
   The QC Report opens:



Thermo Fisher Scientific
QC Report

Analysis

OCAv3_RNA_c1837_2017-06-28-16-37-349

Ion Reporter Version	Launched by	Launched on	Workflow
5.4	Ion User	June 28, 2017 04:04 AM	Oncomine Comprehensive v3 - w3.0 - Fusions - Single Sample r.0

Annotations	Reference
Oncomine Comprehensive Assay v3 Annotations v1.2 r.0	Oncomine Comprehensive RNA v3 Fusions Config v1.1,hg19,Oncomine Comprehensive RNA v3 Fusions v1.1

Samples

OCAv3_RNA

Gender	Relationship	Chip Type	Sample Type
Male	Proband	318	RNA

QC Metrics

This section provides coverage metrics for hotspots, amplicons, and genes are available via export package.

Metric	OCAv3_RNA (Proband)
Fusions/Total	0.013
Number of Fusions	10
Total Mapped Fusion Panel Reads	4337436
Fusion Sample QC	PASS,[TotalMappedFusionPanelReads>500000;MeanReadLength>60]
Fusion Overall Call	POSITIVE,[3pGene=RET,IsoformsDetected=CCDC6-RET.C1R12.COSF1271,CCDC6-RET.C1R11,CCDC6-RET.C1R11.1][3pGene=ALK,IsoformsDetected=EML4-ALK.E6aA20.AB374361,TRMT61B-ALK.T1A9,EML4-ALK.E6bA20.AB374362][3pGene=ROS1,IsoformsDetected=SLC34A2-ROS1.S4R34.COSF1198,SLC34A2-ROS1.S4R32.COSF1196][3pGene=MET,IsoformsDetected=CAPZA2-MET.C1M6][3pGene=PTPN3,IsoformsDetected=ALK-PTPN3.A11P3]
Expression Controls Total Reads	1179307
POOL-1 Expression Control Total Reads	735693
POOL-2 Expression Control Total Reads	443614

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Report generated by Ion User on Jun 30 2017 04:06 PM
ThermoFisher SCIENTIFIC

4. Click **Export** ▸ **QC Package** to download the QC Package to Ion Reporter™ Software.

   - If you use Ion Reporter™ Software in the Thermo Fisher Cloud, open the **Home** tab, then click ✉ to open the notifications list and find, then download the QC package download.
   - If you use Ion Reporter™ Server Software, use the browser to download the file, then find the QC package in the folder used for downloads.

   The downloaded filename is qc-report-*id*.zip, where *id* is a system-generated analysis id.

The following fields are in the `*_coverage_statistics.txt` files:

Column	Description
#Id	The amplicon id or gene name (if any)
Region	Usually the chromosome that contains the amplicon, gene, or hotspot region
Start	Initial position of the amplicon, gene, or hotspot region
End	End position of the amplicon, gene, or hotspot region
No	Number of reads with zero coverage
Low	Number of reads with low coverage
Medium	Number of reads with medium coverage
High	Number of reads with high coverage
TargetSize	Region size (in bases) of the amplicon, gene, or hotspot region
Min	Minimum coverage of any base in the amplicon, gene, or hotspot region
Max	Maximum coverage in the amplicon, gene, or hotspot region
Avg	Average coverage in the amplicon, gene, or hotspot region
1XBases	Number of bases with at least 1X coverage in the amplicon, gene, or hotspot region
AvgBQ	Average base quality value

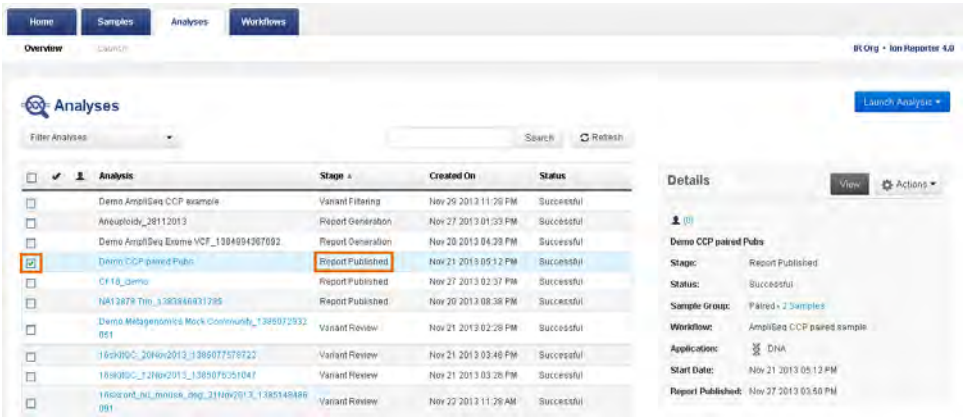
Final report

The final report provides a high level summary on key metrics for a particular analysis to assess the quality of the data. The analysis must be in the "Report Published" stage.

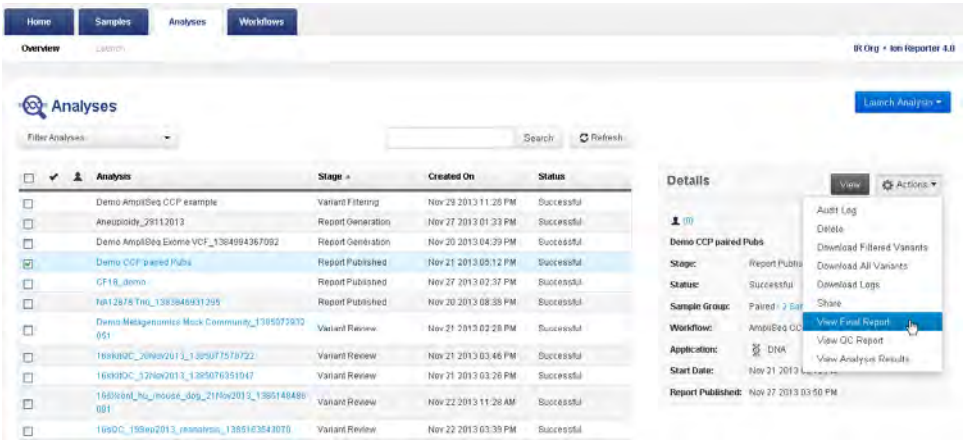
The gene field in this report is comprised of GeneSymbol_ExonNumber_Locus_TranscriptId_Classification.

Follow these steps to access the final report for a completed analysis:

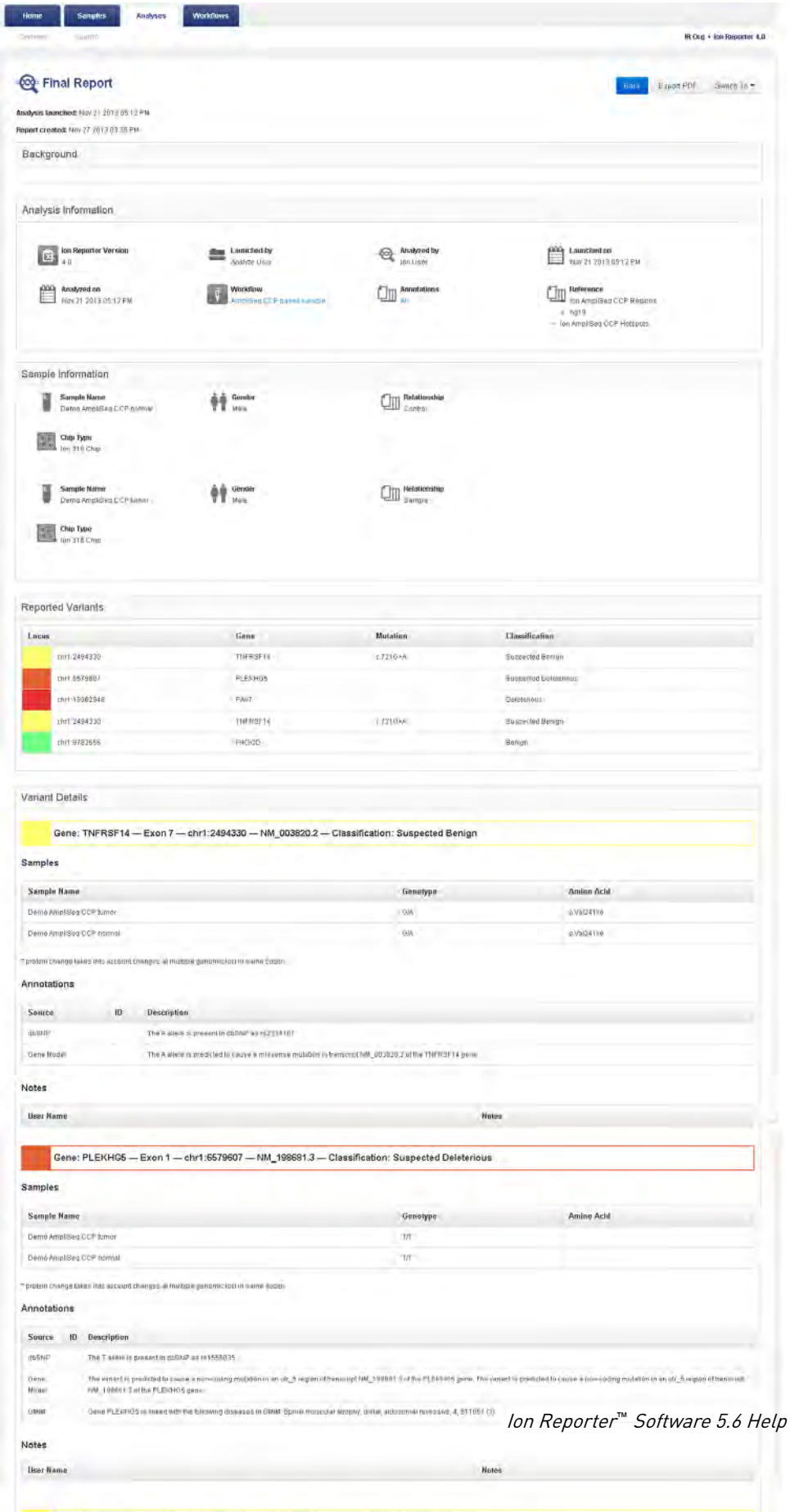
1. Find your analysis in the analysis table and enable its checkbox.



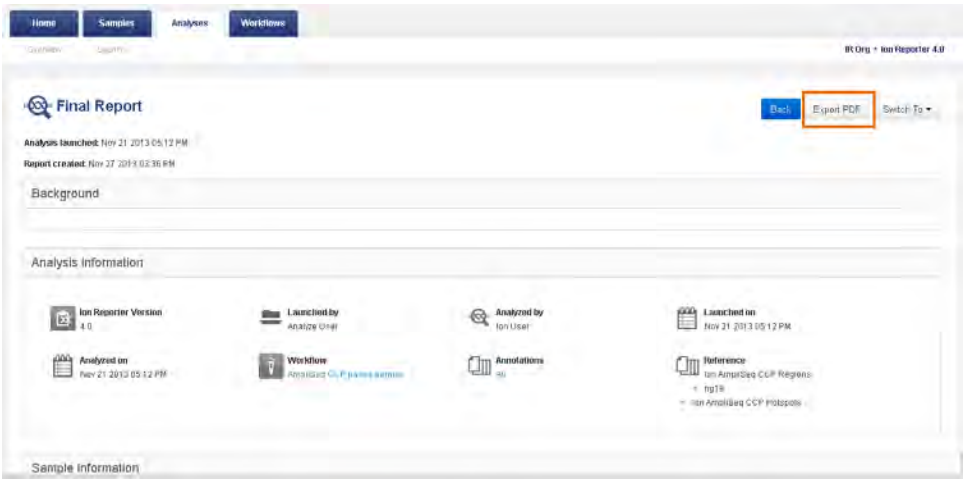
2. In the Actions menu, select **View Final Report**.



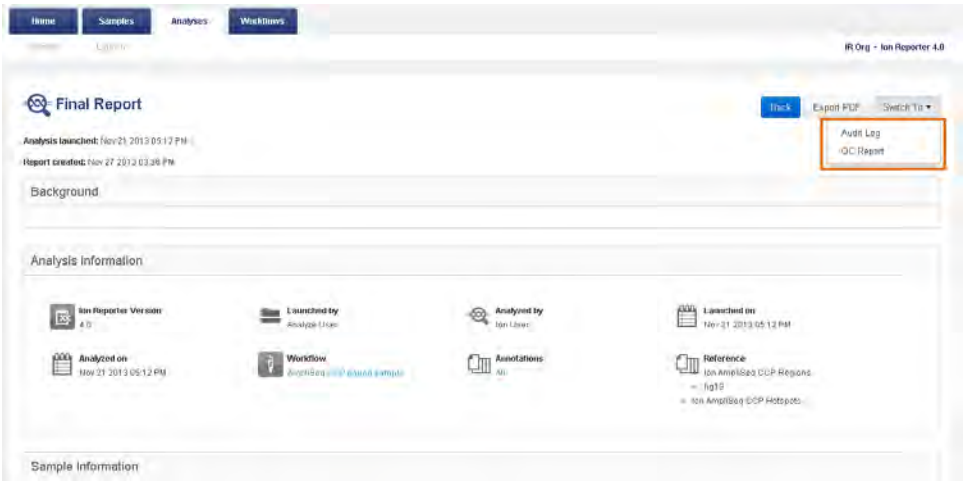
3. The final report opens. Here is an example:



4. (*Optional*) Download a PDF of the final report from the Export menu. Select **PDF**. (The downloaded filename begins with `analysis_report`.)



5. *Optional*) From the Final Report page, use the **Switch To** menu to view either the audit log or the QC report.



Amplicon Coverage Report

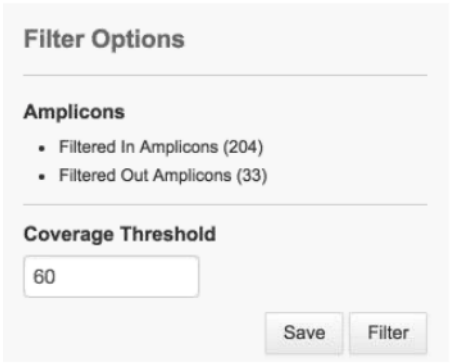
You can now generate a per-amplicon report in Ion Reporter™ software v5.2. You can see which amplicons, SNPs, and bases in an amplicon, and which fall below a certain level of coverage that you set as a threshold for coverage.

Generate Amplicon Coverage Report

1. Go to **Analyses** ▸ **Overview** and select an analysis.
2. Click **Actions** ▸ **View Coverage Report**.

The initial table displays all amplicons. In the **Filter Options** pane, the **Coverage Threshold** default is set to **0**.

3. Enter a threshold value, for example **60**, and click **Filter**. To make this a default filter, click **Save**.



The table now displays amplicons less than 60.

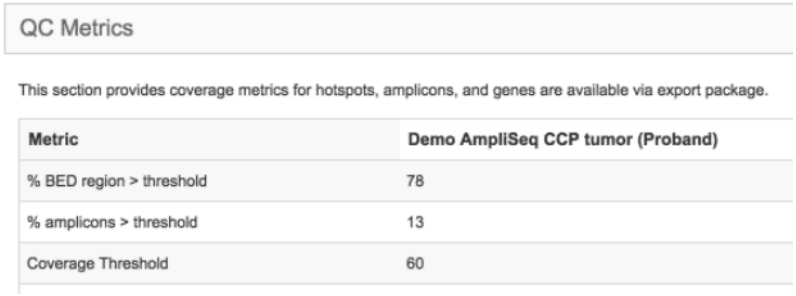
Note: You must save the filter in order for the **% BED region > threshold**, **% amplicons > threshold**, and **Coverage Threshold** metrics to appear in the QC Report.

% amplicons > threshold provides the filtered out percentage.
% BED region > threshold provides the percent greater than threshold.

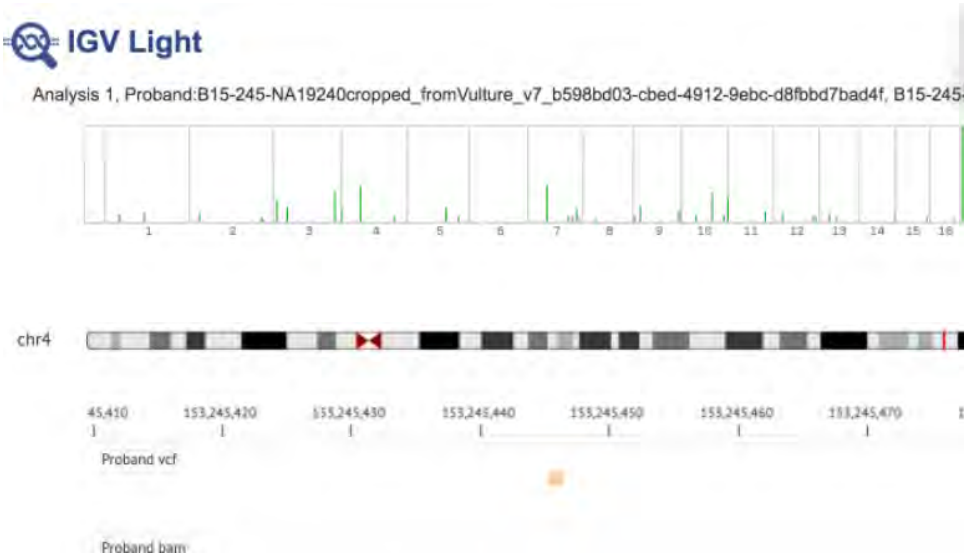
4. To view the results in the QC Report format, click **Switch To ▸ QC Report**.

Note: You can also use the Switch To menu to view the Audit Log or Analysis Results table.

5. Scroll down to the QC Metrics section and you'll see results for **% Bed region > threshold**, **% amplicons > threshold**, and **Coverage Threshold**.



6. To view an amplicon in IGV Light viewer, click on the amplicon link and IGV Light displays the results visually.



Administer Ion Reporter™ Software

This section provides an overview of basic administrative functions for Ion Reporter™ Software. Most of the procedures and functions described in this section require administrative permissions for an organization that you administer. Where indicated, procedures apply to either Ion Reporter™ Software that is installed on local servers or to Ion Reporter™ Software in the Thermo Fisher Cloud.

Create multiple Ion Reporter™ Software server users

Note: This information applies only to Ion Reporter™ Software server.

You can create a spreadsheet that contains user information and then upload it to create multiple Ion Reporter™ Software users.

Note: Use a minimum of two letters for first and last names for user accounts.

- Sign in to Ion Reporter™ Software as an administrative user, then click the **Admin** tab.
- Click **Create User ▸ Batch**, then click **download examples** on the **Import Users** page.
- Open the UserDefinitionTemplate.csv that is downloaded through the browser.
- Enter information for each new user that you want to create into the following columns of the spreadsheet:

  Table 7

Column name	Description
First Name	First name of the account user
Last Name	Last name of the account user
EmailId	Email address of the account user. The email address will be used as the user ID when the user signs in. For example, user@institute.com.
Import Role	Enter 1 to assign the Import role
Analyze Role	Enter 1 to assign the Analyze role
Report Role	Enter 1 to assign the Report role
Administrator	Enter 1 to assign the Administrator role

- Save the spreadsheet to a file directory on your hard drive or in a location that you can get back to.

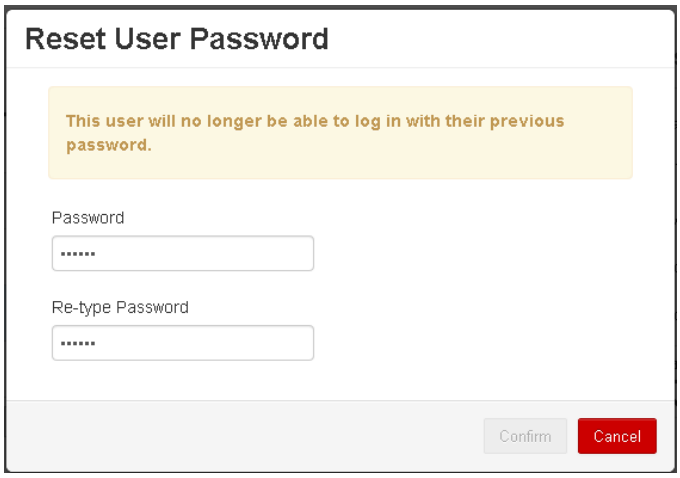
Set or reset the password on a user account

Note: After you set or reset a password, you must notify the user about the new or updated password.

A user becomes locked out after five attempts to log in with an incorrect password. The administrator must reset the password to unlock the account. There are two mechanisms to set or reset the password for a user account.

On the Ion Reporter™ Software server:

1. Sign in to Ion Reporter™ Software, then click the **Admin** tab.
2. Select the user account in the **Users**list (without clicking on the hyperlink for the account), then select **Reset Password** from the **Actions** drop-down menu in the **User Details**.
3. Enter the new password and re-type the password in the **Reset User Password** dialog.



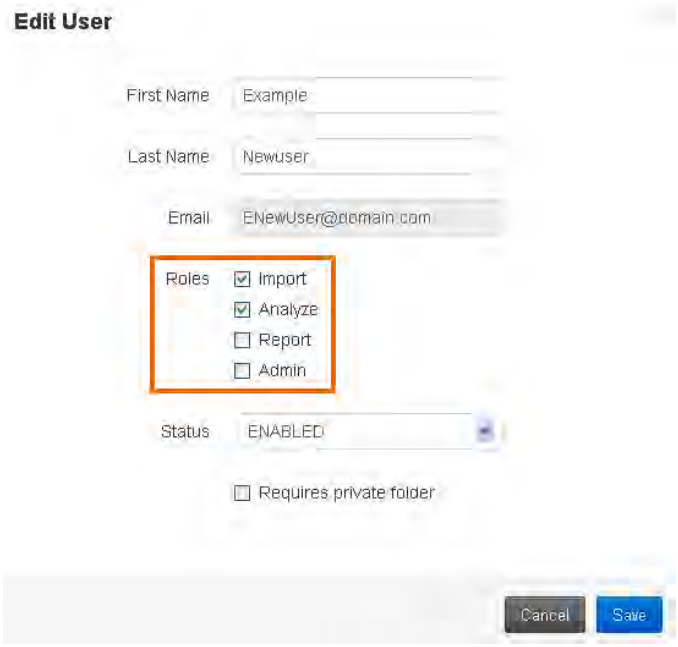
4. If you are certain that you want to change the password, click **Confirm**.
5. Notify the user that the password has been reset, and give the user the new password.

Modify the permissions of a user

Follow these steps to modify the permissions of a user in Ion Reporter™ Software:

1. Sign in to Ion Reporter™ Software and click the **Admin** tab, then click the user account in the **Users** list (without clicking on the hyperlink for the account).
2. Click **Edit** in **User Details**.

3. In the Roles area of the **Edit User** dialog, modify the user permissions.



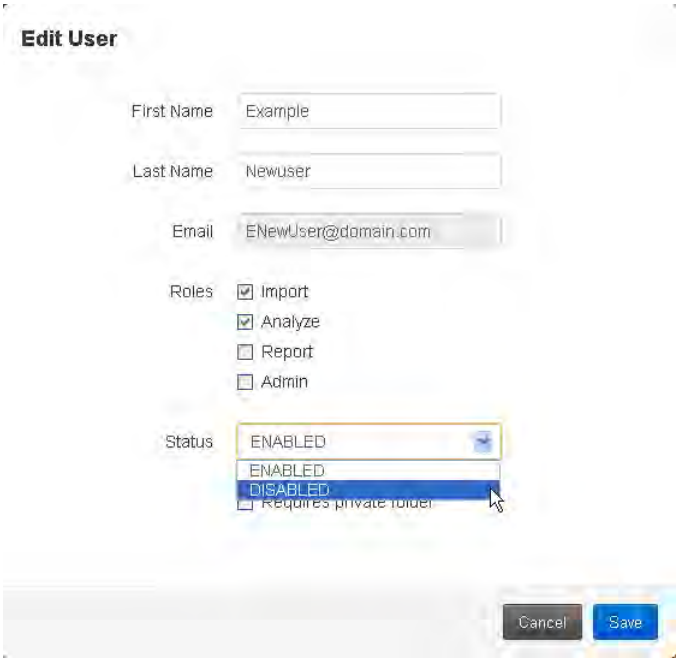
4. *(Optional)* Select **Requires private folder** if you want to give the user the authority to restrict all visibility to their data. For details, see "Enable or disable private folders" on page 313.

5. Click **Save**.

Disable a user

You can disable an Ion Reporter™ Software account. When you disable an account, the email address is not available to be reused for a different user account. If you want to reuse the email address, you must delete the user.

1. Sign in to the Ion Reporter™ Software. Under the **Admin** tab, in the **Users** screen, click the hyperlinked email address of the user.
2. In the **Edit User** dialog, select **Disabled** in the **Status** dropdown list, then click **Save**.



You are returned to the **Users** page. The user account is disabled. You can re-enable the account at any time.

Delete a user

Note: This action can not be undone.

When you delete a user, the email address for that user can be reused for a different user account.

1. Sign in to Ion Reporter™ Software and click the **Admin** tab.
2. Select the user in the table (without clicking on the hyperlink for the account), then click **Actions** ▸ **Delete** in the **User Details**:
3. If you are certain that you want to delete the user, click **Yes** to confirm this deletion.

The user account is deleted.

Manage the API token

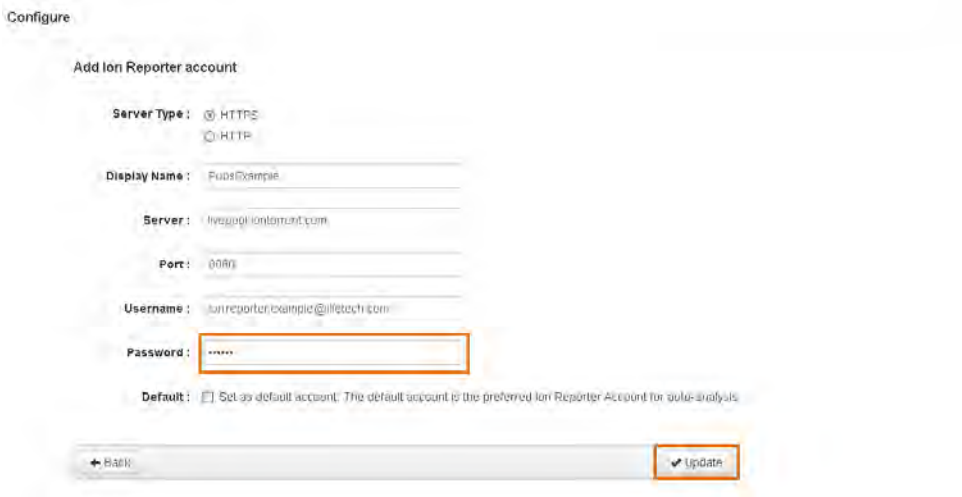
The API token is used to upload data to the server version of Ion Reporter™ Software and access data from the API. These steps can be performed by either an administrative user or a regular user.

The API token is like a password. When you generate a token, it is similar to resetting a password and existing users become locked out.

Note: Under most circumstances, you should not generate a new API token if one already exists.

Two functions of the IonReporterUploader plugin do not work when you regenerate the token. The functions and the steps to recover functionality are as follows:

- IonReporterUploader plugins that are configured with your Ion Reporter™ Software account. To recover, in IonReporterUploader plugin configuration in Torrent Suite™ Software, retype your password, then click **Update** .



- Scripts or code that access the Ion Reporter™ Software API with your previous token. To recover, use your new token with those scripts.

Follow these steps to access your API token:

1. Sign in to Ion Reporter™ Software and click the **Admin** tab.
2. Click **Actions** ▸ **Manage API Token**.
3. In the **Manage API Token** dialog, if the API Token field is blank, click **Generate** to create your token.

   If a token already exists, click **Generate** to generate a new one.

   A new token is created. The functions noted above will no longer work with the new token until you perform the described actions.

Enable or disable private folders

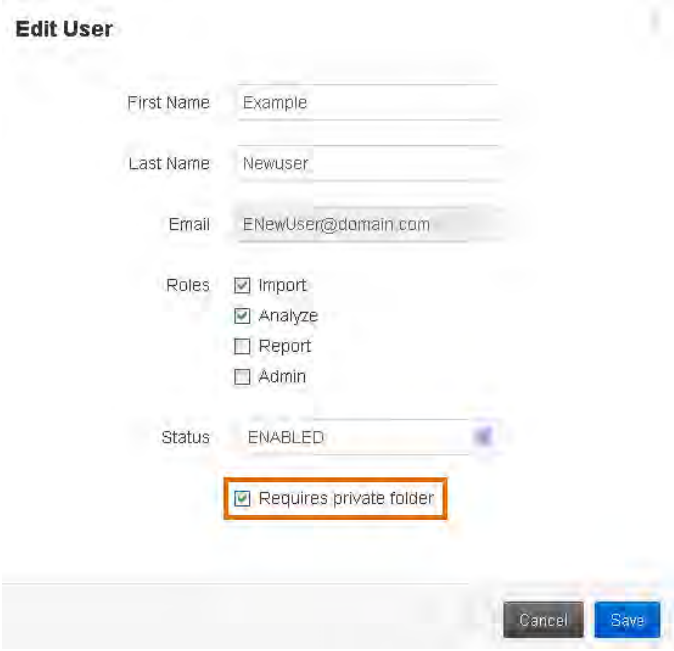
Private folders enable a user to restrict all visibility to their data.

IMPORTANT! If a user stops using a private folder, all data generated in that folder can no longer be accessed.

Note: This functionality is only available for the Ion Reporter™ Software sever. It is not available for Ion Reporter™ Software in the Thermo Fisher Cloud.

Follow these steps to enable or disable a private folder in Ion Reporter™ Software:

1. Sign in to Ion Reporter™ Software as an administrative user, then click on the **Admin** tab.
2. Select the row for the user in the **Users** list, then click **Edit** in the **User Details**.
3. In the **Edit User** window, select or unselect **Requires private folder**, then click **Save**.



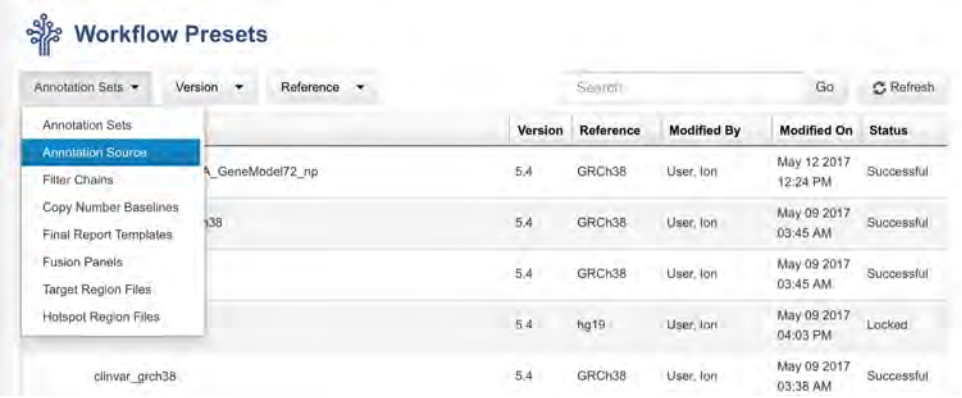
IMPORTANT! If a private folder is disabled anytime after it is enabled, data that was generated in that folder can no longer be accessed. A new private folder is created if you reselect 'Requires private folder', and all data generated in the previous private folder remains inaccessible.

After a private folder is enabled, the data, analysis and workflows created by that user and generated in that folder is visible only to that user.

Delete custom annotation sources

Custom annotation sets created by users are displayed in **Workflow Presets** screen. An administrator can view and delete custom annotation sources created by users.

1. In the **Workflows** tab, click **Presets**.
2. Click **Annotation Sets** ▸ **Annotation Source**.



   A table that lists the custom annotation sources opens. It shows the following information about the annotation: name, version, source version, reference, created by, created on, and source type.

3. Click the custom annotation source that you want to delete in the **Workflow Presets** list, then click **Settings (⚙)** ▸ **Delete**.

   Note: If an annotation source is used in a workflow, it cannot be deleted.

   The annotation source is removed from the **Workflow Presets** list.

Manage system services

Note: This procedure applies only to system services that are related to Ion Reporter™ Software server.

1. Sign in to your Ion Reporter™ Software server with your administrator account.
2. In the **Admin** tab, click the **System Services** subtab.

3. Click **System Services** subtab to display data for servers that are connected to the Ion Reporter™ Software.
   The pane displays the following information.

Parameter	Description
Name	Identifies services.
Status	Running or stopped.
Actions	Click any of the following buttons: • Restart: restart a service. • Start: Start an service. • Stop: Stop an service.

4. Click **Configure Services** subtab, then enter the number of concurrent analyses that can be run.
5. Click **Download All Logs** to down load zip files of logs to your desktop.
6. To power off the IR server, click **Shutdown IR Server** subtab, then click the **Shutdown IR Server** button.

IMPORTANT! All processes are stopped as a result.

Download plugins from Themo Fisher Cloud

The AmpliSeq RNA and RNASeq plugins can be downloaded from the Thermo Fisher Cloud, then installed into the Ion Reporter™ Software. See "Install and uninstall plugins" on page 316 for details to install the plugins.

1. In a web browser, go to **https://apps.thermofisher.com/** , then sign in.
2. Click on the apps icon ( ).
3. In the apps dashboard, click **Plugins**.
4. *(Optional)* Click a category at the top of page.
   The list of plugins is narrowed to only plugins included in the selected category.
5. Click to download the plugin. Enable the checkbox next to indicate that you agree to the End User License Agreement (EULA), then click **Download Plugin**.
   A compressed directory that contains the plugin is downloaded to your local machine.

Install and uninstall plugins

Note: The following steps apply only to Ion Reporter Server.

Only administrators can install plugins for their IR organization.

1. In the **Admin** tab of the **Ion Reporter** screen, select the **Plugins** subtab.
2. Click **Install Plugin**.
   The **Upload Plugin file** dialog appears.
3. Click Select file.
4. Select the file for the plugin, then click **Open**.

   Note: Only .zip files of the plugins are supported.

   The file to be uploaded appears in the file text dialog.
5. Click **Upload**.
   A confirmation appears in the **Upload Plugin file** dialog.
6. Click **Close**.
   - The plugin is added to the list of plugins.
   - Installed plugins are automatically enabled.
7. Select a plugin by clicking the checkbox (left-most column).
8. *(Optional)* to uninstall, click **Actions**, then select **Uninstall**.

See "Run a plugin as part of a workflow" on page 129 for instructions on how to run a plugin.

See “Guidelines to develop Ion Reporter plugins“ on page 421 for information on how to create custom plugins for Ion Reporter™ Software.

Troubleshooting

This section provides some basic information on how to troubleshoot any issues you may be having with Ion Reporter™ Software. If you do not find the answer to your question here, please contact Support.

Provide feedback

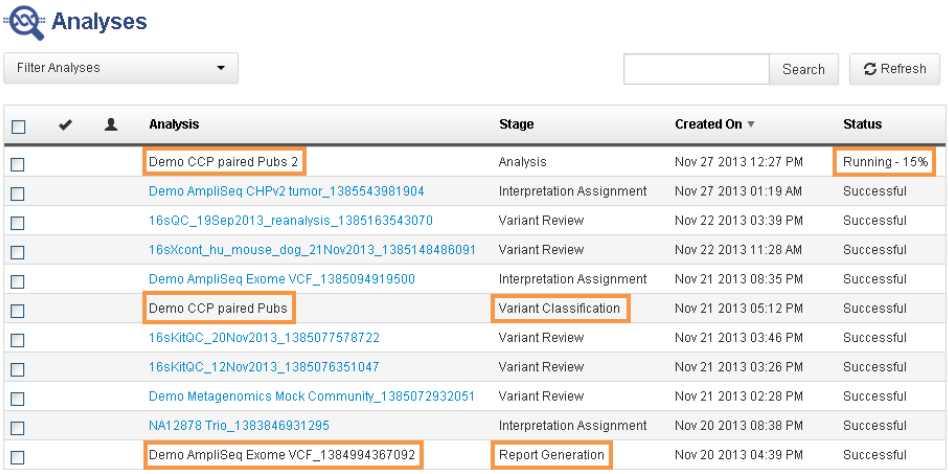
Information on how you can provide feedback on Ion Reporter™ Software can be found in the FAQ section of the help guide.

Contact support

Information on how you contact support for any questions on Ion Reporter™ Software can be found in the FAQ section of the help guide.

If you cannot open the analysis

Sometimes the analysis name is in plain text and does not provide a link to open the analysis.



This scenario can happen for the following reasons:

- The analysis is still running. The analysis can be edited when the processing is complete.
- The next stage is Variant Classification and you do not have the required Report Role permissions.
- The interpretive report is already published for the analysis, and editing is not possible. In this case, the stage is "Report Generation".

If someone else is already editing the analysis

If someone else is already editing the analysis, you see a checkmark in the "being edited" column of the analysis table in the Analysis Overview tab.

You can still open the analysis in view-only mode. A banner at the top of the Analysis Results page names the user who is editing the analysis and notes your view-only mode.

Annotation sources

Ion Reporter™ Software provides a variety of annotation sources from a wide range of public resources. You also have the option to import your own annotations sources if you want to add custom annotation data. Both types of annotation sources are for use directly in Ion Reporter™ Software.

Annotation sources available in Ion Reporter™ Software

pIon Reporter™ Software provides several annotation sources are derived from public and private annotation databases for hg19. Ion Reporter™ Software also provides factory-shipped annotation sets. An annotation set is a collection of annotation sources that you can use to annotate variants in your analyses. You can use the factory-shipped annotation sets or create custom annotation sets.

To add custom annotation sources to an annotation set, see "Import new or custom annotation sources" on page 326.

Table 8 Annotation sources included in Ion Reporter™ Software

Annotation	Description	Source
5000 exomes	Population frequency information from the 5000 exomes project	NHLBI ESP
Allele coverage	Number of reads supporting the called allele	Ion Reporter™ Software
Allele frequency	Frequency of the allele observed from the raw data	Ion Reporter™ Software
Amino acid change	Amino acid change	Ion Reporter™ Software
Genetic category	Genetic category **Note:** For use with trio workflows only.	Ion Reporter™ Software
ClinVar	Assessment of impact of the variant observed from NCBI ClinVar database	ClinVar
Coding	Nucleotide change	Ion Reporter™ Software
Copy number	The copy number ploidy state	Ion Reporter™ Software
COSMIC	Catalog of somatic mutations in tumor tissue	COSMIC

Annotation	Description	Source
Coverage	Total coverage for a variant	Ion Reporter™ Software
Custom	See "Import new or custom annotation sources" on page 326for more information	Ion Reporter™ Software
Cytogenetic band	The cytogenetic band where the CNV was detected **Note:** For use with aneuploidy workflows only.	Ion Reporter™ Software
DRA	Disease Research Area	Ion Reporter™ Software
dbSNP	Single Nucleotide Polymorphism database	**dbSNP**
DGV	Database of Genomic Variants: A curated database of human genomic structural variation	**DGV**
DrugBank	List of drugs known to target the gene(s) affected by the variant	**DrugBank** **Note:** When you create an hg19 annotation set, do not use the annotation source DrugBank version 20150107. Use instead annotation source DrugBank version 1 or DrugBank version 20161212. If you use the DrugBank version 20150107 in an hg19 annotation set, you will not be able to create filter chain of DrugBank for any analysis that uses the annotation set.
ExAC	Exome Aggregation Consortium: Database catalog of variant frequencies	**ExAC**
Father genotype	Genotype of father **Note:** For use with trio workflows only.	Ion Reporter™ Software
FATHMM scores	Prediction of the functional consequences of a variant. In Ion Reporter™ Software, FATHMM scores are available for COSMIC version 77 and later. These scores are populated when "COSMIC" is chosen as a part of the annotation preset that is used.	**COSMIC**
Gene models	Set of genes the variant overlaps	**RefSeq** **Ensembl**

Annotation	Description	Source
Gene panel	Filter variants based on specific amplicon in an AmpliSeq panel. For example, Ion AmpliSeq CCP.	Ion Reporter™ Software
Gene set	Focus on variants found within a specific set of genes	Ion Reporter™ Software
Genetic category	The genetic category. For example, compound heterozygote of the variant identified. **Note:** For use with trio workflows only.	Ion Reporter™ Software
Genotype	Genotype of the sample in each position	Ion Reporter™ Software
Gene Ontology	Standardized ontology for gene and gene products. For example, functional role or localization.	GO
Grantham score	A measure of evolutionary distance. See "Grantham score" on page 342.	Ion Reporter™ Software
Hotspot information	If variant overlaps a hotspot file	Ion Reporter™ Software
Location	Position of the variant. For example, exonic.	Ion Reporter™ Software
Locus	Position of the variant	Ion Reporter™ Software
MAF	Population frequency information from the 1000 genomes project. MAF numbers are provided by the dbSNP in Ion Reporter™, which gets the MAF numbers from 1000 genomes. Therefore, the version of dbSNP annotation sources used within the Ion Reporter™ analysis may impact these MAF values.	1000 Genomes
Mother genotype	Genotype of mother **Note:** For use with trio workflows only.	Ion Reporter™ Software
MyVariants	A personal knowledge base of genomic variants (Formerly, VariantKB database)	Ion Reporter™ Software
Named Variants	An Ion-supplied list of known variants in the CFTR gene panel	Ion Reporter™ Software

Annotation	Description	Source
OMIM	Online Mendelian Inheritance in Man®	**OMIM**
p-value	*p*-value of the variant call	Ion Reporter™ Software
Pfam	Protein domain families in the coded protein	**Pfam**
PhylopP	Measure of conservation of the protein across a wide range of organisms	**Cornell University**
PolyPhen-2	Prediction of the functional effect of a variant on a protein	**Harvard University**
Reference	The reference allele (hg19)	Ion Reporter™ Software
Reference / variant coverage	Individual strand coverage information for the reference and variant calls	Ion Reporter™ Software
SIFT	Prediction of the functional effect of a variant on a protein	**JCVI**
Size	Size of the variant	Ion Reporter™ Software
Transcript set	Preferred transcripts used to determine coding regions of genes. **Note:** If you include a transcript file, only transcripts that are present in your selection of canonical transcripts are reported. Other transcripts are filtered out.	**RefSeq** canonical; **Ensembl** canonical
Type	Type of variant. For example, SNP, Indel, CNV, and so on.	Ion Reporter™ Software
Variant effect	The effect of the variant on the coding sequence. For example, missense or stoploss.	Ion Reporter™ Software

Annotation sources for the Ion GRCh38 reference

The following annotation sources are available for the Ion GRCh38 reference:

Name	Description
GRCh38_clinvar_20160203	GRCh38 CLINVAR version 20160203
GRCh38_cosmic_75	GRCh38 COSMIC version 75
GRCh38_dbsnp_146	GRCh38 DBSNP version 146
GRCh38_dgv_20150723	GRCh38 DGV version 20150723

Name	Description
GRCh38_ensgeneScores_5	GRCh38 Ensembl Gene Functional canonical transcripts scores version 5
GRCh38_ensgene_79	GRCh38 ENSEMBL version 79 gene model
GRCh38_esp6500_20151203	GRCh38 5000 EXOMES version 20151203
GRCh38_namedVariants_20151113	GRCH38 Named Variants version 20151113
GRCh38_pfam_29	GRCh38 PFAM version 29
GRCh38_phylop_20151118	GRCh38 PHYLOP version 20151118
GRCh38_refgeneScores_5	GRCh38 Refgene Functional canonical transcripts scores version 5
GRCh38_refgene_72	GRCh38 REFSEQ version 72 gene model
drugbank_20150107	DRUGBANK version 20150107
go_20151216	GENE ONTOLOGY version 20151216
omim_20160128	OMIM version 20160128

In addition, the following canonical transcripts sets are included for GRCH38 ENSEMBL v79 and REFSEQ v72 gene models:

- GRCH38 ENSEMBL v79 gene model UCSC genome browser canonical transcript set
- GRCH38 REFSEQ v72 gene model UCSC genome browser canonical transcript set

Transcripts included in GRCh38 RefSeq canonical transcripts file

In UCSC's curated canonical transcripts set, there were some discrepancies where there were multiple transcripts for a given gene. We removed the shorter transcripts and kept the transcript with the "appris_principal_1" tag in the Ion Reporter™ Software refseq canonical transcript set. This tag is used by gencode to mark the primary transcript of a particular gene. In the cases, where the appris_principal_1 was missing, we chose the longest transcript in order to have only one canonical transcript for a given gene. In cases where two transcripts were the same length, we used alphabetical order.

The UCSC transcripts for the seven genes in this table are replaced with the transcripts in the New transcript column in Ion Reporter™ Software:

Gene	Current transcript	New transcript
FGFR2	NM_022970.3	NM_000141.4
FGFR3	NM_001163213.1	NM_000142.4
ABL1	NM_007313.2	NM_005157.4
ARAF	NM_001256196.1	NM_001654.4
CHEK2	NM_001005735.1	NM_007194.3

Gene	Current transcript	New transcript
GNAS	NM_080425.2	NM_000516.4
TP53	NM_001276760.1	NM_000546.5

Functional Annotator annotates only supported contigs in GRCh38 reference

The Functional Annotator in Ion Reporter™ Software can annotate only variants that map to the following contigs as provided in the GRCh38.fasta and GRCh38.fasta.fai files:

Table 9 Contigs (sequences) supported by Ion Reporter™ Software

Contig Name	Length (bps)
chr1	248956422
chr2	242193529
chr3	198295559
chr4	190214555
chr5	181538259
chr6	170805979
chr7	159345973
chr8	145138636
chr9	138394717
chr10	133797422
chr11	135086622
chr12	133275309
chr13	114364328
chr14	107043718
chr15	101991189
chr16	90338345
chr17	83257441
chr18	80373285
chr19	58617616
chr20	64444167
chr21	46709983
chr22	50818468
chrX	156040895

Contig Name	Length (bps)
chrY	57227415
chrM	16569
chr22_KI270879v1_alt	304135

There are two kinds of variants that we do not support, currently:

1. Variants that map to a contig we do not support
2. Variants that map to a masked region

In particular, chr22_KI270879v1_alt is completely masked except for 269,814 - 279,355, which contains only the GSTT1 gene. No amino acid changes are provided for the masked regions. However, gene and transcript information is provided.

GRCh38 dbsnp

The GRCh38 dbsnp version in Ion Reporter™ Software 5.2 is dbsnp version 146 for both Ion Reporter™ Software 5.2 and 5.4. However, since the UCSC genome browser did not have the UCSC common genes available for dbSNP v146 when Ion Reporter™ Software 5.2 was released, UCSC common genes version 144 are used to set the UCSC common genes in Ion Reporter™ Software 5.2.

Import new or custom annotation sources

You can use your own annotation source files in Ion Reporter™ Software. If you have annotation data that you want to use, you can import the files into a custom annotation set.

There are four types of annotation sources that you can import into Ion Reporter™ Software. The types of annotation sources and the file formats required for each annotation source are as follows:

Note: Only one version of an annotation source can be used in an annotation set.

Annotation source type	Description	Import format
Genomic region	A set of regions	BED
Gene set	A set of genes	TXT
Preferred transcript set	A set of transcripts	TXT
VariantDB	A set of variants	VCF

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Annotation Set**.

3. Select an annotation type from the drop-down list:

Option	Description
Genomic region	See for more details.
Gene set	See for more details.
Preferred transcript set	See for more details.
VariantDB	See for more details.

   - Click **Create New**.

4. Enter a descriptive name for the custom annotation set.
5. (Optional) Enter a description for the custom annotation set.
6. Click **Select File**.
7. Browse to and select your custom annotation source file, then click **Choose**.
8. Click **Done**.
9. The new custom annotation source is added to the custom annotation set.

Genomic region

With a genomic region annotation source, you provide annotation information for specific regions of interest in the genome. Add your custom annotations in the fourth column, as key-value pairs separated by semi-colons.

The information in your input genomic regions file is used in the following ways in your analysis results:

- In the Analysis Review screen and in the downloaded TSF variant files, information from the fourth column is added to matching variants in your analysis results.
- In the Analysis Review screen, you can create a filter based the first key-value pair in the fourth column. (Only the first key-value pair can be used as a filter.)

Here is an example file:

```
#CHROM    Start    End    Annotation values
chr1    000000    000001    fld1=abc;fld2=123;fld3=this
chr1    000001    000002    fld1=abd;fld2=124;fld3=that
chr1    000002    000003    fld1=abe;fld2=125;fld3=this
chr1    000003    000004    fld1=abf;fld2=126;fld3=that
chr1    000004    000005    fld1=abg;fld2=127;fld3=this
chr1    000005    000006    fld1=abh;fld2=128;fld3=that
```

The hit level for a genomic region annotation source is not configurable and is always set to overlap.

To import a genomic region custom annotation, see “Import new or custom annotation sources“ on page 326

Gene set

With gene sets, you provide annotation information for specific genes of interest. A gene set annotation source file is a two-column tab-separated file of gene names and your categories for those genes.

Notes about the format for a gene set annotation source file:

- This annotation source file uses a .txt format.
- The file includes an optional column header. The second column name is used as the annotation key name. If the header line is not provided, the default annotation key name of Name is used.
- The header line is not required. If the header line is not provided, the default annotation key name of Name is used.
- Subsequent lines list comma-separated gene symbols, a tab character, and any annotation string.

The information in your input gene sets file is used in the following ways in your analysis results:

- In the Analysis Review screen and in the downloaded TSF variant files, information from the second column is added to matching variants in your analysis results.
- In the Analysis Review screen, you can create a filter based on the second column name. You can filter your results on any of the values that appear in the second column.

Here is an example file:

```
#Geneset5    Disease
Gene1,Gene2  disease1
Gene3        disease2
Gene4,Gene5,Gene6    disease3
Gene7,Gene8  disease4
```

To import a gene set custom annotation, see “Import new or custom annotation sources“ on page 326.

Transcript set

With a transcript set, you provide annotation information for specific transcripts of interest. A transcript set limits the annotations applied to your variant calls to those that match your list of preferred transcripts.

Notes about transcript sets:

- The file's contents are in the format: GENE_NAME transcript_accession_id1, transcript_accession_id2, ..., with a tab character between the gene name and the first transcript id.
- Gene names must be specified as HGNC gene symbols.
- A tab character is required after the gene name.
- A comma and a space character are required between transcripts.
- If gene name is missing in the file, all transcript annotations for that gene are preserved.

- Transcripts can be specified as RefSeq or Ensembl® accession ids with version. However, if the accession id contains no dot version, then all versions of the transcript are matched. For example, Gene1 NM_0000006 will be matched with all versions of this transcript . This annotation source uses a TXT format with tab characters. In Ion Reporter™ Software 5.4 and later, even if the version numbers of the custom transcripts do not match, the variant will be annotated as long as the transcript names match.

Here is an example file:

```
GENE REFSEQ_AND_ENSEMBL_TRANSCRIPTS
Gene1 NM_00000006.2, NM_000000005.3, ENST000000000007.3
Gene2 NM_00000001.1, NM_000000007.1, NM_0000000004.5,
NM_000000008.3, ENST000000000006.4
```

RefGene GeneModel and Ensembl GeneModel transcript versions

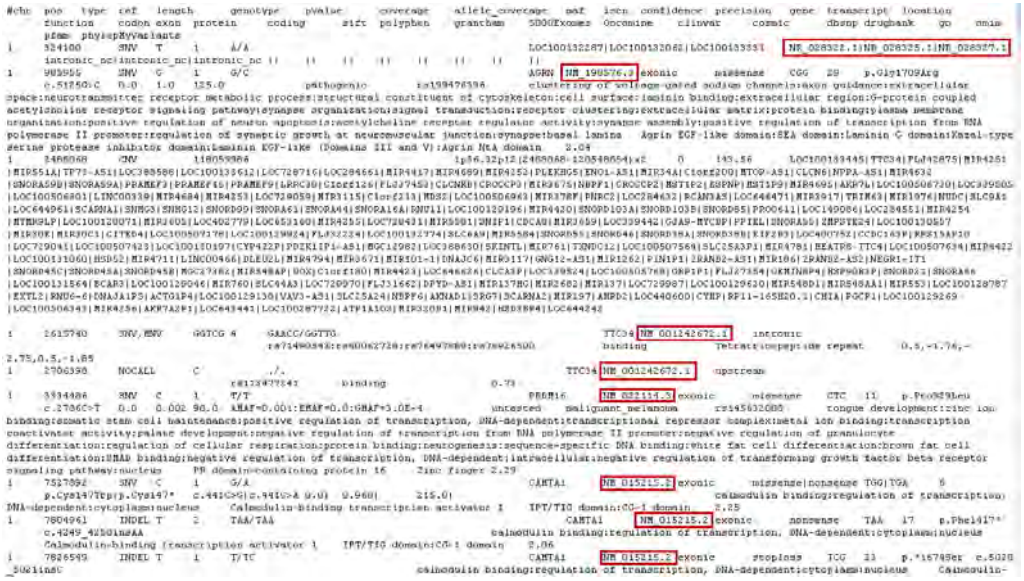
You can view the details about the annotations that Ion Reporter™ Software supports for a particular gene model transcript in the list of **Variant Details**.

Variant Details: chr1:7527892

Variant Details | Notes

Annotation Source	Annotation Value
Amino Acid Change	p.Cys147Trp ...(2)
Coding	c.441C>G ...(2)
Codon	TGA ...(2)
Exon	6
Gene	CAMTA1
Gene Ontology	calmodulin binding ...(4)
Genotype	G/A
Length	1
Location	exonic
Locus	chr1:7527892
OMIM	Calmodulin-binding transcription activator 1
PFAM	IPT/TIG domain ...(2)
PhyloP	2.25
Ref	C
Transcript	NM_015215.2
Type	SNV
Variant Effect	nonsense, missense

And in the TSV variants file (in the Variants/*sample_name* subdirectory):



Use these version numbers with the transcript IDs in your preferred transcript file.

VariantDB files

VariantDB files allow you to provide custom annotation information for specific variants of interest.

Note: Custom variantDBs must have unique names. For example, name and version number should be combined to ensure unique names.

```
Name: variantdbA_v1     Version: v1
Name: variantdbA_v2     Version: v2
Name: variantdbA_v3     Version: v3
```

An example file is shown below. This file is tab-delimited and the header line is required.

```
##fileformat=VCFv4.1
                  #CHROM     POS     ID     REF     ALT     QUAL
FILTER     INFO
                  chr1     124535436    COSM00001    TG
AA   .    .    AMPID=AMPL495041;TEMP_ID=0
                  chr1     124535494    COSM00002    G
T    .    .    AMPID=AMPL495041;TEMP_ID=1
                  chr1     128808434    COSM00003    T
A    .    .    AMPID=AMPL30014;TEMP_ID=2
                  chr1     124597624    .            T
G    .    .    AMPID=AMPL30014;TEMP_ID=3
                  chr1     136671158    .            TT
CA   .    .    AMPID=AMPL30014;TEMP_ID=5
                  chr1     141128903    COSM00006    TTG
CTT  .    .    AMPID=AMPL30014;TEMP_ID=6
```

We recommend that the custom input file provided to VariantDB be left-aligned. Left alignment is used to normalize the positions of ambiguous indels that can be placed at multiple positions.

The information in your input VariantDB file is used in the following ways in your analysis results:

- In the downloaded variants TSV file, the content in your ID, REF, ALT, and INFO fields are added to the variant.
- In the Analysis Review screen, the content in your ID and INFO fields are added to the variant.
- In the Analysis Review screen, you can create a filter that is based on the content in your ID field. If the content of the ID field does not contain a value (contains only a period), then the first key-value pair of your INFO field is used.

Further information on VCF format:

- **Official specification of VCF (Variant Call Format) version 4.1:**
  http://www.1000genomes.org/wiki/Analysis/Variant%20Call%20Format/vcf-variant-call-format-version-41
  In VCF format files, missing values are represented by dots. The content must be tab-separated. Ensure that no extra or hidden characters are added to the VCF files, which may occur when they are opened in programs like Excel or Word, or when emailed as an unzipped attachment.
- **Mandatory headers required when creating a VariantDB file:** The following three headers must be present in the first three lines of the VCF file (FORMAT and Sample columns are optional in VCF files):

```
##fileformat=VCFv4.1

##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
                          #CHROM    POS    ID    REF
ALT    QUAL    FILTER    INFO    FORMAT    Sample
```

- **Hit-level information in a VariantDB file:** You can adjust the hit level of each VariantDB file individually by including this information in the header. The following hit-level parameters can be included in the VCF header.
  - ##**HITLEVEL=overlap** matches all annotations whose loci overlap with variant.
  - ##**HITLEVEL=locus** matches all annotations whose loci start at the variant locus.
  - ##**HITLEVEL=allele** matches all annotations that are 'locus' matches plus have at least one allele in common with variant.
  - ##**HITLEVEL=genotype** matches all annotations that are 'allele' matches where the genotypes also match.
  - ##**HITLEVEL=auto** matches the most specific hit level possible, which could be any of the hit levels listed above.

- **Mandatory columns required in the VCF file when creating a VariantDB:** Providing FORMAT and SAMPLE fields is not mandatory according to the official VCF specification. However, in order to perform a "genotype" hit level match in Ion Reporter™ Software, you must specify a GT (genotype) for the variant in the FORMAT column.

  An example of a variant with a GT field of 0/1 in the FORMAT field of a VCF file is given below:

```
chr1    141128903   COSM00006   TTG CTT .   .
AMPID=AMPL30014;TEMP_ID=6    GT    0/1
```

  If only an "overlap" or "locus" or "allele" match is needed, you do not need to specify a GT field. However, the missing values must be represented by dots in the appropriate columns. For example:

```
chr1    141128903   COSM00006   TTG CTT .   .
AMPID=AMPL30014;TEMP_ID=6...
```

  If the "auto" hit level match is chosen, Ion Reporter™ Software will try to find the most specific hit level match possible. However, if no GT value is supplied, the most specific hit level possible will be an allele match, as there is no GT value to do an allele or genotype level match.

- **How to filter on VariantDB:**
  - **Option 1:**

    Ion Reporter™ Software automatically exposes a filter on the first INFO key of the VariantDB VCF file if such a key is specified and if the ID field of the VCF file is missing.
  - Consider the example below:

```
##fileformat=VCFv4.1

##FORMAT=<ID=GT,Number=1,Type=String,Description="Genoty
pe">
                                    #CHROM    POS    ID
REF    ALT    QUAL    FILTER    INFO    FORMAT    Sample
                                        chr1
124535436   .    TG  AA  .   .
AMPID=AMPL495041;TEMP_ID=0...
                                        chr1
128808434   .    T   A   .   .
AMPID=AMPL30014;TEMP_ID=2...
```

    If the above VCF file with two variants is used in order to make a VariantDB in Ion Reporter™ Software, you will be able to filter on the AMPID field, since the AMPID key is the first INFO key present in the INFO field of the VCF file and the ID fields are missing (represented by dots).

– **Option 2:**

If the INFO field is not populated, filtering will be automatically enabled on the ID column.

Consider the example below, in which the INFO field is missing and represented with a dot:

```
##fileformat=VCFv4.1

##FORMAT=<ID=GT,Number=1,Type=String,Description="Genoty
pe">
                                    #CHROM    POS    ID
REF    ALT    QUAL    FILTER    INFO    FORMAT    Sample
                                    chr1    124535436
COSM00001   TG  AA  .   .   ....
                                    chr1    141128903
COSM00006   TTG CTT .   .   ....
```

If the VCF file above with two variants is used in order to make a VariantDB in Ion Reporter™ Software, you will be able to filter on the ID field, since the INFO field of the VCF file is not populated.

See also the sections on "MyVariants" for marking and tracking of variant annotation beyond the VariantDB annotation presets in workflows.

Annotation sources and scores

This section provides more detail about select annotation sources.

P-value scores

The P-value represents the probability that the variant call is incorrect. The range is from 0.00001 to 0.99999, with numbers approaching 1 being the least confident (that the call is incorrect), and numbers close to zero being the most confident (that the call is incorrect).

P-values closer to 0.0 represent more confidence that the variant call is correct. P-values closer to 1.0 represent less confidence that the variant call is correct.

The p-value reported by Ion Reporter™ Software is a logarithmic transformation of the Phred quality score value made by the VariantCaller. For example, a VariantCaller quality score of 20 is associated with a p-value of 0.01. A VariantCaller quality score of 30 is associated with a p-value is 0.001.

Pfam annotations

Ion Reporter™ Software has two pfam versions: version 26 and version 30. Refer to the following site for more information on PFAM:

http://pfam.xfam.org/

Pfam consist of parts A and B. Ion Reporter™ Software uses Pfam-A. "A is curated and contains well-characterized protein domain families with high quality alignments, which are maintained by using manually checked seed alignments and HMMs to find and align all members", according to this site:

Pfam: a comprehensive database of protein domain families based on seed alignments

See also the following link for information about Pfam:

The Pfam protein families database:: R.D. Finn, J. Mistry, J. Tate, P. Coggill, A. Heger, J.E. Pollington, O.L. Gavin, P. Gunesekaran, G. Ceric, K. Forslund, L. Holm, E.L. Sonnhammer, S.R. Eddy, A. Bateman. Nucleic Acids Research (2010) Database Issue 38:D211-222

The next sections describe interpretation guidelines for annotation scores in the SIFT, Grantham, and PolyPhen columns.

Note: Pfam annotations for all domains of the gene's protein are added for any variant.

IMPORTANT! The interpretations listed here are the recommendations of the respective external sites.

Annotation versions and calculations

This section provides details about the annotation scores used in Ion Reporter™ Software and how these scores are calculated.

SIFT versions

- **SIFT version** -- 5.1.1
- **Protein database** -- UniprotTrEMBL, downloaded on July 11, 2011. Updated on March 14, 2014.
- **BLAST version** -- 2.2.25. Updated to 2.2.26.
- **BLIMPS version** -- 3.9

Polyphen-2

- **Version** -- PolyPhen-2 v2.1.0r367, using the stand-alone default installation instructions and usage
- **BLAST version** -- 2.2.25
- **Protein database** -- Uniref100, downloaded on August 12, 2011 from ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/uniref/uniref100/uniref100.fasta.gz

Codon differences in chromosome M

Chromosome M has its own genome that is different in 4 codons from the other chromosomes.

Ion Reporter™ Software uses the Homo sapiens mitochondrion, complete genome NCBI Reference Sequence: NC_012920.1.

The new rCRS chrM assembly has 16569 bases. The previous version had 16571 bases.

The 2 coding tables, one for standard chromosomes and one for chromosome M, are listed below. These tables are from the following NCBI site:

http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi#SG2

The Standard Code (transl_table=1) for chromosomes 1-22, X and Y (NUCLEAR)

By default all transl_table in GenBank flatfiles are equal to id 1, and this is not shown. When transl_table is not equal to id 1, it is shown as a qualifier on the CDS feature.

```
TTT F Phe      TCT S Ser      TAT Y Tyr      TGT C Cys
 TTC F Phe      TCC S Ser      TAC Y Tyr      TGC C Cys
```

```
TTA L Leu      TCA S Ser      TAA * Ter      TGA * Ter
TTG L Leu i    TCG S Ser      TAG * Ter      TGG W Trp

CTT L Leu      CCT P Pro      CAT H His      CGT R Arg
 CTC L Leu      CCC P Pro      CAC H His      CGC R Arg
CTA L Leu      CCA P Pro      CAA Q Gln      CGA R Arg
CTG L Leu i    CCG P Pro      CAG Q Gln      CGG R Arg

ATT I Ile      ACT T Thr      AAT N Asn      AGT S Ser
 ATC I Ile      ACC T Thr      AAC N Asn      AGC S Ser
ATA I Ile      ACA T Thr      AAA K Lys      AGA R Arg
ATG M Met i    ACG T Thr      AAG K Lys      AGG R Arg

GTT V Val      GCT A Ala      GAT D Asp      GGT G Gly
 GTC V Val      GCC A Ala      GAC D Asp      GGC G Gly
GTA V Val      GCA A Ala      GAA E Glu      GGA G Gly
GTG V Val      GCG A Ala      GAG E Glu      GGG G Gly
```

The Vertebrate Mitochondrial Code (transl_table=2) for chromosome M.

```
TTT F Phe      TCT S Ser      TAT Y Tyr      TGT C Cys
 TTC F Phe      TCC S Ser      TAC Y Tyr      TGC C Cys
TTA L Leu      TCA S Ser      TAA * Ter      TGA W Trp
TTG L Leu      TCG S Ser      TAG * Ter      TGG W Trp

CTT L Leu      CCT P Pro      CAT H His      CGT R Arg
 CTC L Leu      CCC P Pro      CAC H His      CGC R Arg
CTA L Leu      CCA P Pro      CAA Q Gln      CGA R Arg
CTG L Leu      CCG P Pro      CAG Q Gln      CGG R Arg

ATT I Ile i    ACT T  Thr      AAT N Asn      AGT S  Ser
ATC I Ile i    ACC T Thr      AAC N Asn      AGC S Ser
ATA M Met i    ACA T Thr      AAA K Lys      AGA * Ter
ATG M Met i    ACG T Thr      AAG K Lys      AGG * Ter

GTT V Val      GCT A Ala      GAT D Asp      GGT G Gly
 GTC V Val      GCC A Ala      GAC D Asp      GGC G Gly
GTA V Val      GCA A Ala      GAA E Glu      GGA G Gly
GTG V Val i    GCG A Ala      GAG E Glu      GGG G Gly
```

The differences from the Standard Code are the following:

	Code 2	Standard
AGA	Ter *	Arg R
AGG	Ter *	Arg R
AUA	Met M	Ile I
UGA	Trp W	Ter *

Note: In these tables, an asterisk represents a STOP codon.

Background information

The UCSC website has this note about chrM:

"Note on chrM:

Since the release of the UCSC hg19 assembly, the Homo sapiens mitochondrion sequence (represented as "chrM" in the Genome Browser) has been replaced in GenBank with the record NC_012920. We have **not**replaced the original sequence, NC_001807, in the hg19 Genome Browser. We plan to use the Revised Cambridge Reference Sequence (rCRS) in the next human assembly release."

The **IGSR: The International Genome Sample Resource** has this entry:

Which reference assembly do you use?

The reference assembly the 1000 genomes project has mapped sequence data that has changed over the course of the project.

For the pilot phase we mapped data to NCBI36. A copy of our reference fasta file can be found on the **ftp site**.

For the phase1 and phase 3 analysis we mapped to GRCh37. Our fasta file which can be found **here** called human_g1k_v37.fasta.gz, it contains the autosomes, X, Y and MT but no haplotype sequence or EBV.

Other links related to chrM:

Original sequence, NC_001807:

http://www.ncbi.nlm.nih.gov/nuccore/NC_001807.4?report=genbank

The updated Mitochondrial sequence, NC_012920 can be found here:

http://www.ncbi.nlm.nih.gov/nuccore/NC_012920

Effect of Ensembl and RefSeq sources on Polyphen and SIFT scores

The annotation set configuration pages allow you to specify Polyphen and Sift in an annotation set with RefSeq 63 and Ensembl® 74. Functional Scores v4 target RefSeq 63 and Ensembl® 74. Functional scores v3 target RefSeq 53 and Ensembl® 65.

Genetic Category Type variants

This help page describes the variant categories that defined by trio workflows. During these workflows, each variant called in the proband sample is assigned all categories that apply. A variant might be assigned one, none, or many categories.

During your review of analysis results, the Genetic Category Type filter applies to these categories.

HasDeNovoNonRefAllele

An allele of a proband variant is *de novo* if it does not appear in the genotype of either of the proband's parents at this variant position.

A proband variant is assigned the category HasDeNovoNonRefAllele if all of the following are true:

- The variant has an allele that:
  - Is not the same as the reference allele.
  - Does not appear in the father's genotype.
  - Does not appear in the mother's genotype.
- The coverage of the father's genotype at this variant position is at least denovo_nonref_allele_min_father_coverage.
- The coverage of the mother's genotype at this variant position is at least denovo_nonref_allele_min_mother_coverage.

Note: The coverage parameters help avoid false-positive calls that might arise simply because the sequencing coverage is inadequate to call an accurate genotype. Without coverage information, the absence of a variant call for a parent is only absence of evidence, and we cannot exactly determine that the proband's allele is *de novo*. To establish evidence of absence, we require that the parents be covered well enough that variants are called if actually present. Therefore we require that the position in both parents meets a coverage threshold.

HasDeNovoRefAllele

A proband variant is assigned the category HasDeNovoRefAllele if all of the following are true:

- The variant has an allele that:
  - Is the same as the reference allele.
  - Does not appear in the father's genotype.
  - Does not appear in the mother's genotype.
- The coverage of the father's genotype at this variant position is at least denovo_ref_allele_min_father_coverage.
- The coverage of the mother's genotype at this variant position is at least denovo_ref_allele_min_mother_coverage.

See also HasDeNovoNonRefAllele.

IsNewlyHomozygousNonRef

A proband variant is newly homozygous if it is homozygous but genotypes of both of the parents are heterozygous.

A proband variant is assigned the category IsNewlyHomozygousNonRef if all of the following are true:

- The variant is homozygous.
- The variant's allele is not the same as the reference.
- The father's genotype is heterozygous at this variant position.
- The mother's genotype is heterozygous at this variant position.

- The variant's functional annotations include at least one specified by newly_homozygous_nonref_functional_types.
- The frequency of the allele in the population is at most newly_homozygous_nonref_max_population_allele_frequency.<

This situation can indicate a recessive disease or condition, in which the parents carry the same deleterious mutation but are unaffected because they are heterozygous. The trio analysis later examines the variant's functional annotations to help determine if the variant is deleterious.

IsNewlyHomozygousRef

A proband variant is newly homozygous if it is homozygous but genotypes of both of the parents are heterozygous.

A proband variant is assigned the category IsNewlyHomozygousRef if all of the following are true:

- The variant is homozygous.
- The variant's allele is the same as the reference.
- The father's genotype is heterozygous at this variant position.
- The mother's genotype is heterozygous at this variant position.
- The frequency of the allele in the population is at most newly_homozygous_nonref_max_population_allele_frequency.

IsNewlyHeterozygousNonRef

A proband variant is heterozygous non-ref if it is heterozygous and neither allele is the reference allele. The variant is *newly* heterozygous non-ref if the genotypes of both parents contain the reference allele.

A proband variant is assigned the category IsNewlyHeterozygousNonRef if all of the following are true:

- The variant is heterozygous.
- Neither of the variant's alleles is the same as the reference.
- The father's genotype contains the reference allele at this variant position.
- The mother's genotype contains the reference allele at this variant position.
- The variant's functional annotations include at least one specified by newly_homozygous_nonref_functional_types.
- The frequency in the population of at least one of its alleles is at most newly_homozygous_nonref_max_population_allele_frequency.

This situation can indicate a recessive trait or condition, in which the parents carry the same deleterious mutations but are unaffected because they are heterozygous-ref.

Note: The parameters newly_homozygous_nonref_functional_types and newly_homozygous_nonref_max_population_allele_frequency are used in both the IsNewlyHomozygousNonRef and IsNewlyHeterozygousNonRef categories.

InCompoundHeterozygote

A compound heterozygote is a pair of putatively deleterious variants on the same gene.

A proband variant is assigned the category InCompoundHeterozygote if both of the following are true:

- The variant's functional annotations include at least one of the types in compound_heterozygote_functional_types.
- The variant is in the same gene as another such variant.

The variants can be either homozygous or heterozygous. The trio analysis later determines whether a variant is putatively deleterious by examining the existing functional annotations associated with the variant.

InTransPhaseCompoundHeterozygote

A trans-phase compound heterozygote is a compound heterozygote in which one variant has a non-reference allele on the paternal homolog, and the other variant has a non-reference allele on the maternal homolog.

A trans-phase heterozygote is a pair of deleterious proband variants in a gene where:

- One of the two variants has a non-reference allele that is present in the father's genotype, and whose other allele is present in the mother's genotype, *and*
- The other variant has a non-reference allele that is present in the mother's genotype, and whose other allele is present in the father's genotype.

To determine the InTransPhaseCompoundHeterozygote category, for each gene, the trio analysis creates two lists of proband variants, *fromFather* and *fromMother*.

Both of the following apply to each variant in the *fromFather* list:

- Its functional annotations include at least one in compound_heterozygote_functional_types.
- It has a non-reference allele that is present in the father's genotype, and whose other allele is present in the mother's genotype at this variant's position.

Similarly, both of the following apply to each variant in the *fromMother* list:

- Its functional annotations include at least one in compound_heterozygote_functional_types.
- It has a non-reference allele that is present in the mother's genotype, and whose other allele is present in the father's genotype at this variant's position.

Any variant in the *fromFather* list paired with any variant in the *fromMother* list forms a trans-phase heterozygote pair, and any trans-phase heterozygous pair of variants has one variant in the *fromFather* list and the other in the *fromMother* list.

A proband variant is assigned the category InTransPhaseCompoundHeterozygote if either of the following are true:

- The variant appears in the *fromFather* list and there is at least one other variant in the *fromMother* list, or
- The variant appears in the *fromMother* list and there is at least one other variant in the *fromFather* list.

HasMaleMaternalX

A proband variant is assigned the category HasMaleMaternalX if all of the following are true:

- The proband is male.
- The variant is on the X chromosome.
- The variant is hemizygous (non-reference).
- The father's genotype at this variant's position is hemizygous reference.
- The mother's genotype at this variant's position is heterozygous.
- The mother's genotype includes the proband variant's allele.
- The variant's functional annotations include at least one type in maternal_x_functional_types.
- The frequency of the proband's allele is less than maternal_x_max_population_allele_frequency.

The following explain why these conditions apply to this category:

- This category detects possible causative variants inherited from the mother on the single X-chromosome of a male proband (conditions 1 and 2 above).
- The father is not affected, so when a variant is causative, the father, with only one X-chromosome, cannot also have that variant at this position (condition 4).
- If the variant is *de novo*, it is assigned the categoryHasDeNovoNofRefAllele. For the category HasMaleMaternalX, a variant allele is inherited from the unaffected mother (conditions 5 and 6).
- The variant is detrimental (condition 7).

HasUnknownX

A proband variant is assigned the category HasUnknownX if all of the following are true:

- The proband is male.
- The variant is on the X chromosome.
- The variant is hemizygous (non-reference).
- The father's genotype at this variant's position is hemizygous reference.
- The mother's genotype does not include the proband variant's allele.
- The variant's functional annotations include at least one in unknown_x_functional_types.
- The frequency of the proband's minor allele is less than unknown_x_max_population_allele_frequency.

InconsistentWithFather

A proband variant is assigned the category InconsistentWithFather if all of the following are true:

- Neither of the variant's alleles appear in the father's genotype at this variant position.
- The variant has coverage at least consistencyMinCoverage.
- The father's genotype has coverage at least consistencyMinCoverage at this variant position.

InconsistentWithMother

A proband variant is assigned the category InconsistentWithMother if all of the following are true:

- Neither of the variant's alleles appear in the mother's genotype at this variant position.
- The variant has coverage at least consistencyMinCoverage.
- The mother's genotype has coverage at least consistencyMinCoverage at this variant position.

InconsistentWithParents

A proband variant is consistent with its parents if one of its alleles is present in the father's genotype at this variant position and its other allele is present in the mother's genotype at this variant position.

A proband variant is assigned the category InconsistentWithParents if all of the following are true:

- The variant has coverage at least consistencyMinCoverage.
- The father's genotype has coverage at least consistencyMinCoverage at this variant position.
- The mother's genotype has coverage at least consistencyMinCoverage at this variant position.
- The variant is not consistent with it parents.

Gene Category Type and Variant Effect filter types can be applied to the same variants

The Gene Category Type (GCT) has a functional type parameter similar to 'Variant Effect' which you can set when creating a custom trio workflow. Both this functional type selection and Variant Effect will be applied if used together in a filter chain. For example, if a variant has a GCT functional type of indel, it may have a Variant Effect type of frameshift or non-frameshift. The functional annotation types differ for these two filter types.

The GCT functional filter types are as follows:

- snp
- indel
- synonymous
- missense
- stoploss
- stopgain
- splicejunction
- utr

The Variant Effect filter types are as follows:

- unknown
- synonymous
- missense
- nonframeshiftInsertion

- nonframeshiftDeletion
- nonframeshiftBlockSubstitution
- nonsense
- stoploss
- frameshiftBlockSubstitution

Genetic Category Type and MAF filter

If a variant is not a minor allele, MAF filter will not be effective, because MAF filter only applies if the variant is a minor allele. Users may have a threshold setting for MAF filter which will not be effective if variant is not a minor allele.

Grantham score

The Grantham score attempts to predict the distance between two amino acids, in an evolutionary sense. A lower Grantham score reflects less evolutionary distance. A higher Grantham score reflects a greater evolutionary distance. Higher Grantham scores are considered more deleterious:

- The more distant two amino acids are, the less likely the amino acids are to be substituted with one another.
- The more distant two amino acids are, the more damaging is their substitution.

The distance scores published by Grantham range from 5 to 215. A substitution of isoleucine for leucine, or of leucine for isoleucine, has a score of 5 (and is predicted to be tolerated). A substitution cysteine for tryptophan, or of tryptophan for cysteine, has a score of 215. Any variation involving cysteine has a high or very high Grantham score (and is predicted to be deleterious).

For more information on Grantham scores, see the following sites:

- Abstract for **Amino acid difference formula to help explain protein evolution**.
- Article for Grantham R.
  "**Amino acid difference formula to help explain protein evolution**", Science. 1974 Sep 6;185(4154):862-4.

HGVS genomic codes

This table shows the genomic code patterns used in the Analyze Role Review Samples page and the Report Role Interpret Results page.

Note: HGVS c. notations for intronic, upstream, and downstream variants in genes are shown in the **Functional** tab on the **Variant Review** screen. HGVS c. notations are not shown for intergenic variants.

	CHR	POS	REF	ALT	GT	Genomic	Coding	Protein
Homozygous SNP	3	124535434	G	A	1/**1**	chr1:124535434**G>A**	c.1531G>A	p.Ala511Thr
Heterozygous SNP	4	90545103	A	T	0/1	chr2:90545103**A>T**;[=]	c.1638A>T	p.Lys546Asn
Insertion	4	Insertion of bases "AAT" between the positions 61310513 and 61310514	G	GAAT	0/1	chr7:61310513_61310514ins**AAT**;[=]	c.1663_1664insAAT	NA
Deletion	17	Deletion of bases "GGT" in positions 61917157, 61917158,61917159	AGGT	A	0/1	chr7:61917157_61917159del**GGT**;[=]	c.737_739delGGT	NA
MNV	Y	Replacing bases "CC" in positions 50367679 and 50367680 with bases "TT"	CC	TT	0/1	chr7:50367679_50367680delCCinsTT;[=]	c.235_236delCCinsTT	p.Pro79Leu

Homozygous reference call	22	50833853	A	.	0/0	chr11:50833853A	c.=	p.Tyr505Tyr
No call	5	38335801	G	.	./.	chr16:38335801G>?	NA	NA

The Human Genome Variation Society site is **http://www.hgvs.org/mutnomen**.

Ion Reporter™ Software references

- **hg19: This human reference is based on the GRCh37.p5 version of the human genome assembly. The GRCh37.p5 version is described at this web site: https://www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh37.p5.**
- **GRCh38: This human reference is based on the GRCh38.p2 version of the human genome assembly. The GRCh38.p2 version is described at this web site: https://www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh38.p2.**

Ambiguity codes for three positions on Chromosome 3

Three positions on chromosome 3 are marked with

```
'N'
```

in the UCSC version of the genome. These positions have IUPAC ambiguity codes in our version:

Position	IUPAC Ambiguity code in Ion reference	Hard masked character in UCSC hg19
60830534	M	N
60830763	R	N
60830764	R	N

Hard masked PAR regions in chromosome Y

The chromosome Y sequence has the pseudoautosomal regions (PAR) hard masked. This practice is consistent with the 1000 Genome Consortium's decision to hard mask these regions in chromosome Y in order to prevent mis-mapping of reads and issues in variant calling on the gender chromosomes.

The masked Y pseudoautosomal regions are chrY:10001-2649520 and chrY:59034050-59363566. (A related file can be downloaded from **ftp://ftp.ensembl.org/pub/release-56/fasta/homo_sapiens/dna/Homo_sapiens.GRCh37.56.dna.chromosome.Y.fa.gz**.)

The following background information is from the UCSC site **http://genome.ucsc.edu/cgi-bin/hgGateway?org=human&db=hg19**

"The Y chromosome in this assembly contains two pseudoautosomal regions (PARs) that were taken from the corresponding regions in the X chromosome and are exact duplicates:

chrY:10001-2649520 and chrY:59034050-59363566?

chrX:60001-2699520 and chrX:154931044-155260560"

Chromosome M

We use the Cambridge Reference Sequence (rCRS) for chromosome M with the GenBank accession number NC_012920. Ion Reporter™ software uses the UCSC curated Refseq genes which currently do not contain chrM genes. In order to view gene annotation on chrM, use the ENSEMBL gene model which does have chrM genes.

More information is available on the web: **http://genome.ucsc.edu/cgi-bin/hgGateway?org=human&db=hg19**.

PhyloP

PhyloP filter range used in Ion Reporter™ Software corresponds to the values given in the following UCSC Table Browser website:

```
http://genome.ucsc.edu/cgi-bin/hgTables?command=start
```

The following table shows the Table Browser settings and button used to retrieve the phyloP values:

Setting	Value
clade	Mammal
genome	Human
assembly	Feb. 2009 (GRCh37/hg19)
group	All Tables
database	hg19
table	phyloP46wayPlacental
region	genome
output format	data points
file type returned	plain text
button	summary/statistics

The minimum and maximum ranges among all chromosomes that the Table Browser returned, with the rounded values used in the Ion Reporter™ Software phyloP filter, are shown below:

	UCSC Table Browser	Ion Reporter™ Software phyloP filter
Min	-13.796	-14
Max	2.941	3

Phylop score

PhyloP scores measure evolutionary conservation at individual alignment sites. The scores are interpreted as follows compared to the evolution expected under neutral drift:

- Positive scores -- Measure conservation, which is slower evolution than expected, at sites that are predicted to be conserved.
- Negative scores -- Measure acceleration, which is faster evolution than expected, at sites that are predicted to be fast-evolving.

According to the UCSC site below, phyloP scores are useful to evaluate signatures of selection at particular nucleotides or classes of nucleotides (e.g., third codon positions, or first positions of miRNA target sites).

The absolute values of phyloP scores represent -log p-values under a null hypothesis of neutral evolution.

The following range of scores is used in the Ion Reporter™ Software phyloP filter (for how this range is determined, see PhyloP in the Annotation versions and calculations section):

- Min -- -14
- Max -- 3

For more information on phyloP, see the following site. Also click the phyloP link on the left navigation panel in the website.

http://compgen.bscb.cornell.edu/phast/background.php

To find the latest UCSC Genome Browser database search for "UCSC Genome Browser update" in PubMed

PolyPhen-2 score

The PolyPhen-2 score predicts the possible impact of an amino acid substitution on the structure and function of a human protein. This score represents the probability that a substitution is damaging. Ion Reporter™ Software reports the pph2-prob PolyPhen-2 score.

The PolyPhen-2 score ranges from 0.0 (tolerated) to 1.0 (deleterious). Variants with scores of 0.0 are predicted to be benign. Values closer to 1.0 are more confidently predicted to be deleterious. The score can be interpreted as follows:

- 0.0 to 0.15 -- Variants with scores in this range are predicted to be benign.
- 0.15 to 1.0 -- Variants with scores in this range are possibly damaging.
- 0.85 to 1.0 -- Variants with scores in this range are more confidently predicted to be damaging.

PolyPhen-2 and SIFT scores use the same range, 0.0 to 1.0, but with opposite meanings. A variant with a PolyPhen-2 score of 0.0 is predicted to be benign. A variant with a SIFT score of 1.0 is predicted to be benign.

SIFT score

A SIFT score predicts whether an amino acid substitution affects protein function.

The SIFT score ranges from 0.0 (deleterious) to 1.0 (tolerated). The score can be interpreted as follows:

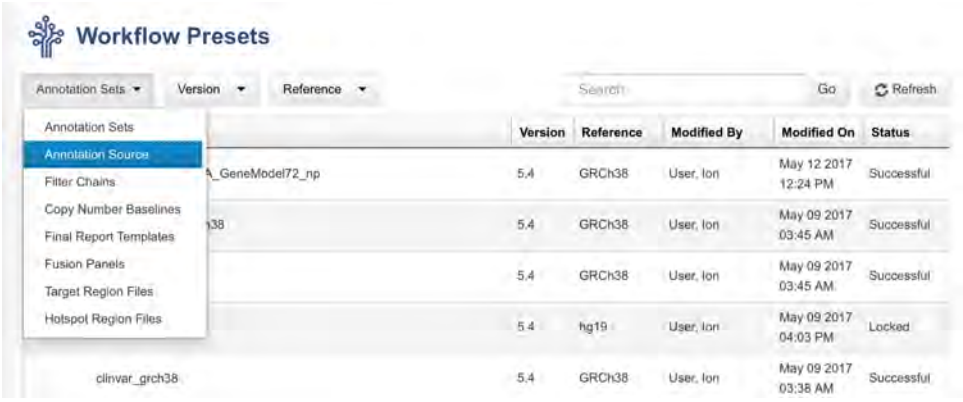
- 0.0 to 0.05 -- Variants with scores in this range are considered deleterious. Variants with scores closer to 0.0 are more confidently predicted to be deleterious.
- 0.05 to 1.0-- Variants with scores in this range are predicted to be tolerated (benign). Variants with scores very close to 1.0 are more confidently predicted to be tolerated.

Note: PolyPhen-2 and SIFT scores use the same range, 0.0 to 1.0, but with opposite meanings. A variant with a PolyPhen score of 0.0 is predicted to be benign. A variant with a SIFT score of 1.0 is predicted to be benign.

Delete custom annotation sources

Custom annotation sets created by users are displayed in **Workflow Presets** screen. An administrator can view and delete custom annotation sources created by users.

1. In the **Workflows** tab, click **Presets**.
2. Click **Annotation Sets** ▸ **Annotation Source**.



   A table that lists the custom annotation sources opens. It shows the following information about the annotation: name, version, source version, reference, created by, created on, and source type.

3. Click the custom annotation source that you want to delete in the **Workflow Presets** list, then click **Settings** (⚙) ▸ **Delete**.

   Note: If an annotation source is used in a workflow, it cannot be deleted.

   The annotation source is removed from the **Workflow Presets** list.

Preferred transcripts in default workflows

The default workflows that are provided in Ion Reporter™ Software include an annotations set with a preferred transcript set, the RefGene canonical transcripts. The default annotation set that includes the default RefSeq canonical transcripts is named **All**.

Default workflows that use the **All** annotation set report only transcripts that are included in the RefGene canonical transcripts. By default, the following RefGene canonical transcripts are included in the **All** annotation set:

- RefGene
- Functional
- Canonical
- Transcript Scores

All other transcripts are filtered out and not reported.

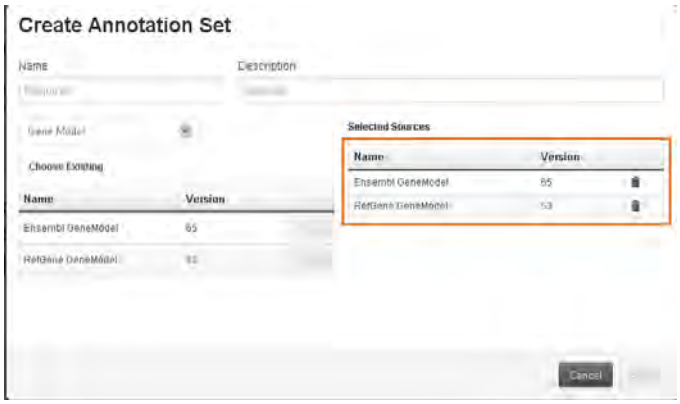
To instead have additional transcripts reported in your analyses, see "Create annotation set without preferred transcript sets" on page 348.

Create annotation set without preferred transcript sets

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset** ▸ **Annotation Set**.
   a. In the **Reference** drop-down menu, select **Gene Model** as the type of annotation source.

b. In the **Choose Existing** tab, click **Use** for either the RefSeq Gene Model or the Ensembl® Gene Model.

Note: Only one gene model can be added to each annotation set.



3. Enter a descriptive name for the custom annotation set.
4. (Optional) Enter a description for the custom annotation set.
5. Click **Save**.
   The new annotation source is listed in the **Workflow Presets** screen.

Oncomine™ Variant Annotator plugin

Ion Reporter™ Software 5.6 for Oncomine™ Assays contains the Oncomine™ Variant Annotator Plugin 2.3. The plugin enables rapid identification of driver gain-of-function or loss-of-function variants in any cancer research sample. The plugin integrates data from more than 5,300 tumor-normal exomes across 48 types of cancer. See the *Oncomine™ Variant Annotator Plugin Annotation Criteria Product Information Sheet* (Pub. No. MAN0014652) on the Thermo Fisher Scientific website for more information.

Note: Do not use the plugin for other panels.

Change amino acid code

Ion Reporter™ Software gives you the option to annotate amino acid changes with the single-letter IUPAC amino acid codes, or the three-letter codes, based on your preference. By default, the three-letter code is used. To use the one-letter IUPAC code, you can change a parameter when you edit a workflow. Then, for example, **Val600Glu** would be used in the annotation and displayed in the analysis results as **V600E**.

1. In the **Workflows** tab, select the workflow of interest, then click **Edit**.
2. Click **Parameters** in the workflow bar.

3. In the **Annotation** section, under **Use IUPAC Single Letter Code for Amino Acid**, select the option that you want to use for amino acid changes:

Option	Description
True	Select to use the single-letter IUPAC code
False	Select to use the three-letter amino acid code

4. Click **Next**.

5. Review your selected options for the edited workflow, then click **Confirm**.

Fusion analyses

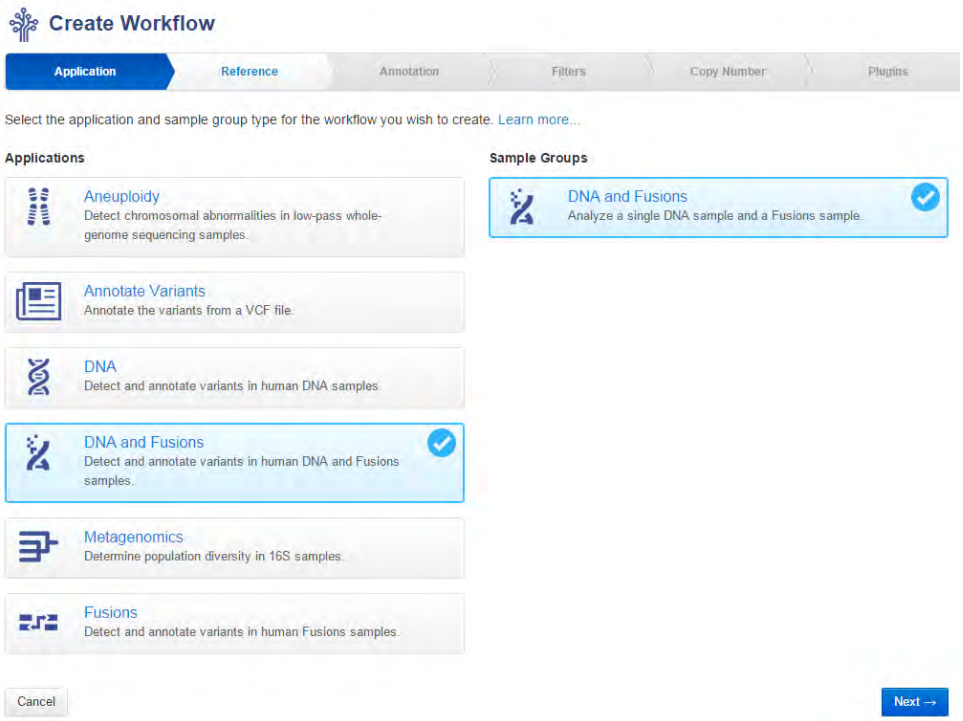
This appendix applies to analyses that are based on the following workflows and panels:

- Workflow: AmpliSeq RNA Lung Fusion single sample
- Panel: Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel
- Workflow: Ampliseq Colon Lung v2 with RNA Lung Fusion single sample
- Panels: Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2 and Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel
- Panels: Custom fusion panels designed by Ion Torrent White Glove team and Ion AmpliSeq™ Designer.
- Oncomine Fusion panels

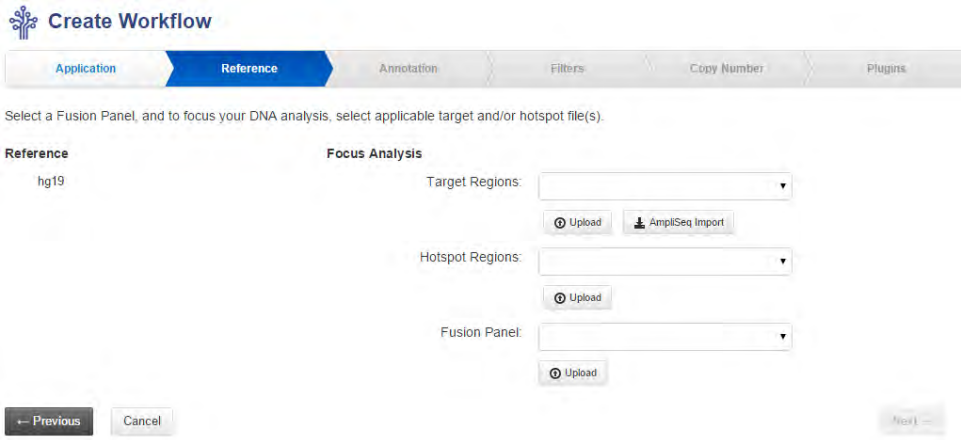
Import fusion panels from Ion AmpliSeq™ Designer

You can import custom and fixed fusion panels from Ion AmpliSeq™ Designer into Ion Reporter™ Software.

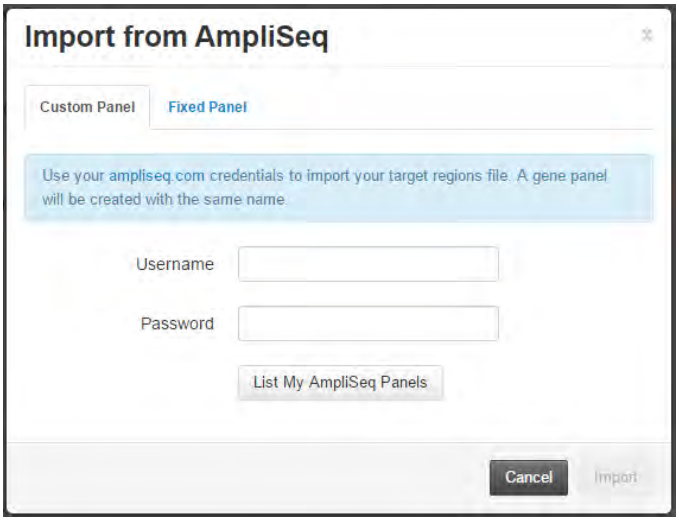
1. In Ion Reporter™ Software, go to **Workflows** ▸ **Create**.
2. Click **DNA and Fusions** and click **Next**.



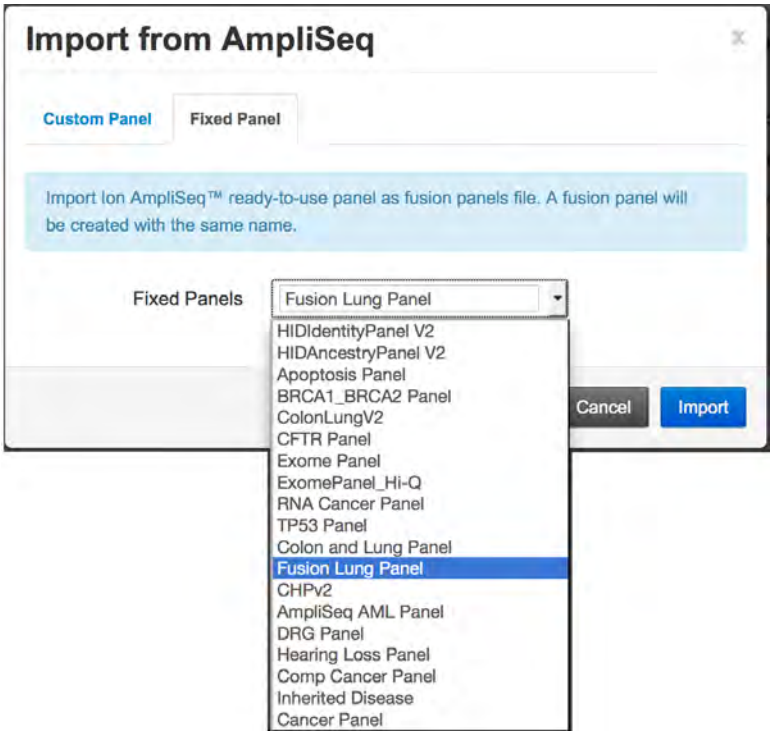
3. On the Reference page, use the AmpliSeq Import button to import your custom or fixed fusion panels and related files from Ion AmpliSeq™ Designer.



4. Enter your username and password and click **List My AmpliSeq Panels** button to access your custom fusion panels.



5. For fixed panels you do not need to enter your username and password, simply select the fixed panel of your choice.



Fusions parameters

Fusion algorithm parameters include:

Parameter Name	UI Group	Allowed Values	Default Value	Description
Sensitivity	Main	Low/Medium/High (Fixed values only one of the three can be applied)	Medium	For High value, the algorithm requires 60% overlap between reads and reference sequence with at-least 50% exact matches in the overlap. For Medium value, the algorithm requires 70% overlap between reads and reference sequence with at-least 66.66% exact matches in the overlap. For Low value, the algorithm requires 80% overlap between reads and reference sequence with at-least 75% exact matches in the overlap.
Minimum Read Counts for Fusions	Main	>=0 Integers only	20	Threshold on minimum number of valid reads aligned to specific fusion isoform sequence in order to call the isoform as Present, provided if the normalized read count is also greater than the threshold.

Parameter Name	UI Group	Allowed Values	Default Value	Description
				Example : If count of a target is >20, the target is called Present.
Minimum Read Counts for Non-Targeted Fusions	Advanced	>=0 Integers only	250	Threshold on minimum number of valid reads aligned to a non-targeted fusion sequence in order to call the fusion as Present. Example : If the Count of a non-targeted isoform is >250, it is reported as Present-non-targeted.
Minimum Read Counts for Controls	Advanced	>=0 Integers only	15	Threshold on minimum number of valid reads aligned to specific expression control sequence required to call it as Present. Example : If the read count of a expression control is >15, it is called Present.
Minimum Normalized Read Counts for Fusions	Main	>=0 Float values.	0	Threshold on minimum normalized read counts threshold required to call a fusion isoform as present.

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum Total Valid mapped reads	Main	>=0 Integers only	20000	Minimum number of total valid mapped reads required to qualify a Sample as Valid and to proceed with the analysis.
Minimum Total Control reads	Advanced	>=0 Integers only	1200	Minimum number of housekeeping control reads required to compute Imbalance scores for 5p3p Assays.
Make calls based on Imbalance Score	Main	True/False Boolean	True	If this flag is set to true, Imbalance scores are used to make Fusion presence, absence, or Nocall calls.
Maximum Imbalance for Negatives	Advanced	Text field String value in specific format as shown in the default value. Verify the user's input using a Regular expression.	ALK=0.001;RET=0.03;ROS1=0.2	If the Imbalance score of any driver gene is less than this value, the sample is called Fusion Negative for that gene.

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum Imbalance for Positives	Advanced	Text Field String value in a specific format as shown in the default value. This verifies the user's input using a Regular expression.	ALK=0.015;RET=0.55;ROS1=0.5	If the Imbalance score of any driver gene is greater than this value, the sample is called Fusion Positive for that gene. However, there is grey zone between maximum and minimum values where scores are called NOCALL. If they are equal, there is no grey zone.
Minimum Isoform Counts for Imbalance	Advanced	Text Field String value in a specific format as shown in the default value. This verifies the user's input using a Regular expression.	ALK=5;RET=5;ROS1=5	If the sum of counts from all the isoforms of that driver gene is greater than this number, thresholds set by *Maximum Imbalance for Negatives with evidence from Isoforms* and *Minimum Imbalance for Positives with evidence from Isoforms* are used for the Imbalance scores.
Maximum Imbalance for Negatives with evidence from Isoforms	Advanced	Text Field String value in a specific format as shown in the default value. This verifies the user's input using a Regular expression.	ALK=0.001;RET=0.3;ROS1=0.15	If the Imbalance score of any driver gene is less than this value, the sample is called Fusion Negative for that gene.

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum Imbalance for Positives with evidence from Isoforms	Advanced	Text Field String value in a specific format as shown in the default value. This verifies the user's input using a Regular expression.	ALK=0.01;RET=0.25;ROS1=0.5	If the Imbalance score of any driver gene is greater than this value, the sample is called Fusion Positive for that gene. However, there is grey zone between Maximum and minimum values where scores are called NOCALL. If they are equal, there is no grey zone.
Estimate max crosstalk	Advanced	>=0 (% values) Float values	0.5	Maximum percentage of spill-over reads that could be seen in any sample due to reasons like Barcode Crosstalk.

Parameter Name	UI Group	Allowed Values	Default Value	Description
Analysis configuration file	Advanced	Path to a tab separated file		A tab separated file specific to each panel that enables users to set individual target specific thresholds for the following properties, as applicable for that type: • Minimum read count • Minimum normalized read count • Minimum wild type ratio • Make calls • Do not report • Max read count negative
Keep Intermediate files	Advanced	True/False Boolean	False	Turn this flag on to keep the intermediate files generated when using the Fusions analysis.
Report non-targeted fusions	Advanced	True/False Boolean	True	If this flagged is turned off, any non-targeted fusions detected are not reported in the output VCF file.
Minimum Read Counts for Gene Expression targets	Advanced	>=0 Integers only	10	Threshold on minimum number of valid reads aligned to specific Gene Expression target in order to call it as Present.

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum Total Valid mapped reads Per Pool	Main	>=0 Integers only	0	Minimum number of total valid mapped reads in each pool (in the case of multi-pool RNA panels) in order to qualify that primer pool as Valid.
Minimum number of Valid pools for SampleQC	Main	>=1 Integers only	2	For multi-pool RNA pools, specify minimum number of pools in a Sample that have to pass QC in order to qualify that Sample as Valid and proceed with the Analysis. Example : If a panel has two pools, use value=2 to specify that both pools needs to have sufficient number of reads in order to qualify that sample. Similarly use value=1 to proceed with the analysis even if one of the pools failed.

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum mean read length for valid SampleQC	Advanced	Integers only	0	If the average read length computed from all the reads in the sample is less than the value specified, that sample is not qualified to be Valid and results are not reported. This is an additional SampleQC metric. Other QC metrics are minimum total valid mapped reads and minimum number of valid pools. For example, a recommended value is 50 bp.
Use pool Specific normalization	Advanced	True/False Boolean	True	For multi-pool RNA panels, use this flag to specify whether read counts are to be normalized to total reads in each pool separately or to total reads in the sample. This also applies to calculation of wild type ratio and normalized count within Gene metrics for RNAExonVariant targets.

Analysis configuration file for gene fusion analysis

A new input Analysis configuration file is available that enables users to set individual assay-specific thresholds for these six properties, as applicable for the type of that individual assay:

- Minimum read count
- Minimum normalized read count
- Minimum wild type ratio
- Make calls
- Do not report
- Max read count negative

The analysis configuration file is a readable and editable tab-separated file which is specific to a fusion panel. For a given panel, the file contains all the targets in the panel as different rows and has 8 columns. The first two columns are the name and type of the target in that row. The **Name** of the assay is the unique identifier and provides mapping between the analysis configuration file and the reference files that are used by the analysis. You can use the other columns to set the six different properties at individual target level.

Editable parameters in the analysis configuration file

Parameter	Description
Min Read Count	This column allows users to set custom minimum read count thresholds for different target(s). If the value is specified as "Use Global Value" for a target (the value in the row) in this column, then the global minimum read count threshold is used. (For example: 20 reads or 40 reads for Fusions as set using the minimum read count for Fusions parameter.) If a user changes the value for any target from "Use Global Value" to an allowed integer value (for example: 50), then that value overrides the global threshold. The new threshold (50 in this example) is used to call that target as Present/Absent. All other targets will still use the global threshold. This column overrides these global parameters at the individual target level (minimum read count for Fusions, minimum read count for Controls, minimum read count for Gene Expression, minimum read count for Non-Targeted fusions). This property is not applicable to 5p3pAssay type targets.

Parameter	Description
Min Normalized read count	This column allows users to set custom minimum normalized read count thresholds for different target(s). If the value is specified as "Use Global Value" for a target (the value in the row) in this column, then the global minimum read count threshold is used. (For example : 0.0 or 0.0015 reads for Fusions.) If a user changes the value for any target from "Use Global Value" to an allowed value (for example : 0.1) , then it overrides the global threshold and the new threshold (0.1 in this example) is used to call the target as Present/Absent. This column can be used to override the minimum normalized read count global parameters at individual target. This property is not applicable to 5p3pAssay type targets.
Make Calls	This column allows user to enable making Present/Absent call for RNAExonVariants at individual target level. This property is not applicable to any other type. By default the workflow parameters are set to not make calls for all the RNAExonVariants. If the value for any RNAExonVariant target (the value in the row) is changed from "Use Global value" to "True", the target will be called Present/Absent based on the read count and wild type ratio.
Min WT Ratio	This column allows users to set custom threshold for minimum wild type ratio for RNAExonVariants at individual target level.

Parameter	Description
Do Not Report	By changing the value to "True" for any target, that target is not displayed in the output files and reports.
Max Read Count Negative	This column allows users to set NoCall range for any Fusion target. The value specified in this column should be always less than or equal to the value for minimum read count as set by global parameter threshold or custom threshold for that target. For example, if a global threshold for minimum read count for Fusion is 40, and the max read count negative is set to 20 for a target (T1)_ , if the count of target T1 is between 20 and 40, it will be called as NoCall. If the count is <20 it is called as Absent; if it is >40 it is called as Present. This is not applicable to 5p3pAssay type targets. If the value for Max Read count Negative is equal to the min read count for any target, then the target is called Absent; if the read count is less than that value, it is called as Present.

Edit the gene fusion analysis configuration file

For Oncomine™ fusion workflows, a default analysis configuration file is provided. You can modify this file to set custom thresholds.

1. Sign in to Ion Reporter™ Software and click on the **Workflows** tab.
2. Search for, then select the Oncomine™ fusion workflow that contains the configuration file that you want to edit, then click **Edit** in the **Details** section.
3. Select a Research Application and Sample Group in the **Edit Workflow** screen, then click **Next**.
4. Click **Parameters** in the workflow bar, then select Fusions, then click the **Advanced** tab and scroll to the **Analysis Configuration File** section.
5. Click **Download** to download the analysis configuration file for the workflow.
6. Save the file to a local directory, then open the file and make edits to any of the following properties:
   - Minimum read count
   - Minimum normalized read count
   - Minimum wild type ratio
   - Make calls

- Do not report
- Max read count negative

See "Editable parameters in the analysis configuration file" on page 362 for more details.

7. Save the file.
8. In the **Analysis Configuration File** section of the **Parameters** workflow bar, click **Upload**.
9. Browse to the updated file and select the file, then click **Upload** in the dialog.

The updated configuration file is added to top of the drop-down list in the **Analysis Configuration File** section.

Fusion sample type

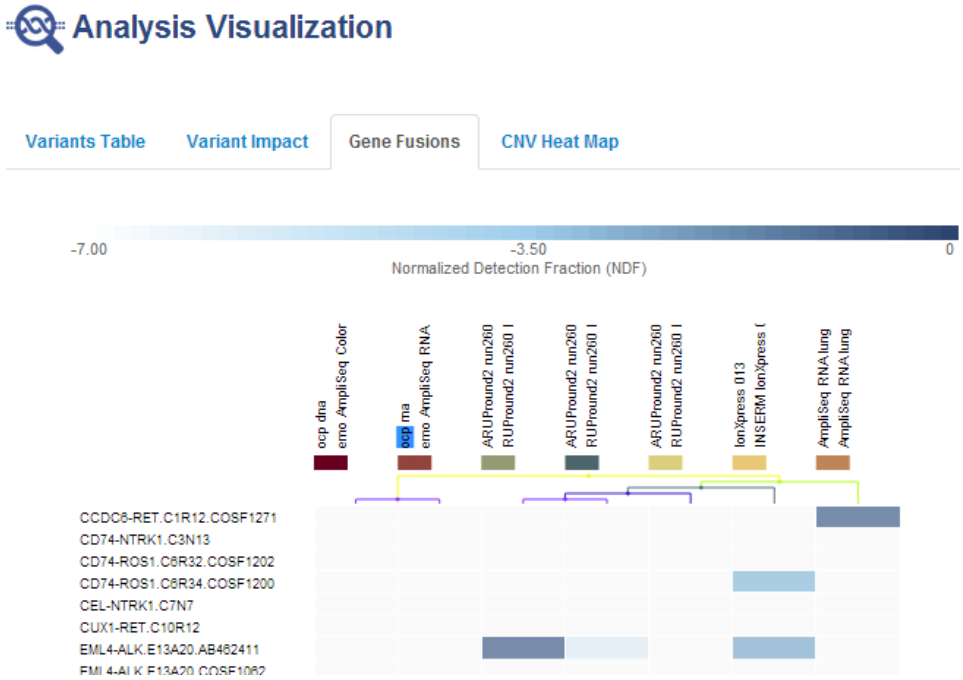
When performing a fusion analysis, the sample type attribute must be set to **RNA**. See "Import and manage samples" on page 34.

Gene fusion analysis results

Two new fields, Fusion Sample QC and Fusion Overall Call, are included in Ion Reporter™ Software.

Analysis results are available in table form:

Analysis results are also available as heat map images:



Use the following guidelines to interpret the fusion calls and other information that is presented in the Analysis Results pages. In addition to fusion type assays, the Analysis Results and Analysis Visualization present information on assays of other types:

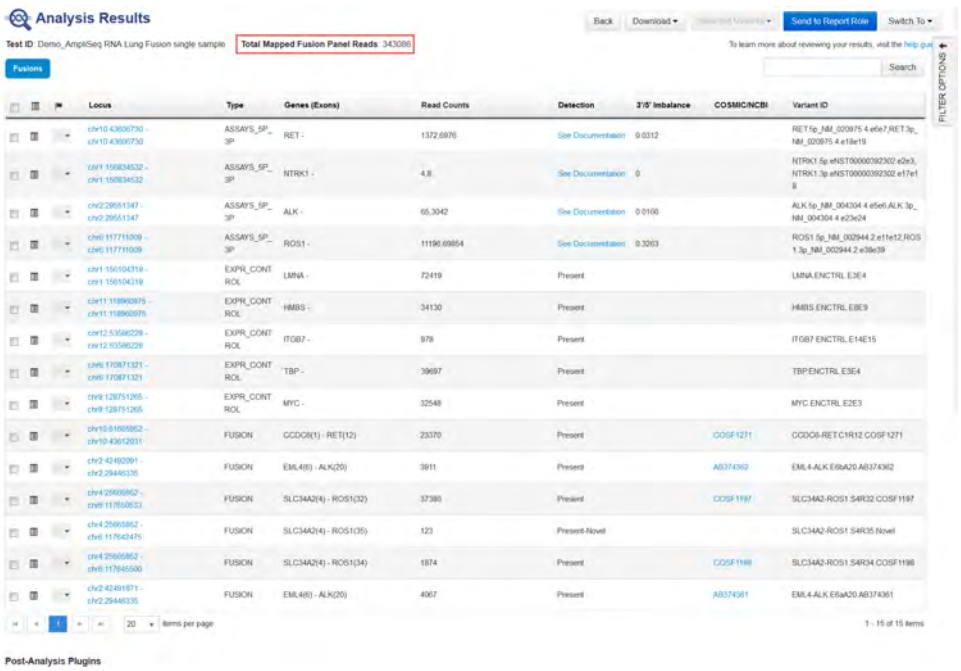
- ASSAYS_5P_3P: These assays provide confirmation for the fusion calls, through the values in the 3'/5' Imbalance column.
- EXPR_CONTROL: Few expression control assays are built into the panel to confirm the success of the sequencing run that the analysis is based on.
- GENE_EXPRESSION: These assays provide confirmation for the Gene expression amplicons that are built into the panel and their level of expression.
- RNA_HOTSPOT: These assays provide confirmation for SNPs or INDELs at a particular position (hot spot).
- RNAExonVariant : This is a new type of assay added in Ion Reporter™ Software 5.4. These assays provide confirmation for the intra-genic events like Exon Skipping deletions, alternate splice variants, expression of wild type transcripts of the gene, and so on.

Note: In the table view, consider closing the Summary Panel on the right, to provide more room for the table columns.

Fusion Sample QC Metrics:

Total Mapped Fusion Panel Reads: gene fusion Sample QC metric

The Total Mapped Fusion Panel Reads is a QC metric that is reported for gene fusion analyses.



This metric reports the sum of all reads that are valid and are assigned to any assay in the panel. Unmapped reads and poorly aligned reads are excluded from this total.

We recommend that you set a total mapped reads for the fusion assay depending upon the expected number of total reads per barcode which is based on the sequencing platform, chip type and number of barcodes per run.

For example : For a sample sequenced using PGM 318 chip multiplexed using 8 barcodes, we expect more than 100000 reads per sample. Our recommended threshold in this case is 20000 minimum total valid mapped reads.

A 20,000 minimum threshold is recommended to avoid the possibility of missing a real fusion (a false negative). 20,000 mapped reads provide acceptably sensitive fusion detection. At that coverage, fusions calls are reliable. However, a real fusion at low abundance may be missed.

Below 20,000 mapped reads, the assay may lack sensitivity, and we recommend repeating the experiment if possible (if sufficient original sample is available). In addition, the 3'/5' Imbalance number is less reliable for very low mapped reads.

The assay is highly sensitive, and if a gene fusion isoform is detected, it is highly likely to be truly present in the sequencing reads. If a fusion is detected in a sample with a low number of total mapped reads, it is highly likely to be a true positive. However, if a sample has a low number of mapped reads, a real fusion at low abundance in the sample relative to the expression control genes may be missed. The limit of detection is lower with larger numbers of mapped reads.

Minimum Mean Read length: gene fusion Sample QC metric

Mean Read Length is a QC metric available for the gene fusion analyses that was added in Ion Reporter™ Software 5.4.

If the mean read length computed from all the reads in the sample is less than the specified threshold, that sample does not pass the QC.

A 50bp minimum mean read length is recommended to avoid the possibility of missing real fusions (false negatives) and reporting false positive fusions.

Minimum total mapped reads per pool

If the panel contains multiple primer pools, it is important to compute QC metrics per each pool separately. Minimum number of total valid mapped reads per pool is an additional QC metric for RNA Fusion panels with multiple primer pools. Total valid mapped reads per pool is the count of all reads assigned to any target in each pool. If any of the pools have total valid mapped reads less than the specified threshold, that pool does not pass QC.

Minimum number of Valid pools: gene fusion Sample QC metric

For multi-pool RNA pools, you can specify the minimum number of pools in a Sample that are required to pass QC to qualify the Sample as Valid, then proceed with the Analysis. If the number of valid pools in any sample is less than the specified threshold, that Sample does not pass QC. Valid pools are determined by the total valid mapped reads per pool.

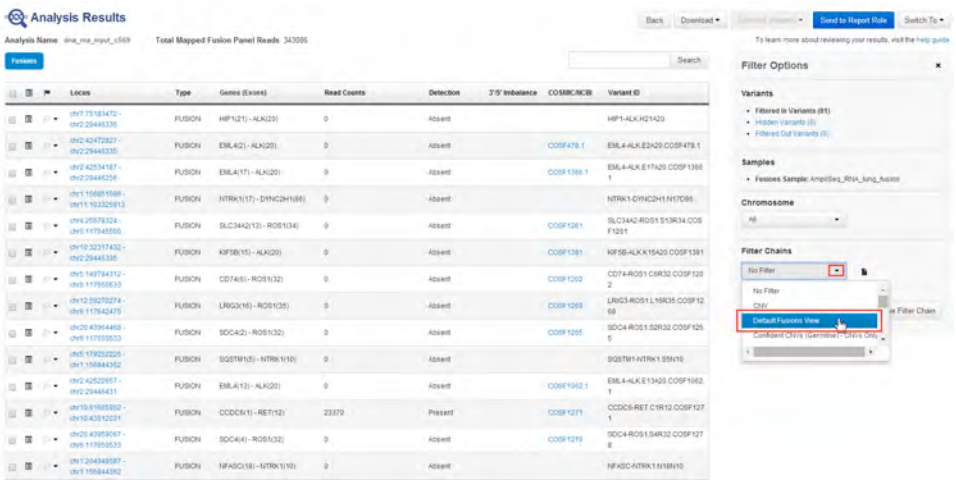
Example: If a panel has two pools, use `value=2` to specify that both pools need to have a sufficient number of reads in order to qualify that sample. Similarly use `value=1` to proceed with the analysis even if one of the pools failed.

Filters

For analyses from the AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample workflow, the first time the Analysis Results page Fusions tab is opened for an analysis, it opens with no filter applied. This view shows targeted fusions that are not found in your sample (and are reported as Absent in the Detection column), as well as other variant types.

For a view that includes only Present fusion calls:

1. Click the **Filter** menu.



2. Select the **Default Fusions View** filter.

Note: The following will always be displayed as Filtered in Variants in the **Type** column, whether the **Default Fusions View** filter is set to Present or Absent:

- EXPR_CONTROL
- ASSAYS_5P_3P
- RNA_HOTSPOT
- GENE_EXPRESSION

Expression controls

A limited number of expression control genes are built into the Ion AmpliSeq™ panel for quality checking purposes. If multiple EXPR_CONTROL calls are reported as Absent in the Detection column, check the Torrent Suite™ Software run report for the sequencing run, as this result could indicate a low-quality run or failed amplification.

FusionSampleOverallCall

The FusionSampleOverallCall allows the user to confirm whether a sample is a No Call, Positive, or Negative. Here is the logic applied:

Fusion Isoforms	RET/ROS1/ALK Imbalance Score	Final Call
Positive	Positive	Positive
Positive	No Call/Negative	Positive
Negative	Positive	Positive
Negative	Positive	Positive
Negative	No Call	No Call
Negative	Negative	Negative

Fusion calls

Fusion calls describe a translocation of genetic material. The Genes (Exons) column reports the donor gene and the partner gene. The exon number for each gene is reported in parentheses. For the donor gene (the first one in the pair), exon number specifies the exon *before* the fusion. For the partner gene (the second one in the pair), exon number specifies the exon *after* the fusion.

For example, in the second row, the Genes (Exons) column reports EML4(6) - ALK(20):

- EML4(6) refers to exon number 6 on the EML4 gene (the donor gene in this example). Exon number 6 in the donor gene indicates that the fusion starts after exon 6.
- ALK(20) refers to exon number 20 in the ALK gene (the partner gene in this example). Exon number 20 in the partner gene indicates that the fusion starts before exon 20.

The Detection column reports whether the fusion meets the required threshold of greater than 20 reads:

- **Fail**: Either no evidence for the fusion is found or fewer than 21 reads support the fusion.
- **Pass**: Greater than 20 reads provide evidence for the fusion.
- **Present-Non-Targeted**: Greater than 250 reads provide evidence for a fusion that is not explicitly included in the Ion AmpliSeq™ panel.
- **No Call**: Not enough evidence to determine if a fusion is present or not.

"Non-targeted" fusion calls

In some samples, non-targeted fusions may be reported, with the designation Present-Non-Targeted in the Detection column. A non-targeted fusion is a fusion for which the panel did not contain a specific primer pair, but it was picked up by a combination of two of the primers used for two different targeted isoforms. The fusion was not among the targeted isoforms and was not explicitly targeted by the panel. If there are a small number of reads providing evidence for such fusions (for example, less than a few hundred reads), these may not be important. If there is very strong evidence for non-targeted fusions, they may be worth following up with more detailed investigation. A literature search or a search of COSMIC or other databases may be helpful in determining whether such fusions have ever previously been observed, and may provide guidance in interpretation.

Multiple fusions in the same gene

Occasionally, when a true positive fusion in a gene pair is detected, a second fusion in the same gene pair may also be reported. Generally, a single fusion has occurred in this pair of genes in this sample, and all the read evidence that covers any exon is reported, which may occasionally be shown as a second fusion, usually describing a different exon in one of the genes. This second fusion may be described as either a targeted fusion (i.e., the panel has a specific assay designed to detect this fusion, so it is designated Present in the Detection column) or a non-targeted fusion (detected, but the panel has no specific assay pair designed for this fusion, so it is designated Present-Non-targeted in the Detection column). Biologically, both of these calls are likely from the same underlying fusion in the gene pair.

Other information reported for fusions:

- The Locus column reports the start and end positions of the fusion transcript.
- The Read Counts column reports the number of reads that provide evidence for the fusion call. For ASSAYS_5P_3P, read counts are displayed in the order 5', 3'.
- The COSMIC/NCBI column provides links at which the fusion is described in these public web sites.
- The Variant ID column reports our internal short-hand for the fusion.

Barcode CrossTalk QC

This module analyzes the reads from all the barcodes on a chip from one run and determines if there are any reads in a particular barcode that could belong to any of the barcodes.

For example, if barcode manufacturers provide us a QC threshold of 0.5%, i.e., there is a chance that up-to 0.5% of the reads in any one barcode (say barcode7) could be mislabeled as that barcode (barcode 7) where in reality those reads are from a different barcode (say barcode 8). Usually for many applications this is not important. But this is very important for applications for like Fusions which require high sensitivity.

For example, if barcode 8 is positive for a fusion involving ROS1 gene with read_count = 100000.

If barcode 7 also has ROS1 read_count = 30. (30 is less than 0.5% of 100000)

Current Fusions algorithm will call both barcode 7 and barcode 8 as positive, because the read count in both these samples is >20 (our default threshold).

Barcode 7 is false positive and barcode 8 is true positive.

Barcode crosstalk is not the only source of the contamination, these types of reads could also be seen due to sample-level contaminations as well.

In order to identify these reads, this module needs to look at the reads from all barcodes. The maximum estimated percentage of crosstalk is by default 0.5%, but there is a parameter exposed in user interface that users can change.

This module generates as qcInfo file per barcode and summary file for the entire chip. These files are generated before launching Fusions calling module on any one of the barcodes and are passed to the Fusions module.

Partner genes in the Ion AmpliSeq™ RNA Fusion panels

This panel contains the following four acceptor (driver) genes (in addition to many donor genes):

- ALK
- NTRK1
- RET
- ROS1

  In analyses that are based on this panel, fusion calls report other genes targeted in this panel that fused to these partner genes.

RNAExonVariant

RNAExonVariant assays provide measurement of expression for Intragenic events like Exon Deletions, Exon skipping events, Alternate Splice transcripts and Wild type transcripts. For these assays, two additional metrics are reported (including read count, normalized read count, RPM, and so on):

- **Ratio to Wild Type**
  - For all the RNAExonVariant assays that belong to a Gene with at-least one RNAExonVariant type assay. It is designed to amplify the wild type transcript of that gene and report this value.
  - Ratio to Wild type of a RNAExonVariant Vi = (Read count of Vi) / (Mean read count of all Wild Type RNAExonVariants for that gene)
- **Norm Count within Gene**

  For all the RNAExonVariant type assays, this normalized count is reported.

  Norm count within Gene for RNAExonVariant Vi = (Read Count of Vi) / (Sum of read counts of all RNAExonVariants of the same Gene).

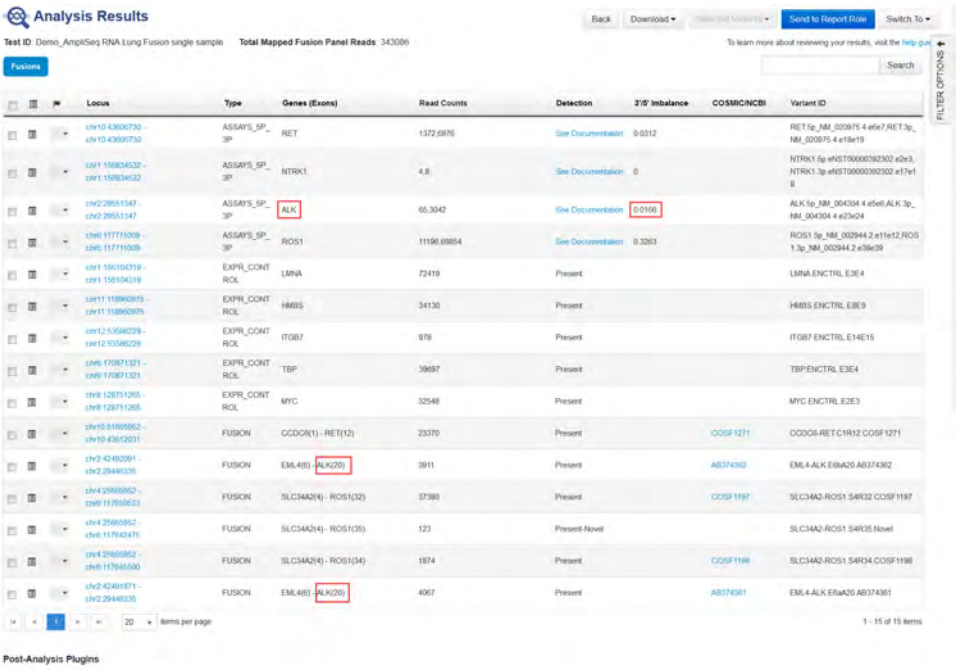
  Detection column for RNAExonVariant will have " N/A " as the value for all the RNAExonVariants.

Assays_5P_3P

5' and 3' assays provide confirmation for the fusion calls. This score is reported in the **3'/5' Imbalance** column.

Four assays are built into the Ion AmpliSeq™ RNA fusion panels. Each assay corresponds to one of the acceptor genes in the panel, and that corresponding acceptor gene is reported in the **Genes (Exons)** column.

For example, the assay reported in the third column names ALK in the **Genes (Exons)** column. The 3'/5' Imbalance score for this assay reflects the strength of the calls for all fusions with the ALK driver gene (in rows 10 through 15 in this example).



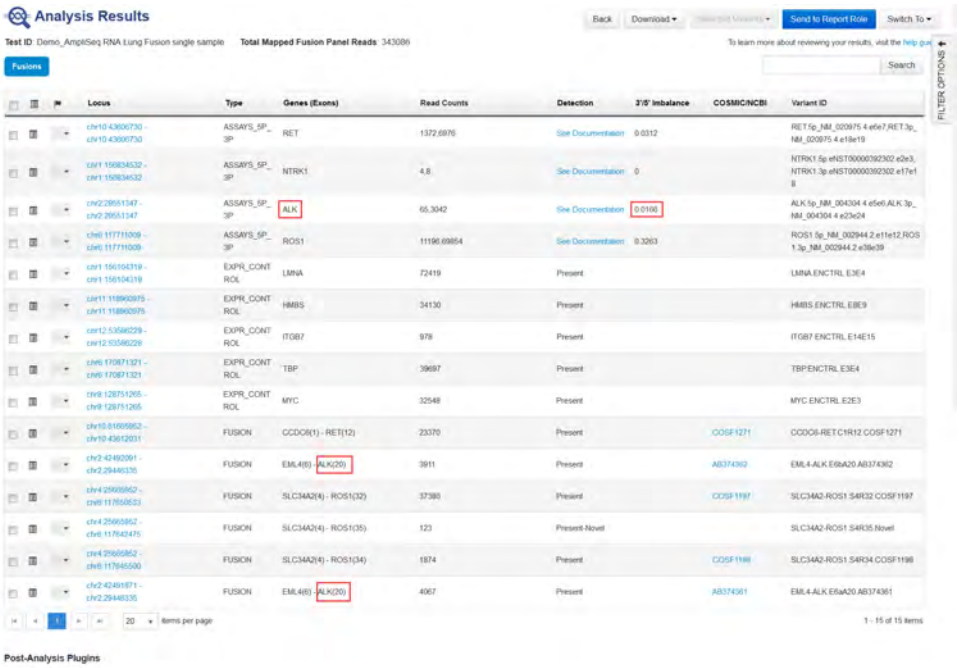
(The **Genes (Exons)** column does not report exon numbers for assays rows.)

Imbalance values for assay calls

The Imbalance value is a metric that we calculate to provide a measurement of the strength of fusion calls that involve ALK, RET, or ROS1 driver genes.

We investigated several metrics and found Imbalance values to be a strong predictor of true fusions calls. We continue to search for predictors for fusions that involve NTRK1 driver genes.

Imbalance values are reported in the 3'/5' Imbalance column. An assay's Imbalance value applies to all fusion calls with the driver gene that is reported in the assay's Genes (Exons) column.



An Imbalance value is calculated by subtracting the number of 5' reads from the number of 3' reads, and dividing the result by the sum of all EXPR_CONTROL reads:

- (3' reads - 5' reads) / (sum of all EXPR_CONTROL reads)

  Note: The column header "3'/5' Imbalance" in the Analysis Results pages implies that the Imbalance value is a direct ratio of the 3' and 5' reads. The value is not a direct ratio of 3' and 5' reads.

- Imbalance values are interpreted as follows:
  - **Higher Imbalance values** (0.025 or greater) indicate a greater likelihood that the fusion *is* present in your sample.
  - **Lower Imbalance values** (either close to zero or negative) indicate a greater likelihood that the fusion *is not* present in your sample.

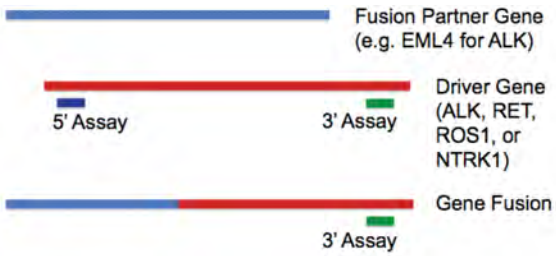
  Note: Imbalance values provide information that is supplementary to the Detection calls of "Present" or "Absent". If a fusion call is "Absent", then we recommended that you check the Imbalance value to see if there is supplemental evidence of the presence of a fusion.

Interpret 3'/5' Imbalance values in Ion Reporter™ Software

The 3'/5' Imbalance values reporter in Ion Reporter™ Software generates analysis results for Ion AmpliSeq™ gene fusions, such as the Ion AmpliSeq™ RNA Lung Fusion workflow. The imbalance values reporter measures the difference in expression between the 5' assay and the 3' assay of each driver gene. Samples that do not contain a fusion are expected to have similar expression of the 5' assay compared to the 3' assay of the driver gene. Samples that contain a fusion are often expected to have elevated expression of the 3' assay compared to the 5' assay (see the following figure). The 3'/5' Imbalance assay is therefore included for two alternate purposes:

The 3'/5' Imbalance values provide a measurement of the strength of fusion calls that involve ALK, RET, or ROS1 driver genes, whether included or not included in the Ion AmpliSeq™ RNA Fusion panel.

1. To confirm presence of a fusion from the ALK, RET, or ROS1 driver genes included in the panel, or



2. If none of the fusions targeted by the panel is detected, to provide evidence of a fusion other than those targeted by the panel but still including ALK, RET, or ROS1 driver genes.

The following approaches interpret the 3'/5' Imbalance values for each gene are reliable only under the conditions that are described in the following sections: *Read number impact on calculation sensitivity*, Sensitivity, and *High 5' expression*. Most samples that are tested for the 3'/5' Imbalance assay have been lung tumor tissue. Threshold settings for other sample types can be sensitive to varying expression of fusion gene and expression control genes.

Note: We currently do not have sufficient data to determine if the 3'/5' Imbalance value is a predictor for the NTRK1 gene.

Gene	No Evidence of a Fusion	Uncertain	Strong Evidence of a Fusion
ALK	⩽0.001	0.001-0.015	⩾=0.015
RET	⩽0.03	0.03-0.55	⩾=0.55
ROS1	⩽0.2	0.2-0.5	⩾0.5

For Oncomine Focus Panel, the following table lists the imbalance score thresholds:

Gene	No Evidence of a Fusion	Uncertain	Strong Evidence of a Fusion
ALK	⩽0.001	0.001-0.0015	⩾=0.0015
RET	⩽0.03	0.3-0.55	⩾=0.55
ROS1	⩽2.1	2.1-2.1	⩾2.1

Imbalance value calculation and predictions

Note: The label 3'/5' Imbalance in the Analysis Results pages relays that the imbalance value is a direct ratio of the 3' and 5' reads.

A 3'/5' Imbalance value is calculated by subtracting the number of 5' reads from the number of 3' reads, and dividing the result by the sum of all EXPR_CONTROL reads:

(3' reads - 5' reads)/(sum of all EXPRE_CONTROL reads)

We find that imbalance values are a good predictor of the presence of a fusion:

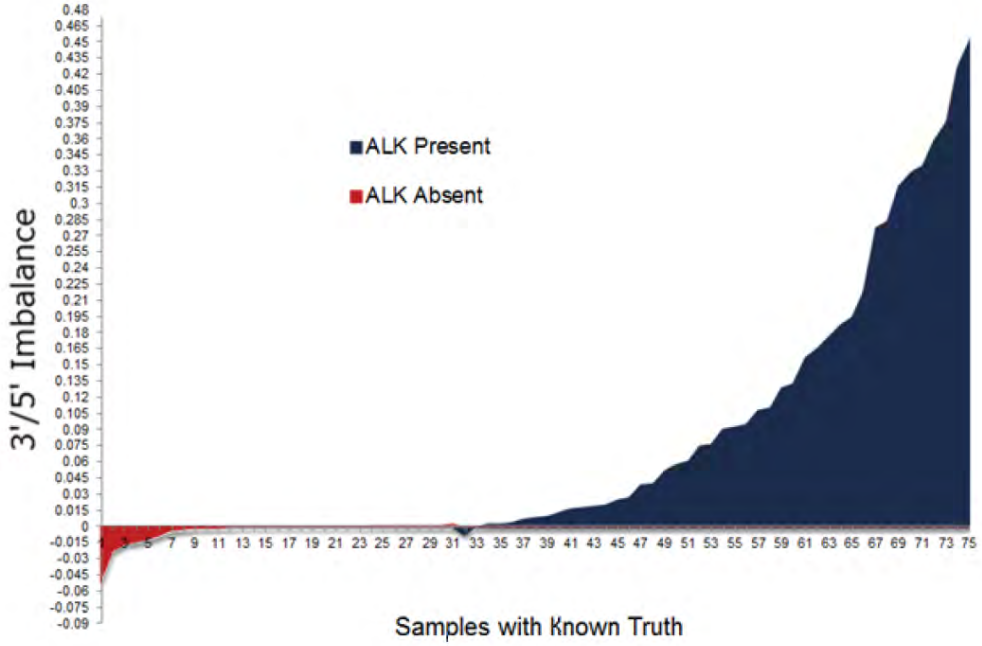
- In normal samples (without a fusion), imbalance values are very low (either close to zero or negative).
- In samples that contain a fusion, imbalance values are higher, with gene-specific thresholds shown in the above table.

In some cases, lower imbalance values are also good predictors of the presence of fusions, as shown in the plots linked below.

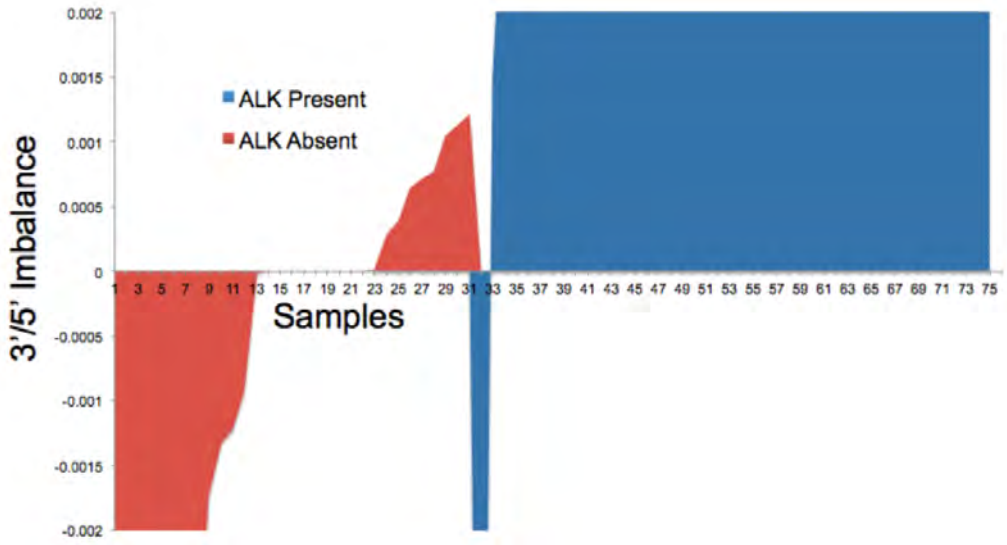
Note: These guidelines apply to samples that meet the criteria described in the sections: Minimum number of reads, Sensitivity, and High 5' expression. The values in the plots below have been based on results obtained from 75 FFPE lung cancer research samples previously tested with other technologies like FISH, IHC, and RT-PCR.

Example plot for ALK driver gene

The image linked here shows the correlation between low imbalance values and samples that do not contain a fusion (in red). Samples that contain a fusion (in blue) all contain higher imbalance values.



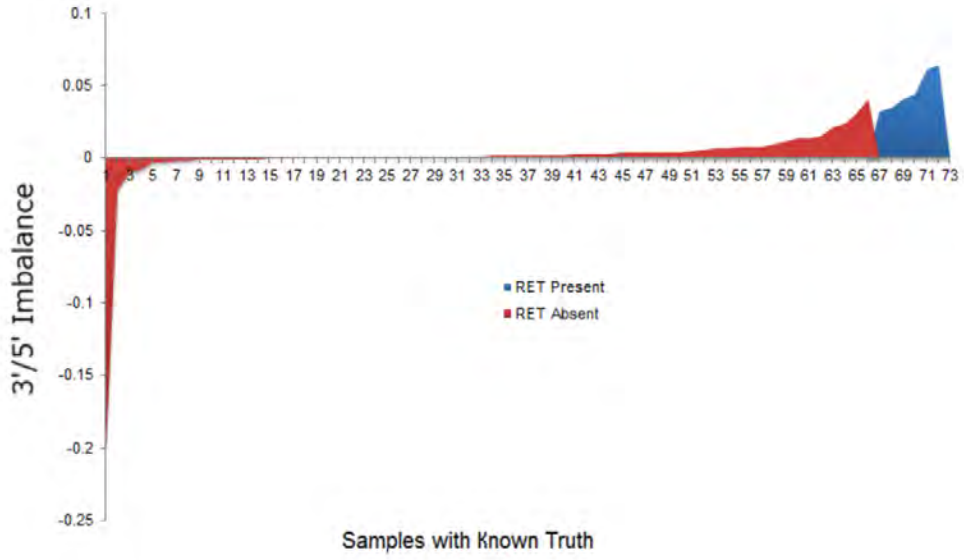
Zoomed in view:



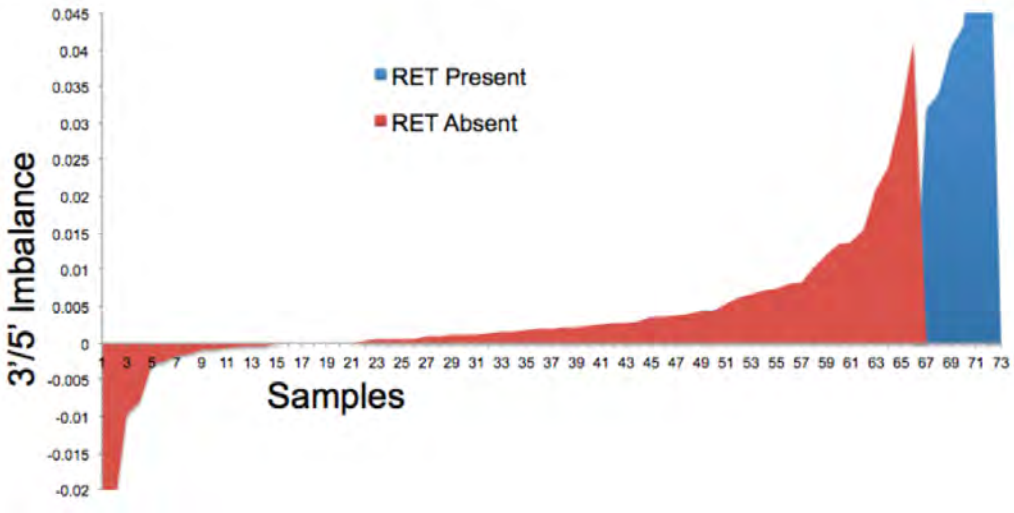
- A 3'/5' Imbalance value of 0.025 or greater indicates the likely presence of an ALK fusion.
- Below 0.001, there is no evidence that an ALK fusion is present.
- The range of 3'/5' Imbalance values between 0.001 and 0.025 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance values, the more evidence there is that a fusion is present.

Example plot for RET driver gene

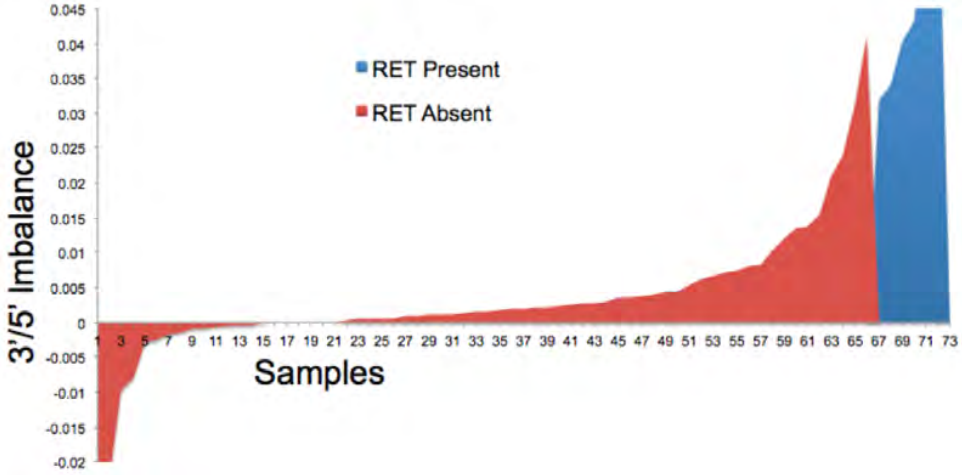
The image linked here shows the correlation between low imbalance values and samples that do not contain a fusion (in red). Samples that contain a fusion (in blue) all contain higher imbalance values.



Zoomed in view:



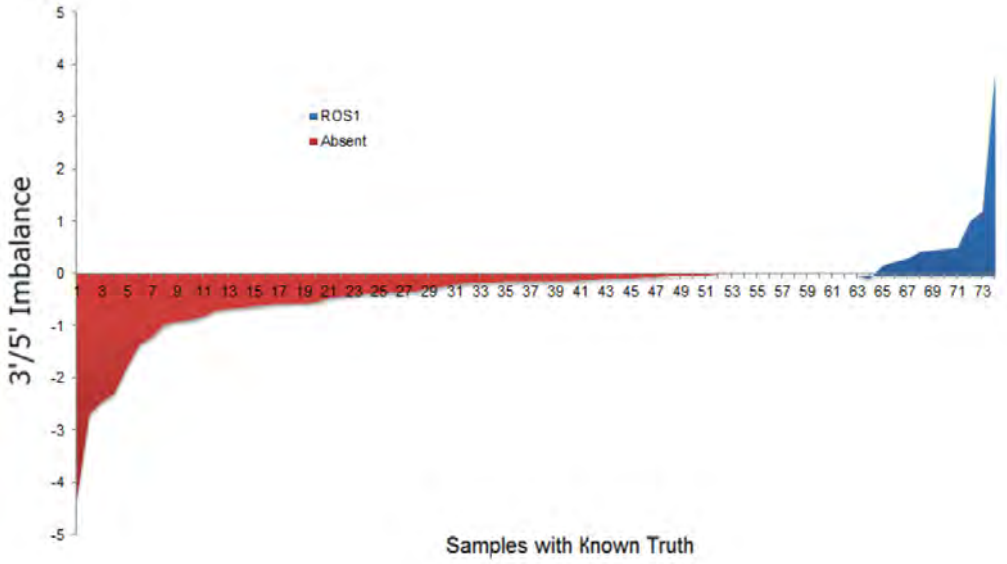
- A 3'/5' Imbalance value of 0.045 or greater indicates the likely presence of a RET fusion.



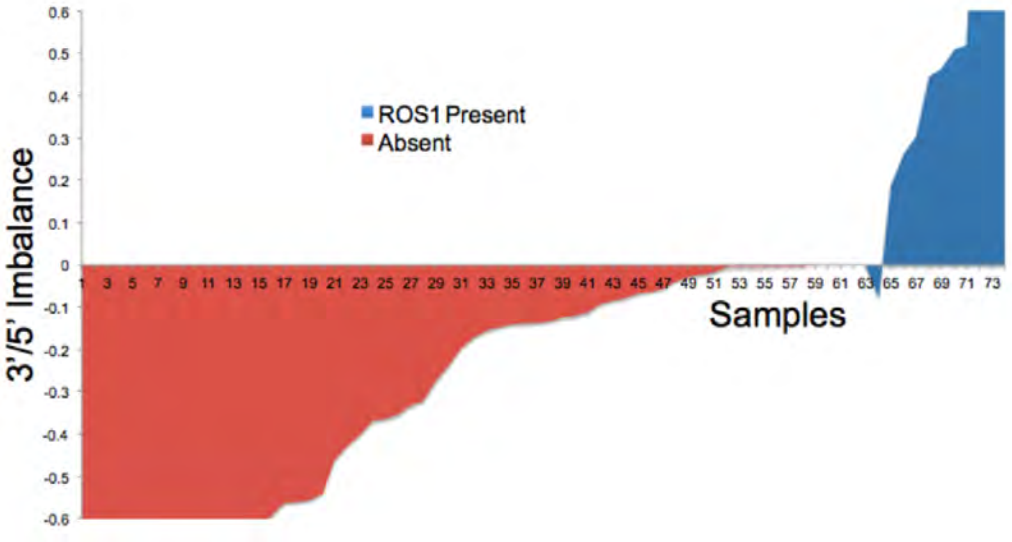
- With a 3'/5' Imbalance value of 0.03, there is no evidence that a RET fusion is present.
- The range of 3'/5' Imbalance values between 0.045 and 0.03 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance values, the more evidence there is that a RET fusion is present.

Example plot for ROS1 driver gene

The image linked here shows the correlation between low imbalance values and samples that do not contain a fusion (in red). Samples that do contain a fusion (in blue) all contain higher imbalance values.



Zoomed in view:



- A 3'/5' Imbalance value of 0.5 or greater indicates the likely presence of a ROS1 fusion.
- With a 3'/5' Imbalance value of 0.2, there is no evidence that a ROS1 fusion is present.
- The range of 3'/5' Imbalance values between 0.2 and 0.5 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance value, the more evidence there is that a ROS1 fusion is present.

Read number impact on calculation sensitivity

- At above 150,000 mapped reads, the workflow is very sensitive.
- Between 20,000 and 150,000 mapped reads, the more reads, the more sensitive the workflow is.
- With 20,000 or few mapped reads, we recommend that you rerun the sequencing experiment, if possible. At around 20,000 or fewer reads, the potential for false negatives (missing a real fusion) increases.

Sensitivity

The workflow is very sensitive for fusion detection and can possibly pick up noise if there are problems with, for instance, the sample or library preparation.

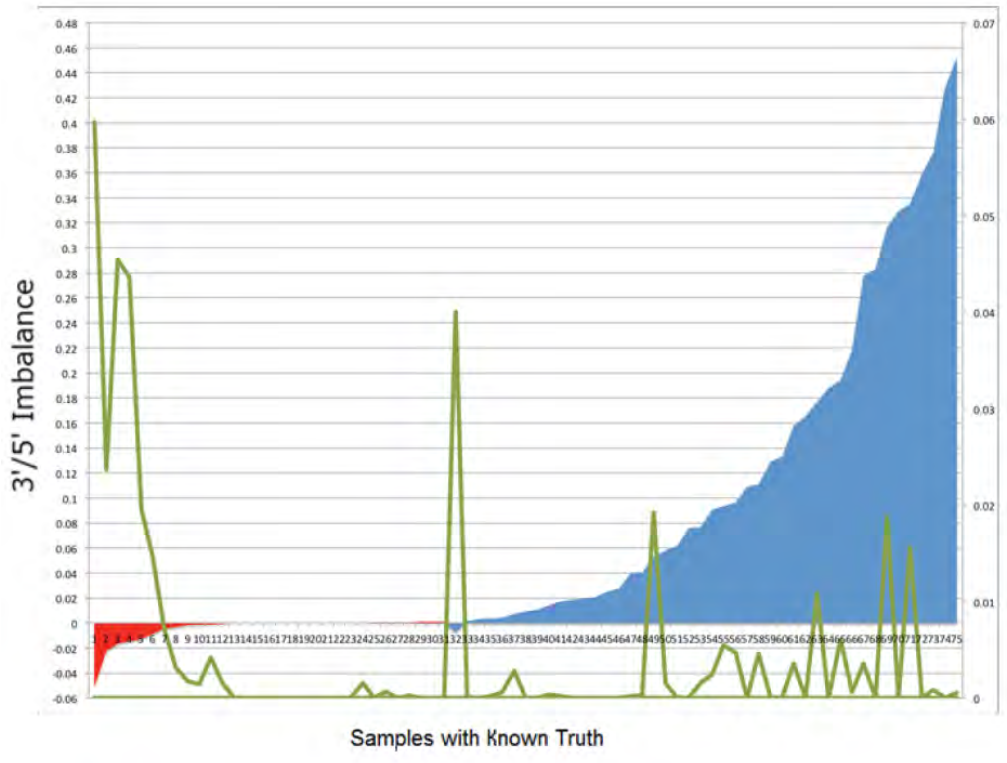
Please check for High 5' expression and also check for the Minimum number of reads before assuming that a fusion call of 'Present' is confirmed.

High 5' expression

If the 5' read count is very high compared to the EXPR_CONTROL read count, the 3'/5' Imbalance value is not a reliable predictor. With high 5' read counts, the potential for false negatives (missing a real fusion) increases.

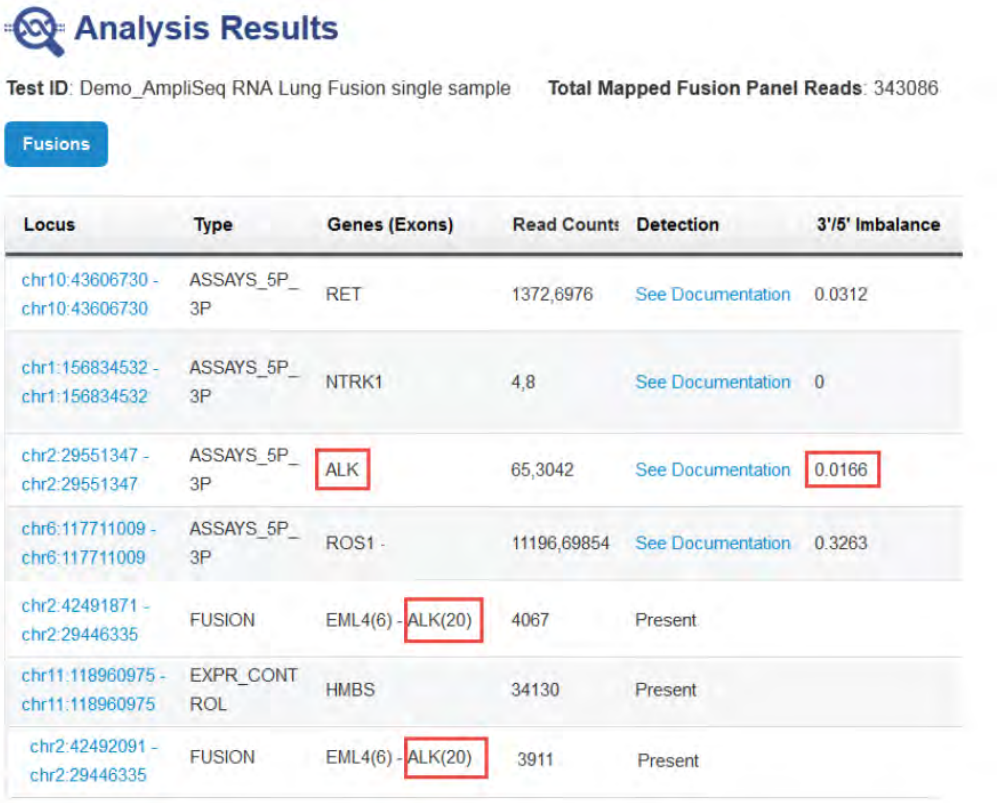
The plot linked here shows a false negative in sample 32. The green line tracks the 5' expression as 5' read counts divided by EXPR_CONTROL read counts. Note the spike of high 5' expression at the false negative for sample 21.

We have not seen a high 5' expression to be associated with a false positive.



How do I find the 3'/5' Imbalance value for a specific fusion?

Imbalance values are reported for ASSAY_5P_3P calls. The ASSAY_5P_3P's Imbalance value applies to all fusions with the driver gene that appears in the ASSAY_5P_3P's Genes (Exons) column:



Variant table information

Detection column and detection thresholds

The Detection column reports Present for calls that are supported by read evidence. The threshold for read evidence support varies by call type:

- **Fusions**: Greater than 20 supporting reads are required to report Present.
- **Expression controls**: Greater than 15 supporting reads are required to report Present.

The Detection column reports the following values:

- **Absent**: Either no evidence for the call is found or fewer than the minimum threshold number of reads support the call.
- **Present**: Greater than the minimum threshold number of reads provide evidence for the call.
- **Present-Novel**: Greater than the minimum threshold number of reads provide evidence for a fusion call that is not explicitly included in the Ion AmpliSeq™ panel.

Variant ID column

The variant ID column reports our shorthand identifier for the fusion variant (or assay or expression control). Each target in the Ion AmpliSeq™ panel has a unique variant ID.

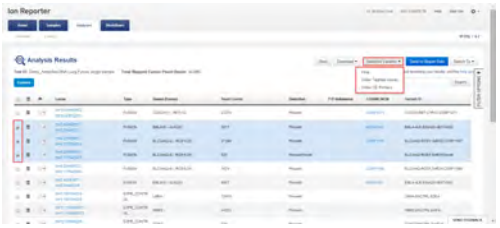
An identifier contains information that is available in other fields in the results table.

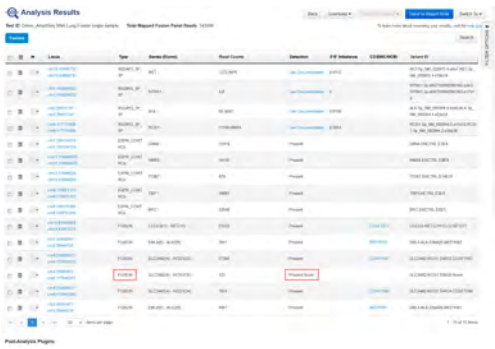
Information	Description, example
Partner gene name	ALK
Gene and exon number	A13, for exon number 13 in the ALK gene
COSMIC ID, NCBI GenBank ID	COSF1198, AB374361
Non-Targeted	Appended to the Variant ID for fusion calls that are not targeted by the Ion AmpliSeq™ panel. Example: SLC34A2-ROS1.S4R35.Non-Targeted

Examples of Variant IDs for fusion calls (these examples are from demo data):

- **EML4-ALK.E6bA20.AB374362**:
  - **EML4**: Donor gene
  - **ALK**: Partner gene
  - **E6**: Exon number 6 in the ELM gene ("E" for "ELM")
  - **b**: The second fusion (ordered by locus) with the same value in the Genes (Exons) column
  - **A20**: Exon number 20 in the ALK gene ("A" for "ALK")
  - **AB374362**: GenBank ID
- **SLC34A2-ROS1.S4R35.**: Non-Targeted
  - **SLC34A2**: Donor gene
  - **ROS1**: Partner gene
  - **S4**: Exon number 4 in the SLC34A2 gene ("S" for "SLC34A2")
  - **R35**: Exon number 35 in the ROS1 gene ("R" for "ROS1")
  - **Non-Targeted**: Not included in the Ion AmpliSeq™ panel

Descriptions of the variant table for fusion analyses

Column	Description
Checkbox	Enable checkboxes of variants, then use the **Selected Variants** menu to hide those variants or to order TaqMan® assays or CE primers for those variants. 
Variant Detail icon	Opens the Variant Detail popup for the variant (or assay or expression control).
My Variants flag	Not used (the My Variants annotation source does not apply to fusion analyses).
Locus	The start and end points of the variant (or assay or expression control).
Type	Fusion call or other panel target.
Genes (Exons)	For fusion calls, reports the donor and acceptor genes (in that order, separated by a hyphen). The exon number for each gene is given in parentheses. For expression controls and 5' and 3' assays, the acceptor gene is reported.
Read Counts	For fusion calls and expression controls, reports the number of reads that support the call. For 5' and 3' assays, reports the separate reads counts for the 5' assay and the 3' assay (in that order, separated by a comma).

Column	Description
Detection	For fusion calls and expression controls, uses Present to report that the expected variant or control is found in your sample, or reports Absent when the expected variant or control is not found. Fusion variants that are called in your sample but not explicitly included in the Ion AmpliSeq™ panel are reported as Present-Novel. See also Detection column and detection thresholds -- minimum read thresholds are different for fusion calls and for expression controls. 
3'/5' Imbalance	For assay calls, reports a measurement of the strength or quality of the related fusion calls.
COSMIC/NCBI	For fusion calls, reports the associated COSMIC ID or NCBI GenBank ID (if any).
Variant ID	Reports the short-hand identifier for the variant (or assay or expression control).

Interpret a fusion call

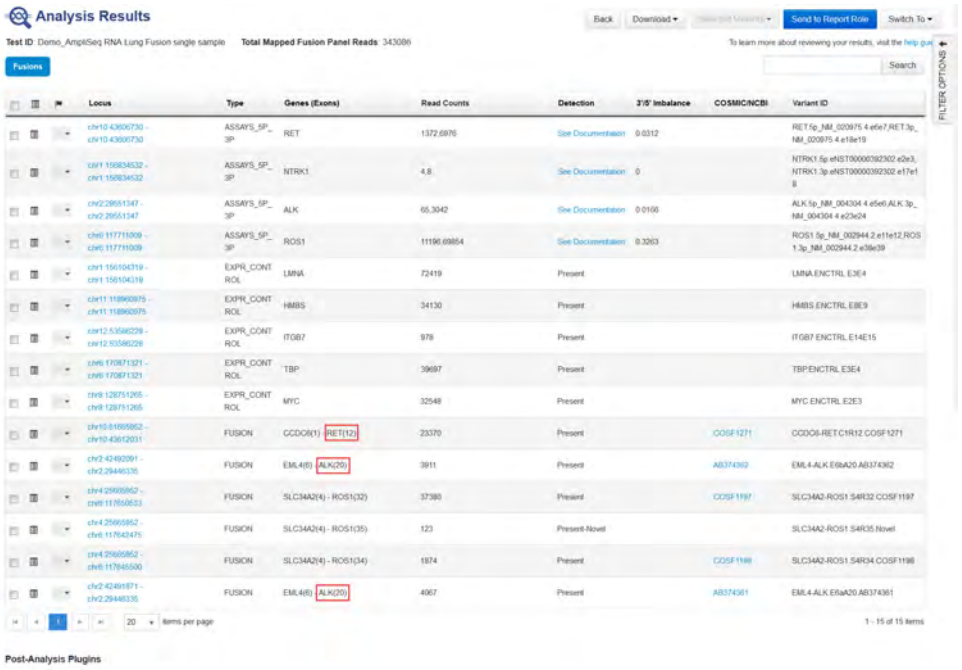
The analysis results provide several ways to confirm a fusion call, including Imbalance values, visualization, and Normalized Detection Fractions.

Imbalance values

Imbalance values are good predictors of fusion calls that involve ALK, RET, or ROS1 driver genes. We have not found Imbalance values to be predictors of fusion calls that involve a NTRK1 driver gene.

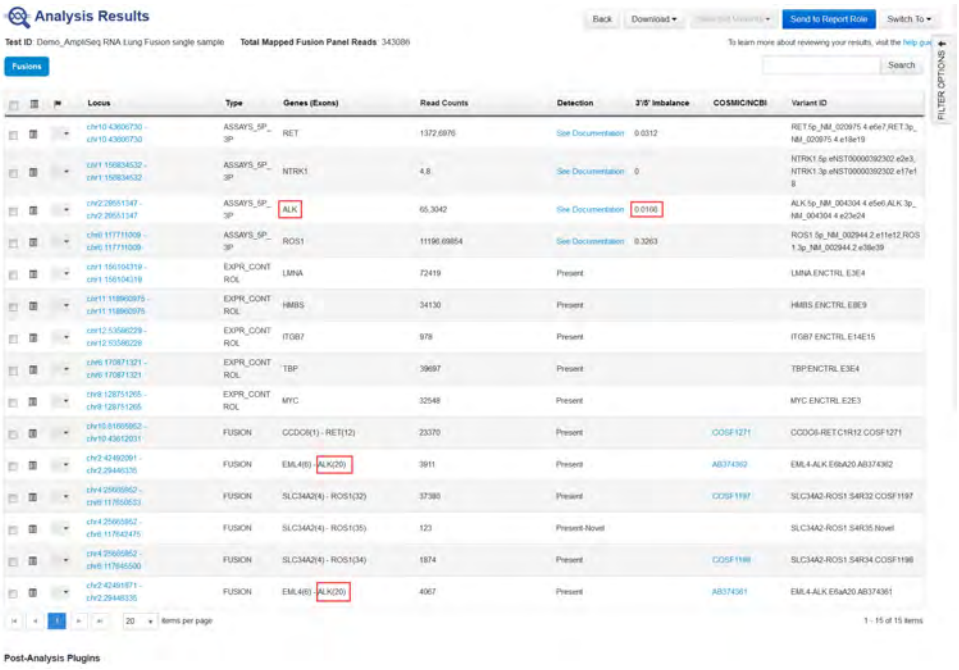
Follow these steps to find the Imbalance value for a specific fusion call:

1. Find the fusion's driver gene (the second gene listed in the Genes (Exons) column.



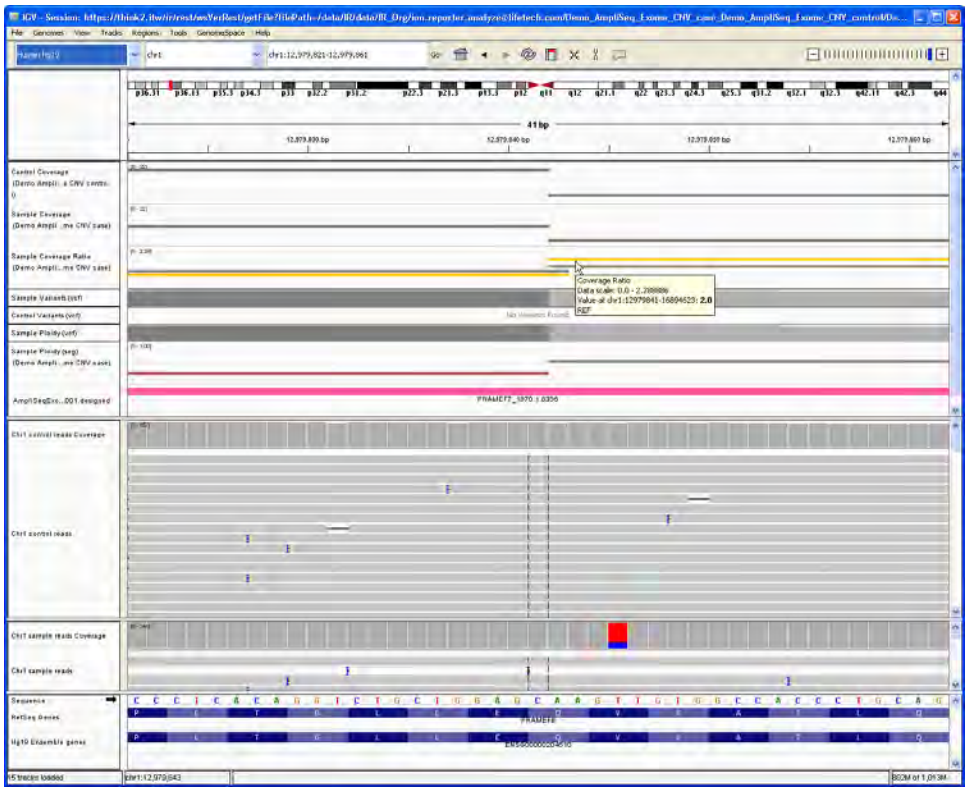
2. Find the ASSAYS_5P_3P entry that reports that driver gene in its Genes (Exons) field.

3. Use the Imbalance value for that ASSAYS_5P_3P entry to interpret the fusion calls for all fusions whose driver gene matches the ASSAYS_5P_3P's Genes (Exons) field.



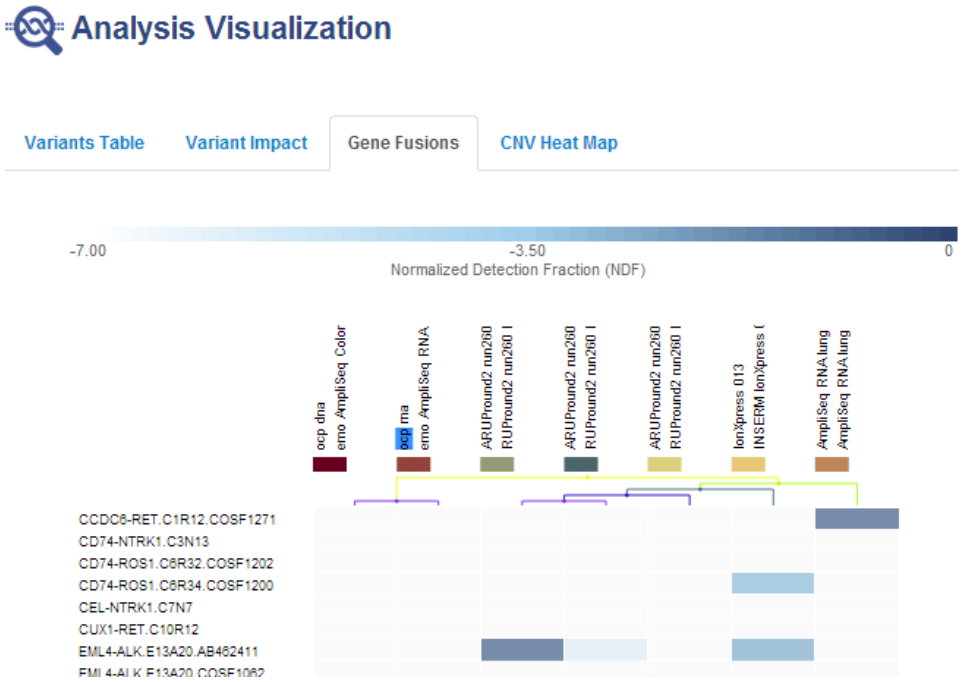
Visualize with IGV

Use IGV to evaluate both fusion calls and the expression controls.



Visualize with heatmaps

Use heatmaps to see a one-page comparison of fusion calls across multiple analyses.



Normalized Detection Fractions (NDF)

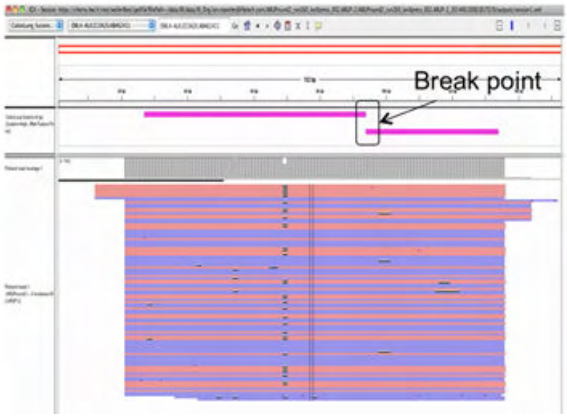
Normalized Detection Fractions provide the color-coding of calls in heatmaps.

Normalized Detection Fractions are interpreted as follows:

- **Higher values** indicate a greater likelihood that the fusion call is correct and that the fusion *is* present in your sample.
- **Lower values** indicate a greater likelihood that the fusion *is not* present in your sample.

Visualize fusion results with IGV

An IGV visualization of a fusion call includes bars for the 3' gene and the 5' gene, coverage for each of the bases, strand information, and each read with its start point and end point.



Notes about the IGV visualization:

- The grey line in the coverage track shows the coverage of each base.
- Reads that are marked in red are on the positive strand.
- Reads that are marked in blue are on the negative strand.
- Zoom in to see individual bases.

These are the characteristics of an IGV visualization of a strong fusion call:

- The 3' ends of reads all line up at the end of the 3' primer.
- The 5' ends of reads all line up at the end of the 5' primer.
- The reads cover the full length of the fusion.

Points to consider when using IGV to evaluate a fusion call:

- What is the percent of overlap between the alignment and the 5' insert? How good is the alignment in the overlapped region?
- What is the percent of overlap between the alignment and the 3' insert? How good is the alignment in the overlapped region?

Points to consider when using IGV to evaluate an expression control or assay call (ASSAY_5P_3P or 3'/5' Imbalance):

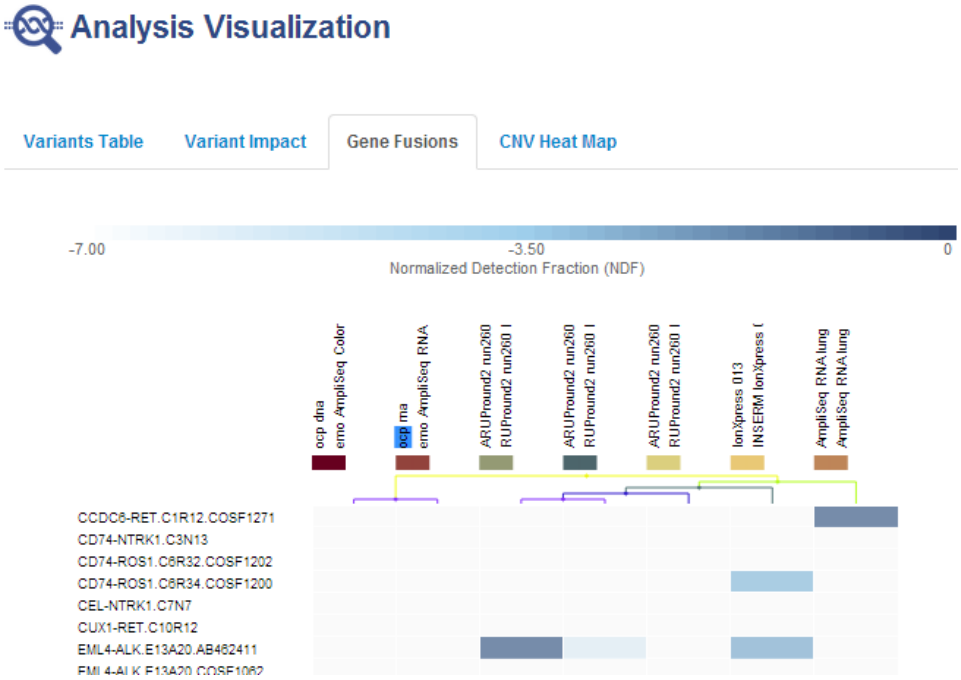
- What is the percent of overlap between the alignment and the insert?
- How good is the alignment in the overlapped region?

Follow these steps to visualize your results with IGV:

1. In the Analysis Results page, click the Locus of a fusion variant.
2. Windows users: save the file `igv.jnlp`. Then double click `igv.jnlp` to open the IGV browser.

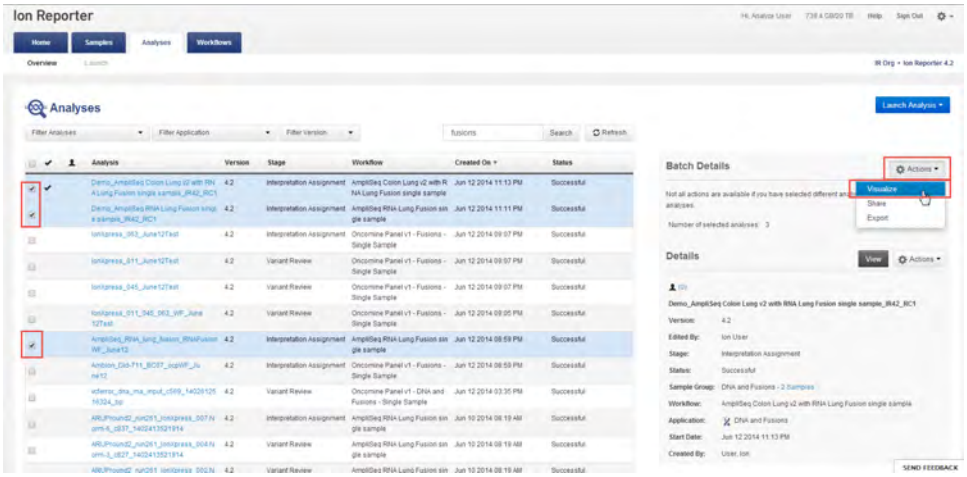
Visualize and compare fusion results of multiple analyses with heatmaps

With heatmap visualizations, you can see the presence or absence of fusions in your samples, and you can compare the fusion calls across multiple analyses.



Follow these steps to visualize your results of multiple fusion analyses:

1. In the Analysis Overview page, enable the checkboxes of the analyses with the results you want to visualize.



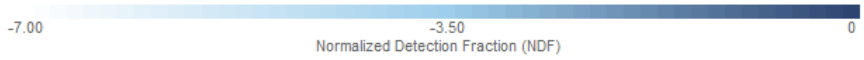
2. In the Action menu on the right, click the **Visualize** option.

   Note: The Visualize menu option is only available when you select more than one analysis.

3. In the Analysis Visualization page, click the **Gene Fusions** tab.

Notes about heatmap visualizations:

- Interpret each cell according to the color scale above the chart. Cells with the rightmost color are the strongest fusion calls.

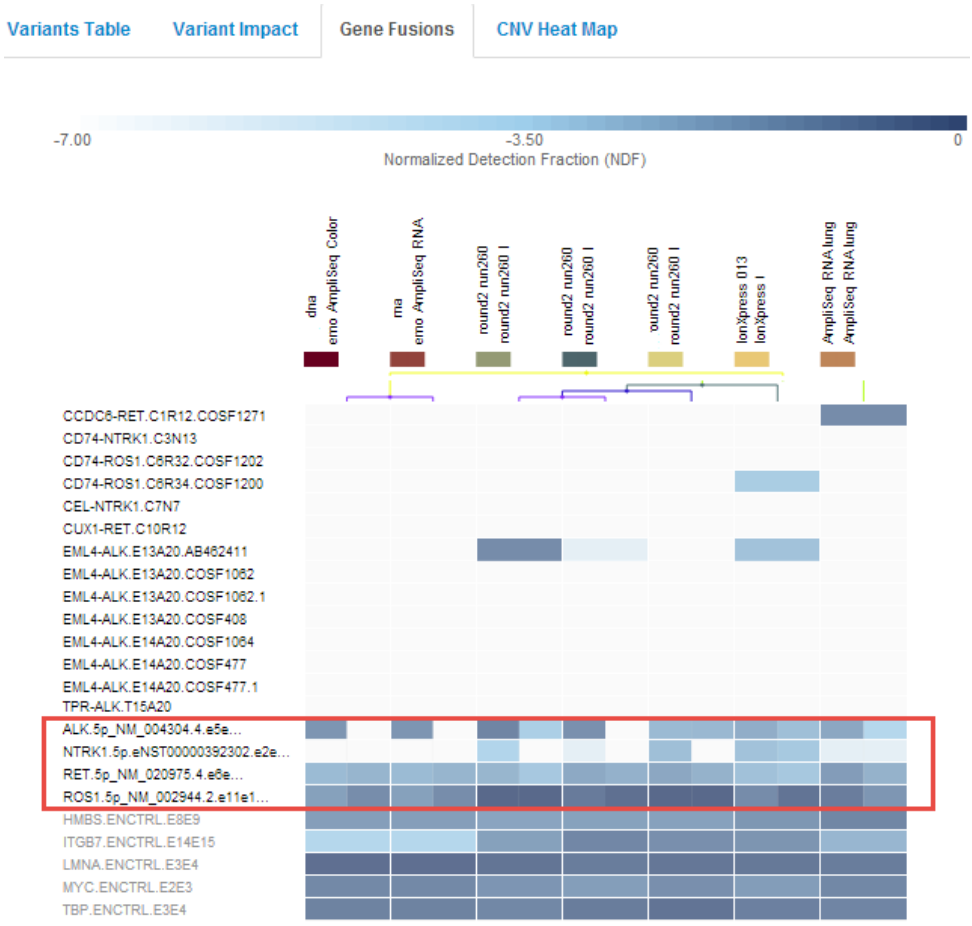


- Normalized Detection Fraction (NDF) values are calculated as follows (for a fusion F in sample S):

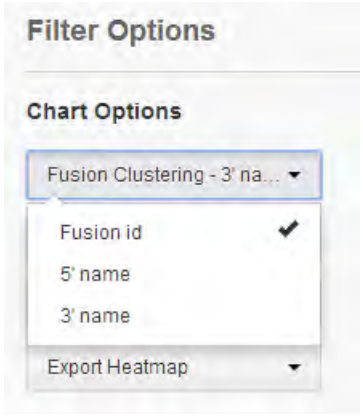
```
log10((read count of F) / (total read count in S))
```

- The left side lists each of the fusion isoforms in the Ion AmpliSeq™ Panel.
- For fusions heat maps, NDF is used as the score for every fusion-sample pair to generate the heat map. Thereafter hierarchical clustering is conducted for clustering fusions and samples within the heat map.

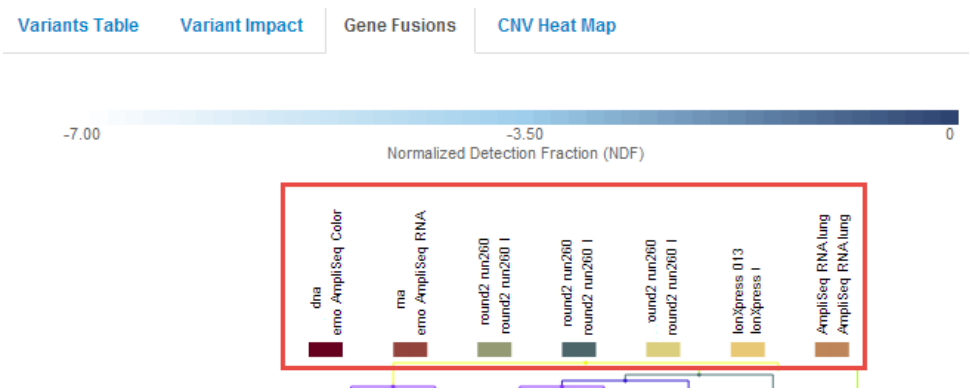
- By default, expression control and ASSAY_5P_3P calls are listed close to the bottom of the chart.



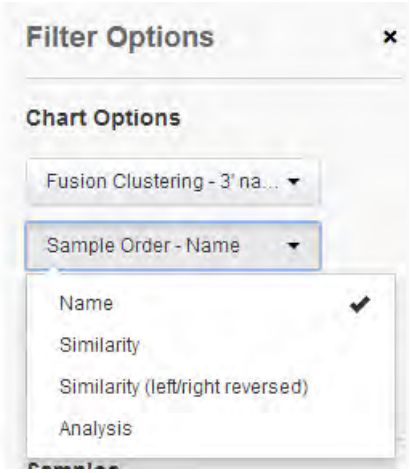
- You can change the order of the fusions (the list on the left side) through the Filter Order menu on the right (under Chart Options).



- Along the top, each sample is listed.



- You can change the order of the samples list (the list above the chart) through the Sample Order menu on the right (under Chart Options).



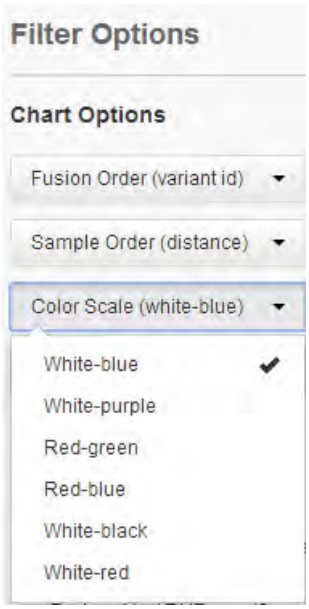
The following ordering options are supported:

- **Name**: Alphabetically by sample name
- **Similarity**: By how similar the samples are in the fusion calls made (presented from fewest fusions to most fusions)
- **Similarity (reversed)**: By how similar the samples are in the fusion calls made (presented from the most fusions to the fewest fusions)
- **Analysis**: Alphabetically by analysis name

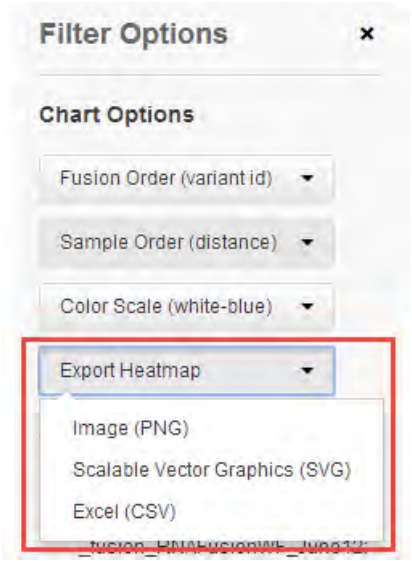
- Samples are also listed in the panel to the right.

- Click the **Selected Analyses** link to see the list of analyses in the heatmap.

- You can change the order of the color scheme used in the chart through the Color Scale menu on the right (under Chart Options).

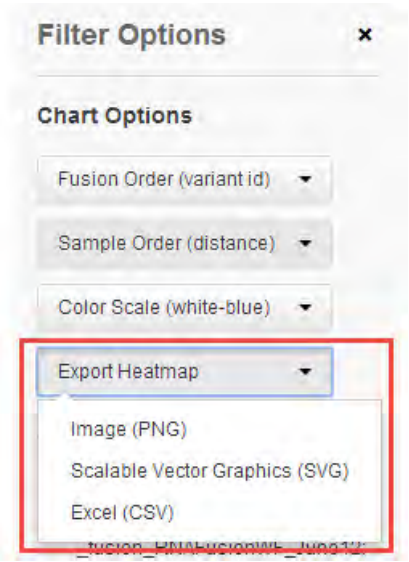


- White or blank cells indicate no reads (in the default color scheme).
  (Many cells in the example heatmap show no reads. Only an example block of no-reads cells is outlined in this image. With red-green and red-blue color schemes, red indicates a no-reads cell.)
- You can export a heatmap as an image or as a comma-separated text file.



Export a heatmap

Use the Export Heatmap menu on the right (under Chart Options) to export a heatmap.



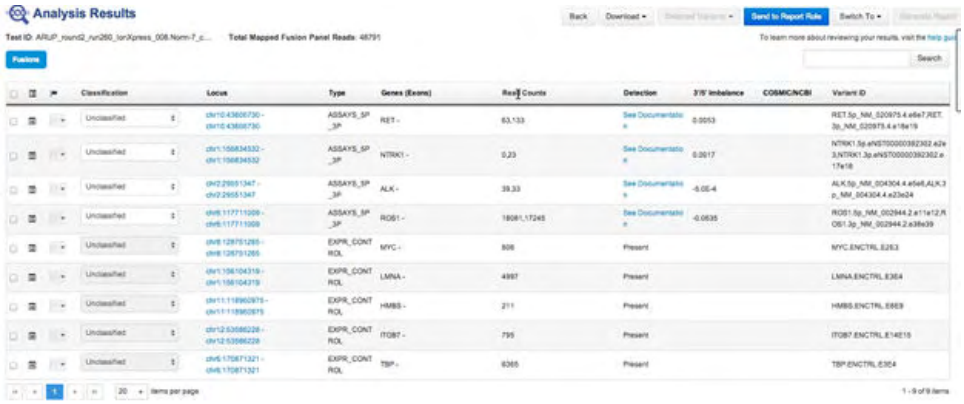
Supported export formats are:

- **PNG**: Portable Network Graphics format
- **SVG**: Scalable Vector graphics format
- **CSV**: A comma-separated text file, readable by spreadsheet programs or as a text file

Note: You can download CSV file in Chrome or Firefox; however, you may need to manually change the file name and add a .csv extension on it. In testing, we found the Safari browser could not download a CSV file.

Example of an analysis with no fusions called

This image shows an example of the Analysis Results table for a run in which no fusions are called.



Note: When you sort on the Gene column, you may believe you are getting inconsistent results. However, the sort is occurring on both of the genes involved in the fusion. The lexicographically smaller gene is used for an ascending sort, while the lexicographically larger gene is used for a descending sort.

Exon deletion thresholds for Oncomine Fusion panels

Oncomine Fusion Panel	Exon Deletion Assay	Type	Present/Absent Threshold	Instrument/Chip	Ion Reporter Software Workflow
Oncomine ™ Comprehensive Fusion Panel	EGFRvIII	Fusion	120	Ion PGM ™ System / Ion 318 ™ Chip	Oncomine Comprehensive w2.2 - Fusions
					Oncomine Comprehensive w2.2 - DNA and Fusions
Oncomine ™ Focus Fusion Panel	MET exon 14	Fusion	120	Ion PGM ™ System / Ion 318 ™ Chip	Oncomine Focus w2.2 - Fusions
	EGFRvIII	Fusion	120		Oncomine Focus w2.2 - DNA and Fusions
Oncomine ™ Focus Fusion Panel for Ion 520 ™ Chip	MET exon 14	Fusion	120	Ion S5 ™ System / Ion 520 ™ Chip	Oncomine Focus - 520 - w2.2 - Fusions
	EGFRvIII	Fusion	120		Oncomine Focus - 520 - w2.2 - DNA and Fusions
Oncomine ™ Comprehensive & Focus Fusion panel for Ion 540 ™ Chip	MET exon 14	Fusion	1000	Ion S5 ™ System / Ion 540 ™ Chip	Oncomine Comprehensive v1 - 540 - w2.2 - DNA and Fusions
	EGFRvIII	Fusion	1000		Oncomine Comprehensive v2 - 540 - w2.2 - DNA and Fusions - Single Sample
Oncomine ™ Comprehensive v3 Panel	MET exon 14	RNAExonVariant	1000	Ion S5 ™ System / Ion 540 ™ Chip	Oncomine Comprehensive v3 - w3.0 - DNA and Fusions - Single Sample
	EGFRvIII	RNAExonVariant	1000		Oncomine Comprehensive v3- w3.0 -Fusions - Single Sample
	Others (9 other genes have similar assays)	RNAExonVariant	Present/Absent calls are not made		

Web services API

Ion Reporter™ Software contains a powerful web services API that allows you to automate pulling key pieces of information from the system.

Use the API

This section provides an example of how to leverage the API built into Ion Reporter™ Software.

Notes:

- These examples show use with the hosted Ion Reporter™ Software instance. If you are running a local version on your own Ion Reporter™ Server System, then replace the hosted URL

  ```
  https://ionreporter.thermofisher.com/ir
  ```

  with the URL root of your local instance.
- Example (invalid) authentication tokens are shown.
- If the JSON download process converts "@" symbols to "%40", do not change the text. Just copy the files into your browser as is.

Get data upload path with authentication

The getDataUploadPathWithAuth code flow is used by Ion Reporter Uploader (IRU) and returns the current data upload path, based on server.properties configuration.

URL: /api/v1/getDataUploadPathWithAuth

HTTP Method: POST

Request Form Parameters: auth. String. *Mandatory.* Valid **API** Token.

```
http://ionreporter.thermofisher.com/api/v1/
getDataUploadPathWithAuth
```

Request Headers Content Type: application/x-www-urlencoded

Returns: Returns the current data upload path of the organisation given by the user's API token.

```
/data/IR/data/IR_Org/data/IRU_Uploads
```

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl Command:

```
curl -X POST -d
"auth=NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5N
zVjODU3YjdlMWM4Mjk3OGYzODE4Yw" --header "Content-Type:
application/x-www-form-urlencoded" -k https://<your server
name>/api/v1/getDataUploadPathWithAuth
```

Check if sample exists

The isSampleExist code flow is used by Ion Reporter Uploader (IRU) when importing a sample. It checks if the sample with the given name exists or not.

URL: /api/v1/isSampleExists

HTTP Method: POST

Request Form Parameters: **sampleName**. String. *Mandatory*. Valid sample Name.

```
http://ionreporter.thermofisher.com/api/v1/isSampleExists
```

Request Headers: **Content Type**: application/x-www-form-urlencoded; **Authorization**: API Token.

Returns: Returns true or false depending on whether sample with given name already exists in the organisation given the user's API token in headers.

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k https://your server name/api/v1/isSampleExists
-H "Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d
"sampleName=Poo_C03-740_DNA_009_think3_IRUCLI"
```

Get user details

The getUserDetails code flow is used by IRU and gets user details based on user-name and password.

URL: /api/v1/getUserDetails

HTTP Method: POST

Request Form Parameters:

userName. String. *Mandatory*. Valid user name.

password String. *Mandatory*. Valid password.

```
http://ionreporter.thermofisher.com/api/v1/getUserDetails
```

Request Headers: Content Type: application/x-www-urlencoded

Returns: Returns user details.

```
{
  "tokendate": "2013-12-08 15:39:37.0",
  "firstname": "Ion",
  "eulaAccepted": "true",
  "userStatus": "ENABLED",
  "orgname": "IR Org",
  "exporttokendate": "2015-11-05 03:25:12.0",
  "exporttoken":
"49bbe49a8307ba61ef28ee9a9122a98347576488c975c857b7e1c82978f381
8c",
  "lastname": "User",
  "token": "wVcoTeYGfKxItiaWo2lngsV/
r0jukG2pLKbZBkAFnlPbjKfPTXLbIhPb47YA9u78"
}
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k https://your server/api/v1/getUserDetails -H
"Content-Type:application/x-www-form-urlencoded" -d
"userName=ion.reporter@lifetech
.com&password=IonPass123"
```

Get user details with authentication

The getUserDetailsWithAuth code flow used by IRU and returns user details based on API token.

URL: /api/v1/getUserDetailsWithAuth

HTTP Method: POST

Request Form Parameters: String. *Mandatory*. Valid user API token.

```
http://ionreporter.thermofisher.com/api/v1/
getUserDetailsWithAuth
```

Request Headers: Content Type: application/x-www-form-urlencoded

Returns: Returns the user details.

```
{
  "tokendate": "2013-12-08 15:39:37.0",
  "firstname": "Ion",
  "eulaAccepted": "true",
  "userStatus": "ENABLED",
  "orgname": "IR Org",
  "exporttokendate": "2015-11-05 03:25:12.0",
  "exporttoken":
"49bbe49a8307ba61ef28ee9a9122a98347576488c975c857b7e1c82978f381
8c",
  "lastname": "User",
  "token": "wVcoTeYGfKxItiaWo2lngsV/
r0jukG2pLKbZBkAFnlPbjKfPTXLbIhPb47YA9u78"
}
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k https://<your server name>/api/v1/
getUserDetailsWithAuth -H "Content-Type:application/x-www-form-
urlencoded" -d
```

```
"auth=NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5N
zVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get available storage space

The getAvailableStorageSpace code flow returns the available storage space in bytes of the organization associated with the API token in headers.

URL: /api/v1/getAvailableStorageSpace

HTTP Method: POST

Request: http://ionreporter.thermofisher.com/api/v1/getAvailableStorageSpace

Request Headers: **Content Type**: application/x-www-form-urlencoded; **Authorization**: API token

Returns: Returns the available storage space in bytes of the organisation given the user's API token in headers.

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k https://<your server name>/api/v1/
getAvailableStorageSpace -H "Content-Type:application/x-www-
form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get available cancer types

The getAvailableCancerType code flow returns the list of all available cancer types defined in attribute-controlled vocabulary.

URL: /api/v1/getAvailableCancerType

HTTP Method: POST

Request: http://ionreporter.thermofisher.com/api/v1/getAvailableCancerType

Request Headers: **Content Type**: application/x-www-form-urlencoded; **Authorization**: API token

Returns: Returns the available cancer types defined in attribute-controlled vocabulary.

```
["Bladder Cancer","Breast Cancer","Colorectal
Cancer","Endometrial Cancer",
"Esophageal Cancer","Gastric Cancer","Gastrointestinal Stromal
Tumor","Glioblastoma"
,"Head and Neck Cancer","Kidney Cancer","Liver
Cancer","Melanoma","Mesothelioma",
"Non-Small Cell Lung Cancer","Osteosarcoma","Ovarian
Cancer","Pancreatic Cancer",
"Prostate Cancer","Skin Basal Cell Carcinoma","Small Cell Lung
Cancer",
"Soft Tissue Sarcoma","Testicular Cancer","Thyroid
Cancer","Unknown"]
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k https://<your server name>/api/v1/
getAvailableCancerType -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get workflow names

The getWorkflowNamesWithApplicationTypeWithAuth code flow is used from within IRU to get list of IRU-tagged workflows for the given version. If there are no IRU-tagged workflows, all workflows for a specified version are returned.

URL: /api/v1/getWorkflowNamesWithApplicationTypeWithAuth

HTTP Method: POST

Request:
http://ionreporter.thermofisher.com/api/v1/getWorkflowNamesWithApplicationTypeWithAuth

Request Headers: **Content Type**: application/x-www-form-urlencoded;
Authorization: **API token**: Version: IR52

Returns: Returns the list of Workflow details for the version specified in the header

```
[
  {
    "ApplicationType": "Amplicon Sequencing",
    "Workflow": "RK_GSTT1_GRCh38_APR06",
```

```
    "irVersion": "52",
    "tag_isFactoryProvidedWorkflow": "false",
    "irReference": "GRCh38"
  }
]
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k https://<your server name>/api/v1/
getWorkflowNamesWithApplicationTypeWithAuth -H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -H "Version:52"
```

Start an analysis

The startAnalysis code flow is used by IRU to start an analysis for the given setId.

URL: /api/v1/startAnalysis

HTTP Method: POST

Request Form Parameters:

setId. String. *Mandatory*. setId column value of the sample in the when the sample is uploaded through IRU.

containerName. String. *Mandatory*. User specified unique name for the analysis that will show up on Ion Reporter user interface.

```
http://ionreporter.thermofisher.com/api/v1/startAnalysis
```

Request Headers: **Content Type**: application/x-www-form-urlencoded; **Authorization**: API token

Returns: Returns JSON with status and message.

```
{ "status":true, "message":"success"}
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k https://<your server name>/api/v1/
startAnalysis -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d
"setId=4__f535ccde-5c6a-490d-
a1d2-7b81352e6830_2016-6-17_1_13_24&containerName=New_Analysis_
Name"
```

Get .vcf files

The getvcf code flow gets the links to download .vcf files.

URL: /api/v1/getvcf

HTTP Method: GET

Request Query Parameters:

format. String. Optional. Response type, current version supports only json

type. String. Optional. Valid values are "sample" or "analysis"

name. String. Conditional. If type parameter is "sample" then name is Mandatory

id. String. Optional. Id of an Analysis

start_date. String. Optional. Parameter to filter the results by date boundary. Date format should be in yyyy-MM-dd

end_date. String. Optional. Parameter to filter the results by date boundary. Date format should be in yyyy-MM-dd

duration. String. Optional. Parameter to filter the results by days/months.

Valid value starts with "-" followed by numeric value and ends with "d" or "m"

exclude. String. Optional. Parameter to filter the response.

Valid values are "unfilteredvariants" or "filteredvariants"

Sample Request:

```
http://ionreporter.thermofisher.com/api/v1/getvcf?
format=json&name=xyzsampletest
&start_date=2016-01-01&end_date=2016-02-01
```

Request Headers: **Content Type**: application/x-www-form-urlencoded; **Authorization**: API token

Returns: JSON object containing the download information.

Sample Response:

```
[
  {
    "data_links": "http://ionreporter.thermofisher.com/api/v1/
download?
      filePath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/
JohnSmithSample
      /JohnSmithSample_20160429014705727/
JohnSmithSample_c150_2016-04-29-14-16-534.zip",
    "name": "JohnSmithSample_c150_2016-04-29-14-16-534",
    "id": "ff808181545d907901546133 36be0008"
  }
]
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -v -k -X GET "https://<your server name>/api/v1/getvcf?
format=json&name=BRCA_Plus_RR_jul29&start_date=2016-07-25&end_d
ate=2016-07-31" -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Check if analysis is running

The isAnalysisRunning code flow checks if the analysis is running.

URL: /api/v1/isAnalysisRunning

HTTP Method: POST

Request Form Parameters:

Content Type: application/x-www-urlencoded

Response Fields: **userId**. String. *Mandatory*. UserId

```
http://ionreporter.thermofisher.com/api/v1/isAnalysisRunning
```

Request Headers: **Content-Type**: application/x-www-form-urlencoded; **Authorization**: API Token

Returns: Returns JSON with status and message.

```
{ "status":true, "message":"success"}
```

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k https://<your server name>/api/v1/
isAnalysisRunning -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d "userId=100"
```

Unshare analyses

The unsharedAnalyses code flow unshares analyses.

URL: /api/v1/unsharedAnalyses

Method: POST

Request Form Parameters: **userId**. String. *Mandatory*. UserId

```
http://ionreporter.thermofisher.com/api/v1/unsharedAnalyses
```

Request Headers: **Content Type**: application/x-www-urlencoded; **Authorization**: API token

Returns: Returns JSON with status and message

```
{ "status":true, "message":"success"}
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -X POST -k "https://<your server name>/api/v1/
unsharedAnalyses" -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d
"userId=ff8081815547ceb401554d62c0ec0493"
```

download

The download code flow gets the file, creates a zip file and downloads it.

URL: /api/v1/download

HTTP Method: GET

Request Query Parameters:

filePath. String. *Mandatory*. Valid filePath.

type. String. *Optional*. Valid values are "pdf" or "". (by Default creates a zip file and downloads it.)

```
http://ionreporter.thermofisher.com/api/v1/download?type=pdf&
filePath=/data/IR/data/IR_Org/download/pdf/3bf2ffb3-
c55e-4da8-819e-f51a6957ad66/
IR42_AmpliSeq Colon Lung v2 with RNA Lung Fusion single
sample_oct1.pdf"
```

Request Headers: **Content Type**: application/x-www-form-urlencoded;
Authorization: API token

Returns: Downloads the given file.

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -O -v -k -X GET "https://<your server name>/api/v1/
download?filePath=/data/IR/data/IR_Org/data/IRU_Uploads/
2016-7-26_23_8_42/v2/report.pdf" -H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

finalreport

The finalreport code flow gets the analysis details and returns links to the Final Report.

URL: /api/v1/finalreport

Method: GET

Request Query Parameters:

format. String. *Optional*. Response type, current version supports only json

type. String. *Optional*. Valid values are "sample" or "analysis"

name. String. *Conditional*. If **type** parameter is "sample" then **name** is Mandatory

id. String. *Optional*. Id of an Analysis by default. If type is sample, this corresponds to the sample id

start_date. String. *Optional*. Parameter to filter the results by date boundary. Date format should be in yyyy-MM-dd

end_date. String. *Optional*. Parameter to filter the results by date boundary. Date format should be in yyyy-MM-dd

```
http://ionreporter.thermofisher.com/api/v1/finalreport?
fomat=json&type=analysis
&name=IR42_AmpliSeq Colon Lung v2 with RNA Lung Fusion single
sample_oct1
&start_date=2014-01-30&end_date=2016-04-12
```

Request Headers: **Content Type**: application/x-www-form-urlencoded

Authorization: API Token

Returns: JSON object containing download information

```
[
  {
    "summary": "",
    "final_report_template": "Default Final Report Template",
    "report_pdf": "http://ionreporter.thermofisher.com/api/v1/
download?type=pdf&
                filePath=/data/IR/data/IR_Org/download/pdf/
                3bf2ffb3-c55e-4da8-819e-f51a6957ad66/
                IR42_AmpliSeq Colon Lung v2 with RNA Lung
Fusion single sample_oct1.pdf",
    "created_on": "2016-03-24T03:11:16.000-07:00",
    "name": "IR42_AmpliSeq Colon Lung v2 with RNA Lung Fusion
single sample_oct1",
    "id": "ff80818148c803530148cdb524020071",
    "variants": [
      {
        "{\"_id\":[\"com.mongodb.BasicDBObject\",
            {\"c\":1,\"p\":115252204}],\"GT\":\"0/0\"}": {
          "comments": "",
          "gene": "NRAS",
          "annotations": "",
          "locus": "chr1:115252204",
          "classification": "UNCLASSIFIED",
          "exon": "4",
```

```
          "sample": [
            {

"Auto_user_PG1-10-140127__ColonLung_v3_test_627_279_IonXpress_0
03_v1": {
                "mutation": "",
                "protein": ""
              }
            }
          ]
        }
      }
    ],
    "analysis": "http://ionreporter.thermofisher.com/api/v1/
analysis?id=
                        ff80818148c803530148cdb524020071",
    "ion_reporter_version": "4.2",
    "created_by": "Report User"
  }
]
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

```
curl -v -k -X GET "https://<your server name>/api/v1/
finalreport?
format=json&name=BRCA_Plus_RR_jul29&type=analysis&start_date=20
16-07-25&end_date=2016-07-31" -H "Content-Type:application/x-
www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get QC report

The qcreport code flow gets the QC Report.

URL: /api/v1/qcreport

HTTP Method: GET

Request Query Parameters:

format. String. *Optional*. Response type, current version supports only json

type. String. *Optional*. Valid values are "sample" or "analysis"

name. String. *Conditional*. If type parameter is "sample" then name is Mandatory

id. String. *Optional*. Id of an Analysis

start_date. *String*. Optional. Parameter to filter the results by date boundary. Date format should be in yyyy-MM-dd

end_date. String. *Optional*. Parameter to filter the results by date boundary. Date format should be in yyyy-MM-dd

view. String. *Optional*. Valid values are "summary" or ""

```
http://ionreporter.thermofisher.com/api/v1/qcreport?
fomat=json&type=analysis&
name=Demo AmpliSeq Exome
VCF_1460407585676&start_date=2016-01-30&end_date=2016-04-12
```

Request HeadersContent Type: application/JSON; **Authorization**: API token

Returns: JSON object containing download information

```
[
  {
    "stage": "VARIANT_REVIEW",
    "flagged": false,
    "name": "Demo AmpliSeq Exome VCF_1460407585676",
    "id": "ff80818153ed40a401540716785a081a",
    "qc_metrics": {
      "Demo AmpliSeq Exome VCF": {
        "Number of Heterozygous SNVs": " 32451",
        "CNV/Total": " 0.000",
        "Number of Homozygous INDELs": " 1089",
        "Ti/Tv Ratio (SNPs)": " 2.308",
        "MAPD": "N/A",
        "LongDels/Total": " 0.000",
        "Number of Heterozygous INDELs": " 2113",
        "Heterozygotes/Homozygotes": " 1.737",
        "Indels/kb": " 0.001",
        "Number of Heterozygous MNVs": " 0",
        "Number of LongDels": " 0",
        "Number of Homozygous MNVs": " 0",
        "Indels/Total": " 0.059",
        "SNPs/kb": " 0.017",
        "Number of Homozygous SNVs": " 18814",
        "Number of CNVs": " 0",
        "dbSNP concordance": " 0.934"
      }
    },
    "started_by": "Ion User",
    "ion_reporter_version": "5.2",
    "samples": {
      "PROBAND": "Demo AmpliSeq Exome VCF"
    },
    "qc_report_pdf": "http://
ionreporter.thermofisher.com/api/v1/download?type=pdf&
                filePath=/data/IR/data/IR_Org/download/pdf/
                890e4268-46dc-442b-a4ed-aa87bb28f71a/
                ff80818153ed40a401540716785a081a_QC.pdf",
    "start_date": "2016-04-11 13:49:53.0",
    "status": "SUCCESSFUL",
    "qc_package": "http://ionreporter.thermofisher.com/api/v1/
download?
                filePath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/
                Demo_AmpliSeq_Exome_VCF/
```

```
Demo_AmpliSeq_Exome_VCF_20160411134952720/
                analysis_downloads/QC_Bundle/
                qc-report-
Demo_AmpliSeq_Exome_VCF_1460407585676_2016-04-12 04-33.zip"
  }
]
```

Response Fields:

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl command:

```
curl -v -k -X GET "https://<your server name>/api/v1/qcreport?
format=json&name=BRCA_Plus_RR_jul29&type=analysis&start_date=20
16-07-25&end_date=2016-07-31" -H "Content-Type:application/x-
www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get analysis details

The analysis code flow gets analysis information.

URL: /api/v1/analysis

HTTP Method: GET

Request Query Parameters:

format. String. *Optional*. Response type, current version supports only json

type. String. *Optional*. Valid values are "sample" or "analysis"

name. String. *Conditional*. If **type** parameter is "sample" then **name** is Mandatory

id. String. *Optional*. Id of an Analysis

start_date. String. *Optional*. Parameter to filter the results by date boundary. Use date format: yyyy-MM-dd

end_date. String. *Optional*. Parameter to filter the results by date boundary. Use date format: yyyy-MM-dd

duration. String. *Optional*. Parameter to filter the results by days/months.

Valid value starts with "-" followed by numeric value and ends with "d" or "m"

exclude. String. *Optional*. Parameter to filter the response.

Valid values are any single or comma-separated combination of values "reports, unfilteredvariants, filteredvariants"

view. String. *Optional*. Valid values are "summary" or ""

Sample Request:

```
http://ionreporter.thermofisher.com/api/v1/analysis?
format=json&name=xyzsampletest
&exclude=reports,filteredvariants,unfilteredvariants&start_date
=2016-01-01
&end_date=2016-02-01
```

Request Headers: **Content Type**: application/x-www-form-urlencoded;
Authorization: API token

Returns:

```
[
  {
    "reports": {
      "qc": {
        "link": "http://ionreporter.thermofisher.com/api/v1/
download?type=pdf&
                filePath=/data/IR/data/IR_Org/download/pdf/
                30cf8ff3-fe3d-4cac-8f7d-ce45aa7d34e1/
                    ff80818153ed40a401540716785a081a_QC.pdf"
      },
      "final": {}
    },
    "report_published": "",
    "workflow": "Annotate variants single sample",
    "data_links": {
      "filtered_variants": "http://
ionreporter.thermofisher.com/api/v1/download?
                            filePath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/
                            Demo_AmpliSeq_Exome_VCF/

Demo_AmpliSeq_Exome_VCF_20160411134952720/

Demo_AmpliSeq_Exome_VCF_1460407585676.zip",
      "unfiltered_variants": "http://
ionreporter.thermofisher.com/api/v1/download?
                            filePath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/
                            Demo_AmpliSeq_Exome_VCF/

Demo_AmpliSeq_Exome_VCF_20160411134952720/

Demo_AmpliSeq_Exome_VCF_1460407585676.zip"
    },
    "variants_saved": "",
    "samples": {
      "PROBAND": "Demo AmpliSeq Exome VCF"
    },
    "shared_with": [],
    "stage": "Review Variants",
    "flagged": false,
    "name": "Demo AmpliSeq Exome VCF_1460407585676",
    "id": "ff80818153ed40a401540716785a081a",
    "ion_reporter_version": "5.2",
    "started_by": "Ion User",
    "start_date": "April 11, 2016",
```

```
    "status": "SUCCESSFUL"
  }
]
```

Response Fields: JSON object containing the download information.

Example Curl command:

```
curl -v -k -X GET "http://ionreporter.thermofisher.com/api/v1/
analysis?format=json&name=xyzsampletest
&exclude=reports,filteredvariants,unfilteredvariants&start_date
=2016-01-01
&end_date=2016-02-01" -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4GM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Status Code	Reason
401	Header Authorization key is null or empty
400	start_date is after end_date in query params
400	Wrong format of start_date or end_date
400	If type is "sample" and query param name is null or empty
500	Exception on exporting analysis
200	success

Get whole genome view png

Gets the whole genome view png for analysis.

URL: /api/v1/genomeView

HTTP Method: GET

Request Query Parameters:

id. String. *Optional*. **Id** of an Analysis, If **name** parameter is empty then id is Mandatory

name. String. *Optional*. **Name** of an Analysis, If **id** parameter is empty then name is Mandatory.

- If both **id** and **name** is given as input then genome-view png will be displayed for Id, and name won't be considered.
- Whole Genome View image will be displayed as part of the response along with analysis and sample name as the header.
- If no png file available in /outputs folder then response is 200 with following message: "Genome View PNG not found." [with analysis name and sample name]

Version. String. *Conditional*. Application version on which analysis lunched. For analysis with older application versions we have to provide "Version=<IR version>" as parameter, along with name.

Sample Request:

```
https://ionreporter.thermofisher.com/api/v1/genomeView?
id=ff8081815ba8eac4015ba8ead9ce005d
```

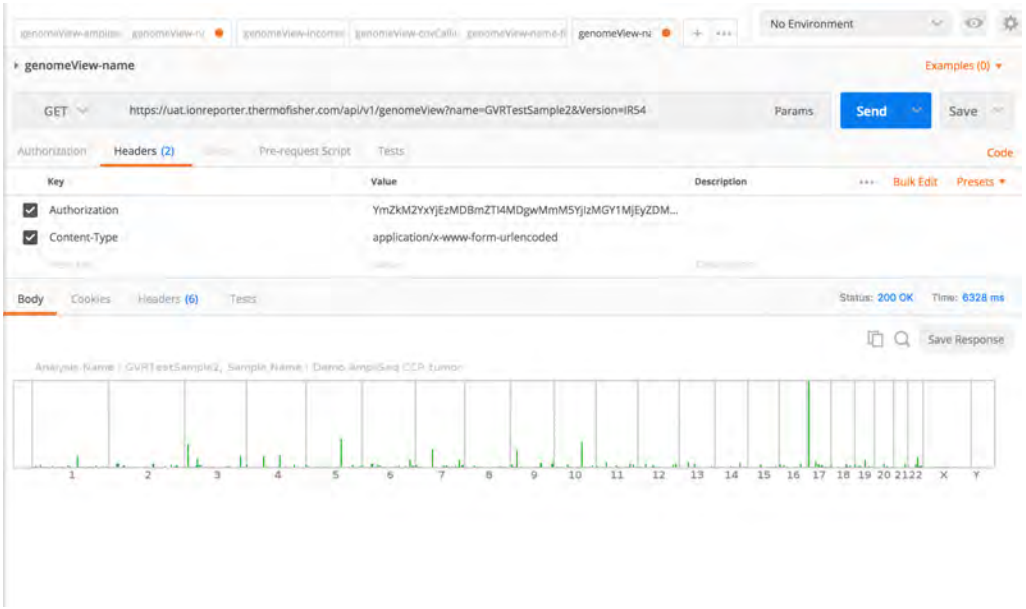
Request Headers: **Content Type**: application/x-www-form-urlencoded
Authorization: API token

Returns: PNG image file for whole genome view of analysis.

Example Curl command:

```
curl -v -k -X GET "https://
uat.ionreporter.thermofisher.com/api/v1/genomeView?
name=GVRTestSample2&Version=IR54"-H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:YmZkM2YxYjEzMDBmZTI4MDgwMmM5YjIzMGY1MjEyZDMyMGI3
ZTU5MmQyZGY4ZmIxZWI5M2ViYzIxZWNhOWU3NQ"
```

Status Codes:



Status Code	Reason
200	Successful response
400	Bad request
401	User is not authenticated
404	Not found
500	Error occurred

Download analysis information

This example uses curl commands to download information about a specific analysis. The output of the first curl command gives us URLs to use in later curl requests.

This curl command requests analysis information for an analysis named IR50_Rc10_Ane_BC18_DeganCases_1014_np:

```
curl --request GET -k -H
"Authorization:ZTRhNWYyNjkzZjhlYjMxMTdmM2FkZGM1ZmQxYmRkNzU2ZTIx
ODk5OGRjZWM5ZDY2MjMzMTBiOGIwZWE5NmNlNg" "https://
baseline.itw/api/v1/analysis?
format=json&name=IR50_Rc10_Ane_BC18_DeganCases_1014_np"
```

The output is in JSON format:

```
[
    {
        "reports": {
            "qc": {
                "link": "https://baseline.itw/api/v1/download?
filepath=/data/IR/data/IR_Org/download/pdf/98ccb759-010f-4c92-
acba-2da2d8aa9f4d/ff8081815067ca42015068f99193000d_QC.pdf"
            },
            "final": {}
        },
        "report_published": "",
        "workflow": "Low-pass whole-genome aneuploidy",
        "data_links": {
            "filtered_variants": "https://baseline.itw/api/v1/
download?filepath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/Ane_BC18_DeganCases/
Ane_BC18_DeganCases_20151014174956618/
IR50_Rc10_Ane_BC18_DeganCases_1014_np___4f776c35-4a9f-4a0f-
a787-6ee28ebb8c7c.zip",
            "unfiltered_variants": "https://
baseline.itw/api/v1/download?filepath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/Ane_BC18_DeganCases/
Ane_BC18_DeganCases_20151014174956618/
IR50_Rc10_Ane_BC18_DeganCases_1014_np.zip"
        },
        "variants_saved": "",
        "samples": {
            "PROBAND": "Ane_BC18_DeganCases"
        },
        "shared_with": [],
        "stage": "Send for Report Generation",
        "flagged": false,
        "name": "IR50_Rc10_Ane_BC18_DeganCases_1014_np",
        "id": "ff8081815067ca42015068f99193000d",
        "ion_reporter_version": "5.0",
        "started_by": "Ion User",
        "start_date": "October 14, 2015",
        "status": "SUCCESSFUL"
    }
]
```

This output contains links to three downloads about this analysis (backslashes have been removed):

- qc: "https://baseline.itw/api/v1/download?filepath=/data/IR/data/IR_Org/download/pdf/98ccb759-010f-4c92-acba-2da2d8aa9f4d/ff8081815067ca42015068f99193000d_QC.pdf"
- unfiltered_variants:" https://baseline.itw/api/v1/download?filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/Ane_BC18_DeganCases/Ane_BC18_DeganCases_20151014174956618/IR50_Rc10_Ane_BC18_DeganCases_1014_np.zip"
- filtered_variants: "https://baseline.itw/api/v1/download?filepath=/data/IR/data/IR_Org/ion.reporter@lifetech.com/Ane_BC18_DeganCases/Ane_BC18_DeganCases_20151014174956618/IR50_Rc10_Ane_BC18_DeganCases_1014_np___4f776c35-4a9f-4a0f-a787-6ee28ebb8c7c.zip"

Take one of these links, remove the backslashes ('\'), and send this URL as the second curl command. Redirect the output to a file. For example, this command downloads the zipped filtered variants file:

```
curl --request GET -k -H
"Authorization:ZTRhNWYyNjkzZjhlYjMxMTdmM2FkZGM1ZmQxYmRkNzU2ZTIx
ODk5OGRjZWM5ZDY2MjMzMTBiOGIwZWE5NmNlNg" -O "https://
baseline.itw/api/v1/download?filepath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/Ane_BC18_DeganCases/
Ane_BC18_DeganCases_20151014174956618/
IR50_Rc10_Ane_BC18_DeganCases_1014_np___4f776c35-4a9f-4a0f-
a787-6ee28ebb8c7c.zip"
```

Download all analyses with one call

To retrieve all analyses in JSON format:

```
curl --request GET -k -H
"Authorization:ODkxY2IxYThhZWNjNDU3MjlmNjdlZDBkYWEwYzdmZTQzODFm
Zjk3NjExNDA0ODVmYjYwMjA4YzQ5MGEwNDEzZg" https://
swanseaone.itw/api/v1/analysis?format=json
```

View=summary

To get a list of all analyses a user has access to without the links to the actual analyses, enter:

```
view=summary
```

Example:

```
webservices_42/rest/api/analysis?format=json&view=summary
```

This approach saves time over the original calling API.

Download QC and Final reports

To retrieve one QC report in JSON format, enter:

```
curl --request GET -k -H
"Authorization:ZDMxNDUwZGYyNjM4NGRlZjY0NjAyNTc1YTE1ZjU2MzFkNjVm
YzQxYjJhOTQwNTYyYWM3OTJmZDY2YTU5NGQwNA"  -O "https://<your
server name>/api/v1/download?filepath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/new_CFTR/new_CFTR_20151106002127527/
new_CFTR_1446798039543.zip"
```

The output in JSON format will resemble this:

```
[
    {
        "reports": {
            "qc": {
                "link": "https://<your server name>/api/v1/
download?filepath=/data/IR/data/IR_Org/download/pdf/
335a1f5e-19f1-4ff4-b80c-af620f1dfe1f/
ff80818150dbc2430150dbe2d9650072_QC.pdf"
            },
            "final": {}
        },
        "report_published": "",
        "workflow": "AmpliSeq CFTR single sample",
        "data_links": {
            "filtered_variants": "https://<your server name>
/api/v1/download?filepath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/new_CFTR/new_CFTR_20151106002127527/
new_CFTR_1446798039543.zip",
            "unfiltered_variants": "https://<your server name>
/api/v1/download?filepath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/new_CFTR/new_CFTR_20151106002127527/
new_CFTR_1446798039543.zip"
        },
        "variants_saved": "",
        "samples": {
            "PROBAND": "new_CFTR"
        },
        "shared_with": [],
        "stage": "Review Variants",
        "flagged": false,
        "name": "new_CFTR_1446798039543",
        "id": "ff80818150dbc2430150dbe2d9650072",
        "ion_reporter_version": "5.0",
        "started_by": "Ion User",
        "start_date": "November 06, 2015",
        "status": "SUCCESSFUL"
    }
]
```

To retrieve all the QC reports in JSON format, enter:

```
curl --request GET -k -H
"Authorization:ODkxY2IxYThhZWNjNDU3MjlmNjdlZDBkYWEwYzdmZTQzODFm
Zjk3NjExNDA0ODVmYjYwMjA4YzQ5MGEwNDEzZg" https://
swanseaone.itw/api/v1/qcreport?format=json
```

To retrieve one Final report in JSON format, enter:

```
curl --request GET -k -H
"Authorization:YTJjNGVmYWYyNDcxNTgyMmU3NzMxZmY0OWVjY2ZhYTA0ZDM2
YTk1OTQ4ZjUxZDlmMzFkZjFjM2UwNzZhNjliMg" "https://
teemo.itw/api/v1/finalreport?
format=json&name=testDNAFusion_c255_1421470231458"
```

To retrieve all Final reports in JSON format, enter:

```
curl --request GET -k -H
"Authorization:ODkxY2IxYThhZWNjNDU3MjlmNjdlZDBkYWEwYzdmZTQzODFm
Zjk3NjExNDA0ODVmYjYwMjA4YzQ5MGEwNDEzZg" https://
swanseaone.itw/api/v1/finalreport?format=json
```

Query by Sample Name API

You can now query by sample name using this API:

```
/api/v1/analysis?format=json&name=[samplename]&type=sample
```

This returns a list of analysis names performed on the sample, ordered by start date.

Guidelines to develop Ion Reporter plugins

A short description for the plugin can be included after the plugin class declaration as shown in the line 4 in the code sample. If you do not include a short description, you will receive a warning message.

The version attribute is mandatory in the plugin script. Define the version in the plugin as given in line 5 in the code example.

The Python class that inherits from the IonPlugin base class should call the main as shown in the sample code in lines 9 and 10.

MyIonPlugin.py

```
1 #!/usr/bin/env python
 2 from ion.plugin import *
 3 class MyIonPlugin(IonPlugin):
 4     '''This is a sample plugin'''
 5     version = '5.4.0.1'
 6     def launch(self):
 7         print "This is a python-based plugin
 8
 9 if __name__ == "__main__":
10     PluginCLI()
```

The above mentioned class is considered as a wrapper class to the plugin script. This above class is used for the IR plugin framework to validate. We would recommend developer to maintain this format and write another class called MyIonPlugin_plugin.py which contains all the main processing. Calling <plugin_name>_plugin.py from <plugin_name>.py is recommended way of structuring.

Example <plugin_name>.py which acts as a wrapper class and which in-turn calls <plugin_name>_plugin.py:

```
1 #!/usr/bin/python
2 # Copyright (C) 2013 Ion Torrent Systems, Inc. All Rights Reserved
3
4 import os
5 import sys
6 from subprocess import *
7 from ion.plugin import *
8
9 class ampliSeqRNA(IonPlugin):
10   '''Whole Transciptome AmpliSeq-RNA Analysis. (Ion supprted)'''
11   version = '5.4.0.6'
12   major_block = True
13   runtypes = [ RunType.FULLCHIP, RunType.THUMB, RunType.COMPOSITE ]
14   runlevels = [ RunLevel.DEFAULT ]
15
16   def launch(self,data=None):
17     plugin = Popen([
18         '%s/ampliSeqRNA_plugin.py' % os.environ['DIRNAME'], '-V', self.version, '-d',
19         'startplugin.json', 'barcodes.json'
20       ], stdout=PIPE, shell=False )
21     plugin.communicate()
22     sys.exit(plugin.poll())
23
24
25 if __name__ == "__main__":
26   PluginCLI()
27
```

At the time of plugin run, Plugin is provided with the startplugin.json and barcodes.json in its running directory which allows the plugin to access these json files as if they are available in their current working directory.

In the code above, lines 18 and 19 show the way how to access these files.

More information on these json files are provided in the sections below.

Naming convention for the plugin zip

-
- IR plugin framework has some validations against how the zip file should be named.
- After compressing the plugin contents into the zip, the zip name should be given as <plugin_name>_<version>.zip.
- For example: From the above code snippet, the plugin zip file name should look like **MyIonPlugin_5.4.0.1.zip.**

How to create plugin zip:

1. Create a folder with pluginname_version.
2. Keep the files/folders in it.
3.
   - Below are the files that every plugin should contain for IR to validate it.
     - **Instance_ir.html**
     - This html file is used by plugin framework to show the plugin configuration page in the plugins tab at the analysis launch time.
     - This html file should contain the parameters that should be configured at the time of launching the plugin.

  - User can modify/configure parameters during workflow creation/analysis launch. When user clicks on the configure icon then it shows instance_ir.html which get the default parameters from database and user can modify those. Please refer the instance_ir.html in ampliSeqRNA plugin for more info.

- **Parameters.json**
  - Plugin framework expects the file called parameters.json in the plugin zip.
  - At the time of installation, plugin framework will save the default values required for the plugin in the database. These default values for those keys to be given in parameters.json.
  - At run time, IR will display plugin's instance_ir.html to configure plugin parameters at the analysis launch time. Once configured, plugin framework will provide these values for the plugin usage under key called "pluginconfig" in startplugin json. Startplugin json will be provided under the plugin results directory. Results directory can be accessed through the environment variable called "RUNINFO_RESULTS_DIR".
  - Parameters.json file should contain all the keys and default values for all these keys. These keys in this file should match the parameters to be configured using instance_ir.html.
  - Parameters.json file should be specific to plugin. ampliSeqRNA plugin parameters.json is different from RNAseq plugin parameters.json.
  - Please refer the default paramaters.json for more information.
- **MyIonPlugin.py**
  - This file name should be the same name (MyIonPlugin.py) as zip file name (MyIonPlugin_5.4.0.1.zip) with no version in it.
  - This python class should implement IonPlugin base class provided by the plugin framework.
  - This IonPlugin base class expects the plugin to have mandatory attribute version, have a method called PluginCLI() method inside __main__ and also to have method definition for launch(self).
- Sample contents in ampliSeqRNA plugin:

```
ionadmin@liverpool:/data/IR/data/IR_Org/apps/IR56/ampliSeqRNA_5.4.0.6$ ls -lts
total 136
 4 drwxr-sr-x 2 iruser irgrp  4096 May 15 22:53 templates
 4 drwxr-sr-x 2 iruser irgrp  4096 May 15 22:53 scripts
20 -rwxr-xr-x 1 iruser irgrp 16616 May 15 22:53 instance.html
12 -rwxr-xr-x 1 iruser irgrp 11610 May 15 22:53 instance_ir.html
 4 drwxr-sr-x 3 iruser irgrp  4096 May 15 22:53 lifechart
 4 -rwxr-xr-x 1 iruser irgrp   288 May 15 22:53 parameters.json
 4 -rwxr-xr-x 1 iruser irgrp  3325 May 15 22:53 plan.html
 4 -rwxr-xr-x 1 iruser irgrp   334 May 15 22:53 README
16 -rwxr-xr-x 1 iruser irgrp 13174 May 15 22:53 run_ampliseqrna.sh
 4 drwxr-sr-x 5 iruser irgrp  4096 May 15 22:53 flot
 0 drwxr-sr-x 2 iruser irgrp   113 May 15 22:53 bed
 4 -rwxr-xr-x 1 iruser irgrp  1025 May 15 22:53 CMakeLists.txt
52 -rwxr-xr-x 1 iruser irgrp 52484 May 15 22:53 ampliSeqRNA_plugin.py
 4 -rwxr-xr-x 1 iruser irgrp   714 May 15 22:53 ampliSeqRNA.py
```

Select the folder and create the zip by right click.

Plugin APIs

Plugin APIs are used to fetch/update the plugin parameters. These api's are part of instance_ir.html so please refer instance_ir.html for more info.

Following are the APIs available:

- To get the reference genome:
  - URL: /plugin-api/v1/referencegenome
- To fetch the already saved parameters:
  - URL: '/plugin-api/v1/results?pluginId=' + <<PLUGINID>>+ '&workflowId=' + <<WORKFLOWID>>
- To fetch the target region's based on reference genome:
  - /plugin-api/v1/targetRegions?genome= <<REFERENCE-GENOME>>
- To fetch the target region's based on reference genome and target region:
  - URL: /plugin-api/v1/targetRegions?genome=<<REF-GENOME>> &targetRegion= <<TARGET-REGION_NAME>>
-

Validation:

Once the plugin is finalized, it needs to be compressed into the .zip file. The zip file name should be same name given to the file <plugin_name>.py. IR would validate this zip to check to see if this plugin has valid version attribute and if this plugin has implemented IonPlugin class.

Below are the validation performed during the plugin installation:

1. Plugin zip should have the at least one python file.
2. Valid python file should import the ion.plugin package.
3. Valid python file should have class name.
4. Valid python file should have version attribute.
5. pluginname_version is used to check the uniqueness. So newly installed plugin should not have the plugin name and version, which is already installed.
6. Plugin zip should have parameters.json inside it with default values otherwise plugin will not generate the proper result.

HTML's for Visualization:

Once the plugin analysis is launched and successful, for the developer to view the plugin results using IR visualization:

- Plugin should generate <plugin_name>.html and <any_name>_block.html (optional) files in the plugin output folder. path: <analysis_root_dir>/outputs/TsPluginActor-00/<Plugin_name>/
- If the _block.html is generated then IR software would show the results in a block like frame as a mini view.
- The frame will have a button called "View Summary" which contains the link to open <plugin_name>.html file in a new tab.

Plugin Results

For the plugin developer to view the results in the backend, the results will be available at the following path: <analysis_root_dir>/outputs/TsPluginActor-00/ <Plugin_name>/

Download logs

For the plugin developer to view the plugin logs in the backend, the logs will be available at the following path: <analysis_root_dir> /log/TsPluginActor-00/ <Plugin_name>/

If the <plugin_name>.log file is generated then user will be able to download logs. IR software would show the "Download Log" in a block header.

Scratch directory:

- For the plugin developer to save any intermediate generated files from that plugin for re-use in subsequent plugin runs, these could be saved in pluginScratch directory.
- Developer should be able to access the environment variable called TSP_PLUGIN_SCRATCH and can create a folder under this scratch directory with your plugin name <plugin_name> to save the results.

Sample and Environment details

At run time, developer is provided with two json files startplugin.json and barcodes.json in the plugin results directory.

Developer can access these json files in the MyIonPlugin.py script in Barcodes json:

- Barcodes json contains the sample information that has been selected at the analysis launch time.
- Barcodes json is mainly used to read the bam_file_path, target_regions_file_path, genome_reference_name, genome_reference_file_path and barcode_name.
- Below is the example barcodes json generated by plugin framework:


```
{
  "bc1" : {
    "genome_urlpath" : "",
    "nucleotide_type" : "",
    "control_sequence_type" : "",
    "barcode_name" : "bc1",
    "sample_id" : "",
    "barcode_type" : "",
    "barcode_annotation" : "",
    "sample" : "SampleCustom",
    "reference_fullpath" : "/data/IR/data/.reference/hg19/
hg19.fasta",
    "target_region_filepath" : "",
    "reference" : "hg19",
    "filtered" : "",
    "barcode_sequence" : "",
    "hotspot_filepath" : "",
    "barcode_index" : "",
    "bam_file" : "1_1_IonXpress_009_rawlib.bam",
    "barcode_adapter" : "",
    "barcode_description" : "",
    "bam_filepath" : "/data/IR/data/IR_Org/data/IRU_Uploads/
20170306_02_12_48/v1/new_ocp_rna/1_1_IonXpress_009_rawlib.bam",
    "aligned" : "",
    "control_type" : "",
    "read_count" : ""
```

```
  }
}
```

- If the sample being used in analysis was uploaded through IRU plugin, then the barcodes json might contain more information like the "read_count", "barcode_adapter", "nucleotide_type", "barcode_sequence" etc.
- Below is the sample barcodes json if the sample is uploaded through IRU plugin:

```
{
  "IonXpress_005" : {
    "read_count" : 1836237,
    "sse_filepath" : "",
    "reference" : "hg19",
    "genome_urlpath" : "",
    "barcode_adapter" : "GAT",
    "sample" : "Dw2-4hr",
    "sample_id" : "",
    "barcode_type" : "",
    "hotspot_filepath" : "",
    "barcode_description" : "",
    "control_type" : "",
    "target_region_filepath" : "",
    "control_sequence_type" : "",
    "bam_filepath" : "/data/IR/data/IR_Org/data/IRU_Uploads/
2017-3-20_11_10_49/v1/Dw2-4hr_RNA_v1/IonXpress_005_rawlib.bam",
    "filtered" : false,
    "barcode_index" : 5,
    "bam_file" : "IonXpress_005_rawlib.bam",
    "aligned" : true,
    "barcode_name" : "IonXpress_005",
    "barcode_annotation" : "",
    "nucleotide_type" : "RNA",
    "barcode_sequence" : "CAGAAGGAAC",
    "reference_fullpath" : "/data/IR/data/.reference/hg19/
hg19.fasta"
  }
}
```

Startplugin json

At the analysis launch time, in UI, user could configure his/her own plugin according to their requirements. These configured parameters will be provided in the startplugin.json under the key called "pluginconfig".

For example: If the user has selected the reference as hg19 in plugin UI (instance_ir.html using plugin api's to get the values from IR DB. See below plugin api's section for more info.), then these values would be provided to the plugin in startplugin json as below:

```
"pluginconfig" : {
 "reference" : "/data/IR/data/.reference/hg19/hg19.fasta"
 }
```

So plugin developer could read the above path as to access the reference file that has been selected in UI.

Startplugin json does contains some IR and plugin meta information like, plugin_dir (plugin zip contents), results_dir (where plugin output should be written).

If the sample being used in IR was uploaded using IRU plugin in TS, then the startplugin json will contain extra meta data like chipType, library, barcodeName, system_type and so on. Given example startplugin json as below if the sample has been uploaded using IRU plugin:


```
{
  "expmeta" : {
    "sample" : "HBR",
    "runid" : "QRIBT",
    "run_name" : "R_2016_08_05_13_17_55_user_S5-00111-181-
LifeLab_DW_08052016_B",
    "run_flows" : 500,
    "output_file_name_stem" :
"R_2016_08_05_13_17_55_user_S5-00111-181-
LifeLab_DW_08052016_B_Auto_user_S5-00111-181-
LifeLab_DW_08052016_B_30836",
    "run_date" : "2016-08-05T20:19:39Z",
    "chiptype" : "530",
    "flowOrder" : "TACGTACGTCTGAGCATCGATCGATGTACAGC",
    "barcodeId" : "IonXpress",
    "analysis_date" : "2016-08-05",
    "chipBarcode" : "DBCD02486",
    "project" : "LifeLab",
    "instrument" : "S5-00111",
    "results_name" : "Auto_user_S5-00111-181-
LifeLab_DW_08052016_B_30836",
    "notes" : ""
  },
  "pluginconfig" : {
    "reference" : "/data/IR/data/.reference/hg19/hg19.fasta",
    "genome" : "hg19"
  },
  "runinfo" : {
    "testfrag_key" : "ATCG",
    "url_root" : "",
    "chipType" : "530",
    "library" : "hg19",
    "sigproc_dir" : "",
    "pluginresult" : 881041,
    "api_url" : "",
    "barcodeId" : "IonXpress",
    "results_dir" : "/data/IR/data/IR_Org/
ion.reporter@lifetech.com/Dw2-4hr_RNA_v1/
Dw2-4hr_RNA_v1_20170321131007199/outputs/TsPluginActor-00/
RNASeqAnalysis",
    "platform" : "s5",
    "pk" : 61178,
    "alignment_dir" : "",
    "api_key" : "",
    "basecaller_dir" : "",
    "net_location" : "",
    "username" : "",
    "library_key" : "TCAG",
    "systemType" : "S5",
    "plugin_name" : "RNASeqAnalysis",
    "tmap_version" : "",
    "report_root_dir" : "/data/IR/data/IR_Org/
ion.reporter@lifetech.com/Dw2-4hr_RNA_v1/
Dw2-4hr_RNA_v1_20170321131007199",
    "chipDescription" : "530",
```

```
    "plugin_dir" : "/share/apps/IR/ionreporter54/apps/IR_Org/
RNASeqAnalysis",
    "raw_data_dir" : "",
    "analysis_dir" : "/data/IR/data/IR_Org/
ion.reporter@lifetech.com/Dw2-4hr_RNA_v1/
Dw2-4hr_RNA_v1_20170321131007199"
  },
  "plan" : {
    "planName" : "LifeLab_DW_08052016_B",
    "username" : "",
    "sequencekitname" : "Ion S5 Sequencing Kit",
    "librarykitname" : "Ion AmpliSeq RNA Library Kit",
    "sampleGrouping" : null,
    "sseBedFile" : "",
    "barcodeId" : "IonXpress",
    "controlSequencekitname" : "",
    "regionfile" : "",
    "runMode" : "single",
    "bedfile" : "",
    "templatingKitName" : "Ion Chef S530 V1",
    "samplePrepKitName" : "",
    "runType" : "AMPS_RNA",
    "runTypeDescription" : "AmpliSeq RNA",
    "reverse_primer" : "",
    "threePrimeAdapter" : "ATCACCGACTGCCCATAGAGAGGCTGAGAC",
    "sampleTubeLabel" : ""
  }
}
```

Related documentation

This appendix provides links to current application notes, white papers, user guides, and other documentation that is related to Ion Reporter™ Software and available at the Thermo Fisher Scientific website.

Advanced algorithm documentation

Ion Reporter™ Software contains many advanced algorithms that have been optimized specifically for Ion Torrent™ semiconductor Sequencing technology. Below are application notes and white papers that provide more background and are available at the Thermo Fisher Scientific website.

- *Variant filtering and prioritization using the Ion AmpliSeq™ Exome trio workflow in Ion Reporter™ Software*, available at **http://tools.thermofisher.com/content/sfs/brochures/Ion-AmpliSeq-Exome-Application-Note.pdf**
- *Metagenomics 16S Algorithm Overview*, available at **http://tools.thermofisher.com/content/sfs/brochures/ion-reporter-16s-metagenomics-algorithms-whitepaper.pdf**.
- *Application Note: CNV detection by Ion semiconductor sequencing*, available at **http://tools.thermofisher.com/content/sfs/brochures/CNV-Detection-by-Ion.pdf**.
- *Application Note: Detection of aneuploidy in a single cell using the Ion ReproSeq PGS Kit*, available at **https://tools.thermofisher.com/CONTENT/SFS/BROCHURES/ANEUPLOIDY-APP-NOTE.PDF**.

Oncomine Assay user guides

The following user guides for Oncomine assays are available at the Thermo Fisher Scientific website.

- *Oncomine Comprehensive Assay version 3 User Guide (Pub. No. MAN0015885)*, available at **http://tools.thermofisher.com/content/sfs/manuals/MAN0015885_OncomineComprehensiveAssay_v3_UG.pdf**.
- *Oncomine Focus Assay Part III: Variant Analysis User Guide (Pub. No. MAN0015821)*, available at **http://tools.thermofisher.com/content/sfs/manuals/MAN0015874_Oncomine_cfDNA_Assay_Part_III.pdf** .
- *Oncomine cfDNA Lung Assays, Part III: Variant Analysis User Guide (Pub. No. MAN0015874)*, available at **https://tools.thermofisher.com/content/sfs/manuals/MAN0015874_Oncomine_cfDNA_Assay_Part_III.pdf**.
- *Oncomine BRCA Research Assay User Guide (Pub. No. MAN0014634)*, available at **http://tools.thermofisher.com/content/sfs/manuals/MAN0014634_OncomineBRCA_UG.pdf**.

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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