iontorrent

Ion Reporter™ Software 5.10

USER GUIDE

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

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C.0	July 23, 2018	Added updates for Ion Reporter Server: • New section on MyVariants • Export and Import a custom workflow topic • Custom BED file troubleshooting section • Step for changing filter when viewing an Oncomine analysis in a TagSeq workflow
B.0	June 25, 2018	Corrected an error.
A.0	June 13, 2018	Updates for new features in Ion Reporter™ Software 5.10. • New features: – Setting to automatically update Classifications and Notes used with MyVariants – Improved algorithm for fusion detection based on multiple imbalance assays – Locus view versus Allele View of variants – Get the latest updates from MyVariants database – Reasons for NOCALL in a gene-level CNV – Create a custom workflow for Ion AmpliSeq™ HD – Analysis workflow templates for Ion AmpliSeq™ HD in Ion Reporter™ Software – Review analysis results for Ion AmpliSeq™ HD panels • Topics updated: – Effects of Ensembl sources on Polyphen and SIFT scores – Status alert emails – Ion Reporter Software Dashboard – Pfam annotations – Documentation and support – Create an Organization on an Ion Reporter Server • Sections updated: – Manage workflows in Ion Reporter™ Software – Web services API – Create and review reports – Filters and filter chains – Visualize results with Ion Reporter™ Software

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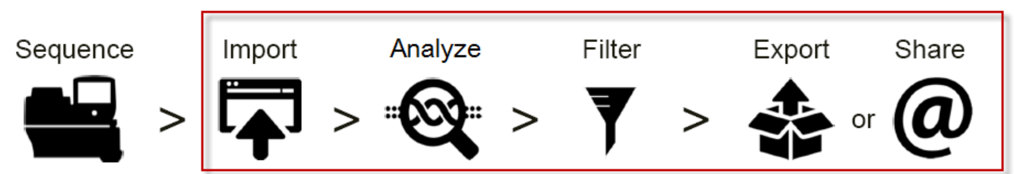
About Ion Reporter™ Software

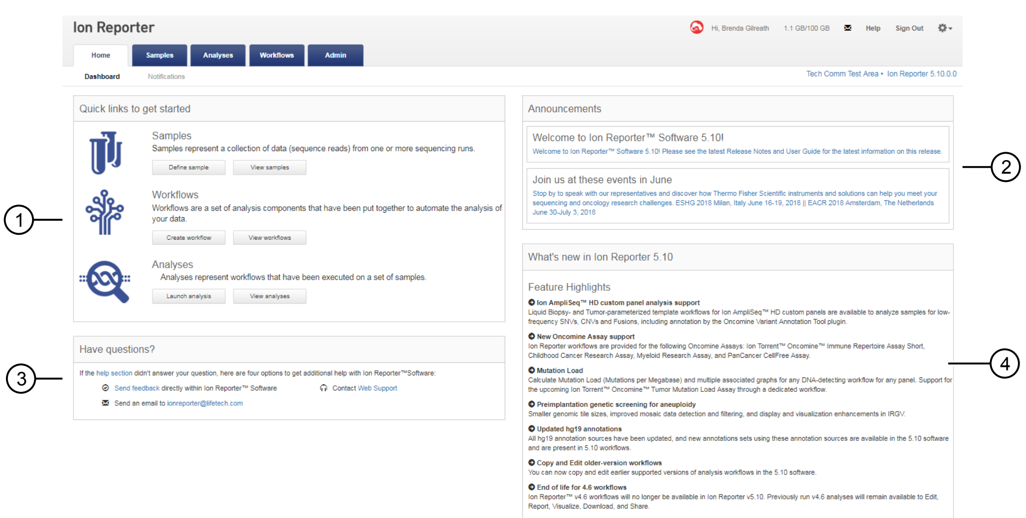


Ion Reporter Software functions in NGS workflow

Ion Reporter™ Software is a suite of bioinformatics tools that streamline and simplify the data analysis, annotation, and reporting of Ion Torrent™ semiconductor sequencing data.

Ion Reporter™ Software dashboard

You can open the dashboard and navigate to various screens of the Ion Reporter™ Software, view important information about the Ion Reporter™ Software, and access Support resources. Click **Home** tab, then click **Dashboard**.



1. Quick links pane
2. Announcements pane
3. Support pane
4. Information pane

The **Quick links** pane includes:

- **Define sample**—Opens the **Define Samples** screen, where you can upload and define samples.
- **View samples**—Opens the **Samples** screen, where you can view, search, sort, or filter samples.
- **Create workflow**—Opens the **Create Workflow** wizard, where you can create a new workflow.
- **View workflows**—Opens the **Workflows** screen, where you can view, search, sort, or filter workflows.
- **Launch analysis**—Opens the **Launch Analysis** wizard, where you can select a workflow, add samples and plugins, and launch an analysis.
- **View analyses**—Opens the **Analyses** screen, where you can view, search, sort, or filter analyses.

The **Announcements** pane includes a link to the latest Ion Reporter™ Software Release Notes and information on upcoming events.

The **Have questions?** pane includes:

- **Help section** link—Opens the software help menu.
- **Send feedback** link—Opens a help topic about how to send feedback regarding your experience with Ion Reporter™ Software.
- Send an email to **ionreporter@lifetech.com**—Opens Microsoft™ Outlook and creates a new email addressed to Ion Reporter™ Software support.
- Contact **Web Support**—Opens the **Services & Support** web page.

The **What's new in Ion Reporter** pane describes new features.

Note: Only the tabs and quick links corresponding to your role are displayed. See "User roles and permissions" on page 21 for more information.

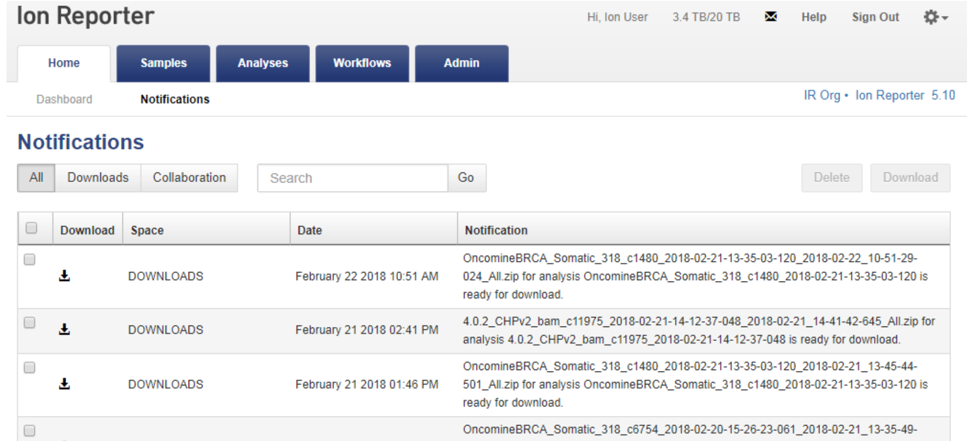
View notifications

The **Notifications** screen lists notifications for files that are generated by the Ion Reporter™ Software and for files that have been shared. Notifications are shown both for files that are shared by you with another user and for files that you share with another user.

From the **Notifications** screen, you can download completed analyses, reports, and files that have been shared with you by another user.

1. To view the **Notifications** screen, select one of the following actions.
   - In the **Home** tab, select **Notifications**.
   - From the home screen, click ✉ in the Ion Reporter™ Software menu bar.

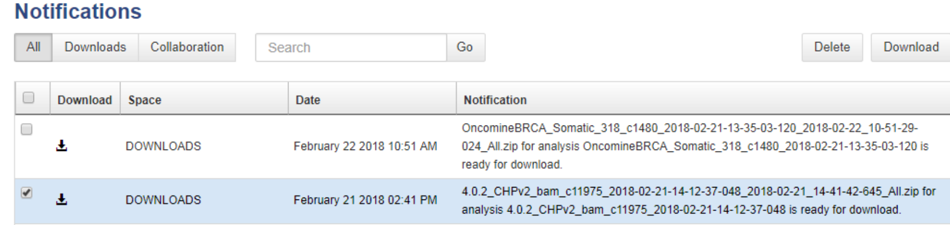
The **Notifications** screen appears.



The filename and status of a given file are shown in the **Notification** column.

2. *(Optional)* Click one of the following to change the list view.

Subtabs for Notifications window	Description
All	Lists notifications for all files that are available for download and files that have been shared.
Downloads	Lists notifications for all files generated within the software that are available for download.
Collaborations	Lists notifications for all files that have been shared.



3. *(Optional)* Enter a term in the search box to search for a file.

4. Select one of the following options.

Option	Description
Download a file	Select one of the following: • Click ⤓ in the row of a notification. • Select the checkbox of a notification, then click **Download**.
Delete the notification	Select the checkbox for a notification, then click **Delete**.

Note: Deleting a notification only deletes the notification from the notifications list and does not delete the file. To delete a file, see the appropriate procedures for that file type.

File type	Topics
Analyses	Analysis files contain the results of sequencing runs. For additional information, See "Analysis results" on page 165 for details.
Samples	Samples files contain a list of all samples from a sequencing run that are to be analyzed. See "Import and manage samples" on page 31 for details.
Reports	Reports are summaries of sequencing runs. For additional information, see "Create and view reports" on page 265 for details.

Ion AmpliSeq panel types

AmpliSeq.com offers four design and ordering options for Ion AmpliSeq™ and Ion AmpliSeq™ HD research panels:

- On -Demand Ion AmpliSeq™ research panels of optimized amplicons for germline analysis. Configurable to a specific human disease area, and ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ research panels of DNA and RNA designs for germline or somatic analysis of any genome, ordered in large reaction packs. Made-to-Order panels were formerly known as Custom Panels.
- Ready-to-Use Ion AmpliSeq™ research panels are predesigned DNA and RNA panels for germline and somatic analysis, ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ HD research panels are ultra high-sensitivity DNA and RNA designs for germline or somatic analysis of the human genome, including featuring dual barcoded amplicons, and bidirectional sequencing.

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 administrator-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 18.)

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 18.

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 17).

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 17.)

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 17 or "Join an existing Ion Reporter™ Software organization on Thermo Fisher Cloud" on page 18.

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.

Create an organization on an Ion Reporter™ Server

The first user on an Ion Reporter™ Server can create an organization. When you create an organization, you automatically are the first administrator-level user of that organization. An organization is more specific than an institute name. It defines a group of users who work together and share samples, data, analyses, and results. You can then begin to enter or import data into the software and invite other users to the organization to share data and results.

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

Note: You can create an organization on an Ion Reporter™ Server when you request a new user account on the server. You are not required to have a user account to make this request.

1. On software sign-in screen click, click **Register new account**.
2. Enter the following information in the **Request Account** dialog box: organization name, organization address, your first and last name, email, phone number.

   Note: If the Ion Reporter™ Server user is connected to the Internet, you must enable the checkbox in the reCAPTCHA dialog box.
3. Click **Submit**.
   A new organization is created and you become the organization administrator. You will receive an email link to set the password.
4. Click on the link from the email and set a password.
5. After the password is set, you can sign in using the new password. The first time you sign in, you must accept the **Terms and Conditions**.

You can now begin using the new Ion Reporter™ Server organization.

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 21.
  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 295.
  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

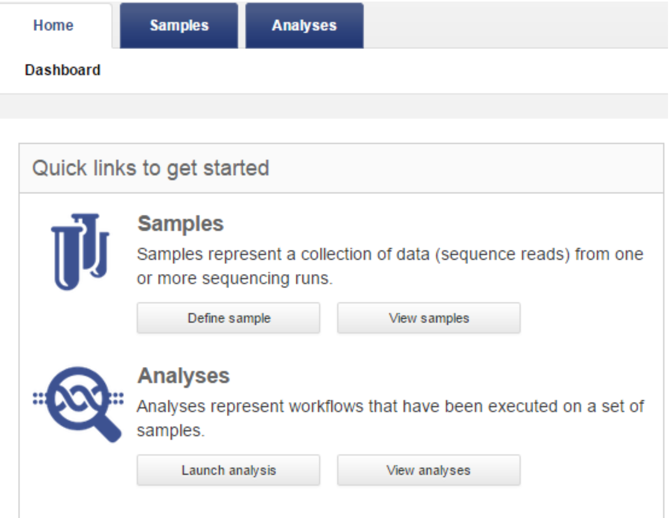
Ion Reporter™ Software grants access to specific functions based on roles that are assigned to users. The roles and functions are as follows:

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

User types

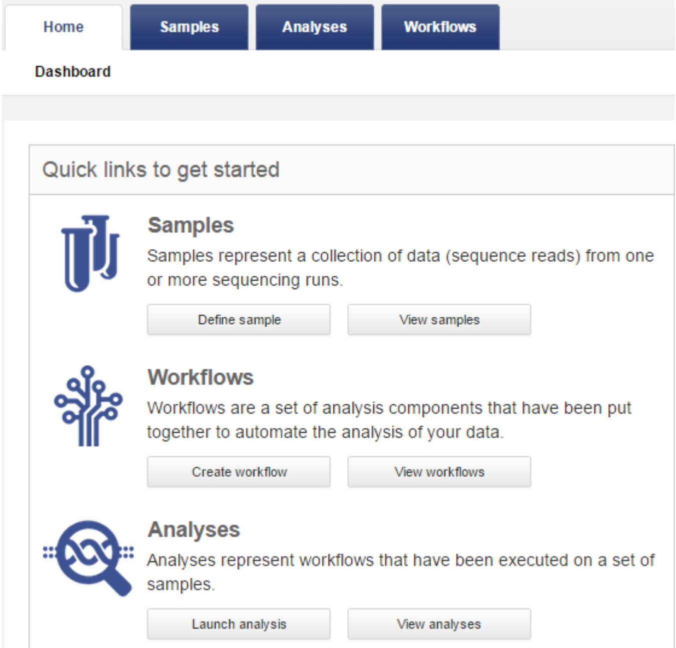
Each role within Ion Reporter™ Software has access to different areas of the software. The dashboard, links, and tabs for the various roles are shown here:

- **Import Role:**



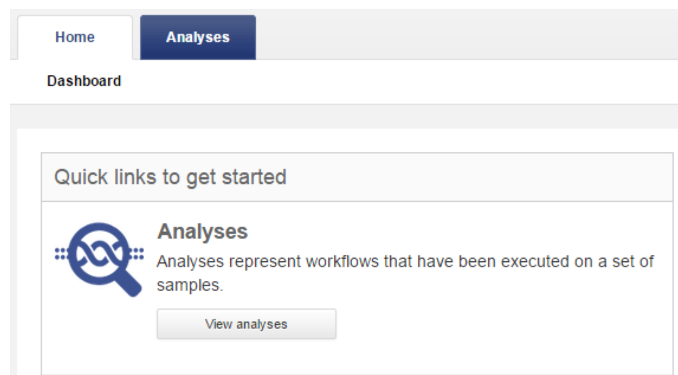
  - View samples
  - Define a sample
  - View analyses list (but not review results)
  - Launch an analysis
  - Access to the Samples and Analyses tabs

- **Analyze Role:**



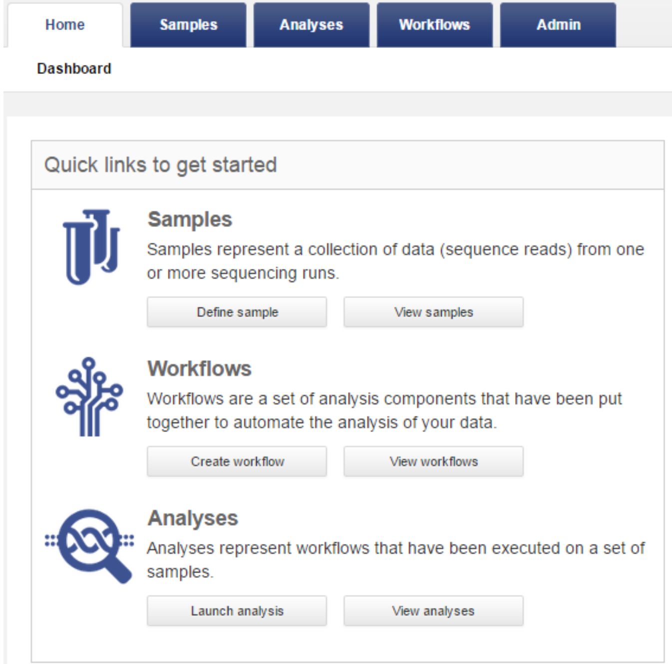
  – View samples
  – Define a sample
  – View workflows
  – Create a workflow
  – View analyses list (and review results)
  – Launch an analysis
  – Access to the Samples, Analyses, and Workflows tabs

- **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

Status alert emails

Ion Reporter™ Software sends an email to the user who is signed in and completes any of the following actions.

- Completes a sequencing run on an instrument that transfers run report files through the IonReporterUploader plugin. See "Integration with Ion Reporter™ Software" on page 35 and *Torrent Suite™ Software 5.10 Help*for more information.

  Note: For Ion Reporter™ Software 5.10 or later, a single email will be sent if multiple samples or barcodes are transferred from a single Torrent Suite™ Software run report. Additional emails will be sent when analyses are deleted from the batch, or if some analyses remain in the Pending or Running state, then complete at a later time.
- Creates an Ion Reporter™ Software account
- Resets a password
- Deletes an account
- Shares an analysis
- Unshares an analysis
- Creates a new organization
- Generates a report generation request

Note: Users with limited storage space will also receive a status email.

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 an 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 request is sent to the person in the report role to generate a report.

GRCh38 human reference

You can use the Ion GRCh38 human reference when you create custom workflows in 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.

AmpliSeq™ Designer currently offers one GRCh38 human reference and related target (BED) file for GRCh38 experiments. Optionally, you can also convert existing coordinates to GRCh38 by using a publicly available lift-over tool, such as **CrossMap**.

Note: If you edit earlier versions of workflows that use the hg19 reference genome, you cannot change the reference genome to Ion GRCh38.

See the following topics for more information about custom workflows:

- "Create a new custom workflow without predefined settings" on page 72
- "Create a Copy Number baseline" on page 124
- "Launch an analysis" on page 135

Requirements for GRCh38 custom BED file

If you create a custom BED file for a custom analysis that uses the GRCh38 reference, consider the following:

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.

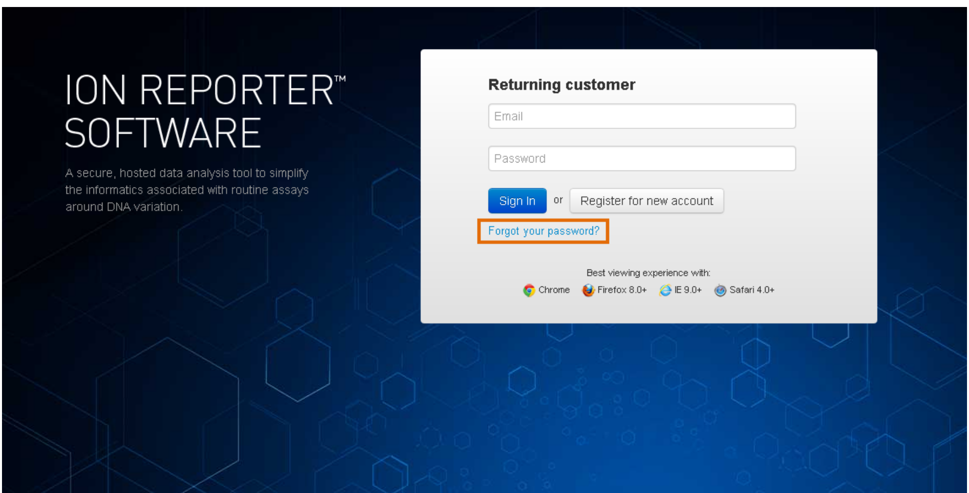
If a URI is desired, define an additional key=value pair in the format referenceURI=http://www.thermofisher.com/GRCh38.p2.fasta .

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?

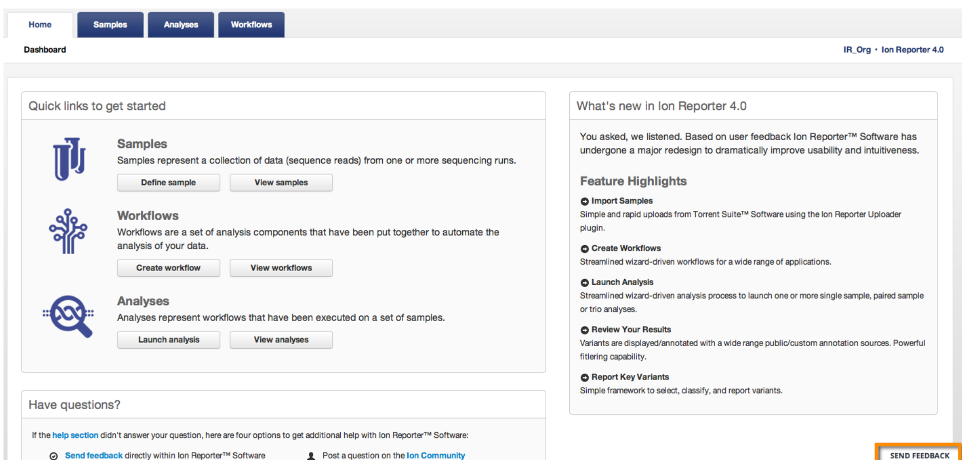
- If you are a server user and have been locked out of your account (system locks you out after five attempts to login in with an incorrect password), you will need to contact your administrator to reset your password. For details on password reset, see "Set or reset the password on a user account" on page 290.
- If you are a cloud user, click the **Forgot your password?** link on the sign in form.



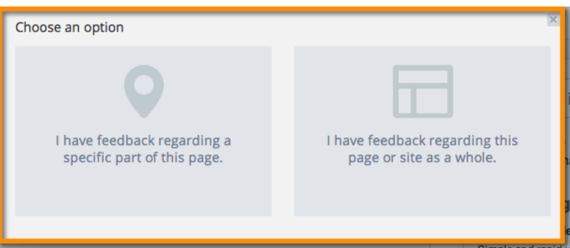
How can I provide feedback?

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

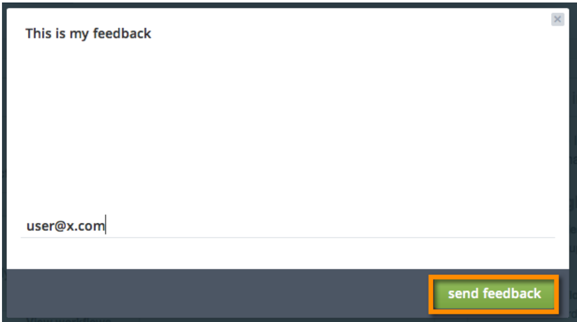
1. Navigate to the page you would like to provide feedback on. In the bottom-right corner of the page you see a **Send Feedback** button.



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**.

Are there any naming restrictions?

- Most names in Ion Reporter™ Software, including those used for analyses, samples, custom workflows, and users, require at least three characters. The name field appears in red with a bold outline if fewer characters are entered.
- Ion Reporter™ Software does not support either a first name or last name of only two characters. If a name has less than 3 characters, you can append the name with an underscore (_) or number.

What special characters are allowed in names?

- Most names in Ion Reporter™ Software, including those used for analyses, samples, custom workflows, users, and other text fields 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.

Set default number of rows displayed in tables

You can set a default number of rows displayed in all tables in the Ion Reporter™ Software.

1. In the upper right corner of the screen, click ⚙ **(Settings)** ▸ **Account Preferences**
2. In the account Preference dialog box, select the number of rows to display in all tables from the **Default rows per page** dropdown menu, then click **Save**.
   The new settings are applied to all tables within the Ion Reporter™ Software.
3. *(Optional)* To override the default setting for a specific table, navigate to the table, then select the number of rows you want to display from the **items per page** dropdown menu located below the table.

Purchasing and ecommerce

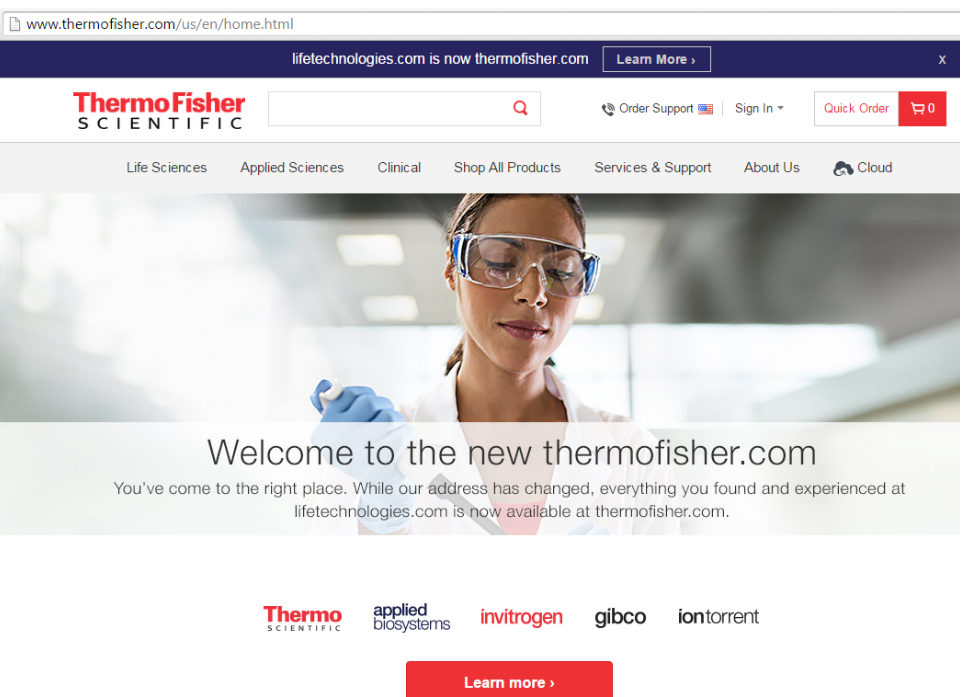
You can use your Thermo Fisher account credentials to manage your Ion Reporter™ Software and related third-party applications.

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

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**.

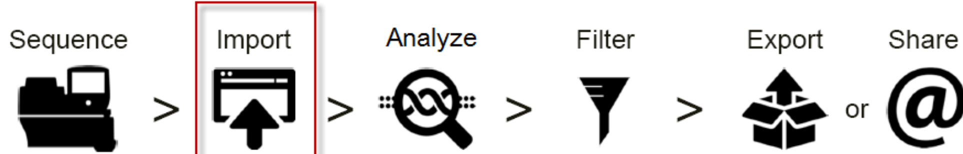
3. Fill out the requested information, then click **Create account**.

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



There are three primary methods to transfer data into Ion Reporter™ Software.

- Use the IonReporterUploader plugin.
- Import a VCF file into Ion Reporter™ Software.
- Use the IonReporterUploader command-line utility.

The following topics explain how to manage samples in Ion Reporter™ Software:

- 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 or BAM file into Ion Reporter™ Software

You can import a VCF or BAM file from an Ion Reporter™ Server and define it as a sample so that its variants can be annotated with Ion Reporter™ Software with the use of 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 the IonReporterUploader command-line utility to upload your VCF files to Ion Reporter™ Software. You can also upload VCF files from an Ion Reporter™ Server. See "Integration with Ion Reporter™ Software" on page 35 for more information.

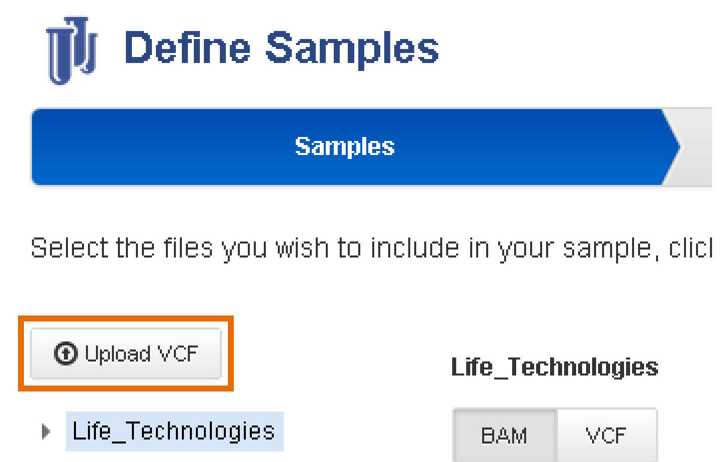
These instructions both import the raw VCF file from your local machine and also define an Ion Reporter™ Software sample from the VCF file.

When you import a VCF file:

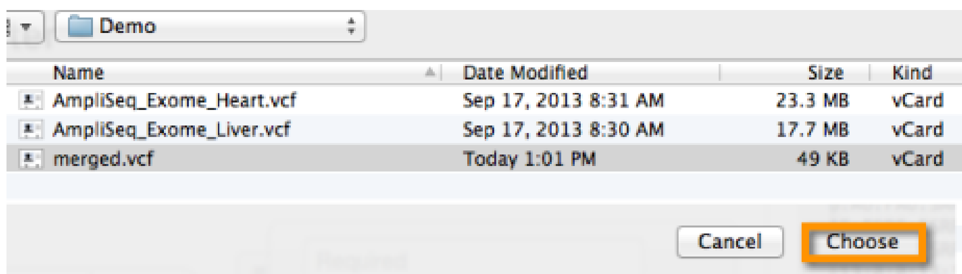
- Annotation-only workflows accept VCF files that conform to the 4.1 VCF standard only.
- Ion Reporter™ Software also accepts a compressed VCF file in .gz format as input for annotation-only workflows. Ion Reporter™ Software unzips the .gz file and works with the resulting VCF file.
- Ion Reporter™ Software 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***

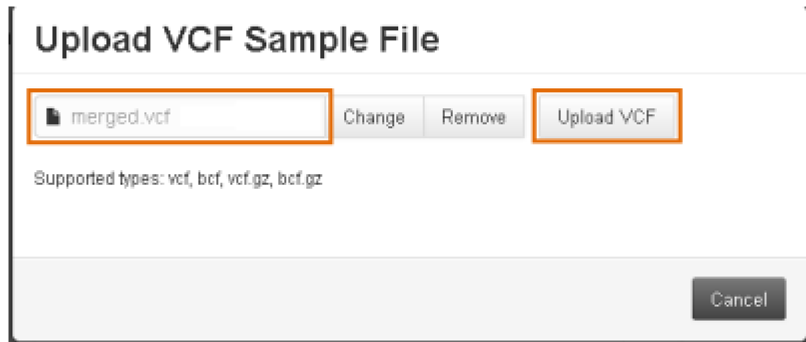
1. In the **Home** tab, click **Define sample**.
2. Click **Upload VCF** in the **Define Samples** screen.



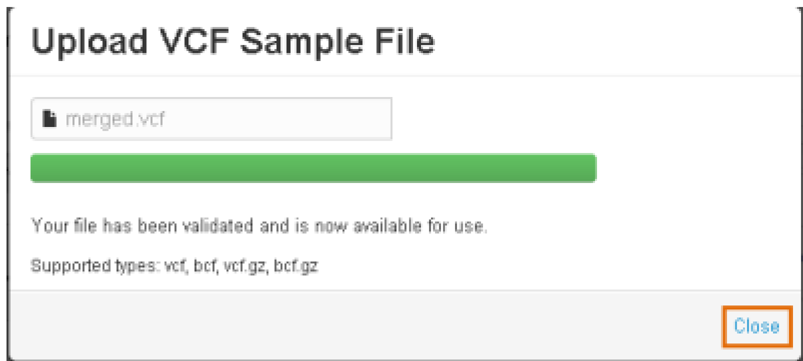
3. In the **Upload VCF Sample File** dialog box, click **Select File**.
4. Browse to the VCF file on your local machine and click **Open** or **Choose** (depending on your browser).



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



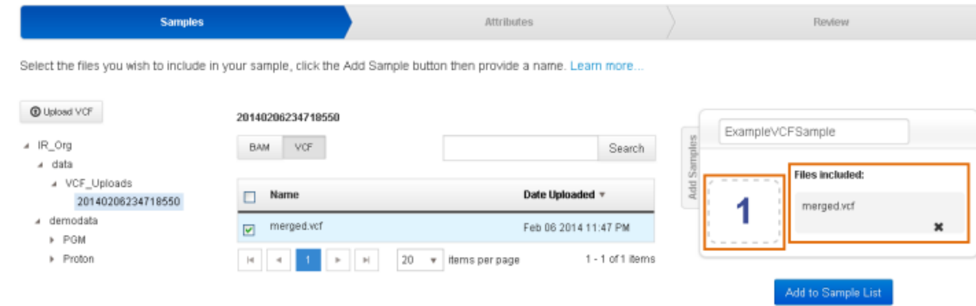
6. Wait while your VCF file is verified.
   When verification is complete, click **Close** .



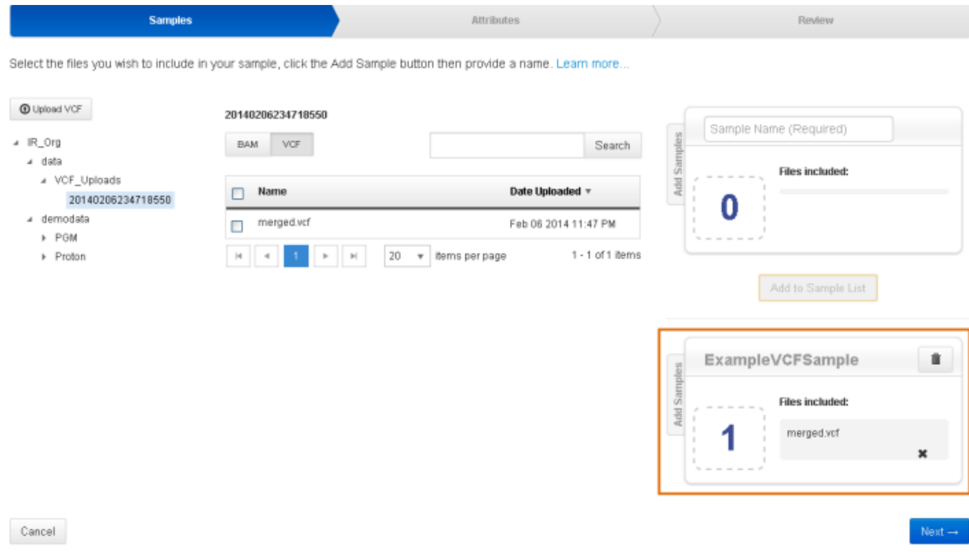
   Your new file is listed in the **Samples** table, and in the left panel as a time-stamp under VCF_Uploads.

7. In the **Samples** table, enable the checkbox to the left of the VCF file name.
   The row is highlighted and the **Add to Sample** tab is enabled.

8. Enter a descriptive (and unique) name for the sample in the **Sample Name** field, then click **Add to Sample**.
   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*.

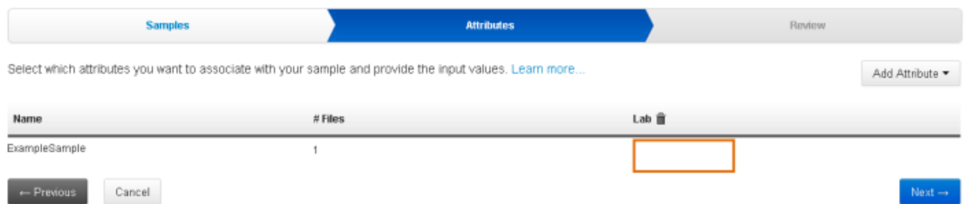


9. Click **Add to Samples List**.
   A new box appears below the **Add to Sample** list. This box contains the data file and sample name part of your sample definition.

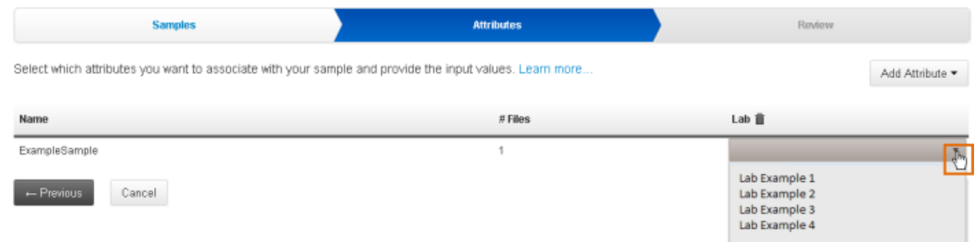


10. Click **Next**.

11. *(Optional)* To set an attribute, click the area under an attribute heading to set the attribute value.

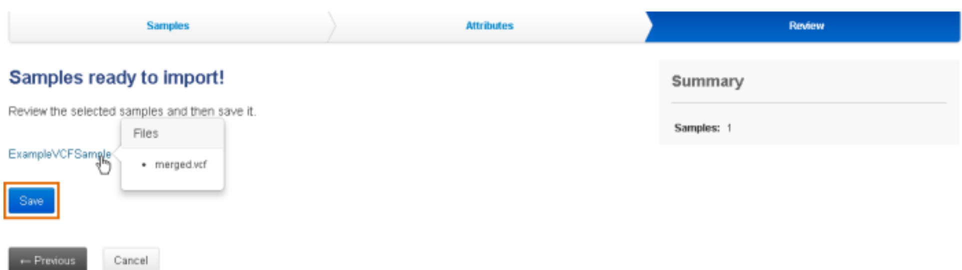


(In this example, Lab is a user-defined attribute. Click the expansion arrow to open the attribute menu and make your selection.

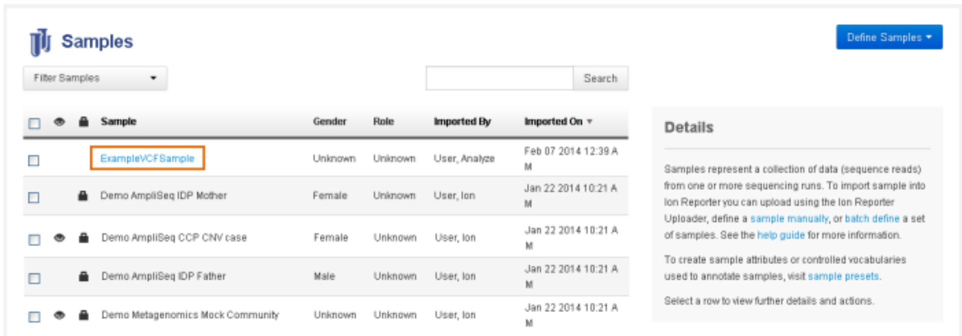


12. Click **Next**.

13. In the summary , 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 screem opens, with your new sample in the sample table. Your new sample is ready to be analyzed.



Integration with Ion Reporter™ Software

Ion Reporter™ Software performs analysis on BAM files that are output from Torrent Suite™ Software. VCF output files, that result from using the variantCaller plugin, can also be transferred and used for Ion Reporter™ Software analyses, provided that an annotation-only workflow is used to process the files in Ion Reporter™ Software.

Note: Ion Reporter™ Software is available under separate license and is not included with Torrent Suite™ Software.

To transfer these BAM and VCF output files to Ion Reporter™ Software, you must add one or more Ion Reporter™ Software accounts. You can add accounts at any time, or when you configure the IonReporterUploader plugin. After an account is configured,

there are several ways that you can transfer files to an organization in Ion Reporter™ Software.

- Torrent Suite™ Software can automatically transfer file from a completed run report to Ion Reporter™ Software and either:
  - Available as analyses in Ion Reporter™ Software. In this case, the output files are transferred to Ion Reporter™ Software and the workflow of your choice for use in Ion Reporter™ Software is automatically launched on your newly transferred samples.
  - Available in Ion Reporter™ Software as BAM and VCF files that can be later defined as samples in Ion Reporter™ Software.
- You can optionally choose to manually upload of data to Ion Reporter™ Software Use this option if, for example, you want to upload data to Ion Reporter™ Software multiple servers. To manually upload data from Torrent Suite™ Software to Ion Reporter™ Software, do one of the following:
  - Run the plugin manually from a completed run report.
  - Select the option to review results from a completed run in Torrent Suite™ Software when you create a Planned Run.

Note: When the IonReporterUploader plugin defines samples from the newly transferred samples for Ion Reporter™ Software, sample relationships for paired and trio samples and sample attributes are also defined. For details, see “Sample gender“ on page 38.

IonReporterUploader command-line utility

You can use the IonReporterUploader command-line utility to transfer results files to Ion Reporter™ Software and to define samples, based on the transferred files. The IonReporterUploader command-line utility uses your login ID to transfer run data from Torrent Suite™ Software to Ion Reporter™ Software. The utility supports these transfer scenarios:

- Transfer a single BAM or VCF file
- Transfer all results files for a Torrent Suite™ Software analysis
- Transfer results files that are in a single flat folder
- Transfer multiple files that are not restricted to a single folder

You can later analyze the samples with Ion Reporter™ Software. You can enter IonReporterUploader command-line utility options through command line arguments, or through a properties file.

Note: IonReporterUploader command-line utility should not be used for uploading samples from references other than hg19 and GRCh38. Although E-coli and animal reference genomes can be used in Torrent Suite™ Software, they are not supported in Ion Reporter™ Software.

The IonReporterUploader command-line utility can be run on any of the following:

- A Torrent Server, Ion S5™ XL Server, or Ion GeneStudio™ S5 Prime Server
- A standard Linux™ computer
- A standard Windows™ computer that uses the Windows™XP operating system or later
- A standard Macintosh™ computer

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.

The IonReporterUploader command-line utility is an alternative to the IonReporterUploader plugin that is included with Torrent Suite™ Software. You can download the IonReporterUploader command-line utility at: **http://iru.ionreporter.thermofisher.com/**. For information on IonReporterUploader plugin, see the Torrent Suite™ Software Help, or the *Torrent Suite™ Software User Guide*.

Download IonReporterUploader command-line utility

This procedure explains how to download and install the IonReporterUploader command-line utility from Ion Reporter™ Software. The procedure may vary, based on the operating system of the target computer. In general, decompress the downloaded directory on your target machine, then copy the directory `IonReporterUploader-cli` to a convenient location.

Ideally, you should download the IonReporterUploader command-line utility onto the computer where it will be run. At a minimum, you must use a computer with the same operating system.

IMPORTANT! Use only the decompression utility available on your local computer. Do not decompress files on a different operating system and copy those files to a computer that uses a different operating system.

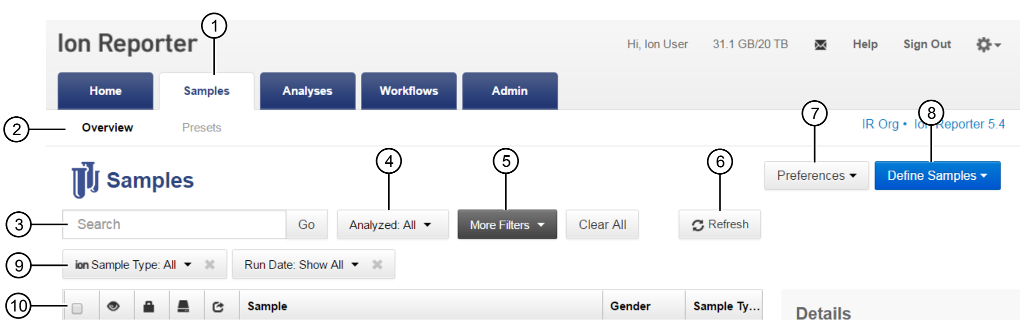
1. Sign in to Ion Reporter™ Software, then click **Settings (⚙) ▸ Download Ion Reporter Uploader**.
2. Click the filename `IonReporterUploader-cli.zip`, then download the file to the target computer.
3. Extract the downloaded `IonReporterUploader-cli.zip` file, then copy the `IonReporterUploader-cli`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

Run IonReporterUploader command-line utility

The IonReporterUploader command-line utility `irucli` is ready to run after you extract it. Run the IonReporterUploader command-line utility from the `IonReporterUploader-cli` bin directory (with the `irucli.bat` or `irucli.sh` script). Instructions to use the IonReporterUploader command-line utility are downloaded with the utility.

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**

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.

Sample gender

Several workflows in Ion Reporter™ Software, especially copy number variation detection and Ion AmpliSeq™ IDP trio, are limited when the sample gender is unknown, and they return unexpected results when the gender is incorrectly specified.

For example, in the Ion AmpliSeq™ IDP trio workflow, when the gender of the proband is not known, variants cannot be assigned in the categories HasMaleMaternalX and HasUnknownX.

If a sample with no gender was transferred from Torrent Suite™ Software to Ion Reporter™ Software, go to the **Sample ▸ Sample Management** screen in Ion Reporter™ Software and edit the sample to specify the gender attribute.

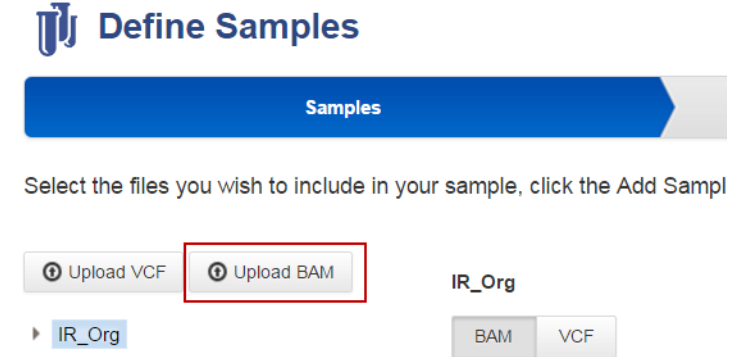
Note:
- You cannot edit samples that have been launched in an Ion Reporter™ Software analysis. Instead, define new samples from the raw data files, and add the correct gender metadata to the new samples.
- If the gender of the sample is not specified or specified as "Unknown", the Integrative Genomics Viewer (IGV) uses female as the gender.

Upload a .bam file as a sample

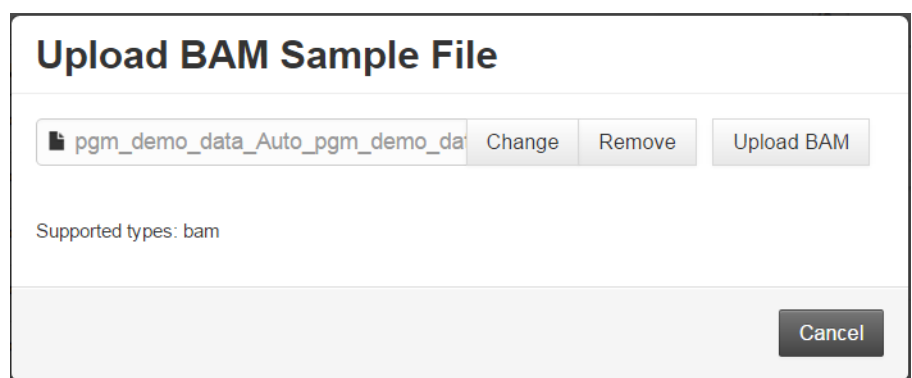
You can upload a .bam file as a single sample.

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.

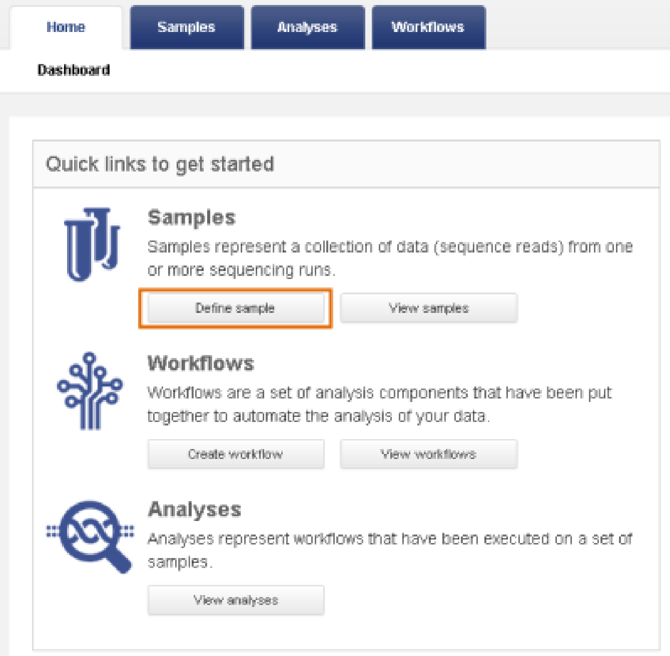
Note: If you used the Ion Reporter™ Uploader plugin or the Ion Reporter™ Command-line Uploader to upload data, the sample definition is done automatically and you do not need to use this procedure.

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

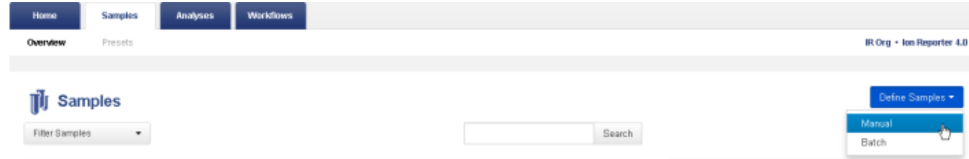
IMPORTANT! Multiple BAM files can be combined into a sample. Multiple VCF files are not supported and mixed file types are not supported.

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

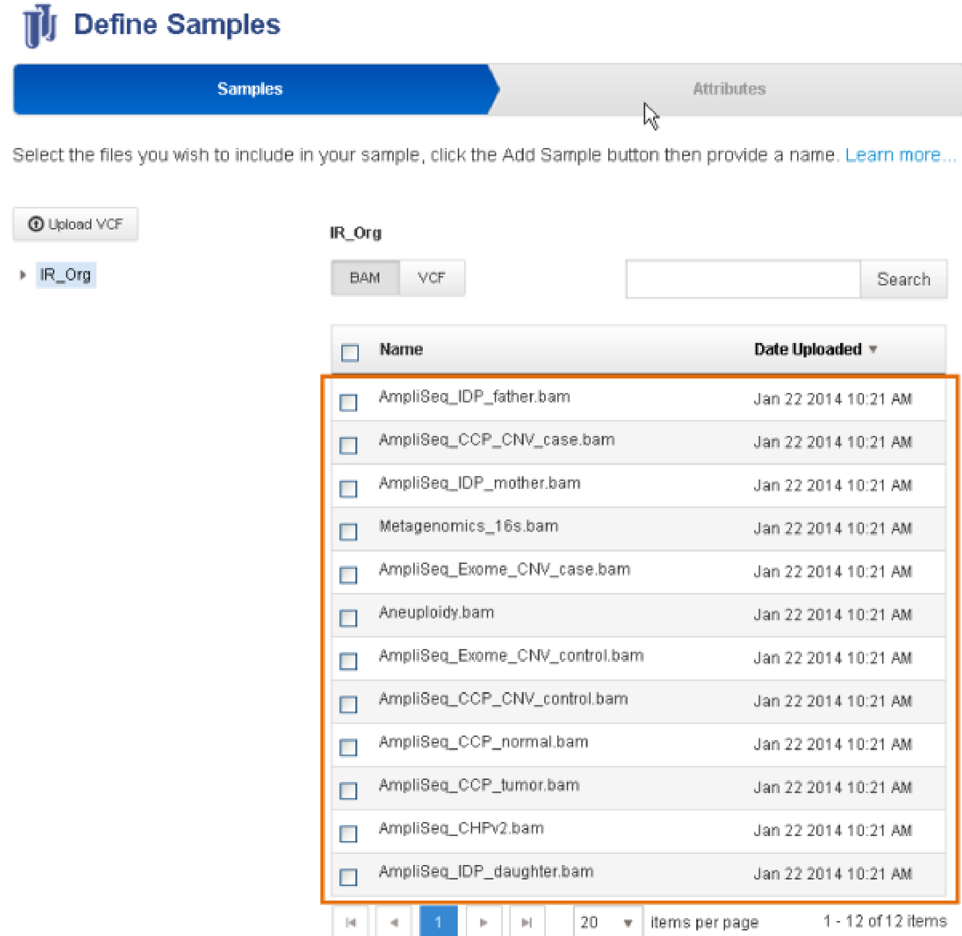
1. Log in to the Ion Reporter™ Software, then do one of the following:
   - Click the Home tab, and then select **Define sample**:



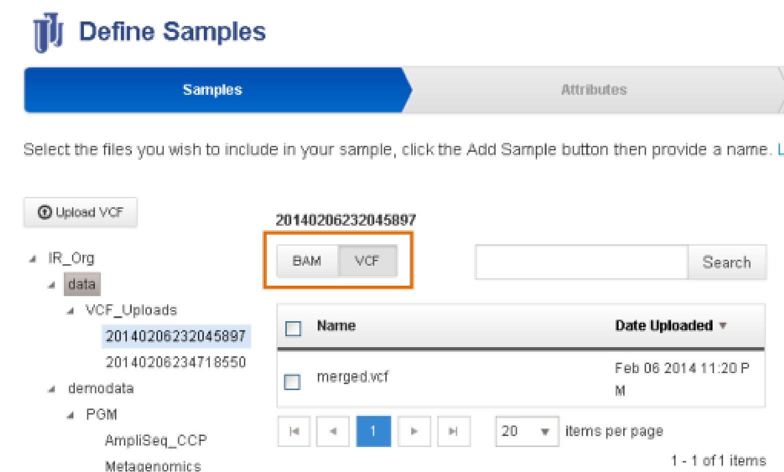
   - Or, click the **Samples** tab, select **Overview** and then click **Define Samples** ▸ **Manual** .



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.

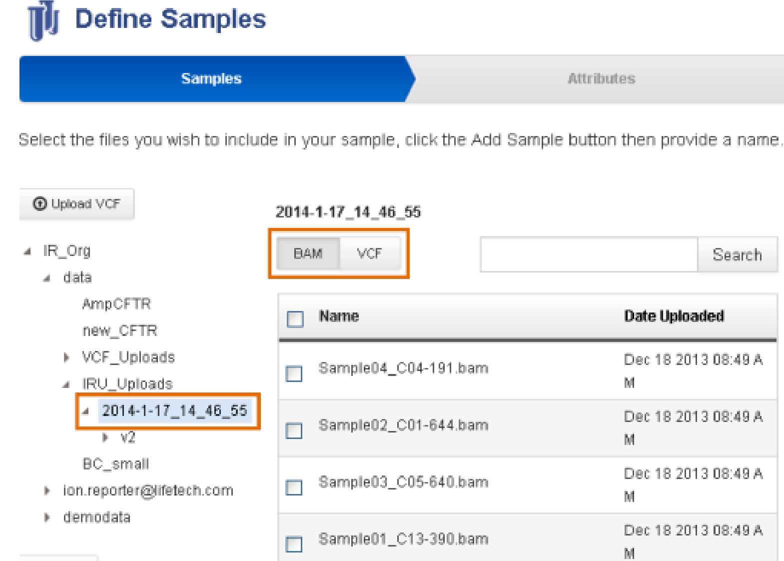


2. Click **BAM** or **VCF** above the list to filter for either .bam or .vcf files. VCF is selected in this example.



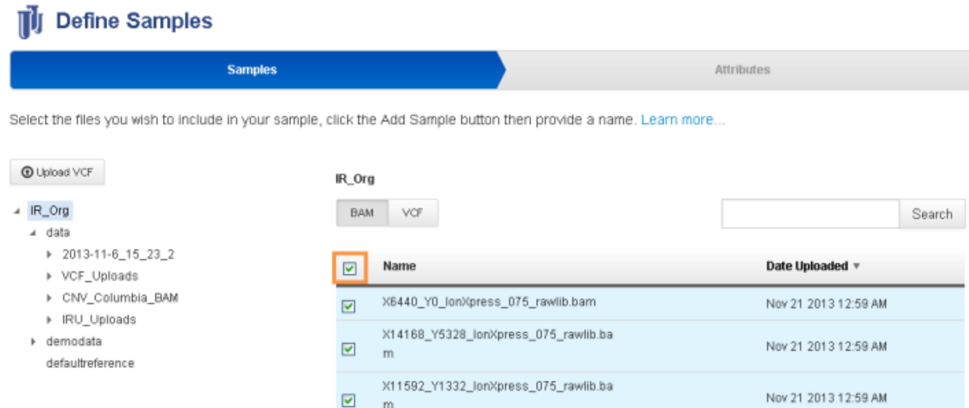
3. To view the files in either your upload directory or the demodata directory, expand the menu on the left that matches your organization name, then expand the data file directory that contains data files of interest.

   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.

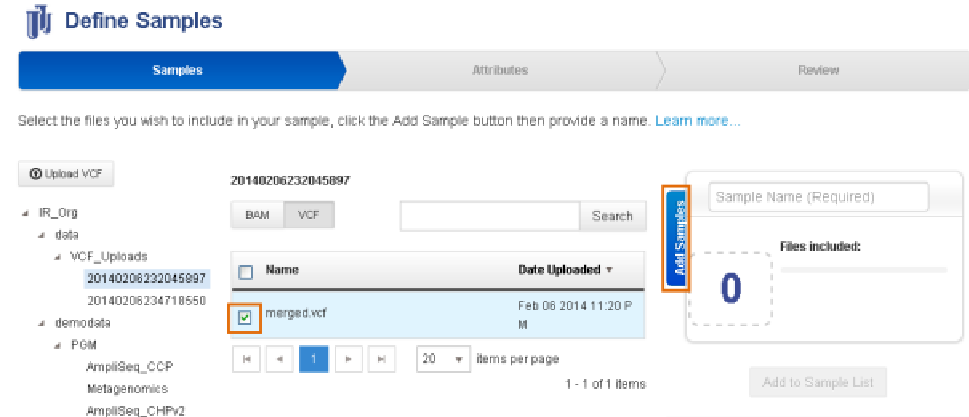


Note: Files transferred by IonReporterUploader appear under data/IRU_Uploads, in time stamp folders. Demo data files appear under the demodata folder.

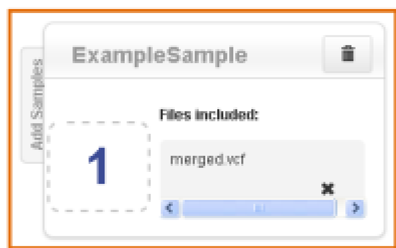
4. Enable the checkbox for each data file that you want to add to your sample, and then click **Add to Sample**. To select all samples in the list, click the checkbox in the header.



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.

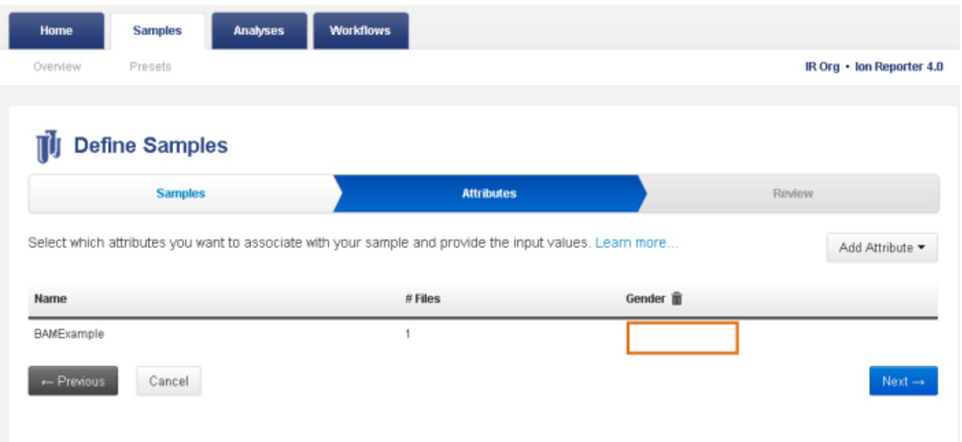


5. Enter a descriptive name for your new sample in the field at the top of the sample list. When the sample box contains the correct data files and the sample name for your sample, click **Add to Sample List**.

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.

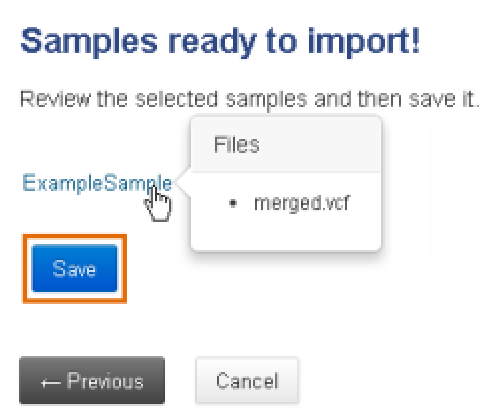
7. Click **Next**.
   - If you have any additional attribute information to associate with the sample, click **Add Attributes**. A new column opens for your attribute.
   - To set an attribute, click the area under an attribute heading to set the attribute value. Gender is selected in this example.



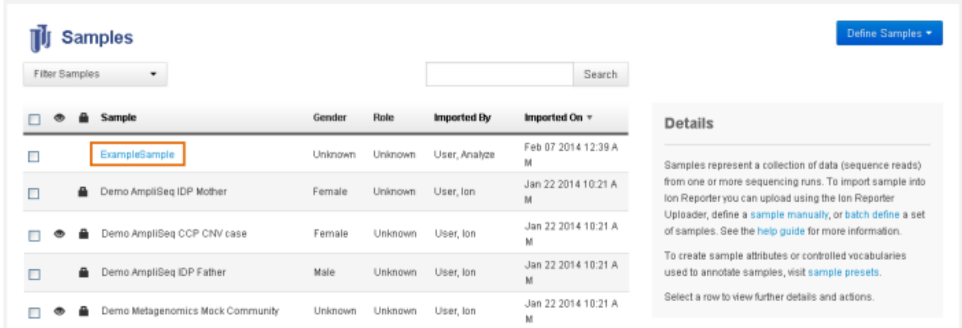
Note: The range for the cellularity attribute is 0 to 100.

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

9. In the Review , 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.

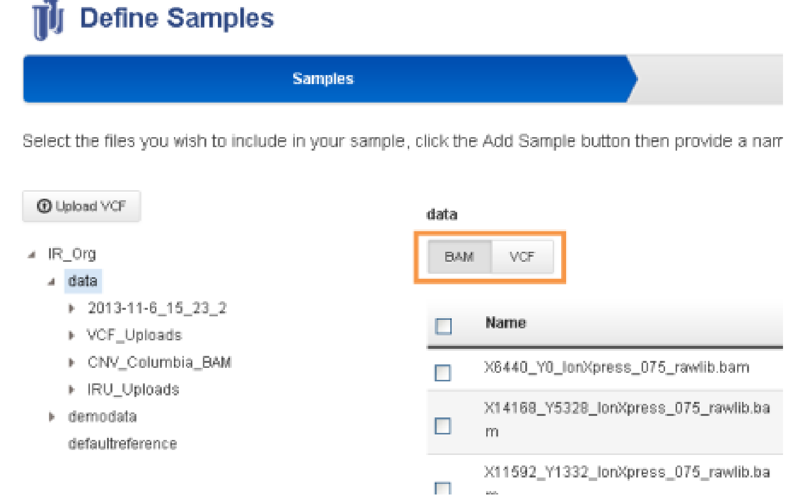


Your new sample is ready to be analyzed.

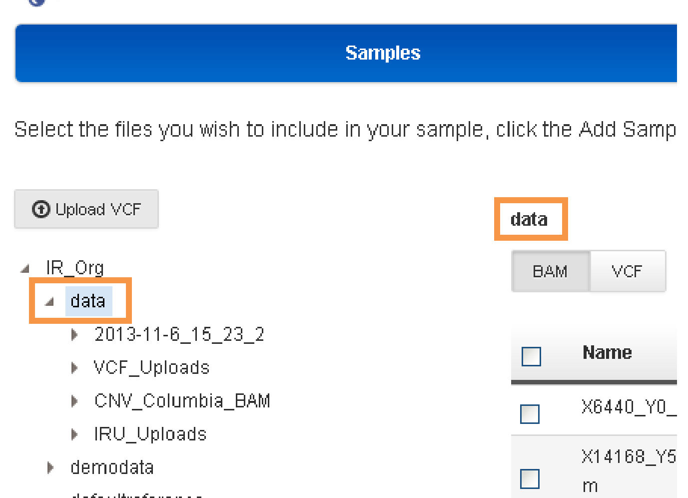
Notes about manual sample definition

Keep the following in mind when defining samples:

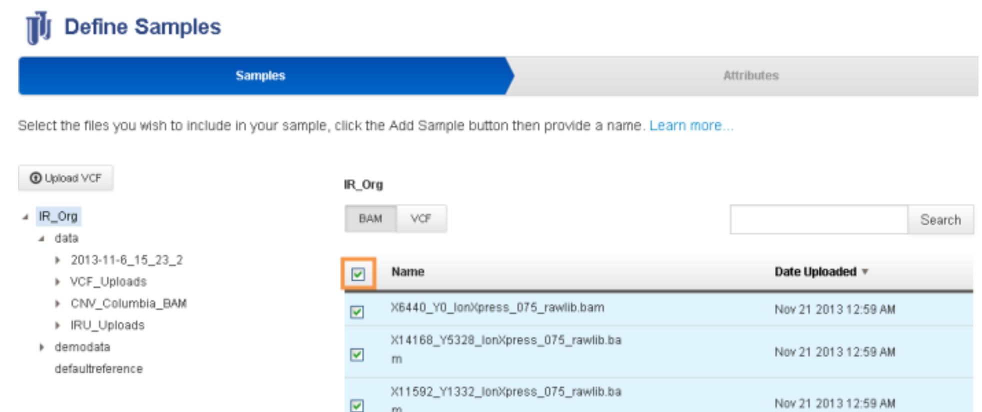
- The table lists either BAM or VCF files (not both at the same time). Click **BAM** or **VCF** to change the list. The file type that is not selected is filtered out and those files do not appear in the table.



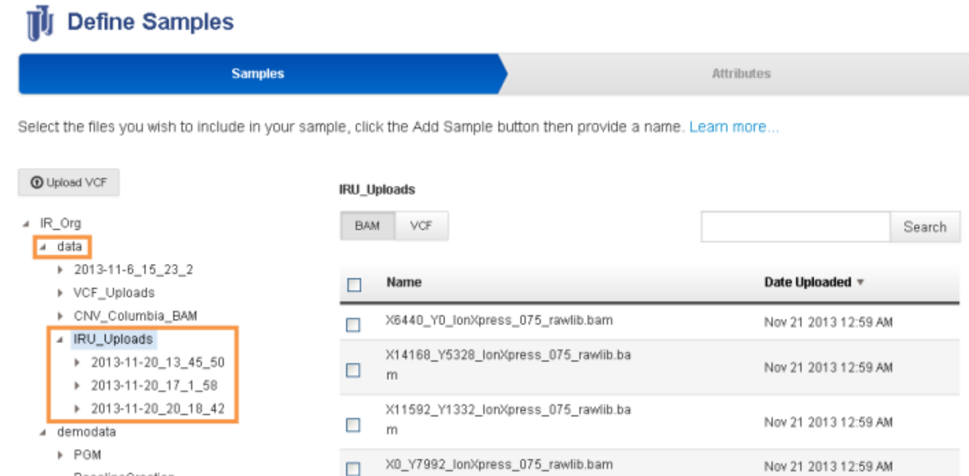
- The data file browser on the left provides a cumulative list of all files at and below the folder that is highlighted in blue. When you click a folder in the data file browser, the list in the table changes to reflect the contents of the selected folder. The selected folder name also appears at the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



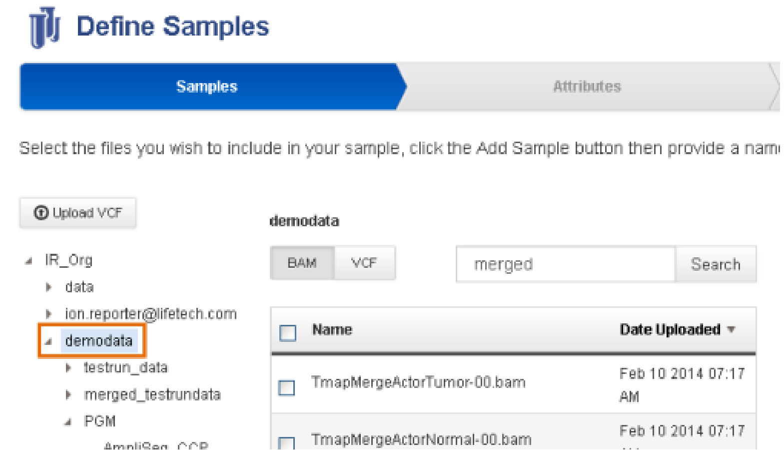
- Click the checkbox in the header to select all samples in the selected directory.



- 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.

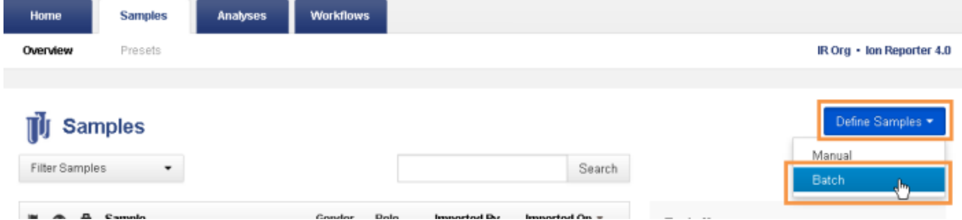


- Multiple BAM files can be combined into a sample. Multiple VCF files are not supported and mixed file types are not supported.
- The range for the cellularity attribute is 0 to 100.

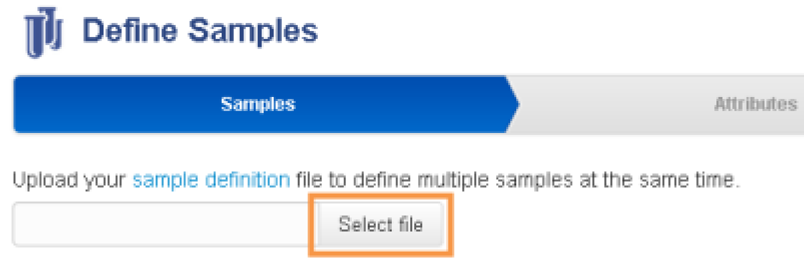
Define a sample via CSV upload

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

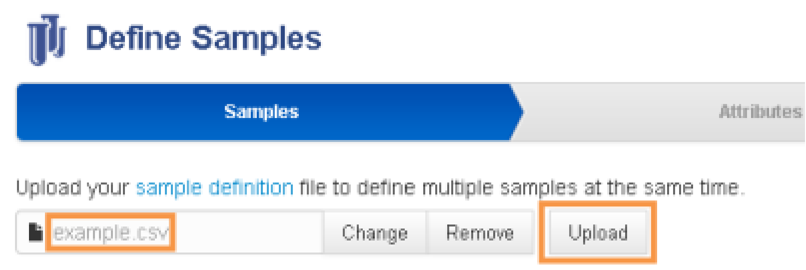
1. Log in to the Ion Reporter™ Software and click the **Samples** tab.
2. Click **Define Samples** ▸ **Batch**



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



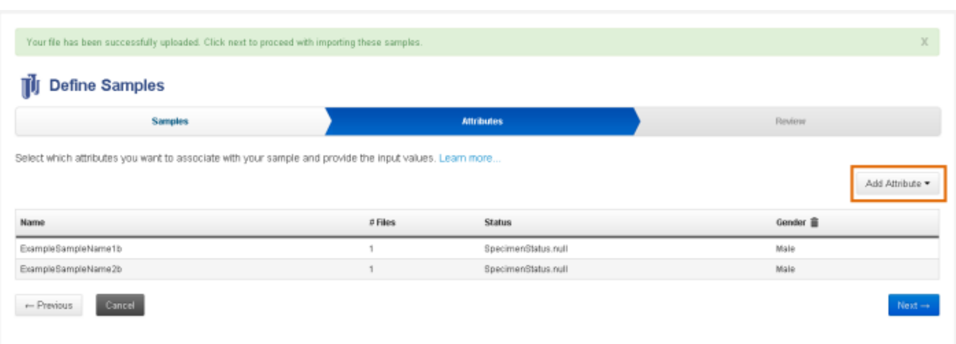
4. Confirm the displayed filename and click **Upload**.



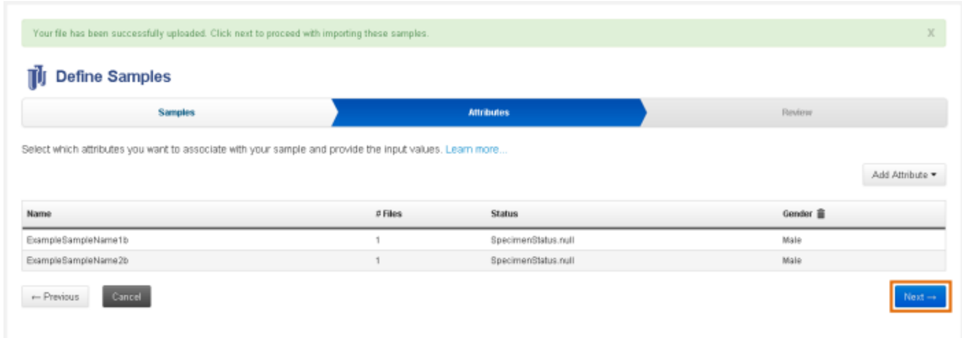
Wait while your file is verified.

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

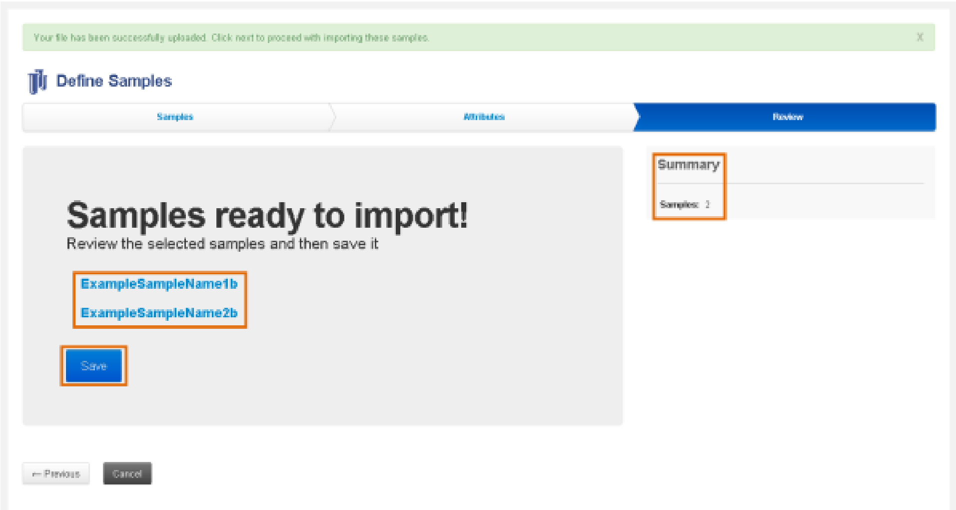
6. (*Optional*) If you have any additional attribute information to associate with the sample, click **Add Attributes**. A new column opens for your attribute.



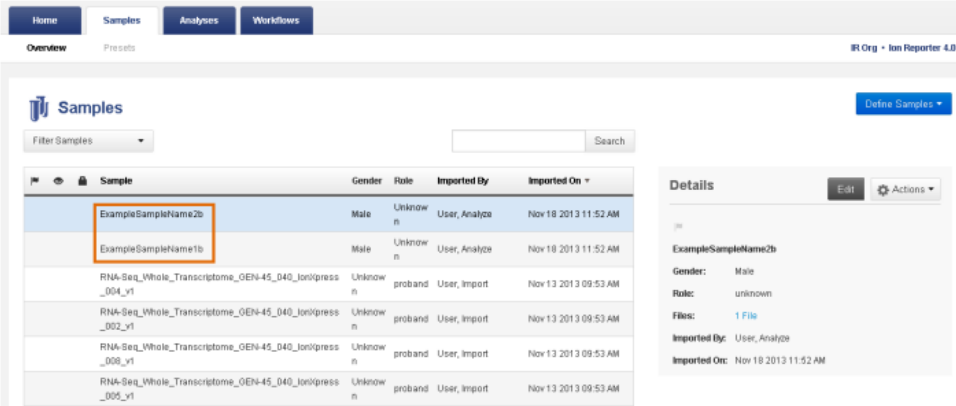
7. When your sample information is complete, click **Next**.



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

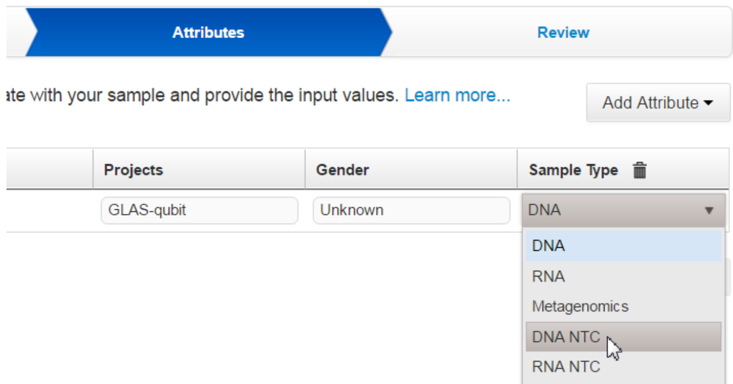
No-template control (NTC) samples can be defined in Torrent Suite™ Software or Ion Reporter™ Software.

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

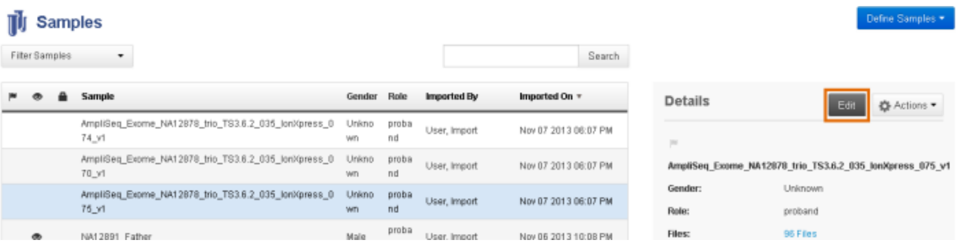
When you edit an existing Ion Reporter™ Software sample, you can add raw data files to the sample, remove raw data files from the sample, or edit the sample attributes.

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:

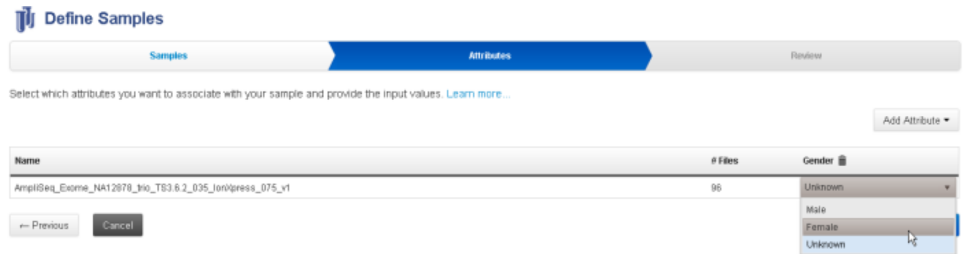
1. Log in to the Ion Reporter™ Software application and click the Samples tab.

2. Select the sample that you want to edit from the table, and then click **Edit** in the sample Details.



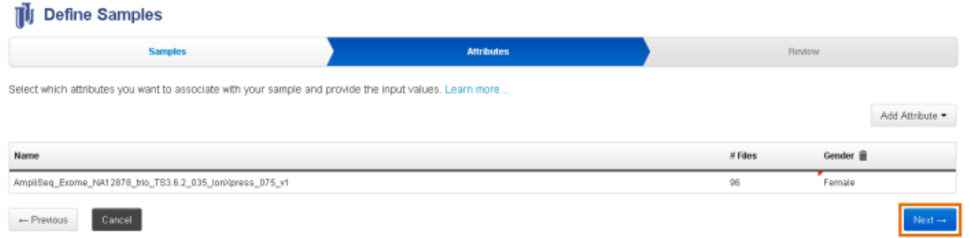
3. Add the raw data files from the table to your sample.

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**.



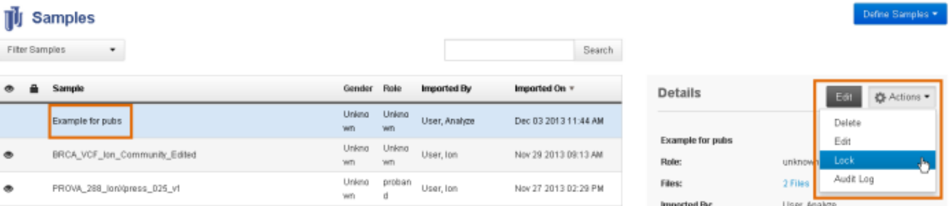
7. In the Review chevron, click **Save** .

Lock a sample

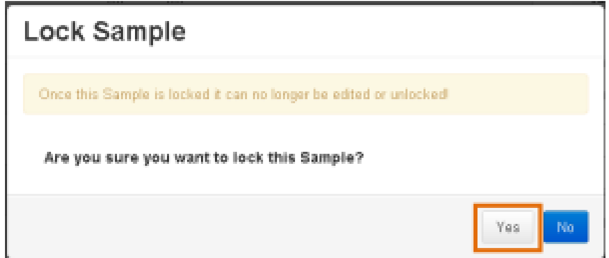
IMPORTANT! After you lock a sample, you are no longer be able to edit the file or attributes associated with it. To edit a sample that has been locked, you must first define a new sample.

Follow these steps to lock a sample:

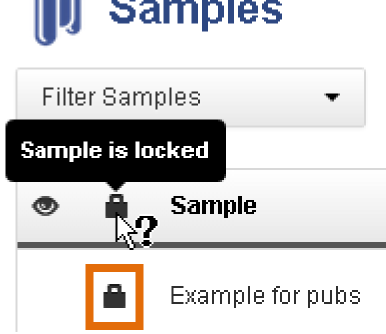
1. Log in to the Ion Reporter™ Software application and click the **Samples** tab.
2. Select the sample in the table, then click **Actions** ⚙ ▸ **Lock** from the in the sample details section.



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 🔒.

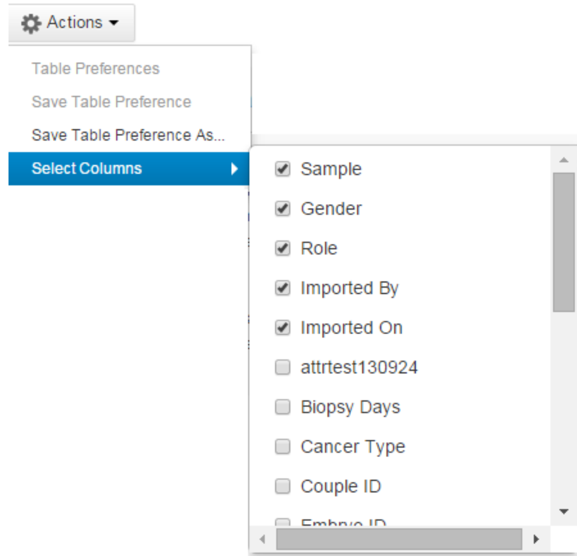


Add sample attributes to Ion Reporter™ Software

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.

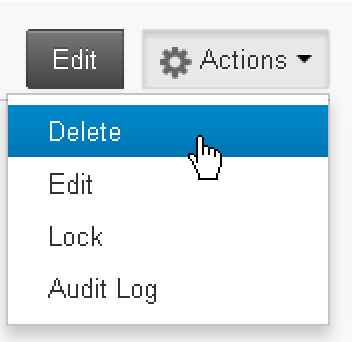


The sample attributes are now added as columns in the Samples table.

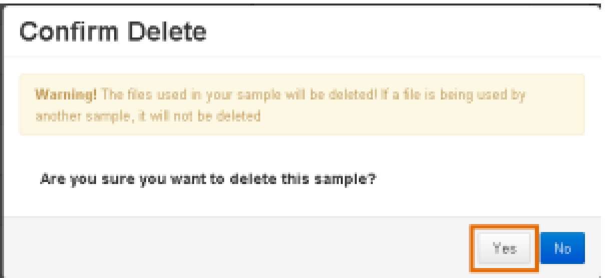
Delete a sample

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.

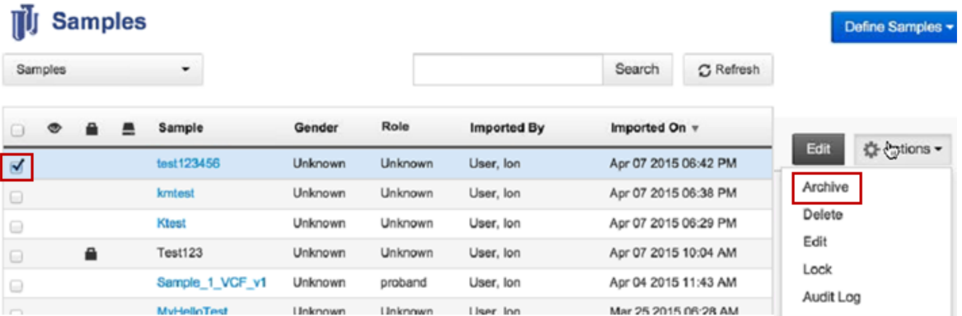


Your sample is deleted and you are brought back to the Samples Overview page.

Archive samples (local server only)

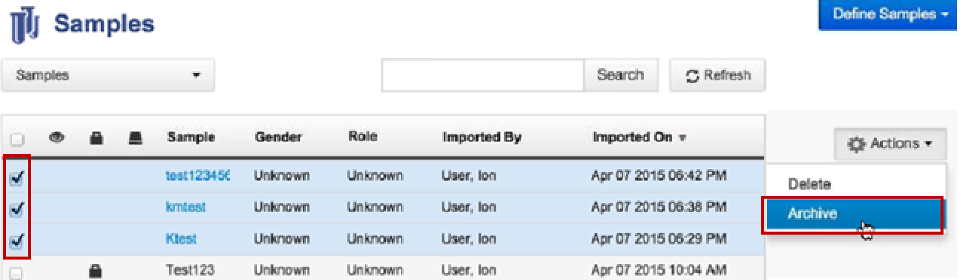
If you have a local Ion Reporter™ Server, you can archive samples to your Torrent Suite™ Software storage device to free up disk space.

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

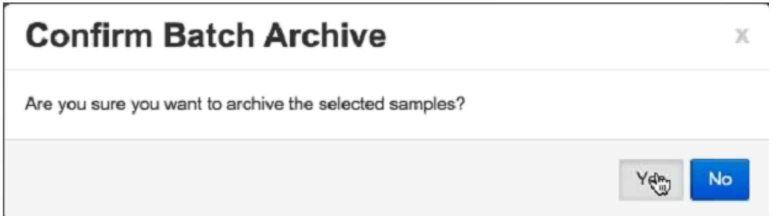


The sample now has an archive icon in its row.

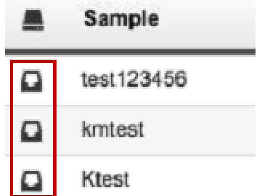
3. You can also select multiple samples and archive them.



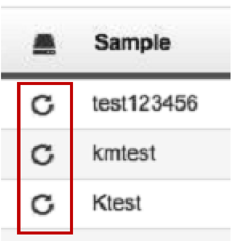
4. Click **Yes** to confirm batch archive.



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.

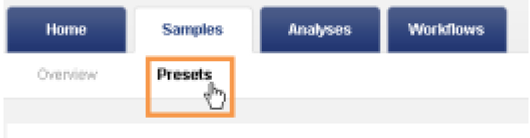
Ion Reporter™ Software supports the following types of attributes:

- 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.

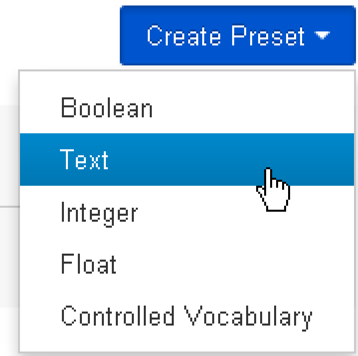
Note: Ion Reporter™ Software gives you the option to encrypt the internal storage for a sample attribute. In the preset form, click the **Encrypted** checkbox.

This example shows a text sample preset:

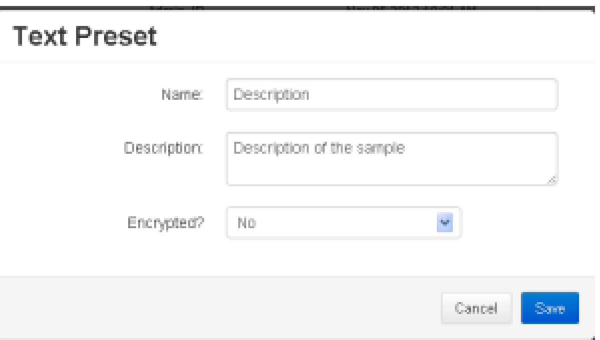
1. Log in to the Ion Reporter™ Software application. Click the **Samples** tab, then select **Presets**.



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

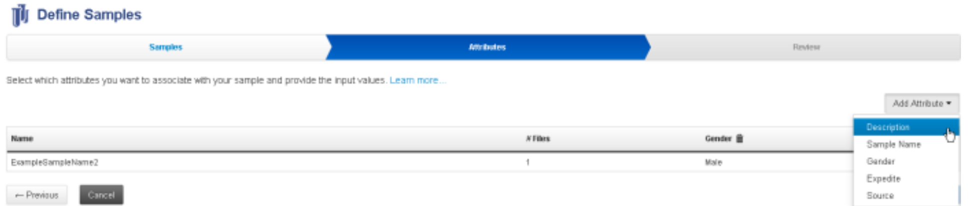


3. Fill out the preset form with the attribute name and your description of the meaning of the attribute. The name that you enter is used for the attribute during sample definition.

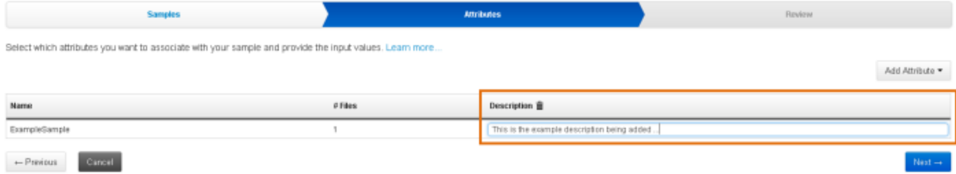


4. *(Optional)* If you want the internal storage for the attribute to be encrypted, click the **Encrypted** checkbox.

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

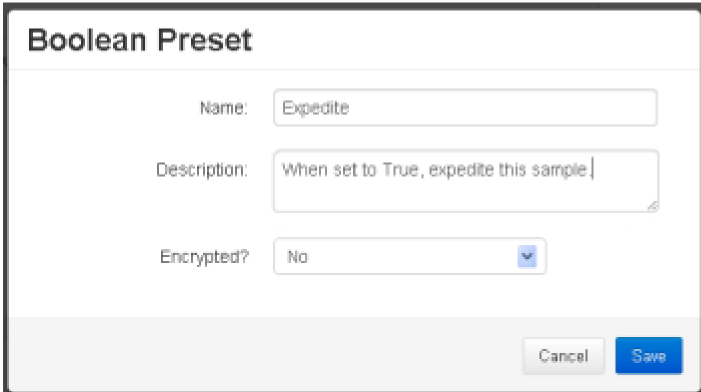


6. To see how the attribute is entered during sample definition, click the image:

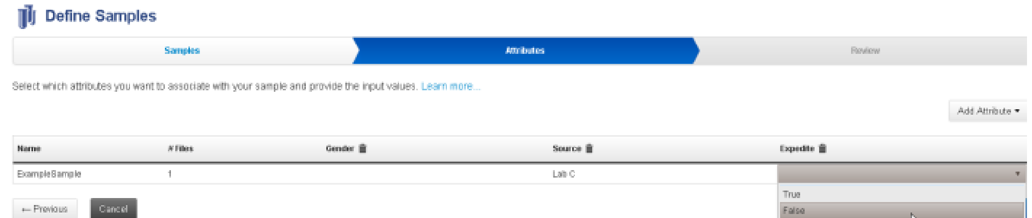


Example boolean preset

This is a completed Boolean preset form:



It appears during sample definition.

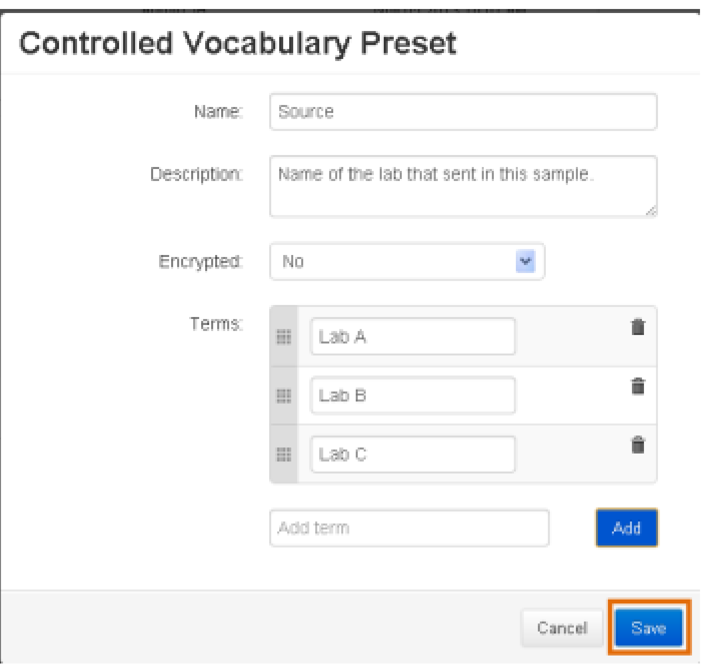


Example Controlled Vocabulary preset

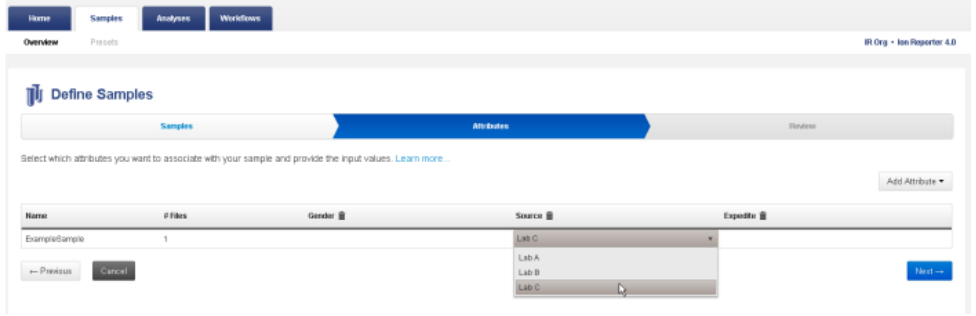
With a Controlled Vocabulary preset, you create a pull-down menu for an attribute. This type of preset gives you control over your sample metadata by preventing misspellings or alternate wordings for common entries.

Click these images to see a Controlled Vocabulary example:

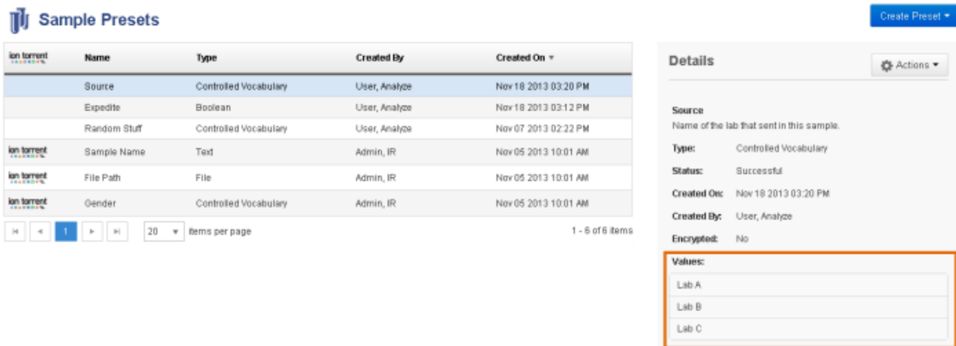
- A completed Controlled Vocabulary preset form:



- How the attribute appears during sample definition:



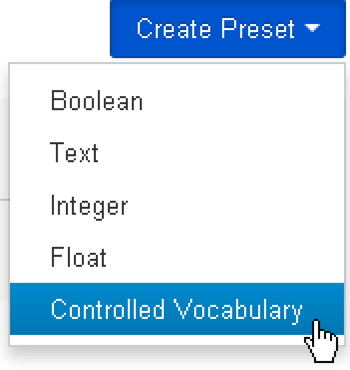
- How the attribute appears in the Samples Presets tab (with the menu options shown in the details Values area):



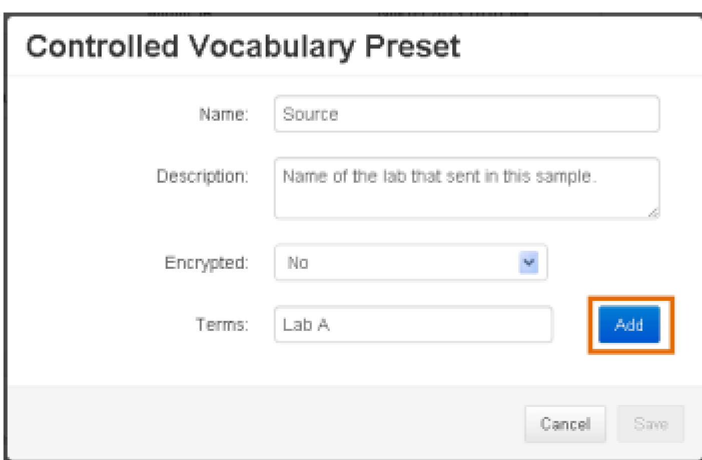
Create a Controlled Vocabulary preset

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

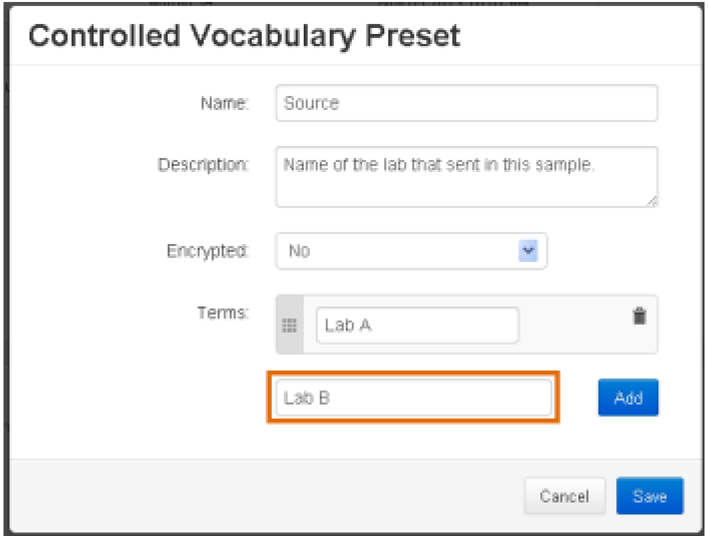
2. Click the **Create Presets** button and select **Controlled Vocabulary**.



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.



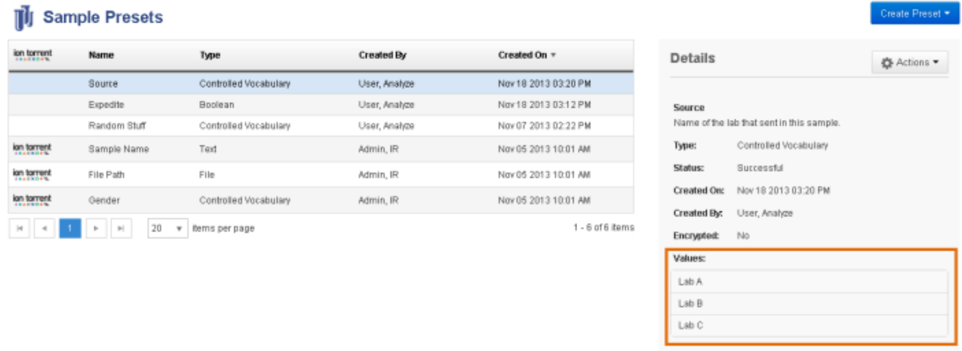
4. Click the **Add** button for an additional Terms field. In the new field, enter the second entry for the attribute's pull-down menu.



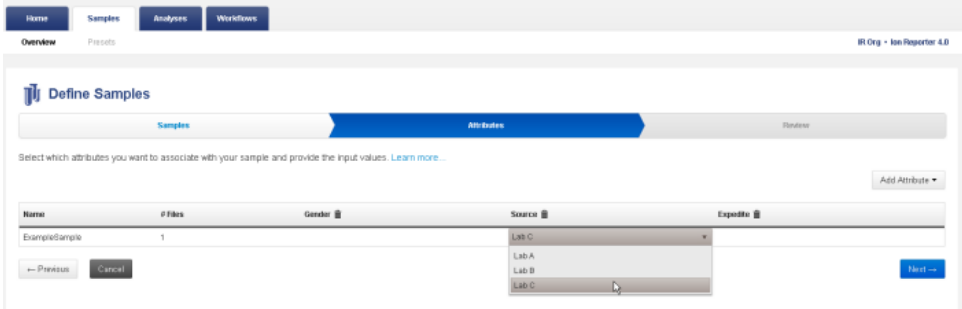
5. Again click the **Add** button for an additional Terms field. In the new field, enter the third entry for the attribute's pull-down menu.

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.



Sort Sample Presets

Once you create Sample Presets, you can sort the **Sample Presets** table to organize the information by sample preset name, file type, user, or date of creation.

1. In the **Samples** tab, click **Presets**.
2. In the **Sample Presets** table, click on a column header to sort the list based on the column category type (numerical, alphabetical, date).
3. Click on the column header again to reverse the order.

Search, sort, or filter Samples

You can search, sort, and filter the **Samples** table to aid you in finding your sample of interest by narrowing the search results.

In the **Samples** tab, click **Overview**, then perform the following actions in the **Samples** table.

To...	Do this...
Search the list	Enter a search term into the **Search** field, then click **Go**. **Note:** The search field is outlined in red if the search string is invalid. The following rules apply to all search fields: • An asterisk (*) is not allowed in the search field. • Searches are not case sensitive. • 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.
Sort the list	Click on a column header to sort the list based on the column category type (numerical, alphabetical, date). Click on the column header again to reverse the order.
Select filter categories	• Click **More Filters**, then select one or more applicable filter categories from the dropdown list. • Click **More Filters**, type in a search string into the **Find Filters** field, then select one or more applicable filter categories. • Click **More Filters**, then click **Select All** to select all filter categories.
Remove filter categories	• Click ✕ next to the filter category dropdown list to remove that specific filter category from the list. • Click **More Filters**, then click on the selected filter category to remove that specific filter category from the list. • Click **More Filters**, then click **Clear** to remove all the selected filter categories from the list.
Select filters within a filter category	• Click on the filter category to expand the dropdown list, then select one or more specific filters. • Click on the filter category to expand the dropdown list, then type in a search string into the **Find Filters** field. Select one or more specific filters. • Click on the filter category to expand the dropdown list, then click **Select All** to select all filters within a specific filter category.
Remove filters from a filter category	• Click on the filter category to expand the dropdown list, then click on the selected filter to remove that specific filter from the filter category. • Click on the filter category to expand the dropdown list, then click **Clear** to remove all filters from a filter category.
Remove all filters and filter categories from the list	Click **Clear All**.

Set Samples table preferences

You can configure the columns displayed in the **Samples** table. You can then save the desired table configuration in table preferences for future use.

IMPORTANT! Table preferences must be saved and applied to individual tables separately. Tables in **Samples** and **Analyses** tabs do not share table preferences.

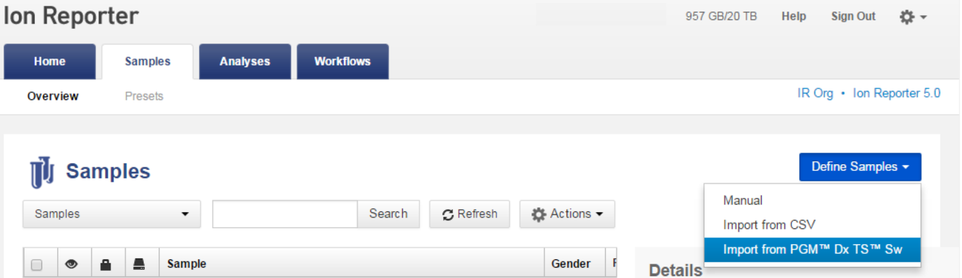
In the **Samples** tab, in the **Overview** screen, click **Preferences**, then select from the following options:

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 preferences 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.
Delete Table Preference	Delete the selected user-defined table preferences from **Table Preferences**.
Restore Defaults	Restore the default table column display.

Import samples from Torrent Server that runs Assay Development Software

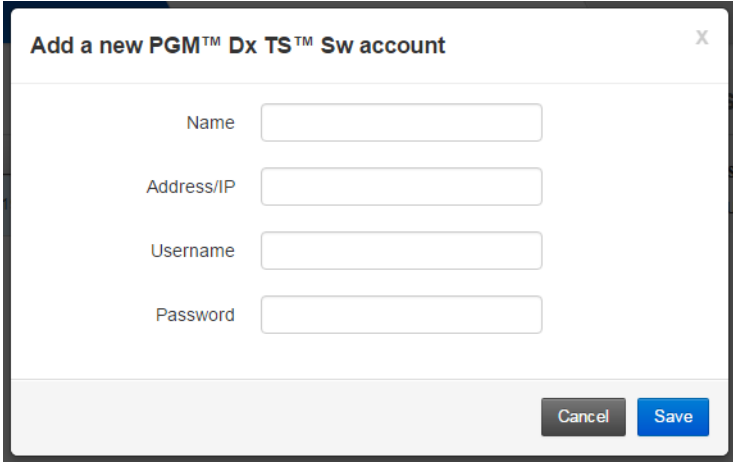
A Ion Reporter™ Server can now import samples from an Ion PGM™ Torrent Server that runs Assay Development Software 5.0, or later.

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

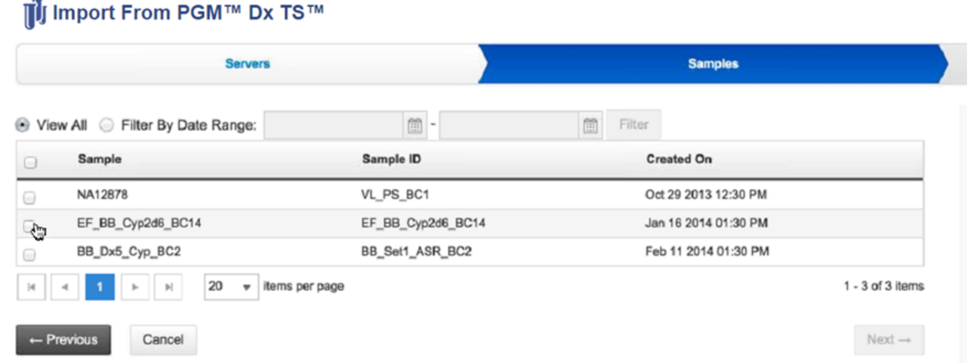


3. On the Servers screen, click **Add Server**.

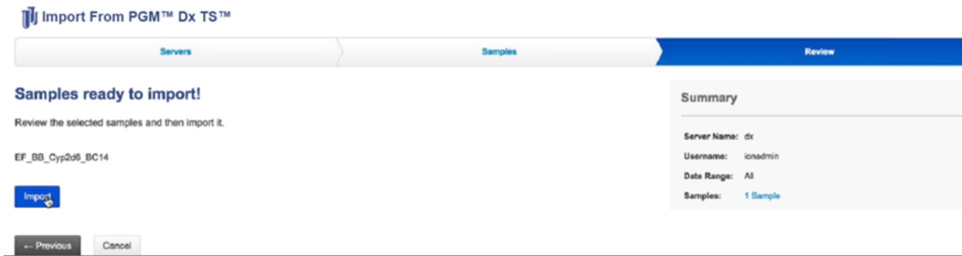
4. Enter name, IP address, username and password. Click **Save**, then click **Next**.



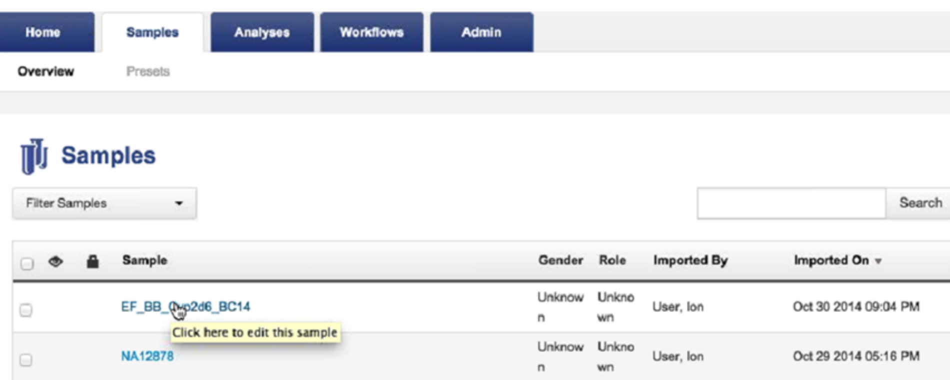
5. Select the samples you want to import and click **Next**.



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



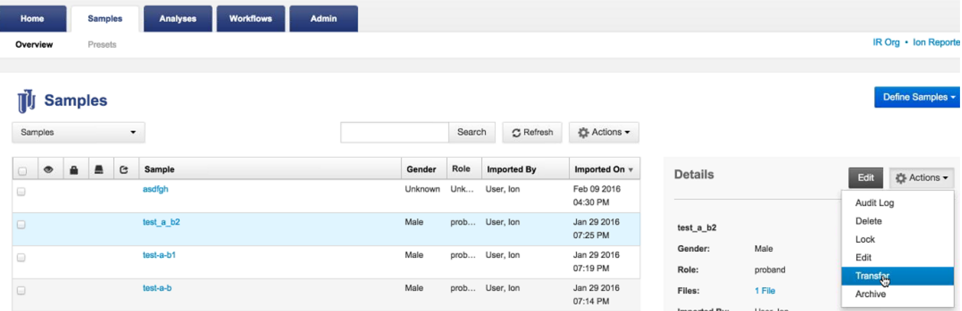
The samples now appear in the **Samples** list on the Ion Reporter™ Server.



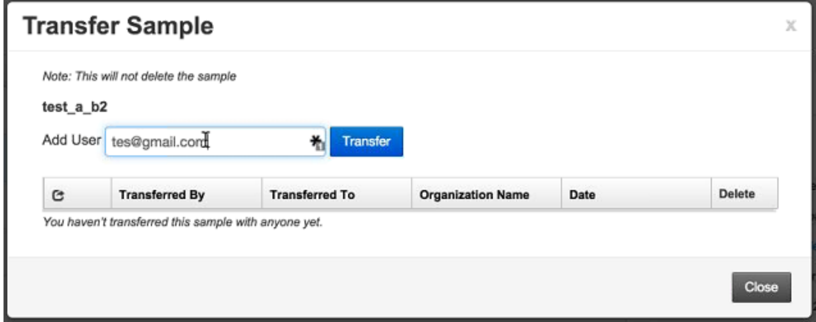
Transfer sample to users in another organization

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.

Congrats! 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**.

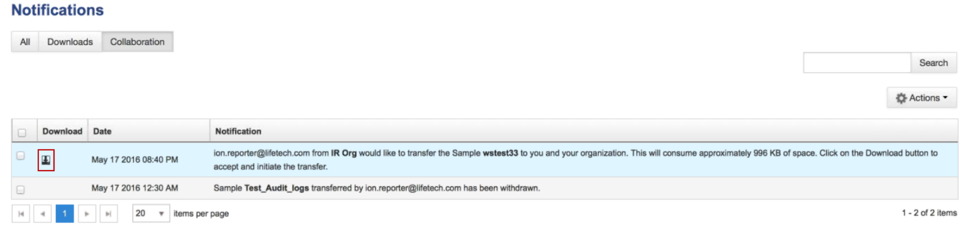
2. In the Batch Transfer dialog, enter the email address of the intended recipient and click **Transfer**.

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.



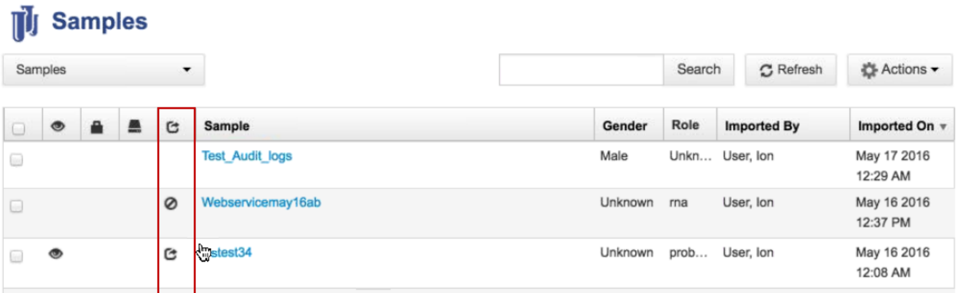
2. Go to the Samples tab to locate the transferred sample.

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.

Manage workflows in Ion Reporter™ Software

Workflows in Ion Reporter™ Software are sets of instructions that determine how Ion Reporter™ Software analysis results are produced.

Ion Reporter™ predefined workflows include settings for common genetic research analysis applications. You can use Ion Reporter™ predefined workflows as is, or you can copy a predefined workflow, customize its settings, then save and reuse it in the future. After an Ion Reporter™ predefined workflow is copied and saved, it is known as a *custom workflow*. You can also copy and edit, or edit a custom workflow.

When a workflow that contains samples is launched, Ion Reporter™ Software generates an analysis. Each analysis contains analysis results in various formats, including tables and visualizations in the software, and data files that can be downloaded.

Note: In Torrent Suite™ Software, you can create a Planned Run that transfers data automatically to the appropriate Ion Reporter™ Server or Ion Reporter™ Software on Thermo Fisher Cloud account and uses one of the available workflows to create an analysis.

All workflows that are available to your Ion Reporter™ Software organization are listed under the **Workflows** tab.

View workflow details in Ion Reporter™ Software

When you select a single workflow that is listed in the **Workflows** table in Ion Reporter™ Software, you can view information about its components in the **Details** pane.

1. Click the **Workflows** tab.
2. In the **Workflows** table, select a workflow.

   Note:

Workflow details are listed in the **Details** section.

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

Ion Reporter™ predefined workflows

Workflows are sets of analysis components that you can use to automate the analysis of your data. Ion Reporter™ predefined workflows are optimized with parameters for use with specific products.

These Ion Reporter™ predefined workflows are organized into the following Research Categories:

Research Category	Description
Exome	For use with AmpliSeq Exome and TargetSeq Exome panels
Metagenomics 16S	For use with Metagenomics 16S panels
Oncology	For use with a variety of Ion AmpliSeq™, Ion AmpliSeq™ HD, and Oncomine™ cancer research assays
Reproductive	For use with Ion ReproSeq™ and other PGS whole genome assays
Immunology	For use with a variety of Ion AmpliSeq™ and Oncomine™ immunology research assays
Annotate Variants	For use with a variety of research assays
Inherited Disease	For use with a variety of Ion AmpliSeq™ and Oncomine™ cancer research assays
Plugin	Inserts plugin code into a run and functions like a workflow

Custom workflows

There are three ways to create custom workflows in Ion Reporter™ Software.

- You can copy an existing workflow, then edit its settings. See “Create a custom workflow from an existing workflow“ on page 71.
- For Ion AmpliSeq™ HD panel files that are imported from **AmpliSeq.com**, you must copy the appropriate Ion AmpliSeq™ HD workflow *template*, then use the make further changes to the custom workflow and save the workflow, before the custom workflow is used for analysis. See “Create a custom workflow for use with Ion AmpliSeq™ HD panels“ on page 73.
- You can also use the wizard to create a custom workflow. See “Create a new custom workflow without predefined settings“ on page 72.

Create a custom workflow from an existing workflow

You can copy and edit a workflow to save time on analysis in Ion Reporter™ Software. This is the recommended way to create a custom workflow.

We recommend that you start with an Ion Reporter™ predefined workflow or a custom workflow to begin with an optimized set of parameters.

When you create a custom workflow, you can change details such as the following:

- variant annotation filters
- final report settings
- analysis plugins
- parameter settings

Note: You can copy Ion Reporter™ predefined workflows and custom workflows from the current software version and from previous versions of the software. When you copy workflows from an earlier version of Ion Reporter™ Software, you must use target regions files, hotspot files, and fusion panel files from the same version of software. You can view the workflow version in the Details pane. See "View workflow details in Ion Reporter™ Software" on page 69 for more information.

1. In the **Workflows** tab, click **Overview**.
2. In the **Workflows** table, click the row for the workflow that you want to copy, then click **(Actions)** ▸ **Copy**.
   The create workflow wizard opens to the **Research Application** step.

   Note: When you copy workflows, some settings and fields are defined by the workflow and remain selected.

3. In the **Research Application** step, confirm the research application and sample group. See "Research Application step" on page 82 for more information, then click **Next**.
4. In the **Reference** step, confirm that the required files are selected. See "Reference step" on page 83 for more information, then click **Next**.
5. In the **Annotation** step, confirm that one annotation set is selected, then confirm or select a MyVariants database is associated with the workflow. See "Annotation step" on page 89 for more information, then click **Next**.
6. In the **Filters** step, confirm or select a filter chain from the dropdown list. See "Filters step" on page 90 and for more information, then click **Next**.
7. In the **Copy Number** step, confirm or select a copy number baseline from the dropdown list. See "Copy Number step" on page 91 for more information, then click **Next**.
8. In the **Plugins** step, confirm or select plugins. See "Plugins step" on page 95 for more information, then click **Next**.
9. In the **Final Report** step, confirm or select the final report template in the dropdown list. See "Final Report step" on page 96 for more information, then click **Next**.

10. In the **Parameters** step, confirm or edit parameters. See “Parameters step“ on page 96 for more information, then click **Next**.

11. In the **Confirm** step, name the workflow, then enter an optional description. See “Confirm step“ on page 120 for more information, then click **Confirm** and **Save Workflow**.

To ensure that the workflow was copied, click the **Workflows** tab, then click **Overview**, and search for workflow name to find the custom workflow that is now listed in the **Workflows** table.

Create a new custom workflow without predefined settings

You can create a workflow in Ion Reporter™ Software that does not use any predefined settings.

Note: If you are creating a new workflow, compare its parameters with those of an Ion Reporter™ predefined workflow to optimize performance.

To create a workflow using the wizard:

1. In the **Workflows** tab, click **Create Workflow**.
   The workflow wizard opens on the **Research Application** step.

2. In the **Research Application** step, select the research application and sample group. See“Research Application step“ on page 82 for more information, then click **Next**.

3. In the **Reference** step, select the required files. See “Reference step“ on page 83 for more information, then click **Next**.

4. In the **Annotation** step, select an annotation set and a MyVariants database. See “Annotation step“ on page 89 for more information, then click **Next**.

5. In the **Filters** step, select a filter chain from the dropdown list. See “Filters step“ on page 90 for more information, then click **Next**.

6. In the **Copy Number** step, select a copy number baseline from the dropdown list. See “Copy Number step“ on page 91 for more information, then click **Next**.

7. In the **Plugins** step, select plugins. See “Plugins step“ on page 95 for more information, then click **Next**.

8. In the **Final Report** step, select the final report template in the dropdown list. See “Final Report step“ on page 96 for more information, then click **Next**.

9. In the **Parameters** step, select parameters. See “Parameters step“ on page 96 for more information, then click **Next**.

10. In the **Confirm** step, name the workflow, and workflow, then enter an optional description. See “Confirm step“ on page 120 for more information, then click **Save Workflow**.

To check that the workflow was created, click the **Workflows** tab, then click **Overview**, and search for workflow name.

Create a custom workflow for use with Ion AmpliSeq™ HD panels

To analyze Ion AmpliSeq™ HD panel files that are downloaded or imported from **AmpliSeq.com**, you must create a custom workflow, then add target regions files, optionally add hotspot files, and add a CNV baseline to the workflow before you use to analyze sample data in Ion Reporter™ Software. For RNA samples, you must add fusion panel files to the custom workflow.

Note: After you create the custom workflow, you can select it in Torrent Suite™ Software, to transfer data automatically to the appropriate Ion Reporter™ Server and use the workflow for the data analysis in Ion Reporter™ Software.

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

   See "Analysis workflow templates for Ion AmpliSeq™ HD in Ion Reporter™ Software" on page 76 for more information about the Ion AmpliSeq™ HD workflow templates.

2. In the list of workflow templates, select the row for the Ion AmpliSeq™ HD workflow template that you want to copy. Click **(Actions)** ▸ **Copy**.

   The create workflow wizard opens to the **Research Application** step with the **Research Application** and **Sample Group** for the selected workflow template. When you copy Ion AmpliSeq™ HD workflow templates, you cannot change these settings.

3. Click **Next**, then in the **Reference** step, select or upload the following files:

Option	Description
Select a file that has been previously uploaded to Ion Reporter™ Software.	Select the and, optionally, **Hotspot Regions** files from the dropdown lists.
Upload the entire package of the Ion AmpliSeq™ HD panel files directly from **AmpliSeq.com**. When you use this option, a target regions file and any available hotspots file for the panel files are uploaded.	Click **AmpliSeq Import**.
Upload a target regions file that was previously uploaded from **AmpliSeq.com** and is located on a computer directory.	1. Click **Upload** under the **Target Regions** dropdown list. 2. Navigate to and select the file, then click **Open**. 3. Select **Ion AmpliSeq™ HD**, then click **Upload**.
(Optional) Upload a hotspots file that was previously uploaded from **AmpliSeq.com** and is located on a computer directory.	1. Click **Upload** under the **Hotspots Regions** dropdown list. 2. Navigate to and select the file, then click **Open**. 3. Select **Ion AmpliSeq™ HD**, then click **Upload**.
If you use a panel that detects fusions, upload a FASTA file that was previously uploaded from **AmpliSeq.com** and is located on a computer directory.	1. Click **Upload** under the **Fusion Panel** dropdown list. 2. Navigate to and select the file, then click **Open**. 3. Select **Ion AmpliSeq™ HD**, then click **Upload**.

4. In the **Annotation** step, confirm or select an annotation set, then confirm or select a MyVariants database. See “Annotation step“ on page 89 for more information, then click **Next**.

5. In the **Filters** step, select a filter chain from the **Filter Chains** dropdown list.

Option	Description
Called Variants and Controls	This is the default filter chain for the workflow. Use this for analysis results that report all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Variant types include: SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL_ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3ITD.
Called Hotpot Variants and Controls	Select this filter chain for analysis results that report all hotspot variants that pass the filter and are not called as reference or NOCALL. Filter variant types include: SNP and INDEL.
Variant Matrix Summary	Select this filter chain for analysis results that replicate data that shown for Ion AmpliSeq™ HD analysis results in the Variant Matrix Summary. Variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants. Use this filter chain if you want to export only the variants that are shown in the Variant Matrix Summary view of the analysis results to Oncomine™ Knowledgebase Reporter.

6. **IMPORTANT!** To add a Copy Number baseline, contact your Field Bioinformatics Specialist (FBS).

   In the **Copy Number** step, select a Copy Number Baseline from the **Baseline** list, if applicable, then click **Next**.

7. In the **Plugins** step, Click **Next**.

   Note: In the **Plugins** step, the OVAT plugin is automatically selected. This plugin adds annotations for driver mutations with Oncomine™ Gene Class and Oncomine™ Variant Class information. This plugin is included by default with the Ion AmpliSeq™ HD workflow templates. If you import the VCF file of analysis results into Oncomine™ Knowledgebase Reporter, these annotations are included in a report that is generated from that software.

8. In the **Final Report** step, confirm or select the final report template that is selected in the dropdown list. See “Final Report step“ on page 96 for more information, then click **Next**.

9. In the **Parameters** step, make any required changes, then click **Next**.

   Note: If you are using the Ion AmpliSeq™ HD test panel, consult your field support representative and other training materials for more information. If you designed a white glove panel, consult with your white glove representative or field support representative to decide if parameter changes are required for the workflow.

10. In the **Confirm** step, name the workflow, then enter an optional description, then click **Confirm** and **Save Workflow**.

To ensure that the workflow was copied, click the **Workflows** tab, then click **Overview**, and search for the custom workflow name.

Analysis workflow templates for Ion AmpliSeq™ HD in Ion Reporter™ Software

Before Ion AmpliSeq™ HD data can be analyzed in Ion Reporter™ Software, you must copy the appropriate Ion AmpliSeq™ HD analysis workflow template, and add the Ion AmpliSeq™ HD panel files and, optionally, a CNV baseline to the workflow.

IMPORTANT! Ensure that the workflow template name that you use matches the type of Ion AmpliSeq™ HD panel files that you include in your analysis. For example, if you use panel files for a liquid biopsy fusions single sample, ensure that you use copy the AmpliSeq™ HD for Liquid Biopsy w2.1 - Fusions - Single Sample workflow template to create the custom workflow.

To filter for Ion AmpliSeq™ HD template workflows, or copy and edited Ion AmpliSeq™ HD workflows that include saved panel files, search for **Target: AmpliSeq HD**.

To filter for all TagSeq workflows, search for **Research Application: Oncology – Liquid Biopsy**, or search for **Target: TagSequencing**.

To filter for Liquid Biopsy and Tumor TagSeq workflows, but *only* the Liquid Biopsy Ion AmpliSeq™ HD workflows (copy and edited workflows that include saved Ion AmpliSeq™ HD panel files), search for **Research Category: Oncology – Liquid Biopsy**.

To filter for Tumor Ion AmpliSeq™ HD workflows, you can use a combination of the **Workflow** filters **Research Category: Oncology – Solid Tumor**, and **Target: AmpliSeq HD**.

Ion AmpliSeq™ HD Workflow Template	Description
AmpliSeq™ HD for Tumor w2.1 - DNA - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) variants (SNPs, INDELs and CNVs) from targeted DNA libraries using Ion AmpliSeq™ HD technology. This is compatible with DNA that is purified from tumor research samples. Released with: Ion Reporter™ Software 5.10. Workflow Version: 2.1.
AmpliSeq™ HD for Tumor w2.1 - Fusions - Single Sample	Detects and annotates gene fusions from targeted RNA libraries using Ion AmpliSeq™ HD technology. This is compatible with RNA purified from tumor research samples. Released with: Ion Reporter™ Software 5.10. Workflow Version: 2.1.
AmpliSeq™ HD for Tumor w2.1 - DNA and Fusions (Single Library) - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) variants (SNPs, INDELs, CNVs and fusions) from targeted nucleic acid libraries using Ion AmpliSeq™ HD technology. This is compatible with DNA and RNA purified together from tumor research samples. Released with: Ion Reporter™ Software 5.10. Workflow Version: 2.1.
AmpliSeq™ HD for Tumor w2.1 - DNA and Fusions (Separate Libraries) - Single Sample	Detects and annotates low frequency (to 0.5% limit of detection) somatic variants (SNPs, INDELs and CNVs) from targeted DNA libraries, as well as gene fusions from matching targeted RNA libraries using Ion AmpliSeq™ HD technology. This is compatible with DNA and RNA purified separately from tumor research samples. Released with: Ion Reporter™ Software 5.10. Workflow Version: 2.1.

Ion AmpliSeq™ HD Workflow Template	Description
AmpliSeq™ HD for Liquid Biopsy - w2.1 - DNA - Single Sample	Detects and annotates low frequency (to 0.1% limit of detection) variants (SNPs, INDELs and CNVs) from targeted DNA libraries using Ion AmpliSeq™ HD technology. This is compatible with DNA purified from cell-free liquid biopsy research samples. Released with: Ion Reporter™ Software 5.10. Workflow Version: 2.1.
AmpliSeq™ HD for Liquid Biopsy w2.1 - Fusions - Single Sample	Detects and annotates gene fusions from targeted RNA libraries using Ion AmpliSeq™ HD technology. This is compatible with RNA purified from cell-free liquid biopsy research samples. Released with: Ion Reporter™ Software 5.10. Workflow Version: 2.1.
AmpliSeq™ HD for Liquid Biopsy w2.1 - DNA and Fusions (Single Library) - Single Sample	Detects and annotates low frequency (to 0.1% limit of detection) variants (SNPs, INDELs, CNVs and fusions) from targeted nucleic acid libraries using Ion AmpliSeq™ HD technology. This is compatible with DNA and RNA purified together from cell-free liquid biopsy research samples. Released with: Ion Reporter™ Software 5.10. Workflow Version: 2.1.
AmpliSeq™ HD for Liquid Biopsy w2.1 - DNA and Fusions (Separate Libraries) - Single Sample	Detects and annotates low frequency (to 0.1% limit of detection) somatic variants (SNPs, INDELs and CNVs) from targeted DNA libraries, as well as gene fusions from matching targeted RNA libraries using Ion AmpliSeq™ HD technology. This is compatible with DNA and RNA purified separately from cell-free liquid biopsy research samples. Released with: Ion Reporter™ Software 5.10. Workflow Version: 2.1.

Create a custom workflow enabled for Mutation Load

Mutation load is a calculation of non-synononomous mutations per megabase (MB). You can enable any DNA – single sample, or DNA and Fusions – single sample workflow to include the mutation load calculation in addition to the other analysis results expected from that workflow.

A workflow that is enabled for Mutation Load calculates mutations per megabase (MB) and adds graphics and other information about the mutations to Ion Reporter™ Software analysis results and visualizations.

To enable Mutation Load, you must edit a workflow, then set a parameter in the workflow.

Note: You can copy and edit any Ion Reporter™ predefined workflow, and you can also edit an existing custom workflow if is not locked.

1. In the **Workflows** tab, click **Overview**.
2. In the list of workflows, select the row for the DNA – single sample, or DNA and Fusions – single sample workflow that you want to copy. Click ⚙ **(Actions)** ▸ **Copy**.
   The create workflow wizard opens to the **Research Application** step with the **Research Application** and **Sample Group** for the selected workflows. When you copy workflow templates, you cannot change these settings.
3. Proceed to the **Parameters** step. See "Steps in workflow creation" on page 81 for more information.

4. In the **Parameters** step, find the **Mutation Load Calculation Filter Chain** parameter and change the value in the dropdown list to **Mutation Load (Somatic SNVs)**.

5. Enter a name for the workflow.

6. *(Optional)* Enter a description of the workflow.

7. Click **Save Workflow**.
   The custom workflow is saved and is added to the **Workflows** table.

To ensure that the workflow was saved, click the **Workflows** tab, then click **Overview**, and search for workflow name.

Create a custom aneuploidy No Gender workflow

To create a new workflow for use with Ion ReproSeq™ aneuploidy research, you can copy and edit an Ion Reporter™ predefined workflow. For example, you can copy the Ion ReproSeq™ No Gender PGS w1.1 workflow, then 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, and save the changes.

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

Note: To enable mosaicism detection and/or smoothing in a No Gender workflow, copy and edit the ReproSeq No Gender w1.1 workflow as explained in this procedure. You cannot enable gender hiding in other Ion ReproSeq™ workflows because the **Hide called gender** setting is locked as **False** (that is, gender is called). This setting cannot be set to **True**.

See “CNV and aneuploidy research“ on page 131 for more information about Ion ReproSeq™ workflows for use with aneuploidy research.

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

2. In the list of workflows, select **Reproductive** from the **Research Category** dropdown list, then select **Aneuploidy** from the **Research Application** list. Select the workflow of interest, then click ⚙ **(Actions) Copy**.

3. Click **Next** to proceed to the the **Parameters** step. See “Steps in workflow creation“ on page 81 for more information.

4. Click the **Cnv Finding** link in the **Parameters** step, then change the **CNV Sensitivity** setting to **Custom**.

   Note: Low sensitivity results in fewer false positives, but more false negatives. High sensitivity results in fewer false negatives, but more false positives. High sensitivity is required to make segmental aneuploidy calls of ~20 Mbp. Custom sensitivity allows you to change the CNV Transition Penalty parameter to detect even smaller segmental CNV events more sensitively. See “Improve detection of small segmental CNV events“ on page 79 for further information. Will need to include it as a shared topic in our book for this crossref to work.

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

6. Scroll to **Analysis (applies to all CNV finding algorithm types except VCIB CNV algorithm)**, and change **CNV Transition Penalty** to **-2**.

7. Scroll to the **Analysis (applies only to Aneuploidy workflows)** section, then make desired changes.
   In this example, select **True** under **Enable Mosacism Detection** to make this change.
   To 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 shows Y chromosome data only if the sample is male.

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

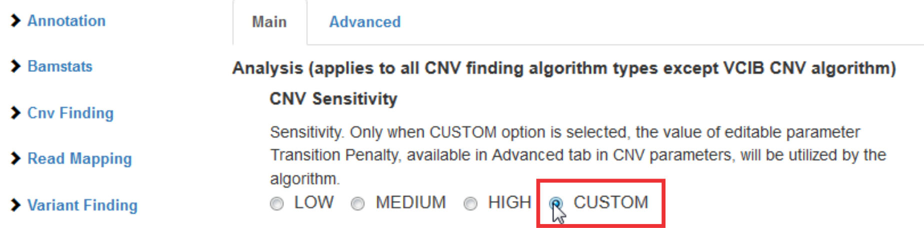
9. Rename the workflow, then click **Save Workflow**.
   The custom workflow is added to the list of available workflows.

Improve detection of small segmental CNV events

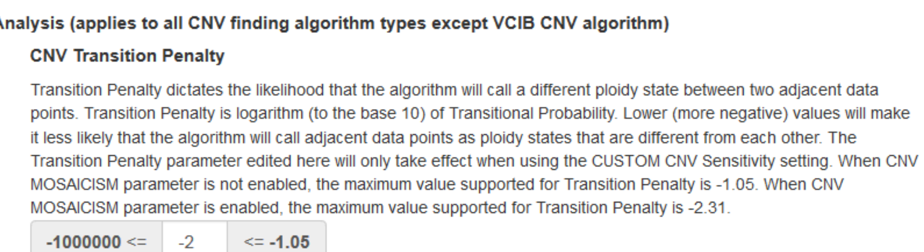
You can improve the detection of small segmental CNV events if you adjust the CNV Transition Penalty parameter in Ion ReproSeq™ workflows for use with aneuploidy research. The value of this parameter in mosaic and non-mosaic workflows that are set at high sensitivity is −3. The following values are the highest allowable for this parameter:

- −1.05 for non-mosaic workflows
- −2.31 for mosaic workflows

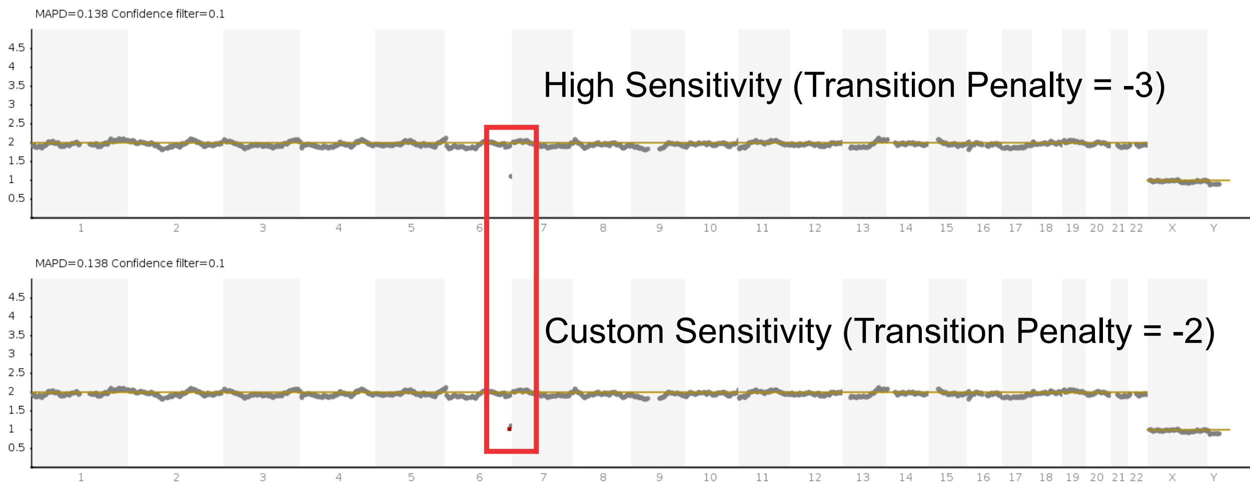
1. In the **Create Workflow** or **Edit workflow** screen, navigate to **Parameters ▸ CNV Finding**. Under the **Main** tab, select **Custom** for **CNV Sensitivity**.



2. Click **Advanced**, scroll to the CNV Transition Penalty parameter, then enter the desired value.



3. In the **Confirm** step, click **Save Workflow**.
   Example of increased sensitivity: a 7.9-Mb deletion is called on chromosome 6 with Transition Penalty set to −2.



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.

Edit custom workflows

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

Note:

- You cannot edit Ion Reporter™ predefined workflows or locked workflows. After a workflow is locked, that action cannot be undone.
- To edit a workflow that is created in a version earlier than Ion Reporter™ Software 5.2, you must select a MyVariants database in the **Annotation** step to proceed.

1. In the **Workflows** tab, select the workflow that you want to edit, then click ⚙ **(Actions)** ▸ **Edit** in the **Details** section.

2. Proceed through the steps in "Create a new custom workflow without predefined settings" on page 72.

Steps in workflow creation

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

Some workflows have fewer setup steps than others.

Research Application step

In the Research Application step, the research application and sample group settings affect the options that are available in subsequent workflow steps. This step applies to all workflow types.

Note: If you start with an existing workflow, you cannot change the research application.

1. In the **Research Application** step, select one of the following options:

Table 2 Research applications in Ion Reporter™ Software

Research Application	Description
Aneuploidy	Detect human chromosomal large structural abnormalities in low-pass whole-genome sequencing research samples
Annotate Variants	Annotate the variants from a VCF file for research use
DNA	Detect and annotate variants in human DNA research samples
DNA and Fusions	Detect and annotate variants in human DNA and Fusions research samples
Immune Repertoire	Detect and analyze T cell receptor beta (TCRB) rearrangements for research use
Metagenomics	Determine population diversity in polymicrobial research samples using detection of 16S gene variable regions
Mutation Load [1]	Calculates Mutation Load and displays associated graphs and tables in human DNA samples **Note:** If you want Mutation Load results to be included in DNA or DNA and Fusions Single Sample results, you must enable the Mutation Load Calculation filter chain parameter. See "Annotation parameters" on page 97 for more information.
Oncology-Liquid Biopsy	Detect and annotate variants in human research samples
Fusions	Detect and annotate gene fusions in human DNA research samples

[1] The Mutation Load Research Application applies only the Oncomine™ Tumor Mutation Load - w1.0 - DNA - Single Sample workflow that was released with Ion Reporter™ Software 5.6.

2. Select a sample group type.

Table 3 Sample groups in Ion Reporter™ Software

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

3. Click **Next**.

Reference step

Presently, all Ion Reporter™ predefined workflows that use a human reference are designed to work with the hg19 human reference. Custom Ion AmpliSeq™ DNA panels can be designed to work with the GRCh38 human reference. This Reference step applies to DNA, DNA and Fusions, and Metagenomics workflows.

1. In the **Reference** step, select a human reference **GRCh38** or **hg19**.

   Note: If you change the reference, you also must change the target regions, hotspots, and fusion panels to the appropriate files for the new reference.

2. Under **Focus Analysis**, select, or upload the following:

Option	Description
Target Regions file	• Select from dropdown list. • If not already loaded, click **Upload** to upload from your desktop, or click **AmpliSeqImport** to import directly from ampliseq.com. See "Import panel files from AmpliSeq.com" on page 85 for more information.
Variant Detection Type	• Somatic – Use this option to detect somatic variants at low allele frequencies. • Germline – Use this option to detect germline variants that are expected to be present at allele frequencies of 50 to 100 percent.
Hotspots file	• Select from dropdown list. • If not already loaded, click **Upload** to upload from your desktop.
Fusions panel file	• Select from dropdown list. • If not already loaded, click **Upload** to upload from your desktop, or click **AmpliSeqImport** to import directly from ampliseq.com. See "Import panel files from AmpliSeq.com" on page 85 for more information.

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

Note: When you upload a Target Regions file, you must select the file type in the **Upload Target Regions File** dialog box. The software does a validation check to ensure that the file is compatible.

3. Click **Next**.

Import panel files from AmpliSeq.com

You can import panel files for your Made-to-Order, On-Demand, or Ready-to-Use research panels from AmpliSeq.com into Ion Reporter™ Software during workflow creation. See “Ion AmpliSeq panel types“ on page 16 for more information.

Note: Only ordered and quoted panel files are available for import into Ion Reporter™ Software.

1. In the **Research Application** step, select an appropriate research application and sample group for your panel, then click **Next**.
2. In the **Reference** step, import a Target Regions file:

Option	Description
Custom Panel	1. Click **AmpliSeq Import** under the Target Regions field. The **Import for AmpliSeq** dialog box appears. 2. Select the **Custom Panel** tab for Ion AmpliSeq™ or Ion AmpliSeq™ HD Made-to-Order panel files. 3. Enter your user name and password and then click **List My AmpliSeq Panels**. 4. Select the panel files that you want to import from the dropdown list, then click **Import**.
Fixed Panel	1. Click **AmpliSeq Import** under the Target Regions field. The **Import for AmpliSeq** dialog box appears. 2. Select the **Fixed Panel** tab for On-Demand or Ready-to-Use panel files. 3. Select the panel file that you want to import from the dropdown list. 4. Click **Import**.

3. If your analysis includes fusions, import your Fusion Panel files.

Option	Description
Custom Panel	1. Click **AmpliSeq Import** under the Fusion Panel field. The **Import for AmpliSeq** dialog box appears. 2. Select the **Custom Panel** tab for Ion AmpliSeq™ or Ion AmpliSeq™ HD Made-to-Order fusion panel files. 3. Enter your user name and password and then click **List My AmpliSeq Panels**. 4. Select the fusion panel file that you want to import from the dropdown list, then click **Import**.
Fixed Panel	1. Click **AmpliSeq Import** under the Fusion Panel field. The **Import for AmpliSeq** dialog box appears. 2. Select the **Fixed Panel** tab for On-Demand or Ready-to-Use fusion (RNA) panel files. 3. Select the fusion panel file that you want to import from the dropdown list. 4. Click **Import**.

4. Complete the remaining steps for workflow creation. See "Create a new custom workflow without predefined settings" on page 72 for more information.

Your target regions and fusion panel files are added to the list of files selectable in the dropdown lists in the Reference step. See "Reference step" on page 83 for more information.

Ion AmpliSeq panel types

AmpliSeq.com offers four design and ordering options for Ion AmpliSeq™ and Ion AmpliSeq™ HD research panels:

- On -Demand Ion AmpliSeq™ research panels of optimized amplicons for germline analysis. Configurable to a specific human disease area, and ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ research panels of DNA and RNA designs for germline or somatic analysis of any genome, ordered in large reaction packs. Made-to-Order panels were formerly known as Custom Panels.
- Ready-to-Use Ion AmpliSeq™ research panels are predesigned DNA and RNA panels for germline and somatic analysis, ordered in small reaction packs.
- Made-to-Order Ion AmpliSeq™ HD research panels are ultra high-sensitivity DNA and RNA designs for germline or somatic analysis of the human genome, including featuring dual barcoded amplicons, and bidirectional sequencing.

Select reference and primers for Metagenomics workflow

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

1. In the **Reference** step, select one or both reference databases to map data against, then click **Next**.

2. In the **Primers** step, select a primer option, then click **Next**.

Option	Description
Use No Primers	Select if no primers were used in library preparation.
Use Custom Primers	Select to provide your own primers.
User Default Primers	Select to use proprietary default primers.

Note: You can 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.

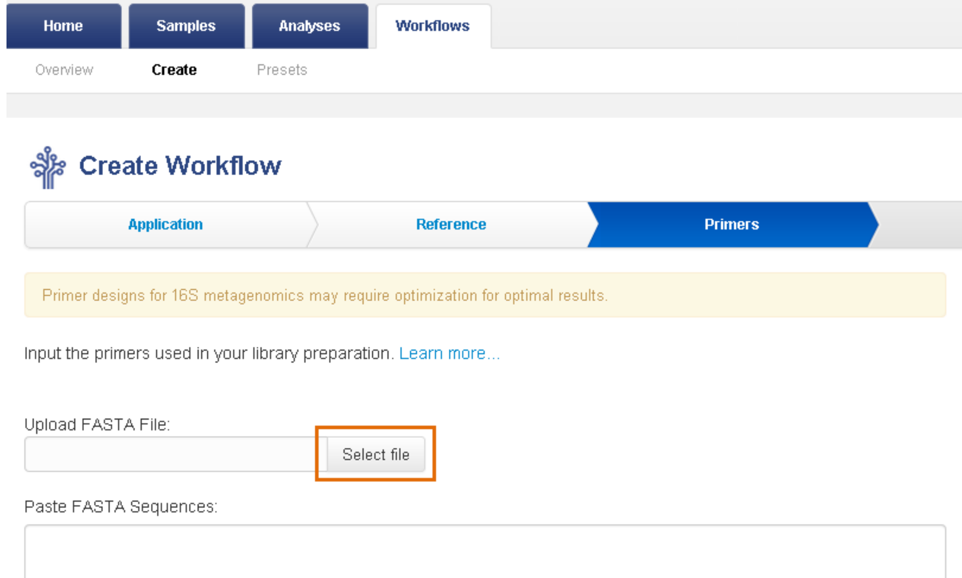
Custom primers for Metagenomics workflows

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.

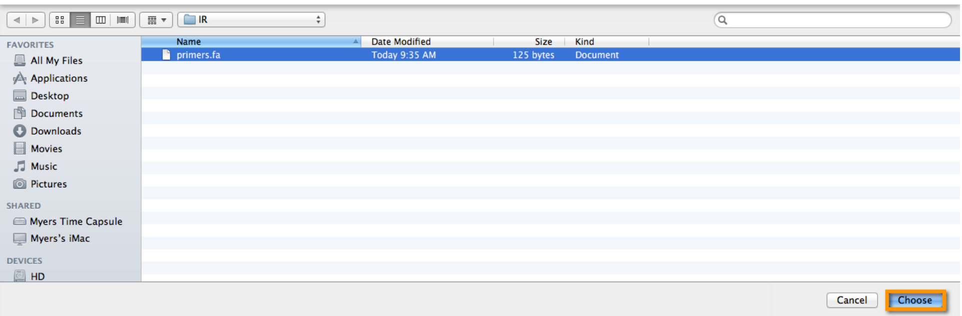
```
>MyFavoriteV5_forward    ACTCGGTCCARACTGAGACT
>MyFavoriteV5_Rev    TTACCGRGGCGTATGCGG
>MyFavoriteV8_Fwd    CCARAACTCGGTCTGSGACT
>MyFavoriteV8_r    RGGCGTATGCSTACCGGG
```

To upload the FASTA file that contains primer sequences:

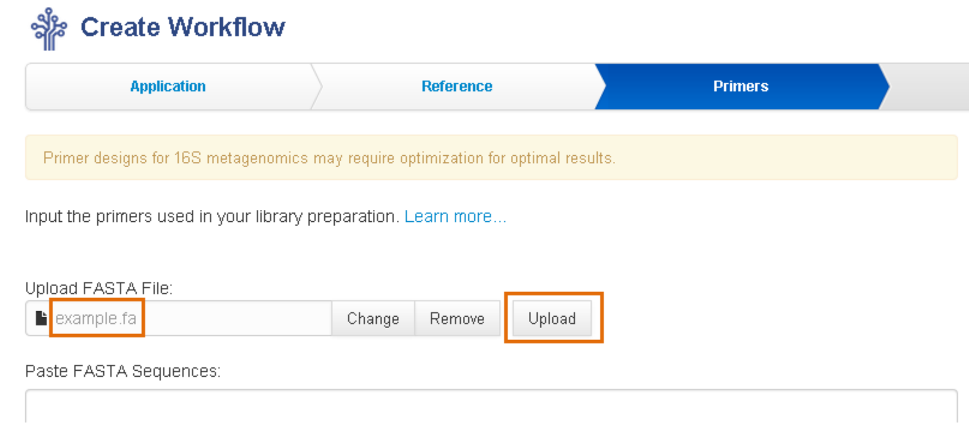
1. Click the **Select file** button.



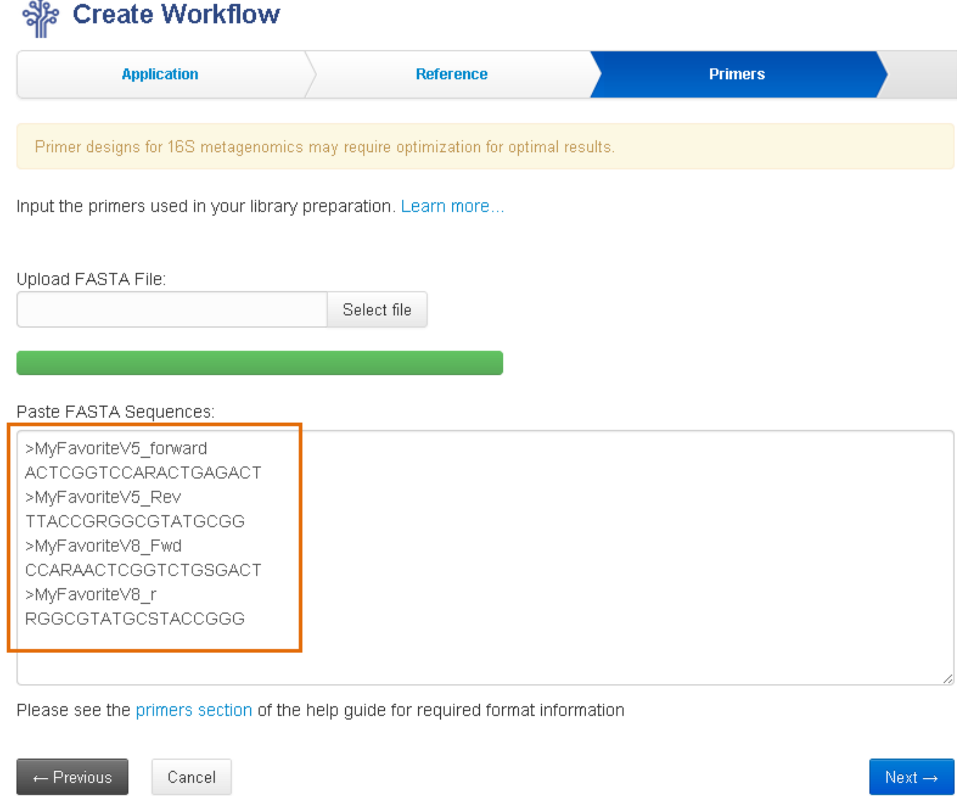
2. Navigate to the folder containing your FASTA file, select it, and click the **Choose**, **Open**, or **Save** button.



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. The primer sequences are uploaded and you can optionally edit them in the text box field. Alternatively, you can paste primer sequences directly into the text box.



5. Click the **Next** button.
6. Enter a Workflow Name, an *(optional)* description, then click **Save Workflow**.

Annotation step

You can select annotation sets when setting up your workflow in Ion Reporter™ Software. Annotation sets contain various annotation sources that provide information about the biological meaning of variants and allow variant sorting and prioritization. Ion Reporter™ Software provides many annotation sources that are derived from public and private annotation databases for hg19 and GRCh38.

1. In the **Annotation** step, under **Annotation Set**, select from the following options:

Option	Description
Dropdown list	Select an existing annotation set from the dropdown list. Annotation source details are shown. Select **All** to select all the annotation sets relevant to your workflow type. Select **Aneuploidy** to select all annotation sets relevant for aneuploidy research. Otherwise, select an individual annotation set from the dropdown list.
Workflow Presets link	Create a new annotation set from Workflow Presets screen. See “Create annotation set workflow presets“ on page 123 for more information.

2. (*Optional*) under **MyVariants Database**, select from the following options:

Option	Description
Dropdown list	Select an existing MyVariants database from the dropdown list. MyVariants database details are shown.
Create here link	Create a new MyVariants database. See "Create a MyVariants database" on page 181 for more information.

3. Click **Next**.

Filters step

You can apply a default filter chain during workflow creation in Ion Reporter™ Software. Filter chains are sets of filters that you apply to variants identified in your analysis. Filters allow you to remove or include variants in your analysis results. See "Filters and filter chains" on page 421 for more information.

1. In the **Filters** step, select a default filter chain from the dropdown list.
   After you select a filter chain, the filters that are contained in the filter chain are shown.

2. (*Optional*) Click **Workflow Presets** link to configure a filter chain. See "Create a custom filter chain" on page 423 for more information.

3. Click **Next**.

This establishes the default filter chain for the workflow. All future analyses that are run through this workflow will use this filter chain.

Change the default filter chain for a workflow

A workflow might have a default filter chain that is associated with it. You can change the default filter chain for an Ion Reporter™ Software workflow to ensure when the workflow is launched, that filter chain is applied. See

Note: Only one default filter chain can be associated with a workflow.

1. In the **Filters** step, select a different filter chain from the dropdown list.

2. Proceed through the remaining steps of the workflow wizard. See "Copy Number step" on page 91 for more information.

3. Click **Save**.

The newly saved filter chain is now the default filter chain for the workflow. All future analyses that are run through the new version of the workflow will use the new filter chain. Previously run analyses that were run through older versions of the workflow are not affected by this change.

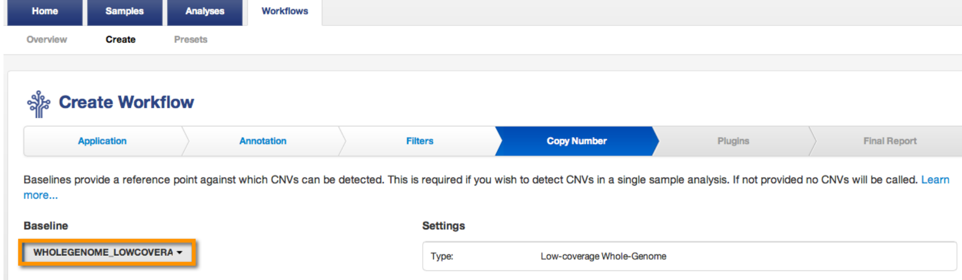
Copy Number step

When you want to identify copy number variants (CNVs), you can select a copy number baseline to apply to the workflow. Workflows that use copy number baseline controls can provide better copy number detection than paired sample workflows. The Copy Number baseline step is present only in DNA, aneuploidy, and some Oncomine™ workflows.

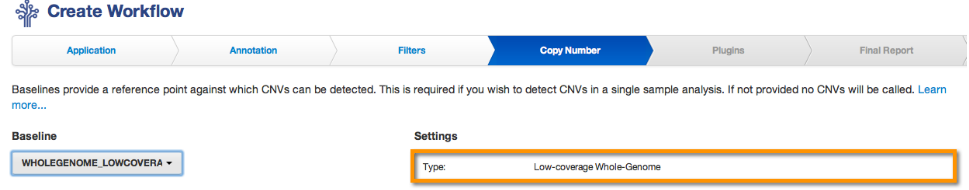
Note: Copy number baseline choices are limited to copy number baselines that were created using the same regions file that is selected in the **Reference** step of the workflow bar.

1. In the **Copy Number** step, select from the following:

Option	Description
No Baseline - Don't call CNVs	If you do not want to identify CNVs, select this option.
Copy number baselines	Select an existing copy number baseline from the dropdown list.



2. The settings for this baseline are displayed.



Note: Alternatively, you can click the **Workflow Presets** link and configure a new copy number baseline preset. See "Create a Copy Number baseline" on page 124 for more information.

3. Click **Next**.

Variability Correction Informatics Baseline (VCIB)

A Copy Number Variation baseline, called the Variability Correction Informatics Baseline (VCIB), is available for some Oncomine™ assays and Ion AmpliSeq™ HD assays.

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

- Create a new VCIB CNV baseline. See “Create a CNV baseline“ on page 452 for more information.
- Use an existing VCIB CNV baseline as is. See “Use VCIB CNV baseline“ on page 452 for more information.
- Augment an existing VCIB CNV baseline. See “Augment (add Samples to) an existing VCIB CNV baseline“ on page 453 for more information.

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.

See the user guide for the assay that you use for details about CNV call results.

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.

Use gender to call gains from expected copy number changes

The following approach applies to analyses performed on Oncomine™, Ion AmpliSeq™, or Ion AmpliSeq™ HD cancer research panels that use the Variability Correction Informatics Baseline (VCIB).

A copy number baseline has a gender, either male or female. A sample also has a gender: male, female or unknown (unknown is interpreted as female). Expected genomic copy number regions for these genders are defined in the ploidy files supplied by Ion Reporter™ Software within workflows.

For example, create a copy number baseline with "male" normal human samples and chromosome X non-Pseudo Autosomal Regions (non-PAR) amplicons in the panel. The baseline copy number for the non-PAR chromosome X amplicons is expected to be 1. If a male sample is run using this baseline the relative copy number to the baseline should be 1:1 and is expected (normal) and should be reported accordingly. A female sample using this baseline should have a relative copy number of 2:1 as is expected (normal) and should be reported accordingly. Using a ploidy file, each amplicon is assigned to the baseline expected copy number found in the baseline ploidy file (male), in this example non-PAR chromosome X amplicons would have a copy number of 1. Running a sample with a female ploidy file, the non-PAR chromosome X amplicons has a copy number of 2, so the final copy number relative to the baseline is adjusted appropriately.

More precisely, if for amplicon *****, expected baseline copy number = ***B*** and expected sample copy number = ***S***, after variability corrections, set ***l2r***=log2 ratio of sample observation to baseline.

Final CN* = 2**(*lrr*) * *B

For purposes of reporting at the CNV_ID level, S and B are used to determine how different Final CN is from expected for that sample. The actual algorithm is proprietary.

Note: Torrent Suite™ Software hg19 FASTA hard masks all PAR region values in chromosome Y. We expect this to occur in other genome builds, otherwise the above ploidy scheme will not work as expected. The variantCaller consensus expects reads to have mapping quality value scores >0 and such reads will not be counted if the build does not omit such duplicate regions. This will have implications for assays designed to determine copy number variation in smaller homologous regions, such as SMN1 and SMN2.

To use this approach:

1. Create a normal male and chrM PAR CNV baseline. See “Create a CNV baseline“ on page 452 for more information.

2. Add the CNV baseline to an Oncomine™, Ion AmpliSeq™, or Ion AmpliSeq™ HD workflow. See “Copy Number step“ on page 91 for more information.

   Note: The name of the Target Regions BED file in the CNV baseline must match the Target Regions BED file used in the workflow.

3. Launch the analysis. See “Confirm and launch an analysis“ on page 146 for more information.

4. Visualize the analysis. See “Visualize DNA and Fusion analysis results“ on page 237 or “Visualize variants in an analysis run with an Ion AmpliSeq™ HD workflow“ on page 238 for more information.

Copy number gains are reported on the **Analysis Results** screen and the **Analysis Visualization** screen, **Variant Matrix** tab.

Reasons for NOCALL in a gene-level CNV

CNV_IDs are used to identify one or more amplicons that represent a single genomic region; most often CNV_IDs are gene symbols.

The CNV algorithm looks for heterogeneity and trends at the CNV_ID level which may potentially be indicative of data artifacts, and annotates the calls for CNV_IDs displaying these effects as SUSPECT as it is unclear whether this the data for this CNV_ID indicates a true biological focal amplification.

The annotation SEVERE_GRADIENT indicates an increasing or decreasing trend in calculated copy number above a certain threshold (range of values > 4*MAPD or > 0.5) across adjacent amplicons. This is calculated as a moving average and is compared to the local MAPD of the CNV_ID.

The annotation DIFFERENT_MEAN_SIGNAL relies on a calculation which looks at differences in groups of amplicons co-located within a CNV_ID. The amplicons are divided into two groups and if the mean difference in the log read ratio is greater than 0.5 this flag is raised.

The annotation LOCAL_AVERAGE_SIGNAL_VARIATION is made if any pairwise mean in log2 read ratio of adjacent amplicons within a CNV_ID has a value of less than -4 and another such pairwise mean has a value greater than -2. A CNV_ID with partial total loss usually is flagged by this heuristic.

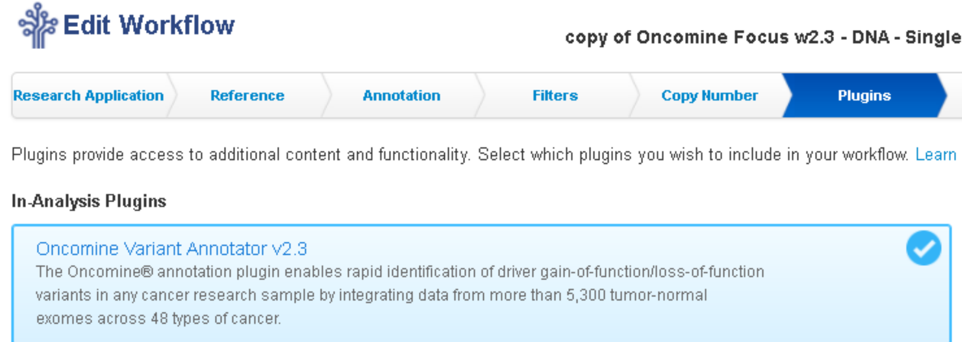
Plugins step

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 a custom workflow. This step applies to the workflows under the following research application categories: DNA, DNA and Fusions, Fusions, Annotate, and Aneuploidy.

Note: Functionality for theOncomine™ Variant Annotator plugin is highly dependent on the panel that is used for the assay, so only data from supported panels should be launched with 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 and all Ion AmpliSeq™ HD workflows.



2. Click **Next**.

Run a plugin as part of a workflow

You can 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
  - RNASeq
- 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 298).
- See "Guidelines to develop Ion Reporter™ Software plugins" on page 413 for more information on creating your own plugins.

1. In the **Workflows** tab, select **Overview**.

2. Select the checkbox in the row of a workflow, then click **Actions ▸ copy**.

   Note: You can also create a new workflow by selecting the default plugin workflow (Run Plugin w1.0). The default plugin workflow is a template for running plugins as part of a workflow. See "Guidelines to develop Ion Reporter™ Software plugins" on page 413 for more information.

3. In the **Edit** wizard, select the **Plugins** tab.

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 146).

5. *(Optional)* Click **(Settings)** to configure plugin.

6. Click **Next** to advance through the wizard steps or click the **Confirm** tab.

7. Enter the name for the workflow.

8. *(Optional)* Enter a description.

9. Click **Save Workflow**.
   The saved workflow now appears in the list of workflows in the **Workflows** screen.

Final Report step

You can set up your final report during workflow creation in Ion Reporter™ Software to reduce report setup time. This step applies to DNA, Fusions, Annotate, and Aneuploidy workflows.

1. In the **Final Report** step, select one of the following options:
   - **Default Final Report Template** – to create a standard final report. You must enter an organization name, at minimum.
   - **No Final Report Template** – no report is automatically created.
   - A **custom report template** – a custom report template is applied.
   - Click the **Workflow Presets** link – to create a custom report template. See "Create a report template with Workflow Presets" on page 127 for more information.

2. Click **Next**.

Parameters step

You can modify parameters in the Parameters step during workflow creation in Ion Reporter™ Software. Parameter categories include: annotation, bamstats, CNV finding, read mapping and variant mapping. All workflow types allow some parameter custom settings. We recommend that you **use the default settings** unless you are an advanced user.

The modules that you can customize vary by workflow.

1. In the **Parameters** step, select the parameter type on the left and modify the parameters if needed.
2. Click the **Next** button.

Annotation parameters

You can modify the following annotation parameters to optimize your analysis results when you create or edit Ion Reporter™ Software workflows.

Parameter Name	Description
Functional Annotations For All Alleles	Flag to include functional annotations for genotype-positive alleles only (false) or all reported alleles (true) for variants. **Allowed values:** True or False **Suggested trial value:** False
Use IUPAC Single Letter Code for Amino Acid	Use True for IUPAC single letter code for amino acid. Use False for three letter code. **Allowed values:** True or False. **Suggested trial value:** False
Mutation Load Calculation Filter Chain	Filter chain to enable the generation of mutation load analysis results. By default, the Mutation Load Calculation Filter Chain parameter is disabled (set to None) in most workflows. To enable mutation load calculations on DNA samples, you must also edit, or copy and edit, either: any DNA – Single Sample, or DNA and Fusions – Single Sample workflow. See "Create a custom workflow enabled for Mutation Load" on page 77 for more information. **Note:** Unlike other Ion Reporter™ Software filter chains, the Mutation Load Calculation Filter Chain generates *final* analysis results, and cannot be used to change the variants that are included in the analysis results. That is, Mutation Load results that are generated as a result of using this filter chain do not change after an analysis is complete. **Suggested trial value:** Mutation Load (Somatic SNVs)
Annotation Statistics and Reporting General Options	**Allowed values:** Select filter from the dropdown list or None **Suggested trial value:** None

Parameter Name	Description
dbSNP Hit Level	Flag to control specificity of dbSNP annotations. 'overlap' matches all annotations whose loci overlap with variant. 'locus' matches all annotations whose loci start at variant locus. 'allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'auto' hit level matches the most specific hit level possible to the annotation which could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** Overlap, locus, allele, or auto **Suggested trial value:** Overlap
COSMIC Hit Level	Flag to control specificity of COSMIC annotations. 'Overlap' matches all annotations whose loci overlap with variant. 'Locus' matches all annotations whose loci start at variant locus. 'Allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'Auto' hit level matches the most specific hit level possible to the annotation which could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** Overlap, locus, allele, or auto **Suggested trial value:** Locus
ClinVar Hit Level	Flag to control specificity of ClinVar annotations. 'Overlap' matches all annotations whose loci overlap with variant. 'Locus' matches all annotations whose loci start at variant locus. 'Allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'Auto' hit level matches the most specific hit level possible to the annotation which could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** Overlap, locus, allele, or auto **Suggested trial value:** Allele

Parameter Name	Description
VariantDB Hit Level	Flag to control specificity of VARIANTDB annotations. 'Overlap' matches all annotations whose loci overlap with variant. 'Locus' matches all annotations whose loci start at variant locus. 'Allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'Genotype' matches all annotations that are 'allele' matches where the genotypes also match. 'Auto' hit level matches the most specific hit level possible to the annotation which could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** Overlap, locus, allele, or auto **Suggested trial value:** Locus
ExAC Hit Level	Flag to control specificity of ExAC annotations. 'Overlap' matches all annotations whose loci overlap with variant. 'Locus' matches all annotations whose loci start at variant locus. 'Allele' matches all annotations that are 'locus' matches plus have at least one allele in common with variant. 'Auto' hit level matches the most specific hit level possible to the annotation which could be any of the overlap, locus, allele or genotype hit levels. **Allowed values:** Overlap, locus, allele, or auto **Suggested trial value:** Overlap

Parameter Name	Description
Gene Extension Size	Gene extension is the number of bases upstream and downstream of a transcript's start and end positions that should include the regulatory and control regions. **Allowed values:** 0 to unlimited **Suggested trial value:** 1,000
Splice Site Size	The 5' splice site of an exon is the small intronic region immediately upstream, which depends on the strand. Its size in bases is given by splice site size. **Allowed values:** 0 to unlimited **Suggested trial value:** 2 **Note:** Prior to Ion Reporter™ Software 5.10, we defined splicesite_5 and splicesite_3 as exon-centric. This was contrary to the common convention of splice site nomenclature that was intron-centric and would exchange the splicesite_5 and splicesite_3 designations. In Ion Reporter™ Software 5.10, splicesite_5 and splicesite_3 refers to the intron locations. In earlier releases of the software, you might have seen a 3' splice site in your results; you will now see a 5' splice site and vice versa.

Bamstats parameters

You can change Bamstats parameters for samples in Ion Reporter™ Software.

Parameter Name	Description
Main tab	
Maximum Coverage	Maximum Coverage of a locations in the reference. Locations with coverage more than the maximum coverage values will be ignored during coverage calculations. **Allowed values:** 100 to 1,000,000 **Suggested trial value:** 100,000
Maximum read length	Maximum read length **Allowed values:** 700 to 20,000 **Suggested trial value:** 700
Advanced tab	

Parameter Name	Description
Max mapping qv	Maximum mapping quality value. Any alignment with mapping quality value more than the specified value will be ignored. **Allowed values:** 0 to 255 **Suggested trial value:** 255
Max mismatches	Maximum mismatches allowed in the alignments. Any alignment with more then the specified number of mismatches will be ignored while generating reports related to number of mismatches. **Allowed values:** 0 to 500 **Suggested trial value:** 500
Max base qv	Max base quality values. Any base with base quality value more then the specified value will be ignored while generating reports. **Allowed values:** 0 to 100 **Suggested trial value:** 100
Maximum Medium Coverage (QC)	Max Medium Coverage value. Any base with coverage value more then the specified value will be ignored while generating reports. Value of Maximum Medium Coverage (QC) should be greater than Maximum Low Coverage (QC). **Allowed values:** 2 to unlimited **Suggested trial value:** 1,000
Minimum Target Overlap Forward	The fraction of an alignment that must be overlapped by a target in order to be classified as on target. **Allowed values:** 0.000001 to 1 **Suggested trial value:** 0.000001

Parameter Name	Description
Maximum Low Coverage (QC)	Max Low Coverage value. Any base with coverage value more then the specified value will be ignored while generating reports. Value of Maximum Low Coverage (QC) should be less than Maximum Medium Coverage (QC). **Allowed values:** 1 to unlimited **Suggested trial value:** 500
Maximum Target Overlap Reverse	The fraction of an alignment that must be overlapped by a target in order to be classified as on target. **Allowed values:** 0.0001 to 1 **Suggested trial value:** 0.5

CNV Finding parameters

You can adjust Copy Number Variant (CNV) finding parameters to optimize your analysis results in Ion Reporter™ Software.

Parameter Name	Description
Main tab	
Analysis (applies to all CNV finding algorithms)	
CNV Sensitivity	Sensitivity. Only when CUSTOM option is selected, the value of editable parameter Transition Penalty, available in Advanced tab in CNV parameters, will be utilized by the algorithm. **Allowed values:** Low, Medium, High, or Custom **Suggested trial value:** Medium
CNV Somatic	IMPORTANT: The somatic gene-level CNV calling parameter is for beta use only, and requires .bed files and a copy number informatics baseline containing gene and pooling information. Not for use in Aneuploidy workflows. **Allowed values:** True or False **Suggested trial value:** False
Advanced tab	
Gender calling	

Parameter Name	Description
CNV Gender Caller Enable Flag. (Do not enable for non-aneuploidy workflows. For other workflows, called gender results may be inaccurate)	Flag to indicate whether Gender caller should be invoked. **Allowed values:** True or False **Suggested trial value:** False
CNV Gender Threshold	Specifies threshold ratio of chrY to Autosomes for taking male/female call. **Allowed values:** 0 to unlimited **Suggested trial value:** 7
CNV Gender Min Mapping QV	Specifies min mapping qv of reads to consider in gender calling. **Allowed values:** 0 to 255 **Suggested trial value:** 30
CNV Gender Min Autosomes Count	Specifies min number of required filtered reads in autosomes. **Allowed values:** 0 to unlimited **Suggested trial value:** 25,000
CNV CHRM to Autosomes Ratio Min Mapping QV	Specifies min mapping qv of reads to consider in calculating chrM A Ratio. **Allowed values:** 0 to 255 **Suggested trial value:** 30
Sample Filtering (applies only to VCIB CNV finding algorithm)	
Read Count	User to enter a threshold number (integer). **Allowed values:** 10,000 to unlimited **Suggested trial value:** 100,000
min-mapping-qv	Minimum mapping quality value required for a read to be counted. **Allowed values:** 0 to unlimited **Suggested trial value:** 0
Percent Non Zero Amplicons. Percent of reads aligning to an amplicon in the target regions file.	User to enter a threshold number (integer). **Allowed values:** 50 to 100 **Suggested trial value:** 60
MAPD threshold. Sample will fail if MAPD is above this threshold.	User to enter a threshold number (float). **Allowed values:** 0 to unlimited **Suggested trial value:** 0.5
Analysis (applies only to VCIB CNV finding algorithm)	

Parameter Name	Description
NPC	Number of Principal Components used for correction. **Allowed values:** 0 to 12 **Suggested trial value:** 4
CNV Shift Type	Methods of CNV shift: 'Median Amplicon' where median Copy Number of autosomal amplicons is set to 2; or 'Median Gene' where median Copy Number of the autosomal genes is set to 2; or 'VALUE_BASED_ON_MEDAMP' where the amount of subtraction in log2 ratio to the result of the CNV Shift method used; or 'MAXGENE' where the log2-normalized counts for each gene in the panel is adjusted by first setting the median of the highest counts gene to the expected normal value (log2N=0) and maintaining the relative copy number of the two BRCA genes. MAXGENE ensures that at least one gene is normal copy number internally, minimizing FP calls due to slight differences in copy number between the genes in germline samples; or 'FLATGENE' where the log2normalized counts for each gene is adjusted by setting median of each gene to the expected normal value (log2N=0) independently of the other gene. FLATGENE ensures that both genes have normal copynumber internally, making calling germline exon deletion variants possible in somatic whole gene deletion samples. **Allowed values:** MEDGENE, MEDAMP, VALUE_BASED_ON_MEDAMP, MAXGENE, or FLATGENE **Suggested trial value:** MEDGENE
CNV Shift Value	The amount of subtraction in log2 ratio to the result of the CNV Shift method used. May be used to fix an error in the CNV Shift method. Shift Value is only used when the Shift Type is set to VALUE_BASED_ON_MEDAMP. **Allowed values:** -2 to 2 **Suggested trial value:** 0
Analysis (applies only to Aneuploidy workflows)	

Parameter Name	Description
Enable Mosaicism Detection	Enable Mosaicism Detection **Allowed values:** True or False **Suggested trial value:** False
Enable Smoothing	Enable Smoothing **Allowed values:** True or False **Suggested trial value:** False
Set Tile Size for Aneuploidy Workflow	Set Tile Size for Aneuploidy Workflow. The tileSize used for creating the Aneuploidy Baseline must match the tileSize selected here. **Allowed values:** 1 to 10,000,000 **Suggested trial value:** 2,000,000
Hide called gender	Hide gender called by CNV gender calling. **Allowed values:** True or False **Suggested trial value:** False
Analysis	
Plot Y chromosome for Female or Unknown Gender?	Plot Y chromosome for Female or Unknown Gender. **Allowed values:** True or False **Suggested trial value:** False
Analysis (applies only to Exon Deletions)	
Cutoff for non-integer CN calls	Specifies the cutoff for making CN #calls that are not precisely of integer values. **Allowed values:** 0 to 0.50 **Suggested trial value:** 0.30
Max Calls	Specifies the number of non-contiguous exon variant calls above which the sample will fail QC. **Allowed values:** 0 to 47 **Suggested trial value:** 4
Min Quality	Specifies the quality score below which a CNV variant is classified as a NOCALL. **Allowed values:** 0 to 100 **Suggested trial value:** 10
Analysis (applies only to Liquid Biopsy and Ion AmpliSeq™ HD)	

Parameter Name	Description
Minimum Tag Family Size	Minimum number of reads with the same tag required to form a functional family. **Allowed values:** 0 to unlimited **Suggested trial value:** 0
Max Fold Difference for Loss	Maximum fold difference relative to reference for calling a loss. **Allowed values:** 0 to 1 **Suggested trial value:** 0.85
Min Fold Difference for Gain	Minimum fold difference relative to reference for calling a gain. **Allowed values:** 1 to unlimited **Suggested trial value:** 1.15
P-value for maximum calls	P-value for maximum calls. **Allowed values:** 0 to 1 **Suggested trial value:** 0.00001
Analysis (applies to all CNV finding algorithm types except VCIB CNV algorithm)	
CNV Transition Penalty	Transition Penalty dictates the likelihood that the algorithm will call a different ploidy state between two adjacent data points. Transition Penalty is logarithm (to the base 10) of Transitional Probability. Lower (more negative) values will make it less likely that the algorithm will call adjacent data points as ploidy states that are different from each other. The Transition Penalty parameter edited here will only take effect when using the CUSTOM CNV Sensitivity setting. When CNV MOSAICISM parameter is not enabled, the maximum value supported for Transition Penalty is -1.05. When CNV MOSAICISM parameter is enabled, the maximum value supported for Transition Penalty is -2.31. **Allowed values:** -1,000,000 to -1.05 **Suggested trial value:** -8
Analysis (applies only to VCIB CNV finding algorithm when custom panel is focal amplication)	
CNV Gain Threshold	Threshold value (greater than or equal to 0) for calling GAIN in autosomes **Allowed values:** 0 to unlimited **Suggested trial value:** 4

Parameter Name	Description
CNV Gain Threshold XY	Threshold value (greater than or equal to 0) for calling GAIN in X or Y for males. **Allowed values:** 0 to unlimited **Suggested trial value:** 3
Gain Confidence Level	Confidence level value (greater than 0 and less than 1) to be used to compare to gain-threshold or gain_threshold_xy. **Allowed values:** 0 to 1 **Suggested trial value:** 0.05
Analysis (applies only to Hidden Gender Aneuploidy Workflow)	
Male Normal Ploidy Lower Bound	Male Minimum Normal Ploidy for Hidden Gender Aneuploidy Worflow. **Allowed values:** 0 to 100,000 **Suggested trial value:** 0.8
Male Normal Ploidy Upper Bound	Male Maximum Normal Ploidy for Hidden Gender Aneuploidy Worflow **Allowed values:** 0 to 100,000 **Suggested trial value:** 1.2
Female Normal Ploidy Lower Bound	Female Minimum Normal Ploidy for Hidden Gender Aneuploidy Worflow. **Allowed values:** 0 to 100,000 **Suggested trial value:** 1.8
Female Normal Ploidy Upper Bound	Female Maximum Normal Ploidy for Hidden Gender Aneuploidy Worflow **Allowed values:** 0 to 100,000 **Suggested trial value:** 2.2

Read Mapping parameters

You can adjust read mapping parameters to optimize your analysis results.

Variant Name	Description
Main tab	
Tmap Mapped Files Enable Re-map	Flag to indicate whether mapepd Bam files should be remapped. **Allowed values:** True or False **Suggested trial value:** False

Variant Name	Description
Tmap Realignment Enable Flag	Flag to indicate whether Bam Realignment should be invoked. **Allowed values:** True or False **Suggested trial value:** False
Advanced tab	
Mismatch Penalty	Specifies the mismatch penalty. **Allowed values:** 1 to 100 **Suggested trial value:** 3
Soft Clipping Type	Specifies the type of soft-clipping to perform. **Allowed values:** 0 to 3 **Suggested trial value:** 2
Gap Open Penalty	Specifies the gap open penalty. **Allowed values:** 1 to 100 **Suggested trial value:** 5
Match Score	Specifies the match score. **Allowed values:** 1 to 100 **Suggested trial value:** 1
Gap Extension Penalty	Specifies the gap extension penalty. **Allowed values:** 1 to 100 **Suggested trial value:** 2
Context	Realign with context-dependent gap scores. **Allowed values:** True or False **Suggested trial value:** False
Maximum Amplicon Overrun Large INDEL Rescue	The maximum number of bases allowed for a read to overrun the end of the amplicon. **Allowed values:** 0 to unlimited **Suggested trial value:** 6
Flow Score Penalty	Specifies the flow score penalty. **Allowed values:** 1 to 100 **Suggested trial value:** 2
Max adapter bases for soft clipping	Specifies to perform 3' soft-clipping (via -g) if at most this # of adapter bases were found. **Allowed values:** 0 to unlimited **Suggested trial value:** 2147483647

Variant Name	Description
Local Band Width	Specifies the Local band width. **Allowed values:** 1 to 100 **Suggested trial value:** 50
Do Repeat Clip	Trim repetitive sequence at the ends of alignment. **Allowed values:** True or False **Suggested trial value:** False
End Repair	Specifies to perform 3' end repair. **Allowed values:** 0 to unlimited **Suggested trial value:** 0
Use BED file	Use bed file to capture INDELs near amplicon edges. **Allowed values:** True or False **Suggested trial value:** False
Output Filter	Specifies the output filter for the mapping. **Allowed values:** 0 to 3 **Suggested trial value:** 1
Maximum INDEL Size to Rescue	The maximum INDEL size to rescue with one large INDEL algorithm. **Allowed values:** 0 to unlimited **Suggested trial value:** 30

Variant Finding parameters

You can change the Variant Finding parameters to optimize your analysis results in Ion Reporter™ Software.

Parameter Name	Description
Main tab	
Liquid Biopsy and Ion AmpliSeq™ HD	
ampliseq-hd	Sets defaults for counting reads in liquid biopsy runs with ampliseq-hd using "tvc consensus". **Allowed values:** 0 to 1 **Suggested trial value:** 0

Parameter Name	Description
Min Family Size	Minimum number of reads with same Unique Molecular Tag required to form a functional family. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 0 to unlimited **Suggested trial value:** between 3 and 7
min-mapping-qv	Minimum mapping quality value required for a read to be counted. **Allowed values:** 0 to unlimited (integer) **Suggested trial value:** 20 (TagSeq); 0 (other)
Poisson	Use Poisson parameter estimation to estimate count of functional families. **Allowed values:** 0 to 1 **Suggested trial value:** 0
SNP Min Var Coverage	Minimum number of variant supporting functional families required to make a SNP call. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 2 to 10 **Suggested trial value:** 2
MNP Min Var Coverage	Minimum number of variant supporting functional families required to make a MNP call. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 2 to 10 **Suggested trial value:** 2
INDEL Min Var Coverage	Minimum number of variant supporting functional families required to make a INDEL call. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 2 to 10 **Suggested trial value:** 2

Parameter Name	Description
Hotspot Min Var Coverage	Minimum number of variant supporting functional families required to make a hotspot call. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 2 to 10 **Suggested trial value:** 2
Indel Func Size Offset	Require family of size >= (min_tag_fam_size + this value) to be functional for calling HP-INDEL. Impact: Increasing values make variant calls less sensitive but more specific. **Allowed values:** 0 to 4 **Suggested trial value:** 2
Tag Sim Max Cov	Check the similarity of UID of variant families if the variant molecular coverage is less than or equal to this value. Related VCF field: TGSM. **Allowed values:** 0 to unlimited **Suggested trial value:** 20
Minimum Family Coverage per Strand	Minimum required coverage of reads on each strand in a bi-directional molecular tag family. **Allowed values:** 0 to unlimited **Suggested trial value:** 0
Analysis	
Data Quality Stringency	Filter: Phred-scaled minimum average evidence per read or no-call. Related VCF field: MLLD. **Allowed values:** 0 to unlimited **Suggested trial value:** 6.5
Downsample to Coverage	Reduce coverage in over-sampled locations to this value. **Allowed values:** 1 to unlimited **Suggested trial value:** 4000
SNP Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited **Suggested trial value:** >=3

Parameter Name	Description
SNP Min Variant Score	Filter: Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited **Suggested trial value:** 10
SNP Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals) **Suggested trial value:** 0.0005 to 0.005 (TagSeq), 0.01 to 0.2 (other)
SNP Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited **Suggested trial value:** 2 (TagSeq), 5 to 20 (other)
SNP Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1.0 (decimals) **Suggested trial value:** 0.95
INDEL Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited **Suggested trial value:** >=3
INDEL Min Variant Score	Filter: Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** >=10
INDEL Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals) **Suggested trial value:** 0.0005 to 0.005 (TagSeq), 0.05 to 0.2 (other)
INDEL Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** 2 to 10,000 (TagSeq), 15 to 30 (other)

Parameter Name	Description
INDEL Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1.0 (decimals) **Suggested trial value:** 0.95
Hotspot Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited (integers **Suggested trial value:** 3
Hotspot Min Variant Score	Phred-scaled evidence that the reads support a variant above minimum frequency. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** >=10
Hotspot Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1.0 (decimals) **Suggested trial value:** 0.0005 to 0.005 (TagSeq); 0.01 to 0.2 (other)
Hotspot Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** 2 to 10,000 (TagSeq); 5 to 20 (other)
Hotspot Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1.0 (decimals) **Suggested trial value:** 0.95
Prediction Precision	Number of pseudo-data-points suggesting our predictions match the measurements without bias. **Allowed values:** 0 to unlimited (decimals) **Suggested trial value:** 1.0
Outlier Probability	Prior probability that a read comes from some other distribution. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 0.005 to 0.01

Parameter Name	Description
Heavy Tailed	How heavy the t-distribution tails are to allow for unusual spread in the data. **Allowed values:** 0 to unlimited (decimals) **Suggested trial value:** 3
Filter Unusual Predictions	Filter: predictions are distorted to fit the data more than this distance (relative to the size of the variant). Related VCF fields: FWDB, REVB [RBI = sqrt(FWDB ^ 2 + REVB ^ 2)]. **Allowed values:** 0 to unlimited (decimals) **Suggested trial value:** 0.3 (30% of variant change size)
Filter Insertion Predictions	Filter: observed clusters deviate from predictions more than this amount (relative to the size of the variant). Related VCF fields: VARB, REFB. **Allowed values:** 0 to unlimited (decimals) **Suggested trial value:** 0.2 (20% of variant change size)
Filter Deletion Predictions	Filter: observed clusters deviate from predictions more than this amount (relative to the size of the variant). Related VCF fields: VARB, REFB. **Allowed values:** 0 to unlimited (decimals) **Suggested trial value:** 0.2 (20% of variant change size)
HP Max Length	Filter: homopolymer length involved in an in/del. Related VCF field: HRUN. **Allowed values:** 1 to unlimited (integers) **Suggested trial value:** 8
Do SNP Realignment	Realign reads in the vicinity of SNP candidates. Impact: True = do not realign, False = realign. **Allowed values:** True or False **Suggested trial value:** PGM: (germline) True, (somatic) False; Proton: (germline) False, (somatic) False.

Parameter Name	Description
Suppress Recalibration	Recalibration values from pipeline used or not (experimental). No related fields, changes basecalling behavior. Allowed values: True = allow recalibration, False = don't allow recalibration. **Allowed values:** True or False **Suggested trial value:** False
SSE Probability Threshold	Filter out variants in motifs with error rates above this. **Allowed values:** 0 to 1 **Suggested trial value:** 0.2
MNP Min Cov Each Strand	Filter: Minimum coverage required on each strand. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** >=3
MNP Min Variant Score	Filter out MNPs with a QUAL score less than or equal to this Phred-scaled value. **Allowed values:** 0 to unlimited **Suggested trial value:** 10
MNP Min Allele Freq	Frequency cutoff for supporting a variant. **Allowed values:** 0 to 1 **Suggested trial value:** 0.0005 to 0.005 (TagSeq); 0.01 to 0.2 (other)
MNP Min Coverage	Total coverage required of reads or no-call. **Allowed values:** 0 to unlimited (integers) **Suggested trial value:** 2 to 10,000 (TagSeq); 5 to 20 (other)
MNP Strand Bias	Filter: proportion of variant alleles comes overwhelmingly from one strand. Related VCF field: STB. **Allowed values:** 0.5 to 1 **Suggested trial value:** 0.95
MNP Strand Bias Pval	Filter out mnps with pval below this [1.0] given strand bias > mnp-strand-bias. **Allowed values:** 0 to 1 **Suggested trial value:** 1

Parameter Name	Description
SNP Strand Bias Pval	Filter out snps with pval below this [1.0] given strand bias > snp-strand-bias. **Allowed values:** 0 to 1 **Suggested trial value:** 1
INDEL Strand Bias Pval	Filter out indels with pval below this [1.0] given strand bias > indel-strand-bias. **Allowed values:** 0 to 1 **Suggested trial value:** 1
Hotspot Strand Bias Pval	Filter out hotspot variants with pval below this [1.0] given strand bias > hotspot-strand-bias. **Allowed values:** 0 to 1 **Suggested trial value:** 1
Position Bias Reference Fraction	Skip position bias filter if (reference read count)/(reference + alt allele read count) less than or equal to this. **Allowed values:** 0 to 1 **Suggested trial value:** 0.05
Position Bias	Filter out variants with position bias relative to soft clip ends in reads > position-bias. **Allowed values:** 0 to 1 **Suggested trial value:** 0.75
Position Bias Pvalue	Filter out if position bias is above the Position Bias given pval less than Position Bias Pval. **Allowed values:** 0 to 1 **Suggested trial value:** 0.05
Use position bias	Enable the position bias filter. **Allowed values:** True or False **Suggested trial value:** False
Indel As HPindel	Apply indel filters to non HP indels. **Allowed values:** True or False **Suggested trial value:** False
Do MNP Realignment	Realign reads in the vicinity of candidate mnp variants. **Allowed values:** True or False **Suggested trial value:** True

Parameter Name	Description
Realignment Threshold	Maximum allowed fraction of reads where realignment causes an alignment change. **Allowed values:** 0 to 1 **Suggested trial value:** 1
FD Nonsnp Min Var Cov	Override min_var_coverage of the flow-disrupted variants that are not SNPs (0 to disable the override). Impact: Decreasing values make variant calls less specific but more sensitive. **Allowed values:** 0 to 10 **Suggested trial value:** 1
Read Mismatch Limit	Do not use reads with number of mismatches (where 1 gap open counts 1) above this value. **Allowed values:** 0 to unlimited **Suggested trial value:** 5 (TagSeq), 0 (other)
Min Cov Fraction	Do not count reads with fraction of covering any amplicons below this threshold. **Allowed values:** 0 to 1 **Suggested trial value:** 0.9 (TagSeq), 0 (other)
Use Input Allele Only	Only consider provided alleles in the hotspots file. 0 = generate de novo candidates, 1 = hotspots only. **Allowed values:** 0 to 1 **Suggested trial value:** 0
Advanced tab	
Analysis	
Allow Indels	Enable indels in FreeBayes hypothesis generator. Allowed values: True = generate indel hypotheses, False = don't generate. **Allowed values:** True or False **Suggested trial value:** True
Allow SNPs	Enable SNPs in FreeBayes hypothesis generator. Allowed values: True = generate SNP hypotheses, False = don't generate. **Allowed values:** True or False **Suggested trial value:** True

Parameter Name	Description
Allow MNPs	Enable MNPs in FreeBayes hypothesis generator. Allowed values: True = generate MNP hypotheses, False = don't generate. **Allowed values:** True or False **Suggested trial value:** True
Allow Complex	Enable complex variants in FreeBayes hypothesis generator. Allowed values: True = generate MNP hypotheses, False = don't generate. **Allowed values:** True or False **Suggested trial value:** False
Minimum mapping qv	Minimum mapping QV value required for reads to be allowed into the pileup (both FreeBayes and evaluator). **Allowed values:** 0 to unlimited (integer) **Suggested trial value:** 4
Read SNP Limit	Read mismatch limit on number of mismatches: filter potential mis-mapped reads. **Allowed values:** 0 to unlimited (integer) **Suggested trial value:** 10
Read Max Mismatch Fraction	Read maximum mismatch fraction of mismatches in length of read: filter potential mis-mapped reads. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 1
Generate Min Alt Allele Freq	Generate variants with at least this frequency in the pileup. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 0.02 to 0.15
Generate Min Indel Alt Allele Freq	Generate INDEL variants with at least this frequency in the pileup. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 0.02 to 0.15
Generate Min Coverage	Generate variants in locations with at least this depth of coverage. **Allowed values:** 0 to unlimited **Suggested trial value:** 6

Parameter Name	Description
Kmer Len	Size of the smallest k-mer used in assembly. Impact: Increasing values make indel calls less sensitive but more specific. **Allowed values:** 5 to unlimited (integers) **Suggested trial value:** 11 to 30
Min Var Count	Minimum support for a variant to be evaluated. Impact: Increasing values make indel calls less sensitive but more specific. **Allowed values:** 1 to unlimited (integers) **Suggested trial value:** 3 to 30
Short Suffix Match	Minimum assembled sequence match on both sides of the variant. Impact: Increasing values make indel calls less sensitive but more specific. **Allowed values:** 2 to unlimited (integers) **Suggested trial value:** between 4 and the value given to the kmer_len parameter
Min INDEL Size	Minimum size INDEL reported by assembly. Impact: Increasing values make indel calls less sensitive but more specific. **Allowed values:** 1 to unlimited (integers) **Suggested trial value:** 2 to 30
Max HP Length	Variants containing HP larger than this are not reported. Impact: Increasing values make indel calls more sensitive but less specific. **Allowed values:** 1 to unlimited (integers) **Suggested trial value:** 2 to 11
Min Var Freq	Minimum frequency of the variant to be reported. Impact: Increasing values make indel calls less sensitive but more specific. **Allowed values:** 0 to 1 (decimals) **Suggested trial value:** 0.1 to 0.4
Relative Strand Bias	Variants with strand bias above this are not reported. Impact: Increasing values make indel calls more sensitive but less specific. **Allowed values:** 0 to 1(decimals) **Suggested trial value:** 0.6 to 1.0

Parameter Name	Description
Output MNV	Enables reporting of complex variants. Impact: 1 = report complex variants, 0 = don't report. **Allowed values:** 0 to 1(decimals) **Suggested trial value:** 0
Variant View	Represent variants with Locus view or Allele view **Allowed values:** Locus view or Allele view **Suggested trial value:** your preference
Analysis SSE File	Analysis strand specific error file. Select a mask from the dropdown list or upload a mask from Upload.

Import custom variantCaller parameters

If you would like to use custom variantCaller plugin 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 variantCaller parameters from Torrent Suite™ Software and store the JSON file on your computer or network.
2. In the **Parameters** step, select **Variant Finding**.
3. Click **Select File** and browse to your variantCaller parameters JSON file and click **Import**.
   The variantCaller 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 step

The **Confirm** step is the final step that you must complete to create a workflow in Ion Reporter™ Software. All workflows include a confirmation step.

1. Enter a name for the workflow.
2. *(Optional)* Enter a description of the workflow.
3. Click **Save Workflow**.
   The workflow is saved and is added to the **Workflows** table.

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

Upload a target regions BED file workflow preset

You can upload a target regions BED file from a copied workflow and then create a target regions BED file workflow preset for use in other workflows.

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. For **Reference**, select **GRCh38** or **hg19**.
4. In the **Target Regions** section, click **Upload**.
5. 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, then click **Upload**.
Ion Reporter™ Software uploads and verifies your BED file. When verification is complete, the progress bar changes to green, and a message confirms the files are ready for use.

d. Click **Close**.

The new BED file preset appears in the **Target Regions** section of the Reference step.

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

7. *(Optional)* When you have run a set of samples to validate that your preset works as you intended, select the preset in the **Workflow Presets** table, then click **Actions ▸ Lock** to lock the workflow. You cannot undo a lock action.

Upload a hotspot regions BED file preset

You can upload a hotspots BED file from a copied workflow and then create a hotspots BED file workflow preset for use in other workflows.

IMPORTANT! Use only BED file names that do not contain spaces. If a file name include 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** step, in the **Hotspot Regions** section, click **Upload**.

4. In the **Upload Hotspot File** dialog box:

   a. Click **Select File**, then browse to your BED file.

   b. Confirm that the correct BED file name appears in the display field, then 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 step.

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

6. *(Optional)* When you have run a set of samples to confirm that your new preset works as you intended, select the preset in the **Workflow Presets** table, then click **Actions** ▸ **Lock** to lock the workflow. You cannot undo a lock action.

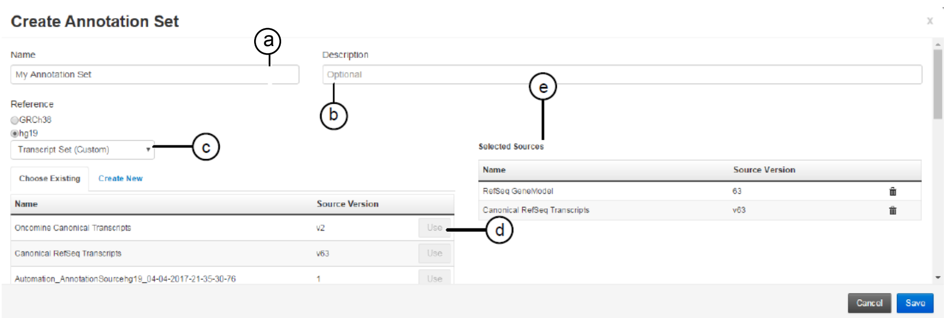
Create annotation set workflow presets

You can create custom annotation set workflow presets for use in your Ion Reporter™ Software analyses.

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 an Ion Reporter™ Software 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** step 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.

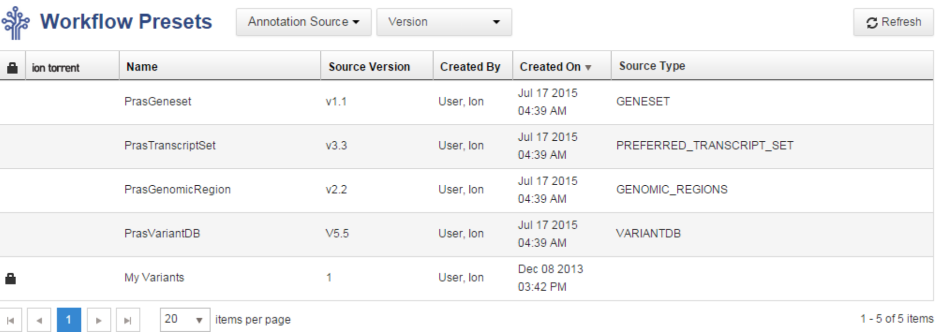
View custom annotation sources

Annotation sets created by users are displayed in the Annotation Source dropdown list. You can mix customer annotation sources with Ion Reporter™ Software predefined annotation sources to create your own custom annotation set. See "Create annotation set workflow presets" on page 123 for details.

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 a Copy Number baseline

You can create a Copy Number Variant (CNV) baseline workflow preset in Ion Reporter™ Software for use in your analyses. These presets are used in the Copy Number step 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.
- 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 "Configure Baseline Type" on page 125, "Select samples" on page 125, and "Confirm baseline" on page 126 for more informaiton.

Configure Baseline Type

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

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

To configure your baseline type:

1. Select the baseline type, then select the target regions file from the dropdown list.
2. Click **Next**.

Select samples

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

Samples that are used in CNV baselines:

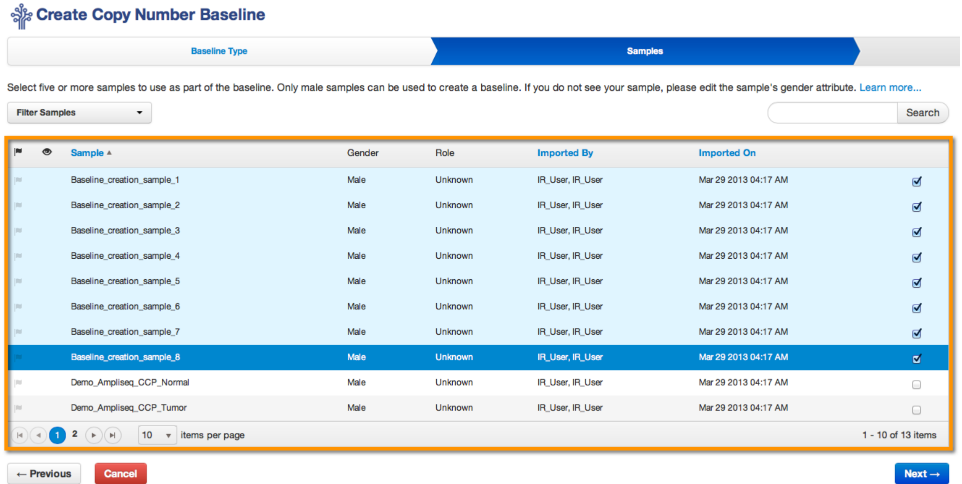
- Use only male samples in the baseline creation process in Ion Reporter™ Software 4.x.x.
- Although at least five samples are required in the baseline creation, ten or more samples are recommended for satisfactory results. (The use of more samples for baseline calculation substantially reduces sample-to-sample 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 38 for information on how to upload or define a sample.
- See "Gender information requirements" on page 38 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.

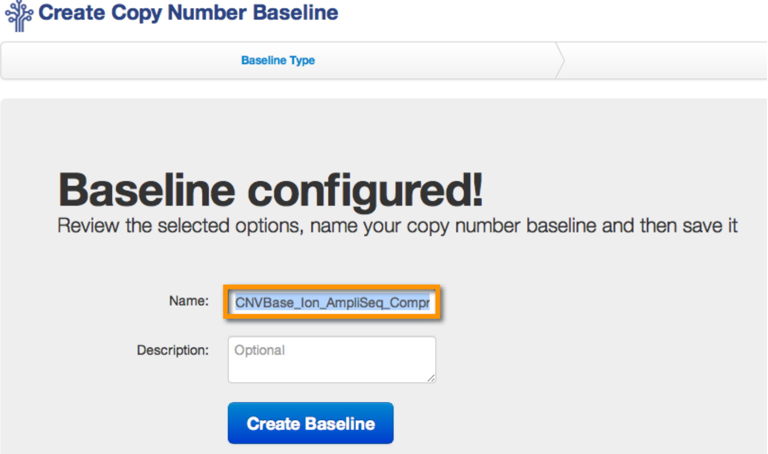


2. Click **Next**.

Confirm baseline

Use the **Confirm** step to name your baseline and review before you launch the baseline creation.

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** step of creating a workflow.

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

Create a report template with Workflow Presets

You can predefine your report template as an Ion Reporter™ Software workflow preset. You can also create a more flexible report template from your analysis results. See "Create a report template" on page 267 for more information.

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset ▸ Final Report Template**.
3. In the **Create Final Report Template** dialog box, do the following:

   Note: For optional sections, either enter content for the section or click the **Exclude** to remove the section from the report template.

   a. *(Required)* Enter a name for the report template.

   b. *(Optional)* Enter a description.

   c. *(Optional)* Click **Upload Logo**. The **Upload Header Logo** dialog box appears. Click **Select File** to browse to your logo file. Acceptable formats are: PNG, GIF, and JPG.

   d. *(Optional)* Enter an Organization name.

   e. *(Optional)* Enter the organization address.

   f. *(Optional)* Enter other standard information for the **Background**, **Disclaimer**, and **Sign-Off** sections.

      Note: If you want the **Sign Off** section to list more than one required approver, click **Add** to add an extra approver.

4. Click the **Save** to create your report template preset.
5. Next, add your report template to a workflow. See "Add a report template to an Ion Reporter™ Software workflow" on page 272 for more information.
6. Launch an analysis from your workflow. See "Launch an analysis" on page 135 for more information.

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 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

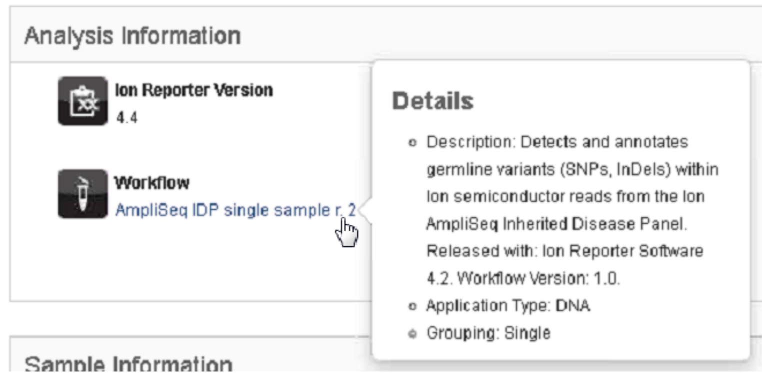
The information that you enter in the Header section also is fixed and appears as-is for every analysis (in workflows that use this template). You specify the content for the Header section.

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. The number increases if you copy or edit either an Ion Reporter™ predefined workflow or custom workflow and use the name of the previous workflow as the name for the new workflow.
   You can copy Ion Reporter™ predefined workflows and custom workflows from the current version of the software or from previous versions of the software.
   When you hover over the workflow version, the revision numbers appear at the end of the workflow name (r.#). This revision level also appears on the QC Report and Audit log.



Lock a workflow

You can lock a workflow to ensure that the workflow settings are not changed.

Note: After a workflow is locked, it cannot be edited or unlocked. However, it can be copied.

1. Sign in to Ion Reporter™ Software, then click the **Workflows** tab.
2. Select the workflow that you want to lock, then click **Actions** ▸ **Lock**.
3. Click **Yes** to confirm that you want to lock the workflow.

The workflow is now locked and cannot be edited.

Search, sort, or filter Workflows and Workflow Presets

You can search, sort, and filter the **Workflows** and **Workflow Presets** tables to help you in finding the information within the tables more easily.

In the **Workflows** tab:

- Click **Overview**, then perform the following actions in the **Workflows** table.

  Note: You can also perform the following actions in the **Workflows** table that is located in the **Launch** screen of the **Analyses** tab.

- Click **Presets**, then perform the following actions in the **Workflow Presets** table.

To...	Do this...
Search the list	Enter a search term into the **Search** field, then click **Go**. **Note:** The search field is outlined in red if the search string is invalid. The following rules apply to all search fields: • An asterisk (*) is not allowed in the search field. • Searches are not case sensitive. • 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.
Sort the list	Click on a column header to sort the list based on the column category type (numerical, alphabetical, date). Click on the column header again to reverse the order.
Select filters within a filter category	Click on the filter category to expand the dropdown list, then select a filter from the list.
Remove filters from a filter category	Click on the filter category to expand the dropdown list, then select **Show All** at the top of the list.

Ion Reporter™ predefined workflows

Tumor-normal pair research

Ion Reporter™ predefined workflows for tumor-normal research generate analysis results that identify reads of both a tumor sample and reads of the related normal sample. These workflows are optimized to find somatic variants, which appear in the tumor sample and *do not appear in the normal sample*. These workflows also perform a statistical evaluation of the likelihood that the tumor allele is *not present in the normal sample* and calculates a P-value that represents the statistical confidence of that call.

At each position within a variant in the tumor research sample, the evidence for that allele in the normal sample is examined as part of the analysis. If the tumor allele is detected in the reads of the normal research sample in levels that are higher than the error rate, it is not considered to be a tumor-specific (somatic) variant and therefore the variant is rejected (not called).

Tumor variants in which the same position in the normal sample has no coverage, or has low coverage, are designated as non-confident. Non-confident variants are not assigned a P-value, and are 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. This occurs with variants for which both of these conditions are true:

- The allele frequencies for the variant 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 the variant might appear *not only* in the tumor sample, but also in the normal sample. This could indicate that either a germline variant or a systematic error is present in both samples.

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

The tumor-normal pair Ion Reporter™ predefined workflows is run on a pair of research samples from the same individual. Ideally both research samples are sequenced on the same chip.

Note: When you create a custom workflow for an AmpliSeq Exome tumor-normal pair analysis, we recommend that you do one of the following to ensure that the correct parameters that are applied:

1. Make a copy of the Ion Reporter™ predefined workflow or a custom workflow for use with AmpliSeq Exome tumor-normal pairs and edit any desired parameters. See “Create a custom workflow from an existing workflow“ on page 71 for more information.
2. Use the predefined BED file to create a new custom workflow. See “Create a new custom workflow without predefined settings“ on page 72 for more information.

It is *not* recommended that you import the AmpliSeq Exome panel BED file through either the Import function in Ion Reporter™ Software , or with a manual import.

Flow Space Alternate Allele Calculation (FAO) calculation

Flow Space Alternate Allele Calculation (FAO) is calculated with the following formula.

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.

CNV and aneuploidy research

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

These workflows contain a CNV detection module, and corrects read coverage for GC bias. Corrected coverage is compared to a baseline coverage from control samples of regions with known correct ploidy (ploidy of 2).

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

The following information applies to the Ion Reporter™ predefined workflows for CNV and aneuploidy research and to custom workflows that you create from Ion Reporter™ predefined workflows:

- The input data are 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.
- These workflows identify regions of the genome that are duplicated or deleted. The variant length detectable is typically from ~10 Mb up to a whole chromosome.
- These workflows overcome the variations in coverage that are typical with amplified data.
- With these workflows, 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 predefined 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 133 and “Create a custom aneuploidy No Gender workflow“ on page 78 for more information.

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 "Create a custom aneuploidy No Gender workflow" on page 78 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.

Analyses in Ion Reporter™ Software

Analyses are comprised of samples and an Ion Reporter™ Software workflow that you want use to generate analysis results. You can review and interpret analysis results in Ion Reporter™ Software, when you generate reports and use the visualization tools IRGV and IGV.

The first time you select samples and a workflow to run the samples through, you *launch* the analysis. After the analysis is successfully launched, you can return to the analysis to open or rerun the results, or launch a new analysis based on changes that you make.

You can launch a single analysis, or multiple analyses.

To launch a single analysis, you will either:

- Review the details for a workflow, then select the workflow that you want to use in the analysis.
- Launch an analysis from the list of analyses in Ion Reporter™ Software.

To launch multiple analyses, you can select multiple analyses in Ion Reporter™ Software, or create a CSV file that describes the analyses and upload that file to Ion Reporter™ Software. See "Launch analyses by batch" on page 157 for details.

Launch an analysis

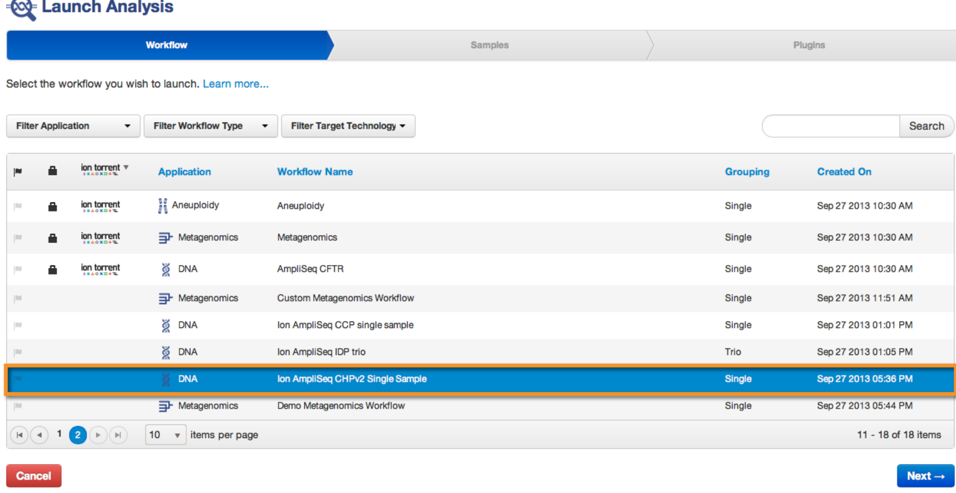
Use one of the following methods to launch a single analysis:

- You can review the details for a workflow, then select the workflow that you want to use in the analysis that you will launch.
  a. In the **Workflows** tab, select the row that contains the workflow that you want to use in an analysis.
     Information for the selected workflow is shown in the Details pane.
  b. Click **Actions ▸ Launch Analysis**.
     The **Launch Analysis** screen opens to the **Samples** step.
  c. Proceed to "Choose samples" on page 139.
- You can launch an analysis from the list of analyses in Ion Reporter™ Software:
  a. In the **Analyses** tab, click **Launch Analysis ▸ Manual**.
     The **Launch Analysis** screen opens to the **Workflow** step.
  b. Proceed to "Select a workflow" on page 136.

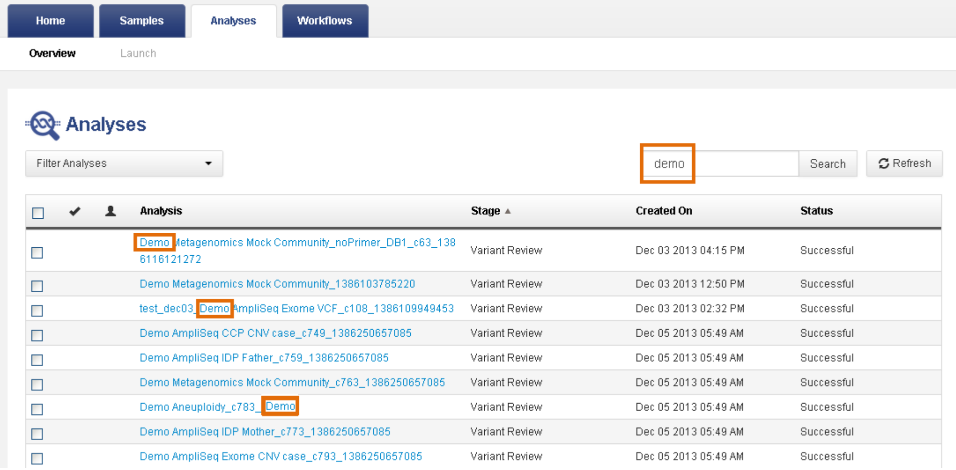
Select a workflow

In the Workflow chevron, you select a workflow to launch. If you have previously selected a workflow, proceed to “Choose samples“ on page 139

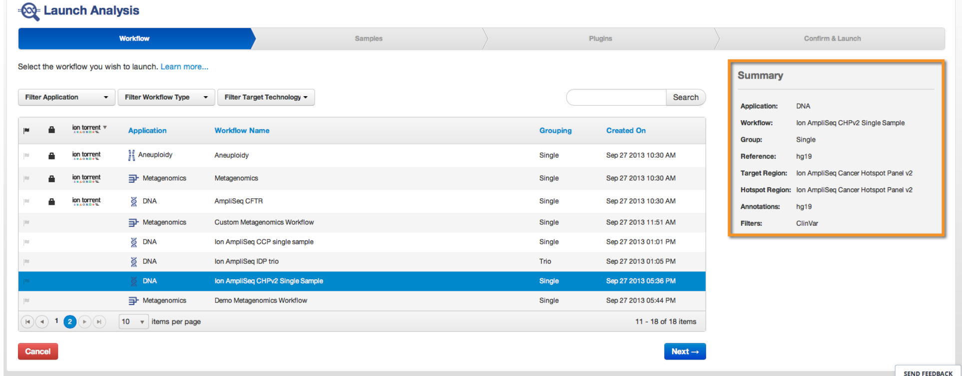
1. In the workflow table, select the workflow that you want to launch. Click a row to highlight a workflow.



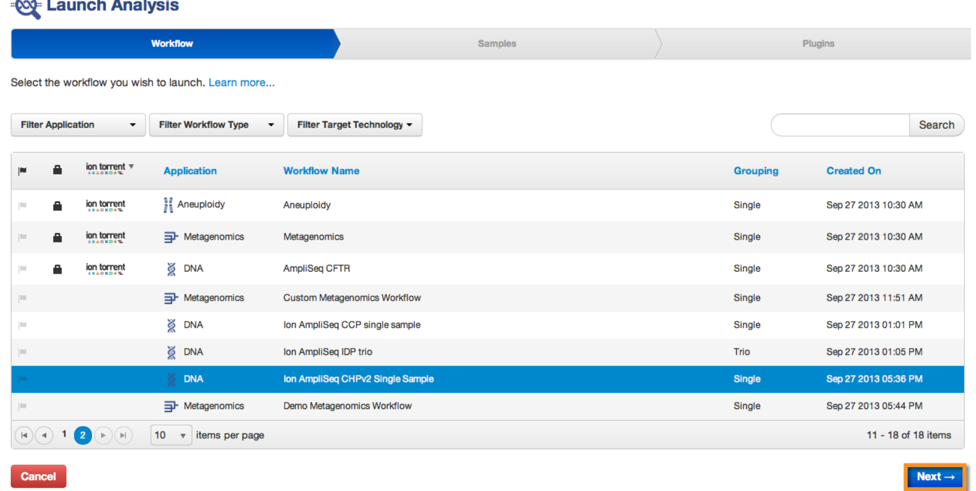
Note: To find a workflow in the table, you can use**Search**. Enter a string such as "CCP", "Exome", "IDP", "tumor", "TargetSeq", or your own custom workflow name and then click **Search** to get a list of results:



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



3. Click the **Next** button.



Proceed to “Choose samples“ on page 139.

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	—

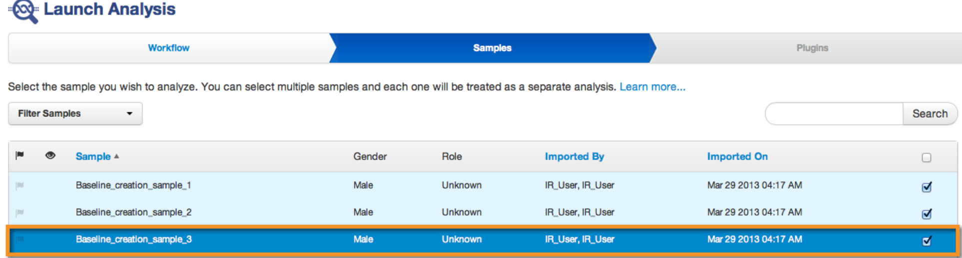
Choose samples

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

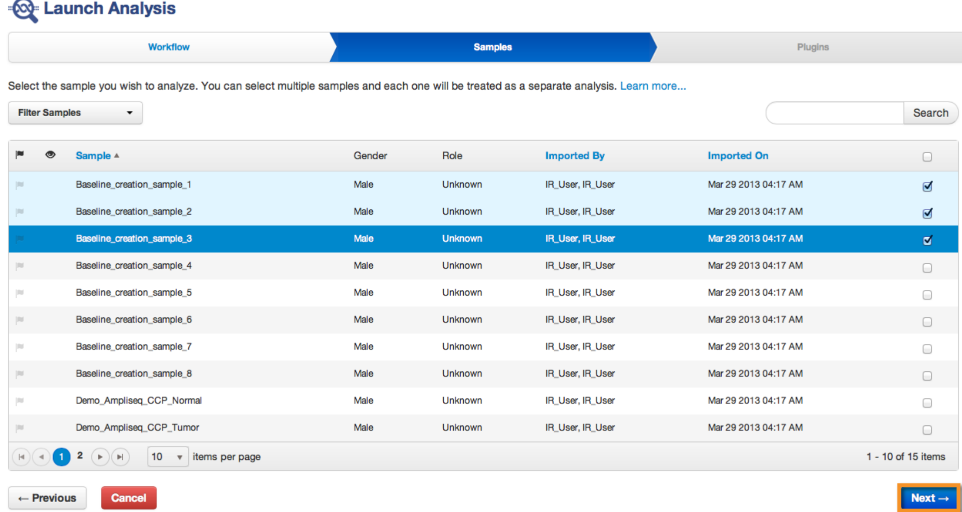
Select a single-sample or multi-sample analyses

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**.



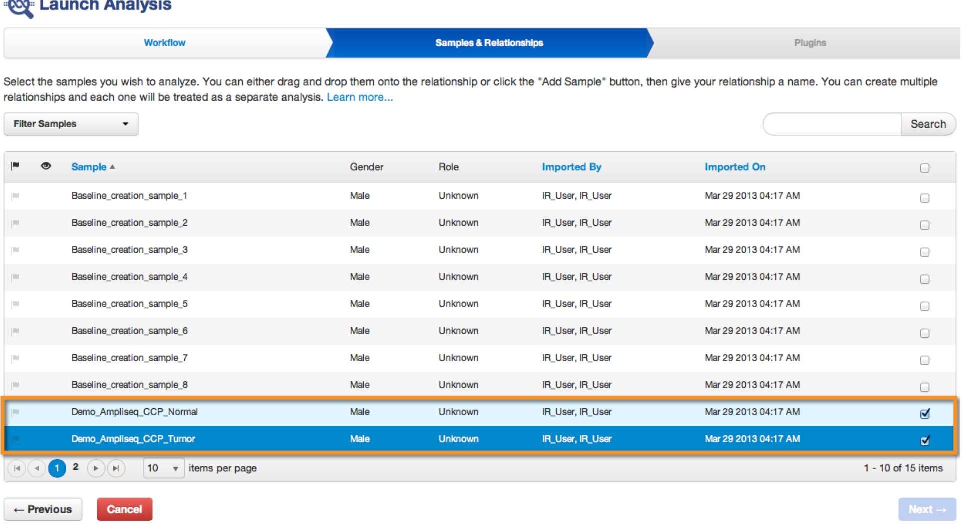
Note: For DNA workflows, if you select multiple samples on this page, Ion Reporter™ Software creates a separate analysis for each sample.

Select paired-sample or tumor-normal analyses

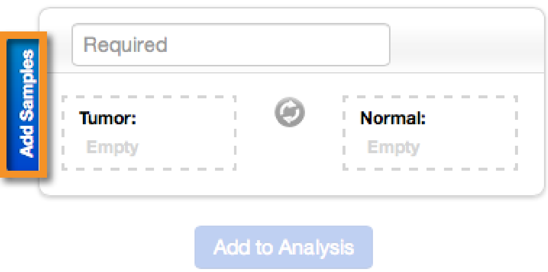
Note: For demo data samples with the tumor-normal workflow, use only the Demo AmpliSeq CCP normal and Demo AmpliSeq CCP tumor data sets.

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.

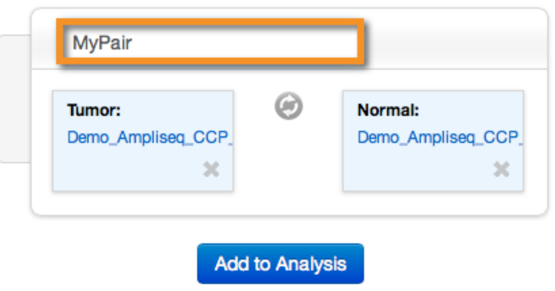
   This activates the **Add Samples** button on the pair window.

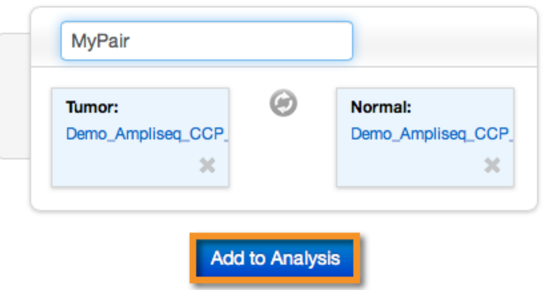
2. Click **Add Samples** to add the samples to the pair.

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.

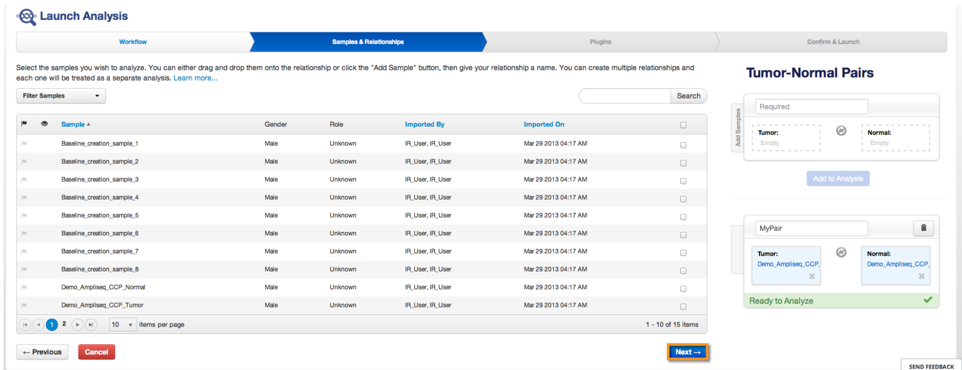


5. Click **Add to Analysis**.



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

7. Click **Next**.

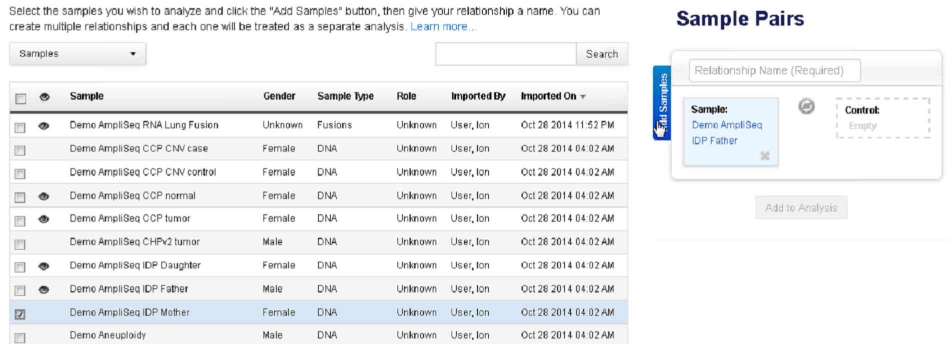


Control sample for CNV workflows

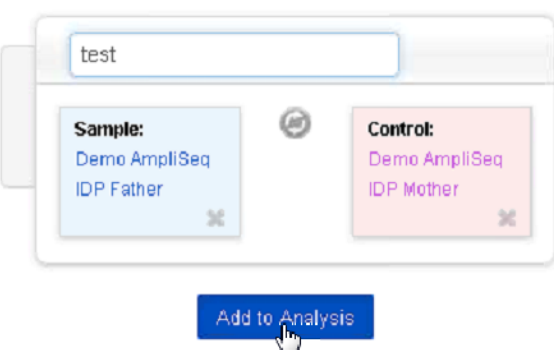
CNVs are reported based on their copy number relative to the control sample used. For best results, choose a control sample with no known CNVs in any region that is covered by the Ion AmpliSeq™ panel used. If most or all test samples are reported as having a CNV in the same region, one possible cause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

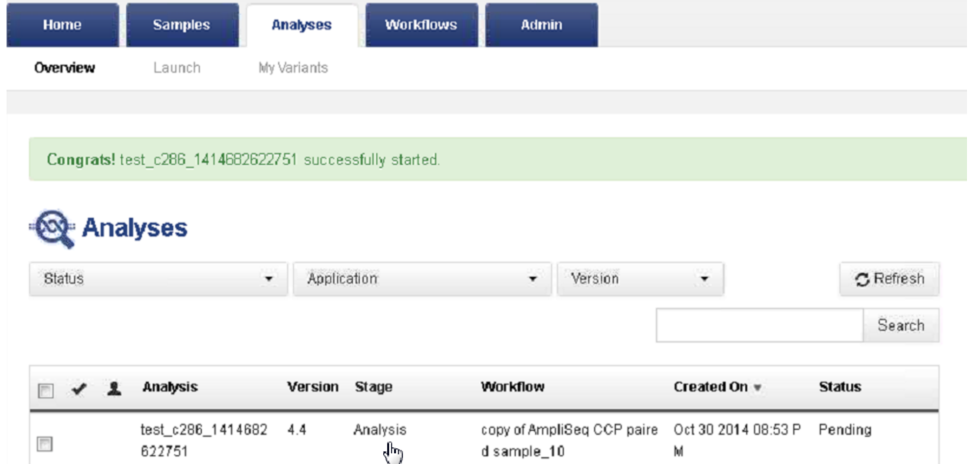


2. Name the sample pair and click **Add to Analysis**.



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.

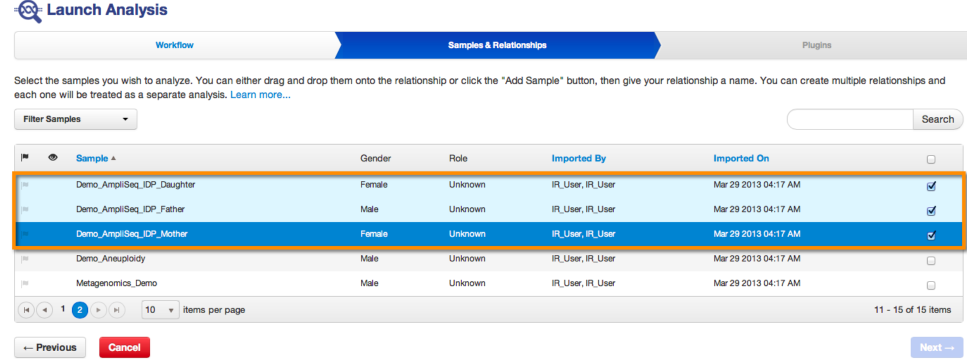
6. Verify there is a message that states the analysis started successfully:



Select Trio analyses

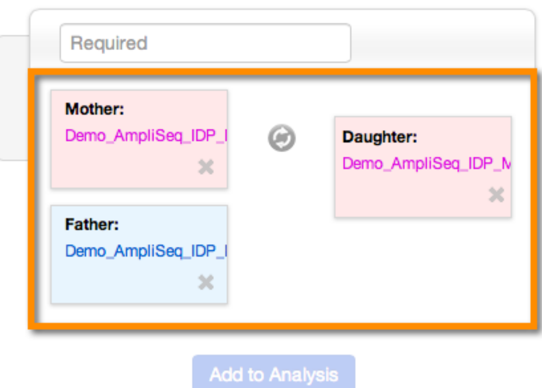
Follow these steps to select samples for trio workflows:

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.



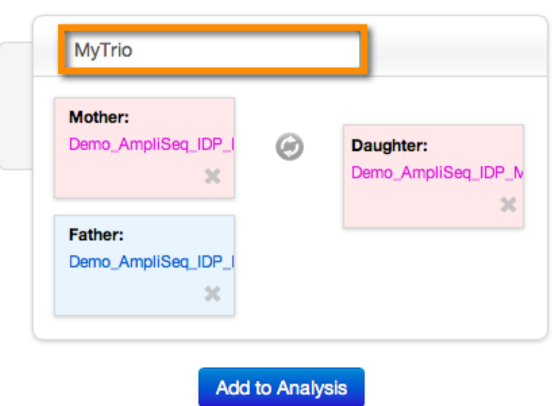
This activates the **Add Samples** button on the trio window.

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

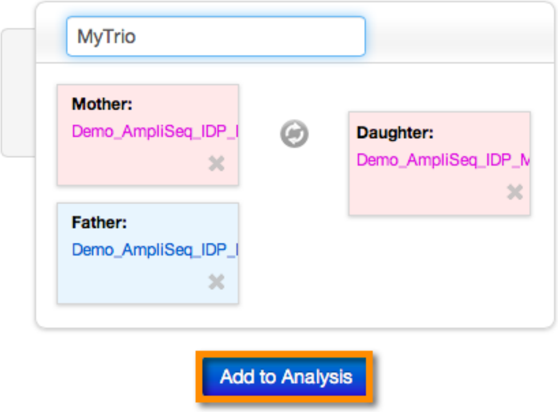


3. If the samples were placed in the incorrect roles, click the swap icon to switch the placement of the samples.

4. Give your trio a descriptive name.



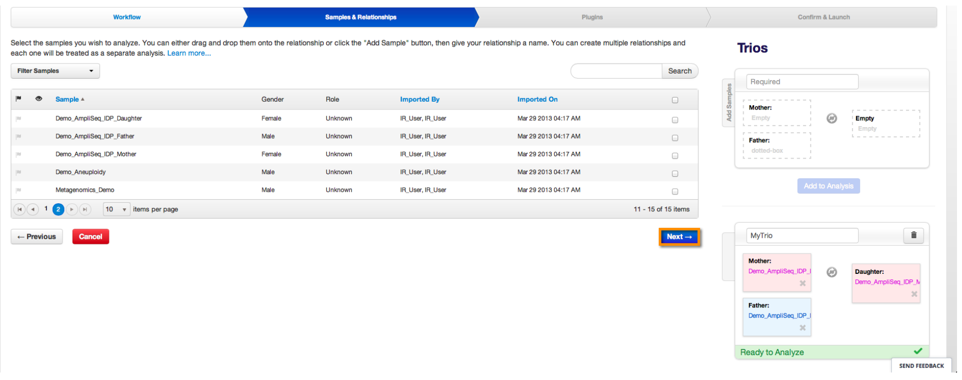
5. Click **Add to Analysis**.



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**.



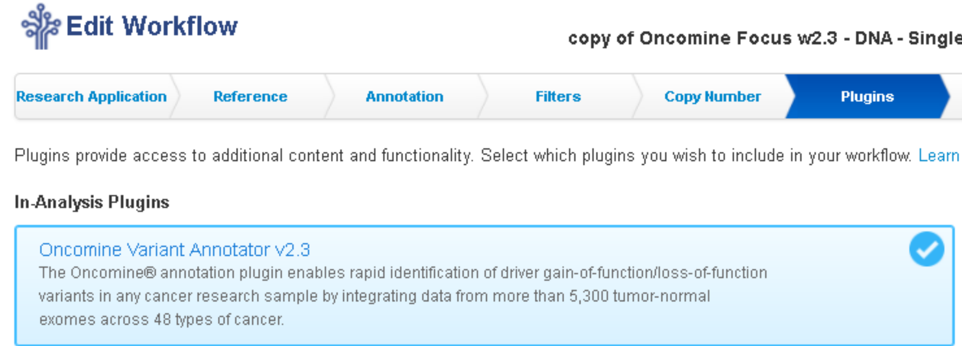
Add plugins

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**.

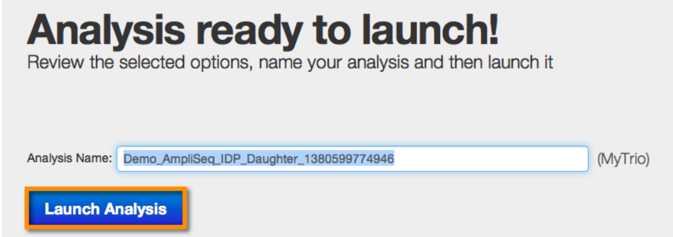
Confirm and launch an analysis

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

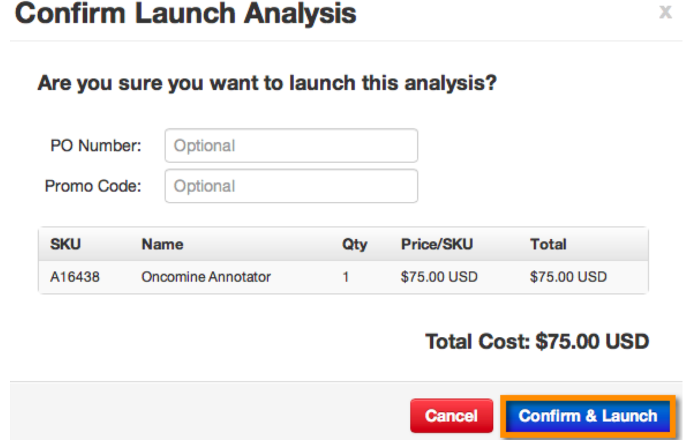
1. Provide a name for your analysis or use the default name. Optionally, add a description.

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

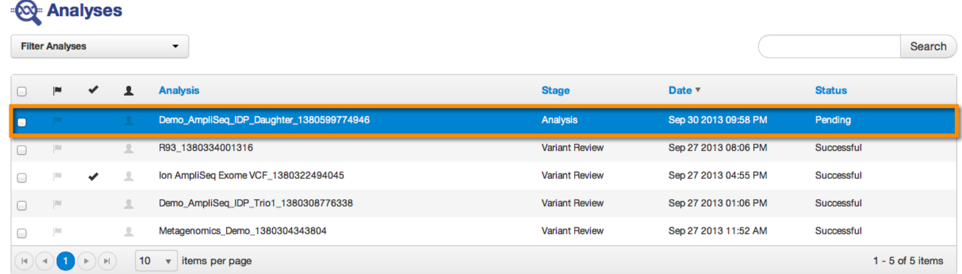
2. Click **Launch Analysis**.

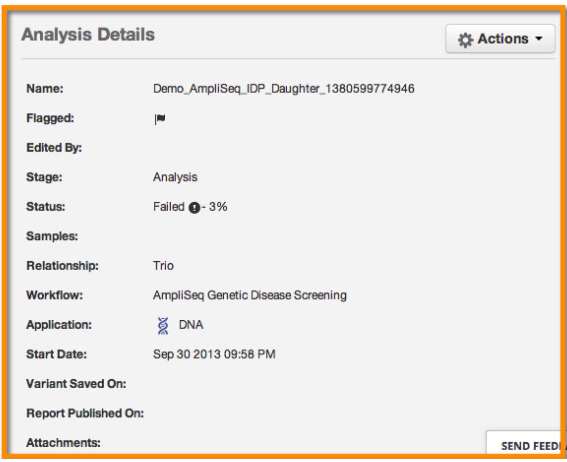
3. If you have included a plugin in your analysis, you are presented with a cost breakdown and confirmation screen. Click **Confirm & Launch**.



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

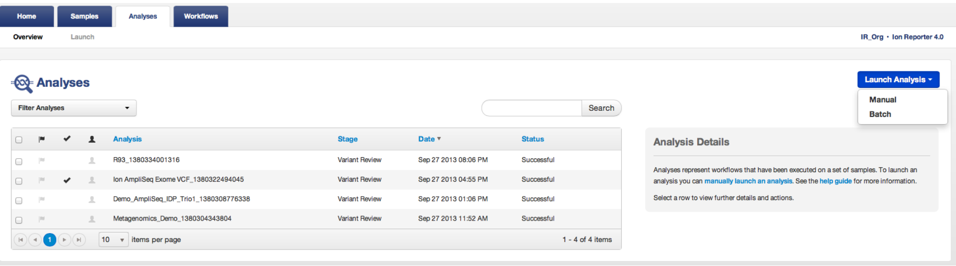
This technique analyses multiple samples, or multiple sets of related samples, using separate analyses with the same workflow. For example, with one use of the wizard, you can launch several trio workflows, or several paired workflows -- and each analysis has its own related samples.

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

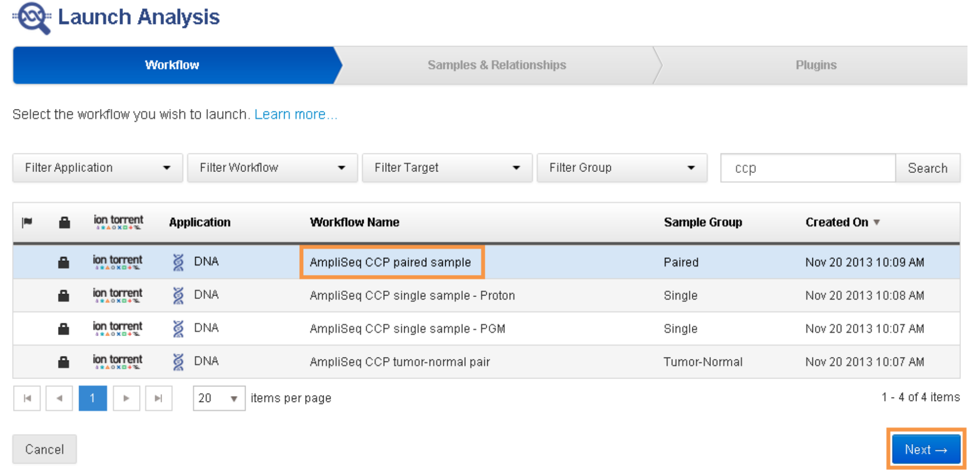
Follow these steps to launch multiple analyses with the analysis wizard:

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

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

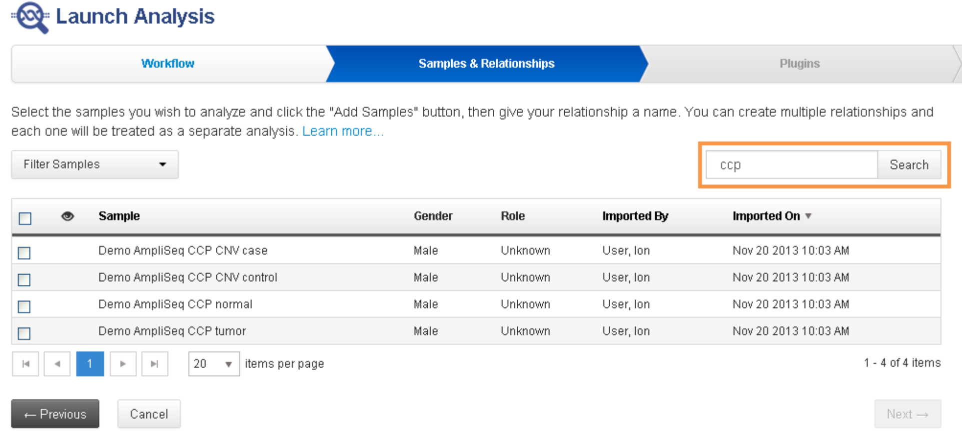


3. Select your workflow.

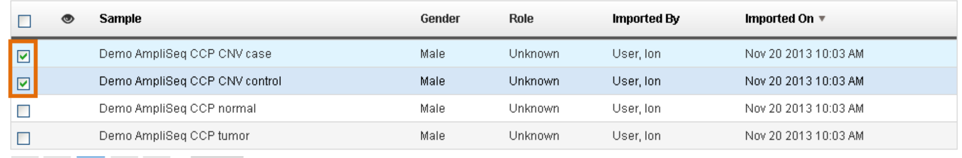


Click the **Next** button.

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.

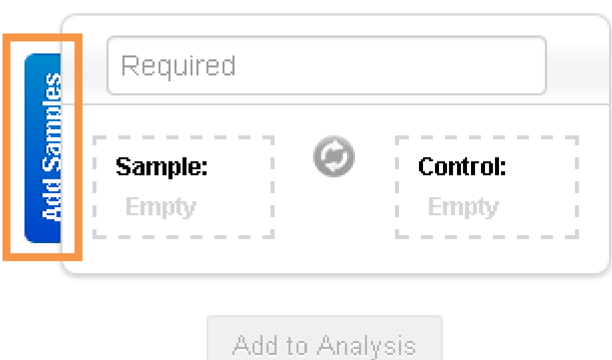


5. Enable the checkboxes for the samples in your first analysis.

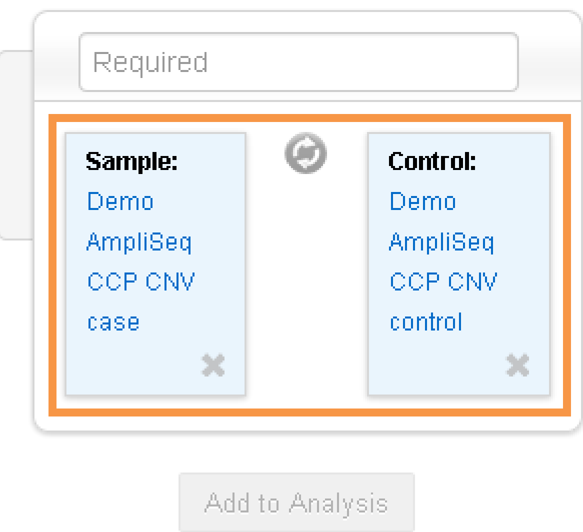


Follow the same steps as you would to analyse these samples in a single analysis:

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

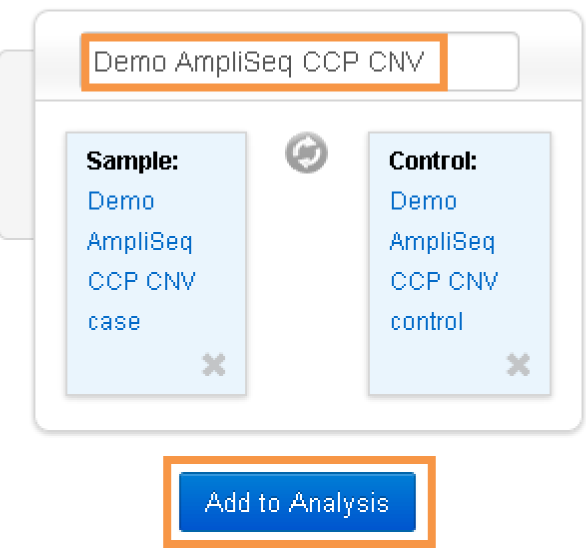


b. Your samples are added to the placeholders in the samples area.



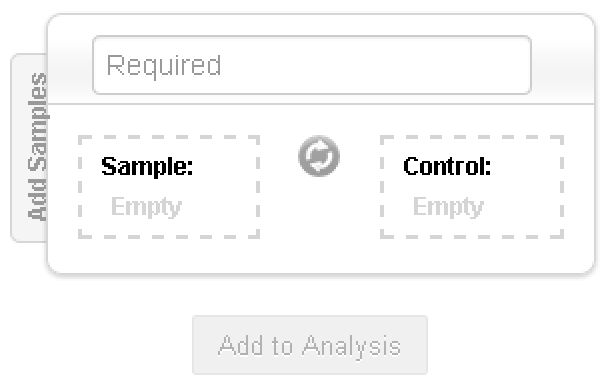
c. If the samples are placed in incorrect roles, swap them by clicking the swap icon .

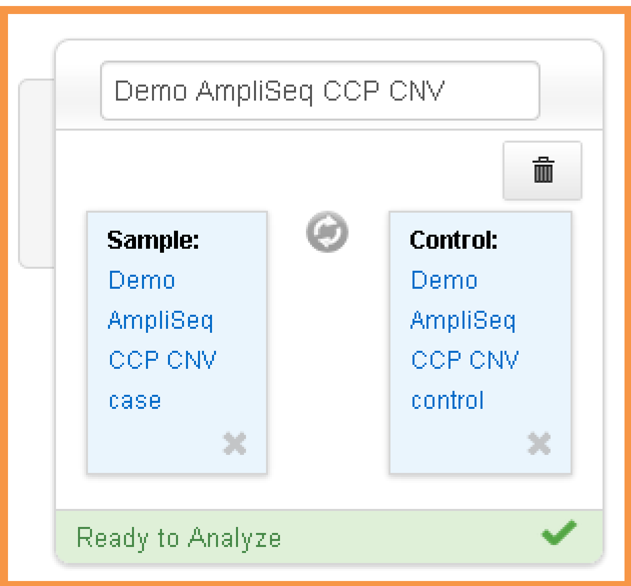
d. In the text field above your samples, enter a name for this set of samples.



Click the **Add to Analysis** button.

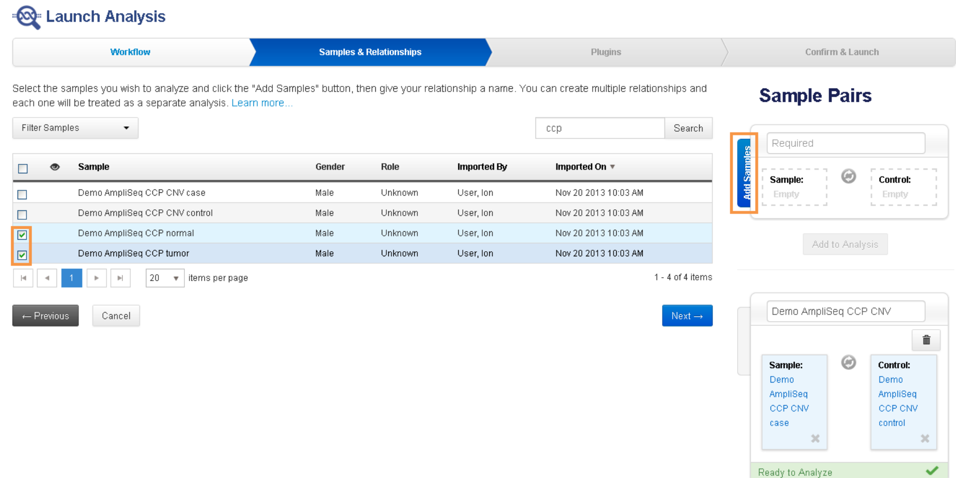
e. Your samples are added to the Ready-to-Analyze area.



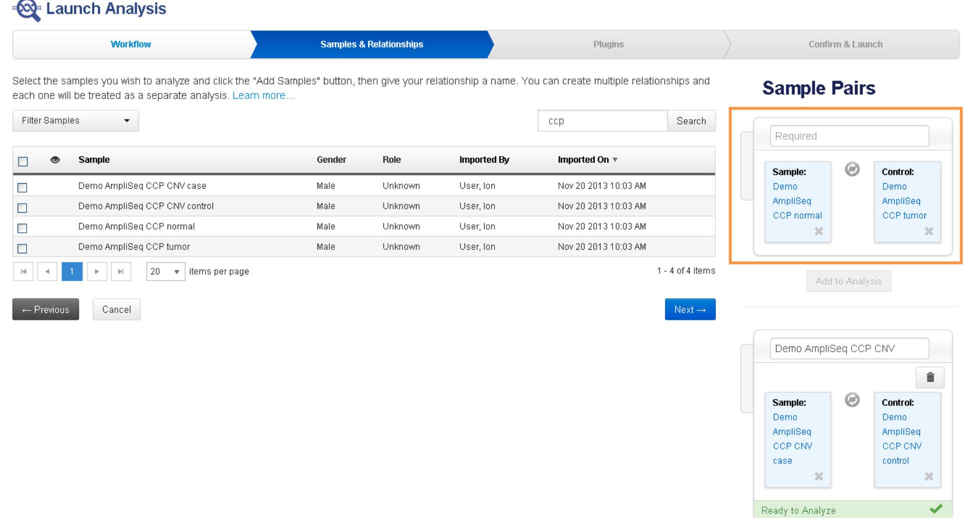


The Ready-to-Analyze area represents one separate analysis.

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.

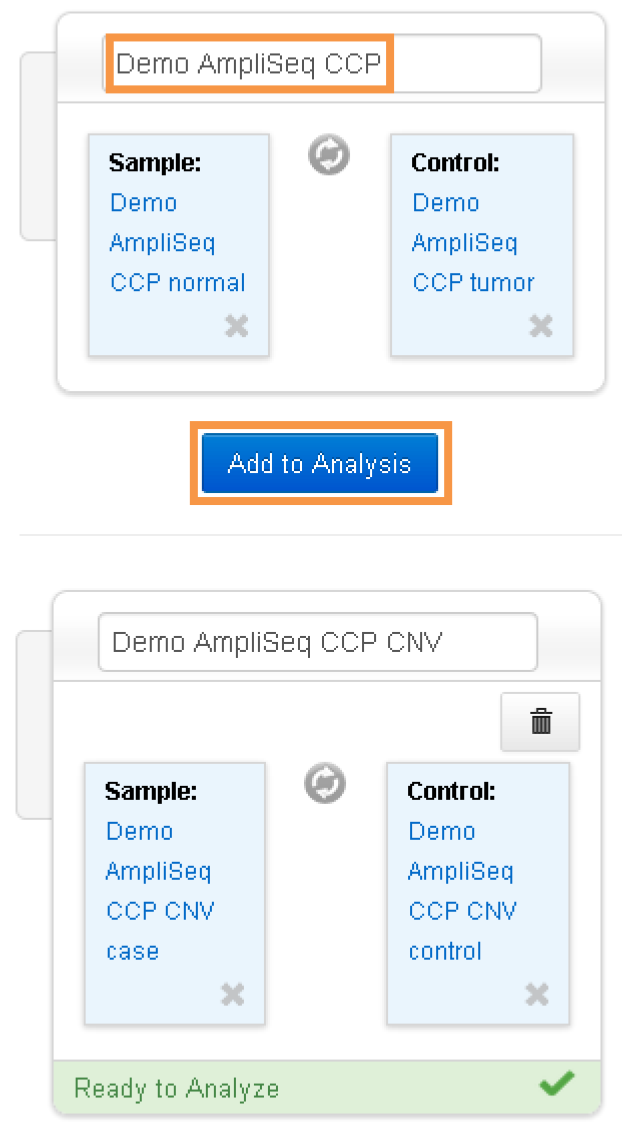


   b. Your second set of samples are added to the placeholder area.



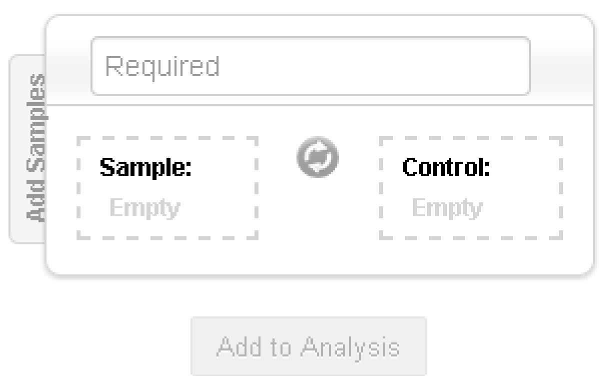
   c. If necessary, swap the sample relationship with the swap icon .

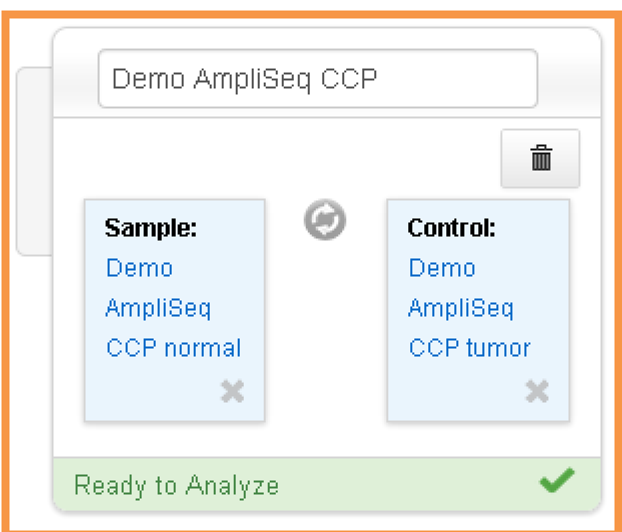
d. In the text field above your samples, enter a name for this set of samples.

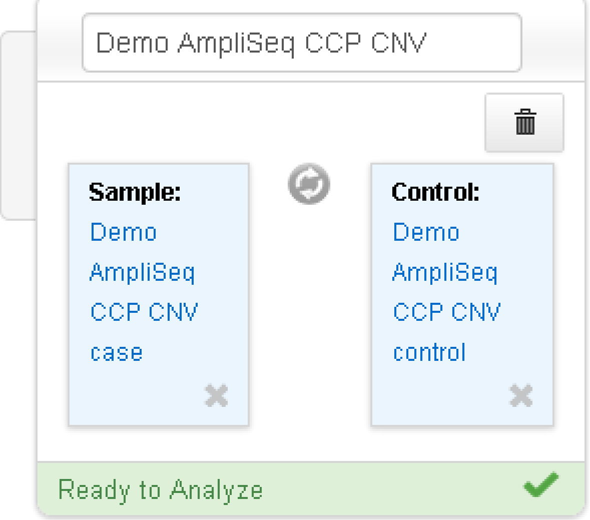


Click the **Add to Analysis** button.

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



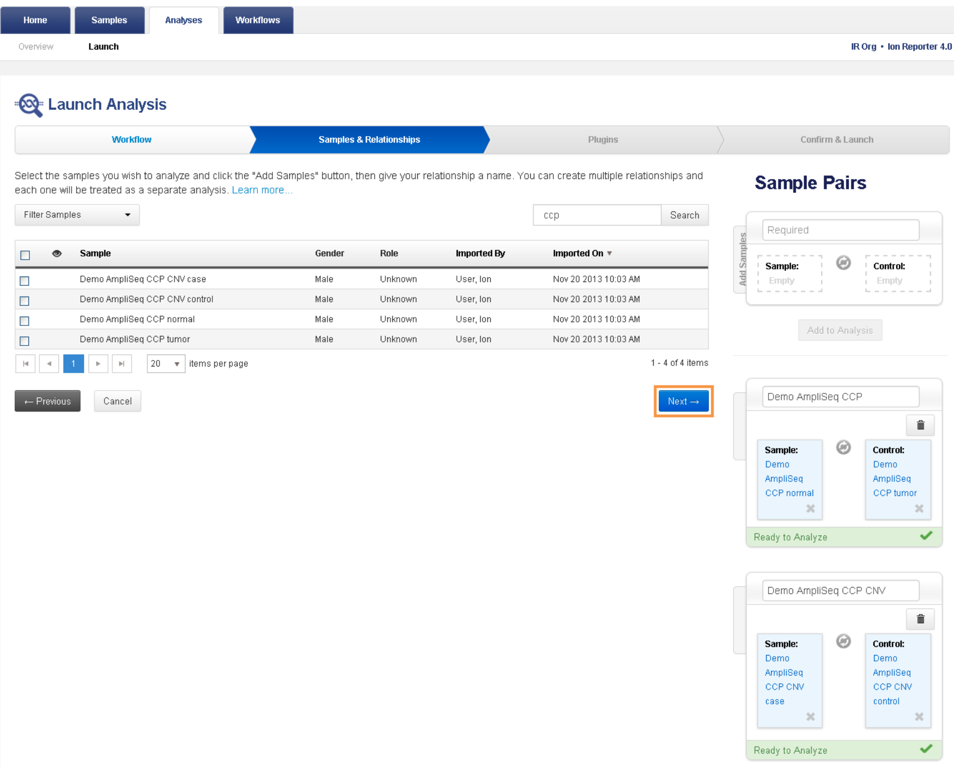




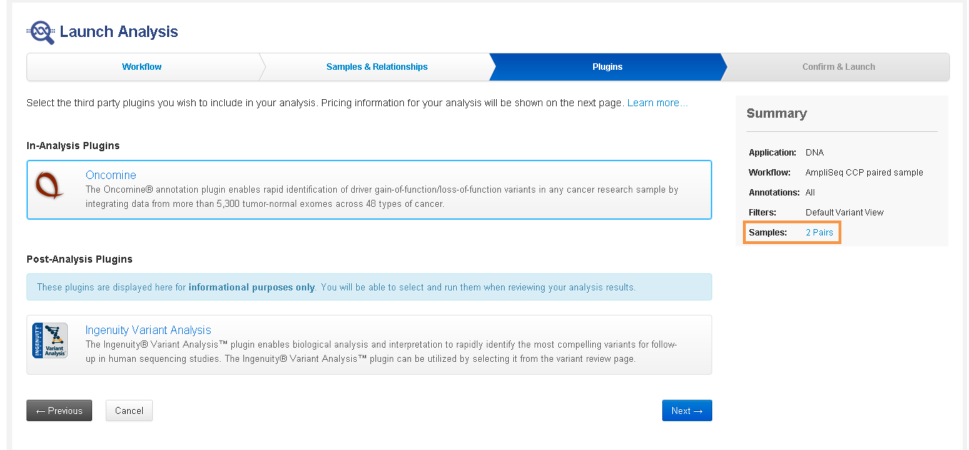
This new Ready-to-Analyze area represents a second separate analysis.

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.



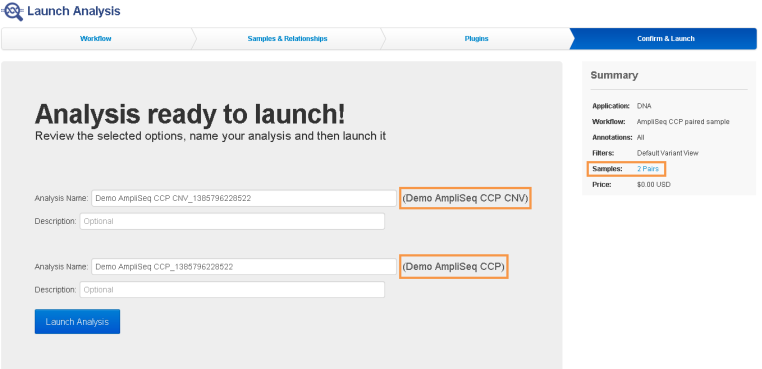
9. In the Plugin chevron, select any in-analysis plugin that you want to be included in these analyses. (If you select a plugin, it is added to all of your analyses being launched now.)



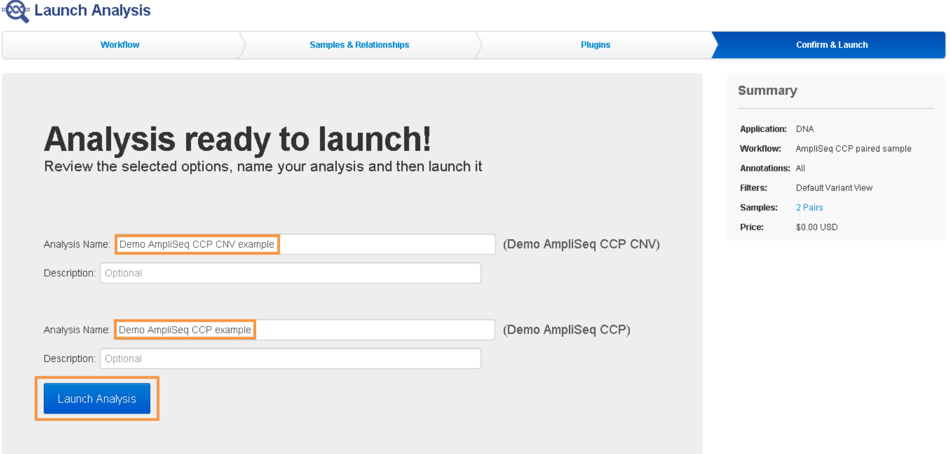
Note: In the Summary panel, "Samples: 2 pairs" refers to the two analyses.

10. Click the **Next** button.

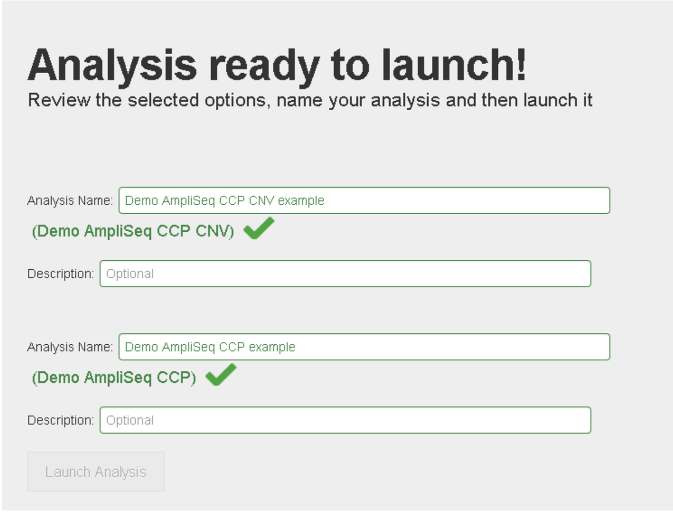
11. In the Confirm & Launch chevron, each analysis is named with the name that you gave to the related samples.

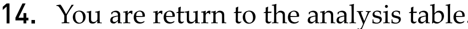


12. You can rename your analyses. Click the **Launch Analysis** button.

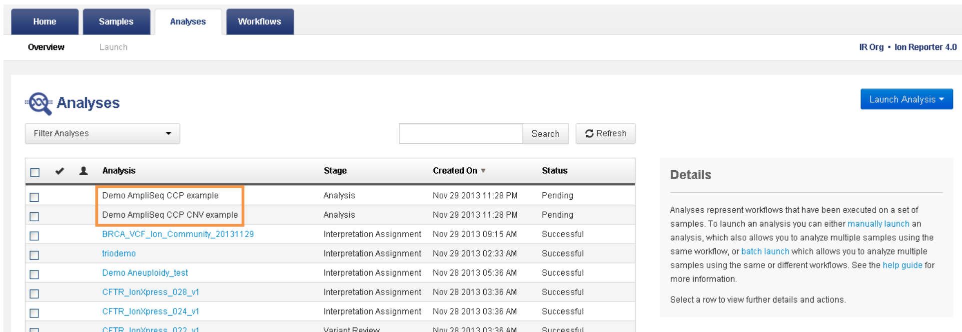


13. Each analysis that is successfully launched is shown with a large checkmark.





14. You are return to the analysis table.



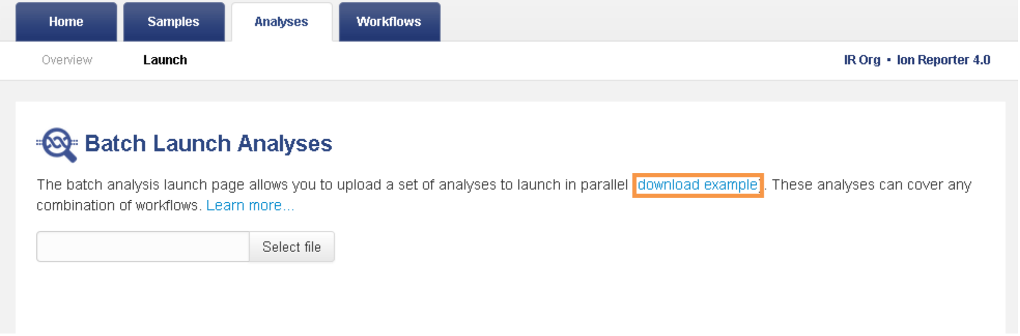
Click on an analysis to see more details in the analysis details section on the right.

Launch analyses by batch

You can launch multiple analyses through a CSV file that you upload to Ion Reporter™ Software.

1. Prepare CSV file to launch analyses by batch:
   a. In the **Analyses** tab, click **Overview**, then click **Launch Analysis ▸ Batch**.

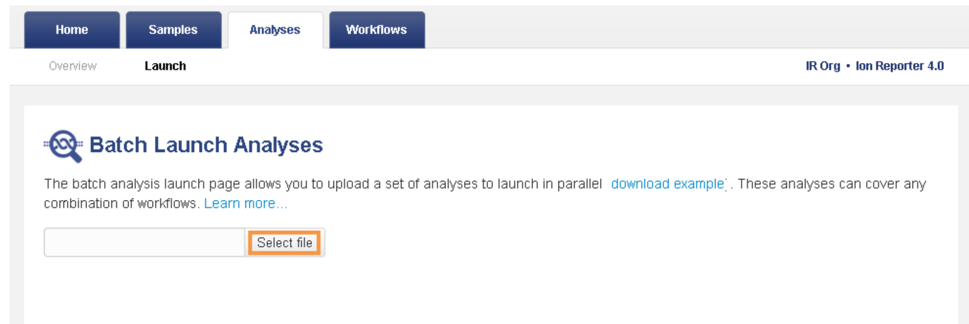
   b. Click **download example** to download the example CSV file.



   c. Enter information about the analyses that you want to launch by batch into the example CSV file.

   d. Save the CSV file to a directory that you can access from Ion Reporter™ Software.

2. Click **Select file** in the **Batch Launch Analyses** screen, navigate to your CSV file, and then click **Open**.



3. Ensure that the correct file name appears, then click **Upload**.
   The file is verified and the analyses are created. If the analyses are successfully created, the analyses are listed in the **Batch Launch Analyses** screen.

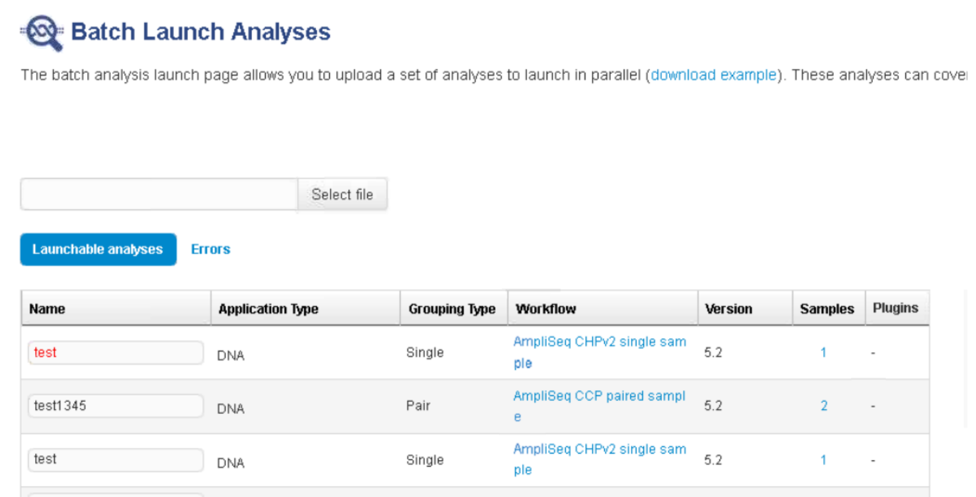
4. Click **Start Batch Analyses**.

The analyses that you launched by batch are added to the list in the **Analyses** screen.

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**.



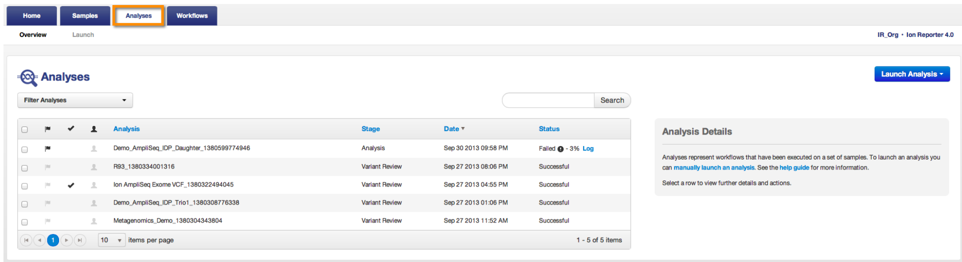
2. You can now proceed with your batch analyses work.

Delete an analysis

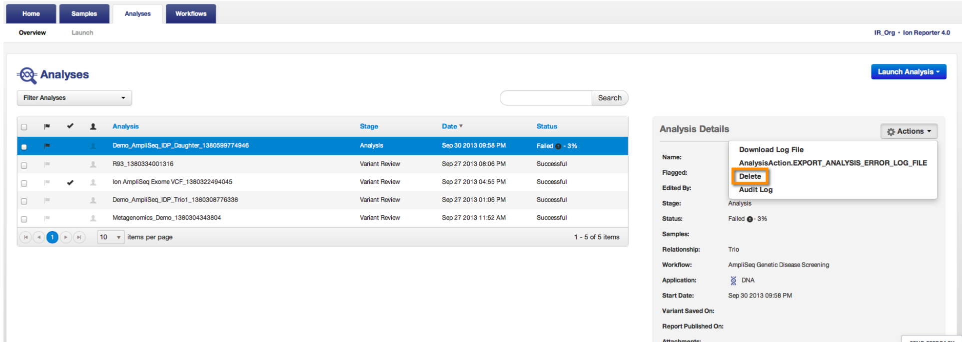
IMPORTANT! When an analysis is deleted, the action cannot be undone.

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.

Copy analyses to Thermo Fisher Cloud storage

You must be a user of Ion Reporter™ Software on Thermo Fisher Cloud to use this procedure.

You can copy analyses in VCF format from successful Ion Reporter™ Software analyses to the Thermo Fisher Cloud for storage and to share analyses with other users. In addition, you can upload your analyses to other software applications on the Thermo Fisher Cloud, such as Oncomine™ Knowledgebase Reporter Software. See "Increase storage space on Thermo Fisher Cloud" on page 160 for more information.

1. In the **Analyses** tab, click **Overview**.
2. Select the row that contains the successful analysis that you want to copy to the Thermo Fisher Cloud.
3. In the **Details** pane, click **Actions ▸ Copy to DataConnect**.
4. In the **Export to Data Manager** dialog box, click **Confirm**.
   A message at the top of the **Analyses** page confirms that the files have been copied to the Thermo Fisher Cloud.
5. To view the files, sign in to the Thermo Fisher Cloud and then click **View my files ▸ Personal Files**.

   Note: If you use higher capacity sequencing chips, you are granted a one-time increase of 1 TB storage space of Thermo Fisher Cloud storage space. This increase is reflected in your Ion Reporter™ Software account immediately after you copy an analysis to the Thermo Fisher Cloud from Ion Proton™ instruments, or from Ion S5™ or Ion GeneStudio™ S5 instruments that use Ion 540™ or Ion 550™ chips.

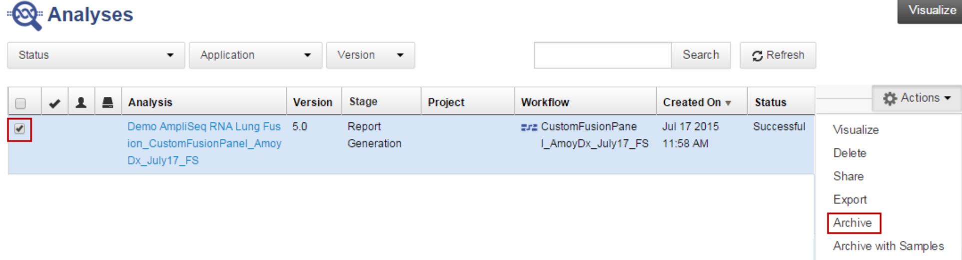
Increase storage space on Thermo Fisher Cloud

You can purchase extra storage space as a user of Ion Reporter™ Software on Thermo Fisher Cloud, and the administrator of the Ion Reporter™ Software organization can assign the storage subscription to the organization. When a subscription is assigned to an organization, all members of the organization have access to the storage space. See Thermo Fisher Cloud Help for more information.

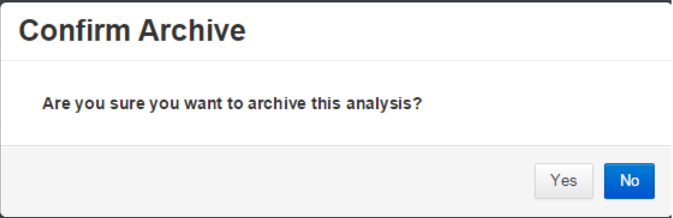
Archive analyses

If you have an Ion Reporter™ 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.

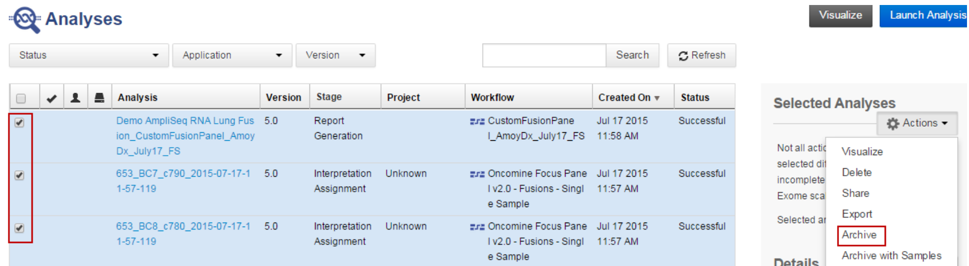


3. Click **Yes** to confirm you want to archive.



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**.

Search, sort, or filter Analyses

You can search, sort, and filter the **Analyses** table to aid you in finding your analysis results of interest.

In the **Analyses** tab, click **Overview**, then perform the following actions in the **Analyses** table.

To...	Do this...
Search the list	Enter a search term into the **Search** field, then click **Go**. **Note:** The search field is outlined in red if the search string is invalid. The following rules apply to all search fields: • An asterisk (*) is not allowed in the search field. • Searches are not case sensitive. • 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.
Sort the list	Click on a column header to sort the list based on the column category type (numerical, alphabetical, date). Click on the column header again to reverse the order.
Select filter categories	• Click **More Filters**, then select one or more applicable filter categories from the dropdown list. • Click **More Filters**, type in a search string into the **Find Filters** field, then select one or more applicable filter categories. • Click **More Filters**, then click **Select All** to select all filter categories.
Remove filter categories	• Click ✕ next to the filter category dropdown list to remove that specific filter category from the list. • Click **More Filters**, then click on the selected filter category to remove that specific filter category from the list. • Click **More Filters**, then click **Clear** to remove all the selected filter categories from the list.
Select filters within a filter category	• Click on the filter category to expand the dropdown list, then select one or more specific filters. • Click on the filter category to expand the dropdown list, then type in a search string into the **Find Filters** field. Select one or more specific filters. • Click on the filter category to expand the dropdown list, then click **Select All** to select all filters within a specific filter category.
Remove filters from a filter category	• Click on the filter category to expand the dropdown list, then click on the selected filter to remove that specific filter from the filter category. • Click on the filter category to expand the dropdown list, then click **Clear** to remove all filters from a filter category.

To...	Do this...
Remove all filters and filter categories from the list	Click **Clear All**.

Set Analyses table preferences

You can configure the columns displayed in the **Analyses** table. You can then save the desired table configuration in table preferences for future use.

IMPORTANT! Table preferences must be saved and applied to individual tables separately. Tables in **Samples** and **Analyses** tabs do not share table preferences.

In the **Analyses** tab, in the **Overview** screen, click **Preferences**, then 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 preferences 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.
Delete Table Preference	Delete the selected user-defined table preferences from **Table Preferences**.
Restore Defaults	Restore the default table column display.

Analysis results

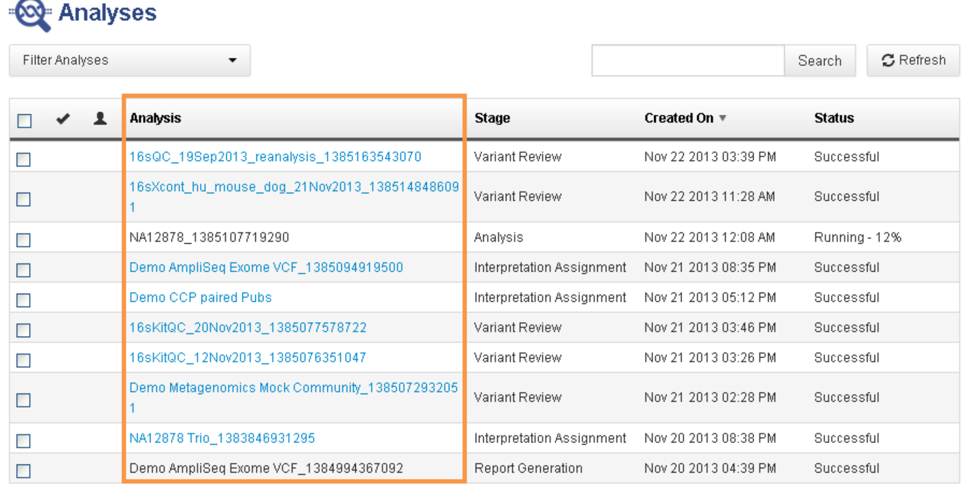
This section describes how to sort and display your analysis results. Analysis results are displayed under the **Analyses** tab in the **Overview** screen. Click on an analysis in this screen to display the results.

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.



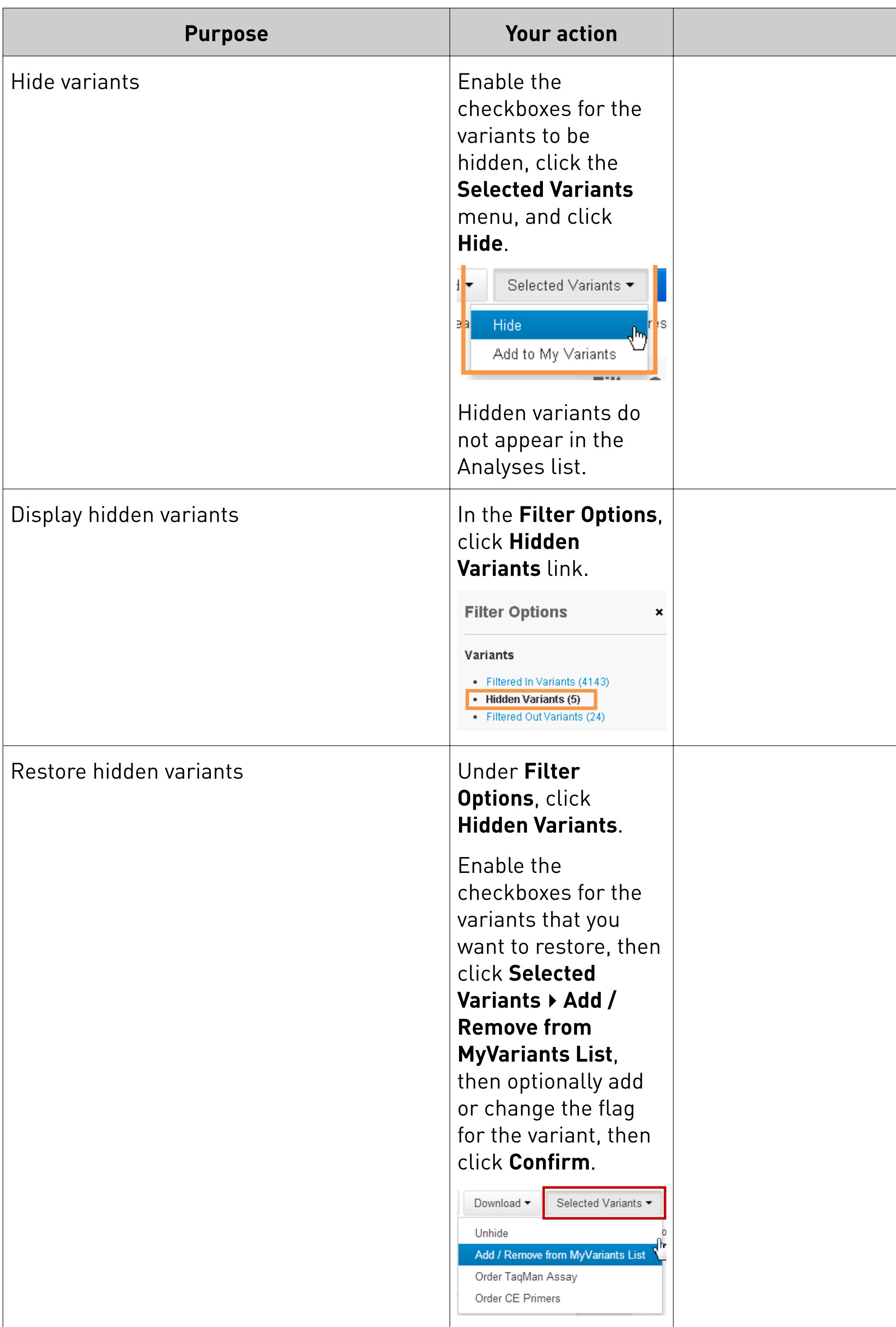
This action opens the analysis in edit mode.

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 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 "Filters and filter chains" on page 421 for more information.	
View the variant in IGV	Click on the variant position link in the Locus column. See "Visualize DNA and Fusion analysis results" on page 237.	

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 MyVariants database	Click the **(Flag) ▸ Important** The selected variants (with flags now in orange) are added to the MyVariants annotation source. See "Apply flags to variants of interest" on page 178 for more information.	
Remove one or more variants from the MyVariants database	Click the flag menu in the MyVariants column and click the **None** option. Or, enable its checkbox and click **Remove from MyVariants** in the Selected Variants menu. See "Edit a MyVariants database" on page 179 for more information.	



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**. Send to Report Role Do you want to send this analysis "Demo CCP paired Pubs" for report generation? After confirming, it will be sent to the report roles in your organization. • You can no longer edit this analysis or filter variants after the analysis is sent. Cancel Send 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.

Apply a filter chain to Analysis Results

You can apply a filter chain to the analysis results to further refine your view to only variants of interest in Ion Reporter™ Software. You can only apply one filter chain at a time. See "Filters and filter chains" on page 421 for more information.

Note: When you apply a filter chain to analysis results, the list of variants is filtered in real-time. You must save your filter chain to have the filter chain immediately alter the heat maps, other visualizations, and exported results of your variants of interest. If you do not save your filter chain on the **Analysis Results**, then the heat maps and other multiple analysis visualizations show the entire results.

1. In the **Analyses** tab, click **Overview**.
2. Select the link to your analysis of interest.
   The **Analysis Results** screen appears.
3. In the **Filter Options** pane, **Filter Chains** dropdown list, click a filter chain to apply it.
   The filtered variants results change.

4. Review the Variants counts.
   - **Filtered In Variants –** Number of variants that will be included in the final report, if you choose to publish it at this point.
   - **Hidden Variants –** Number of variants that have been explicitly hidden.
   - **Filtered Out Variants –** Number of variants that are removed from the final report, if you choose to publish it at this point, because of filter chain settings.
5. *(Optional)* If you want to save these changes to your analysis results, click **Save Filter Chain**.
   Save Filter Chain ensures that when reopening the same analysis, the newly saved filter chain is applied to the analysis results.

Show variants for a single chromosome

You can refine analysis results to show only those variants called on a single chromosome in Ion Reporter™ Software.

1. In the **Analyses** tab, click **Overview**.
2. Select the row of your analysis of interest.
   The **Analysis Results** screen appears.
3. In the right pane, click the **Chromosome** menu and select the chromosome of interest.

The number of **Filtered In Variants** reflects only the variants on the selected chromosome. Because variants called on other chromosomes are considered to be "not displayed" instead of "filtered out", their variant totals are not added to the **Filtered Out Variants** for the analysis results.

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.

Locus View versus *Allele View* of variants

When you set workflow parameters, you can set the view that is used for variants in analysis results. The options are for this setting are variants per position, **Locus View**, or variants per allele, **Allele View**. See "Parameters step" on page 96 for more information on how to set this view. The VCF files for both the Locus and Allele views include one line for each variant row that is displayed in the Ion Reporter™ Software variant table.

Locus view is the default variant view that appears in analysis results in Ion Reporter™ Software, which is a locus-centric view. Allele View is an allele-centric view that displays each allele as a single row in the Ion Reporter™ Software and in an individual line in the VCF file output. In Locus View, there can be multiple alternate alleles for a given locus in each line or row.

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 Locus View VCF file and the Allele View 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, the Allele View 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 Allele View VCF file.

Allele subset information

Beginning with Ion Reporter™ Software, a data column labeled **Subset** can be used to determine whether alleles are present at a variant locus in either analysis results that are viewed as either Allele View or Locus View.

In Locus View, the following applies:

The subset column indicates that an allele, which is part of a larger variant that has been called, is present (the superset). The format is subset index (superset index). The larger variant can be found by using the value of the superset index counting into the alleles listed in the **Observed Allele** column. The subset id can be a hotspot id or a number. If it is a number and not a hotspot id then it is also an index counting in the alleles listed in the **Observed Allele** column. The subset allele always will match one of alleles without a genotype. A special case is that two superset indices might be present, in which case both indices are displayed and are separated by a foward slash (/).

In Allele View, the following applies:

The subset column indicates that an allele, which is part of a larger variant that has been called, is present (the superset). The format is "SupersetID" which will be a name for the superset variant. A special case is that two superset indices might be present, in which case both indices are displayed and are separated by a foward slash (/).

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.

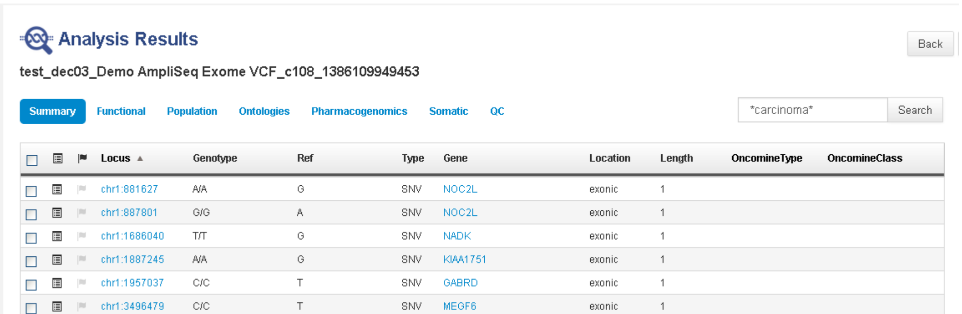
"vbk" in the table above stands for MyVariants.

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

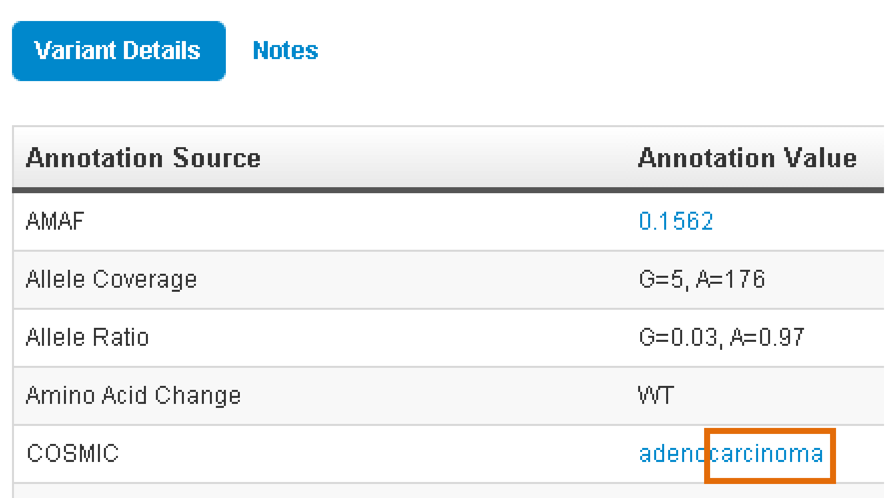
- An asterisk (*) in the search field is only allowed on the Analysis Results page.
- An asterisk (*) is required for some searches here, but is not allowed for other searches. The differences are due to how the different types of information are stored.
- The asterisk is a search wildcard. Without the 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*.

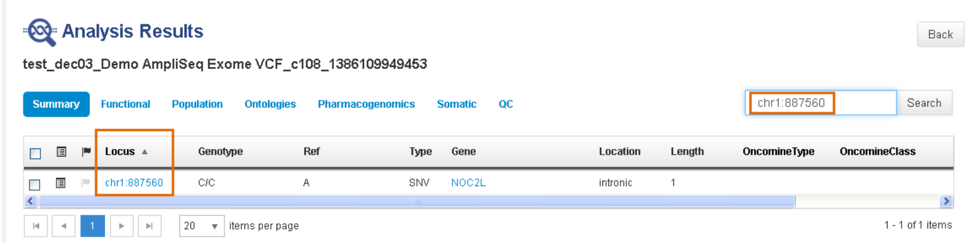


This search lists all variants that have an OMIM, COSMIC, ClinVar, DrugBank, and so on, annotation that contains "carcinoma" anywhere in the annotation. Click the detail icon for one of the matched variants to open its variant detail card. Scroll down to fine the entries that contain "carcinoma".

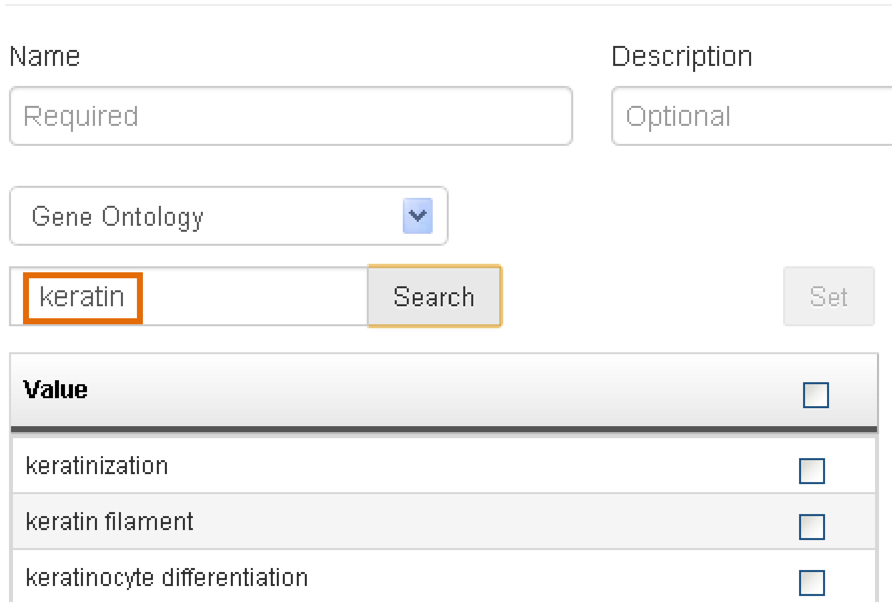


Searches without asterisks

- 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.

MyVariants

You can label variants as relevant to your research in Ion Reporter™ Software and either track significant variants or ignore insignificant variants, such as known false positives. You can then share the data about these variants with other researchers through a TSV file or PDF report that you create in Ion Reporter™ Software. MyVariants complements variant annotations already applied by the various annotation sources in Ion Reporter™ Software.

When you apply MyVariants flags, **Important** or **Ignore**, to the variants that are included in analysis results from Ion Reporter™ predefined workflows, the variants are automatically added to the default MyVariants database for your organization in Ion Reporter™ Software. This database is named **MyVariantsDb_hg19** and all users in the organization can apply it to their analysis results.

You can also apply notes and classifications to the MyVariants. See "Classifications and Notes" on page 177 for more information.

Note: Each workflow can be associated with only one MyVariants database.

Custom MyVariants databases can be created for specific research projects or individual researchers. For example, you might want to create a separate MyVariants database for breast cancer research and another for lung cancer research. The same variants can have different flags and classifications in the different research areas.

MyVariants table

You can view MyVariants when you click the **Analyses** tab, then click **MyVariants**. The MyVariants table includes specific information such as genome location and mutation type. The columns are:

Information type	Description
(Variant Flag)	MyVariant flag options: • Important—To identify variants that are relevant to the research. • Ignore—To denote known false positives or variants that are not associated with the research area. • None—To remove a variant marked as Important or Ignore from the MyVariants database. This column can be edited.
(MyVariants Notes)	Information that researchers add to the MyVariants in note format. This column can be edited.
Locus	Variant location on a specific chromosome. This column can be sorted.
Classification	Standard classifications: Unclassified, Unknown, Benign, Suspected Benign, Suspected Pathogenic, and Pathogenic. This column can be edited.
Gene(s)	Gene or genes where variant is found. This column can be sorted.

Information type	Description
Genotype	Genotype sequence (ATCG). This column can be sorted.
Ref	Reference sequence. This column can be sorted.
Type	Type of variant or call, such as SNV, CNV, Fusion, or REF. This column can be sorted.
Analysis Name	Name of analysis where the variant was identified.
Sample Name	Name of sample where the variant was identified.
Observed Allele	Observed allele variation. This column can be sorted.
Copy Number	Copy number variation. This column can be sorted.
CNV Subtype	Copy number subtype. This column can be sorted.
Variant ID	Variant ID. This column can be sorted.

MyVariants database

A MyVariants database is a repository of identified variants that is shared by an entire Ion Reporter™ Software organization, a specific research project, or an individual researcher. When you label the variants that are included in analysis results from Ion Reporter™ predefined workflows as **Important** or **Ignore**, the variants are automatically added to the default MyVariants database for your organization in Ion Reporter™ Software. This database is named **MyVariantsDb_hg19**. You can also create a different MyVariants database for specific research project and then select this MyVariants database when you create a workflow.

Other ways to use MyVariants databases include:

- Export MyVariants between different MyVariants databases with a TSV file. See “Export MyVariants“ on page 182 and “Import MyVariants“ on page 182 for more information.
- Use a MyVariants database to create a variety of filter chains to narrow analysis results. See “MyVariants filter“ on page 449 for more information.
- Apply the latest MyVariants information to older analysis results. See “Get the latest updates from MyVariants database“ on page 180 for more information.
- Create a MyVariants report in PDF format. See “Create a PDF report of MyVariants“ on page 182 for more information.

Classifications and Notes

Variants that use the **Important** or **Ignore** flags are often variants to which you might want to also add **Classifications** and **Notes**. Ion Reporter™ Software includes an optional setting to automatically add the previous **Classifications** and **Notes** to a new occurrence of a variant when that variant is subsequently found in a new set of analysis results. The workflow used for the analysis must be associated with a MyVariants database. For example, you might decide that a variant is Important and Benign, and you might want to include a note about why this is the case. The setting to automatically add **Classifications** and **Notes** to MyVariants ensures that the variant is always marked Important and Benign. MyVariants **Flags**, **Classifications** and **Notes** are all stored in the MyVariants database.

The setting to automatically add variant **Classifications** and **Notes** is disabled by default. See "Automatically add classifications and notes to variants" on page 177 for more information.

IMPORTANT! The latest **Classifications** and **Notes** are added to a variant and the MyVariants database when this setting is enabled. If you change the **Classifications** and **Notes** in the future, the variants for subsequent analyses will reflect those changes.

Automatically add classifications and notes to variants

You can set a preference to automatically add notes and classifications to MyVariants in Ion Reporter™ Software.

1. Under the **Analyses** tab, in the **MyVariants** screen, click **Preferences**.
2. In the **Preferences** dialog box, select one or both options to be added to variants.

Option	Description
Notes	Details about the variant that were added by a user.
Classifications	Classification options include: Unclassified, Unknown, Benign, Suspected Benign, Suspected Pathogenic, and Pathogenic.

Note: Notes are added automatically if no other note is available for the variant. Classifications are added only if the classification is not updated earlier than the variant.

Apply flags to variants of interest

You can contribute to the MyVariants databases for your organization by applying flags to variants in analysis results in Ion Reporter™ Software. We recommend that you first apply classifications and notes before selecting the MyVariants flags.

1. Click the **Analyses** tab to open the list of analyses, then click an analysis of interest.
   The **Analysis Results** screen appears in Summary view.
2. (*Optional*) In the **Classification** column, select appropriate classifications for every variant of interest. Options include:
   - Unclassified
   - Unknown
   - Benign
   - Suspected Benign
   - Deleterious
   - Suspected Deleterious
3. (*Optional*) In the (**Note**) column, click the **(Plus)** sign, then click **Notes** tab, and enter a note about the variant. Then click **Add Note**.
4. In the **(Flag)** column, click the **(Flag)** dropdown list and select the appropriate flag.

Option	Description
Important	Select Important and the flag icon becomes orange in the row of the variant. This also adds the variant to the MyVariants database. Important is used to track variants that are relevant to the research.
Ignore	Select Ignore and the flag icon becomes gray in the row of the variant. This also adds a variant to the MyVariants database. Ignore is often used to denote known false positives or false negatives, or variants that are not associated with the research area.
None	All variants are marked None by default. Select None to remove an Important or Ignore flag and to remove a variant from the MyVariants database.

5. To check that the variants were added, click **Analyses ▸ MyVariants**. The latest additions appear at the top of the table. See "Annotation step" on page 89 for more information.

Variants labeled as **Important** or **Ignore** are added to the default MyVariants database, **MyVariantsDb_hg19**, if the analyses were run through Ion Reporter™ predefined workflows. To add variants to a different MyVariants database, create or select a custom MyVariants database in the Annotation step when you create, copy, or edit a workflow. See "Annotation step" on page 89 for more information.

Search, sort, and filter MyVariants

You can search, sort, and filter the **MyVariants** table to aid you in finding the variant of interest by organizing the table by locus, gene(s), sample name, and other variant characteristics.

In the **Analyses** tab, click **MyVariants**, then perform the following actions in the **Analyses** table.

To...	Do this...
Search the list	Enter a search term into the **Search** field, then click **Go**. **Note:** The search field is outlined in red if the search string is invalid. The following rules apply to all search fields: • An asterisk (*) is not allowed in the search field. • Searches are not case sensitive. • 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.
Sort the list	Click on a column header to sort the list based on the column category type (numerical, alphabetical, date). Click on the column header again to reverse the order.
Filter the list by category	Click on the filter category to expand the dropdown list, then select a specific filter.
Filter the list by date of analysis	• Click next to the **From :** and **To :** fields above the variants table, select the date range from the calendar menu or enter the dates in the fields in mm/dd/yyyy format, then click **Go**. • Delete **From :** and **To :** field entries to remove the date range filter.

Edit a MyVariants database

You can change MyVariants flags in a MyVariants database in Ion Reporter™ Software. However, keep in mind your edits may impact MyVariants flags set by other members of your organization if you are sharing a MyVariants database.

Note: If you delete an analysis in which you originally labeled a variant as Important or Ignore, the MyVariants database is unaffected and the variant flags, notes and classifications remain.

1. In the **Analyses** tab, click **MyVariants**.
   The MyVariants table appears.

2. If you want to select a specific MyVariants database, select from the database dropdown list.

3. Review the MyVariants flags.

4. To change a MyVariants flag, click the flag dropdown list and select an appropriate option.

Option	Description
None	Removes the existing Important or Ignore flag from the table and also removes the variant from the MyVariants database for all analyses.
Important	Changes the existing flag to Important and stores the variant in the MyVariants database.
Ignore	Changes the existing flag to Ignore and stores the variant in the MyVariants database.

Get the latest updates from MyVariants database

You can get the latest additions, deletions, or changes to your organization's MyVariants database by synchronizing your analysis results to the MyVariants database in Ion Reporter™ Software. For example, you can open older analysis results and apply the updates for any MyVariants that were added, deleted, or changed, to your older analysis results.

Note: In previous versions of Ion Reporter™ Software, updates to MyVariants flags were made automatically when a user opened analysis results. In Ion Reporter™ Software 5.10, updates to the MyVariants flags are made only when an analysis is successfully completed or when **MyVariants** is clicked.

1. In the **Analyses** screen, click **Overview**.
2. Click the link of a new or existing analysis to open the **Analysis Results**.
3. Click **MyVariants** to download flags from the MyVariants database into the analysis results.
4. *(Optional)* Manually edit **Important** or **Ignore** flags within your analysis results.

View the variants in a MyVariants database

You can view a list of variants contained in a specific MyVariants database in Ion Reporter™ Software.

1. In the **Analyses** tab, click **MyVariants**.
2. In the database dropdown list, select the MyVariants database of interest.
3. View the list.

View a MyVariants database associated with a workflow

You can determine which MyVariants database is selected for a workflow in Ion Reporter™ Software.

1. In the **Workflows** tab, click **Overview**.
2. Click in the row of a workflow of interest.
   The **Details** pane appears.
3. In the **Details** pane, look for **MyVariants Database**.

   Note: If MyVariants Database is not in the Details pane, then no MyVariants database was selected during workflow creation. To change the MyVariants database, select a different MyVariants database in the Annotation step of

workflow creation or revision. See "Associate MyVariants database with a workflow" on page 182 for more information.

Create a MyVariants database

You can create a MyVariants database for use with specific research projects when you create a workflow. This may be useful if different projects have conflicting interpretations of specific variants. For example, if a researcher is studying breast cancer in which certain gene mutations are suspected to cause tumors, and another user is studying lung cancer where this same gene mutation is not suspected to cause tumors, then two different databases might be required.

1. In the **Workflows** tab, click **Create**.
2. In the **Research Application** step, select the research application and sample group, then click **Next**. See "Research Application step" on page 82 step for more information.
3. In the **Reference** step, select the required files, then click **Next**. See "Reference step" on page 83 for more information.
4. In the **Annotation** step, under MyVariants Database, click **Create here**. See "Annotation step" on page 89 for more information.
    a. In the **Create MyVariant Database** window, enter a Database Name and click **Save**. The newly created database is now available for selection in the Annotations step.
    b. Select the new MyVariants database, then click **Next**.
5. In the **Filters** step, select a filter chain from the dropdown list, then click **Next**. See "Filters step" on page 90 for more information.
6. In the **Copy Number** step, select a copy number baseline from the dropdown list, then click **Next**. See "Copy Number step" on page 91 for more information.
7. In the **Plugins** step, select plugins. Then click **Next**.
8. In the **Final Report** step, select the final report template in the dropdown list. Then click **Next**. See "Final Report step" on page 96 for more information.
9. In the **Parameters** step, select parameters, then click **Next**.
10. In the **Confirm** step, name the workflow and enter an optional description, then click **Save Workflow**.

To check that the workflow was created, click the **Workflows** tab, then click **Overview**, and search for the workflow name.

Associate MyVariants database with a workflow

You can apply MyVariants already defined in a MyVariants database to your analysis results in Ion Reporter™ Software by selecting a MyVariants database when you create your workflow. MyVariants allow you to track variants that are important to your research and to ignore other variants that are not relevant to your research. See "Annotation step" on page 89 for more information.

Note: Each workflow can be associated with only one MyVariants database.

To check that the MyVariants database was selected, see "View a MyVariants database associated with a workflow" on page 180 for more information.

Export MyVariants

You can export a MyVariants database in order to share it with another user, project or organization in Ion Reporter™ Software.

1. In the **Analyses** tab, click **MyVariants**.
2. Click **MyVariants**, then select **Show All**, **Important**, or **Ignore**.
3. Click **Export TSV**.
   A compressed file appears.
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: 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.

Import MyVariants

You can import MyVariants from a TSV file exported from another MyVariants database, see "Export MyVariants" on page 182 for more information.

1. In the **Analyses** tab, click **MyVariants**, then click **Import MyVariants**.
2. In the **Upload MyVariants File** screen, click **Select file** and browse to your VCF or TSV file. Then click **Submit**.

Create a PDF report of MyVariants

You can create PDF report of your MyVariants in Ion Reporter™ Software.

1. In the **Analysis** tab, click **Overview**.
2. Click an analysis link to open the **Analysis Results** screen.
3. Select the MyVariants (those denoted by orange or gray flags) to include in the report. You can also use filter chains to filter your analysis results for only flagged variants. See "MyVariants filter" on page 449 for more information.
4. Click **Generate Report**. If a report template is associated with the workflow, use the **Select Final Report Template** dialog box to:
   - Select a template, then click **OK.**
   - Click **Create Report Template** to create a new template. See "Create a report template" on page 267 for more information.

5. Enter required information into the sections. See “Final report template sections“ on page 270 for more information.

6. (*Optional*) Scroll to **Reported Variants** section, then click **Select Columns**. Rearrange columns, if necessary. Click **Next**.
   A preview of the PDF report opens. Click **Previous** to go back and change the report if needed.

7. When you are satisfied with the report sections, click **Lock and Publish**, then review the PDF review. If edits are needed click **Cancel**, then make your edits.

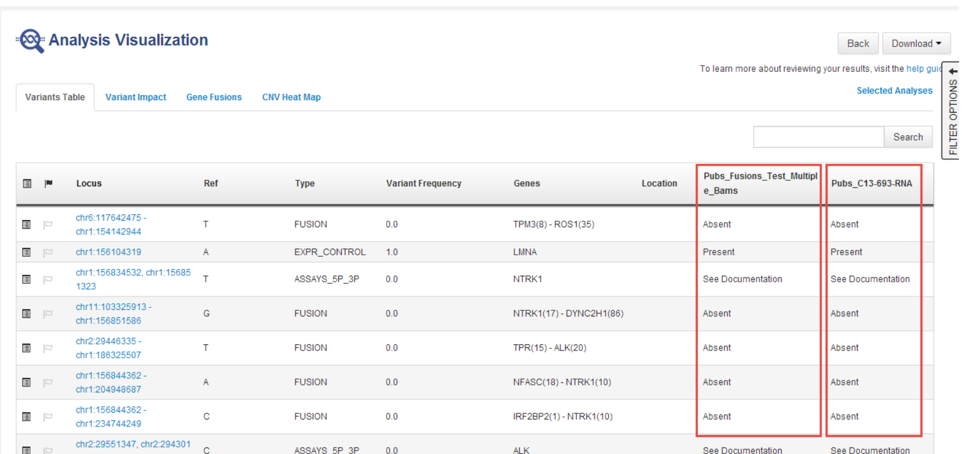
8. Click **Publish** to create the final report.

9. Click **Download** to download the published report.

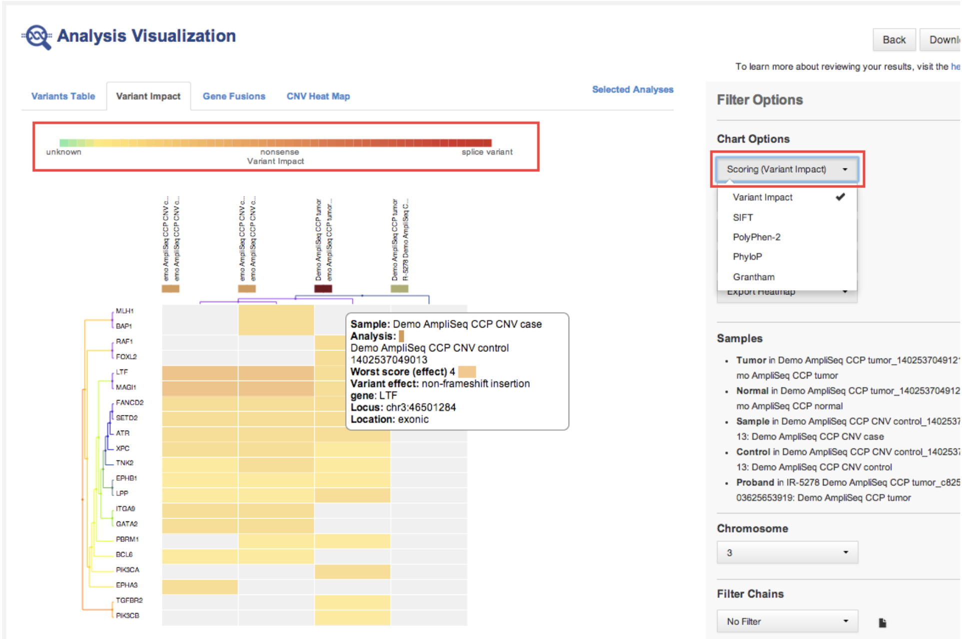
Compare results of single or multiple analyses

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

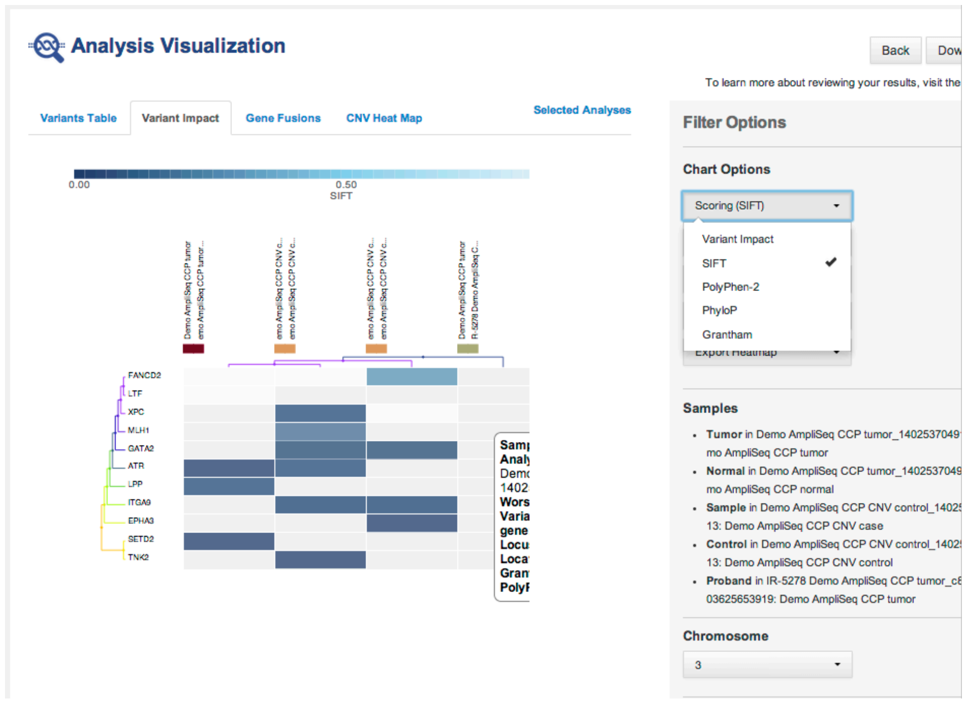
- Table view, with side-by-side columns to compare variant calls in different analyses:



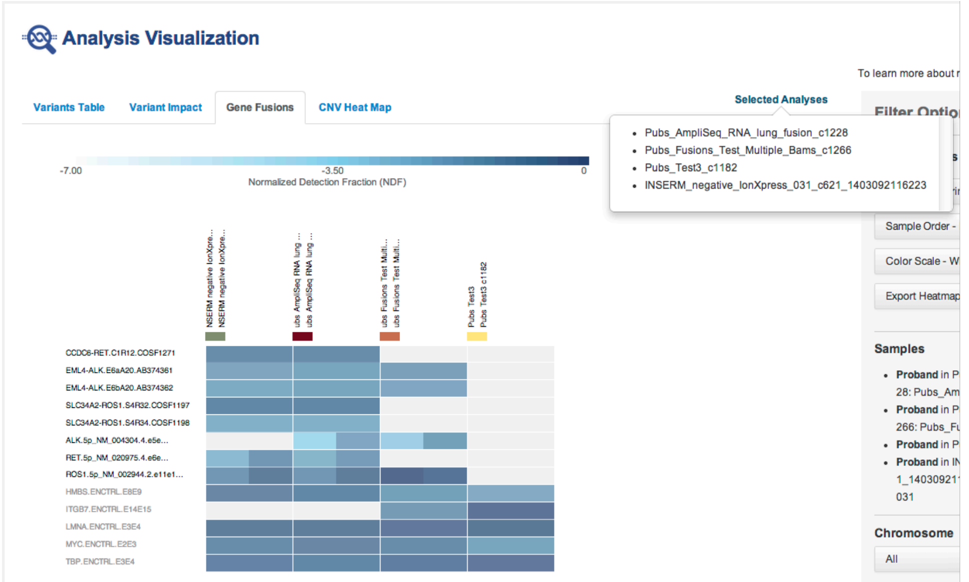
- Variant Impact heat map that is based on the predicted variant impact:



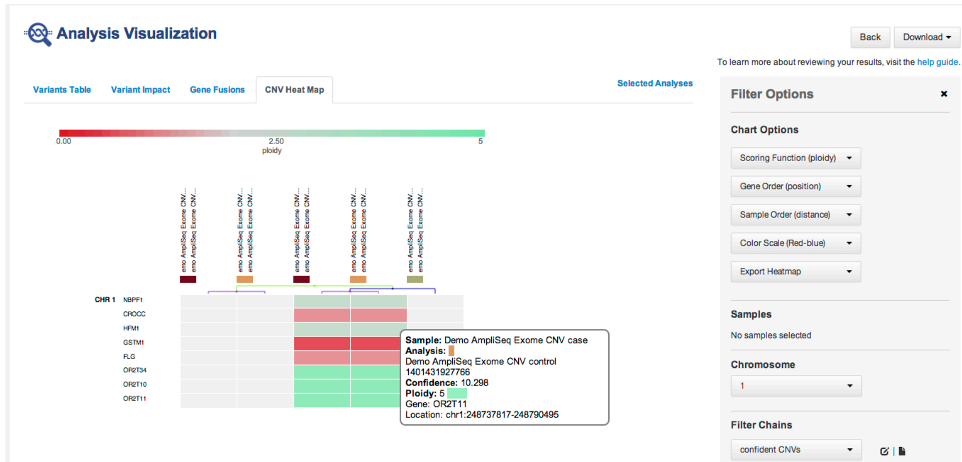
Variant Impact heat map that is based on other scoring:



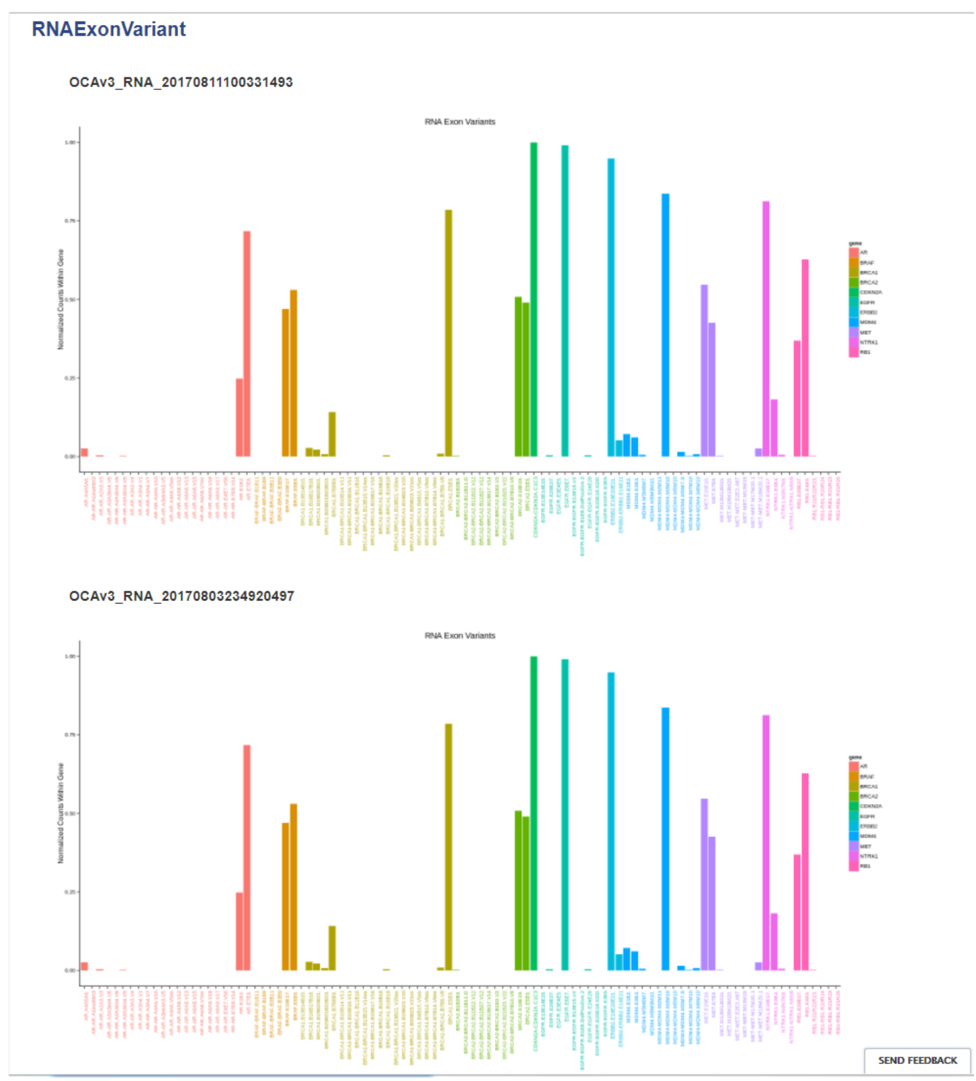
- Gene Fusions heat map:



- CNV heat map:



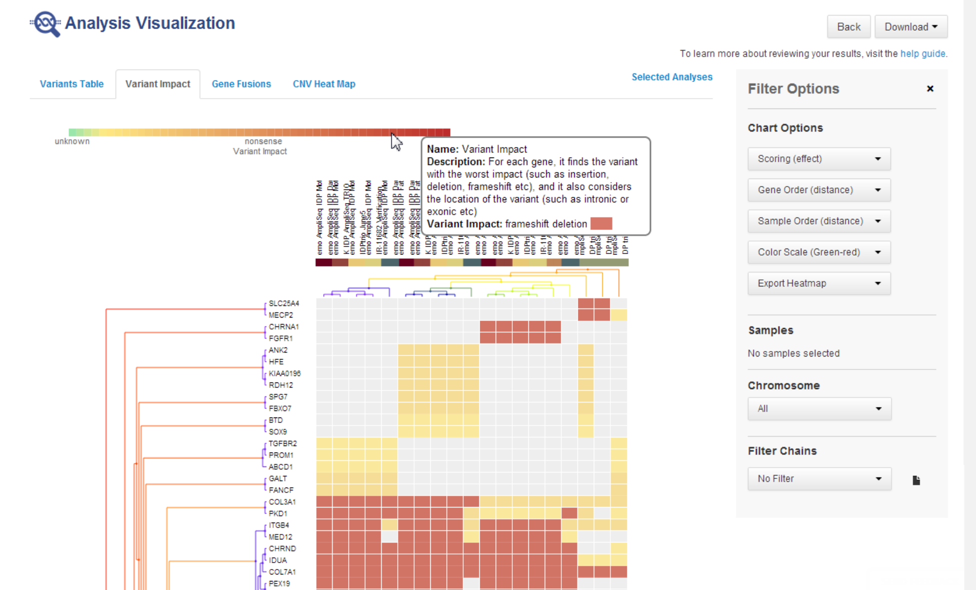
- RNA Exon Variants:



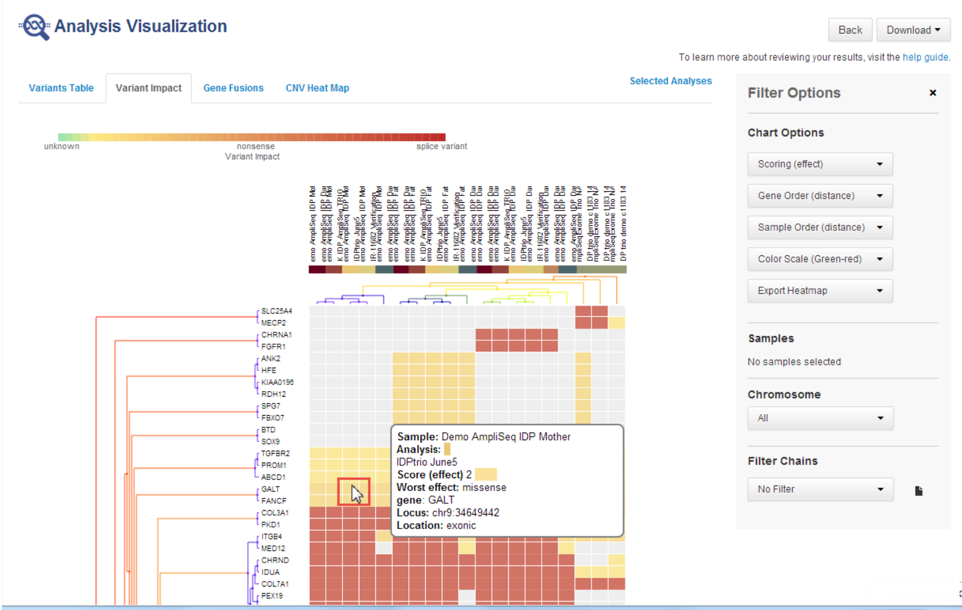
Note:

- 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

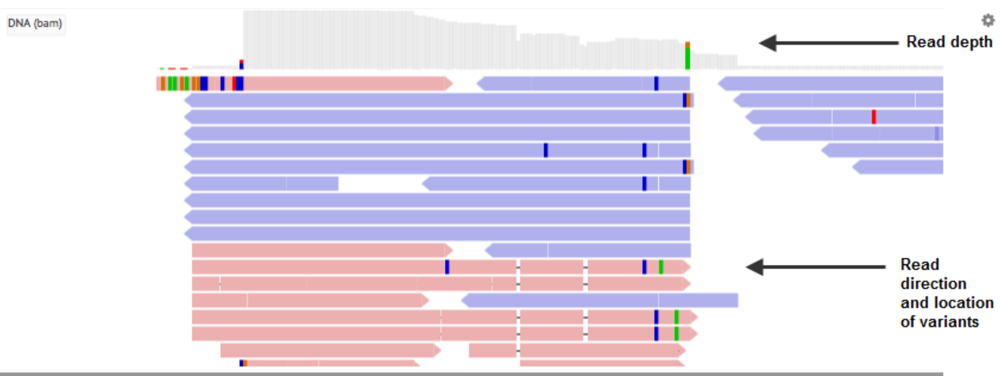
Therefore the counts in the Venn diagram (as well as the counts for the Total, Filtered-In, and Filtered-Out) represent the following:

- 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.

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.

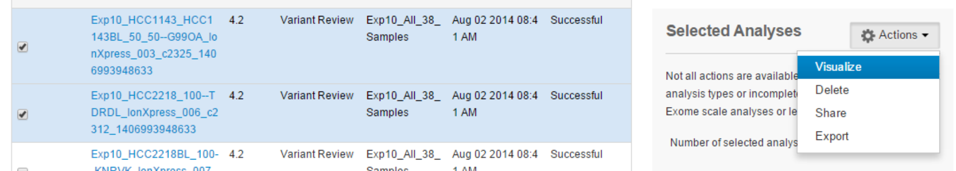


Compare multiple analyses and download TSV file

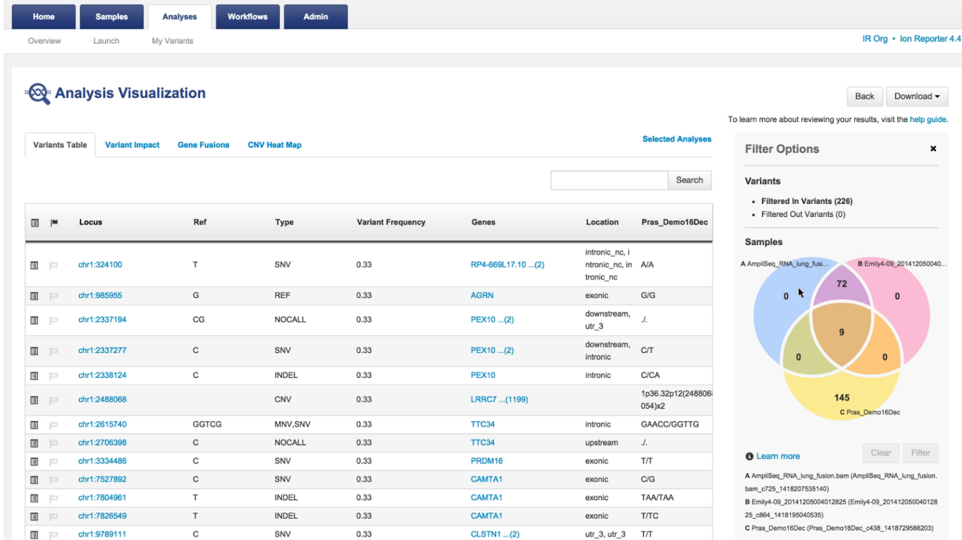
Follow these steps to compare multiple analyses:

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.

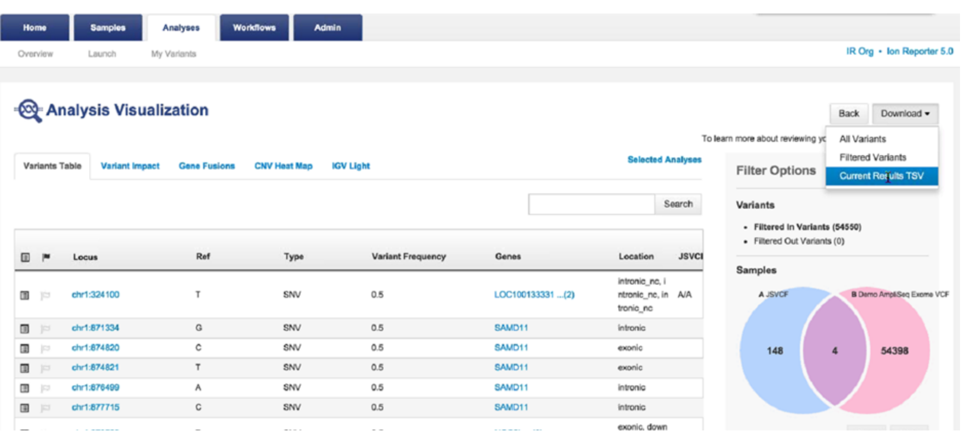
6. Then use the Filter button to update the results to include only toggled components.

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.

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

Note: In the Variants Table view, analysis columns are ordered from left to right starting with the first analysis that you selected. The annotation data from the variants in the first selected analysis is what populates the Location column of the table.

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:

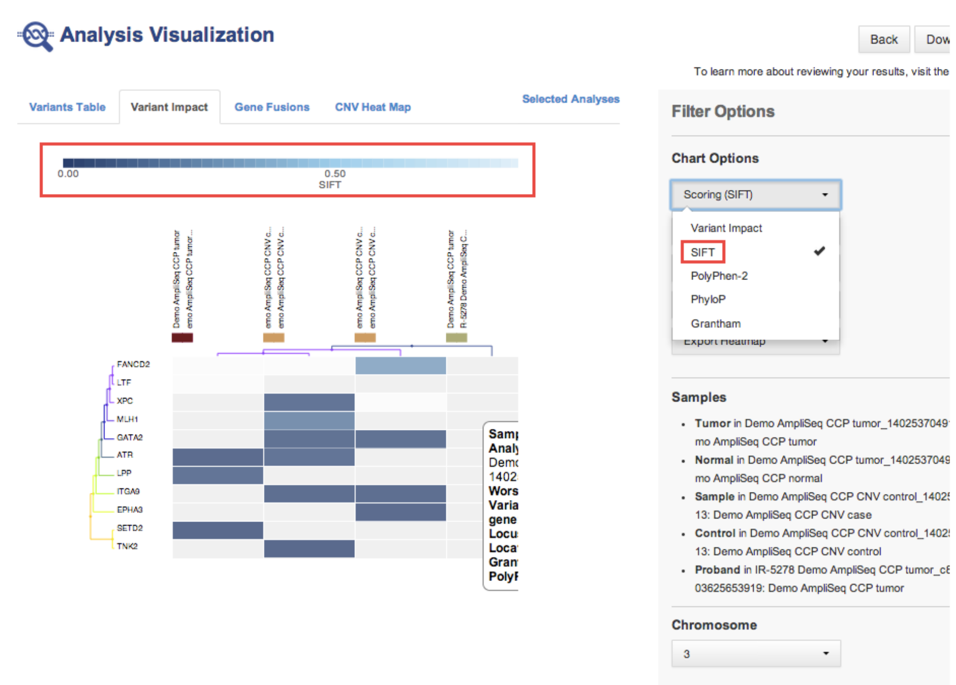
- unknown: 0
- synonymous: 1
- missense: 2
- non-frameshift block substitution: 3
- non-frameshift insertion: 4
- non-frameshift deletion: 4
- nonsense: 5
- stop-loss: 6
- frameshift block substitution: 7
- frameshift insertion: 7
- frameshift deletion: 7
- splice variant: 8

Set the Scoring menu to a different option, such as SIFT or PolyPhen2 , to base the legend and the heat map on those scores.

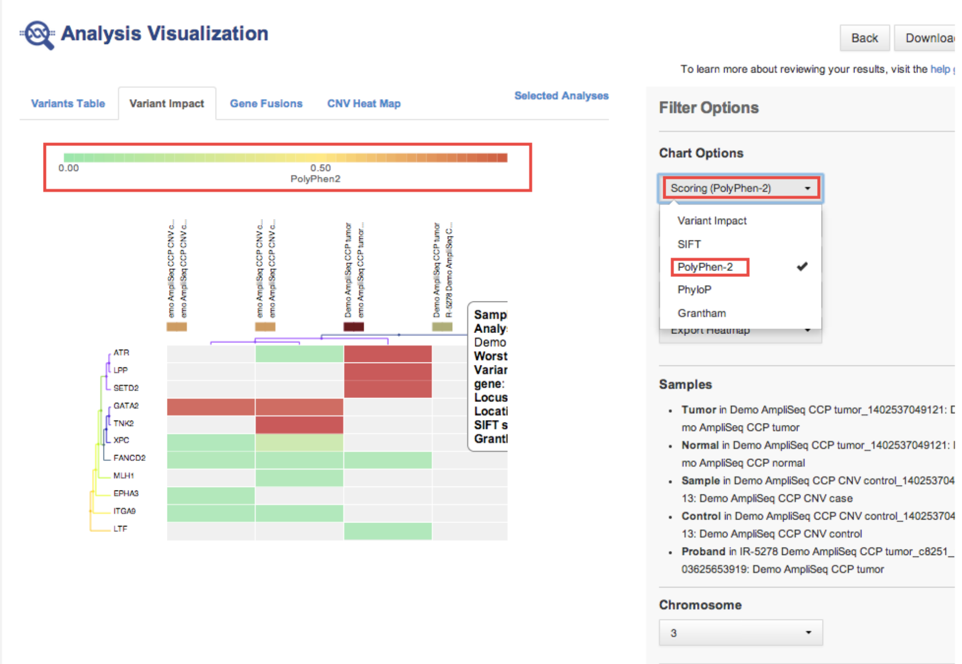
Clustering in Variant Impact heatmap For variant impact heat maps, based on the scoring criteria selected from chart options, most deleterious score is picked for every gene-sample pair to generate the heat map. Thereafter hierarchical clustering is conducted for clustering genes and samples within the heat map.

Chart options

Set the Scoring menu to a different option, such as SIFT

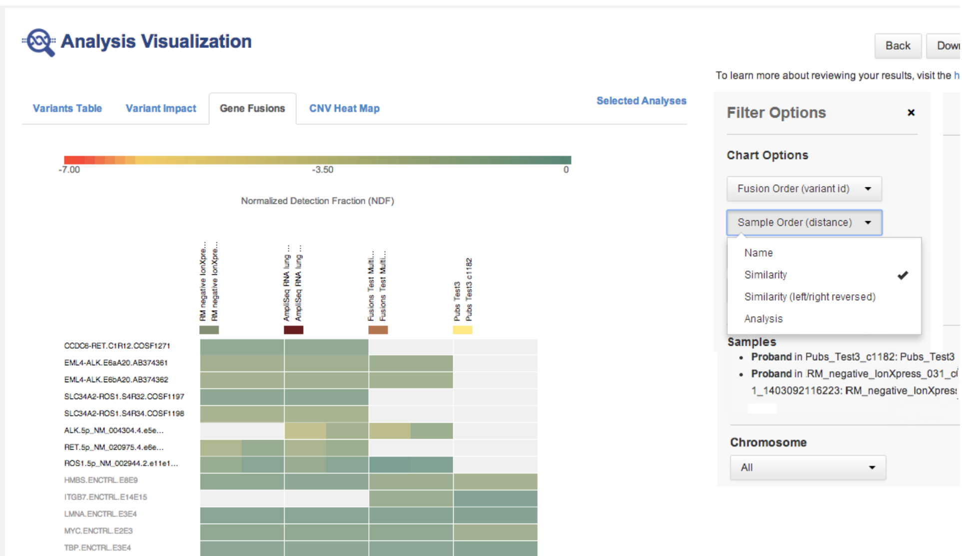


or PolyPhen2



, to base the legend and the heat map on those scores.

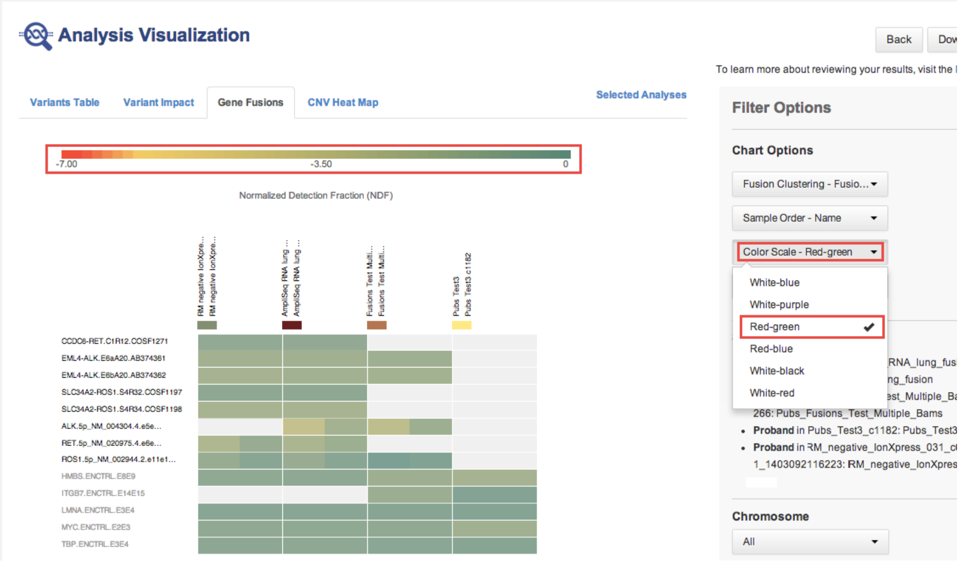
Set the Sample Order menu to base the presentation order of the sample on name or on their similarity in terms of variants found.



The following ordering options are supported:

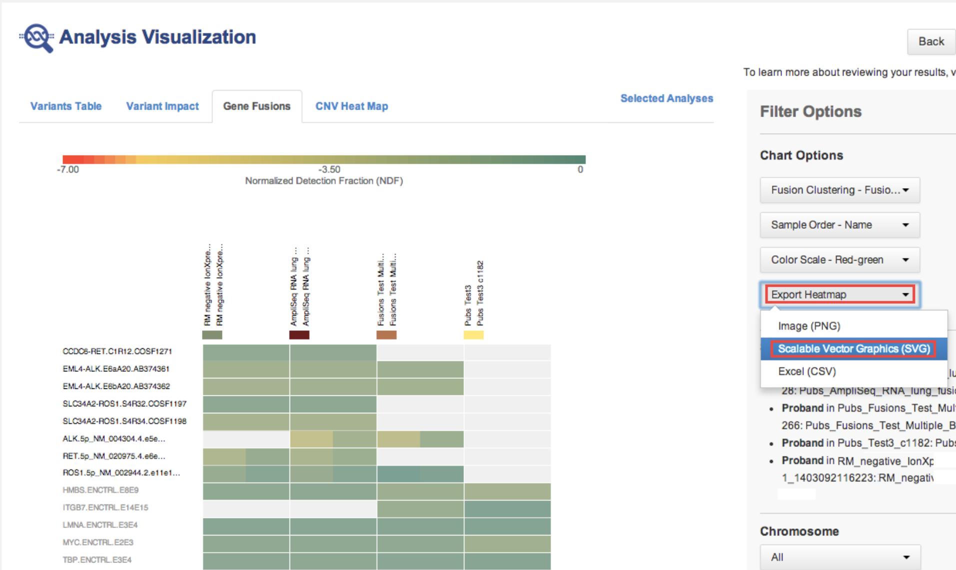
- Name: Alphabetically by sample name
- Similarity: By how similar the samples are in the fusion calls made (presented from fewest fusions to most fusions)
- Similarity (reversed): By how similar the samples are in the fusion calls made (presented from the most fusions to the fewest fusions)
- Analysis: 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).

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.

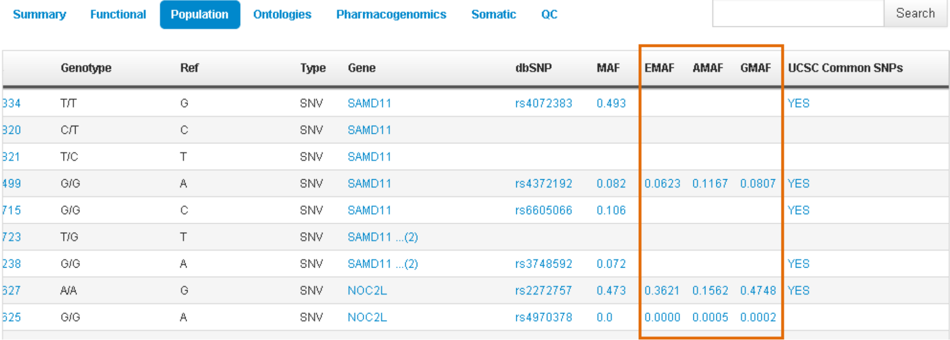
After the Whole Genome View of each non-aneuploidy analysis, the various data tracks are loaded in this order by default:

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)

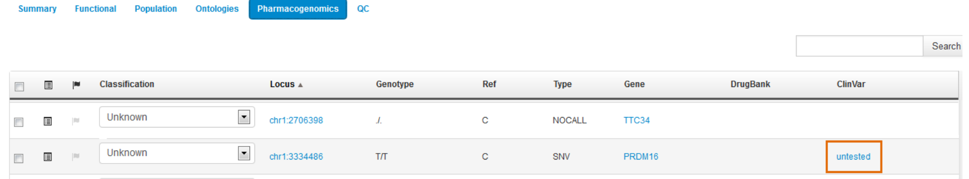
Links to external databases

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:



- 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".

Download a variants file

If you want to analyze the Ion Reporter™ Software data in downstream software, such as Oncomine™ Knowledgebase Reporter, or examine VCF files directly, you can download a compressed directory of files that includes variants results in a VCF file, provided that you use a successful analysis. You can choose between two types of directory.

- **Filtered variants:** This directory contains a VCF file and other files, all of which contain variants that were *filtered* from the analysis, and are based either the filter chain used by default in the Ion Reporter™ Software workflow, or the filter chain that is saved to the workflow.
  - **All variants:** A VCF, and other files, all of which contain *all* variants included in the analysis.

The compressed directory includes auxiliary files that have other results set information about variants that is not contained in the VCF file.

Downloads of results files can be automated with the web services API that is included with Ion Reporter Software. See "Web services API" on page 380 for more information.

Note: This does not apply to analyses that do not include a VCF file in the results package, such as the Immune Repertoire and Metagenomics workflows. Also, if you run a plugin as a workflow Ion Reporter™ Software, then the compressed directory does not include a VCF file if the plugin does not generate a VCF file.

Download filtered variants in an analysis

1. In the **Analyses** tab, click **Overview**, then select the analysis that contains the filtered variants.

2. Click **Actions ▸ Download Filtered Variants**.
A compressed directory that contains the following folders is downloaded: The set of files will depend on what types of analysis results are available, based on how data was filtered, what workflow was used, and whether plugins were used, and so on.

Folder	Description of contents
CNV_VCIB	Contains `cn_results.png`, a file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. Open the file with a text editor.
QC	
Variants	Contains VCF and TSV files for the CNV variants. You can open these files in Microsoft™ Excel™.
Workflow_Settings	Contains folders with: • A text file that describes settings used for the analysis. Open the file with a text editor. • Configuration files

3. To download the compressed directory, click the **Home** tab, then click **Notifications**, and then click **Downloads**.
The file is named `analysis name_Filtered.zip`

You can extract the files, or upload them to a downstream software that uses VCF files, such as OKR.

Download all variants in an analysis

1. In the **Analyses** tab, click **Overview**, then select the analysis of interest.
2. Click **Actions ▸ Download All Variants**.
A compressed directory that contains the following folders is downloaded:

Folder	Description of contents
CNV_VCIB	Contains `cn_results.png`, a file that represents the CNV amplicons in the X scale and log2 ratio in Y scale. Open the file with a text editor.
QC	
Variants	Contains VCF and TSV files for the CNV variants. You can open these files in Microsoft™ Excel™.
Workflow_Settings	Contains folders with: • A text file that describes settings used for the analysis. Open the file with a text editor. • Configuration files
RESULTS	If you are using the Mutation Load Research Application or a workflow that is enabled for Mutation Load: • TSV files that contain post-filter and somatic variants. • PDF report that contains mutation load results. • File named `statistic.txt`, which contains mutation load statistics. • PNG files that contain: – allele frequency distribution of germ-line and somatic variants – allele frequency distribution of only somatic variants – bar plot of signature type and context of somatic mutations – pie chart of substitution type of somatic mutations – pie chart of signature pattern of somatic mutations

3. To download the compressed directory, click the **Home** tab, then click **Notifications**, and then click **Downloads**.
The file is named `analysis name_All.zip`.

You can extract the files to view them individually, or upload VCF files to a software that requires VCF files, such as Oncomine™ Knowledgebase Reporter Software.

Download BAM files

You must be a user of Ion Reporter™ Software on an Ion Reporter™ Server to complete this procedure. You cannot download BAM files from Ion Reporter™ Software on Thermo Fisher Cloud.

You can download a BAM file that is generated by Ion Reporter™ Software to review the file, or open it and inspect the data with a visualization tool outside of Ion Reporter™ Software. Files generated by the software are mapped to a reference sequence. If a BAM file is not mapped to a reference sequence Ion Reporter™ Software will add the mapping, or if the files requires additional reference mapping, those reference sequences will be added. A *mapped BAM* in Ion Reporter™ Software might originate in Torrent Suite™ Software, or it might be a BAM that you import. A *processed BAM* is transferred to Ion Reporter™ Software as output from the variantCaller in Torrent Suite™ Software.

You can download either types of these BAM files:

1. In the **Analyses** tab, click **Overview**, then select the analysis of interest.
2. Select one of the following options:
   - Click **Actions ▸ Download Processed BAM** to download the BAM file that was sent from Torrent Suite™ Software.
   - Select **Actions ▸ Download Mapped BAM** if you want to process, or use, the BAM file outside of Ion Reporter™ Software.

Download analysis logs

You can download two logs that contain details about each analysis.

1. In the **Analyses** tab, click **Overview**, then select the analysis of interest.
2. Click **Actions ▸ Download Analysis Logs**.
   Two log files are downloaded:

   Table 4

File name	Description
analysis	This file contains details about the algorithmic pipeline that was used in the workflow and details about the analysis.
summary	This file contains details about the analysis process and the start and finish time for each algorithmic module.

Edit analysis results

Add 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 ▤ .

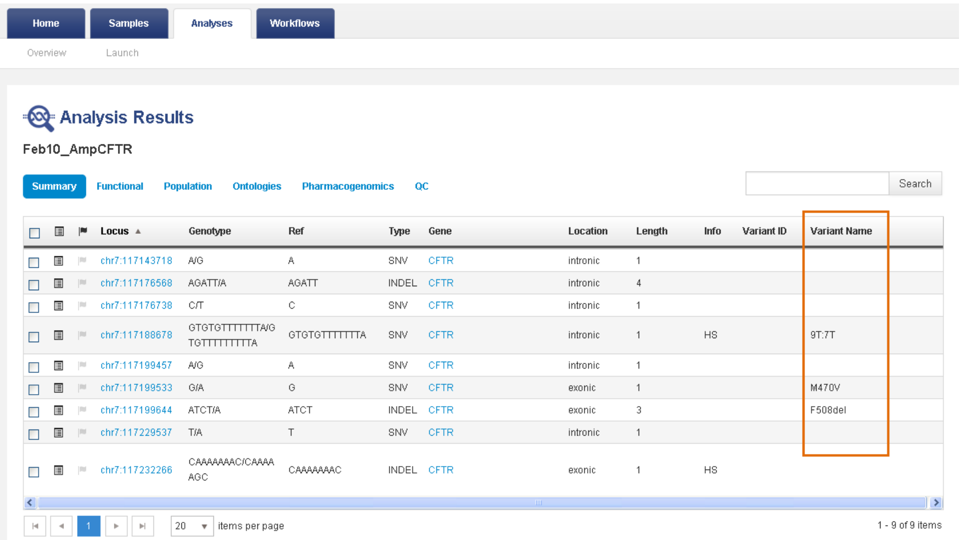
CNV subtypes in the Analysis Results screen

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

CFTR analysis results

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

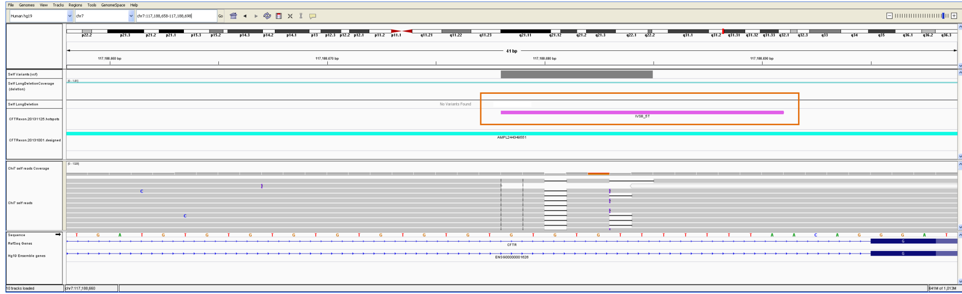
- The **Variant Name** column reports matches with the Named Variants annotation source.



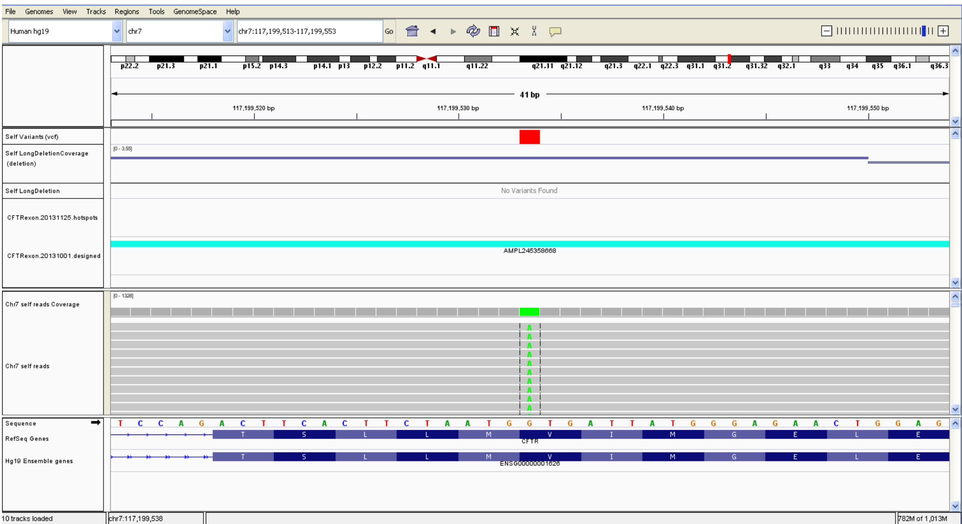
Locus	Genotype	Ref	Type	Gene	Location	Length	Info	Variant ID	Variant Name
chr7:117143718	A/G	A	SNV	CFTR	intronic	1			
chr7:117176568	AGATT/A	AGATT	INDEL	CFTR	intronic	4			
chr7:117176738	C/T	C	SNV	CFTR	intronic	1			
chr7:117188678	GTGTGTTTTTTTA/G TGTTTTTTTTA	GTGTGTTTTTTTA	SNV	CFTR	intronic	1	HS		9T:7T
chr7:117199457	A/G	A	SNV	CFTR	intronic	1			
chr7:117199533	G/A	G	SNV	CFTR	exonic	1			M470V
chr7:117199644	ATCT/A	ATCT	INDEL	CFTR	exonic	3			F508del
chr7:117229537	T/A	T	SNV	CFTR	intronic	1			
chr7:117232266	CAAAAAAAC/CAAAA AGC	CAAAAAAAC	INDEL	CFTR	exonic	1	HS		

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

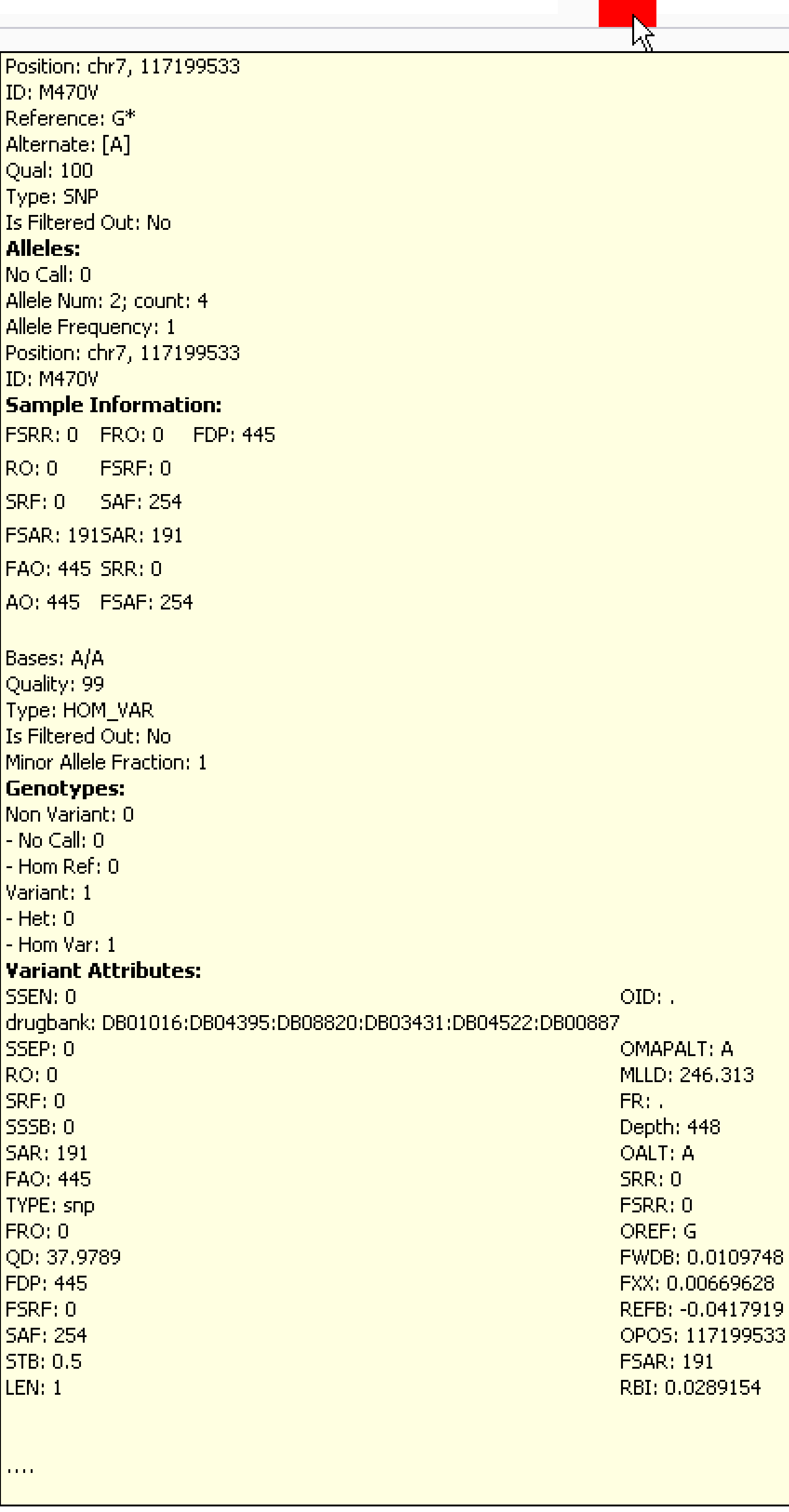
- The following IGV visualization for CFTR analysis results include a named variant:



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**.

Review analysis results for Ion AmpliSeq™ HD panels

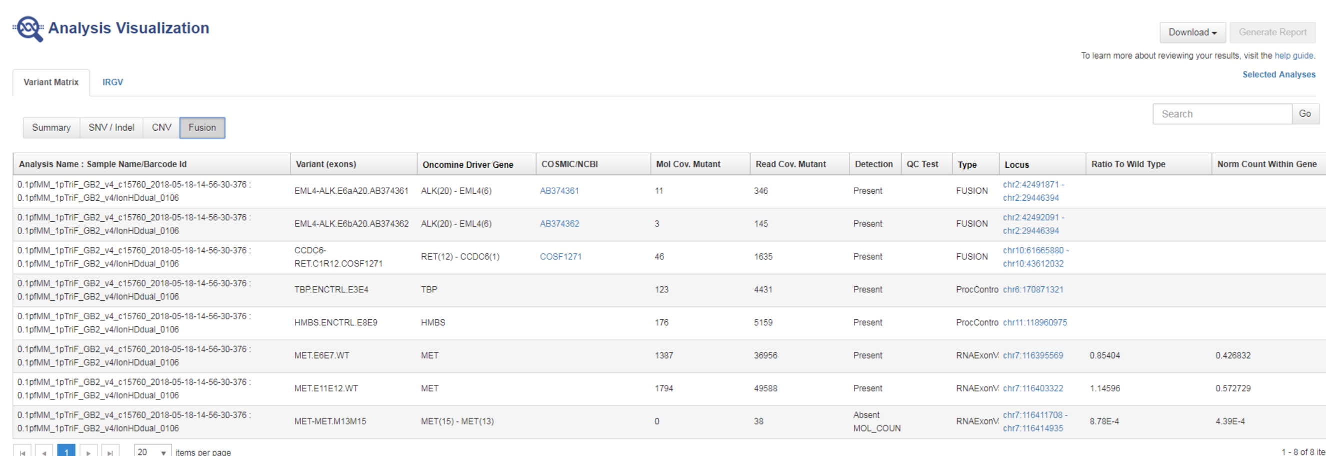
1. In the **Analyses** tab, in the **Analysis Results** table, click the link in the **Analysis** column to open an analysis of interest.

2. *(Optional)* In the **Filter Options** pane, select a different filter chain from the dropdown list.

Option	Description
Called Variants and Controls	This is the default filter chain for the Ion AmpliSeq™ HD workflows. Use this for analysis results that report all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Variant types include: SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL_ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3ITD.
Called Hotpot Variants and Controls	Select this filter chain for analysis results that report all hotspot variants that pass the filter and are not called as reference or NOCALL. Filter variant types include: SNP and INDEL.
Variant Matrix Summary	Select this filter chain for analysis results that generate a visualization that contains the same set of variants that are included in the Analysis Results table. This filter chain allows results to be exported. Import the exported results file into Oncomine™ Knowledgebase Reporter (OKR) to include the variants shown in the Variant Matrix Summary view in OKR reports. Variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants.

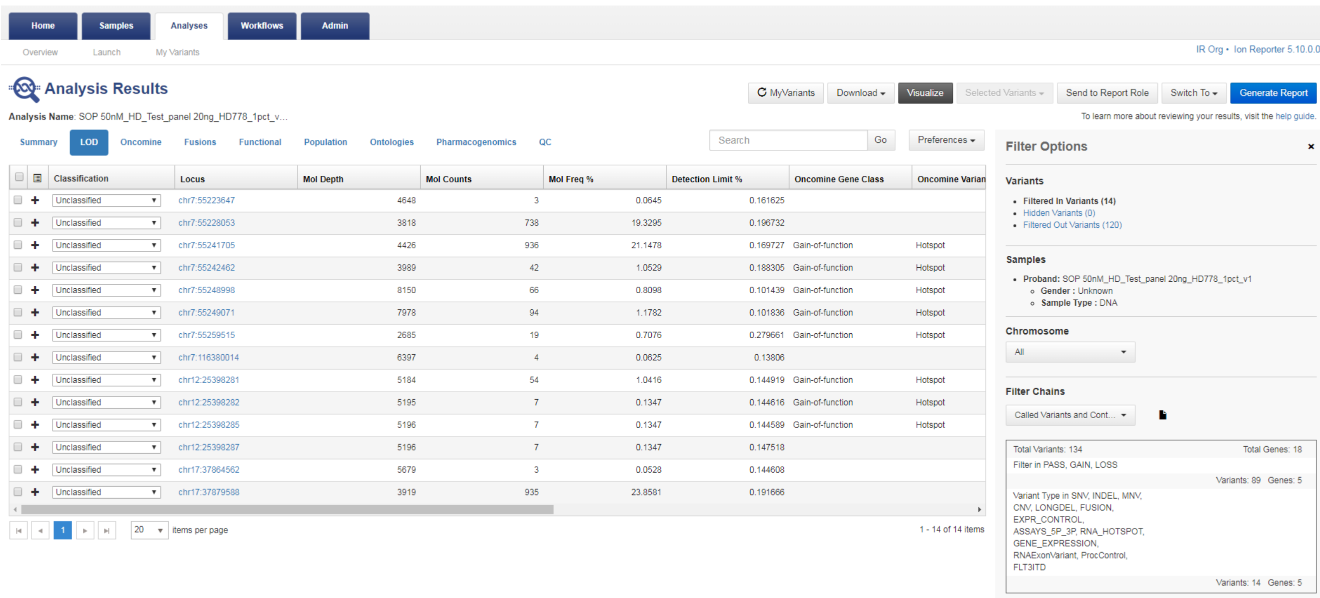
3. If you wish to show only OVAT annoted variants, then apply the **Oncomine Variants (5.10)** filter chain. If you wish to export filtered variants using any filter chain, be sure to save that filter chain.

Example of **Variant Matrix** data view, **Fusions** screen.



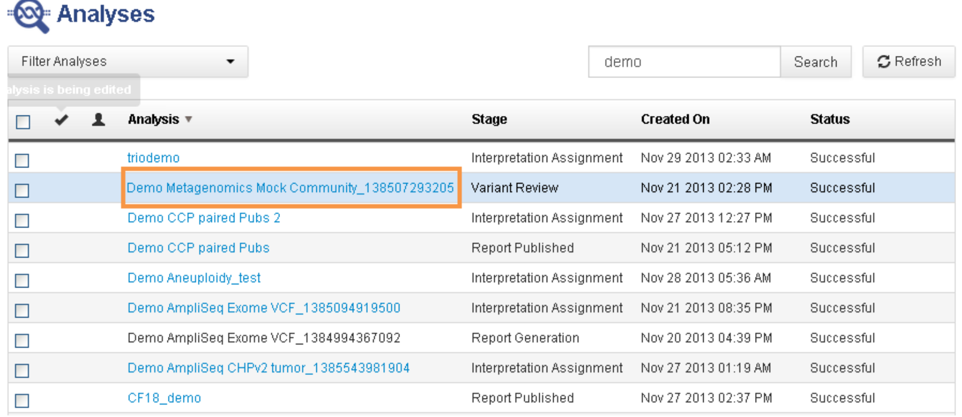
Analysis Name : Sample Name/Barcode Id	Variant (exons)	Oncomine Driver Gene	COSMIC/NCBI	Mol Cov. Mutant	Read Cov. Mutant	Detection	QC Test	Type	Locus	Ratio To Wild Type	Norm Count Within Gene
0.1pfMM_1pTriF_GB2_v4_c15760_2018-05-18-14-56-30-376 : 0.1pfMM_1pTriF_GB2_v4/IonHDdual_0106	EML4-ALK.E6aA20.AB374361	ALK(20) - EML4(6)	AB374361	11	346	Present		FUSION	chr2:42491871 - chr2:29446394		
0.1pfMM_1pTriF_GB2_v4_c15760_2018-05-18-14-56-30-376 : 0.1pfMM_1pTriF_GB2_v4/IonHDdual_0106	EML4-ALK.E6bA20.AB374362	ALK(20) - EML4(6)	AB374362	3	145	Present		FUSION	chr2:42492091 - chr2:29446394		
0.1pfMM_1pTriF_GB2_v4_c15760_2018-05-18-14-56-30-376 : 0.1pfMM_1pTriF_GB2_v4/IonHDdual_0106	CCDC6-RET.C1R12.COSF1271	RET(12) - CCDC6(1)	COSF1271	46	1635	Present		FUSION	chr10:61665880 - chr10:43612032		
0.1pfMM_1pTriF_GB2_v4_c15760_2018-05-18-14-56-30-376 : 0.1pfMM_1pTriF_GB2_v4/IonHDdual_0106	TBP.ENCTRL.E3E4	TBP		123	4431	Present		ProcContro	chr6:170871321		
0.1pfMM_1pTriF_GB2_v4_c15760_2018-05-18-14-56-30-376 : 0.1pfMM_1pTriF_GB2_v4/IonHDdual_0106	HMBS.ENCTRL.E8E9	HMBS		176	5159	Present		ProcContro	chr11:118960975		
0.1pfMM_1pTriF_GB2_v4_c15760_2018-05-18-14-56-30-376 : 0.1pfMM_1pTriF_GB2_v4/IonHDdual_0106	MET.E6E7.WT	MET		1387	36956	Present		RNAExonV	chr7:116395569	0.85404	0.426832
0.1pfMM_1pTriF_GB2_v4_c15760_2018-05-18-14-56-30-376 : 0.1pfMM_1pTriF_GB2_v4/IonHDdual_0106	MET.E11E12.WT	MET		1794	49588	Present		RNAExonV	chr7:116403322	1.14596	0.572729
0.1pfMM_1pTriF_GB2_v4_c15760_2018-05-18-14-56-30-376 : 0.1pfMM_1pTriF_GB2_v4/IonHDdual_0106	MET-MET.M13M15	MET(15) - MET(13)		0	38	Absent MOL_COUN		RNAExonV	chr7:116411708 - chr7:116414935	8.78E-4	4.39E-4

Example of **Analysis Results** screen, **LOD** view, and **Filter Options** pane.



Metagenomics 16S analysis results

The results page contains the following sections and features:

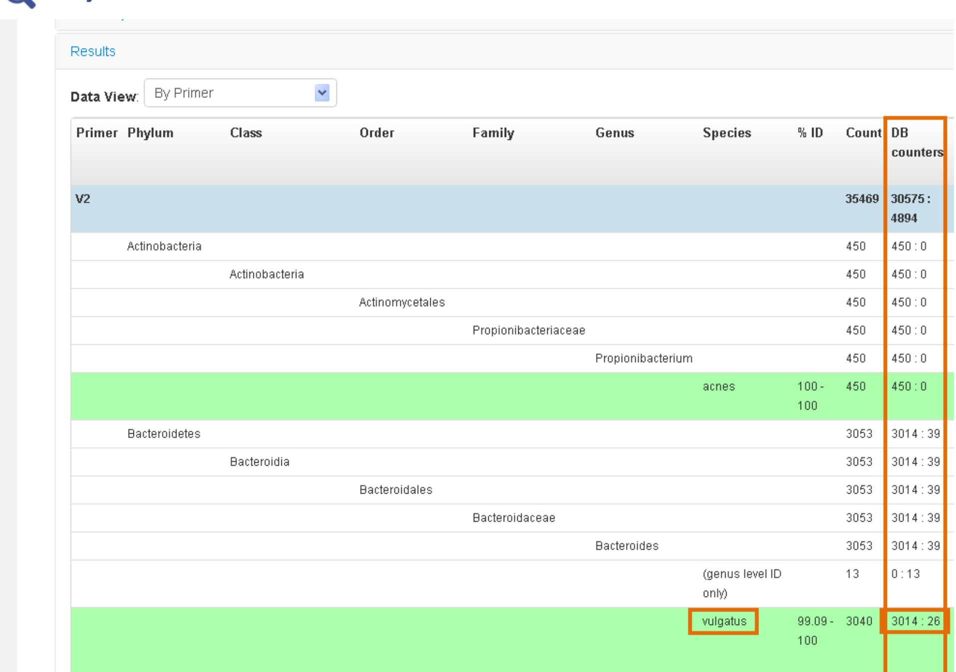


Analysis	Stage	Created On	Status
triodemo	Interpretation Assignment	Nov 29 2013 02:33 AM	Successful
Demo Metagenomics Mock Community_138507293205	Variant Review	Nov 21 2013 02:28 PM	Successful
Demo CCP paired Pubs 2	Interpretation Assignment	Nov 27 2013 12:27 PM	Successful
Demo CCP paired Pubs	Report Published	Nov 21 2013 05:12 PM	Successful
Demo Aneuploidy_test	Interpretation Assignment	Nov 28 2013 05:36 AM	Successful
Demo AmpliSeq Exome VCF_1385094919500	Interpretation Assignment	Nov 21 2013 08:35 PM	Successful
Demo AmpliSeq Exome VCF_1384994367092	Report Generation	Nov 20 2013 04:39 PM	Successful
Demo AmpliSeq CHPv2 tumor_1385543981904	Interpretation Assignment	Nov 27 2013 01:19 AM	Successful
CF18_demo	Report Published	Nov 27 2013 02:37 PM	Successful

- 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.


Analyses

Results

Data View: By Primer

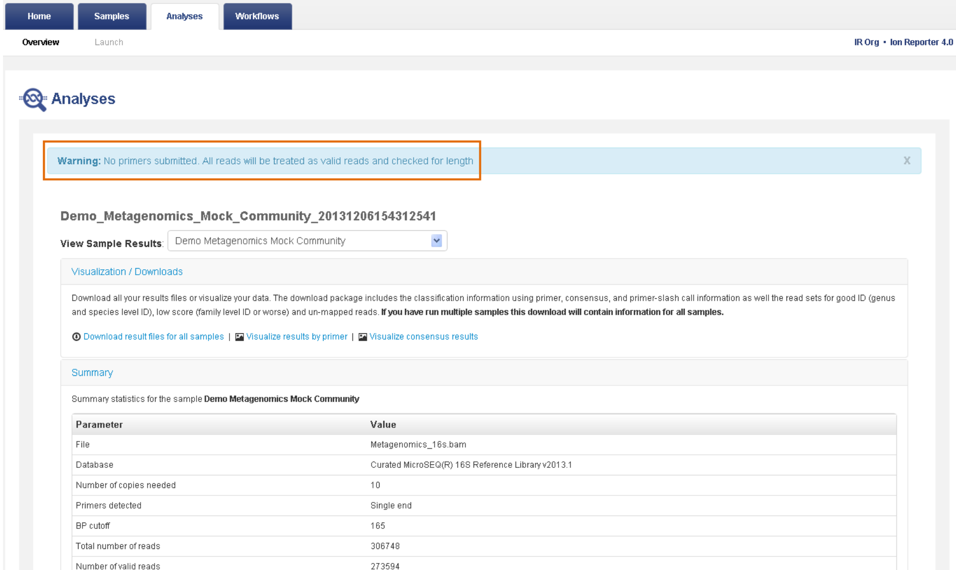
Primer	Phylum	Class	Order	Family	Genus	Species	% ID	Count	DB counters
V2								35469	30575 : 4894
	Actinobacteria							450	450 : 0
		Actinobacteria						450	450 : 0
			Actinomycetales					450	450 : 0
				Propionibacteriaceae				450	450 : 0
					Propionibacterium			450	450 : 0
						acnes	100 - 100	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
```

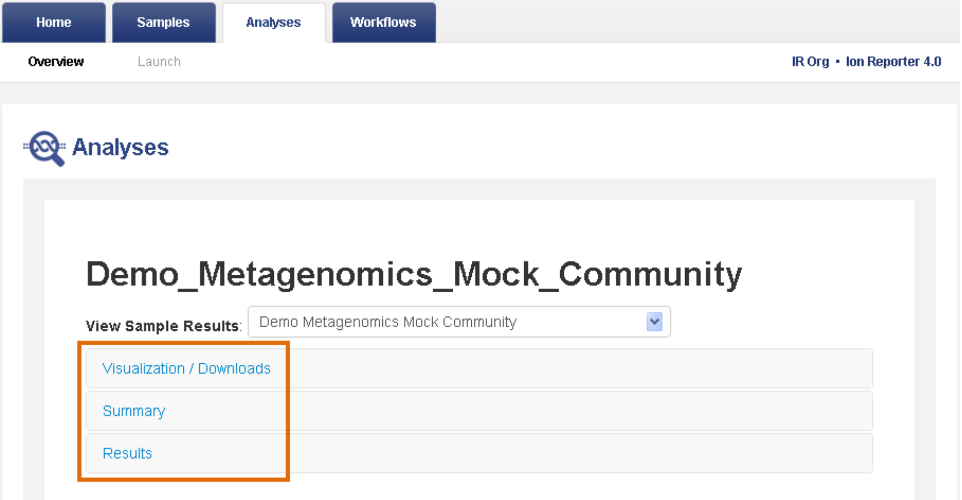
means that 3014 reads matched sequences in the MicroSEQ™ database and 26 matched sequences in the Greengenes database.

Note: When primer information is missing in the metagenomics workflow, a warning message appears during analysis review.

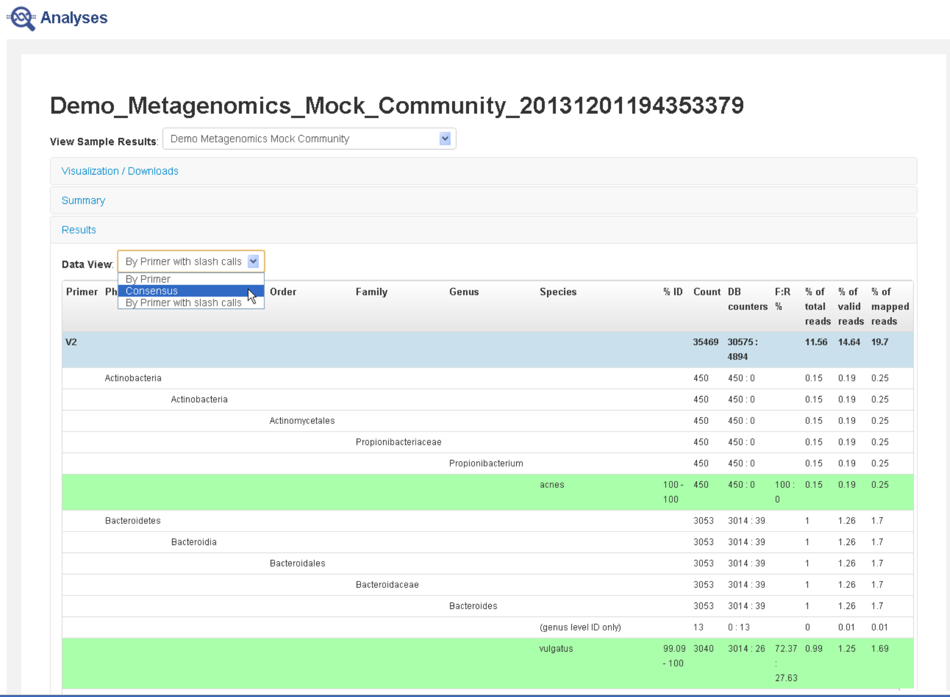


Steps to interpret your metagenomics 16S™ analysis results

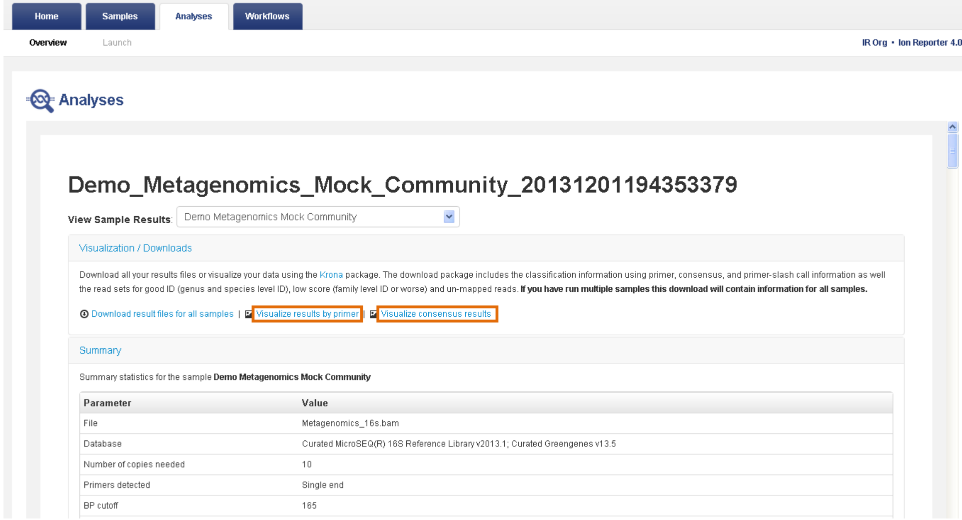
1. Log in to the Ion Reporter™ Software application and click on the **Analysis** tab.
2. Click on the name of your metagenomics analysis in the analysis table.
3. Click on the Visualization / Downloads, Summary, or Results section headers to collapse the section.



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™ Software to other, external databases.

Alpha-beta diversity results

The 16S Metagenomics workflow in Ion Reporter™ Software includes alpha diversity calculations and beta diversity calculations.

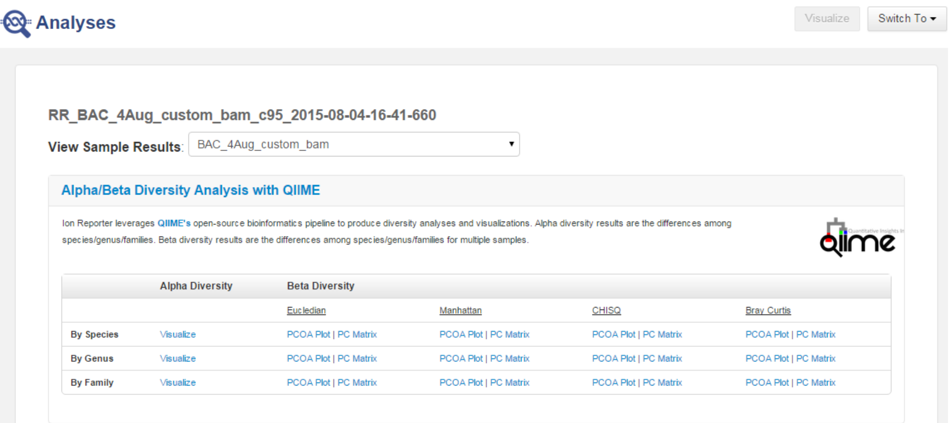
Alpha diversity results describe the diversity in a single sample at the Species, Genus, and Family levels. Beta diversity results describe the diversity between multiple samples at the Species, Genus, and Family levels. For results interpretation guidance, see the QIIME online help at **qiime.org**.

Note: If you only run one sample, Ion Reporter™ Software 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.

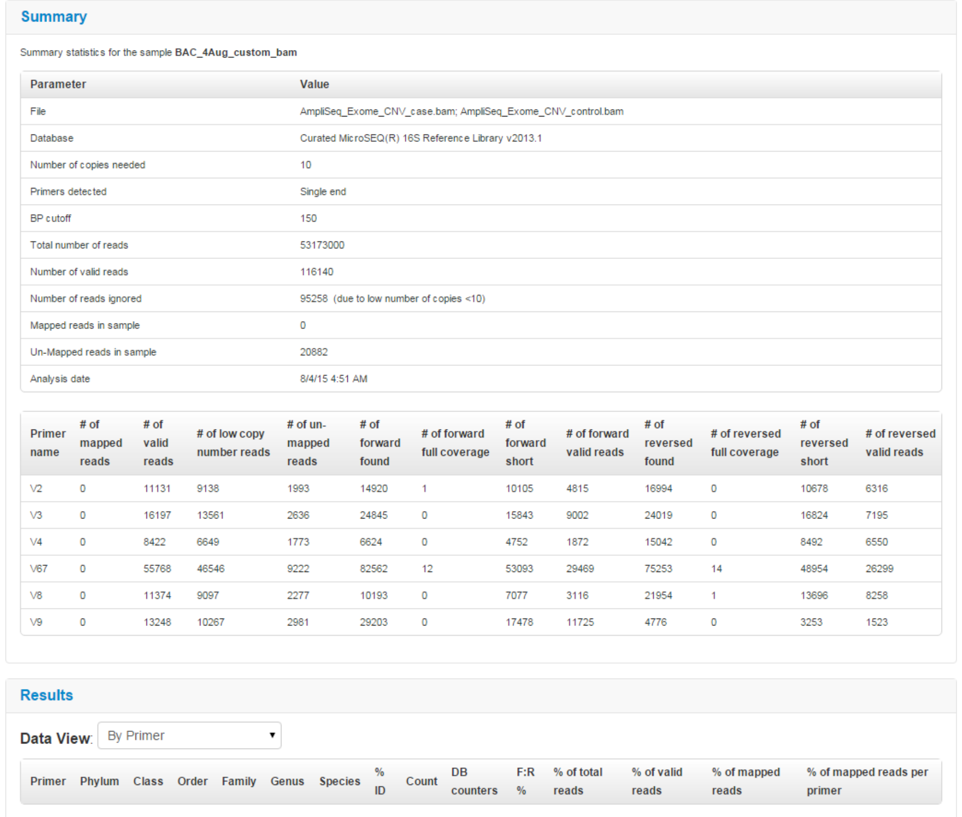
To review results:

1. In the **Analyses**, click **Overview**, then select the analysis of interest.
   An Analyses summary opens.



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	55768	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

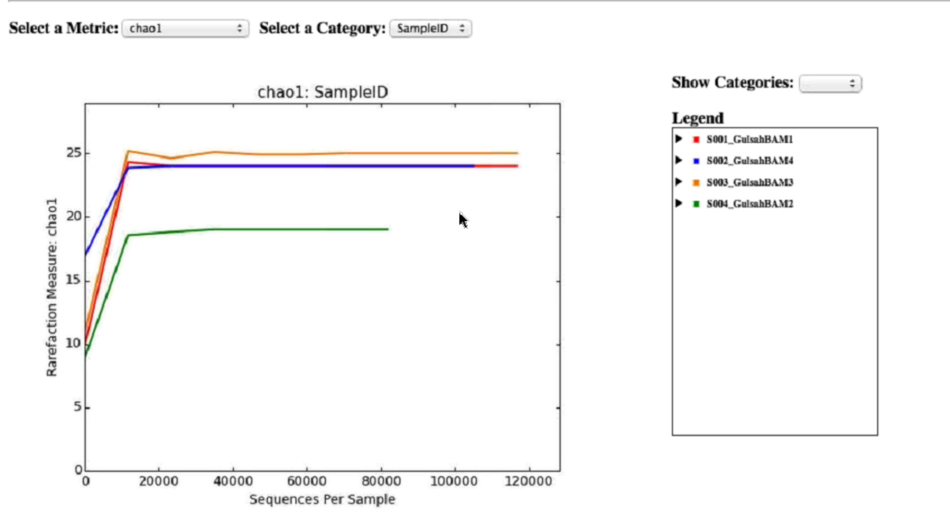
Data View: By Primer

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.

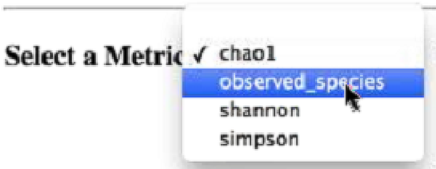
Ion Reporter Metagenomics 16S Alpha Diversity Analysis QIIME Results for Species:



If the lines for some categories do not extend all the way to the right end of the x-axis, that means that at least one of the samples in that category does not have that many sequences.

SampleID	Seqs/Sample	chao1 Ave.	chao1 Err.	observed_species Ave.	observed_species Err.	shannon Ave.	shannon Err.	simpson Ave.	simpson Err.
S001_GulsahBAM1	10.0	9.870	nan	6.000	nan	2.419	nan	0.792	nan
S001_GulsahBAM1	11682.0	24.300	nan	23.400	nan	3.250	nan	0.857	nan
S001_GulsahBAM1	23354.0	24.000	nan	24.000	nan	3.254	nan	0.858	nan
S001_GulsahBAM1	35026.0	24.000	nan	24.000	nan	3.250	nan	0.858	nan

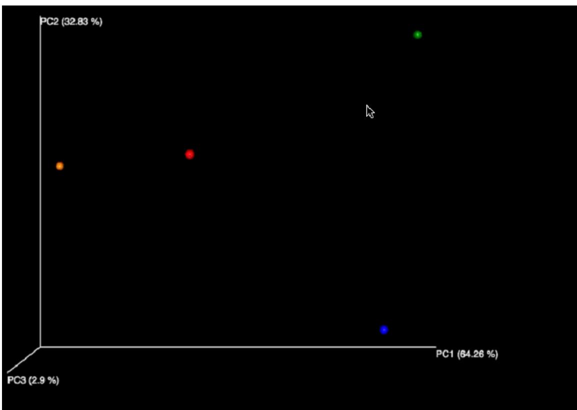
4. Select your metrics.



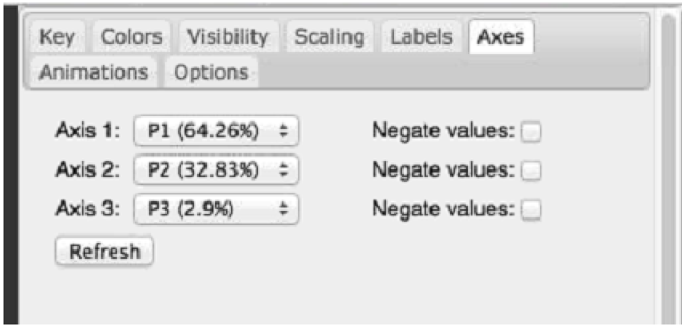
5. Repeat for Genus and Family links under Alpha Diversity Results to see the genus and families that are in the sample.

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.

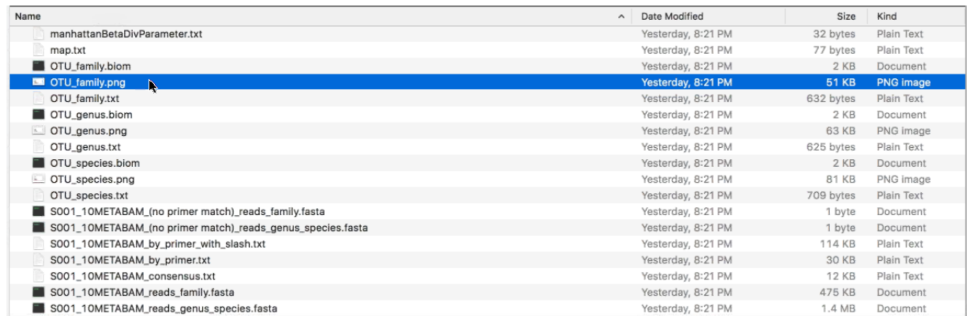


Visualize OTU results with R-scripts graphs

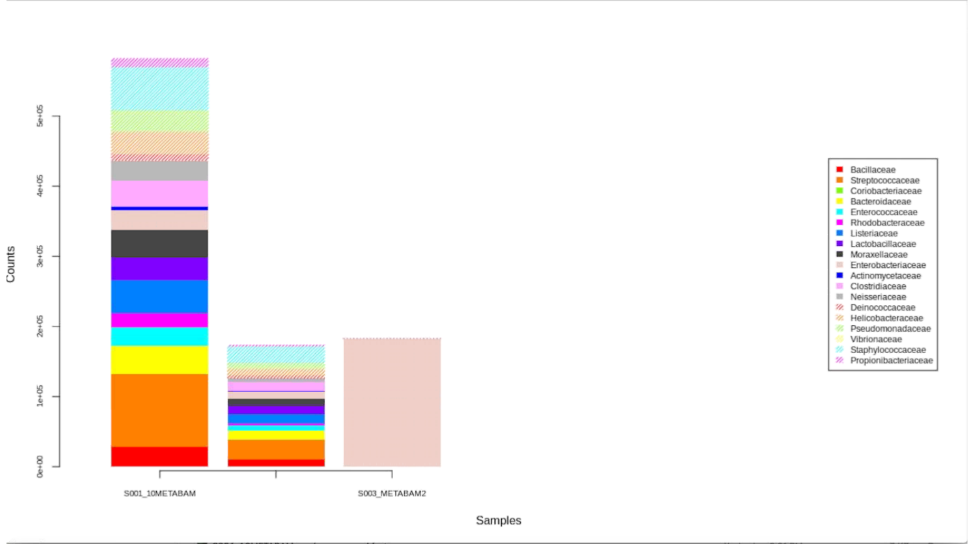
In Ion Reporter™ Software, OTU files are displayed graphically. For instance, in the example below, the `OTU_family.txt` file is rendered graphically in the `OTU_family.png` file.

1. Download your results.

2. Click on the PNG file of the results.



The data is displayed visually.

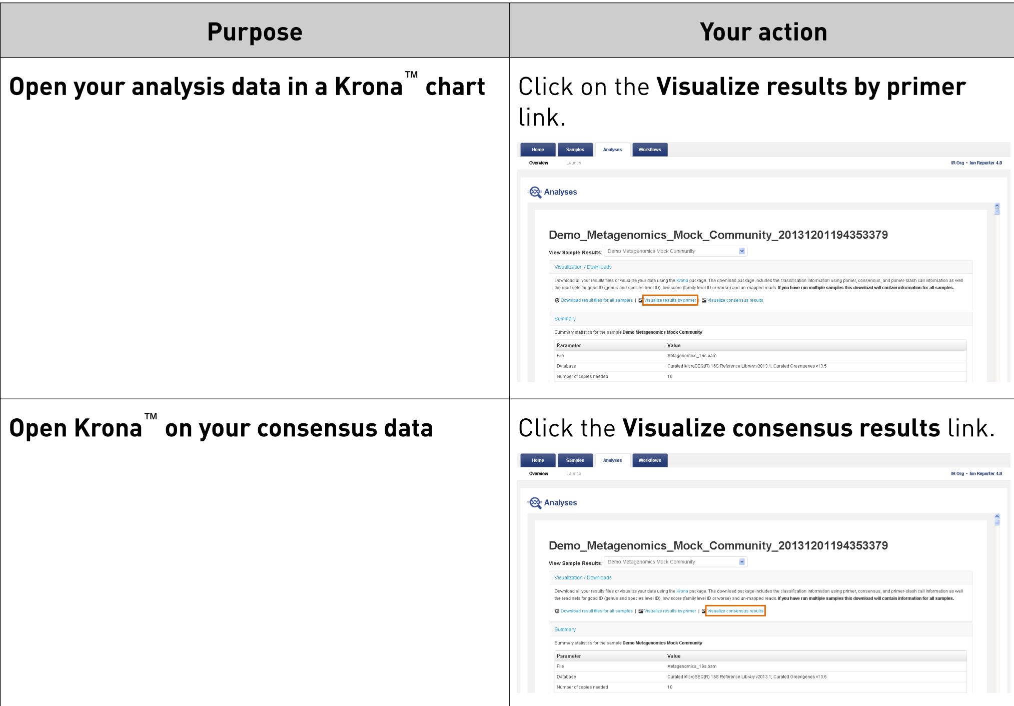


Visualize metagenomics 16S results with Krona charts

The Krona™ visualization package enables you to browse through your data using interactive zoomable pie charts. Krona™ documentation is available on their **github site**.

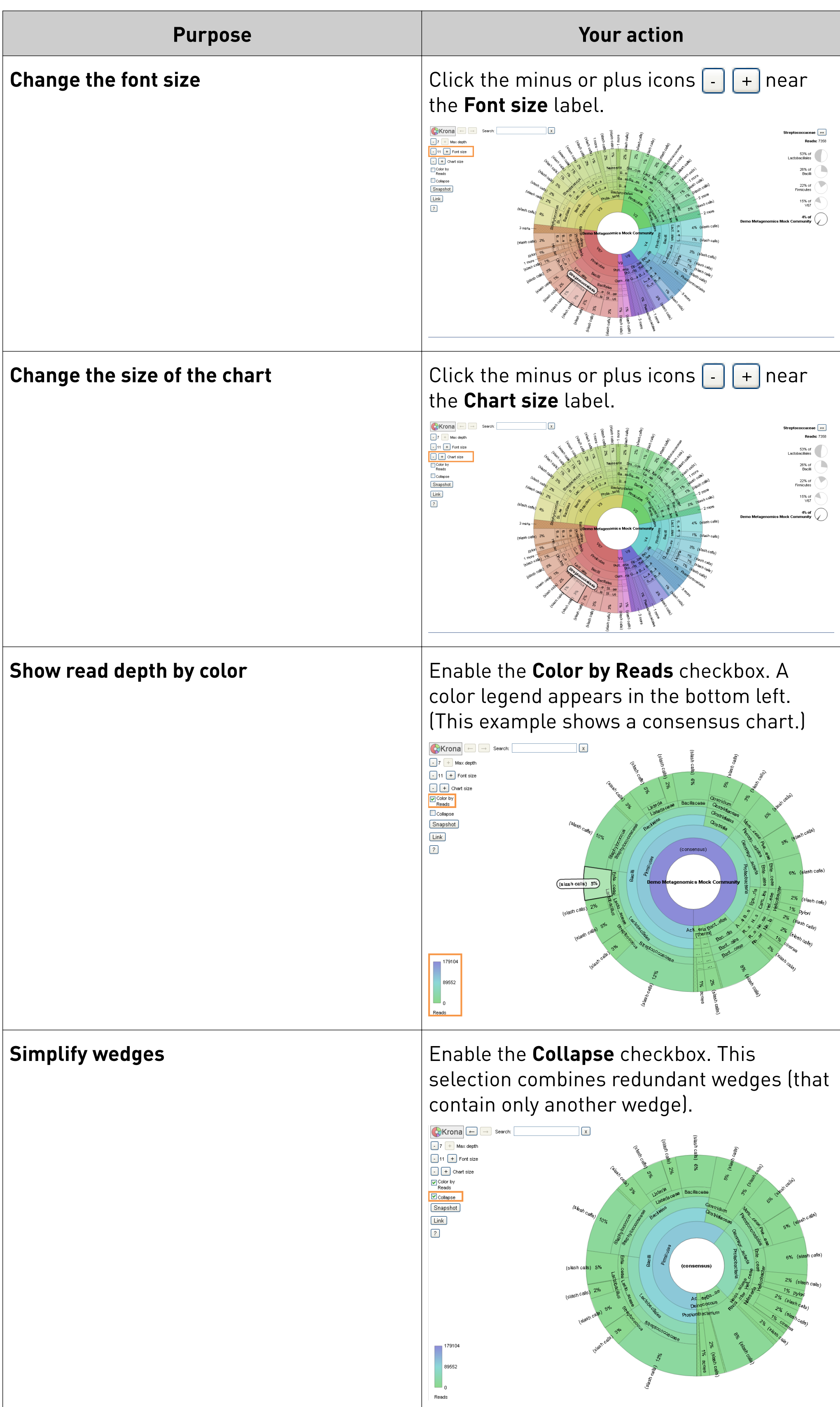
Note: The Snapshot button does not work on the Krona visualization page in the Metagenomics workflow. Krona™ documentation states that their charts are best viewed with the Firefox™ browser.

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.
Open Krona™ on your consensus data	Click the **Visualize consensus results** link.

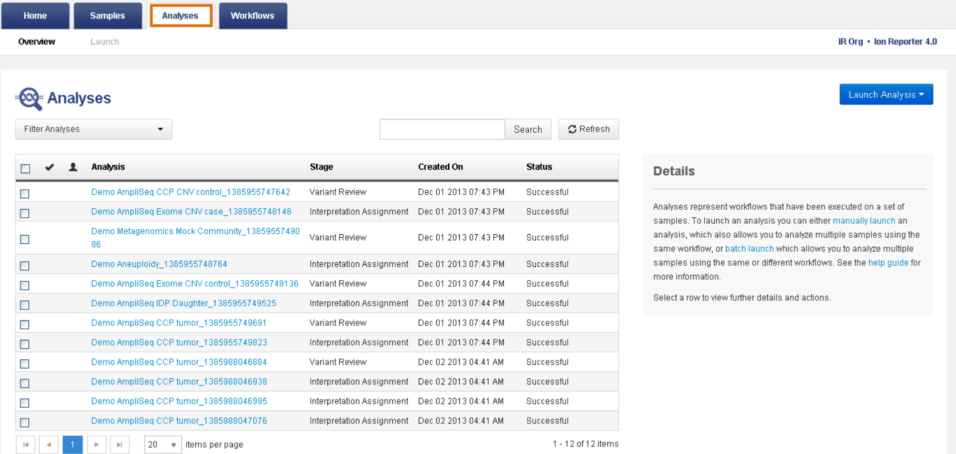
Purpose	Your action
Display percentages for one area of the chart	Click on that area of the chart. The percentages for that area are displayed in the top right of the page.
Make that area the new focus of the chart	Click the expand box in the top right of the page.
Return to the previous chart focus	Click the back arrow [←] near the Krona™ logo in the top left of the page. (Only one go-back is supported at a time.)
Change the number of circles shown in the chart	Click the minus or plus icons [-] [+] near the **Max depth** label.



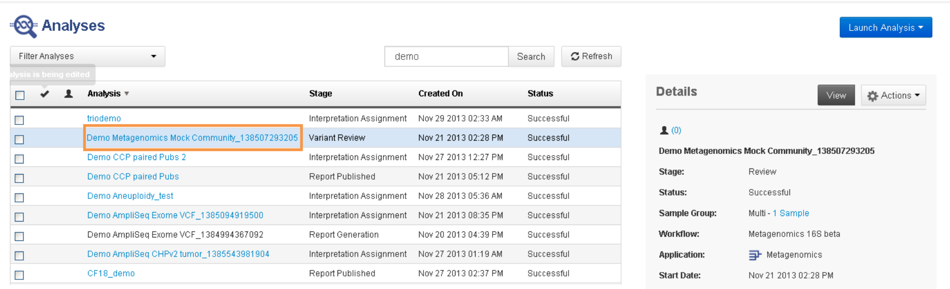
Purpose	Your action
Change the font size	Click the minus or plus icons [-] [+] near the **Font size** label.
Change the size of the chart	Click the minus or plus icons [-] [+] near the **Chart size** label.
Show read depth by color	Enable the **Color by Reads** checkbox. A color legend appears in the bottom left. (This example shows a consensus chart.)
Simplify wedges	Enable the **Collapse** checkbox. This selection combines redundant wedges (that contain only another wedge).

Purpose	Your action
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.)
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.

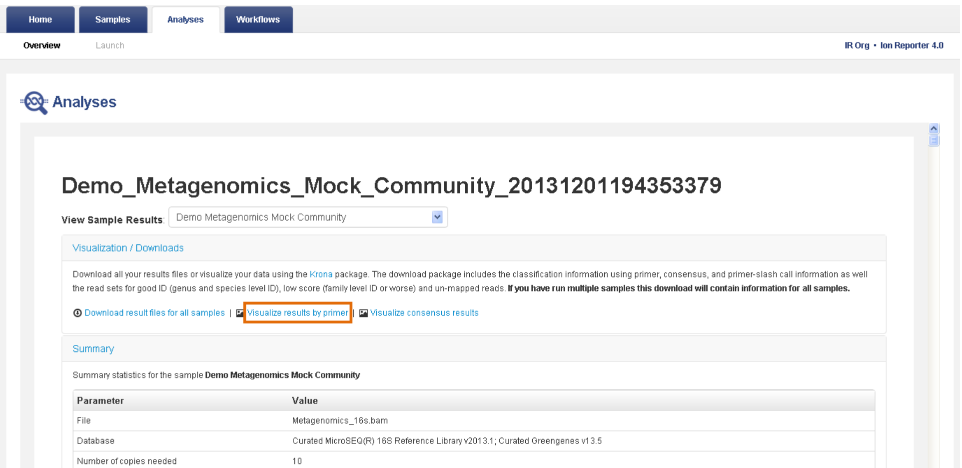
1. Log in to the Ion Reporter™ Software application and click on the **Analysis** tab.



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 Oncomine™ TCR Beta 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.	

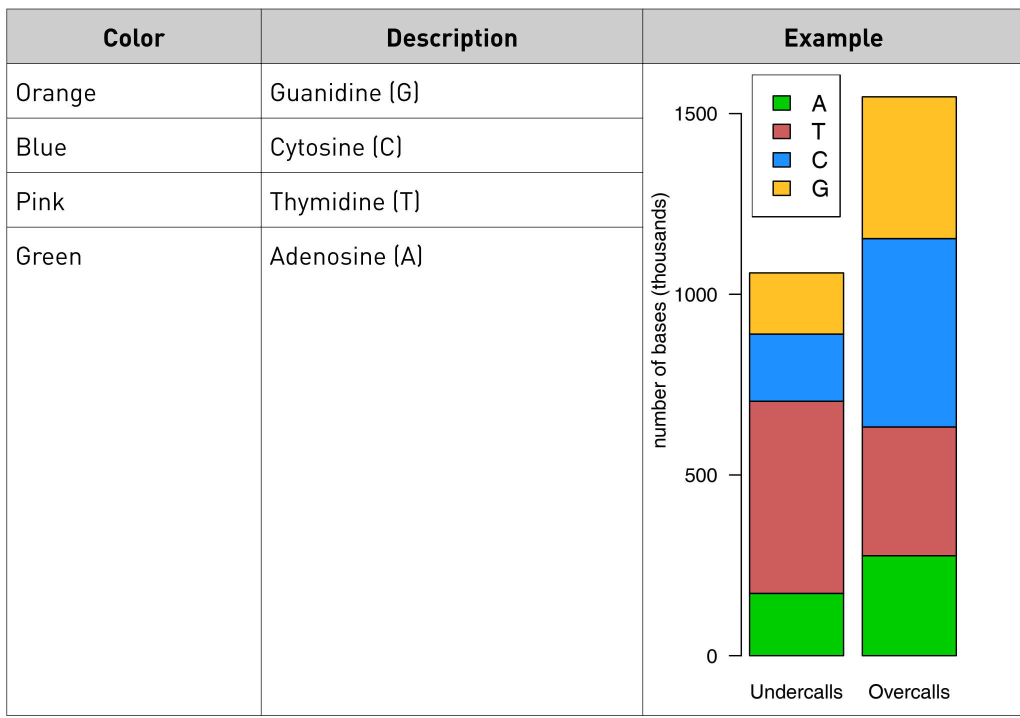
Proportion of full length, quality trimmed and reads lacking P1-key, by read classification

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 Oncomine™ TCR Beta 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 Oncomine™ TCR Beta-LR Assay on Jurkat Total RNA should detect a single clonotype (a single spot on spectratyping plot).

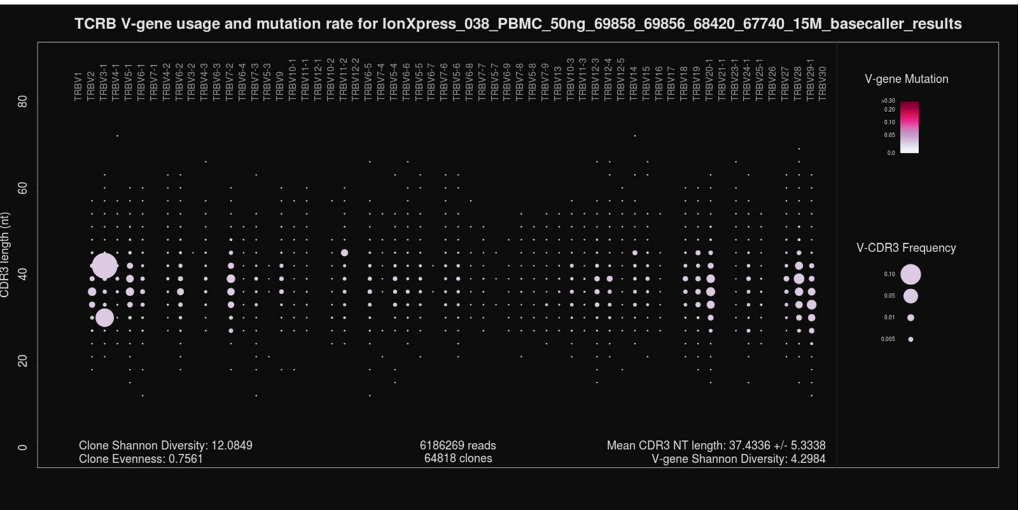


Figure 1 TCRB V-gene usage and mutation rate

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.

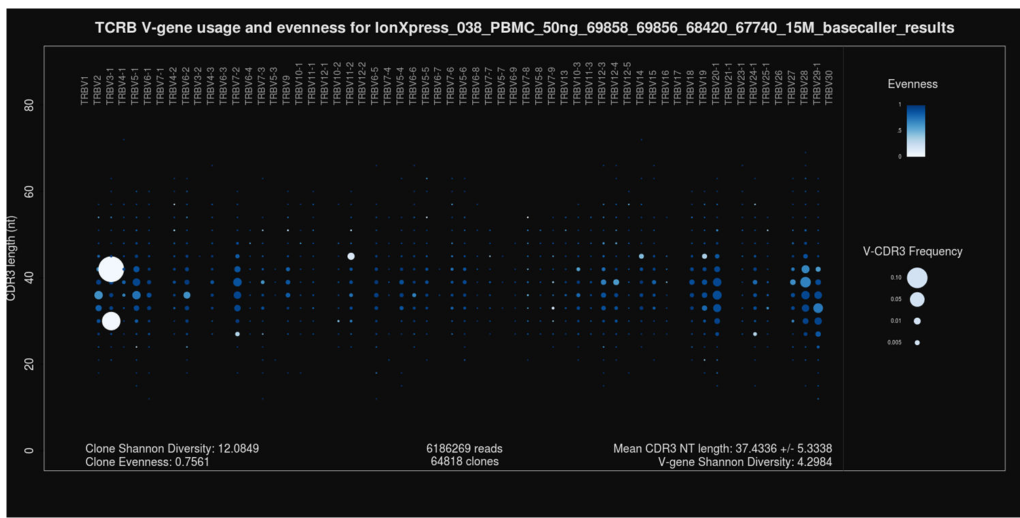


Figure 2 TCRB V-gene usage and evenness

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.

TCRB V-gene usage and Shannon Diversity for IonXpress_038_PBMC_50ng_69858_69856_68420_67740_15M_basecaller_results

Shannon Diversity

V-CDR3 Frequency

CDR3 length (nt)

Clone Shannon Diversity: 12.0849
Clone Evenness: 0.7561
6186269 reads
64818 clones
Mean CDR3 NT length: 37.4336 +/- 5.3338
V-gene Shannon Diversity: 4.2984

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.

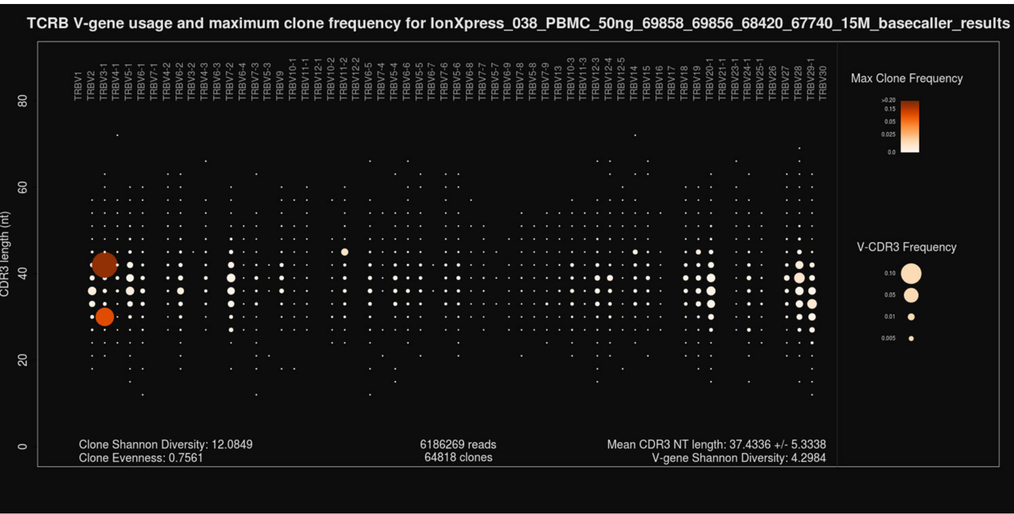


Figure 4 TCRB V-gene usage and largest clone frequency

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 Oncomine™ TCR Beta 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.

Note: Variable gene and allele identification is most accurate when using long-amplicon sequencing covering all three CDR domains.

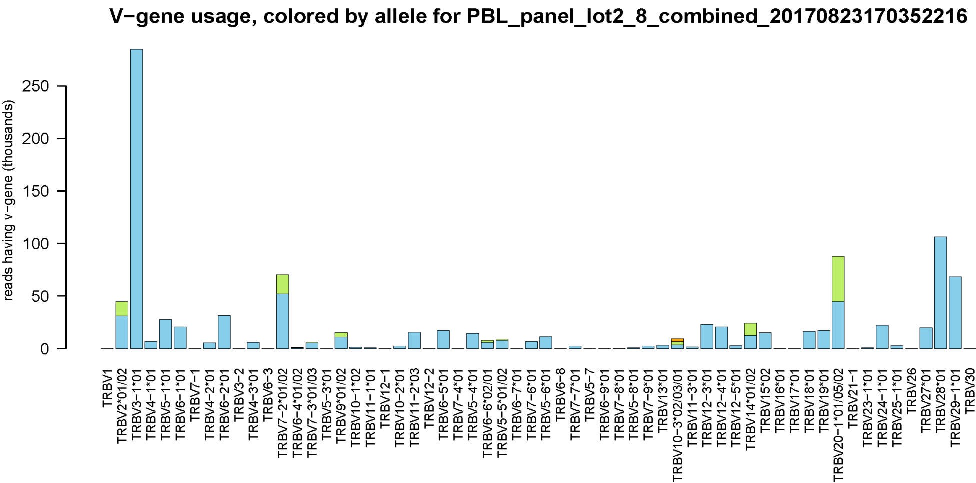


Figure 6 V-gene usage highlighting alleles

Color segments within each bar indicate the frequency of particular variable gene alleles, arranged by frequency from rarest (top) to most common (bottom).

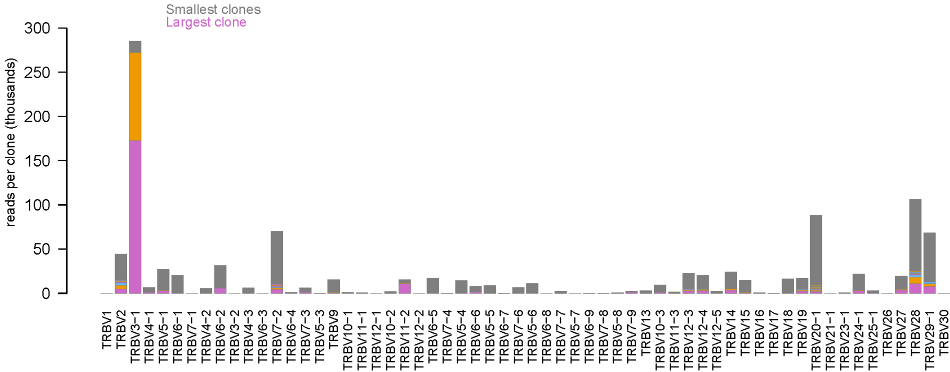


Figure 7 V-gene usage highlighting clone sizes

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 Oncomine™ TCR Beta Assay results are represented graphically. Select **VJ- gene usage heatmap** from the **Views** dropdown list.

Note: Variable gene and allele identification is most accurate when using long-amplicon sequencing covering all three CDR domains.

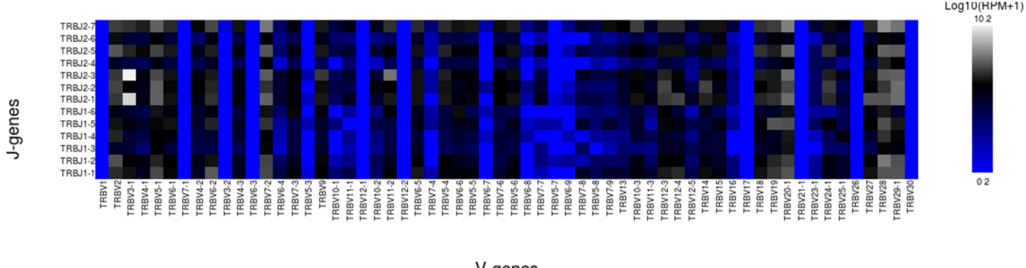
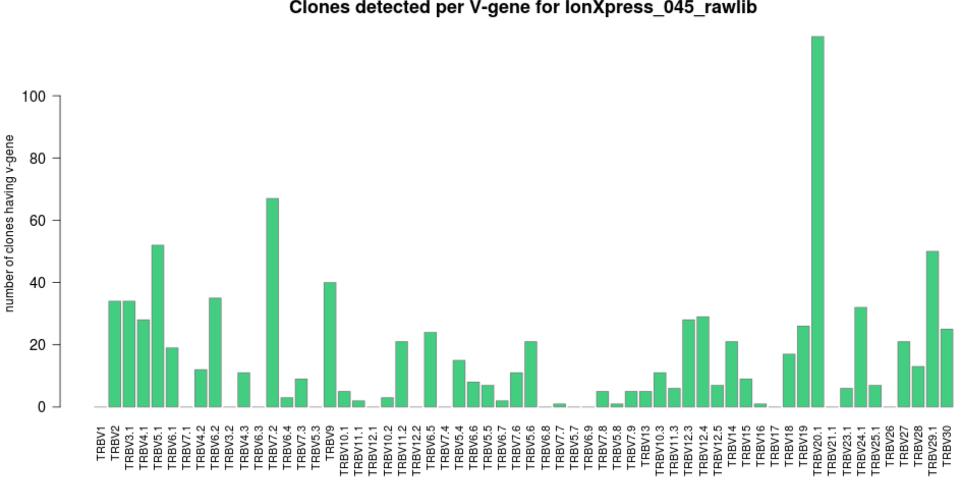


Figure 8 VJ-gene usage heatmap

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 TCRβ locus.

Clones detected per variable gene

The Oncomine™ TCR Beta 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 Oncomine™ TCR Beta Assay results are represented graphically. Select **CDR3 histogram** from the **Views** dropdown list. The histogram indicates the distribution of identified clones with a given CDR3 nucleotide length.

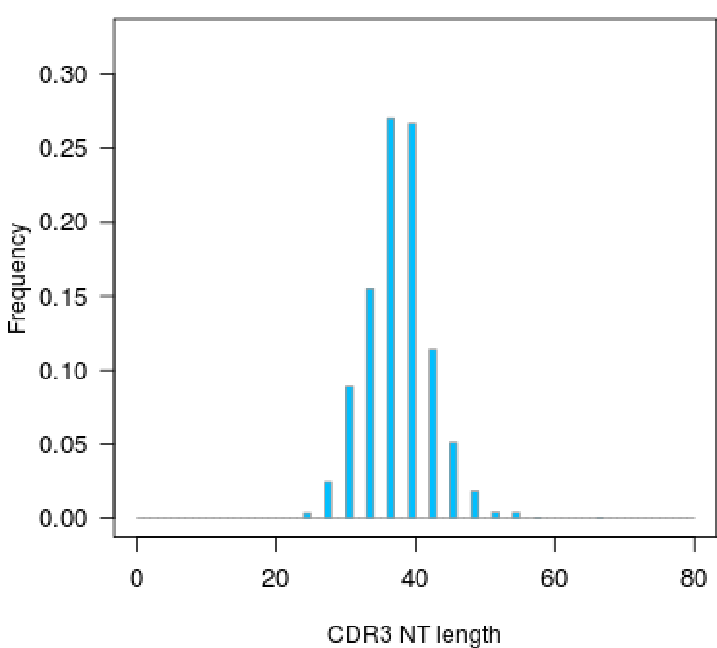


Figure 9 Relative frequency (Y-axis) of identifed clones with a given CDR3 nucleotide length (X-axis)

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 5 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 6 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).

Visualize analysis results with Ion Reporter™ Software

You can visualize any human DNA or RNA analysis type with Ion Reporter™ Software for research use. You can also visualize multiple analyses in a single browser tab that is useful for comparisons.

Note: The maximum number of analysis results that can be included in a single visualization depends on the size of the analyses results data.

Ion Reporter™ Genomic Viewer is a streamlined version of the Integrative Genomics Viewer (IGV) that is developed by the Broad Institute. Ion Reporter™ Genomic Viewer is included with Ion Reporter™ Software. You can set preferences that specify which viewer opens by default and which elements are included in IRGV visualizations. See "Set IRGV or IGV as default viewer" on page 259 and "Set Ion Reporter™ Genomic Viewer preferences" on page 259 for more information.

Multiple analyses and multiple analysis types in Ion Reporter™ Genomic Viewer

You can view multiple analyses at a time and multiple types of analyses in Ion Reporter™ Genomic Viewer. The image below is a Whole Genome View of an aneuploidy, Oncomine™, and cancer hotspot analyses. 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.

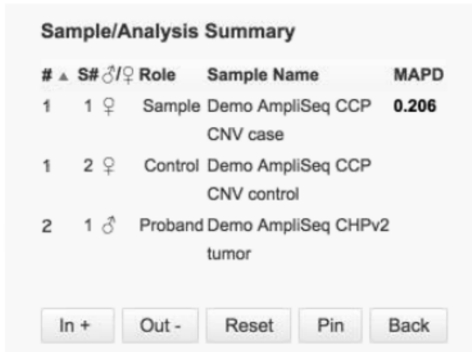
The first Whole Genome View includes CNV calls, a MAPD call, and a Confidence Filter threshold. Blue and red bars in the whole genome view represent CNV calls. Blue bars represent calls above the expected normal ploidy value (Copy Number

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 bars represent 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.

This summary table is from a CNV analysis:

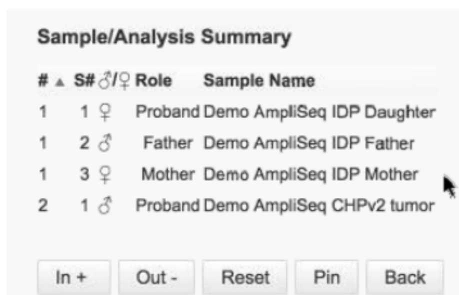


Sample/Analysis Summary

# ▲	S#	♂/♀	Role	Sample Name	MAPD
1	1	♀	Sample	Demo AmpliSeq CCP CNV case	**0.206**
1	2	♀	Control	Demo AmpliSeq CCP CNV control	
2	1	♂	Proband	Demo AmpliSeq CHPv2 tumor	

In + | Out - | Reset | Pin | Back

This summary table is from a Trio analysis:

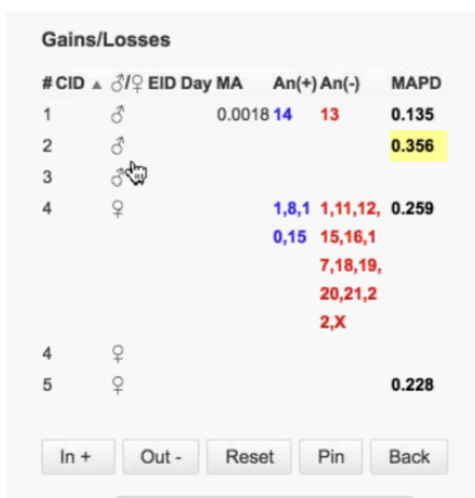


Sample/Analysis Summary

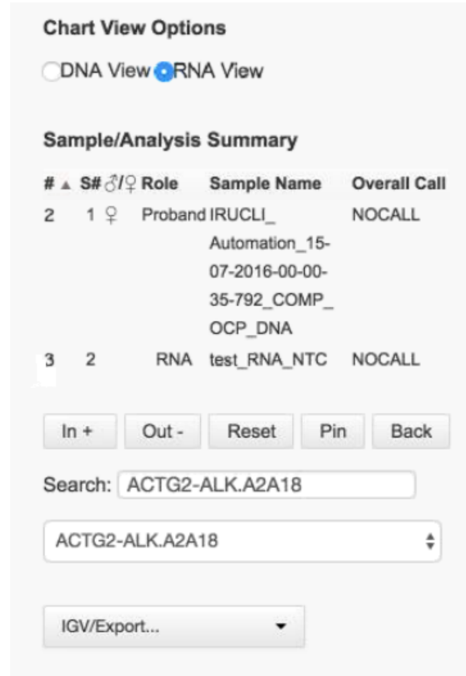
# ▲	S#	♂/♀	Role	Sample Name
1	1	♀	Proband	Demo AmpliSeq IDP Daughter
1	2	♂	Father	Demo AmpliSeq IDP Father
1	3	♀	Mother	Demo AmpliSeq IDP Mother
2	1	♂	Proband	Demo AmpliSeq CHPv2 tumor

In + | Out - | Reset | Pin | Back

This summary table is from an aneuploidy analysis:



This summary table is from the RNA View option in a DNA analysis:



Visualize aneuploidy analysis results

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.

Note: You must have IRGV set as the default viewer for this procedure. See "Set IRGV or IGV as default viewer" on page 259 for more information.

1. Under the **Analyses** tab, click **Overview**.

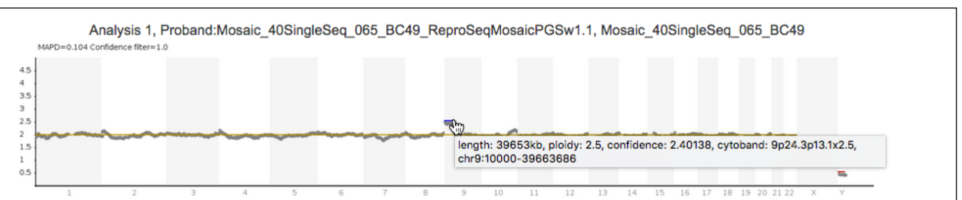
2. Select one or more analyses then click **Actions** ▸ **Visualize**.

   The **Analysis Visualization** screen opens to the **IRGV** tab. A copy number histogram for each analysis selected appears, with ploidy maps for selected chromosomes or chromosome regions, and karyograms that show copy number gains and losses.

   Above the whole genome view for each analysis is a metric known as Productive Reads. It is the sum of mapped reads from a sample that align to the autosomes and chrX in females or to the autosomes and chrX + chrY in males. If the Remove Duplicates CNV finding parameter is set to True, then the Productive reads metric excludes duplicates. Productive reads are the specific reads that are used to establish the relative coverage of tiles across the sample.

Note: If you select multiple analyses generated from workflows with different filter chains with different confidence settings, the software will use the filter chain with the lowest confidence setting and apply that filter chain across the group. You can adjust the Filter Chain and Mapd filters with the 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.

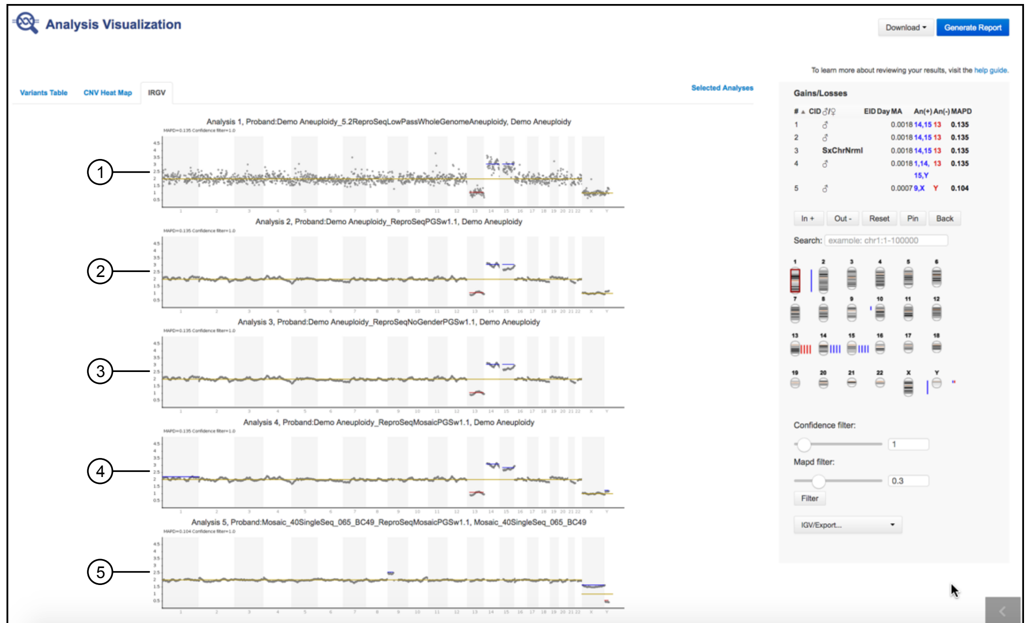


Smoothing, No Gender, and Mosaicism results in IRGV

This image shows five aneuploidy analyses run in various Ion Reporter Software aneuploidy workflows that show how smoothing, No Gender, and Mosaicism appear in Ion Reporter™ Genomic Viewer visualizations. Visualization of aneuploidy detection is enhanced by tile-by-tile data points that are displayed as easier-to-see

circles in the whole-genome views. All results from Ion Reporter™ Genomic Viewer 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.



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 an 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.
3. An analysis of the Demo Aneuploidy sample run through the ReproSeq No Gender workflow. Use of the 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. A gender call is SxChrAbnrml when the sex chromosomes deviate from normal male or normal female by either whole-chromosome or subchromosomal aneuploid events. Aneuploidies on autosomes do not affect 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 ploidy 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 An analysis of a spiked-in sample that was mixed to have a chr9p event of relative copy number ploidy of 2.5 and 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. The mixing of two samples of different gender can be seen in the observed ploidies of the X and Y chromosomes.

Visualize DNA and Fusion analysis results

You can visualize analysis results from a DNA and Fusions sample.

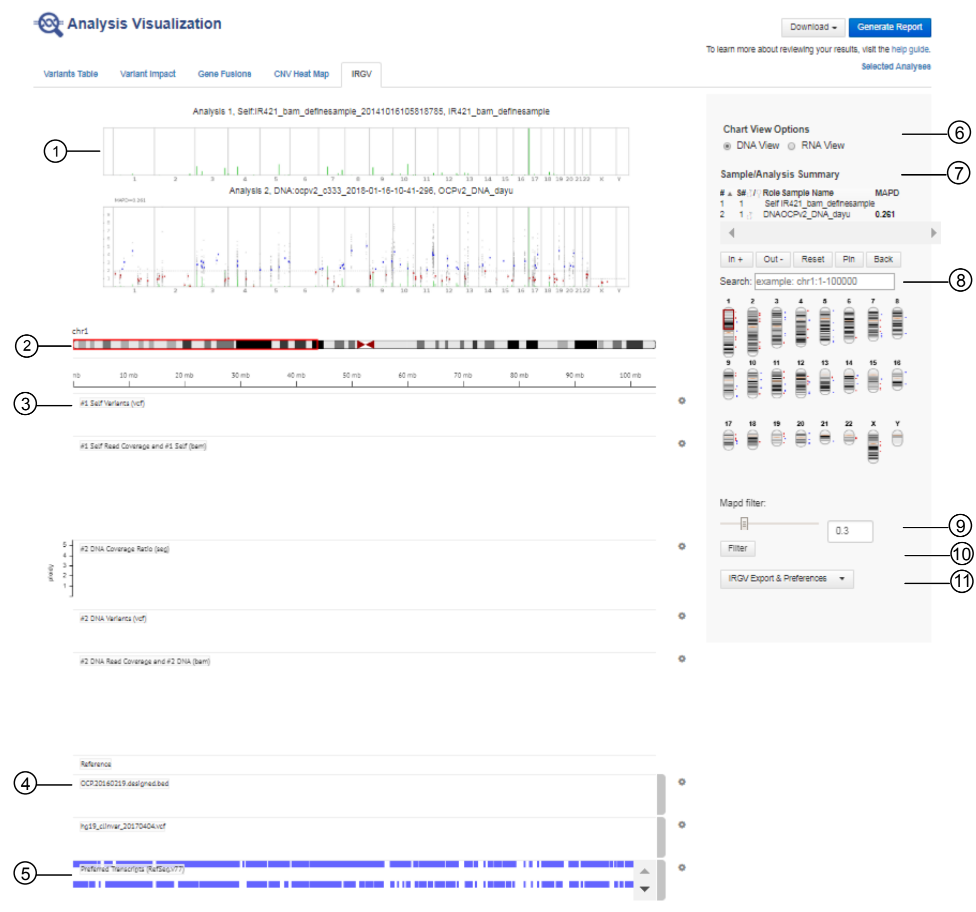
1. Under the **Analyses** tab, click **Overview**.

   Note: You can also launch Ion Reporter™ Software from the heatmap views (variant impact, CNV, fusion plots) in a visualization and from the **Analysis Results** table and the coverage analysis table. However, the preferences used for IGV Browser track loading will differ depending on where the IGV software is launched from.

2. Select one or more analyses and click **Visualize**.
   The tab that opens depends the type of workflows that you selected and the number of analyses that are selected.

3. Click the **IRGV** tab and to see a visualization of the results.

   ① Analysis Whole Genome View – Provides a genome-wide perspective of the data .
   ② Selected Chromosome bar – Click on the Whole Genome View image to see the variant location on the selected chromosome.
   ③ Proband Coverage Ratio. Type in the chromosome coordinates, or click on the Karyo view to see discrete data for Copy Number calls.
   ④ Reference – Lists the genomic reference used for the analysis.
   ⑤ Shows annotation source results. Click ⚙ (Settings) to rearrange the annotation source tracks.
   ⑥ Chart View Options – Toggle between a DNA or an RNA view. Select RNA for fusion analyses to get a drop-down menu to search for individual fusions in the panel.
   ⑦ Sample/Analysis Summary – Sort by Sample number and rearrange the analysis rows on the left.
   ⑧ Search – Enter chromosome coordinates or gene names to find data.
   ⑨ Filter chain – You can apply a filter chain and immediately view its results. You cannot save the results of the applied filter chain to the visualization. You can also create a new filter chain. See “Filters and filter chains“ on page 421 for more information.
   ⑩ MAPD filter – An analysis samplewide metric of noise. You can adjust it to determine when a particular sample has met the threshold that was set for the MAPD filter.
   ⑪ IGV/Export – Use multiple export options, including an export to Integrative Genomics Viewer (IGV), and access to preferences. See “Visualize variants with Integrative Genomics Viewer (IGV)“ on page 252 and “Set Ion Reporter™ Genomic Viewer preferences“ on page 259 for more information.



Use **In+** and **Out -** to zoom in and out on the selected chromosome.

Visualize variants in an analysis run with an Ion AmpliSeq™ HD workflow

Ion Reporter™ Software analyses are performed automatically on uploading of the data files from the Torrent Suite™ Software. You can view a summary of data about the identified variants, and toggle to other views that provide more details about the same variants.

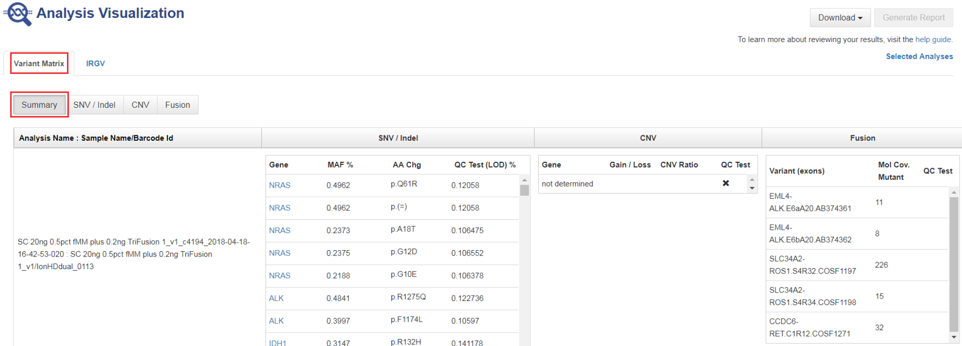
1. In the **Analyses** tab, click **Overview**.
   The **Analyses** table lists all the available analyses results.

2. In the **Analyses** table, click the column headers to sort the results, or use the available filters to limit the list of analyses. For more information, see “Search, sort, or filter Analyses“ on page 163.

3. Select the checkbox in the row of the analysis you want to visualize or select two or more analyses if you want to visualize a side-by-side comparison of multiple results.

4. Click **Visualize**.

The **Analysis Visualization** screen opens to the **Variant Matrix** tab, displaying the **Summary** screen that shows all the identified SNVs/INDELs, CNVs, and Fusions.



Note: A **none detected** result indicates that down to the displayed limit of detection (LOD), no variants were observed in the sample within or above the LOD range.

5. Review detailed variant data.

- In the **Variant Matrix** tab, in the **Summary** screen, click on the gene name in the **Gene** column to access the HGNC report for that gene.
- Click **SNV/INDEL**, **CNV**, or **Fusion** to view detailed analysis metrics. See "Detailed analysis metrics" on page 255 for a description of each metric.
- In the **SNV/INDEL**, **CNV**, or **Fusion** screen, click the link in the **Locus** column to view specific variants in the Ion Reporter™ Genomic Viewer (IRGV) in a separate window.

Figure 10 Example SNV/Indel visualization in IRGV

1. Variant density overview, illustrated as copy number (Y-axis) at a specific position on the chromosome (X-axis).
2. Displayed chromosomal region; use the search field to view a different chromosomal region.
3. Proband variant position on the displayed chromosomal region
4. Proband read coverage tracks

Note:

- Ion AmpliSeq™ HD analyses group consensus reads into families. A family is a group of reads that are associated with the same DNA molecule before library amplification. Each family is identified using the molecular tags, and consensus reads with the same molecular tags are grouped into the same family. In IRGV data view, the color of the consensus reads is used to indicate a family. Side by side consensus reads with the same color belong to the same family.
- Within each read track, each nucleotide variant is indicated by a different color. T, A, C, and G are red, green, blue, and orange, respectively. An "I" denotes insertion, and white color with a dash indicates deletion.

Figure 11 Example fusion visualization in IRGV

① Fusion target track
② 5′ (top) and 3′ (bottom) gene track
③ Read coverage track
④ Use the dropdown list or search for another fusion variant

- Alternatively, click the IRGV tab to open IRGV, then search for variants or fusions at specific positions on the chromosome.

Visualize mutation load analysis results

To visualize Mutation Load analysis results in Ion Reporter™ Software, the workflow launched for the analysis must be any DNA – single sample, or DNA and Fusions – single sample workflow that has Mutation Load enabled. See "Create a custom workflow enabled for Mutation Load" on page 77 for more information.

1. Click the **Analyses** tab.

2. In the **Workflow** filter category, select any DNA – single sample, or DNA and Fusions – single sample workflow that has Mutation Load enabled to limit the list of results to mutation load analyses.

3. Click the name for the analysis of interest to view the analysis results.
   You can view the analysis name and the value for the Mutation Load (Mutations/Mb) in the analysis results screen.

4. Click **Visualize**.
   The **Analysis Visualization** screen opens to the **Mutation Load** tab.

   Note: Alternatively, to go directly to the **Analysis Visualization**, select the row of the analysis, then in the **Details** pane, click ⚙ **Actions ▸ Visualize** .

5. Select either the **Sample Results** or **Sample QC** tab, then select the graphical representation of the data from the **Views** dropdown list.

Sample Results tab

The Mutation Load results are represented graphically. On the **Sample Results** tab, select one of the following from the **Views** dropdown list.

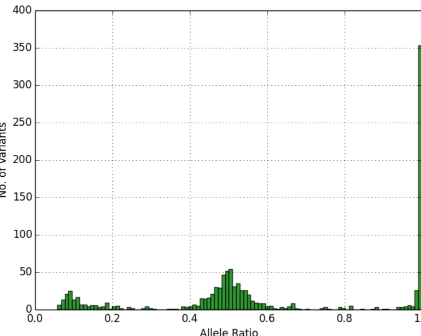


Figure 12 Allele ratio of Germ-line and Somatic Variants

A histogram showing frequency distribution of allele ratio for total called germ-line and somatic variants.

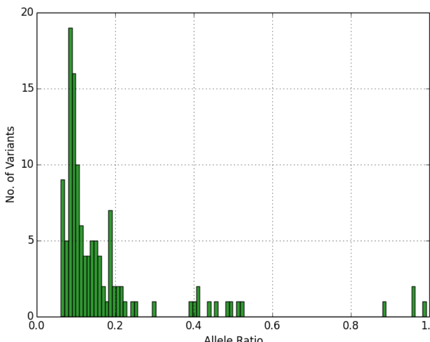


Figure 13 Allele ratio of Only Somatic Variants

A histogram showing frequency distribution of allele ratio for only somatic mutations.

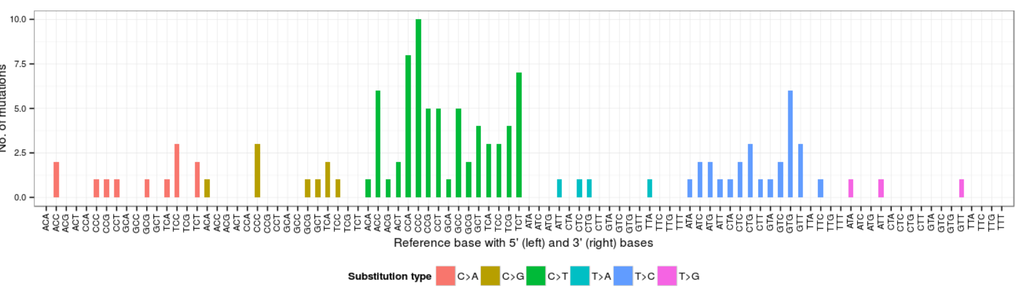


Figure 14 Substitution Type and Context of Somatic Mutations

Somatic mutations can be divided into 6 base substitution classes (that is, C>A, C>G, C>T, T>A, T>C, T>G) based on their substitution type. After incorporating information on the bases immediately 5′ and 3′ to each mutated base, there are 96 possible mutation types in this classification. These 96 mutation types are represented on the x-axis, and variant frequency for mutation type on the y-axis. Bars for each substitution class are grouped and displayed with different color.

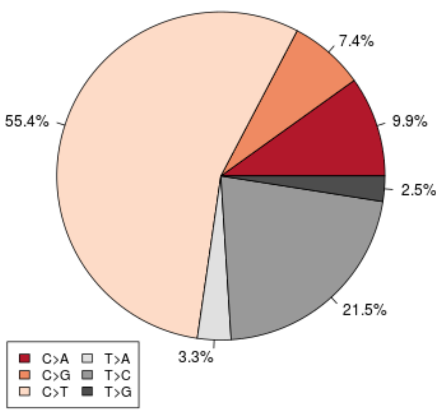


Figure 15 Substitution type of Somatic Mutations

A pie chart dividing somatic mutations into 6 base substitution classes (that is, C>A, C>G, C>T, T>A, T>C, T>G) based on their substitution type.

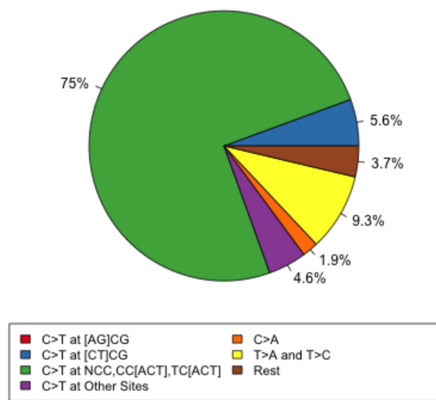


Figure 16 Signature Pattern of Somatic Mutations

A pie chart dividing somatic mutations in groups consistent with specific mechanisms.

In Figure 15, a small fraction of multiple signature types can be observed in the sample. However, significant dominance of a single signature pattern often correlates to the respective tumor type. For example, 89.9% of the variants detected (sum of blue, green, and yellow) are an observed UV damage signature in this hypothetical melanoma sample.

- High C>T at <u>C</u>pG is consistent with spontaneous deamination of 5-methylcytosine.
- High C>T at <u>C</u>pC, Cp<u>C</u>, Tp<u>C</u>, T>A, and T>C is consistent with UV damage.
- High C>A is consistent with smoking damage.
- High C>T (site independent) is consistent with FFPE processing.

Note: Underlined bases represent the reference base being substituted (for example, <u>C</u>pG in the first bullet is same as to [ACGT]CG).

Sample QC tab

The sample QC tab lists the:

QC metric	Definition
Average Coverage	The average read depth across covered nucleotide bases.
Number of Calls	The number of variants identified in the sample.
Estimated SNP proportion consistent with Deamination (mainly FFPE)	The proportion of identified mutations that are likely the result of FFPE sample deamination during processing.

Download a Report

- In the **Mutation Load Results** screen, click **Download Report**.
  A two-page PDF report of the results including copies of the graphs is automatically downloaded. See "Example Mutation Load report" on page 245 for an example report.
- In the lower right corner, click **Download Variant Details TSV**.
  A tab separated values list of each variant detected is automatically downloaded. Open the file with a TSV compatible viewer to see details of each individual variant that contributed to the tumor mutation load count.

Example Mutation Load report

Mutation Load
Analysis Report

Mutation Load (Mutations/Mb): 51.93

Analysis

ILS36557PT4_v1_c13528_2018-06-08-13-06-48-226

Ion Reporter Version	Launched by	Launched on	Workflow
5.10	Ion User	June 08, 2018 01:06 PM	Oncomine Comprehensive v3 - w3.2 - DNA - Single Sample_TMBEnabled r.0

Annotations	Reference	Copy Number Baseline
Oncomine Comprehensive Assay v3 Annotations v1.2 r.0	Oncomine Comprehensive DNA v3 Mask v1.1, Oncomine Comprehensive DNA v3 Regions v1.1, hg19, Oncomine Comprehensive DNA v3 Hotspots v1.1	Oncomine Comprehensive DNA v3 540 Assay Baseline v2.0

Samples

ILS36557PT4_v1

Gender	Relationship	Chip Type	Percentage Cellularity
Unknown	Proband	540	40

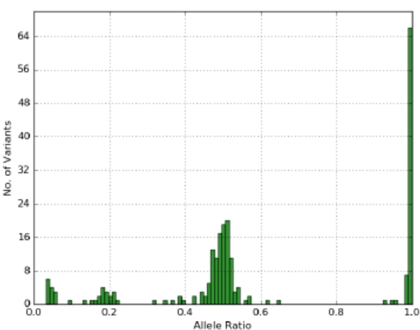
Sample Type
DNA

QC Metrics

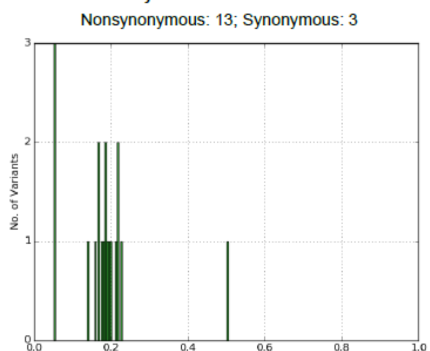
Average Coverage	Total Variants Called	Estimated SNP proportion consistent with Deamination (mainly FFPE)
3538.0	1,642	2

Analysis Results

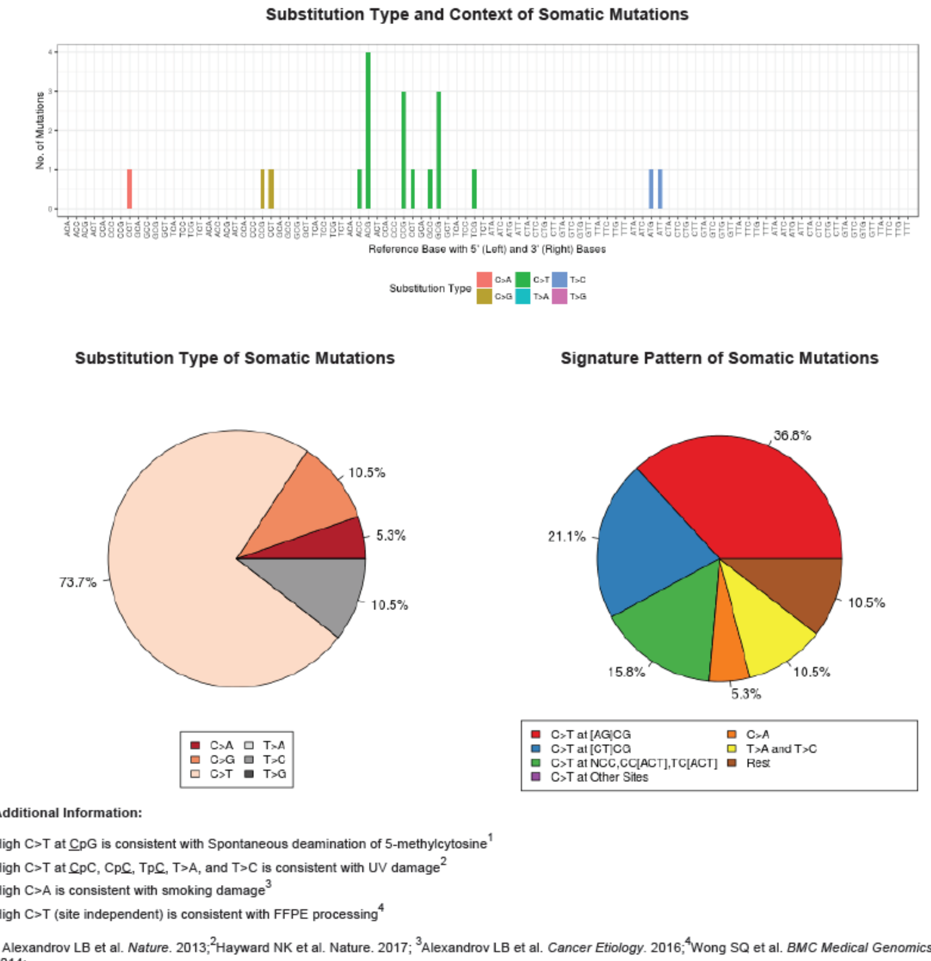
Germ-line and Somatic Variants: 1,642



Only Somatic Variants: 19
Nonsynonymous: 13; Synonymous: 3



Number of Somatic Variants Present in COSMIC: 0



Visualize identified variants in an Oncomine™ analysis from a TagSeq workflow

You can view a summary of data about the identified variants, and toggle to other views that provide more details about the same variants in Ion Reporter™ Software.

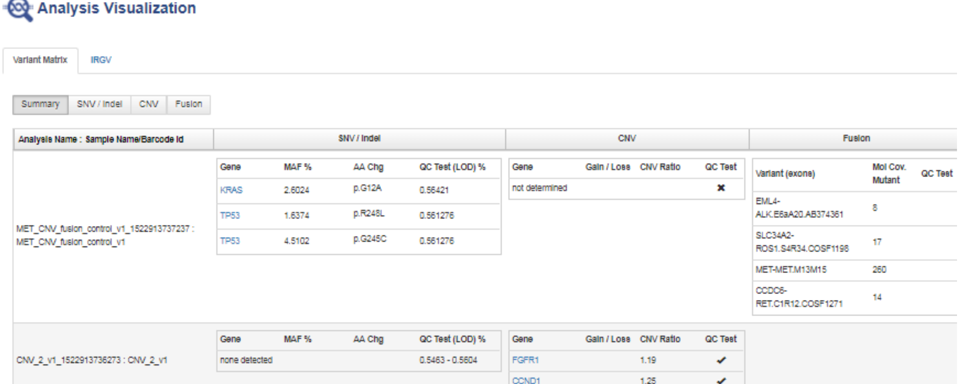
1. Under the **Analyses** tab, click **Overview**.
2. Click the **Analyses** tab.
3. Click the column headers to sort the results, or use the available filters to limit the list of analyses.

4. Click the checkbox adjacent to each analysis of interest; select two or more analyses to visualize a side-by-side comparison of multiple results. Then click **Visualize**.

   Note: Alternatively, select the analyses, then click ⚙ **Actions ▸ Visualize** next to **Selected Analyses**.

5. The **Analysis Visualization** screen opens to the **Variant Matrix** tab with a **Summary** of all the identified SNVs, CNVs, and Fusions (Lung only).

   Note: Click a hyperlinked **Gene** name to be redirected to the HGNC report for that gene.

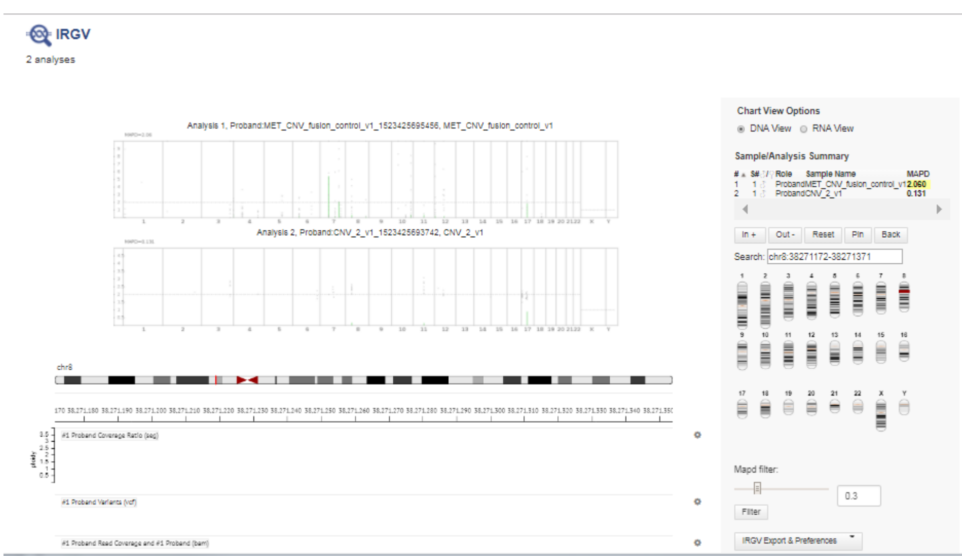


Example visualization of an Oncomine™ assay used with a TagSeq workflow.

A none detected result indicates that down to the displayed limit of detection (LOD), no variants were observed in the sample within or above the LOD range.

6. Click **SNV/INDEL**, **CNV**, or **Fusion** (Lung only) to view detailed analysis metrics.

7. In the **SNV/INDEL**, **CNV**, or **Fusion** detailed view, click the link in the **Locus** column to view specific variants in the Ion Reporter™ Genomic Viewer (IRGV).



Example CNV variant result in the Ion Reporter™ Genomic Viewer.

Note:
- The IRGV viewer displays CNVs as ploidy assuming 100% tumor cellularity whereas we report CNVs as fold difference.
- Customer generated baseline creation for CNV analysis is not supported in this version of the Ion Reporter™ Software. Contact your local service representative for assistance creating a new CNV baseline.

Use filter chain to change analysis results

You can review the results of an Oncomine™ analysis run through a TagSeq workflow and apply different filter chains to alter the results.

1. In the **Analyses** tab, click the link in the **Analysis** column to open an analysis of interest.
   The analysis results appear with the Oncomine™ Variants filter applied.

2. *(Optional)* In the **Filter Options** pane, select a different filter chain from the dropdown list.

Option	Description
Called Variants and Controls	This is the default filter chain for the TagSeq workflows. Use this filter chain for analysis results that report all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Variant types include: SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL_ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3ITD.
Called Hotpot Variants and Controls	Select this filter chain for analysis results that report all hotspot variants that pass the filter and are not called as reference or NOCALL. Filter variant types include: SNP and INDEL.
Variant Matrix Summary	Select this filter chain for analysis results that generate a visualization that contains the same set of variants that are included in the Analysis Results table. This filter chain allows results to be exported. Import the exported results file into Oncomine™ Knowledgebase Reporter (OKR) to include the variants shown in the Variant Matrix Summary view in OKR reports. Variant types include: SNV/INDEL, CNV, fusions, and RNAExonVariants.

3. Review results.

View analysis results for a single sample

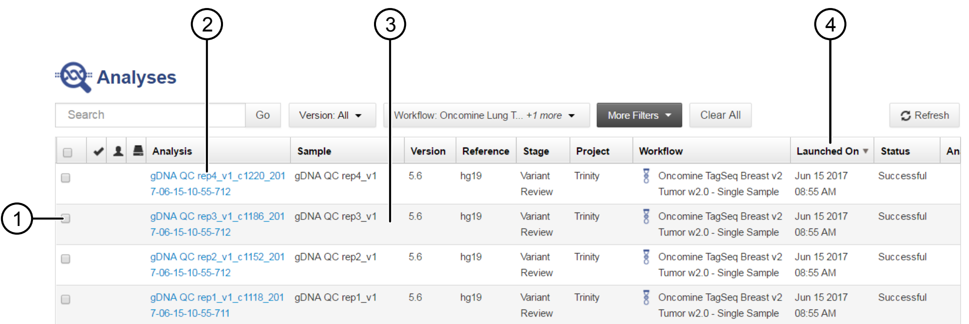
Use this procedure to view and manage the extended analysis results of a single sample.

Note: Analyses that are performed as described in this procedure generate a variants table that does not have optimized TagSeq specific filters applied. These filters are only applied when you generate results analysis as described in "Visualize identified variants in an Oncomine™ analysis from a TagSeq workflow" on page 246.

1. Click the **Analyses** tab, then click **Overview**.

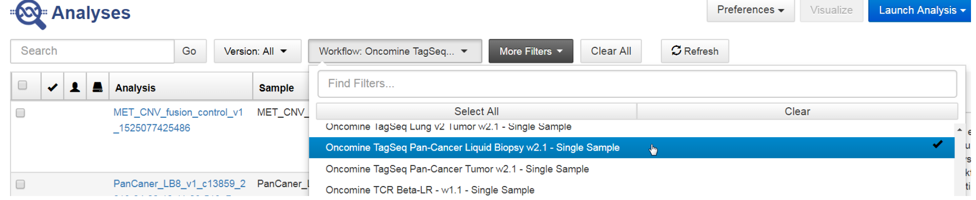
2. In the **Analyses** screen you can:

To	Action
Select an analysis	Click the checkbox.
Open an Analysis Results screen.	Click the hyperlink (in the Analysis column).
View details	Click anywhere in the analysis' row, except on the hyperlink.
Sort	Click column headers to sort the analyses based on the column contents.



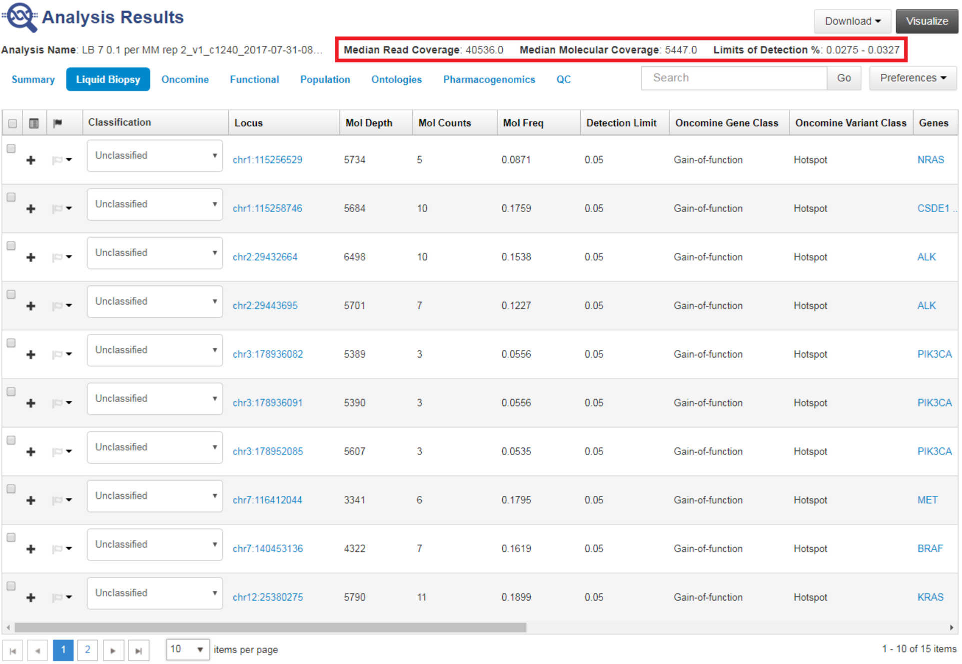
① Select analysis
② Open Analysis Results screen
③ View details
④ Sort

3. To view and manage the extended Oncomine™ Cell-Free Research Assay results, use the available filters to limit the list of analyses (for example, select your workflow from the **Workflow** dropdown list), then click the hyperlink in the **Analysis** column.



4. In the **Analysis Results** screen, sort or filter the data using the Oncomine™-specific annotations. See the software help menu for more options.

5. Review the results in the **Median Read Cov**, **Median Mol Cov**, and **LOD %** columns.



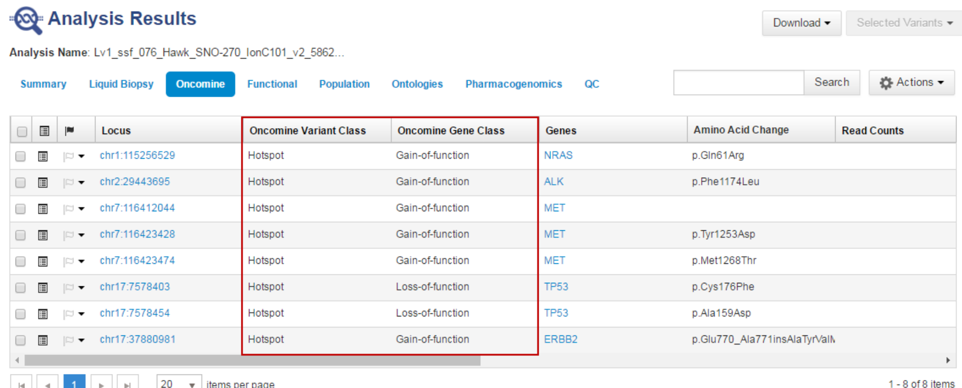
Column	Description
Median Read Coverage	Reports median coverage across targets. Median Molecular Coverage reports median number of individual interrogated DNA molecules across targets.
Median Molecular Coverage	Directly influences the limit of detection in a sample run. We always require two independent molecular families to identify a variant for it to be called. Lower median molecular coverage values result in less sensitive detection of variants at 0.1% frequency, although still sufficient for sensitive detection of variants with higher frequency. For example, Median Molecular Coverage of 700 is sufficient for accurate detection of variants at 0.5% frequency.
LOD %	A segment (e.g., 0.02–0.03) where 0.02 represents the median value across all targets, and 0.03 represents the limit of detection (LOD) for the 80th percentile targets. If both numbers are ≤0.1% then the sequencing run is of acceptable quality for 0.1% LOD.

For sensitive variant detection down to 0.1% frequency, we see optimal results when targeting a Median Read Coverage >25,000, Median Molecular Coverage >2,500, and both numbers of the LOD % segment are ≤0.1.

6. In the **Liquid Biopsy** tab, view **Mol Depth**, **Mol Counts**, and other columns.

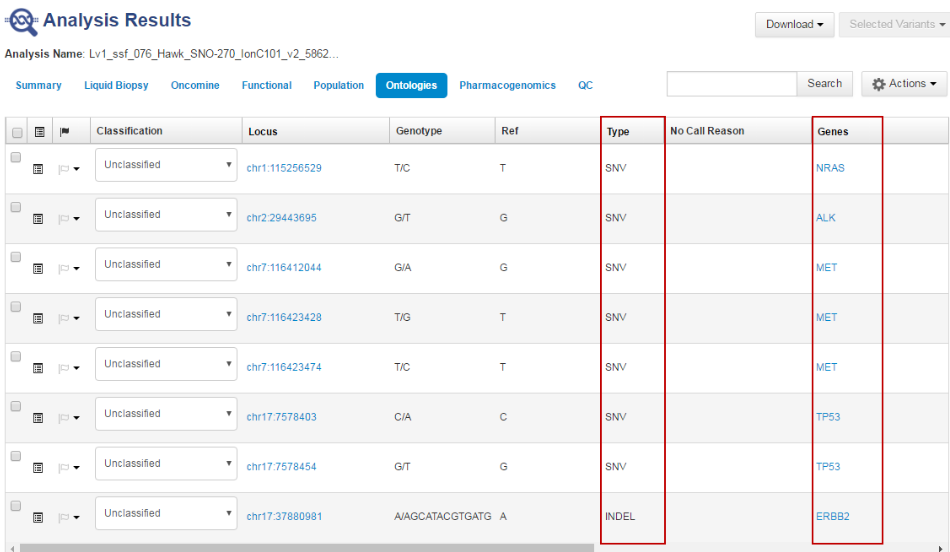
Column	Description
Molecular Depth	Reports number of interrogated DNA molecules containing target. It defines limit of detection at hotspot position in a particular run and sample. For instance, if molecular depth is ≥1,500, you can have high confidence that no variant is present at 0.2%. If molecular depth is ≥2,500, you can have high confidence that no variant is present down to 0.1% LOD. For reference calls, Molecular Depth provides measurable metric that serves as confirmation for variant absence among a large number of interrogated molecules.
Molecular Counts	Reports the number of detected DNA molecules containing variant allele.

7. In the **Oncomine** tab, click the column headers to sort the list of variants by **Oncomine Variant Class** and **Oncomine Gene Class**.



Reference calls display chromosomal position with empty value in amino acid change field.

8. In the **Ontologies** tab, click the column headers to sort the list by variant **Type** or **Genes** to analyze your results.



Visualize variants with Integrative Genomics Viewer (IGV)

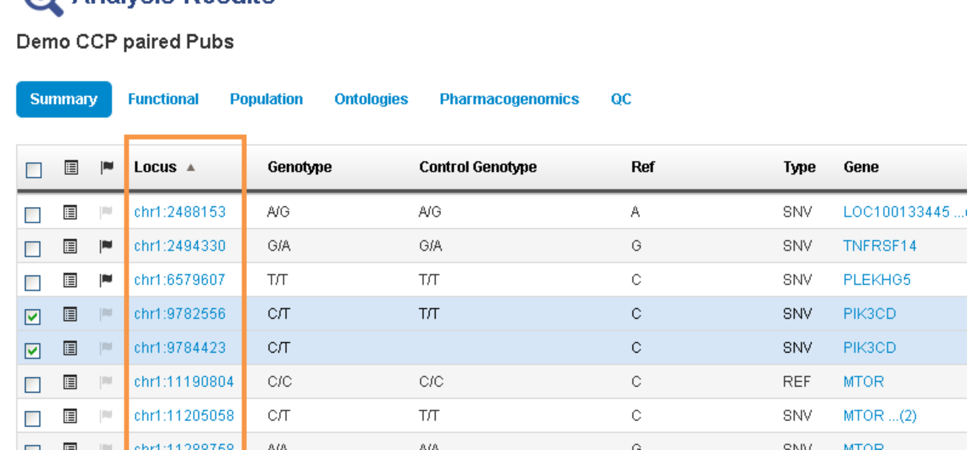
You can visualize data from Ion Reporter™ Software with the Broad Institute Integrative Genomics Viewer (IGV) **http://software.broadinstitute.org/software/igv/home**).

Note: Hotspots files that are in shown in a track in IGV, might include Hotspot annotations that 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.

1. Sign in to the Ion Reporter™ Software and click on the **Analysis** tab.
2. Click on the name for the analysis of interest in the **Analyses** table.

3. In the **Analysis Results** screen, click on the locus for a variant of interest.



Locus	Genotype	Control Genotype	Ref	Type	Gene
chr1:2488153	A/G	A/G	A	SNV	LOC100133445 ...
chr1:2494330	G/A	G/A	G	SNV	TNFRSF14
chr1:6579607	T/T	T/T	C	SNV	PLEKHG5
chr1:9782556	C/T	T/T	C	SNV	PIK3CD
chr1:9784423	C/T		C	SNV	PIK3CD
chr1:11190804	C/C	C/C	C	REF	MTOR
chr1:11205058	C/T	T/T	C	SNV	MTOR ...(2)
chr1:11288758	A/A	A/A	G	SNV	MTOR
chr1:11301714	G/G	G/G	A	SNV	MTOR

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).

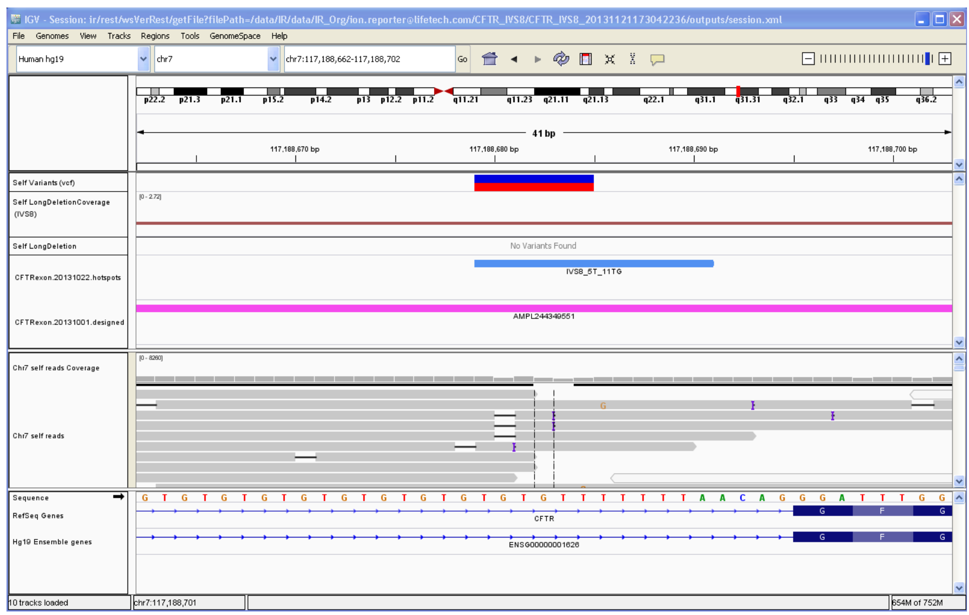


Figure 17 Example of Ion Reporter™ Software in an IGV visualization

Note: When different analyses that use the same samples or the same panel files (but different algorithm versions, for example) are visualized in IGV, the reads, read counts, BED file, and other tracks will load only once into the browser. This occurs only if exact duplicate tracks are detected by the software; any differences in results data or input tracks between multiple analyses will trigger any and all unique tracks to be loaded separately for visualization of the requested analyses.

Navigate from variant to variant in IGV and Ion Reporter™ Genomic Viewer

You can use the links in the **Analysis Results** table and coverage analysis table to move from location to location in the same Ion Reporter™ Genomic Viewer, (IRGV), tab or in the same IGV browser tab or window.

1. Click on a locus link to open a visualization in 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 to the next variant in the visualization.

Note: In some web browser versions, this functionality may be blocked for IGV. If you click on subsequent variants and the next variant's locus is not selected in the visualization, 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.

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:

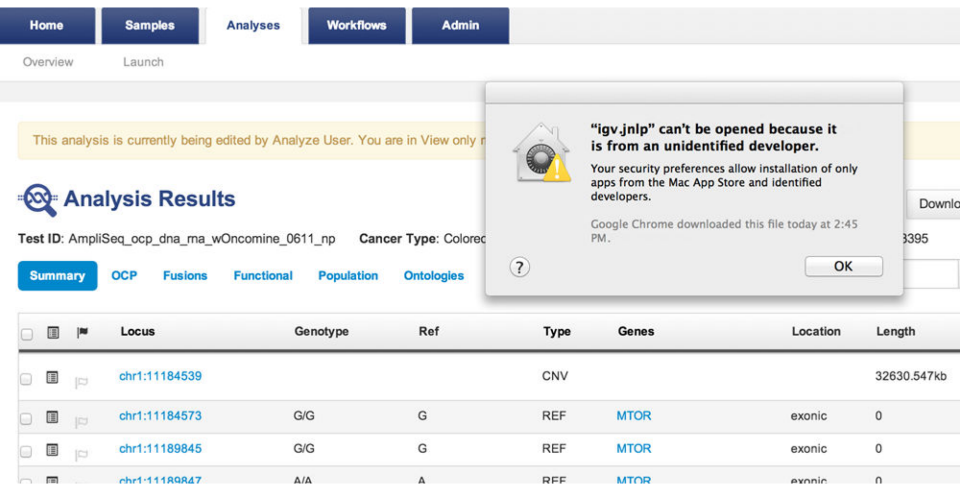
```
Errors were encountered loading the session: Unable to parse
header with error: Your input file has a malformed header:
unknown column name 'CHR'; it does not match a legal column
header name.
```

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**.

Detailed analysis metrics

Metric	Description
Sample ID	Name of the sequenced Sample imported from a sequencing run.
SNV/INDEL	
Gene	HGNC reviewed official gene symbol.
AA Chg	Amino acid change resulting from non-synonymous DNA variant.
Mutant Frequency %	Frequency of mutant allele expressed as a percentage.
Oncomine Variant Class	Variant class annotation as defined using Oncomine™ Variant Annotator (OVAT).
Oncomine Gene Class	Variant gene functional annotation as defined using Oncomine™ Variant Annotator (OVAT).
Info	HS (targeted hotspot) or PN (potentially novel TP53 variant). *De novo* variant calls available for the breast panel only.
Genotype	Genotype measured associated with a DNA variant call.
Ref Allele	Reference allele as defined in the human genome reference (hg19).
Mut Molecular Cov.	Molecular coverage of the mutant allele.
WT Molecular Cov.	Molecular coverage of the wild type allele from the reference genome.
Depth	Total read coverage across amplicon containing SNV/INDEL hotspot locations. Count of chip-level reads aligned at this locus that participate in variant calling.
QC Test (LOD) %	Quality control check for SNV/INDEL target regions based on molecular coverage.
Transcript ID	NCBI accession number for the transcript representing the gene target being measured.
Locus	Chromosome and position of detected variant. Click the hyperlink to open the Ion Reporter™ Genomic Viewer to the specified locus.
CNV	
Gene	Gene locus targeted for CNV measurement.

Metric	Description
Gain/Loss	Detected copy number gain or loss.
CNV ratio	Ratio of measured CNV gene locus coverage relative to coverage on non-CNV loci.
p-value	Significance of CNV ratio measurement.
Med. Mol Cov. Gene	Median molecular coverage of targeted CNV gene.
Med. Mol Cov. Ref	Median molecular coverage of non-CNV reference loci.
Med. Read Cov. Gene	Median read coverage of targeted CNV gene.
Med. Read Cov. Ref	Median read coverage of non-CNV reference loci.
QC Test	Assay quality control as determined by amplicon coverage uniformity and number of amplicons remaining after outlier removal.
Valid CNV Amplicons	Number of CNV amplicons remaining after outlier removal.
CNV Locus	Chromosomal location of CNV gene being targeted.
Fusion	
Variant (exons)	Name of fusion targeted and respective acceptor and donor exons.
Oncomine Driver Gene	Cancer driver gene descriptions as reported by Oncomine™ Variant Annotator (OVAT).
COSMIC/NCBI	COSMIC mutation or NCBI accession number.
Mol Cov. Mutant	Median molecular coverage across fusion amplicon.
Read Cov. Mutant	Median read coverage across fusion amplicon.
Detection	Detection status from assay.
QC Test	Assay quality control measured from expression detection of housekeeping genes.
Type	Assay type (e.g., Fusion, RNA exon variant (exon skipping), Proc Control).
Locus	Chromosomal locations of targets included in assay.
Ratio To Wild Type	Ratio molecular for exon skipping assay relative to wild type control amplicons.
Norm count Within Gene	Exon skipping assay coverage normalized to molecular coverage of wild type (WT) MET control amplicons. (Lung panel only)

Quality control (QC) thresholds

QC Test	Detection threshold
SNV/Indel	
A limit of detection (LOD) is calculated and displayed for each variant call. LOD is determined by the level of molecular amplicon coverage. If no variant call is detected, the LOD range is displayed across entire amplicon.	Molecular coverage must be at least 2 with a minimum detection cutoff frequency of 0.035% and 0.05% for lung and breast panels, respectively.

QC Test	Detection threshold
CNV	
The MAPD metric is a measure of read coverage noise detected across all amplicons in a panel. Higher MAPD typically translates to lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. MAPD score is viewable in downloadable VCF file or review of the **Analysis Results** of a single sample extended analysis.	To make a CNV call the following criteria must be met: • MAPD <0.4 • P-value $<10^{-5}$ • CNV Ratio for a copy number gain must be >1.15 • CNV Ratio for a copy number loss must be <0.85 **Note:** The CNV Ratio call thresholds were derived empirically using plasma samples from healthy donors with normal CNV status.
Fusions/Exon Skipping[1]	
• Fusions—Panel includes 2 process control target genes, TBP and HMBS. At least 1 control must have a molecular count of >2 to pass QC. • MET Exon Skipping—Panel includes 2 MET Wild Type control amplicons (gene name has WT at the end). At least 1 of these controls must have a molecular count >2 to pass QC.	Fusion and Exon Skipping amplicons must have >2 molecular counts to be reported.

[1] These variant types are included in the Oncomine™ Lung cfNA Assay, derived from RNA reverse-transcribed into cDNA during library preparation.

Visualization interpretation guidance

Table 7 Oncomine™ Lung Cell-Free Total Nucleic Acid Research Assay

Metric	Description
Copy Number Variation	
CNV Ratio	Should be interpreted as the fold amplification (gain) as detected by the assay. CNV specific amplicon (MET) coverage levels are compared to non-CNV amplicon coverage.
P-value	Significance of CNV Ratio measurement based on amplicon coverage variability (MAPD level) and magnitude of the pairwise coverage differences between the CNV and non-CNV amplicons. High coverage variability will result in less significant p-values. See page 256 for QC and CNV calling rules.
Fusion detection	
Nomenclature	Each reported fusion target follows a specific naming convention such that the 5'- and 3'-genes are reported along with donor and acceptor exon numbers. Lastly, a COSMIC ID or NCBI transcript accession number is added to the end of each target name. For example, EML4-ALK.E13A20.COSF463 identifies the EML4-ALK fusion variant with exon 13 of EML4 fused to exon 20 of ALK.

Metric	Description
Fusion QC genes	Two non-fused process control genes (HMBS and TBP) that have been shown to be consistently detected in cell-free nucleic acid extracts are included in the assay to inform quality of fusion variant calls.
Analysis detail	• Fusion targets are reported as **FUSION** in the **Type** column. • Fusion QC genes are reported as **ProcControl** in the **Type** column. • See page 256 for QC and Fusion calling rules.
MET Exon 14 Skipping Assay	
Nomenclature	• There is one assay specific to the exon 14 skipping detection in the MET gene called MET-MET.M13M15. • Two additional wild type assays are provided to inform the quality of a MET exon 14 skipping variant call. These are named MET.E6E7.WT and MET.E11E12.WT.
Analysis detail	• MET exon 14 targets are reported as **RNAExonVariant** in the **Type** column. • See page 256 for QC and MET exon 14 skipping calling rules.

Table 8 Oncomine™ Breast cfDNA Research Assay v2

Metric	Description
Copy Number Variation	
CNV Ratio	Should be interpreted as the fold amplification (gain) as detected by the assay. CNV specific amplicon (CCND1, ERBB2, FGFR1) coverage levels are compared to non-CNV amplicon coverage.
P-value	Significance of CNV Ratio measurement based on amplicon coverage variability (MAPD level) and magnitude of the pairwise coverage differences between the CNV and non-CNV amplicons. High coverage variability will result in less significant p-values. See page 256 for QC and CNV calling rules.
De novo **(non-hotspot) variant calling in TP53**	
Analysis detail	• Panel includes approximately 80% coverage of the TP53 gene. • These variants are reported as **PN** (potentially novel) in the **Info** column. If the variant is reported as **HS** in the **Info** column, this variant is a hotspot specifically targeted by the breast panel. • These variant calls must be at a frequency of ≥0.5% to be reported in the analysis visualization. To view *de novo* TP53 variants at lower frequencies, download a VCF file from the visualization pages.

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.

Note: Integrative Genomics Viewer (IGV) is the genomic viewer made by the Broad Institute. IRGV is the faster loading genomics viewer that is built into Ion Reporter™ Software.

1. Click ⚙ **(Settings)** ▸ **Account Preferences**.
2. In the **Account Preferences** dialog box, in the **IGV launch preference** row, select **IGV or IRGV**, then click **Save**.
   A visualization opens when you perform the following actions.

To view . . .	Do this . . .
A visualization of one or more analyses in **IRGV**.	1. In the **Analyses** tab, in the **Overview** screen, select the checkbox in for the analysis you want to visualize, then click **Visualize** in the upper right corner of the screen. The visualization opens in the **Analysis Visualization** screen in **IRGV**.
A visualization of a gene of interest in a separate browser tab.	1. In the **Analysis Visualization** screen, click **Variants Table**, then click on the locus of interest in the **Locus** column. • If the **Account Preference** is set to IRGV, IRGV is launched in a separate browser window. • If the **Account Preference** is set to IGV, a JNLP file is downloaded when you click on the locus in the **Variants Table**. Double-click on the file to launch IGV as a standalone application on your computer.

Set Ion Reporter™ Genomic Viewer preferences

Before you set preferences, you must visualize an analysis in Ion Reporter™ Software.

You can modify the elements that are included in analysis visualizations.

1. Under the **Analyses** tab, click **Overview**.
2. Select one or more analyses in the **Analysis Results** screen, then click **Visualize**.
3. In the open Visualization, click **IRGV/Export & Preferences** ▸ **Show IRGV preferences** .
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** dialog box, then click **OK**.

5. In **Preferences** you can set the following options:

Option	Description
Default mapd value	This value will be used as default value in the mapd filter.
Default sort order [1]	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.
Max Ploidy in Whole Genome View	Maximum value of the Y-axis (ploidy) in the Whole Genome View graphs.

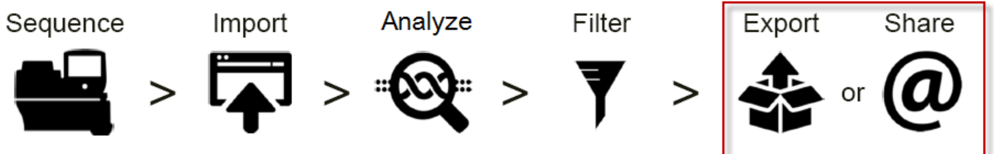
[1] This preference can be set only when multiple analyses are selected.

6. When your edits are complete, click **Save Preferences**.

   Note: The preferences are reset to the default settings if you click **Reset Preferences**.

   The selected preferences are applied to all analyses that you visualize immediately after you save your changes.

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 9

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 in Ion 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.

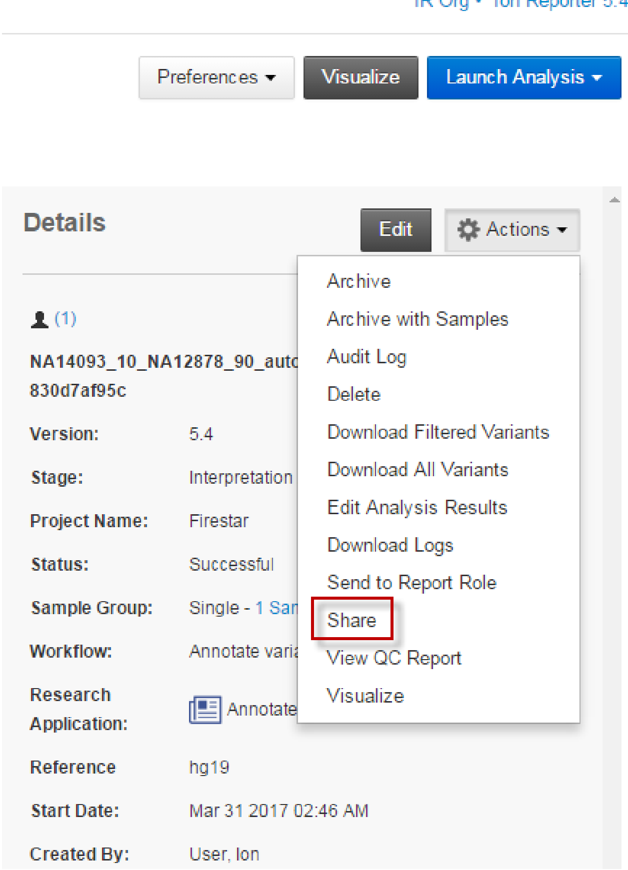
Unshare analyses data

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.

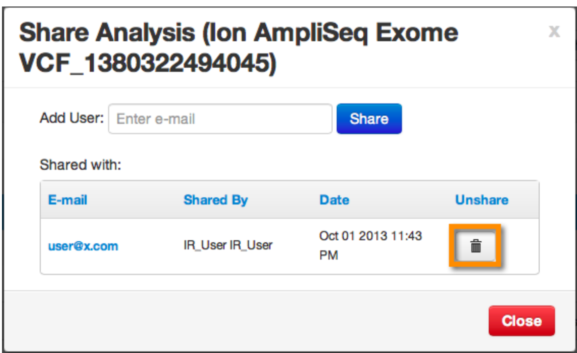
Note: This action can not be undone.

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.

Create and view reports

Ion Reporter™ Software provides several types of reports for various uses in sequencing, quality control, and audits of user activities. To save or distribute a report, you can download the report and files that are related to the report.

Note: You must have Report Role privileges to view and create reports. Otherwise, you can send completed analyses to the person on your team who has the Report Role. See "Send analyses to Report role" on page 288.

- **Final report** – Final reports include information on the analysis and samples, variant details, background information, disclaimers, comments, and signatures. You can use system templates or create custom templates for these reports, and add a report template to an Ion Reporter™ Software workflow to reduce report setup time. For a less detailed report that includes basic information, see "Create a basic report" on page 267.
- **Amplicon coverage report** – Amplicon coverage reports list amplicons, SNPs, and bases in the amplicons. When you view the amplicon coverage report, you can adjust the amplicon coverage threshold to focus your report on only the amplicons that met the threshold that you set.
- **Quality Control (QC) report** – QC reports provide a high-level summary of key metrics that you can use to evaluate the quality of the data for analysis and samples, or amplicon coverage reports. Each report is specific to an individual analysis and the samples used for the sequencing run.
- **Audit log** – Audit logs record user activity such as who launches analyses, the start and end time of analyses, report creation and publication, and edits of analyses, including the addition of annotations, MyVariants flags, Classifications, and notes.

View a published report

You can view a final report of a completed analysis that has been published. These reports include details such as location, type, coverage, and annotations. You can download published reports as PDF files.

Note: A final report of analysis results that has been published cannot be edited. That is, you cannot add annotations, notes, My Variants, or Classifications to the analysis. However, you can generate another report if you reanalyze the results.

1. In the **Analyses** tab, click **Overview**.
2. In the **Stage** column, look for an analysis that is marked as **Report Published**.
3. Select the row or checkbox for the analysis of interest.

4. Click ⚙ **(Actions)** ▸ **View Final Report**.
   A report preview is created.

5. Review the various sections of the report. You can:
   - Click **Download** to download a PDF of the report.
   - Click **Switch To** to view a QC report or audit log. See "View a Quality Control (QC) report" on page 277 and "View audit logs" on page 285

Create a flexible report

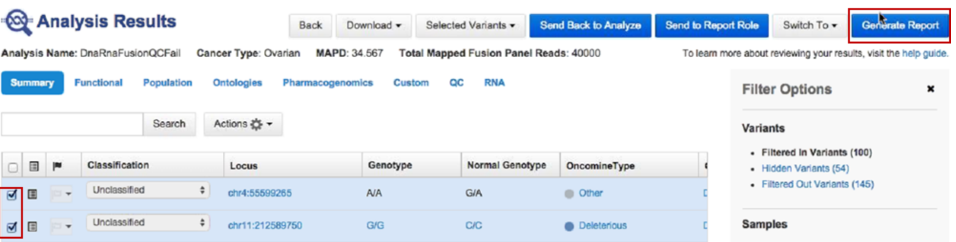
You can customize reports in many ways. You can create a report with an existing report template or a new report template. This flexible approach also allows you to reorder sections, reorder sections of the report, and to remove sections.

Note: Only one final report for each analysis can been published for later use. A report that is published to PDF format cannot be edited. Also, you cannot edit MyVariants, classifications or notes that are associated with the analysis results if a report has been published. To create a different report for the analysis, you must reanalyze the sample using the same workflow, then create and publish the report.

1. In the **Analyses** tab, click **Overview**.

2. Click an analysis link to open the **Analysis Results** screen.

3. Select the variants to be included in the report, by clicking the checkbox next to each variant or click the checkbox at the top of the column to select all variants.



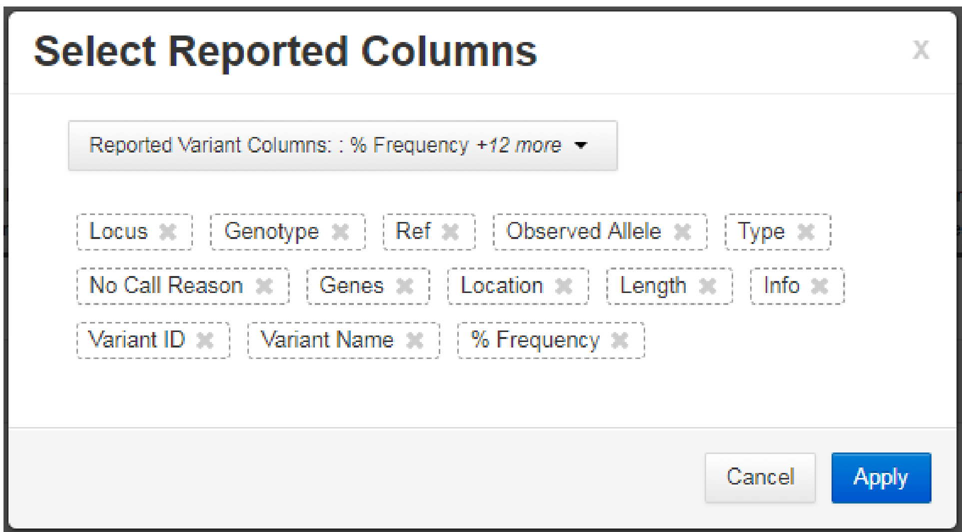
4. Click **Generate Report**. If a report template is associated with the workflow, use the **Select Final Report Template** dialog box to:
   - Select a template, then click **OK**.
   - Click **Create Report Template** to create a new report template. See "Create a report template" on page 267 for more information.

   If a report template is not associated with the workflow, go to the next step.

5. Enter information into the sections. See "Final report template sections" on page 270 for more information.

6. *(Optional)* Scroll to **Reported Variants** section, then click **Select Columns**.
   a. In the dialog box, you can:

   - Delete columns – Click the **x** in the column name to remove that data from the report.
   - Change column order – Rearrange the column names to reflect the position that you want the date to appear in the report.



7. Click **Next**.
   A preview of the PDF report opens. Click **Previous** to go back and change the report.

8. When you are satisfied with the report sections, click **Lock and Publish**, then review the PDF preview. If edits are needed, click **Cancel**, then make your edits.

   IMPORTANT! After a report is published, the analysis used for the report cannot be edited. That is, you can no longer edit MyVariants, classifications or notes that are in the analysis.

9. Click **Publish** to create the final report.

10. Click **Download** to download the published report.

Create a basic report

You can predefine your report template as a Ion Reporter™ Software workflow preset. However, the resulting report is not as customizable as it is in the flexible reporting approach. See “Create a report template with Workflow Presets“ on page 127 for more information.

Create a report template

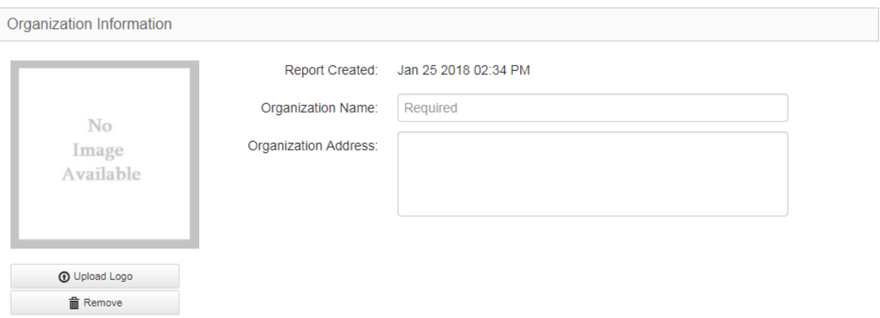
You must have Report role privileges to generate reports or report templates.

You can create a new report template and save it for future analysis reports. This flexible approach allows you to reorder sections, and to remove unnecessary sections.

1. In the **Analyses** tab, click **Overview**.

2. In the **Analysis Results** screen, click **Generate Report**.

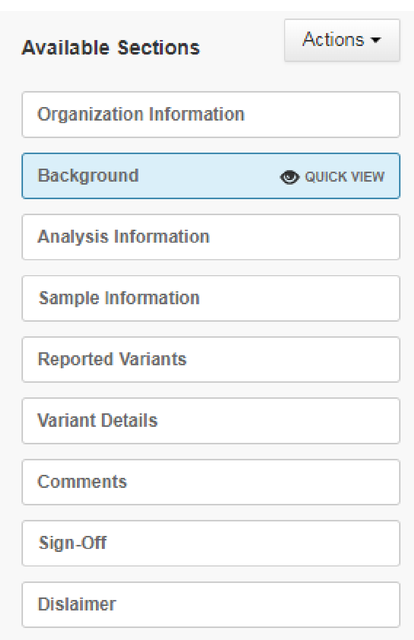
3. In the **Generate Report** screen, in the **Configuration** step, do the following:
   a. (Required) Enter your organization name.
   b. *(Optional)* Click **Upload Logo**. The **Upload Header Logo** dialog appears. Click **Select File** to browse to your logo file. Acceptable formats are: PNG, GIF, and JPG.
   c. *(Optional)* Enter your organization address.
   d. *(Optional)* Enter other standard information for the **Background**, **Disclaimer**, and **Sign-Off** sections.



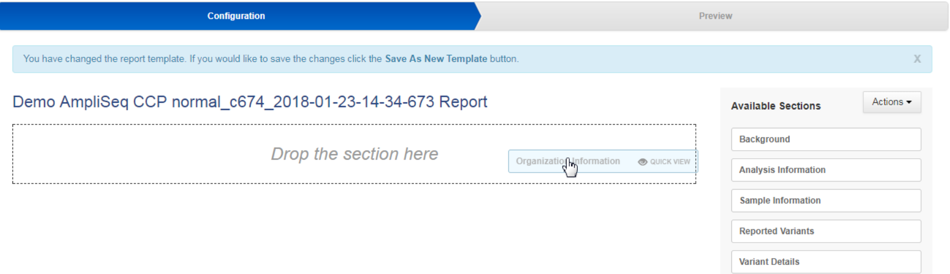
4. Use the up, down, and X icons to move or delete sections. Or, click **Actions**, then select one of the following:

Option	Description
Add all	Add all the default report template sections to your customized template or report.
Remove all	Remove all the default report template section from your customized template or report.
Reset	Return to the default template.

5. For example, to select a limited set of sections, click **Remove all**.
   The sections that you can add to the report are listed under **Available Sections**. See “Final report template sections“ on page 270 for more information.



6. Then click, then drag any of the **Available Sections** back into the Report template.



7. Click **Save As New Template**.

8. Enter a template name, click **Save**.

9. To use the template in future analyses, you must add it to your workflows. See “Add a report template to an Ion Reporter™ Software workflow“ on page 272 for more information.

Final report template sections

The Final report template offers the following sections from which you can use to build your own report templates or reports.

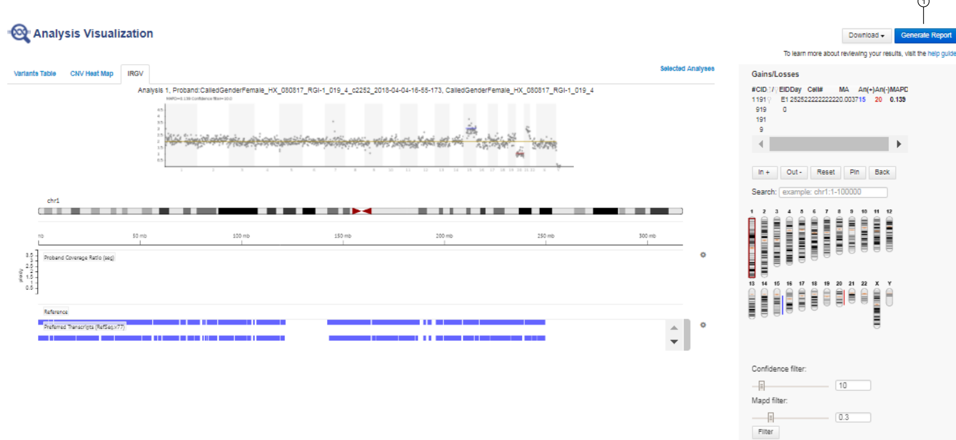
Category	Description
Organizational information	• **Required**: Organizational name. • *(Optional)* Upload a logo and/or enter the address
Background	*(Optional)* Enter background information
Analysis Information	Includes software version number, report generation date, name of person who launched the report, workflow, name of person who analyzed the data, list of annotations, and reference information.
Samples Overview	Includes sample name, gender, relationship and chip type information.
Reported Variants	Generates a table of reported variants and includes columns of variant details.
Variant Details	Includes name and description of variants as well as which samples they came from. In addition, it lists Annotations in a table.
Comments	*(Optional)* General comments
Sign–Off	Affix an author's name and qualification to the report
Disclaimer	*(Optional)* Enter a custom legal disclaimer.

Generate aneuploidy report with Ion Reporter™ Genomic Viewer graphics

When you visualize multiple aneuploidy results in Ion Reporter™ Genomic Viewer (IRGV), you can generate a single PDF report that contains graphics from each results.

1. Under the **Analyses** tab, click **Overview**, then select one or more aneuploidy analyses that you want to visualize in Ion Reporter™ Genomic Viewer.
2. Click**Visualize**.
   The visualization of the data is opened in the **IRGV** tab of the **Analysis Visualization** screen. The visualization includes a copy number histogram for each analysis, ploidy maps for selected chromosomes or chromosome regions, and karyograms that show copy number gains and losses.

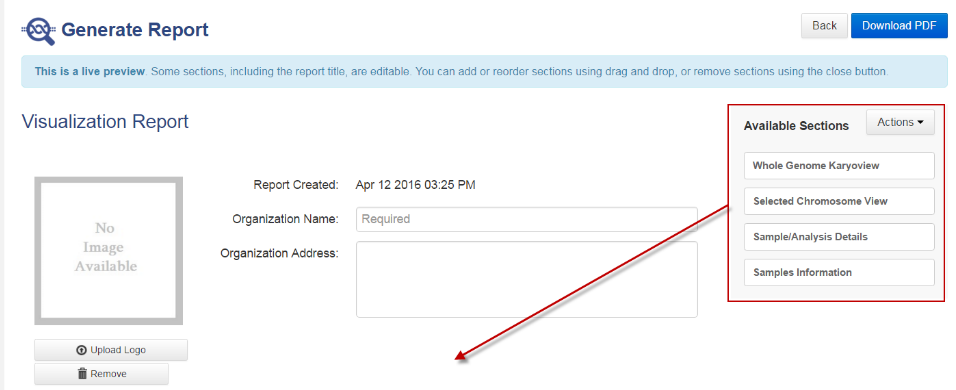
Note: For six or more aneuploidy analyses results, the Whole Genome Karyoview is not able to show Gain and Loss data segments.



① Generate Report for the IRGV visualization

3. Click **Generate Report**.
   A preview of the report is shown. You can customize the report.

4. Drag the title of the graphics that you want to include in the report from the **Available Sections** into the **Visualization Report** preview.



5. Enter an organization name for the report. Enter any other relevant information, then click **Download PDF**.

The report is downloaded to the location used for downloads from the browser. This location depends on your browser settings.

Search for a report template

You can review your existing custom and factory report templates.

1. In the **Workflows** tab, click **Presets**.
2. In the first dropdown list, select **Final Report Templates**. Then, select a template type.

Option	Description
Ion factory templates	Factory templates are labeled ion and cannot be altered. However, you can review the components of the factory templates in the Details pane.
Custom templates	Custom templates and can be added to workflows, deleted, edited, or locked.

Add a report template to an Ion Reporter™ Software workflow

If you know which information that you want to include in a final report for sequencing results that are shown in the **Analysis Results** table, you can add a report template to a Ion Reporter™ Software workflow. Reports published from the analysis results that use the workflow are based on the report template that is associated with a workflow.

1. Create a custom report template or select a system template. See “Create a report template“ on page 267 or “Create a report template with Workflow Presets“ on page 127 for more information.
2. In the **Workflows** tab, click **Create Workflow**.
3. Make the appropriate selections in the **Research Application**, **Reference**, **Annotation**, **Filters**, **Copy Number**, and **Plugins** steps. See “Create a new custom workflow without predefined settings“ on page 72 for more information.
4. In the **Final Report** step, under **Final Report Template**, select the report template that you want to associate with the workflow.
5. Make appropriate selections in the **Parameters** step, then confirm.
   Your workflow with corresponding report template is now available for use when you review analysis results.

Edit a report template

You can edit custom report templates that exist in your account.

1. In the **Workflows** tab, click **Presets**.
2. Select **Final Report Templates** from the dropdown list.
3. Select row of the final report template that you want to edit, then click ⚙ **(Actions)** ▸ **Edit**.
4. You can change the name, select sections to include or exclude, then drag, then drop Reported Variants details.
5. Click **Save**.
   If template is included in an Ion Reporter™ Software workflow, it is now available for use when you review analysis results. If it was not, you must add the template to a workflow. See "Add a report template to an Ion Reporter™ Software workflow" on page 272 for more information.

Lock a report template

You can lock custom report templates so that they cannot be altered.

1. In the **Workflows** tab, click **Presets**.
2. Select **Final Report Templates** from the dropdown list.
3. Select the row of the final report template that you want to lock, then click ⚙ **(Actions)** ▸ **Lock**.
   The Final Report Template now displays a lock icon in its row in the **Workflow Presets** screen. You can now add this report template to a workflow. See "Add a report template to an Ion Reporter™ Software workflow" on page 272 for more information.

Delete a report template

You can delete unwanted custom report templates.

1. In the **Workflows** tab, click **Presets**.
2. Select **Final Report Templates** from the dropdown list.
3. Select row of the final report template that you want to delete, then click ⚙ **(Actions)** ▸ **Delete**.
   The final report template is now removed from the list of available templates in the **Workflow Presets** screen and can no longer be added to an Ion Reporter™ Software workflow.

View and filter an amplicon coverage report

You can view an amplicon coverage report in Ion Reporter™ Software that shows which amplicons in your analysis *do not* have coverage; the report covers all bases to a user-defined coverage depth threshold. In an open coverage report you can alter this threshold, and you can filter the list of amplicons shown in the report based on a threshold that you select.

The following Coverage Quality Control (QC) metrics are also shown in report screen:

- Percent of the total number of amplicons in your panel that have coverage at all bases at a level greater than the threshold you choose (% amplicons > threshold).
- Percent of the total bases defined in your panel regions BED file that have coverage greater than the threshold you have choose (**% BED region > threshold**).

To access the amplicon coverage report in Ion Reporter™ Software:

1. In the **Analyses** tab, click **Overview** and select the row for the analysis of interest.

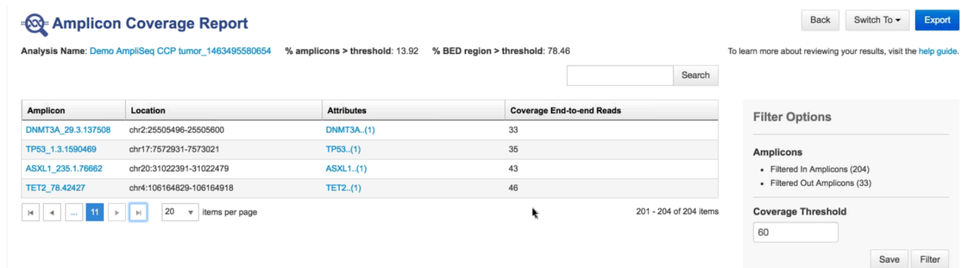
2. Click **Actions ▸ View Coverage Report**.

   Note: Alternatively, you can click the analysis of interest to open the **Analysis Results** table, then click **Switch To ▸ Coverage Report**.

   An **Amplicon Coverage Report** opens. The default value for the threshold is ***0***, so by default, no amplicons are included in the list.

3. In the **Filter Options** section, enter a **Coverage Threshold** value, for example `60`, then click **Filter**. To save this threshold as the default for this amplicon report, click **Save**.

   Note: You must save the filter in order for the **% BED region > threshold**, **% amplicons > threshold**, and **Coverage Threshold** metrics to appear in the **Amplicon Coverage Report**.

   The **% amplicons > threshold** and **% BED region > threshold** change to reflect the **Coverage Threshold** value.

4. Click **Export** to generate an tab-separated CSV file that contains details such as chromosome location, and start and end positions on forward and reverse strands.
   The downloaded file is named `ampliconmetrics.csv` file.

Download amplicon coverage report

You can download amplicon coverage reports in Ion Reporter™ Software. Amplicon coverage reports contain details such as amplicon coverage, chromosome location, and start and end positions.

1. In the **Analysis** tab, click **Overview**.
2. Select the row of the analysis you want to report on.
3. Click **Actions ▸ View Coverage Report**.
4. Click **Export** to generate an tab-separated CSV file that contains details such as chromosome location, and start and end positions on forward and reverse strands.
   The downloaded file is named `ampliconmetrics.csv` file.

Amplicon coverage report metrics

The amplicon coverage report includes the following metrics:

Metric	Description
Region	Amplicon name as defined in the BED file for the amplicon region
Chr	Chromosome
Start	Start coordinate of the amplicon
End	End coordinate of the amplicon
Attributes	Attribute in the BED file for the amplicon
TotalE2E	The number of assigned reads from end to end for the amplicon region
FwdE2E	The number of assigned forward strand reads from from end to end for the amplicon region
RevE2E	The number of assigned reverse strand reads from from end to end for the amplicon region
Total	The total number of reads assigned to this amplicon **Note:** This value is equal to Fwd + Rev.

Metric	Description
Fwd	The number of forward strand reads assigned to this amplicon **Note:** This value may differ from the reads for end to end.
Rev	The number of reverse strand reads assigned to this amplicon **Note:** This value may differ from the reads for end to end.

Open an amplicon coverage report in (IRGV)

To view an amplicon visually, click on an amplicon link in the **Amplicon Coverage Report** screen to view the results in Ion Reporter™ Genomic Viewer (IRGV) or Integrative Genomics Viewer (IGV), depending on your settings. See "Set IRGV or IGV as default viewer" on page 259 for more information.

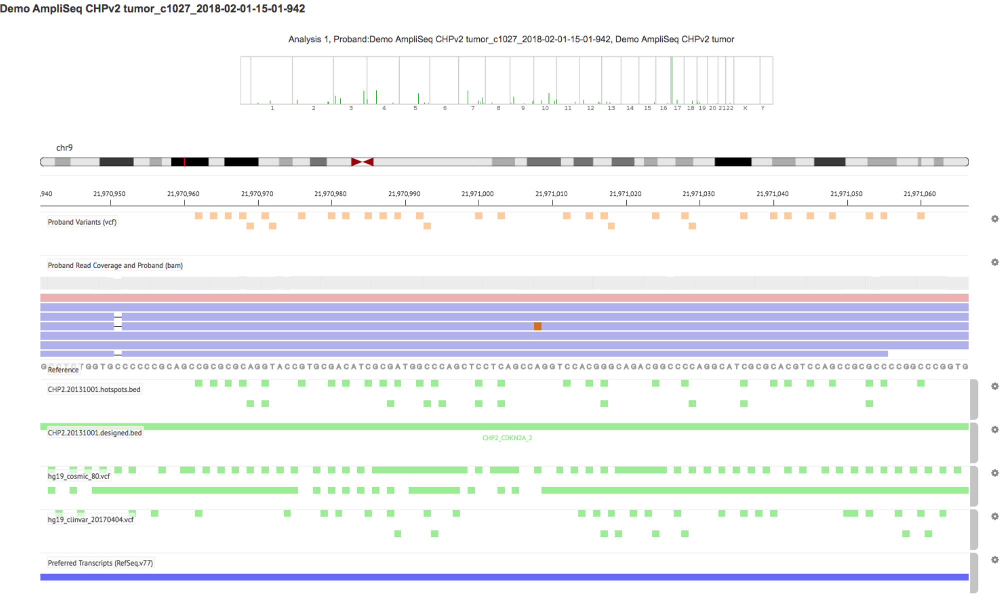


Figure 18 Amplicon visualization in IRGV

View a Quality Control (QC) report

You can view a Quality Control (QC) report for a completed analysis in Ion Reporter™ Software. The QC report provides a high-level summary of key metrics that you can use to evaluate the quality of the data. You can also download a PDF version of the QC report.

1. In the **Analysis** tab, click **Overview**.
2. Select the row for the report of interest, then click ⚙ ▸ **(Actions)** ▸ **View QC Report**.

   Note: Alternatively, you can click the analysis link to open the **Analysis Results** screen, then click **Switch To:** ▸ **QC Report**.

The QC Report opens:

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	
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

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Report generated by Ion User on Aug 28 2017 11:44 PM

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3.
   - To download a PDF of the QC report:
     a. **Export ▸ PDF**.
     b. Download the report from the **Notifications** page. The filename for the PDF is *id*_QC.pdf, where *id* is a system-generated analysis identifier.

        Note: You can also view the QC report in your browser, or find the PDF in the directory that you use for downloads, depending on your browser settings.
   - To download a compressed directory that contains the QC Report PDF, a tab-separated file of the QC metrics, and individual text files of coverage statistics by amplicon, analysis, and gene:
     a. **Export ▸ QC Package**.
     b. Download the compressed directory from the **Notifications** page. The downloaded filename is `qc-report-`*`id`*`.zip`, where *id* is a system-generated analysis identifier.

        Note: You can also view the QC report in your browser, or find the PDF in the directory that you use for downloads, depending on your browser settings.

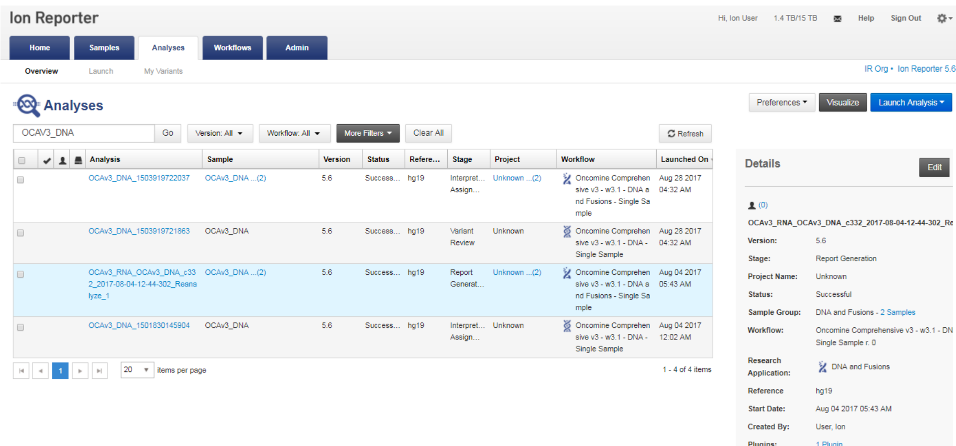
   See "Quality Control (QC) report contents" on page 281 for more information about the downloaded directory.

Download Quality Control (QC) files

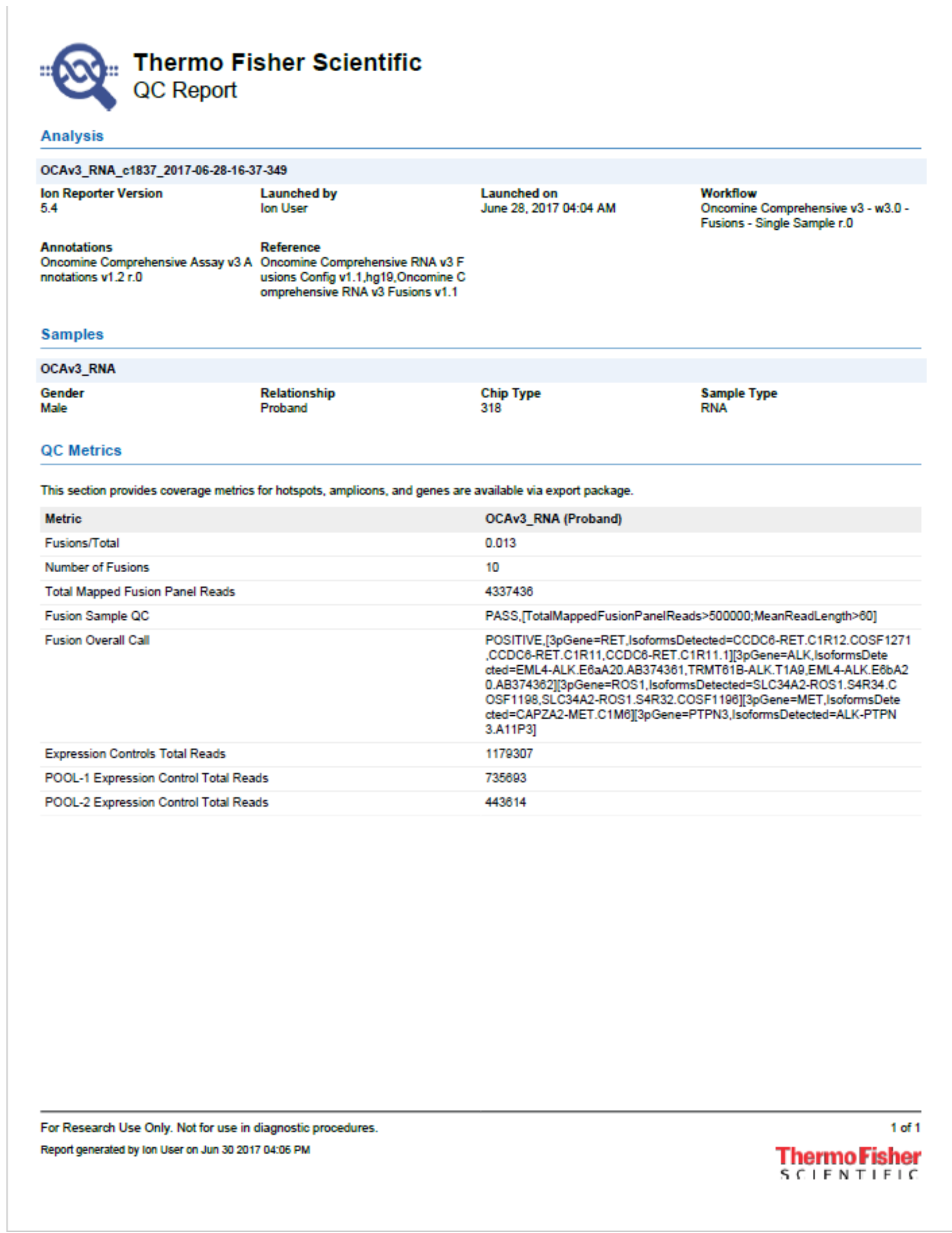
You can download files from a Quality Control (QC) report that you open in Ion Reporter™ Software.

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
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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, download the file through the browser that you use for Ion Reporter™ Software. Then, find the QC package in the folder that is used for downloads.

    The downloaded filename is qc-report-*id*.zip, where *id* is a system-generated analysis identifier.

Quality Control (QC) report contents

The Quality Control (QC) files that you can download contain a compressed directory contains the following files:

- `id_QC.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.

The QC report PDF file for a completed analysis contains a high level summary of the following key metrics:

QC metrics that are available for QC reports are as follows.

QC Metrics field	Description
% BED region > threshold	Percentage of BED regions greater than the threshold you set
% amplicons > threshold	Percentage of amplicons greater than the threshold you set
Coverage Threshold	Coverage adequacy setting
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

QC Metrics field	Description
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
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

QC Metrics field	Description
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
CNV/Total	CNVs divided by total variant count minus no calls)
LongDels/Total	LongDels divided by total variant count minus no calls
Number of CNVs	Number of CNV variants
Number of LongDels	Number of LongDel variants
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.

The following fields are included in each of 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

About the QC package coverage columns

Quality Control (QC) statistics files report coverage distribution. QC report settings can be customized when you create a custom workflow or edit a workflow.

(QC) package coverage distribution is calculated for each region and reported in the No, Low, Medium, and High columns .

Table 10

Coverage	Definition
No	0 to 0 coverage range
Low	1 to Maximum Low Coverage
Medium	Max Low Coverage +1 to Max Medium Coverage
High	Maximum Medium Coverage +1 to Maximum Coverage

View audit logs

You can view and download logs of user activities in Ion Reporter™ Software. These reports contain information regarding who launched or edited analyses.

View audit log for a completed analysis

You can view an audit log for a completed analysis in Ion Reporter™ Software. Information is logged about the activities if the state of an analysis changes. The analysis states are:

- Analysis
- Variant Review
- Interpretation Assignment
- Pending Report
- Variant Classification
- Report Generation
- Report Publishing
- Report Published

Audit logs also record information about analyses, samples and logs.

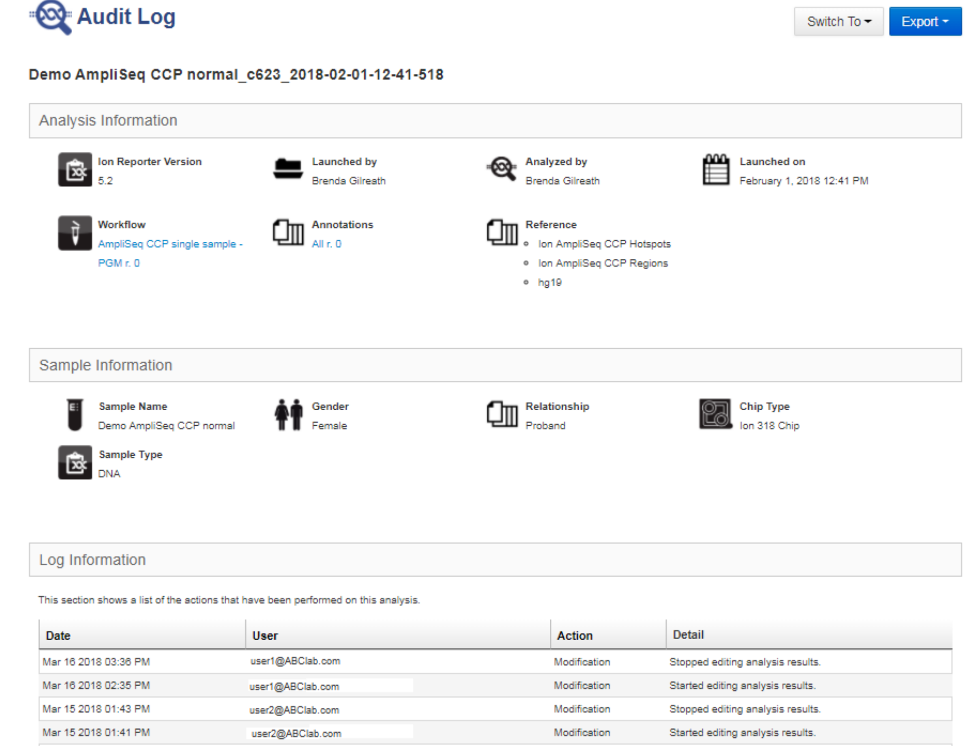
Section	Description
Analysis information	Information on the analysis that was performed, such as: software version, workflow, annotations, reference, launched by, and so on.
Sample information	Information on the samples that are part of the analysis, such as: gender, relationship, type, and so on.
Log information	The individual actions that have been recorded for this analysis including: date, time, user, action, and details on the action performed.

1. In the **Analysis** tab, click **Overview**.
2. Select the row of the analysis that you want to view.

3. Click ⚙ **(Actions)** ▸ **Audit Log**.

   Note: Alternatively, you can click the analysis link to open the Analysis Results screen, then click **Switch To** ▸ **Audit Log**.

   The **Audit Log** opens:



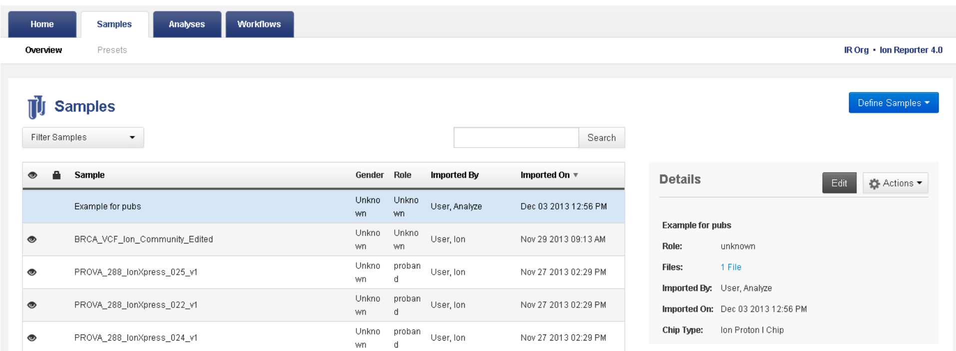
4. (*Optional*) Click **Export** ▸ **PDF**.

View audit log for a sample

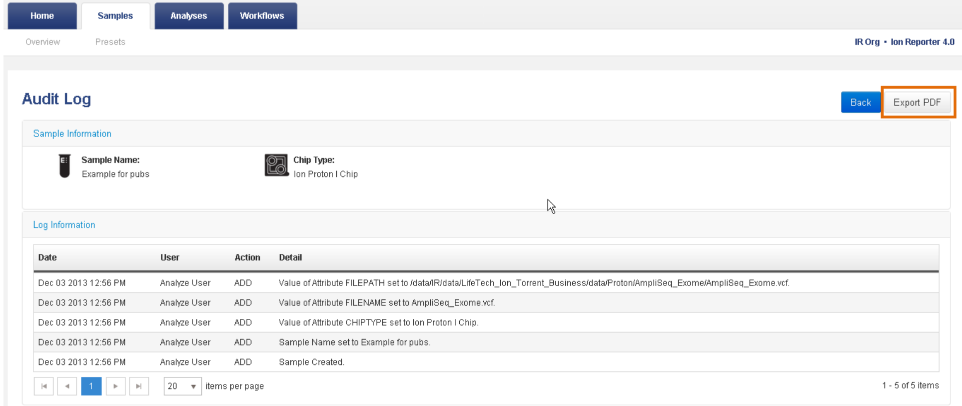
You can view an audit log for samples in Ion Reporter™ Software. The sample audit log allows you to see who has accessed a particular sample and what the user did to a particular sample. Sample audit logs are not available for the pre-installed demo data samples.

Follow these steps to access the sample audit log:

1. In the **Samples** tab, click **Overview**.
2. Select the row of the sample you want to report on.



3. Click **Actions ▸ Audit Log**.
   The audit log opens.
4. (*Optional*) Click **Export PDF**. The downloaded filename is *sample_name*.pdf, where *sample_name* is the name of the sample.



Download an audit log

You can download audit logs of samples and analyses in Ion Reporter™ Software.

1. In the **Samples** or **Analyses** tabs, click **Overview**.
2. Select the row of the Sample or Analysis you want to audit.
3. Click **Actions ▸ Audit Log**.
   The audit log opens.
4. Click **Export ▸ PDF**.
   The downloaded filename is `sample_name.pdf` or `analysis_name.pdf`.

Send analyses to Report role

If you do not have report role access, you can send your analyses to someone in your organization who has Report role access. The person with Report role access can then generate reports.

1. In the **Analyses** tab, select **Overview**.
2. Click an analysis link to open the **Analysis Results** screen.
3. Select the variants to be included in the report, by clicking the checkbox next to each variant or click the checkbox at the top of the column to select all variants.

   IMPORTANT! You can no longer edit the analysis or filter the variants after the analysis results are sent to the Report role.

4. Click **Send to Report Role**.
5. In the **Send to Report Role** window, click **Send**.
   The analysis results are sent to the person in your organization who has the report role function.

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 11

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.

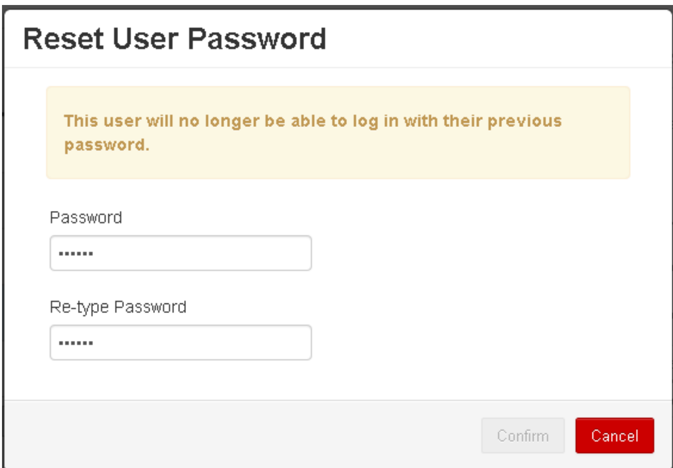
A user becomes locked out after five attempts to log in with an incorrect password. The administrator must reset the password to unlock the account. There are two mechanisms to set or reset the password for a user account.

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.

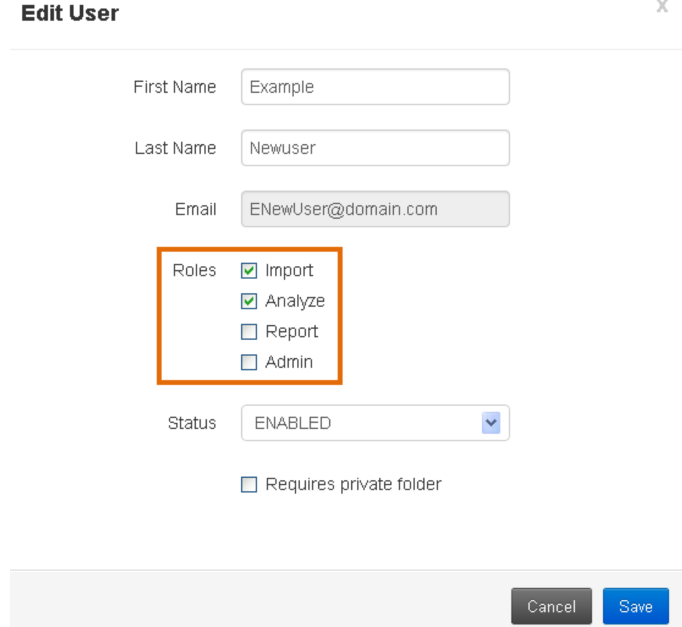
Modify the permissions of a user

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 295.

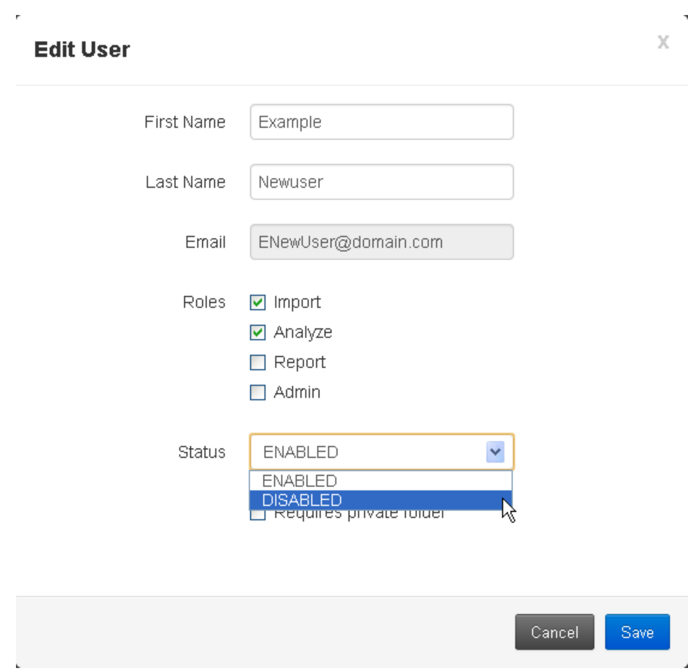
5. Click **Save**.

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.

Download recent user activity

As an administrator, you can track user activity within your organization in Ion Reporter™ Software. For example, you can see who created analyses and who modified samples.

Note: This is different from the audit log report that lists the details of a single analysis. See "Download an audit log" on page 287 for more information.

1. In the **Admin** tab, click **Users**.
2. Click **Download Activity**.
3. In the **Download Activity** dialog box, select a time range. Options include:
   - Today
   - Last 7 Days
   - Last 30 Days
   - Date Range

   Use **Date Range** if you need to search for a specific time range. Enter start and end dates in the mm/dd/yyyy format.
4. Click **Download**.
   A CSV file is created.
5. Open the CSV file and review the user actions.

Column	Description
Name	Name of analysis, workflow, sample or other item created or edited
Type	Type of item created or edited, such as analysis, My Variants, etc.
Created By	First and last name of the user who created an analysis or other element
Created By Email	Email address of the user who created an analysis or other element
Updated By	First and last name of the user who updated an analysis or other element
Updated By Email	Email address of the user who updated an analysis or other element
Updated By Organization	Name of the organization that updated an analysis or other element
Screen	Screen name that was modified
Values	Values that were changed

Manage the API token

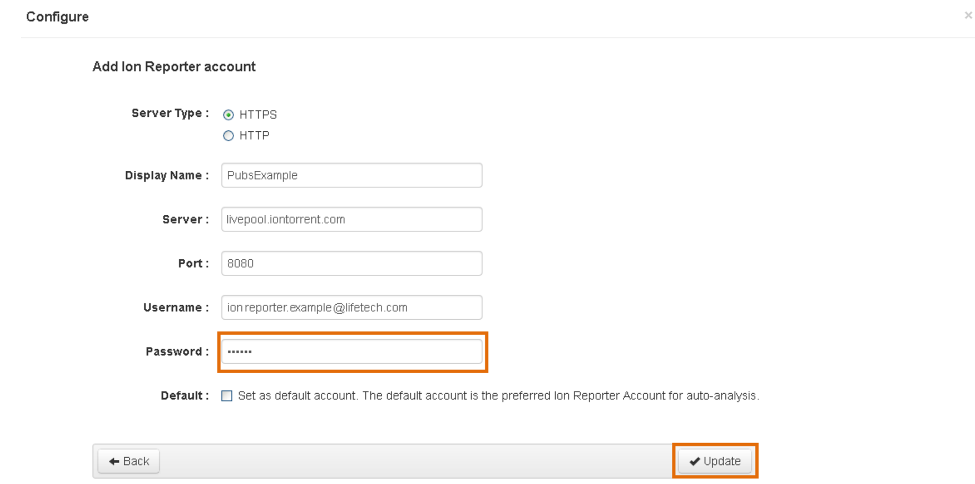
The API token is used to upload data to the server version of Ion Reporter™ Software and access data from the API. These steps can be performed by either an administrative user or a regular user.

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

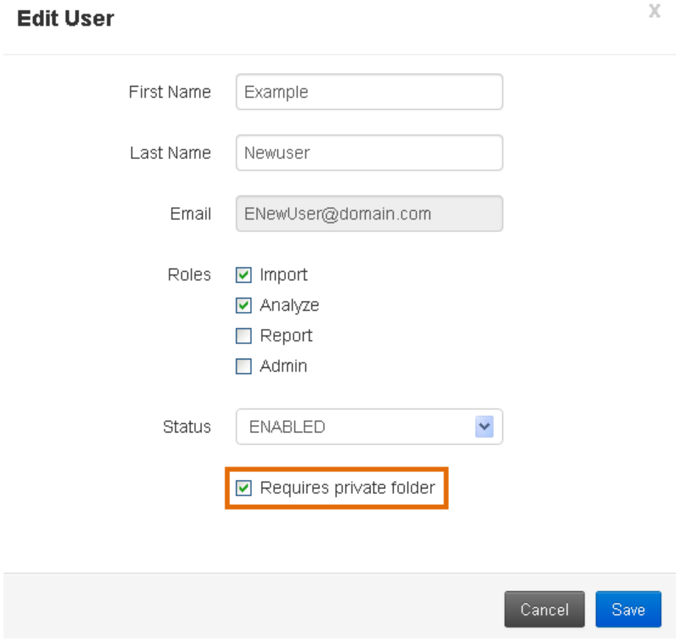
Private folders enable a user to restrict all visibility to their data.

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.

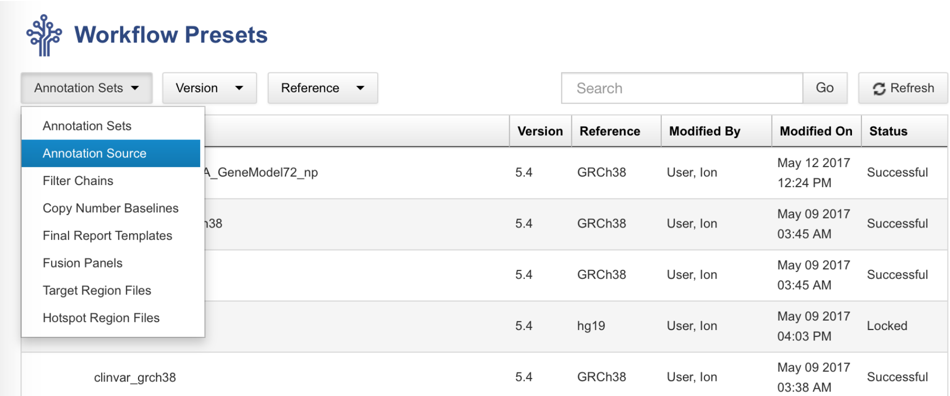
After a private folder is enabled, the data, analysis and workflows created by that user and generated in that folder is visible only to that user.

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.

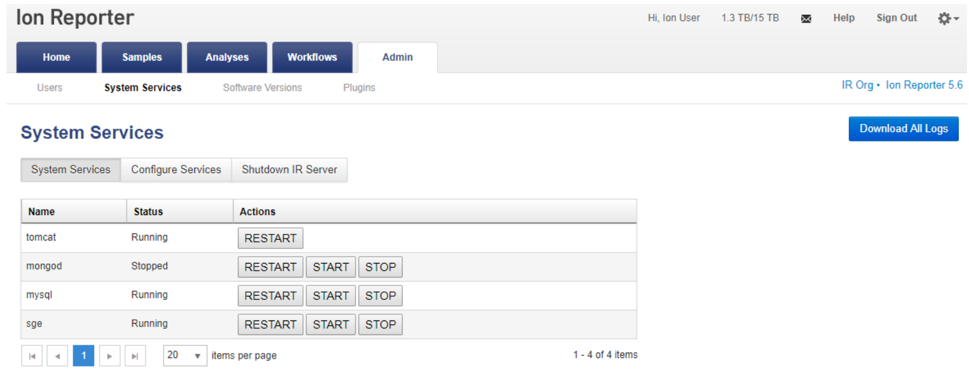
   The annotation source is removed from the **Workflow Presets** list.

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 298 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: Only administrators can install plugins for their IR organization.

1. In the **Admin** tab, click **Plugins**.
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: The plugin files must be contained in a compressed directories in ZIP file format.

   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 95 for instructions on how to run a plugin.

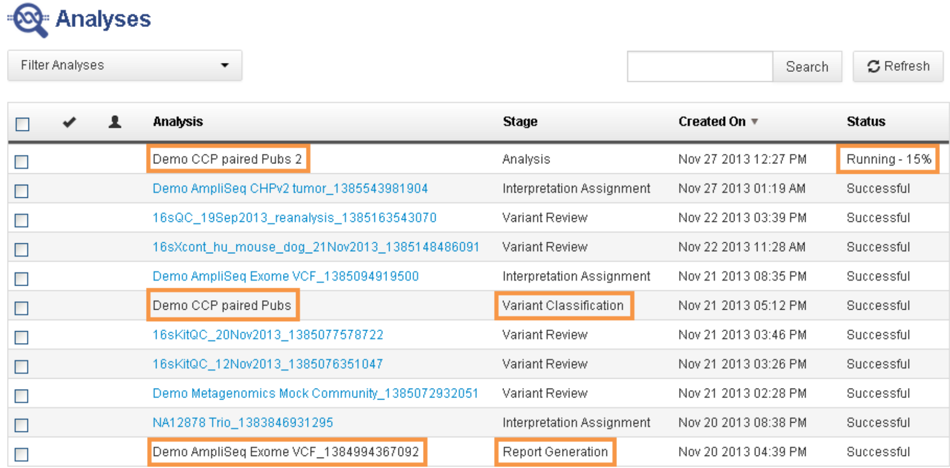
See "Guidelines to develop Ion Reporter™ Software plugins" on page 413 for information on how to create custom plugins for Ion Reporter™ Software.

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.

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.

This analysis is currently being edited by Ion User. You are in View only mode.

Custom BED file causes an error

If you use import a custom BED file into Ion Reporter™ Software, the file is validated. The following errors indicate that there is a problem with the BED file. To resolve an error, review the BED file, and edit the BED file if necessary. Contact your Field Bioinformatics Specialist (FBS) for further assistance with troubleshooting BED files.

Observation	Possible cause	Recommended action
Error parsing track line key=value pairs	There is an error for in the key values that are used in the track line. Example key value pairs are: `track name="HSMv12.1" description="AmpliSeq Pool HSMv12.1" version="Ampliseq.com1.0"` type="bedDetail".	Review the BED file and ensure that the value pairs are correct.

Observation	Possible cause	Recommended action
Error parsing track line, no closing quotes	The closing quotes are missing from the key value pair in the first line (track line) of the BED file. For example: `track name="HSMv12.1" description="AmpliSeq Pool HSMv12.1" version="Ampliseq.com1.0" type=bedDetail`.	Review the track line and add closing quotes.
ionVersion track key is only valid with type=bedDetail	A BED file that includes an `IonVersion` field must also have a `type=bedDetail` field.	Review the first line (track line) of the BED file and ensure that the `type=bedDetail` and the `ionVerison` field are included.
Field OBS cannot be the same as REF	The strings used in the OBS and REF fields cannot be the same. Both fields cannot be `ATC`, for example.	Change `OBS=string` so that it is not the same as the `REF=string`.
Inconsistent number of columns	Every line in the BED file must include the same number of columns.	Review the BED file and ensure that every line in the file has the same number of columns.
BED track line only allowed in the first line: All lines ignored after this one	BED files allow only one track line in the first line of the header. An example track line is: `track name="HSMv12.1" description="AmpliSeq Pool HSMv12.1" version="Ampliseq.com1.0" type=bedDetail"`.	Remove the redundant track line.
Line length exceeds 64K	A line in the BED file exceeds 64K characters.	Review the BED file and reduce the line length so that it is 64K or less.
Unknown chromosome name	A chromosome name in the BED file is not included in the FASTA file.	Review the BED file and provide the correct chromosome name.
Region start not in a valid range	For hotspot BED file, the region start value is not in a targeted BED region.	Remove this line.
Region end not in a valid range	For hotspots BED file, the region end value is not in a target bed region.	Remove this line.
Region start and end in reverse order	The region end has a smaller value than the value for the start region.	Ensure that the value for the region start has a smaller value than the value for the end region.
Detail BED file with type=bedDetail must have between 5 and 14 columns	The numbers of columns in a Detail BED file that includes `type=bedDetail` must have between 5 and 14 columns	Review the number of columns in the BED file and ensure that there are between 5 and 14 columns.
Hotspots BED file must have format type=bed	Information is missing from the header of the BED file.	Add `format type=bedDetail` to the file header of the hotspots BED file.

Observation	Possible cause	Recommended action
BED file without type=bedDetail must have between 3 and 12 columns	The number of columns for a BED file without `type=bedDetail` has the incorrect number of columns	Review the number of columns in the BED file and ensure that there are between 3 and 12 columns.
BED file contains no usable regions	The BED file might be empty, or every line has at least one error.	Ensure that you are using a BED file that is valid and correctly formatted.
REF field contains characters other than ATCGatcg	The alpha-numeric characters ATCG, or atcg, are not used in the REF field.	Find the REF field in the custom BAM file. Ensure that the field is populated with characters that use the format `ATCG` or `atcg`.
OBS field contains characters other than ATCGatcg	The alpha-numeric characters ATCG, or atcg, are not used in the OBS field.	Find the OBS field in the custom BAM file. Ensure that the field is populated with characters that use the format `ATCG` or `atcg`.
ANCHOR field contains characters other than ATCGatcg	The alpha-numeric characters ATCG, or atcg, are not used in the ANCHOR field.	Find the ANCHOR field in the custom BAM file. Ensure that the field is populated with characters that use the format `ATCG` or `atcg`.
REF field does not match content of reference fasta file	The string for the REF sequence does not match the reference file.	Look at the REF=`string` and ensure that string is the as the one at that position of the reference sequence from the FASTA file
Mandatory OBS field not found	A field is missing from the custom BED file.	Add an OBS field to the custom BAM file. Ensure that the field is populated with characters that use the format `ATCG` or `atcg`.

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

Ion 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 a custom annotation source" on page 310.

Table 12 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 a custom annotation source" on page 310for 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 326.	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™ Software, 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 13 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 a custom annotation source

You can use your own annotation source files in Ion Reporter™ Software. If you have annotation data that you want to use, you must first create a new annotation set for the custom annotation source. Then, you can import the files into the new 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. Enter a descriptive **Name** for the custom annotation set.
4. (Optional) Enter a description for the custom annotation set.

5. Select one of the follows custom annotation types from the drop-down list:
   - Genomic Region (Custom)
   - Gene Set (Custom)
   - Preferred transcript set (Custom)
   - VariantDB (Custom)
6. Click the **Create New** tab.
7. Enter a descriptive **Name** for the custom annotation source.
8. Enter a **Version**
9. (Optional) Enter a description for the custom annotation source.
10. Click **Select File**, then browse to and select your custom annotation source file, then click **Open**.
11. Click **Save**.
12. 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 a custom annotation source“ on page 310

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 a custom annotation source“ on page 310.

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, ENST0000000000007.3
Gene2 NM_00000001.1, NM_000000007.1, NM_0000000004.5,
NM_000000008.3, ENST0000000000006.4
```

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):

```
#chr	pos	type	ref	length	genotype	pvalue	coverage	allele_coverage	maf	iscn	confidence	precision	gene	transcript	location
	function	codon	exon	protein	coding	sift	polyphen	grantham	5000Exomes	Oncomine	clinvar	cosmic	dbsnp	drugbank	go	omim
	pfam	phylopMyVariants
1	324100	SNV	T	1	A/A	LOC100132287|LOC100132062|LOC100133331	NR_028322.1|NR_028325.1|NR_028327.1
	intronic_nc|intronic_nc|intronic_nc ||	||	||	||	||	||	||	||
1	985955	SNV	G	1	G/C	AGRN	NM_198576.3	exonic	missense	CGG	29	p.Gly1709Arg
	c.5125G>C	0.0	1.0	125.0	pathogenic	rs199476396	clustering of voltage-gated sodium channels:axon guidance:extracellular
space:neurotransmitter receptor metabolic process:structural constituent of cytoskeleton:cell surface:laminin binding:extracellular region:G-protein coupled
acetylcholine receptor signaling pathway:synapse organization:signal transduction:receptor clustering:extracellular matrix:protein binding:plasma membrane
organization:positive regulation of neuron apoptosis:acetylcholine receptor regulator activity:synapse assembly:positive regulation of transcription from RNA
polymerase II promoter:regulation of synaptic growth at neuromuscular junction:synapse:basal lamina	Agrin EGF-like domain:SEA domain:Laminin G domain:Kazal-type
serine protease inhibitor domain:Laminin EGF-like (Domains III and V):Agrin NtA domain	2.04
1	2488068	CNV	118059986	1p36.32p12(2488068-120548054)x2	0	143.56	LOC100133445|TTC34|FLJ42875|MIR4251
|MIR551A|TP73-AS1|LOC388588|LOC100133612|LOC728716|LOC284661|MIR4417|MIR4689|MIR4252|PLEKHG5|ENO1-AS1|MIR34A|C1orf200|MTOR-AS1|CLCN6|NPPA-AS1|MIR4632
|SNORA59B|SNORA59A|PRAMEF3|PRAMEF15|PRAMEF9|LRRC38|C1orf126|FLJ37453|CLCNKB|CROCCP3|MIR3675|NBPF1|CROCCP2|MST1P2|ESPNP|MST1P9|MIR4695|AKR7L|LOC100506730|LOC339505
|LOC100506801|LINC00339|MIR4684|MIR4253|LOC729059|MIR3115|C1orf213|MDS2|LOC100506963|MIR378F|PNRC2|LOC284632|RCAN3AS|LOC646471|MIR3917|TRIM63|MIR1976|NUDC|SLC9A1
|LOC644961|SCARNA1|SNHG3|SNHG12|SNORD99|SNORA61|SNORA44|SNORA16A|RNU11|LOC100129196|MIR4420|SNORD103A|SNORD103B|SNORD85|PRO0611|LOC149086|LOC284551|MIR4254
|MTMR9LP|LOC100128071|MIR3605|LOC402779|LOC653160|MIR4255|LOC728431|MIR5581|SNIP1|CDCA8|MIR3659|LOC339442|GJA9-MYCBP|PPIEL|SNORA55|ZMPSTE24|LOC100130557
|MIR30E|MIR30C1|CITED4|LOC100507178|LOC100129924|FLJ32224|LOC100132774|SLC6A9|MIR5584|SNORD55|SNORD46|SNORD38A|SNORD38B|EIF2B3|LOC400752|CCDC163P|RPS15AP10
|LOC729041|LOC100507423|LOC100130197|CYP4Z2P|PDZK1IP1-AS1|MGC12982|LOC388630|SKINTL|MIR761|TXNDC12|LOC100507564|SLC25A3P1|MIR4781|HEATR8-TTC4|LOC100507634|MIR4422
|LOC100131060|HSD52|MIR4711|LINC00466|DLEU2L|MIR4794|MIR3671|MIR101-1|DNAJC6|MIR3117|GNG12-AS1|MIR1262|PIN1P1|ZRANB2-AS1|MIR186|ZRANB2-AS2|NEGR1-IT1
|SNORD45C|SNORD45A|SNORD45B|MGC27382|MIR548AP|UOX|C1orf180|MIR4423|LOC646626|CLCA3P|LOC339524|LOC100505768|GBP1P1|FLJ27354|GEMIN8P4|HSP90B3P|SNORD21|SNORA66
|LOC100131564|BCAR3|LOC100129046|MIR760|SLC44A3|LOC729970|FLJ31662|DPYD-AS1|MIR137HG|MIR2682|MIR137|LOC729987|LOC100129620|MIR548D1|MIR548AA1|MIR553|LOC100128787
|EXTL2|RNU6-6|DNAJA1P5|ACTG1P4|LOC100129138|VAV3-AS1|SLC25A24|NBPF6|AKNAD1|SRG7|SCARNA2|MIR197|AMPD2|LOC440600|CYMP|RP11-165H20.1|CHIA|PGCP1|LOC100129269
|LOC100506343|MIR4256|AKR7A2P1|LOC643441|LOC100287722|ATP1A1OS|MIR320B1|MIR942|HSD3BP4|LOC644242

1	2615740	SNV,MNV	GGTCG	4	GAACC/GGTTG	TTC34	NM_001242672.1	intronic
	rs71490543:rs80062728:rs76497889:rs76926500	binding	Tetratricopeptide repeat	0.5,-1.76,-
2.75,0.5,-1.85
1	2706398	NOCALL	C	./.	TTC34	NM_001242672.1	upstream
	rs113477241	binding	0.73
1	3334486	SNV	C	1	T/T	PRDM16	NM_022114.3	exonic	missense	CTC	11	p.Pro929Leu
	c.2786C>T	0.0	0.002	98.0	AMAF=0.001:EMAF=0.0:GMAF=3.0E-4	untested	malignant_melanoma	rs145632008	tongue development:zinc ion
binding:somatic stem cell maintenance:positive regulation of transcription, DNA-dependent:transcriptional repressor complex:metal ion binding:transcription
coactivator activity:palate development:negative regulation of transcription from RNA polymerase II promoter:negative regulation of granulocyte
differentiation:regulation of cellular respiration:protein binding:neurogenesis:sequence-specific DNA binding:white fat cell differentiation:brown fat cell
differentiation:SMAD binding:negative regulation of transcription, DNA-dependent:intracellular:negative regulation of transforming growth factor beta receptor
signaling pathway:nucleus	PR domain-containing protein 16	Zinc finger 2.29
1	7527892	SNV	C	1	G/A	CAMTA1	NM_015215.2	exonic	missense|nonsense	TGG|TGA	6
	p.Cys147Trp|p.Cys147*	c.441C>G|c.441C>A	0.0|	0.968|	215.0|	calmodulin binding:regulation of transcription,
DNA-dependent:cytoplasm:nucleus	Calmodulin-binding transcription activator 1	IPT/TIG domain:CG-1 domain	2.25
1	7804961	INDEL	T	2	TAA/TAA	CAMTA1	NM_015215.2	exonic	nonsense	TAA	17	p.Phe1417*
	c.4249_4250insAA	calmodulin binding:regulation of transcription, DNA-dependent:cytoplasm:nucleus
	Calmodulin-binding transcription activator 1	IPT/TIG domain:CG-1 domain	2.06
1	7826549	INDEL	T	1	T/TC	CAMTA1	NM_015215.2	exonic	stoploss	TCG	23	p.*1674Ser	c.5020
_5021insC	calmodulin binding:regulation of transcription, DNA-dependent:cytoplasm:nucleus	Calmodulin-
```

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.

```
1  Name: variantdbA_v1    Version: v1
2  Name: variantdbA_v2    Version: v2
3  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 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 made by the VariantCaller. For example, a VariantCaller quality score of 20 is associated with a p-value of 0.01. A 30 is associated with a p-value is 0.001.

Pfam annotations

Ion Reporter™ Software has three Pfam versions: version 26, version 30 and version 31. Refer to the following website 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

Note: Pfam annotations for all domains of the gene's protein are added for any variant.

IMPORTANT!

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 the corresponding RefSeq or Ensembl® gene models:

- Functional scores v7 is for the gene model versions RefSeq 83 and Ensembl® 90.
- Functional Scores v6 is for the gene model versions RefSeq 77 and Ensembl® 84.
- Functional Scores v5 is for the gene model versions RefSeq 72 and Ensembl® 79.
- Functional Scores v4 is for the gene model versions RefSeq 63 and Ensembl® 74.
- Functional scores v3 is for the gene model versions 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 613105134	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

Ion Reporter™ Software uses 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. To view gene annotation on chrM use the ENSEMBL gene model, which does have chrM genes.

More information is available at: **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:

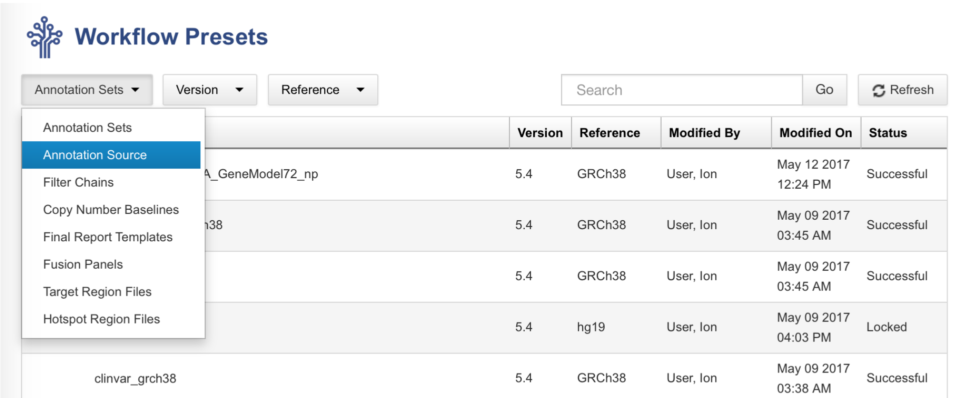
- 0.0 to 0.05 -- Variants with scores in this range are considered deleterious. Variants with scores closer to 0.0 are more confidently predicted to be deleterious.
- 0.05 to 1.0-- Variants with scores in this range are predicted to be tolerated (benign). Variants with scores very close to 1.0 are more 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:

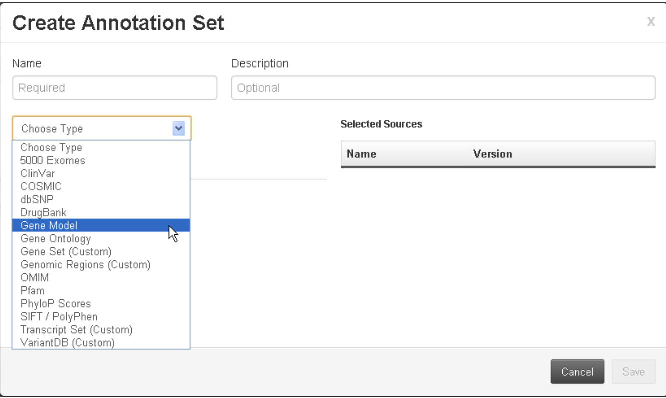
- RefGene
- Functional
- Canonical
- Transcript Scores

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 332.

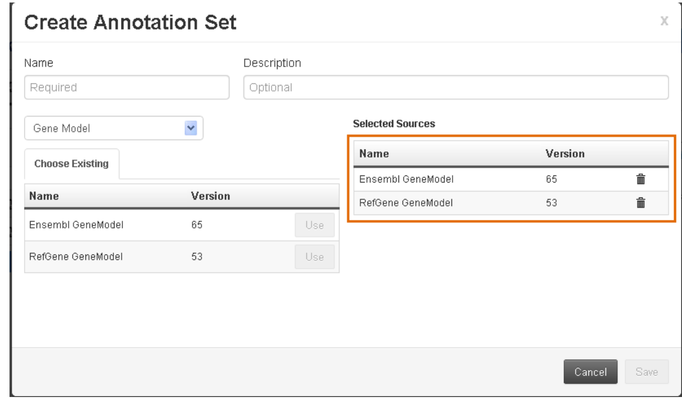
Create annotation set without preferred transcript sets

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 for Oncomine™ Assays contain the Oncomine™ Variant Annotator Plugin. The plugin integrates data from more than 24,000 exomes across solid tumor and hematological cancer types, and annotates driver mutations with Oncomine™ Gene Class and Oncomine™ Variant Class information. Contact your local Support representative, Field Bioinformatics Specialist (FBS), or Clinical Account Consultant (CAC) for more information.

Note: Do not use the Oncomine™ Variant Annotator 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:

- Panels: Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2 and Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel
- Panels: Custom Ion AmpliSeq™ and Ion AmpliSeq™ HD fusion panels designed by Ion Torrent White Glove team and Ion AmpliSeq™ Designer.
- Oncomine Fusion panels for solid tumor, liquid biopsy, heme oncology, and immuno oncology research applications.

For information on importing fusion panels, see "Import panel files from AmpliSeq.com" on page 85.

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. Example : If count of a target is >20, the target is called Present.

Parameter Name	UI Group	Allowed Values	Default Value	Description
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.
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.

Parameter Name	UI Group	Allowed Values	Default Value	Description
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.
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.
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.
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 normalizatio n	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.

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum Molecular Family Consensus Size	Advanced	Integers only	2	Minimum number of reads with same tag required to form a functional family. Suggested value between 3 and 7. Impact: Increasing values make variant calls less sensitive but more specific.
Minimum Molecular Family Count	Advanced	Integers only	2	Minimum number of variant supporting functional families required to make a call. Suggested value between 2 and 10. Impact: Increasing values make calls less sensitive but more specific.
Minimum Family Coverage per Strand	Advanced	Integers only	1	Minimum required coverage of reads on each strand in a bi-directional molecular tag family.
Minimum Number Of PC Amplicons Required To Pass QC	Advanced	Integers only	2	Minimum number of process (or expression) control amplicons containing equal or more families than fusions.min.fam.count required to pass quality control.
Minimum Number Of PC Amplicons Required To Fail QC	Advanced	Integers only	1	Maximum number of process (or expression) control amplicons containing equal or more families than fusions.min.fam.count required to FAIL quality control.

Parameter Name	UI Group	Allowed Values	Default Value	Description
Minimum read counts for RNAExonVariants	Advanced	Integers only	20	Minimum number read counts for RNAExonVariant targets to be called as PRESENT. This value is used only in cases where present/absent calls are made for RNAExonVariant targets.
Minimum Molecular Family Count for RNAExonVariant	Advanced	Integers only	2	Minimum number of variant supporting functional families required to make a call for RNAExonVariant targets. This value is used only in the cases where present/absent calls are made 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.
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.

Parameter	Description
Min WT Ratio	This column allows users to set custom threshold for minimum wild type ratio for RNAExonVariants at individual target level.
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 343 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.

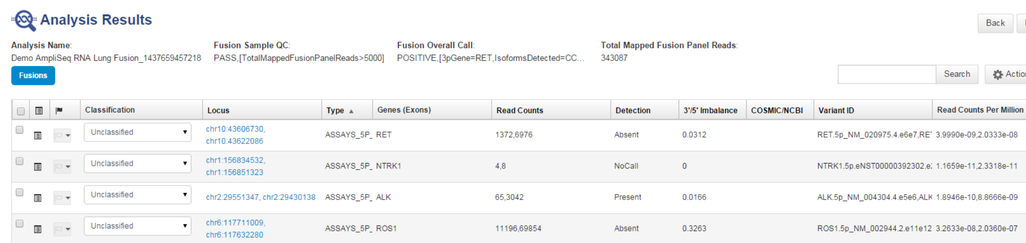
Fusion sample type

When performing a fusion analysis, the sample type attribute must be set to **RNA**. See "Import and manage samples" on page 31.

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:

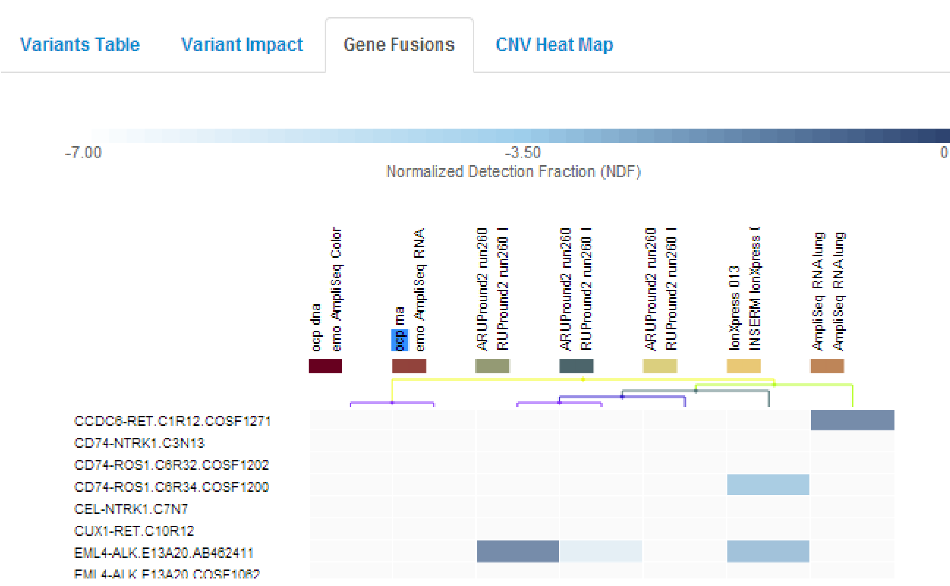


Analysis Results

Analysis Name: Demo AmpliSeq RNA Lung Fusion_1437659457218
Fusion Sample QC: PASS,[TotalMappedFusionPanelReads>5000]
Fusion Overall Call: POSITIVE,[3pGene=RET,IsoformsDetected=CC...
Total Mapped Fusion Panel Reads: 343087

Classification	Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID	Read Counts Per Million
Unclassified	chr10:43606730, chr10:43622086	ASSAYS_5P_	RET	1372,6976	Absent	0.0312		RET.5p_NM_020975.4.e6e7,RE	3.9990e-09,2.0333e-08
Unclassified	chr1:156834532, chr1:156851323	ASSAYS_5P_	NTRK1	4,8	NoCall	0		NTRK1.5p.eNST00000392302.e	1.1659e-11,2.3318e-11
Unclassified	chr2:29551347, chr2:29430138	ASSAYS_5P_	ALK	65,3042	Present	0.0166		ALK.5p_NM_004304.4.e5e6,ALK	1.8946e-10,8.8666e-09
Unclassified	chr6:117711009, chr6:117632280	ASSAYS_5P_	ROS1	11196,69854	Absent	0.3263		ROS1.5p_NM_002944.2.e11e12	3.2633e-08,2.0360e-07

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:

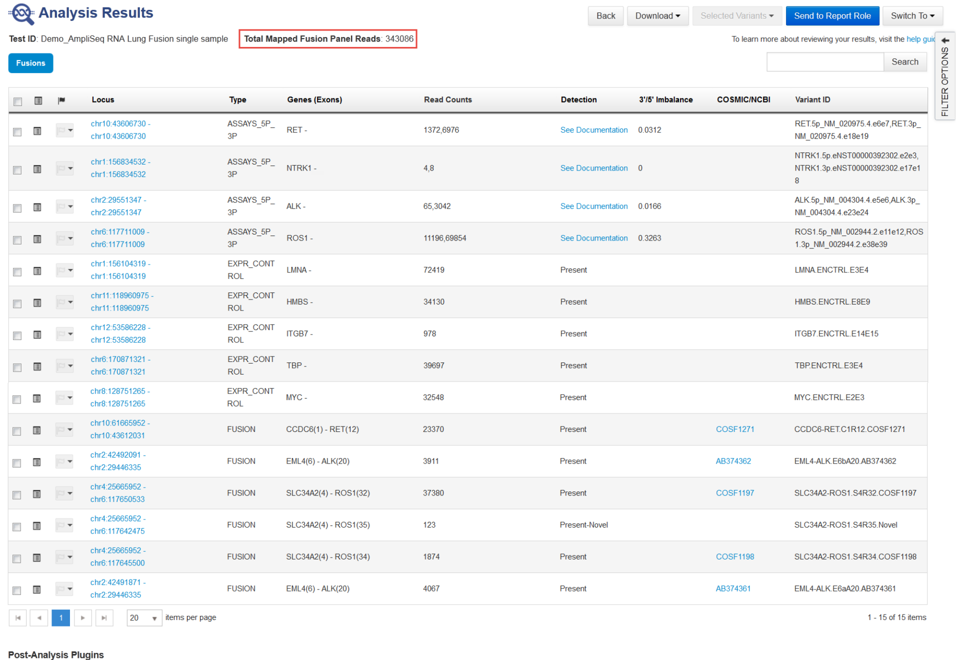
- ASSAYS_5P_3P: These assays provide confirmation for the fusion calls, through the values in the 3'/5' Imbalance column.
- EXPR_CONTROL: Few expression control assays are built into the panel to confirm the success of the sequencing run that the analysis is based on.
- GENE_EXPRESSION: These assays 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.
- ProcessControl: A few control assays are built into the TagSeq and Ion AmpliSeq™ HD panels to help determine if the sample is valid or not, similar to the Expression control assays

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.

Process Controls for TagSeq and Ion AmpliSeq™ HD research panels only

For TagSeq and Ion AmpliSeq™ HD panels only, sample QC is also dependent on the expression of the Process controls. Depending on the number of process control (PC) assays that are detected to be PRESENT in the sample, the sample QC is either qualified as PASS, FAIL, or WARN.

If the number of PC assays Present is greater than the value specified by the `Minimum Number Of PC Amplicons Required To Pass QC` parameter, the sampleQC is PASS.

If the number of PC assays present is less than the value specified by the `Minimum Number Of PC Amplicons Required To Pass QC` parameter, but greater than the value specified by the `Maximum Number Of PC Amplicons Required To Fail QC` parameter, the sampleQC is WARN.

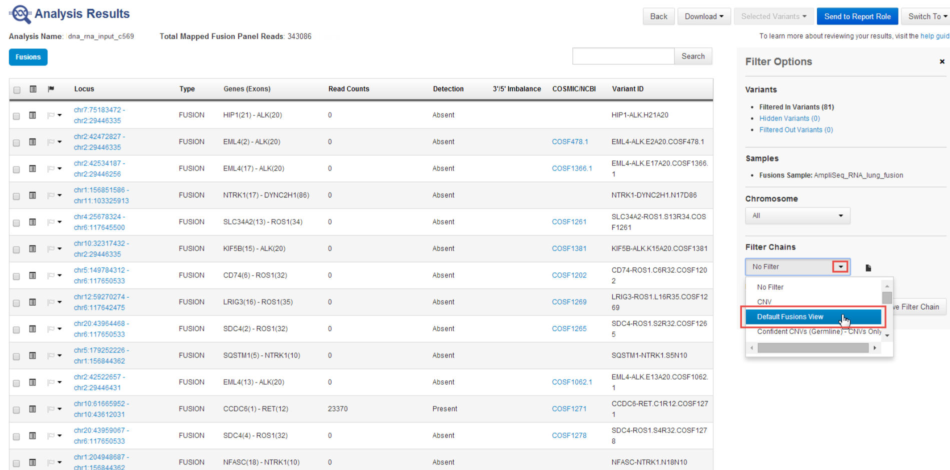
If the number of PC assays present is less than the value specified by the `Maximum Number Of PC Amplicons Required To Fail QC` parameter, the sampleQC is FAIL.

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.

In the case of TagSeq and Ion AmpliSeq™ HD workflows: To make **PRESENT/ABSENT** calls, Minimum molecular count threshold (default value : >=3) is applied for each target along with the minimum read count threshold (default value > =21).

"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):

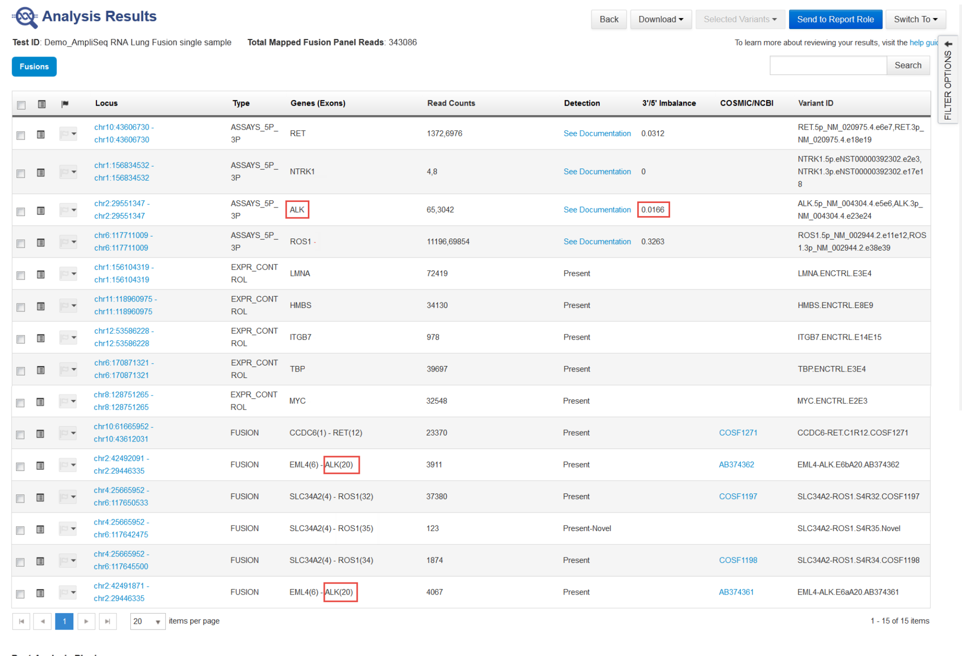
- **Ratio to Wild Type**
  - For all the RNAExonVariant assays that belong to a Gene with at-least one RNAExonVariant type assay. It is designed to amplify the wild type transcript of that gene and report this value.
  - Ratio to Wild type of a RNAExonVariant Vi = (Read count of Vi) / (Mean read count 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).



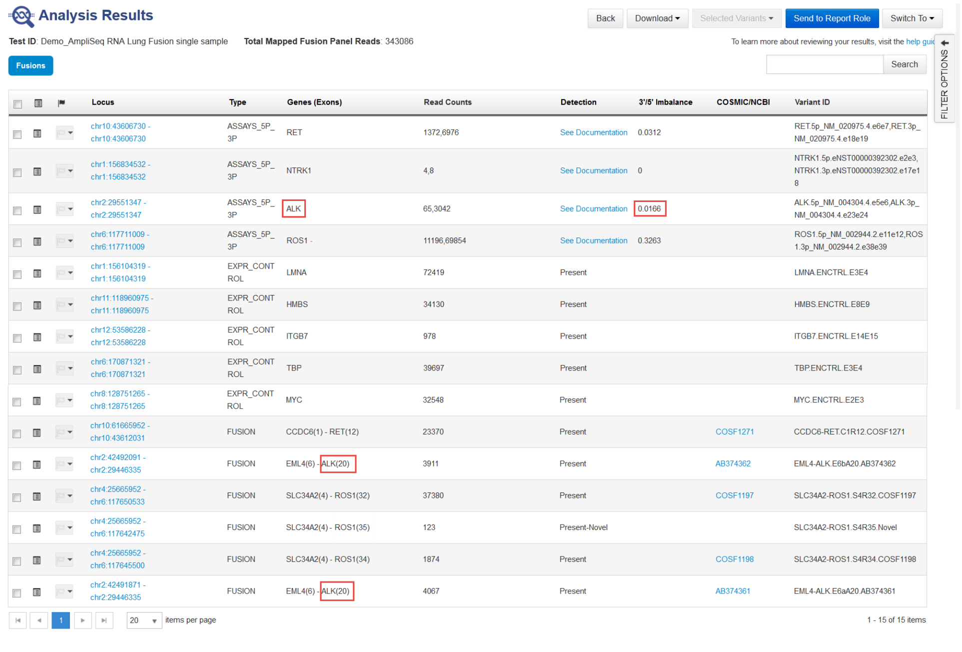
(The **Genes (Exons)** column does not report exon numbers for assays rows.)

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.



Analysis Results

Back | Download | Selected Variants | Send to Report Role | Switch To

Test ID: Demo_AmpliSeq RNA Lung Fusion single sample **Total Mapped Fusion Panel Reads**: 343086

To learn more about reviewing your results, visit the help guid

Fusions | Search | FILTER OPTIONS

Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID
chr10:43606730 - chr10:43606730	ASSAYS_5P_3P	RET	1372,6976	See Documentation	0.0312		RET.5p_NM_020975.4.e6e7,RET.3p_NM_020975.4.e18e19
chr1:156834532 - chr1:156834532	ASSAYS_5P_3P	NTRK1	4,8	See Documentation	0		NTRK1.5p.eNST00000392302.e2e3,NTRK1.3p.eNST00000392302.e17e18
chr2:29551347 - chr2:29551347	ASSAYS_5P_3P	ALK	65,3042	See Documentation	0.0166		ALK.5p_NM_004304.4.e5e6,ALK.3p_NM_004304.4.e23e24
chr6:117711009 - chr6:117711009	ASSAYS_5P_3P	ROS1 -	11196,69854	See Documentation	0.3263		ROS1.5p_NM_002944.2.e11e12,ROS1.3p_NM_002944.2.e38e39
chr1:156104319 - chr1:156104319	EXPR_CONTROL	LMNA	72419	Present			LMNA.ENCTRL.E3E4
chr11:118960975 - chr11:118960975	EXPR_CONTROL	HMBS	34130	Present			HMBS.ENCTRL.E8E9
chr12:53586228 - chr12:53586228	EXPR_CONTROL	ITGB7	978	Present			ITGB7.ENCTRL.E14E15
chr6:170871321 - chr6:170871321	EXPR_CONTROL	TBP	39697	Present			TBP.ENCTRL.E3E4
chr8:128751265 - chr8:128751265	EXPR_CONTROL	MYC	32548	Present			MYC.ENCTRL.E2E3
chr10:61665952 - chr10:43612031	FUSION	CCDC6(1) - RET(12)	23370	Present		COSF1271	CCDC6-RET.C1R12.COSF1271
chr2:42492091 - chr2:29446335	FUSION	EML4(6) - ALK(20)	3911	Present		AB374362	EML4-ALK.E6bA20.AB374362
chr4:25665952 - chr6:117650533	FUSION	SLC34A2(4) - ROS1(32)	37380	Present		COSF1197	SLC34A2-ROS1.S4R32.COSF1197
chr4:25665952 - chr6:117642475	FUSION	SLC34A2(4) - ROS1(35)	123	Present-Novel			SLC34A2-ROS1.S4R35.Novel
chr4:25665952 - chr6:117645500	FUSION	SLC34A2(4) - ROS1(34)	1874	Present		COSF1198	SLC34A2-ROS1.S4R34.COSF1198
chr2:42491871 - chr2:29446335	FUSION	EML4(6) - ALK(20)	4067	Present		AB374361	EML4-ALK.E6aA20.AB374361

1 | 20 items per page | 1 - 15 of 15 items

Post-Analysis Plugins

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.

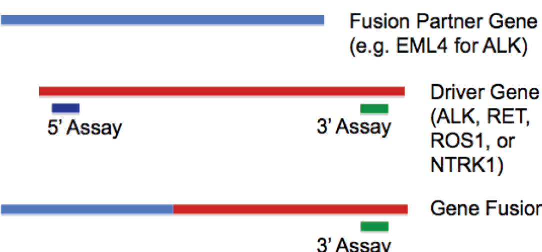
  Note: Imbalance values provide information that is supplementary to the Detection calls of "Present" or "Absent". If a fusion call is "Absent", then we recommended that you check the Imbalance value to see if there is supplemental evidence of the presence of a fusion.

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:

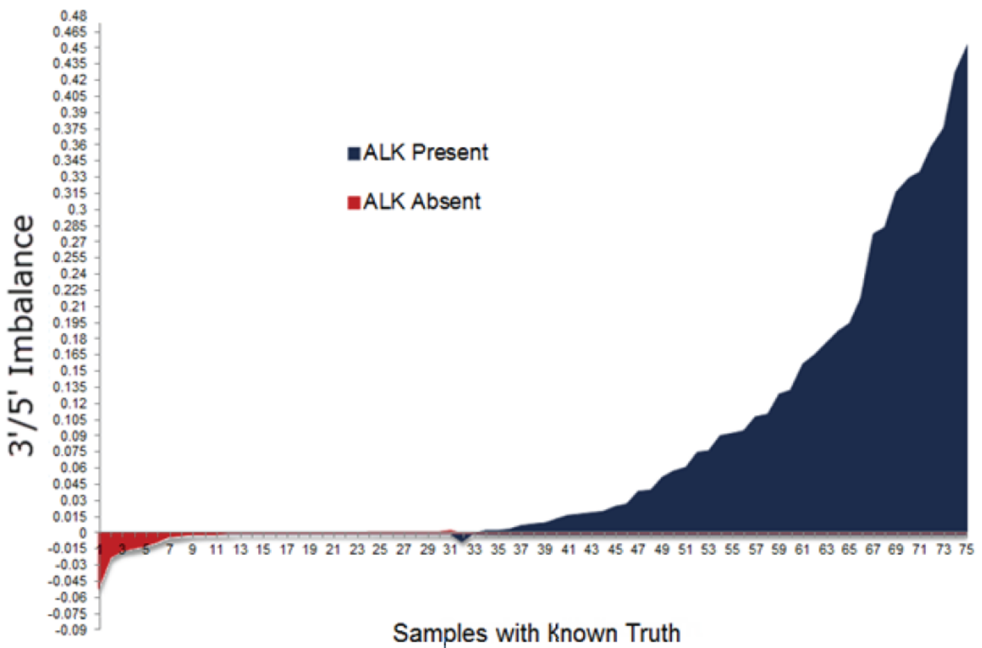
- In normal samples (without a fusion), imbalance values are very low (either close to zero or negative).
- In samples that contain a fusion, imbalance values are higher, with gene-specific thresholds shown in the above table.

In some cases, lower imbalance values are also good predictors of the presence of fusions, as shown in the plots linked below.

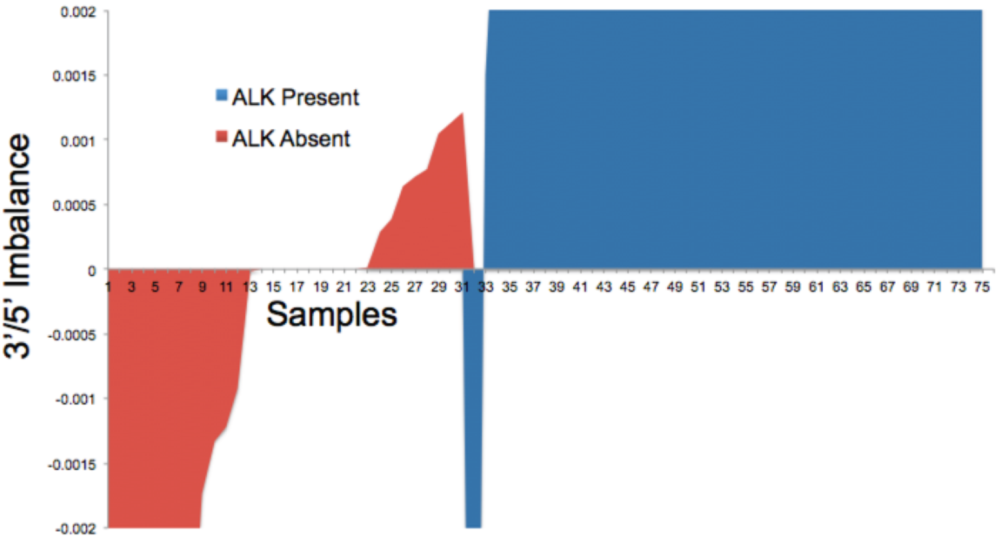
Note: These guidelines apply to samples that meet the criteria described in the sections: Minimum number of reads, Sensitivity, and High 5' expression. The values in the plots below have been based on results obtained from 75 FFPE lung cancer research samples previously tested with other technologies 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.



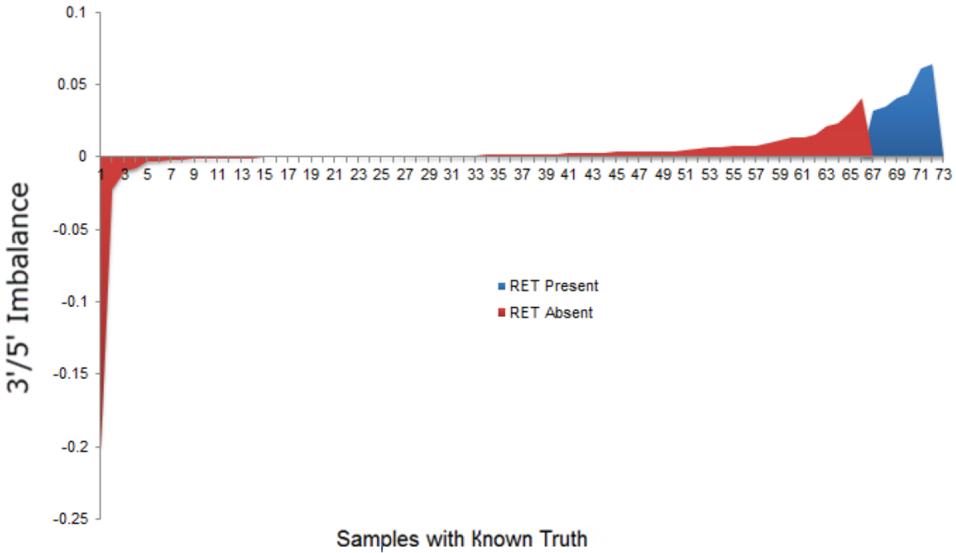
Zoomed in view:



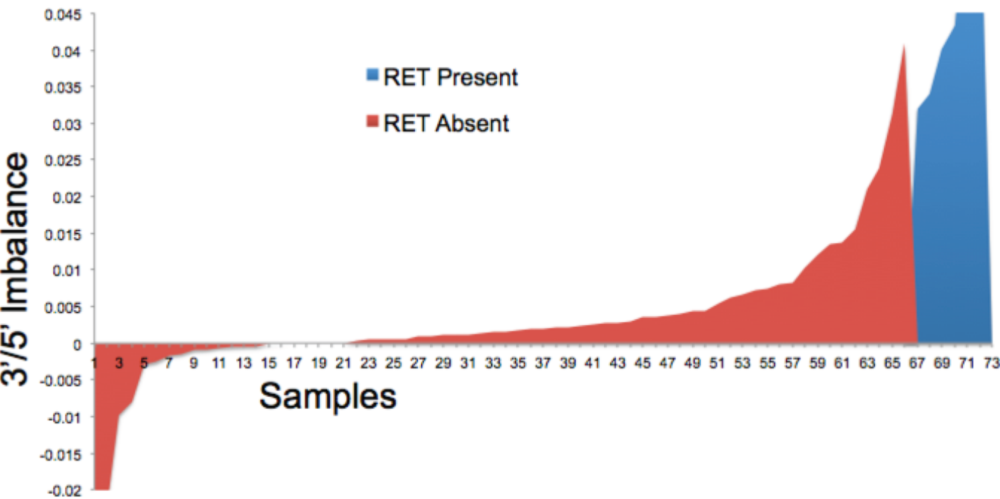
- A 3'/5' Imbalance value of 0.025 or greater indicates the likely presence of an ALK fusion.
- Below 0.001, there is no evidence that an ALK fusion is present.
- The range of 3'/5' Imbalance values between 0.001 and 0.025 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance values, the more evidence there is that a fusion is present.

Example plot for RET driver gene

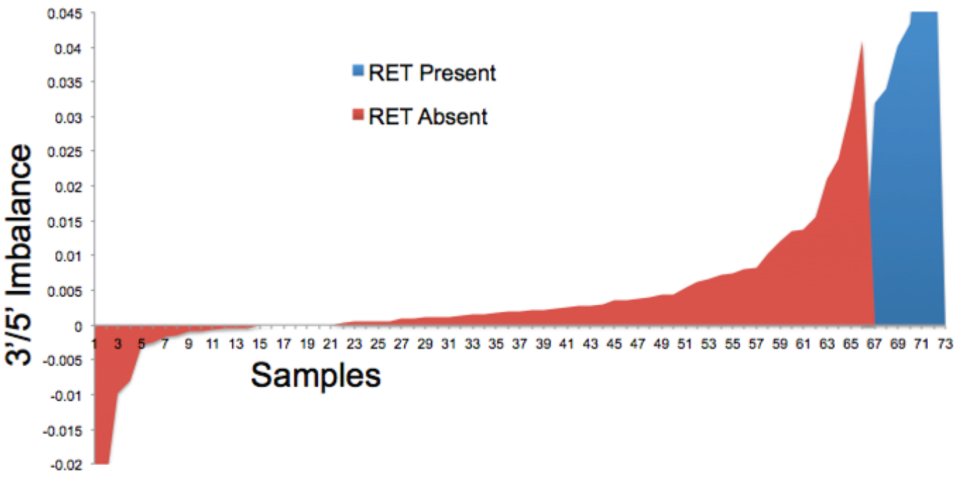
The image linked here shows the correlation between low imbalance values and samples that do not contain a fusion (in red). Samples that contain a fusion (in blue) all contain higher imbalance values.



Zoomed in view:



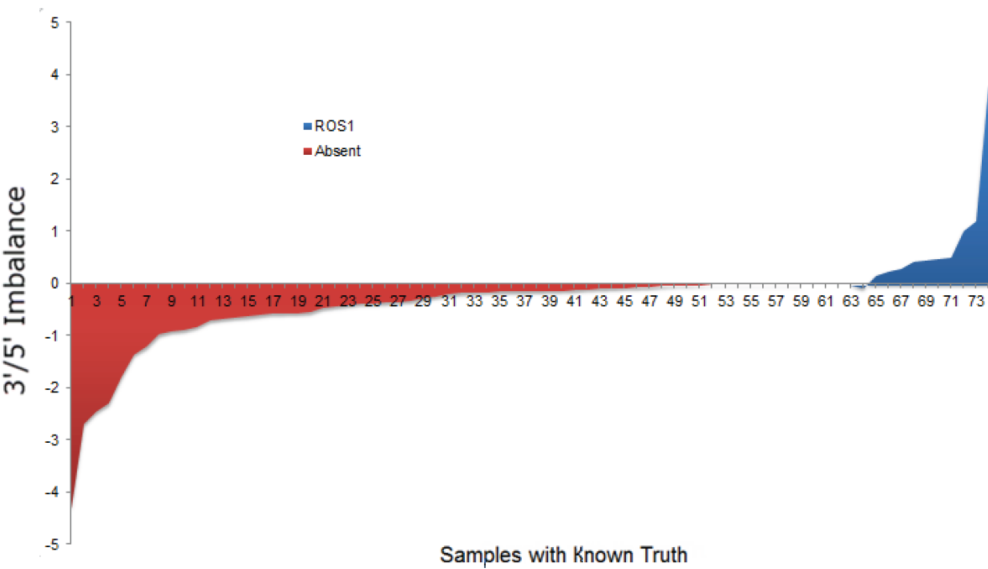
- A 3'/5' Imbalance value of 0.045 or greater indicates the likely presence of a RET fusion.



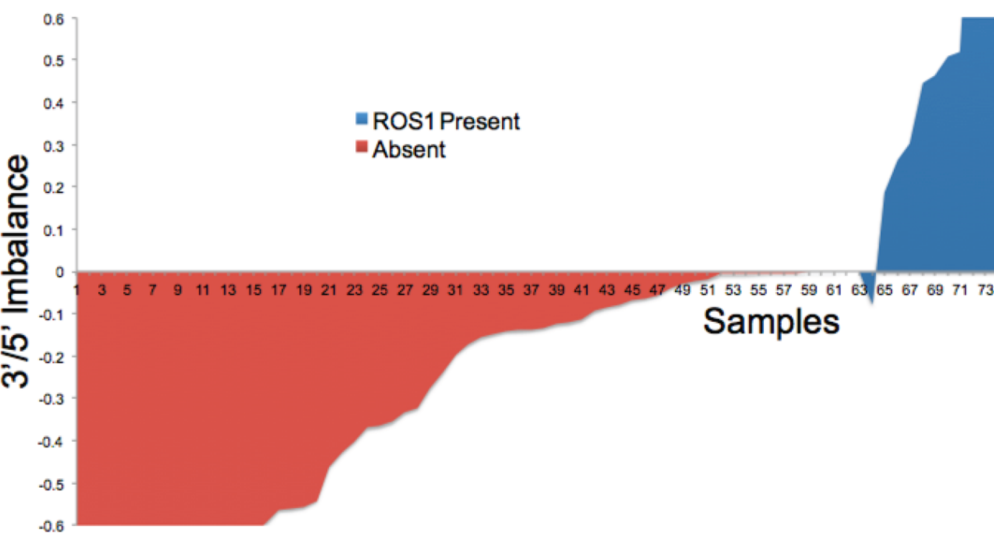
- With a 3'/5' Imbalance value of 0.03, there is no evidence that a RET fusion is present.
- The range of 3'/5' Imbalance values between 0.045 and 0.03 is an area of uncertainty where there is some evidence that a fusion may be present. The higher the imbalance values, the more evidence there is that 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.

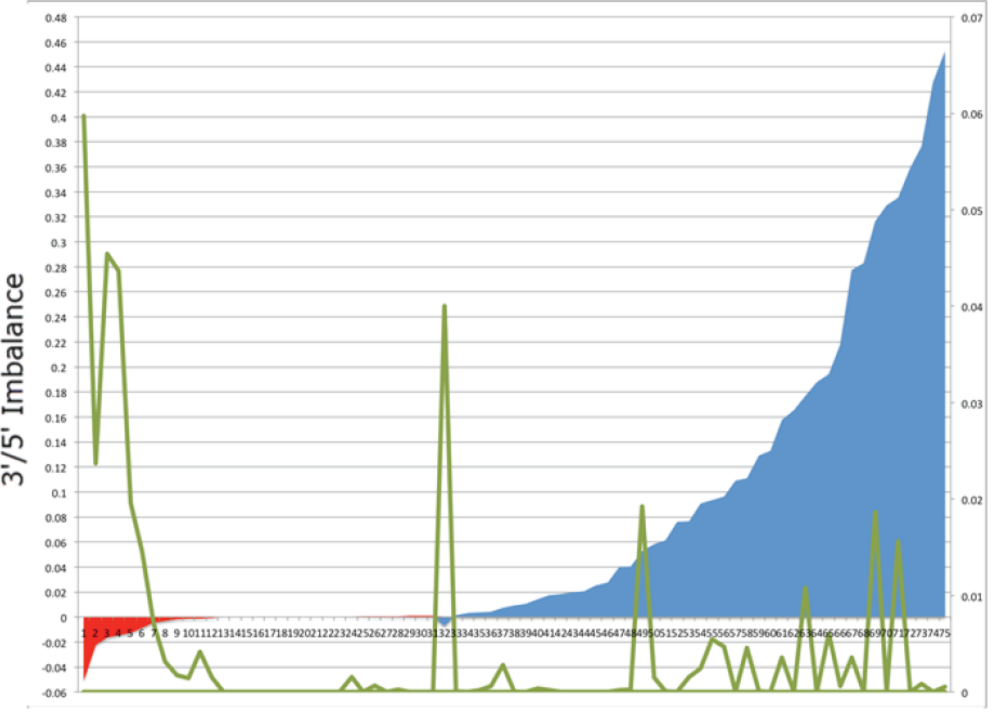
Please check for High 5' expression and also check for the Minimum number of reads before assuming that a fusion call of 'Present' is confirmed.

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:

Test ID: Demo_AmpliSeq RNA Lung Fusion single sample **Total Mapped Fusion Panel Reads**: 343086

Locus	Type	Genes (Exons)	Read Counts	Detection	3′/5′ Imbalance
chr10:43606730 - chr10:43606730	ASSAYS_5P_3P	RET	1372,6976	See Documentation	0.0312
chr1:156834532 - chr1:156834532	ASSAYS_5P_3P	NTRK1	4,8	See Documentation	0
chr2:29551347 - chr2:29551347	ASSAYS_5P_3P	ALK	65,3042	See Documentation	0.0166
chr6:117711009 - chr6:117711009	ASSAYS_5P_3P	ROS1 -	11196,69854	See Documentation	0.3263
chr2:42491871 - chr2:29446335	FUSION	EML4(6) - ALK(20)	4067	Present	
chr11:118960975 - chr11:118960975	EXPR_CONTROL	HMBS	34130	Present	
chr2:42492091 - chr2:29446335	FUSION	EML4(6) - ALK(20)	3911	Present	

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

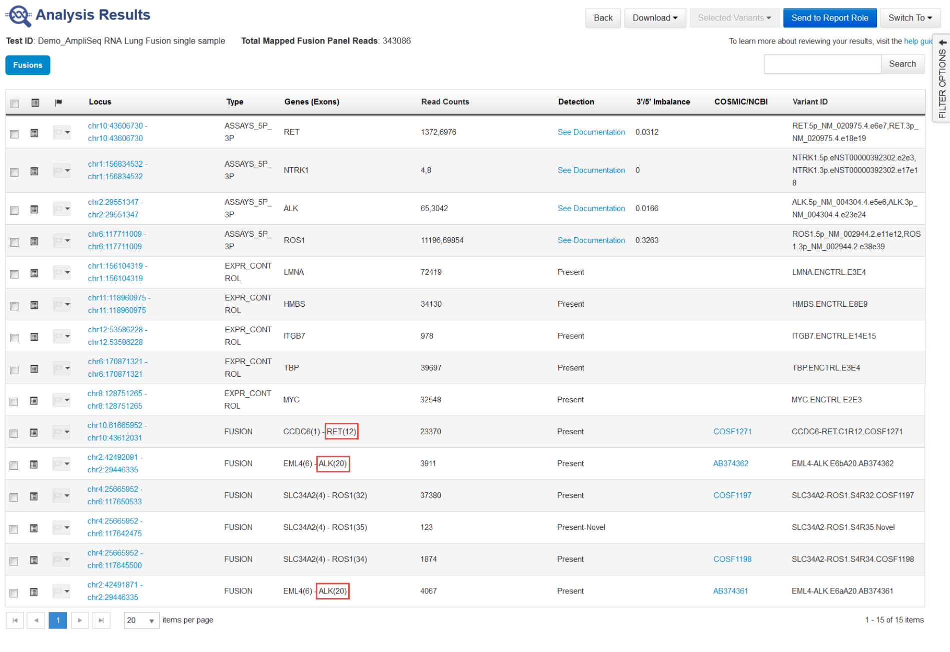
The analysis results provide several ways to confirm a fusion call, including Imbalance values, visualization, and Normalized Detection Fractions.

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.

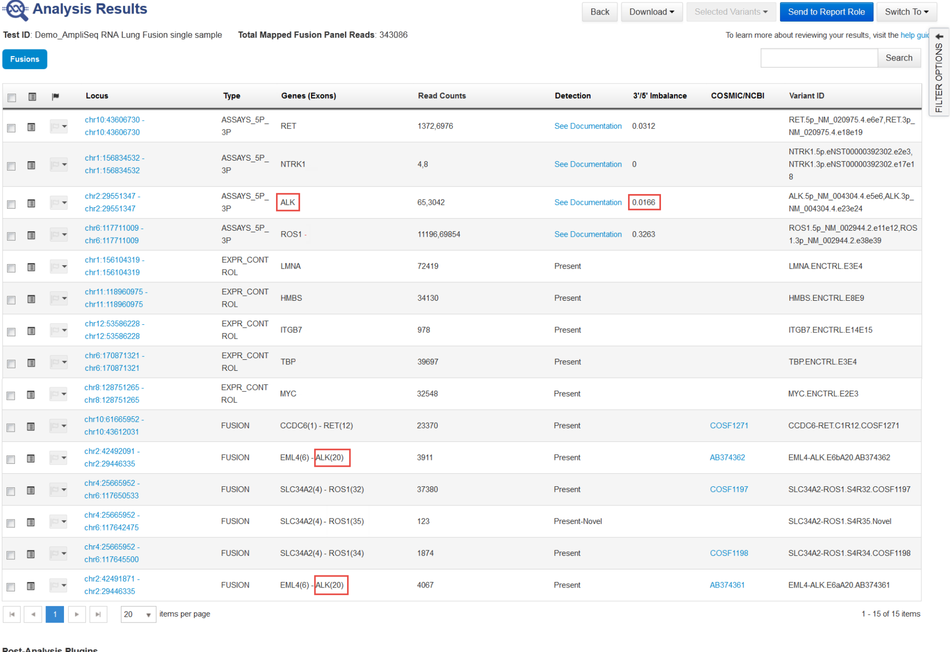
Follow these steps to find the Imbalance value for a specific fusion call:

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.



Analysis Results

Back | Download | Selected Variants | Send to Report Role | Switch To

Test ID: Demo_AmpliSeq RNA Lung Fusion single sample **Total Mapped Fusion Panel Reads**: 343086

To learn more about reviewing your results, visit the help guid

Fusions

Search

FILTER OPTIONS

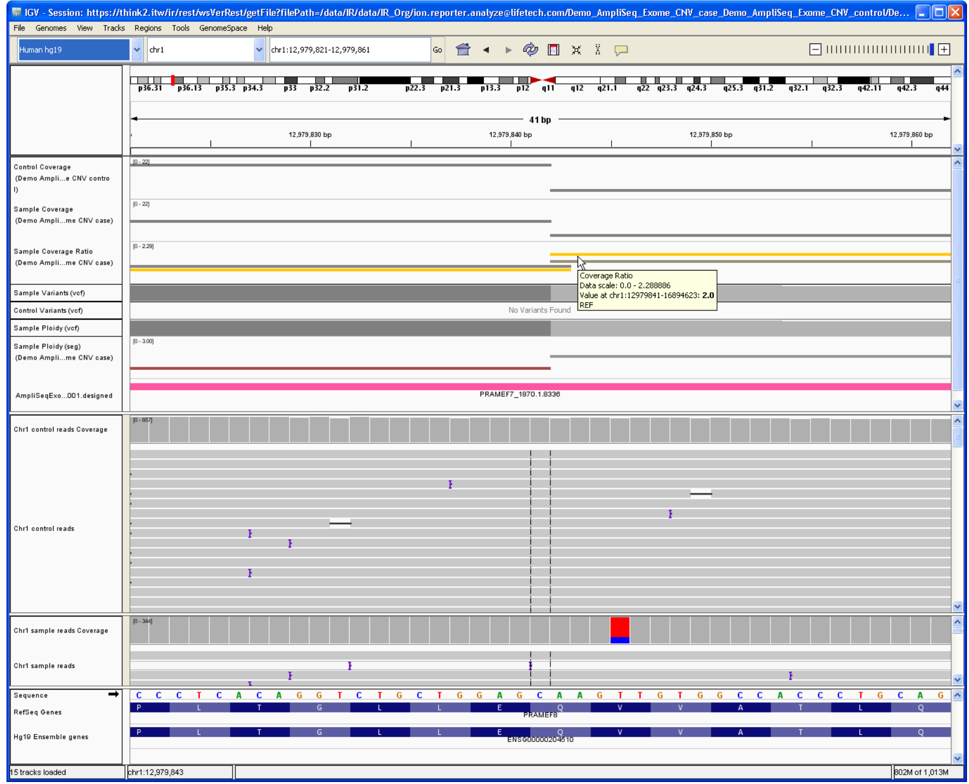
Locus	Type	Genes (Exons)	Read Counts	Detection	3'/5' Imbalance	COSMIC/NCBI	Variant ID
chr10:43606730 - chr10:43606730	ASSAYS_5P_3P	RET	1372,6976	See Documentation	0.0312		RET.5p_NM_020975.4.e6e7,RET.3p_NM_020975.4.e18e19
chr1:156834532 - chr1:156834532	ASSAYS_5P_3P	NTRK1	4,8	See Documentation	0		NTRK1.5p.eNST00000392302.e2e3, NTRK1.3p.eNST00000392302.e17e18
chr2:29551347 - chr2:29551347	ASSAYS_5P_3P	ALK	65,3042	See Documentation	0.0166		ALK.5p_NM_004304.4.e5e6,ALK.3p_NM_004304.4.e23e24
chr6:117711009 - chr6:117711009	ASSAYS_5P_3P	ROS1 -	11196,69854	See Documentation	0.3263		ROS1.5p_NM_002944.2.e11e12,ROS1.3p_NM_002944.2.e38e39
chr1:156104319 - chr1:156104319	EXPR_CONTROL	LMNA	72419	Present			LMNA.ENCTRL.E3E4
chr11:118960975 - chr11:118960975	EXPR_CONTROL	HMBS	34130	Present			HMBS.ENCTRL.E8E9
chr12:53586228 - chr12:53586228	EXPR_CONTROL	ITGB7	978	Present			ITGB7.ENCTRL.E14E15
chr6:170871321 - chr6:170871321	EXPR_CONTROL	TBP	39697	Present			TBP.ENCTRL.E3E4
chr8:128751265 - chr8:128751265	EXPR_CONTROL	MYC	32548	Present			MYC.ENCTRL.E2E3
chr10:61665952 - chr10:43612031	FUSION	CCDC6(1) - RET(12)	23370	Present		COSF1271	CCDC6-RET.C1R12.COSF1271
chr2:42492091 - chr2:29446335	FUSION	EML4(6) - ALK(20)	3911	Present		AB374362	EML4-ALK.E6bA20.AB374362
chr4:25665952 - chr6:117650533	FUSION	SLC34A2(4) - ROS1(32)	37380	Present		COSF1197	SLC34A2-ROS1.S4R32.COSF1197
chr4:25665952 - chr6:117642475	FUSION	SLC34A2(4) - ROS1(35)	123	Present-Novel			SLC34A2-ROS1.S4R35.Novel
chr4:25665952 - chr6:117645500	FUSION	SLC34A2(4) - ROS1(34)	1874	Present		COSF1198	SLC34A2-ROS1.S4R34.COSF1198
chr2:42491871 - chr2:29446335	FUSION	EML4(6) - ALK(20)	4067	Present		AB374361	EML4-ALK.E6aA20.AB374361

1 | 20 items per page | 1 - 15 of 15 items

Post-Analysis Plugins

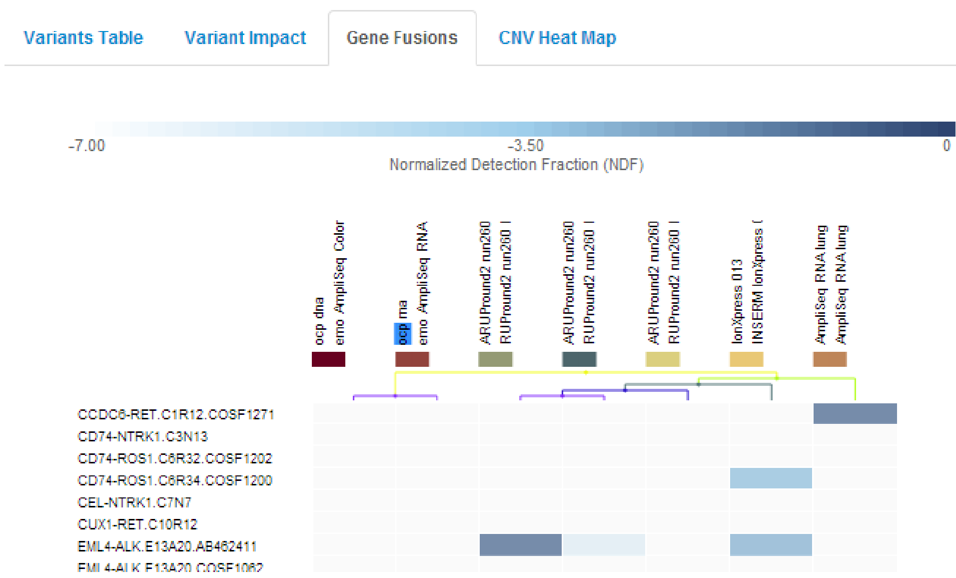
Visualize with IGV

Use IGV to evaluate both fusion calls and the expression controls.



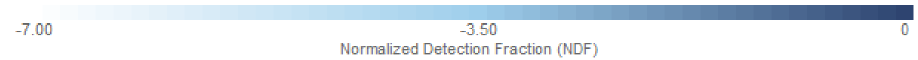
Visualize with heatmaps

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.

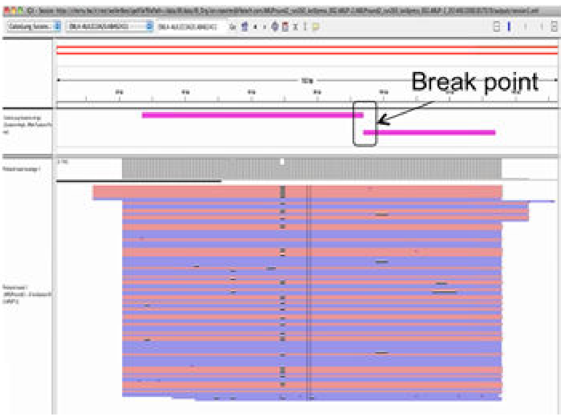


Normalized Detection Fractions are interpreted as follows:

- **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.

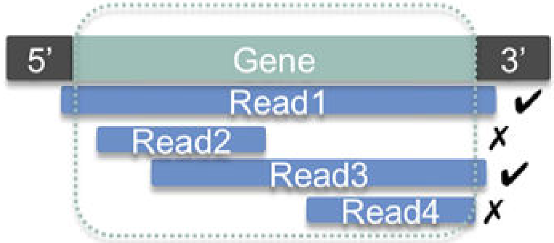
These are the characteristics of an IGV visualization of a strong fusion call:

- 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):



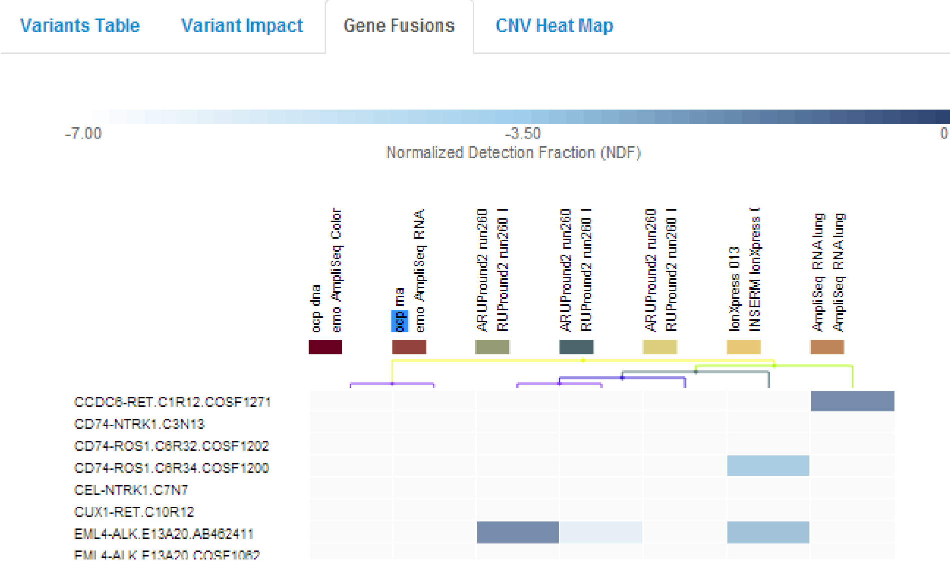
- What is the percent of overlap between the alignment and the insert?
- How good is the alignment in the overlapped region?

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.

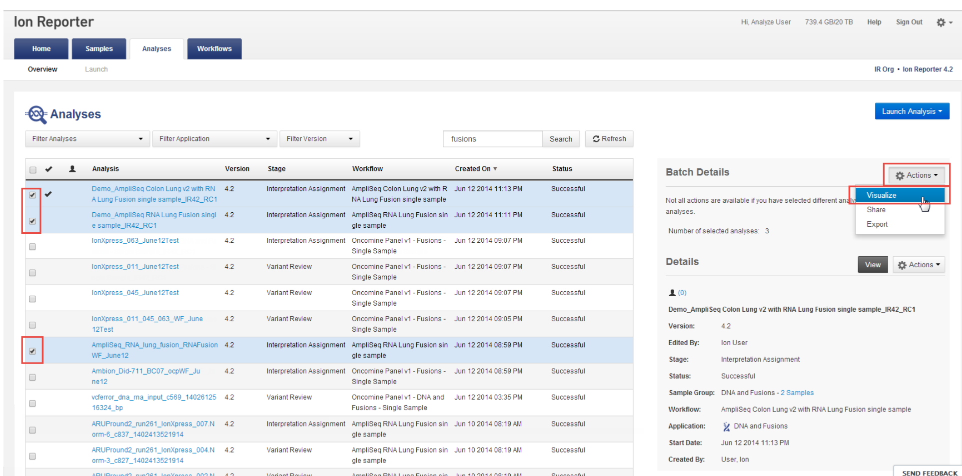
Visualize and compare fusion results of multiple analyses with heatmaps

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.



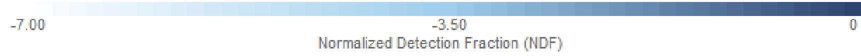
2. In the Action menu on the right, click the **Visualize** option.

   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.

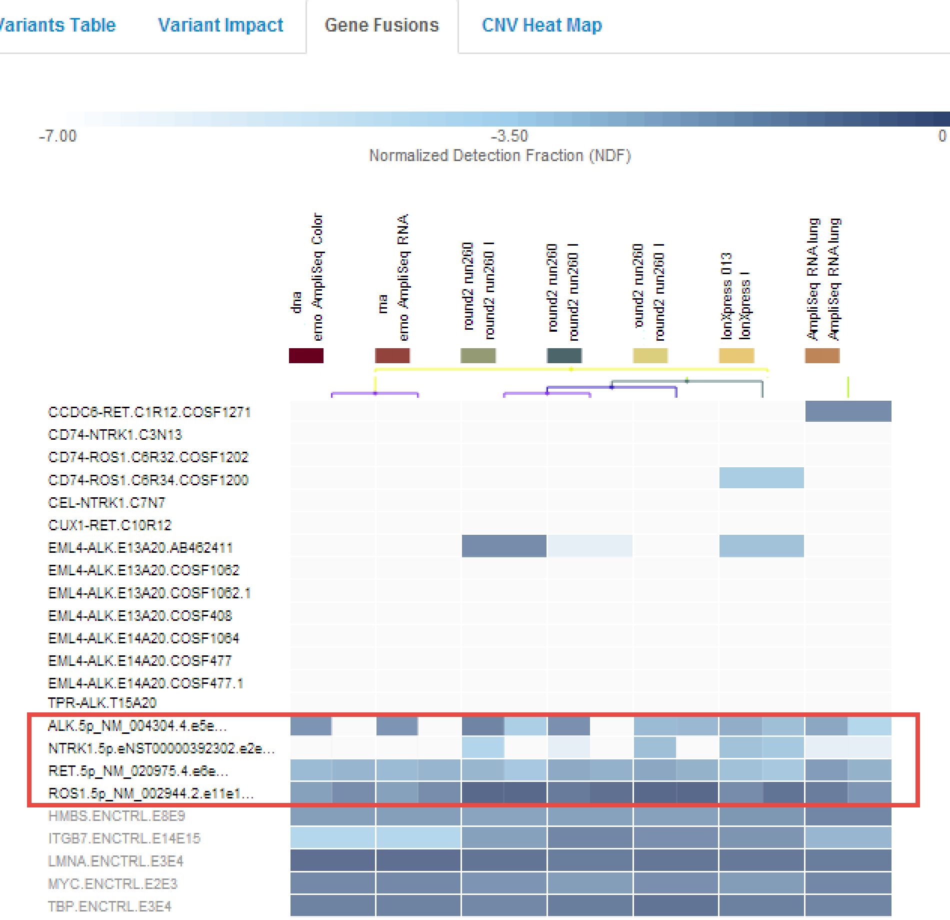


- Normalized Detection Fraction (NDF) values are calculated as follows (for a fusion F in sample S):

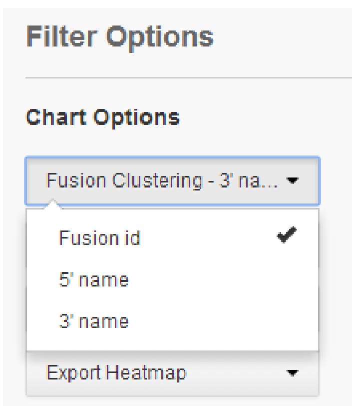
```
log10((read count of F) / (total read count in S))
```

- 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.

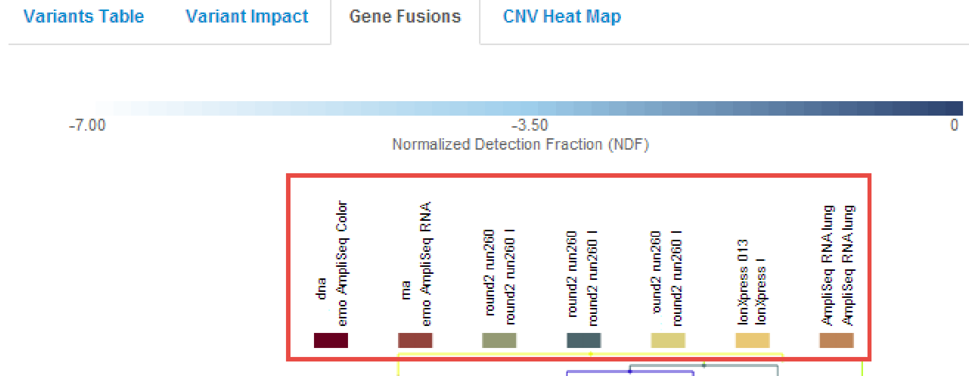
- By default, expression control and ASSAY_5P_3P calls are listed close to the bottom of the chart.



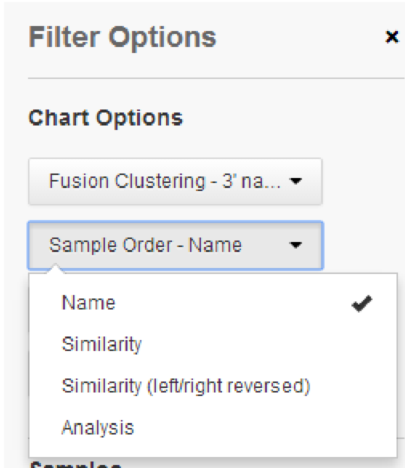
- You can change the order of the fusions (the list on the left side) through the Filter Order menu on the right (under Chart Options).



- Along the top, each sample is listed.



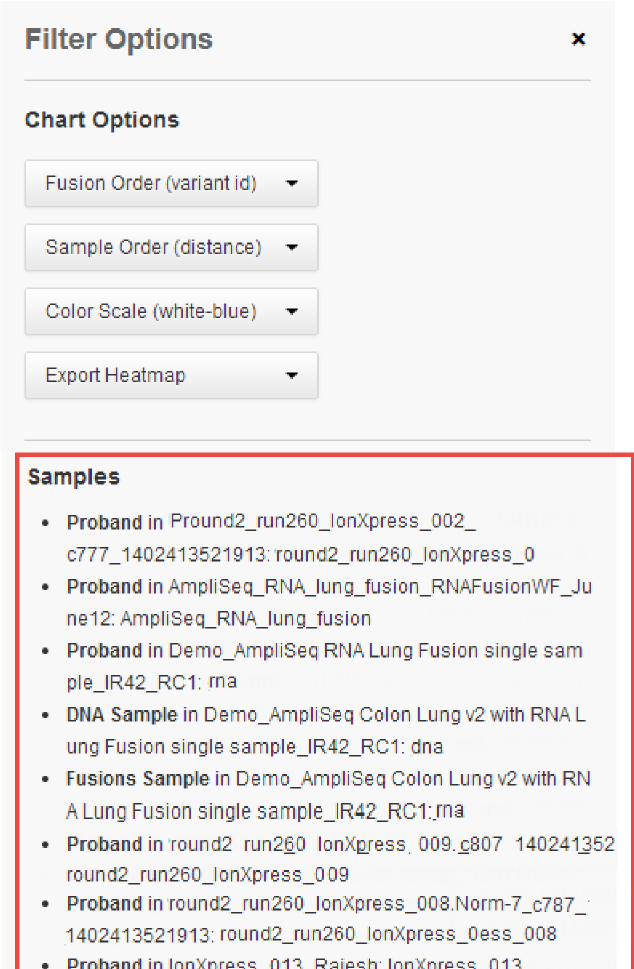
- You can change the order of the samples list (the list above the chart) through the Sample Order menu on the right (under Chart Options).



The following ordering options are supported:

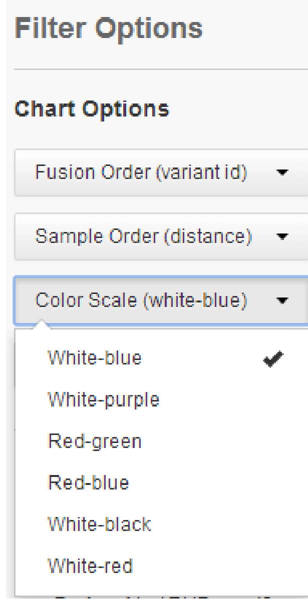
– **Name**: Alphabetically by sample name
– **Similarity**: By how similar the samples are in the fusion calls made (presented from fewest fusions to most fusions)
– **Similarity (reversed)**: By how similar the samples are in the fusion calls made (presented from the most fusions to the fewest fusions)
– **Analysis**: Alphabetically by analysis name

- Samples are also listed in the panel to the right.

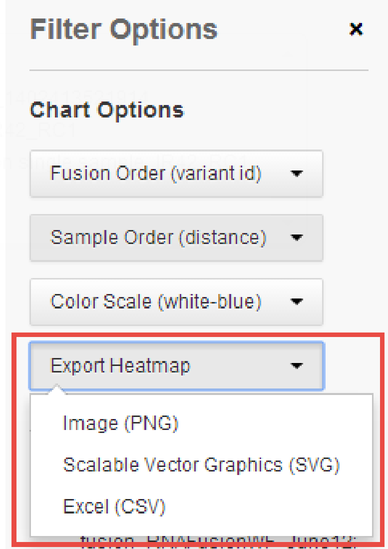


- Click the **Selected Analyses** link to see the list of analyses in the heatmap.

- You can change the order of the color scheme used in the chart through the Color Scale menu on the right (under Chart Options).

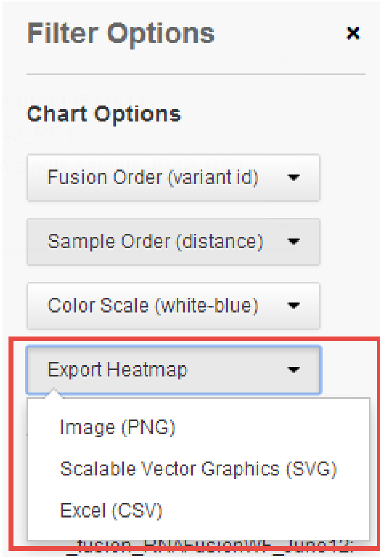


- White or blank cells indicate no reads (in the default color scheme).
  (Many cells in the example heatmap show no reads. Only an example block of no-reads cells is outlined in this image. With red-green and red-blue color schemes, red indicates a no-reads cell.)
- You can export a heatmap as an image 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.



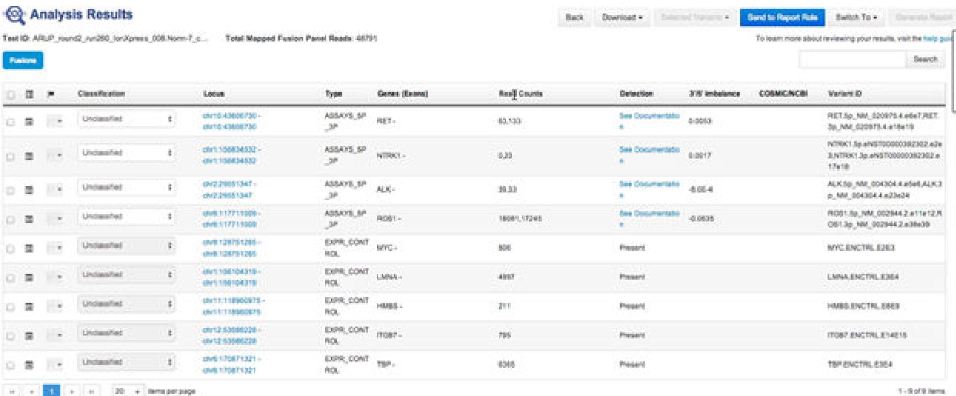
Supported export formats are:

- **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	EGFRvlll	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 has a web services API that allows you to automate returns to retrieve key information from the system.

This section provides examples that demonstrate how to leverage the API.

- These examples are used with the hosted Ion Reporter™ Software instance. If you are running a local version on your own Ion Reporter™ Server, then replace the hosted URL

  ```
  https://ionreporter.thermofisher.com/ir
  ```

  with the URL root of your local instance.
- API tokens that are included in examples are invalid and cannot be used for authentication.
- If the JSON download process converts `@` symbols to `%40`, do not change the text. Instead, copy the JSON files as is into the browser.

Get data upload path with authentication

getDataUploadPathWithAuth	
URL	`/api/v1/getDataUploadPathWithAuth`
Description	Used by theIonReporterUploader plugin to return the current data upload path that is associated with the API token for the user, based on the configuration of the `server.properties` file
Method	`POST`
Request form parameters	`auth` String, *Mandatory*, `Valid API Token`
Example	`https://ionreporter.thermofisher.com/api/v1/getDataUploadPathWithAuth`
Request headers	`Content Type: application/x-www-form-urlencoded`
Returns	`/data/IR/data/IR_Org/data/IRU_Uploads`

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
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

isSampleExists	
URL	`/api/v1/isSampleExists`
Description	IonReporterUploader plugin checks whether there is an existing sample that uses the sample that is given in the call
Method	`POST`
Request form parameters	`sampleName` String *Mandatory*, `Valid sample Name`
Example	`https://ionreporter.thermofisher.com/api/v1/isSampleExists`
Request headers:	• `Content Type`:application/x-www-form-urlencoded • `Authorization: API Token`
Returns	`TRUE` if sample with given name already exists in the organization given the API token for the user in headers; `FALSE` if the sample does not exist

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<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"
```

Query by sample name API call

You can query by sample name using this API call:

```
/api/v1/analysis?format=json&name=[samplename]&type=sample
```

This returns a list of analysis names performed on the sample, ordered by start date.

Get user details

getUserDetails	
URL	`/api/v1/getUserDetails`
Description	Used by IonReporterUploader plugin to get user details based on username and password. Returns the user details.
Method	`POST`
Request form parameters	• **userName**, String, *Mandatory*, Valid username • **password**, String, *Mandatory*, Valid password
Example	`https://ionreporter.thermofisher.com/api/v1/getUserDetails`

<table>
<tr><th colspan="2">getUserDetails</th></tr>
<tr><td>Request headers</td><td>Content Type: application/x-www-urlencoded</td></tr>
<tr><td>Returns</td><td>{
  "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":
"49bbe49a8307ba61ef28ee9a9122a98347576488c975c857
b7e1c82978f3818c",
  "lastname": "User",
  "token": "wVcoTeYGfKxItiaWo2lngsV/
r0jukG2pLKbZBkAFnlPbjKfPTXLbIhPb47YA9u78"
}</td></tr>
</table>

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/getUserDetails -H
"Content-Type:application/x-www-form-urlencoded" -d
"userName=ion.reporter@lifetech
.com&password=IonPass123"
```

Get user details with authentication

getUserDetailsWithAuth

getUserDetailsWithAuth	
URL	`/api/v1/getUserDetailsWithAuth`
Description	Used by IonReporterUploader (IRU) plugin to return user details based on the API token
Method	`POST`
Request form parameters	String, *Mandatory*, Valid user API token
Example	`https://ionreporter.thermofisher.com/api/v1/` `getUserDetailsWithAuth`
Request headers	`Content Type: application/x-www-form-urlencoded`

Returns	`{`
	`"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":`
	`"49bbe49a8307ba61ef28ee9a9122a98347576488c975c857`
	`b7e1c82978f3818c",`
	`"lastname": "User",`
	`"token": "wVcoTeYGfKxItiaWo2lngsV/`
	`r0jukG2pLKbZBkAFnlPbjKfPTXLbIhPb47YA9u78"`
	`}`

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/
getUserDetailsWithAuth -H "Content-Type:application/x-www-form-
```

```
urlencoded" -d
"auth=NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3NjQ4OGM5N
zVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get available storage space

getAvailableStorageSpace	
URL	`/api/v1/getAvailableStorageSpace`
Description	Returns the available storage space in bytes for the organization that is associated with the API token in headers
Method	`POST`
Request	`https://ionreporter.thermofisher.com/api/v1/getAvailableStorageSpace`
Request Headers	• **Content Type**: application/x-www-form-urlencoded • **Authorization**: API token
Returns	`20452031596466`

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://server name/api/v1/
getAvailableStorageSpace -H "Content-Type:application/x-www-
form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get available cancer types

getAvailableCancerType	
URL	`/api/v1/getAvailableCancerType`
Description	Returns the list of all available cancer types defined in attribute-controlled vocabulary
Method	`POST`
Request	`https://ionreporter.thermofisher.com/api/v1/getAvailableCancerType`
Request Headers	• **Content Type**: application/x-www-form-urlencoded • **Authorization**: API token
Returns	`["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://server name/api/v1/
getAvailableCancerType -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw"
```

Get workflow names

getWorkflowNamesWithApplicationTypeWithAuth

getWorkflowNamesWithApplicationTypeWithAuth	
URL	`/api/v1/getWorkflowNamesWithApplicationTypeWithAuth`
Description	Used by IonReporterUploader plugin to get a list of workflows that are tagged with IRU for the given version. Returns the list of workflow details for the version that is specified in the header. If there are no workflows that are tagged with IRU, all workflows for a specified version are returned.
Method	`POST`
Request	`https://ionreporter.thermofisher.com/api/v1/getWorkflowNamesWithApplicationTypeWithAuth`
Request headers	• **Content Type:** application/x-www-urlencoded • **Authorization:** API token • **Version:** `IR56`
Returns	`[ { "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://<server name>/api/v1/
getWorkflowNamesWithApplicationTypeWithAuth -H "Content-
Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -H "Version:52"
```

Start an analysis

startAnalysis	
URL	`/api/v1/startAnalysis`
Description	Used by the IonReporterUploader plugin to start an analysis for the given setId. Returns JSON object with status and message.
Method	`POST`
Request	http://ionreporter.thermofisher.com/api/v1/ startAnalysis
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™ Software user interface. `https://ionreporter.thermofisher.com/api/v1/startAnalysis`
Request headers	• **Content Type**: application/x-www-form-urlencoded • **Authorization**: API token
Returns	`{ "status":true, "message":"success"}`

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://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

getvcf	
URL	`/api/v1/getvcf`
Description	Gets the links to download VCF files. Returns JSON object that contains the download information.
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	`https://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	`[`
	`  {`
	`    "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": "ff808181545d90790154613336be0008"`
	`  }`
	`]`

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -v -k -X GET "https://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

Table 14

isAnalysisRunning	
URL	`/api/v1/isAnalysisRunning`
Description	Checks the status of the analysis. Returns a JSON response object with status and message. The response object can be converted to a JSON object.
Method	`POST`
Request form parameters	• **Content Type**: application/x-www-urlencoded • **Response Fields**: **userId**, String, *Mandatory*, UserId `https://ionreporter.thermofisher.com/api/v1/ isAnalysisRunning`
Request headers	• **Content-Type**: application/x-www-form-urlencoded • **Authorization**: API Token
Returns	`{ "status":true, "message":"success"}`

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k https://<server name>/api/v1/isAnalysisRunning
-H "Content-Type:application/x-www-form-urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d "userId=100"
```

Unshare analyses

unsharedAnalyses	
URL	`/api/v1/unsharedAnalyses`
Description	Unshares analyses. Returns JSON file with status and message.
Method	`POST`
Request form parameters	**userId**, String, *Mandatory*, UserId `https://ionreporter.thermofisher.com/api/v1/unsharedAnalyses`
Request headers	• **Content Type**: application/x-www-urlencoded • **Authorization**: API token
Returns	`{ "status":true, "message":"success"}`

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -X POST -k "https://<server name>/api/v1/
unsharedAnalyses" -H "Content-Type:application/x-www-form-
urlencoded" -H
"Authorization:NDliYmU0OWE4MzA3YmE2MWVmMjhlZTlhOTEyMmE5ODM0NzU3
NjQ4OGM5NzVjODU3YjdlMWM4Mjk3OGYzODE4Yw" -d
"userId=ff8081815547ceb401554d62c0ec0493"
```

Download file

download	
URL	`/api/v1/download`
Description	Gets the file, creates a compressed archive file in ZIP format and downloads it
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.) `https://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 file that is located at the file path that is specified

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
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"
```

Get link to a final report

finalreport	
URL	`/api/v1/finalreport`
Description	Gets the analysis details and returns links to the final report. Returns a JSON object that contains download information.
Method	`GET`
Request form 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

<table>
<tr><th colspan="2">finalreport</th></tr>
<tr><td>Request headers</td><td>Content Type: application/x-www-form-urlencoded
Authorization: API Token
<pre>https://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</pre></td></tr>
</table>

finalreport	
Authorization	API Token
Returns	(see below)


```
[
  {
    "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_2
79_IonXpress_003_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
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
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

qcreport	
URL	`/api/v1/qcreport`
Description	Gets the Quality Control (QC) Report. Returns a JSON object that contains download information.
Method	`GET`
Request form 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 "" `https://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`

<table>
<tr><th colspan="2">qcreport</th></tr>
<tr><td>Request headers</td><td>
Content Type: application/JSON
Authorization: API token</td></tr>
<tr><td>Returns</td><td>

<pre>
[
  {
    "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
</pre>

</td></tr>
</table>

qcreport	
	`04-33.zip" } ]`

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

```
curl -v -k -X GET "https://<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"
```

Download QC and Final reports

To retrieve one Quality Control (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
```

Get analysis details

analysis	
URL	/api/v1/analysis
Description	Gets the analysis details and links to download a Quality Control (QC) report, final report, and compressed archive (ZIP format) of unfiltered and filtered variants files. Returns a JSON object that contains the download information.
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 "" `https://ionreporter.thermofisher.com/api/v1/analysis?format=json&name=xyzsampletest&exclude=reports,filteredvariants,unfilteredvariants&start_date=2016-01-01&end_date=2016-02-01`

analysis	
Request Headers	**Content Type**: application/x-www-form-urlencoded; **Authorization**: API token
Returns	`[{ "reports": { "qc": { "link": "http://dev.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://dev.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://dev.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",`

analysis	

	`"started_by": "Ion User",`
	`"start_date": "April 11, 2016",`
	`"status": "SUCCESSFUL"`
	`}`
	`]`

Response fields

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

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"
```

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
```

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"
```

Get associated BAM files

getAssociatedBamfiles	
URL	`/api/v1/getAssociatedBamfiles`
Description	For use with the Ion Reporter™ Server only. Gets the BAM file or files that are associated with a single sample or analysis, or multiple samples or analyses, and downloads the BAM files using `/downloadBAM api.` Returns a JSON response object that contains the BAM file links. After BAM files are displayed as output as JSON, users can download the BAM file using the `/api/v1/downloadBAM` API call.
Method	`GET`
Request query parameters	• **name**, String, *Mandatory*. Single/multiple names of either sample or analysis • **type**, String, *Conditional*. If type parameter is not provided, name is considered as analysis name, For sample name(s), type is mandatory (For example, type=sample)

getAssociatedBamfiles	
Sample request	`https://dev.ionreporter.thermofisher.com/api/v1/getAssociatedBamfiles?name=multi bam test,Sample_1_v2,test62,1194954B_Bladder_ManLib,Demo AmpliSeq Exome VCF,invalid_sapmle1,Demo AmpliSeq IDP Mother&type=sample`
Analysis request	`http://dev.ionreporter.thermofisher.com/api/v1/getAssociatedBamfiles?name=Demo AmpliSeq IDP Daughter_1518065163079,invalid_name,trio_c1078_2018-03-01-20-35-30-948`
Request headers	• **Content Type:**ion/x-www-form-urlencoded • **Authorization:** API Token

getAssociatedBamfiles	
Returns	• Sample Response


```
[

    {
       "sample": "1194954B_Bladder_ManLib",
        "bam_links": [
            "http://
dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/
Zyklus/data/IRU_Uploads/20170727_08_45_03/
v1/1194954B_Bladder_ManLib/
1_IonXpress_030_R_2016_12_20_13_27_19_user_S5-001
05-348-
C656s2_OCAv3.4_CNVbaseline_ManLibs_Pool6_chip6_Au
to_user_S5-00105-348-
C656s2_OCAv3.4_CNVbaseline_ManLibs_Pool6_chip6_10
74.bam"
        ]
    },
    {
        "sample": "invalid_sapmle1",
        "bam_links": [
            "Sample or Bam file could not be
found for the specified name."
        ]
    },
    {
        "sample": "multi bam test",
        "bam_links": [
            "http://
dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/Zyklus/data/
IRU_Uploads/2017-12-29_1_51_48/v1/e5272-wfa-
l165_v3/rawlib.bam",
            "http://
dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/Zyklus/data/
IRU_Uploads/20171229_14_54_21/v1/
sample_test16/1_IonDx-9_rawlib12.bam"
        ]
    },
    {
        "sample": "Demo AmpliSeq Exome VCF",
        "bam_links": [
            "Sample or Bam file could not be
found for the specified name."
        ]
    }
]
```


• Analysis Response


```
[
    {
        "analysis":
"trio_c1078_2018-03-01-20-35-30-948",
```

getAssociatedBamfiles

```
        "version": "IR510",
        "analysisStatus": "SUCCESSFUL",
        "sampleDetails": [
            {
                "sampleName": "Demo AmpliSeq IDP
Daughter",
                "sampleRole": "proband",
                "inputBam": [
                    "http://
dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/
demodata/PGM/AmpliSeq_IDP/
AmpliSeq_IDP_daughter.bam"
                ],
                "processedBam": [
                    "http://
dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/
uattestsskadmuser@gmail.com/
Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Fath
er_Demo_AmpliSeq_IDP_Mother/
Demo_AmpliSeq_IDP_Daughter_20180301150555171/
outputs/VariantCallerActorProband-00/
merged.bam.ptrim.bam"
                ]
            },
            {
                "sampleName": "Demo AmpliSeq IDP
Father",
                "sampleRole": "father",
                "inputBam": [
                    "http://
dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/
demodata/PGM/AmpliSeq_IDP/
AmpliSeq_IDP_father.bam"
                ],
                "processedBam": [
                    "http://
dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/
uattestsskadmuser@gmail.com/
Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Fath
er_Demo_AmpliSeq_IDP_Mother/
Demo_AmpliSeq_IDP_Daughter_20180301150555171/
outputs/VariantCallerActorFather-00/
merged.bam.ptrim.bam"
                ]
            },
            {
                "sampleName": "Demo AmpliSeq IDP
Mother",
                "sampleRole": "mother",
                "inputBam": [
                    "http://
dev.ionreporter.thermofisher.com:80/api/v1/
downloadBAM?filePath=/shared/data/ShazIndia-Labs/
demodata/PGM/AmpliSeq_IDP/
```

getAssociatedBamfiles	
	AmpliSeq_IDP_mother.bam"], "processedBam": ["http:// dev.ionreporter.thermofisher.com:80/api/v1/ downloadBAM?filePath=/shared/data/ShazIndia-Labs/ uattestsskadmuser@gmail.com/ Demo_AmpliSeq_IDP_Daughter_Demo_AmpliSeq_IDP_Father_Demo_AmpliSeq_IDP_Mother/ Demo_AmpliSeq_IDP_Daughter_20180301150555171/ outputs/VariantCallerActorMother-00/ merged.bam.ptrim.bam"] }] }, { "analysis": "invalid_name", "message": "Analysis could not be found for the specified name." }]

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
401	User is not authenticated
500	Error occurred

Curl commands

```
curl -X GET -k "http://dev.ionreporter.thermofisher.com/api/v1/
getAssociatedBamfiles?name=multi bam test,Sample_1_v2,
test62,1194954B_Bladder_ManLib,Demo AmpliSeq Exome
VCF,invalid_sapmle1,Demo AmpliSeq IDP Mother&type=sample" -H
"Content-Type:application/x-www-form-urlencoded" -H
"Authorization:ODA5YTVlZDcyNjkyNDg5Mjg2YmZiNjI3ODExZWFmM2I3Nzhk
Nzg0MDU5M2U
0YmIxOGU2YjQ2OTRmYmIxZjAxNw"
```

```
curl -X GET -k "http://dev.ionreporter.thermofisher.com/api/v1/
getAssociatedBamfiles?name=Demo AmpliSeq IDP
Daughter_1518065163079,invalid_name,trio_c1078_2018-03-01-20-35
-30-948" -H "Content-Type:application/x-www-form-urlencoded" -
H
"Authorization:ODA5YTVlZDcyNjkyNDg5Mjg2YmZiNjI3ODExZWFmM2I3Nzhk
Nzg0MDU5M2U0YmIxOGU2YjQ2OTRmYmIxZjAxNw
```

Download BAM file

download	
URL	`/api/v1/downloadBAM`
Description	Download BAM file for a given sample or analysis. This procedure applies to Ion Reporter™ Server only.
Method	`GET`

Request query parameters	• **filePath** String. *Mandatory*. Filepath of bam file got using / getAssociatedBamfiles AP
	• **type** String *Optional* Valid values are "pdf" or "" (by Default creates a zip file and downloads it.)
	`https://ir-dev.ionreporter.thermofisher.com:`
	`80/api/v1/downloadBAM?filePath=/shared/data/`
	`ShazIndia-Labs/data/IRU_Uploads/`
	`20180302_01_06_30/v1/CFTR_15KB_deletion/`
	`1_AmpCFTR_contig7_15KBDeletions.bam"`

Request headers	• **Content Type**: application/x-www-form-urlencoded • **Authorization**: API token

Returns	Downloads the given bam file. User can modify the bam file name in curl command instead of "download.bam".
	`(INBL2-0QFG8WP:bamDownLaod_test dasd$ curl -0`
	`download.bam _L -k _X GET "https://juicebox.itw:`
	`443/api/v1/downloadBAM?filePath=/data?IR/data/`
	`IR_Org/ion.reporter@lifetech.com/`
	`Demo_AmpliSeq]_CHPv2_tumor/`
	`Demo_AmpliSeq_CHPv2_tumor_20180319063625805/`
	`outputs/VariantCallerActor-00/`
	`merged.bam.ptrim.bam" -H "Content-`
	`Type:application/x-www-form-urlencoded" -H`
	`"auth:`
	`ZWJjODU2ODIwYWY40Tk2Yjg2MzI5NTU1MzIxZTA0NGVlYTZln`
	`jIyYTJllGJlNDMwMGI3oWM4ZGNhZTNlNzg1Mw" -H`
	`"Connection: close"`
	`% Total % Received %Xferd`
	`Average Speed Time Time Current 100 43.2M 100`
	`43.2M 0 0 186k 0 0:03:58 0:03:58 --:--:-- 242k`
	`[INBL2-0QFG8WP:bamDownLoad_test dasd$ ls -lrt`
	`total 88608 -rw-r-r 1 dasd 1153344739 45363244`
	`Mar 29 21:09 download.bam`
	`INBL2-0QFG8WP:bamDownLoad_test dasd$`

Response fields

Status Code	Response Message
200	Successful response
400	Bad request
404	Not found
401	User is not authenticated
500	Error occurred

Curl command

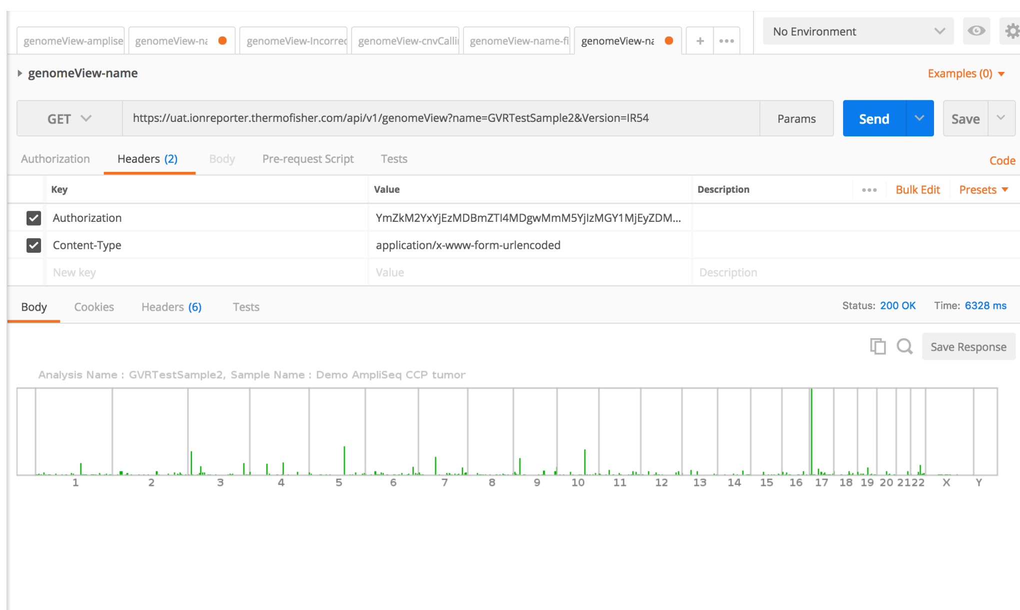
```
curl -o download.bam -L -k -X GET "https://juicebox.itw:
443/api/v1/downloadBAM?filePath=/data/IR/data/IR_Org/
ion.reporter@lifetech.com/Demo_AmpliSeq_CHPv2_tumor/
Demo_AmpliSeq_CHPv2_tumor_20180319063625805/outputs/
VariantCallerActor-00/merged.bam.ptrim.bam" -H "Content-
Type:application/x-www-form-urlencoded" -H "auth:
ZWJjODU2ODIwYWY4OTk2Yjg2MzI5NTU1MzIxZTA0NGVlYTZlNjIyYTJlMGJlNDM
wMGI3OWM4ZGNhZTNlNzg1Mw" -H "Connection: close"
```

Get whole genome view png

genomeView	
URL	`/api/v1/genomeView`
Description	Gets the whole genome view png for analysis. Returns PNG image file for whole genome view of analysis.
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.

genomeView	
Sample request	`https://ionreporter.thermofisher.com/api/v1/genomeView?id=ff8081815ba8eac4015ba8ead9ce005d`
Request headers	• **Content Type**: application/x-www-form-urlencoded • **Authorization**: API token

Returns



Status codes

Status Code	Reason
200	Successful response
400	Bad request
401	User is not authenticated
404	Not found
500	Error occurred

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"
```

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.

Guidelines to develop Ion Reporter™ Software 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" : ""
  }
}
```

Filters and filter chains

A filter chain is a set of filters that Ion Reporter™ Software uses to narrow the list of variants that are included in analysis results. Most Ion Reporter™ predefined workflows include default filter chains, which you can change.

Filter chains are also available in analysis results and visualizations. You can select a different filter chain when you view results to change the variants that are included. If you save the filter chain to an analysis result or visualization, the variants included reflect the use of the filter chain when the analysis results or visualization is subsequently opened. You also can apply the filter chain temporarily, then review the results before you decide whether to save the updated analysis results, or discard the changes. In either case, you can download a VCF file of the variants that are shown in the analysis results.

Ion Reporter™ Software includes predefined filter chains that you can apply to workflows, analysis results or visualizations. You can also create custom filter chains, that are built from predefined filter chains, or one or more filters. The filters are based on public and proprietary annotation sources and data types that are included in Ion Reporter™ Software. See "Annotation source filters" on page 440 and "Data type filters" on page 443 for more information. After you create a custom filter chain, you can add it to a workflow, or apply to analysis results or visualizations.

Note: The Mutation Load Calculation filter chain is a unique filter that can be added to an analysis through the parameters that you add to a workflow.

View filter chain details

You can view the details of predefined and custom filter chains in Ion Reporter™ Software. For example, you can check whether the genome reference version is suited to your research. If it is not, you can build a new filter chain. Custom filter chains are created by combining filters to make a filter chain that meets your specific variant filtering needs.

- When you plan your workflow or create a workflow preset, you can view filter chain details from the **Workflows Presets** screen:
  a. Click **Workflows**, then click **Presets**.
  b. Select **Filter Chains** in the search filter category.
  c. Select the row that contains the filter chain of interest.
  d. In the **Details** pane, view the following details:

Option	Description
Version	Software version when filter was released
Reference	Genome reference that is used in the filter chain
Last Modified By	Person who last set or changed the filter chain settings
Last Modified On	Date that the filter chain was last modified
Created By	Person who created the filter chain
Created On	Date that the filter chain was created
Filter Chains	List of other filter chains included in this filter chain and the order in which they are applied
Filter chain query applied	List of query settings applied

- When you refine analysis results, you can view filter chain details from the **Analysis Results** screen:
  a. Click **Analyses**, the click **Overview**.
  b. Select the link for the analysis of interest.
  c. In the **Analysis Results** screen, view the details of the filter chain that is applied to an analysis.

Option	Description
Filter Chain	Name of filter chain applied. A filter chain from the list might have been applied when the workflow is launched, or applied to the completed analysis results.
Total Variants	Total number of variants that are detected in the analysis.
Total Genes	Total number of genes that are detected in the analysis.
Filter chain details	Description of the applied filters and their condition settings.
Variants	Number of variants Filtered In or Filtered Out.
Genes	Number of genes Filtered In or Filtered Out.

Create a custom filter chain

You can create custom filter chains to refine your analysis results in Ion Reporter™ Software. A filter chain is a set of filters that can be applied to the variants identified in an analysis that are used to narrow the analysis results to only the variants of interest. After you create a custom filter chain, you can associate the filter chain with an Ion Reporter™ Software workflow when you create or edit a workflow. You can also apply the filter chain to analysis results.

1. To create a filter chain, use one of the following procedures to get started:

Option	Description
Workflow Presets	1. In the **Workflows** tab, click **Presets**. 2. Click **Create Preset** ▸ **Filter Chain**.
Analysis Results	1. In the **Analysis** tab, click **Overview**. 2. Click the link of an analysis of interest. The **Analysis Results** screen appears. 3. In the right pane, **Filter Chains** section, click (New).
Visualize	1. In the **Analysis** tab, click **Overview**. 2. Click the link of an analysis of interest. The **Analysis Results** screen appears. 3. Click **Visualize**. 4. In the right pane, **Filter Chains** section, click (New).

2. In the **Create Filter Chain** dialog box, enter a name and, optionally, a description.

3. Select a filter to add from the **Choose Filter** dropdown list. See "Annotation source filters" on page 440 and "Data type filters" on page 443 for more information.

4. Set conditions for the filter, then click **Set**.

5. Click **Save**.

The filter chain can now be selected to be associated as the default filter chain when a workflow is created, or be used change an existing default filter chain. See "Filters step" on page 90 and "Change the default filter chain for a workflow" on page 90for more information. It can also be applied to analysis results or a visualization. See "Apply a filter chain to Analysis Results" on page 169 for more information.

Edit a filter chain

You can change the conditions or remove filters from custom filter chains in Ion Reporter™ Software.

Note: You can only edit custom filter chains. You cannot edit predefined filter chains.

1. To edit a filter chain, use one of the following procedures to get started:

Option	Description
Workflow Presets	1. In the **Workflows** tab, click **Presets**. 2. In the dropdown list, select **Filter Chains**. 3. Select the row of the filter chain you want to modify, then click **Edit**.
Analysis Results	1. In the **Analysis** tab, click **Overview**. 2. Click the link of an analysis of interest. The **Analysis Results** screen appears. 3. In the right pane, Filter Chains section, select the custom filter chain that you want to edit, then click (Edit).
Visualize	1. In the **Analysis** tab, click **Overview**. 2. Click the link of an analysis of interest. The **Analysis Results** screen appears. 3. Click **Visualize**. 4. In the right pane, **Filter Chains** section, click (Edit).

2. In the **Edit Filter Chain** dialog box:

Option	Description
Change name	In the **Name** field, change filter chain name.
Change description	In the **Description** field, change the filter chain description.
Change reference	Under **Reference**, select **GRCh38** or **hg 19**.
Choose filter	In the **Choose Filter** dropdown list, select a different filter or filters.
Modify FilterChain Query.	In the **FilterChain Query** field, change the order filters are applied.
Modify filter conditions.	To modify the conditions of a filter included in a filter chain: 1. Click (Delete). 2. Reselect the filter from the **Choose Filter** dropdown list, set the conditions, then click **Set**.
Delete filters	To remove filters that are included in a filter chain, click (Delete).

3. Click **Save**.

The edited filter chain can now be selected to be associated as the default filter chain when a workflow is created, or be used change an existing default filter chain. See “Filters step“ on page 90 and “Change the default filter chain for a workflow“ on page 90for more information. It can also be applied to analysis results or a visualization. See “Apply a filter chain to Analysis Results“ on page 169 for more information.

Lock a filter chain in Workflow Presets

After you create a custom filter chain, you can lock it. A locked filter chain cannot be changed or unlocked in Ion Reporter™ Software.

1. In the **Workflows** tab, click **Presets**.
2. Select **Filter Chains** in the search filter category.
3. Select the row of the filter chain you want to lock.
4. Click ⚙ **(Actions)** ▸ **Lock**.
   A **Lock Filter Chain** dialog box appears. Once the filter chain is locked, it can no longer be edited or unlocked.
5. Click **Yes** to lock the filter chain.

The filter chain preset now has a lock symbol in its row on the **Workflow Presets** screen.

Delete a filter chain in Workflow Presets

You can delete a custom filter chain from the available filter chains list in Ion Reporter™ Software.

Note: You cannot delete a predefined filter chain.

1. In the **Workflows** tab, click **Presets**.
2. In the dropdown list, select **Filter Chains**.
3. Select the row of the filter chain you want to remove.
4. Click ⚙ **(Actions)** ▸ **Delete**.
   The **Confirm Delete** dialog box appears. Presets that are in use by a workflow cannot be deleted.
5. Click **Yes** to delete the filter chain.

The filter chain is now removed from the **Workflow Presets** table and is no longer available in the **Filters** step when creating a workflow. It is also no longer available from the **Filter Chains** dropdown lists in the **Analysis Results** and **Visualization** screens.

Example custom filter chains

The following topics describe how to build various filter chains. Custom filter chains can be built from the **Create Filter Chain** dialog box, which can be accessed from either:

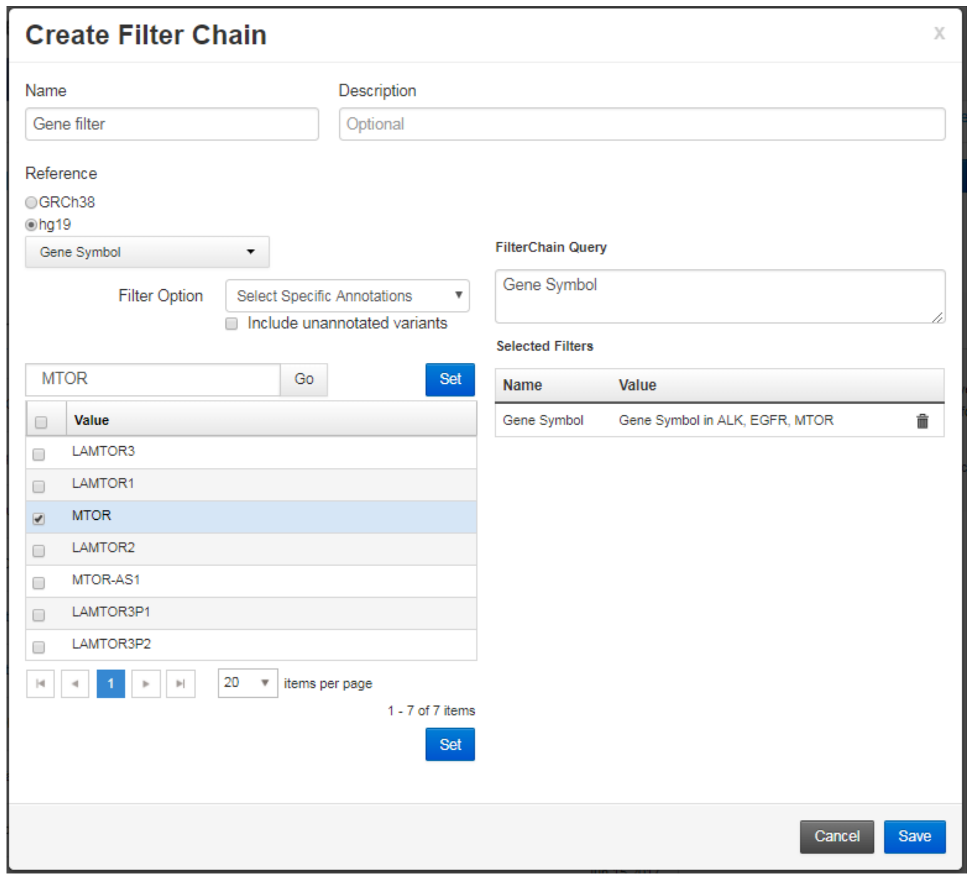
- The **Workflow Presets** screen
- The **Analysis Results** screen, (**New Filter**) icon

Create a gene-level filter chain

You can create a filter chain based on gene symbols to narrow the analysis results to only those genes of interest in Ion Reporter™ Software.

1. In the **Worksflows** tab, click **Presets**.
2. Click **Create Preset ▸ Filter Chain**.
   The **Create Filter Chain** dialog box appears.
3. In the **Create Filter Chain** dialog box, enter a name and an (*optional*) description.
4. In the **Choose Filter** dropdown list, select **Gene Symbol**.
5. Select one or more genes of interest from the list.
   a. Type the gene symbol into the search box, then click **Go**.
   b. Select the gene.
   c. Repeat for additional genes of interest.

6. Click **Set**
   Your genes appear in the **Selected Filters** table on the right.



7. Click **Save**.

Your gene symbol filter chain can now be added to a workflow, or applied to analysis results or a visualization. See "Filters step" on page 90, "Apply a filter chain to Analysis Results" on page 169, and for more information.

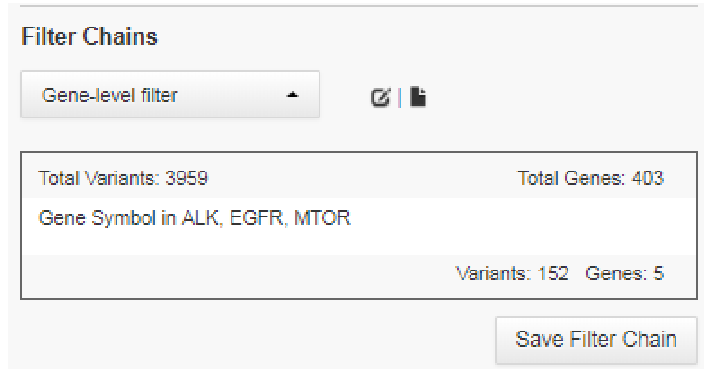


Figure 19 Example results of gene symbol filter chain that is applied to analysis results

Create a filter chain query

You can create filter chains that combine filters and specify the order in which they are applied in Ion Reporter™ Software. The modifiers are AND and OR and brackets ( ) are used to set the order.

Note: The modifiers AND and OR must be in all caps.

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset ▸ Filter Chain**.
   The **Create Filter Chain** dialog box appears.
3. Add the filters to create a chain:
   a. Enter a name and, optionally, a description.
   b. Select your first filter, then set its value to **In**, then click **Set**.
      The filter name appears on the right side of the **Create Filter Chain** dialog box.
   c. Add another filter. Set its value to **In**, then click **Set**.
      The second filter name appears on the right side of the **Create Filter Chain** dialog box.
   d. Add a third filter. Set its value to **In**, then click **Set**.
      The third filter name appears on the right side of the **Create Filter Chain** dialog box.
4. In the **FilterChain Query** field, your selected filters are listed by name. You can change the order in which your filters are applied:
   - Enter brackets ( ) around the filter names you want the software to apply first. All other filters are applied in order that is left to right.

   FilterChain Query

   (dbSNP(138) AND COSMIC(67)) AND Variant Effect

   In this example, the dbSNP and COSMIC filters are applied first, and then the Variant Effect filter is applied.

   - Enter or edit the modifiers. Enter AND to include all filters that are specified, or enter OR to specify that another filter be run if the first filter did not detect any variants.

   FilterChain Query

   dbSNP(138) AND COSMIC(67) OR Variant Effect

   In this example, the dbSNP and COSMIC filters are applied, if no variants were detected, the Variant Effect filter is applied.

5. Click **Save**.

The filter chain can now be added to a workflow, or applied to analysis results or a visualization. See “Filters step“ on page 90, “Apply a filter chain to Analysis Results“ on page 169, and for more information.

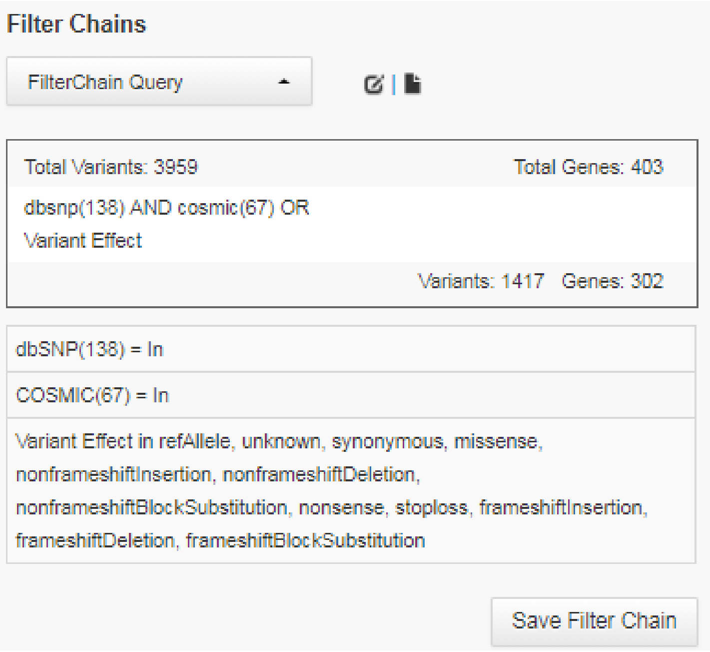


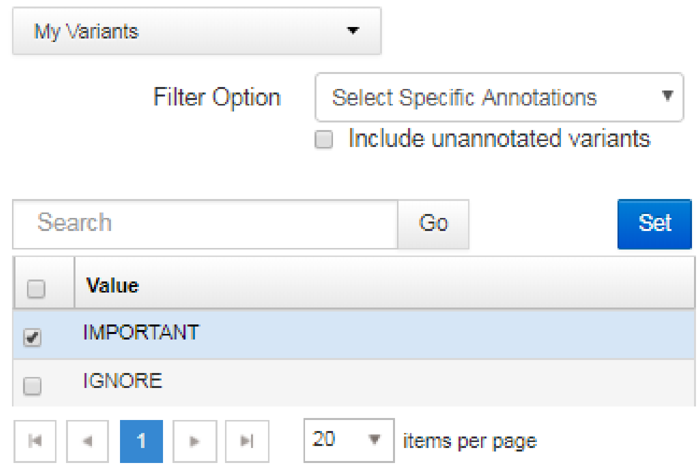
Figure 20 Example results of filter chain query applied to analysis results

Create a MyVariants and Variant Classification filter chain

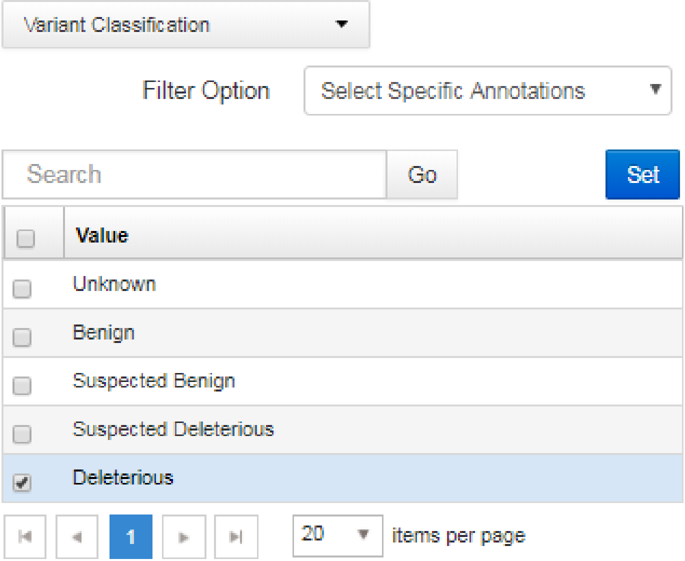
You can create a filter chain to narrow the analysis results to include only the variants that are flagged and classified by members of your Ion Reporter™ Software organization. This example shows how to set up a filter chain for MyVariants that are flagged as Important and classified as Deleterious in Ion Reporter™ Software.

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset ▸ Filter Chain**.
3. In the **Create Filter Chain** dialog box, enter a name and (*optional*) description.

4. In the **Choose Filter** dropdown list, select **MyVariants**.
   a. Select **Important**.



   b. Click **Set**.
5. In the **Choose Filter** dropdown list, select **Variant Classification**.
   a. Select **Deleterious**.



   b. Click **Set**.
6. Click **Save**.

Your filter chain can now be added to a workflow, or applied to analysis results or a visualization. See "Filters step" on page 90, "Apply a filter chain to Analysis Results" on page 169, and for more information.

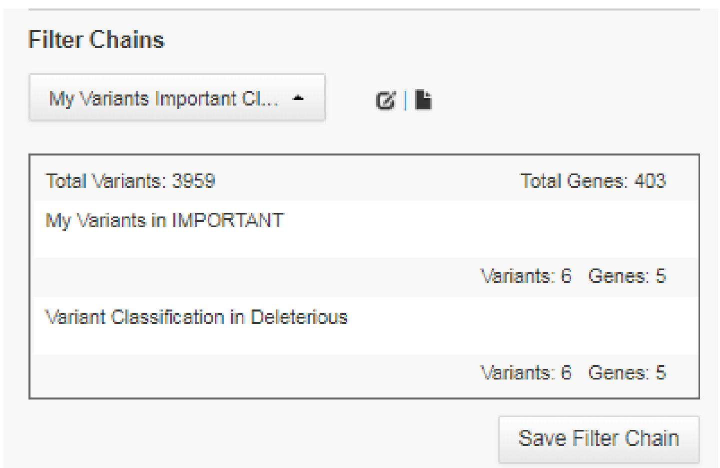
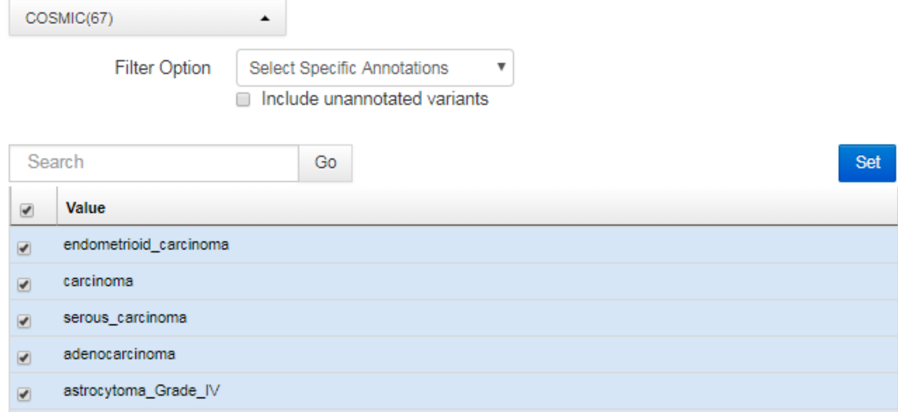


Figure 21 Example results of MyVariants and Variant Classification filter chain applied to analysis results

Create a COSMIC, ClinVar, MAF, and Variant Effect filter chain

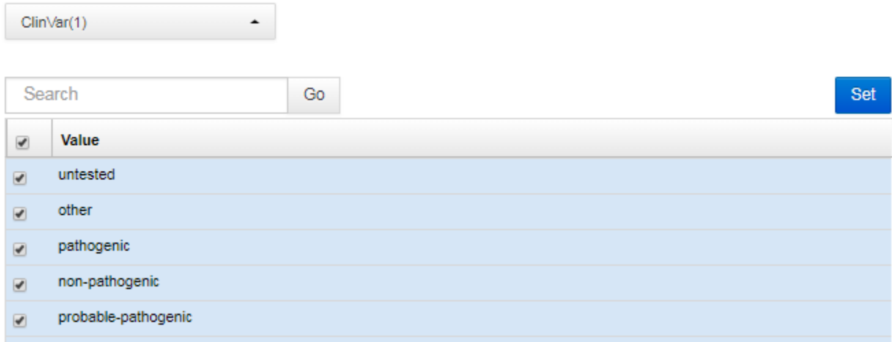
You can create a filter chain that includes both annotation source filters and Ion Reporter™ Software variant data type filters. This example shows how to create a COSMIC, ClinVar, Minor Allele Frequency (MAF), and Variant Effect filter chain.

1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset ▸ Filter Chain**.
3. In the **Create Filter Chain** dialog box, enter a name and, optionally, a description.
4. In the **Choose Filter** dropdown list, select **COSMIC**.
   a. Click top checkbox to select all values.



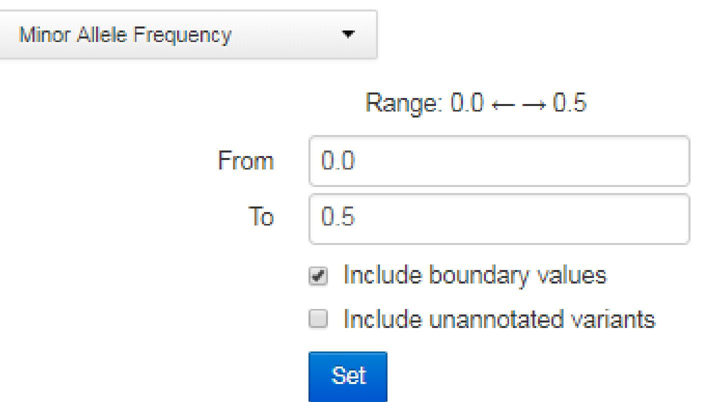
   b. Click **Set**.

5. In the **Choose Filter** dropdown list, select **ClinVar**.
   a. Click top checkbox to select all values.



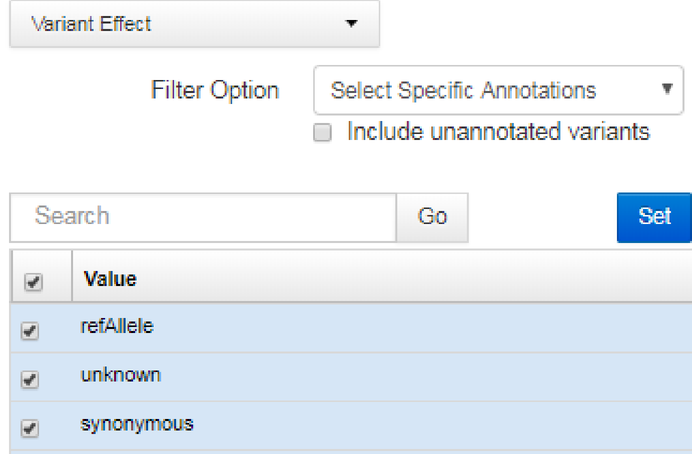
   b. Click **Set**.

6. In the **Choose Filter** dropdown list, select **Minor Allele Frequency**.
   a. Enter range, then select **Include boundary values**.



   b. Click **Set**.

7. In the **Choose Filter** dropdown list, select **Variant Effect**.
   a. Click top checkbox to select all values.



   b. Click **Set**.

8. Click **Save**.

Your filter chain can now be added to a workflow, or applied to analysis results or a visualization. See “Filters step“ on page 90, “Apply a filter chain to Analysis Results“ on page 169, and for more information.

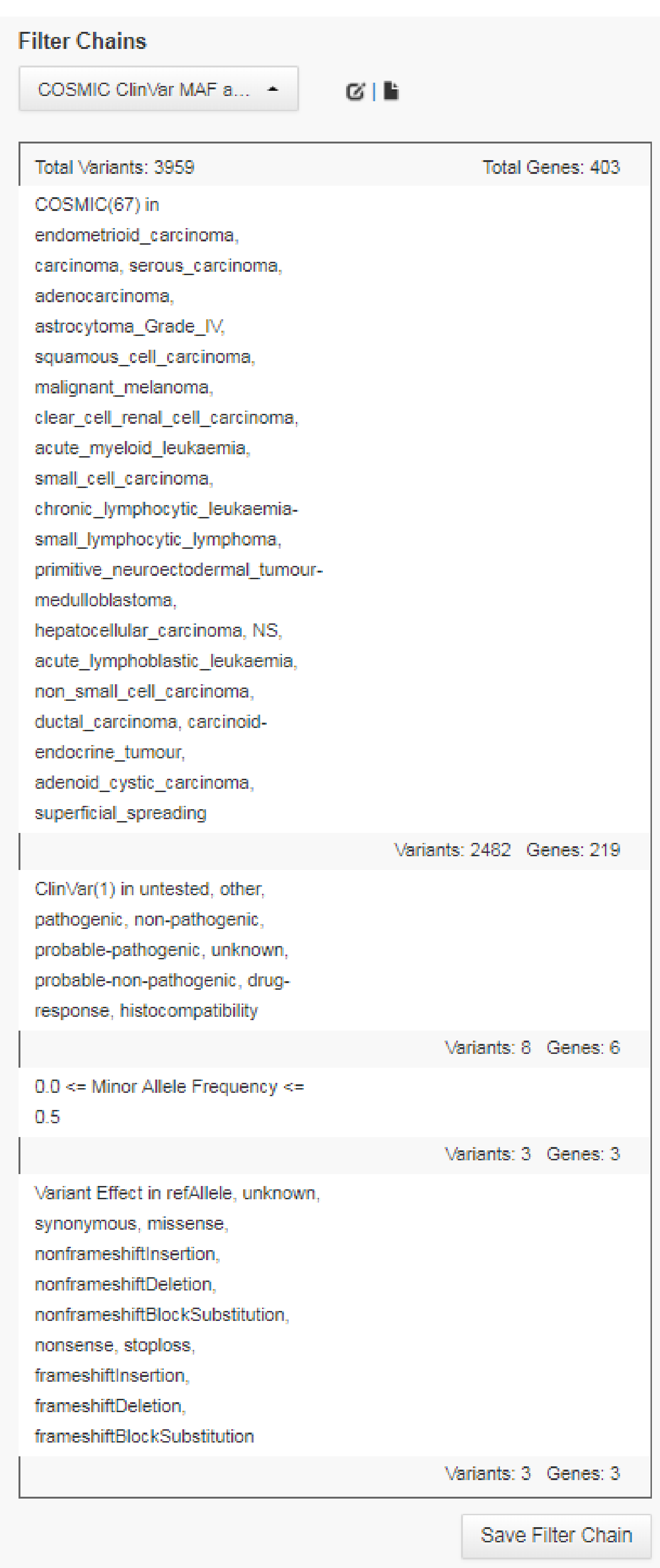
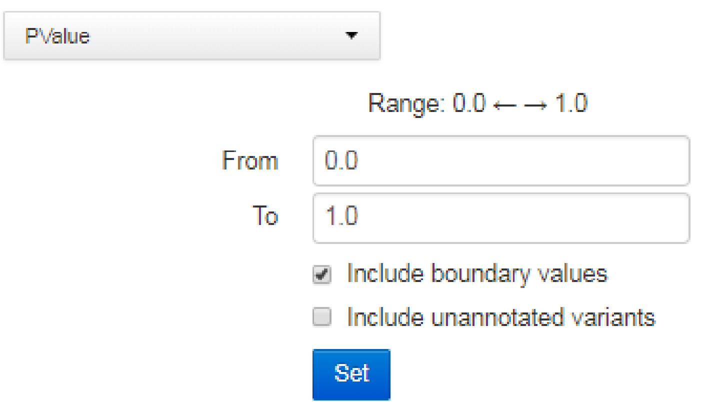


Figure 22 Example results of COSMIC, ClinVar, MAF, and Variant Effect filter chain applied to analysis results

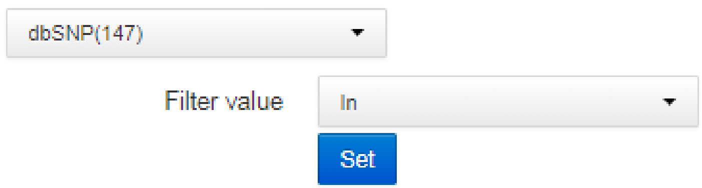
Create PValue, dbSNP, and Variant Type filter chain

You can create a filter chain with multiple filters in Ion Reporter™ Software. This example shows how to create a filter chain with PValue, dbSNP, and Variant Type filters.

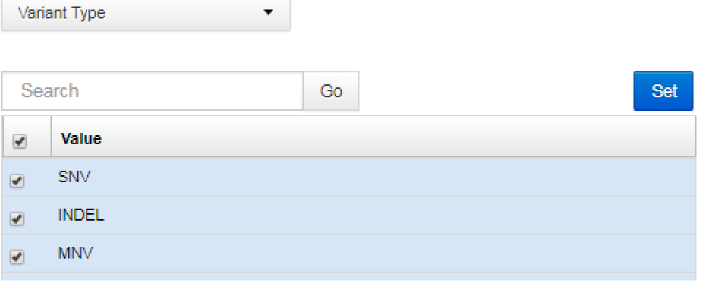
1. In the **Workflows** tab, click **Presets**.
2. Click **Create Preset ▸ Filter Chain**.
3. In the **Create Filter Chain** dialog box, enter a name and an (*optional*) description.
4. In the **Choose Filter** dropdown list, select **PValue**.
   a. Enter a range, then select **Include boundary values**.



   b. Click **Set**.
5. In the **Choose Filter** dialog box, select **dbSNP**.
   a. Set **Filter value** to **In**.



   b. Click **Set**.
6. In the **Choose Filter** dropdown list, select **Variant Type**.
   a. Click the checkbox to select all values.

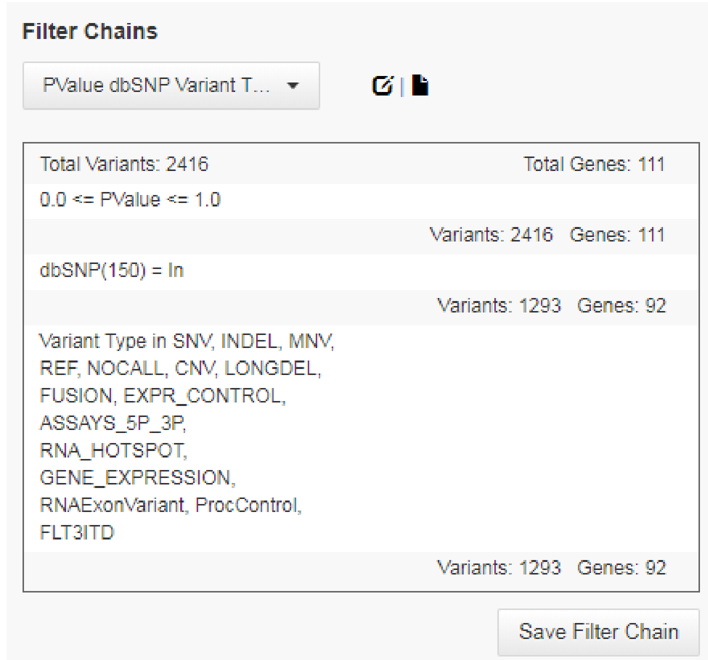


b. Click **Set**.

7. Click **Save**.

Your filter chain can now be added to a workflow, or applied to analysis results or a visualization. See “Filters step“ on page 90, “Apply a filter chain to Analysis Results“ on page 169, and for more information.

Example of this filter that is applied to analysis results:



Predefined filter chains

Ion Reporter™ Software includes the following predefined filter chains.

Filter chain name	Description
AmpliSeq Exome Tumor Normal	Detects all CNVs, Confident somatic variants with Allele Ratios between 0.1 and 1.0, Allele Read Counts between 4 and 1,000,000. PValue between 0 and 5.0E-6. It is the default filter for AmpliSeq Exome tumor-normal pair workflows. The Confidence range is 10.0 to 1.0E7.
Aneuploidy Mosaicism	Detects mosaicism by allowing decimal-level Copy Number Gain or Loss calls with a Confidence score of at least 0.1, while filtering out false positive calls near expected normal Copy Number. This filter is turned on by default in the ReproSeq Mosaic PGS w1.1 and is not selected by default in other predefined aneuploidy workflows.
Called Hotspot Variants and Controls	Reports all hotspot variants that pass the filter and are not called as reference or NOCALL. Variant Types include: SNP and INDEL. Will display the same set of variants in **Analysis Results** view as the **SNP/INDEL** summary table in visualization view.
Called Variants and Controls	Reports all variants (either hotspots or novel) that pass the filter and are not called as reference or NOCALL. Variant Types include: SNV, INDEL, MNV, CNV, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3ITD.
CNVs of Confidence >=0.1 - Germline - CNVs only	For germline analyses, narrows your analysis results to Copy Number variants with a Confidence value >= 0.1.
Confident Germline CNVs - CNV Only	For germline analyses, narrows your analysis results to Copy Number variants with a Confidence value >= 10. Not a default filter.
Confident Somatic Confidence - CNV	Includes 5% Confidence Interval Range and 95% Confidence Interval Range.

Filter chain name	Description
Default DNA and Fusions View	Default filter for the Ampliseq Colon Lung v2 with RNA Lung Fusion single sample workflow. Either fusion detection is present, or variant type is not fusion. Results include: • FUSIONS variants detected as Present • All EXPR_CONTROL markers • All ASSAYS_5P_3P markers • DNA variants of all types
Default Fusions View	Default filter for the AmpliSeq RNA Lung fusion single sample workflow. Either fusion detection is present, or variant type is: • EXPR_CONTROL • ASSAYS_5P_3P • RNA_HOTSPOT • GENE_EXPRESSION
Default Variant View	Default filter chain 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 non-ReproSeq Low-pass whole-genome aneuploidy workflows. Narrows your results to confident variant types, which are not CNVs, but could include: SNV, INDEL, MNV, REF, NOCALL, LONGDEL, FUSION, EXPR_CONTROL, ASSAYS_5P_3P, RNA_HOTSPOT, GENE_EXPRESSION, RNAExonVariant, ProcControl, and FLT3ITD.
Genetic Disease Variants	Narrows your analysis results to genetically-relevant variant types: IsNewlyHomozygousNonRef, HasDeNovoNonRefAllele, HasUnknownX, InTransPhaseCompoundHeterozygote, and HasMaleMaternalX. It is the default filter chain for the AmpliSeq Exome trio and AmpliSeq IDP trio workflows.

Filter chain name	Description
Mutation Load (Somatic SNVs)	Returns results for somatic SNVs based upon dbSNP, 5000Exomes, ExAC, and UCSC Common SNPs annotation source databases. The minor allele frequencies range lies between 0.0 and 1.0E-6. It also filters out variants of homopolymer lengths greater than 7, coverage lower than 60, and allele frequency less than 0.05. The parameter for this filter chain – The Mutation Load Filter Chain parameter – must be enabled for Ion Reporter™ Software to generate mutation load analysis results. By default, mutation load calculation is disabled. You must also copy and edit either: an DNA - Single Sample, or a DNA and Fusions - Single Sample workflow to enable mutation load calculations on DNA samples. Note: Unlike other Ion Reporter™ Software filter chains, the Mutation Load (Somatic SNVs) filter chain generates final analysis results, and cannot be used to change the variants that are included in the analysis results. That is, mutation load results that are generated as a result of using this filter chain do not change after an analysis is complete.
Oncomine BRCA	Default filter for Oncomine BRCA workflows. It removes any variants in the sample ID amplicons, which are not in the BRCA1 and BRCA2 genes.
Oncomine Somatic Variants	Returns results for INDELs and SNV variant types, and minor allele frequencies between 0.0 and 1.0E-6 based on 5000Exomes and ExAC annotation source databases that have homopolymer lengths less than or equal to 7 and allele frequencies between 0.05 and 1.0.
Oncomine Variants, 5% CI CNV ploidy >= gain of 2 over normal	Restricts copy number variants to gains of greater than 2 based on the 5% confidence interval level. It also returns Oncomine-annotated variants.

Filter chain name	Description
Somatic Mutation Load	Returns results for SNV variant types, and minor allele frequencies between 0.0 and 1.0E-6 based on 5000Exomes and ExAC annotation source databases that have homopolymer lengths less than or equal to 7.
Variant Matrix Summary	For visualizations that include the Variant Matrix, such as TagSeq and Ion AmpliSeq™ HD analyses, this filter chain returns results in a visualization that contains the same set of variants that are included in the Analysis Results table. This filter chain allows results to be exported. Import the exported results file into Oncomine™ Knowledgebase Reporter (OKR) to include the variants shown in the Variant Matrix Summary view in OKR reports. Variant types returned are SNV/INDEL, CNV, fusions, and RNAExonVariants.

Annotation source filters

You can create filter chains that identify variants based on the annotations associated with those variants. Ion Reporter™ Software uses annotation sources from public and proprietary genomic databases to apply these annotations to variants during analysis.

Filters that are made from annotation sources are available in the software and you can use these filters build filter chains that narrow the list of variants that appear in the analysis results.

5000Exomes filters

These filters can be added to filter chains to detect minor allele frequencies (MAF) for specific population groups in Ion Reporter™ Software. The filters are derived from the 5000Exomes annotation database. The specific filters are:

- 5000Exomes AfricanAmerican MAF
- 5000Exomes EuropeanAmerican MAF
- 5000Exomes Global MAF

Filter conditions include the ability to set minor allele frequency ranges from 0.0 to 0.5, to include or exclude boundary values in the range, and to include or exclude unannotated variants in the analysis results.

The filter returns minor allele frequency results in samples that align with the 5000Exomes minor allele frequency database.

Clinvar filter

This filter can be added to filter chains in Ion Reporter™ Software to evaluate the impact of variants observed in samples that match those in the National Center for Biotechnology Information (NCBI) ClinVar database.

The impact values that can be included in the filter chain are: Pathogenic, Likely benign, Benign, other, Likely pathogenic, Uncertain significance, not provided, and drug response.

The filter returns all variants with the selected impact values that match those in the NCBI ClinVar database.

COSMIC filter

This filter can be added to filter chains in Ion Reporter™ Software to compare variants to the catalog of somatic mutations in tumor tissue as compiled by the COSMIC database.

Filter conditions include the ability to select all COSMIC values, to select specific annotation values, and to include or exclude unannotated variants.

The filter returns variants that match those in the COSMIC database.

dbSNP filter

This filter can be added to filter chains in Ion Reporter™ Software to compare single nucleotide polymorphism variants in samples against the dbSNP database.

Filter conditions include the ability to select all dbSNP values (In), or to exclude all dbSNP values (Not In).

The filter detects SNP variants that match those in the dbSNP database, which can be included in or excluded from analysis results.

DGV filter

This filter can be added to filter chains in Ion Reporter™ Software to detect human genomic structural variants that match those in the Database of Genomic Variants (DGV).

Filter conditions include the ability to include all DGV variants (In), or to exclude all DGV variants (Not In).

The filter detects human genomic structural variants as defined by DGV, which can be included in or excluded from analysis results.

DrugBank filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants that are correlated with drugs and drug targets listed DrugBank database.

Filter conditions include the ability to select specific values, to include all DrugBank values (In), or to exclude all DrugBank values (Not In). In addition, unannotated variants can be included or excluded.

This filter detects variants that are correlated with drugs and drug targets in DrugBank, which can be included in or excluded from analysis results.

ExAC filters

These filters can be added to filter chains to detect rare gene variants for specific population groups in Ion Reporter™ Software. The filters are derived from the Exome Aggregation Consortium (ExAC) database. The specific filters are:

- ExAC AAF
- ExAC EAAF
- ExAC EFAF
- ExAC ENFAF

- ExAC GAF
- ExAC LAF
- ExAC OAF
- ExAC SAAF

Filter conditions include the ability to set a range from 0.0 to 1.0, to include or exclude boundary values from the ranges, and to include or exclude unannotated variants.

The filter detects rare gene variant results that align with the ExAC database, which can be included or excluded from analysis results.

FATHMM Score filter

This filter can be added to filter chains in Ion Reporter™ Software to calculate Functional Analysis through Hidden Markov Models (FATHMM) Scores for coding variants, non-synonymous single nucleotide variants (nsSNVs), and non-coding variants.

Filter conditions include the ability to set a range from 0.0 to 1.0 for the score, to include or exclude boundary values in the ranges, and to include or exclude unannotated variants.

The filter calculates FATHMM scores for coding variants, nsSNVs, and non-coding variants.

Gene Ontology filter

This filter can be added to filter chains in Ion Reporter™ Software to detect various gene ontologies (functions of specific genes).

Filter conditions include the ability to select all (In), none (Not In), or to select specific annotations, and to include or exclude unannotated variants.

This filter returns gene ontologies of samples based on the selections made.

Gene Symbol filter

This filter can be added to filter chains in Ion Reporter™ Software to report on specific genes.

Filter conditions include the ability to select all (In), none (Not In), or specific gene symbols from the list, and to include or exclude unannotated variants.

This filter returns the gene symbols of genes that are found in your samples.

Minor Allele Frequency filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants with minor allele frequencies that match those in the dbSNP database from the 1000 genomes project.

Filter conditions include the ability to set a range from 0.0 to 0.5 for minor allele frequencies, to include or exclude range boundary values, and to include or exclude unannotated variants.

The filter returns variants in samples that match those in the dbSNP database from the 1000 genomes project.

OMIM filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants that match those in the Online Mendelian Inheritance of Man® (OMIM) database.

Filter conditions include the ability to search for all (In), none (Not In), or specific annotations, and to include or exclude unannotated variants.

The filter returns variants in samples that match those in the OMIM database.

Pfam filter

This filter can be added to filter chains in Ion Reporter™ Software to detect protein domain families in the coded proteins as defined by the Pfam database.

Filter conditions include the ability to include all (In), none (Not In), or specific annotations, and to include or exclude unannotated variants.

The filter returns variants in the samples that match the variants in the Pfam database.

PhyloP Scores filter

This filter can be added to filter chains in Ion Reporter™ Software to measure the conservation of protein across a wide range of organisms in metagenomics 16S analyses.

Filter conditions include the ability to set a score range from -14.0 to 3.0, to include range boundary values, and to include or exclude unannotated variants.

This filter returns PyloP scores for metagenomics 16S samples.

UCSC Common SNPs filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants that match those in the UCSC Genome Browser Common SNPs database.

Filter conditions include the ability to include (In) or exclude (Not In) variants that match the UCSC Common SNPs.

The filter returns variants in samples that match the UCSC Common SNPs database.

Data type filters

You can create filter chains that are based on Ion Reporter™ Software variant data types, such as allele frequency, allele ratio. Many data types used to sort variants in the **Analysis Results** screen are available as filters.

Allele frequency filter

This filter can be added filter chains in Ion Reporter™ Software to report the frequency of alleles observed in raw data.

Filter conditions include the ability to set allele frequency ranges from 0.0 to 1.0, to include or exclude boundary values in the range, and to include or exclude unannotated variants in the analysis results.

The filter returns allele frequencies of alleles observed in raw data.

Allele ratio filter

This filter can be added to filter chains in Ion Reporter™ Software to narrow analysis results to non-reference allele frequencies.

Filter conditions include the ability to set allele ratio ranges from 0.0 to 1.0, to include or exclude boundary values in the range, and to include or exclude unannotated variants in the analysis results.

The filter returns all variants that have at least one non-reference allele reported with a frequency in the selected filter range.

Allele read-count filter

This filter can be added filter chains in Ion Reporter™ Software to set the minimum count for genotype alleles.

Filter conditions include the ability to set allele read-count ranges from 0 to 100,000, to include or exclude boundary values in the range, and to include or exclude unannotated variants in the analysis results.

The filter removes variant candidates that do not have the required numbers of supporting reads from analysis results.

Alternate allele count filter

This filter can be added to filter chains in Ion Reporter™ Software to set the minimum count for alternate alleles.

Filter conditions include the ability to set alternate allele read-count ranges from 0 to 100,000, to include or exclude boundary values in the range, and to include or exclude unannotated variants in the analysis results.

The filter removes variant candidates that do not have the required numbers of reads from analysis results.

CNV Confidence Range filter

This filter can be added to filter chains in Ion Reporter™ Software to return copy number variants (CNV) with confidence levels between 10 and 10,000,000 and other variants.

Filter conditions include the ability to set the CNV confidence range from 10 to 1.0E7, to include or exclude boundary values in the range, and to include or exclude unannotated variants in the analysis results.

The filter returns copy number variants with high confidence levels and other variants.

CNV Confidence Range - CNVs Only

This filter can be added to filter chains in Ion Reporter™ Software to return copy number variants (CNV) with confidence levels between 10 and 10,000,000.

Filter conditions include the ability to set the CNV confidence range from 10 to 1.0E7, to include or exclude boundary values in the range, and to include or exclude unannotated variants in the analysis results.

The filter returns only copy number variants with high confidence levels.

CNV Somatic Confidence Range filter

The CNV Somatic Confidence Range filter can be added to filter chains in Ion Reporter™ Software to make ploidy estimates with lower and upper confidence values on somatic samples. The filter detects extra copies over the expected normal ploidy value (2 for autosomes, 2 for X chromosomes in females, and 1 for X chromosomes in males). The 5-percent lower confidence bound value is the ploidy estimate where there is a 5 percent chance the true ploidy is below that value. The 95 percent upper confidence bound is the ploidy estimate where it is 95 percent certain that the true ploidy is below that value. The lower bound is the most important for gains. The upper bound is most important for losses.

By default, the filter is set to detect gains and losses using the confidence interval values of 5-percent confidence interval for Minimum Ploidy Gain over the expected value and 95-percent confidence interval for Minimum Ploidy Loss under the expected value. The default boundary values are set to 0.0 by default.

If you set the **Minimum Ploidy Gain (5% CI) over expected** to **2.0** (2+2=4) and the **Minimum Ploidy Loss (95% CI) under expected** to **0.0**, you can expect the following example CNV call CI data:

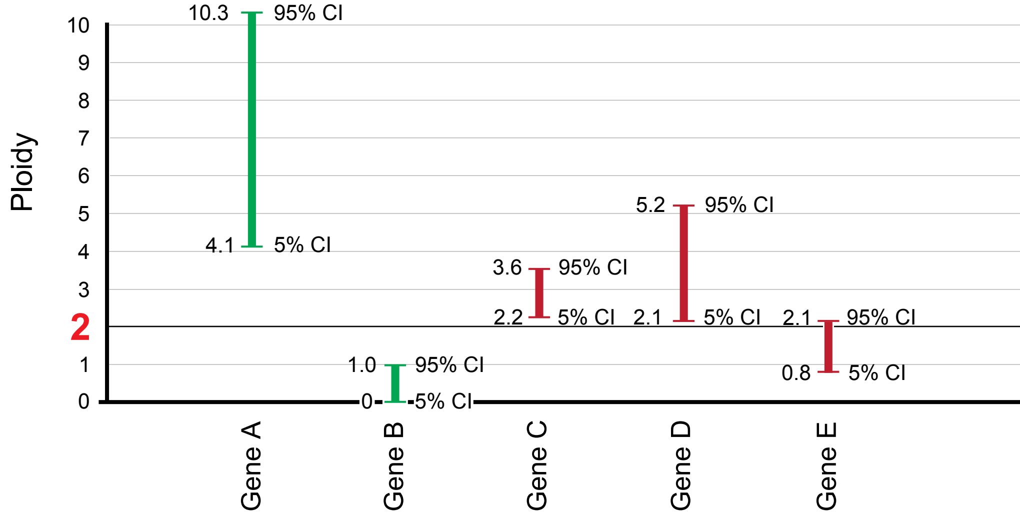
Gene A – A gene with suspected gain of 5% CI = 4.1 and 95% CI = 10.3 filtered in.

Gene B – A gene with suspected loss of 5% CI = 0 and 95% CI = 1.0 filtered in.

Gene C – A gene with suspected gain of 5% CI = 2.2 and 95% CI = 3.6 filtered out (2.2 is less than 4).

Gene D – A gene with suspected gain of 5% CI = 2.1 and 95% CI = 5.2 filtered out (2.1 is less than 4).

Gene E – A gene with 5% CI = 0.8 and 95% CI = 2.1 filtered out (0.8 is less than 4 and 2.1 is greater than 2).



CNV Somatic Confidence - CNVs Only

The CNV Somatic Confidence - CNVs Only filter can be added to filter chains in Ion Reporter™ Software to make ploidy estimates with lower and upper confidence levels.

Filter conditions include the ability to enable and set the range for Minimum Ploidy Gain (5% CI) over expected, to enable and set the Minimum Ploidy Loss (95% CI) under expected ploidy values, and to include or exclude boundary values for either of the lower and upper confidence ranges.

The filter returns only somatic copy number variants within the lower and upper limits of the set confidence ranges.

Confident Somatic Variants filter

This filter can be added to a filter chain to return variants that Ion Reporter™ Software algorithms that are deemed to be confident somatic variants.

Filter conditions include the ability to include the confident somatic variants in the analysis results (In), or to exclude the confident somatic variants in analysis results (Not In).

The filter detects confident somatic variants to include or exclude.

Default DNA and Fusions View filter

This filter can be added to filter chains in Ion Reporter™ Software to detect all the fusion variants, expression control markers, assay 5-prime/3-prime markers, and all DNA variants that the predefined Default DNA and Fusions View filter chain detects.

Filter conditions include the ability to select specific annotations (Present or Absent), to include all default DNA and Fusions view variants (In), or to exclude all Default DNA and Fusions view variants (Not In). In addition, unannotated variants can be included or excluded in the analysis results.

The filter detects the fusion variants, expression control markers, assay 5-prime/3-prime markers, and all DNA variants, which can be included or excluded.

Default Fusion View filter

This filter can be added to filter chains in Ion Reporter™ Software to detect all the fusion variants, expression control markers, and assay 5-prime/3-prime markers that the predefined Default Fusions View filter chain detects.

Filter conditions include the ability to select specific annotations (Present or Absent), to include all Default Fusions View variants (In), or to exclude all Default Fusions View variants (Not In). In addition, unannotated variants can be included or excluded in the analysis results.

The filter detects fusion variants, expression control markers, and assays 5-prime/3-prime markers, which can be included or excluded.

Disease Research Area filter

This filter can be added to filter chains in Ion Reporter™ Software to annotate disease research categories as defined by the Disease Research Area (DRA) annotation source database.

Filter conditions include the ability to select specific annotations, to include all Disease Research Area values (In), or to exclude all Disease Research Area values (Not In). In addition, unannotated variants can be included or excluded.

This filter annotates disease research areas that match the DRA database, and can be included or excluded.

Expected normal ploidy buffer filter

The Expected normal ploidy buffer filter can be added to filter chains in Ion Reporter™ Software to detect mosaic chromosomal aneuploidies in research samples and to set a buffer for normal ploidy values (2 for autosomes, 2 for X chromosomes in females, and 1 for X and Y chromosomes in males) so as to identify the most likely aneuploidy samples. Because of mosaicism detection allows decimal-level Copy Number Gain or Loss calls, ploidy calls do not have to be integers.

By default, the filter is set to filter out all copy number variant segments of gain or loss within 0.2 ploidy value of expected normal. Gains must be above ploidy 2.2 and below ploidy 1.8 to remain filtered in on autosome and female X, and ploidy 1.2 and ploidy 0.8 on male Y. Filter conditions include the ability to change the amount of buffer by adjusting the From and To values. This Expected Normal Ploidy Buffer overrides any Confidence filtering that may be in effect within the buffer zone.

If default settings are applied, ploidy values of 1.8 to 2.2 are filtered out as normal for autosomes and X chromosomes in females, and 0.8-1.2 are filtered out as normal for X and Y chromosomes in males. Values outside this range are detected as chromosomal ploidy samples.

Filtered Coverage filter

This filter can be added to filter chains in Ion Reporter™ Software to review results that meet your coverage threshold.

Filter conditions include the ability to set a coverage threshold and to include or exclude boundary values for the threshold, and include unannotated variants. The default threshold setting is 100.

The filter reports all results with a coverage threshold greater than 100 (or other threshold that is set).

Functional Scores filter

This filter can be added to filter chains in Ion Reporter™ Software to provide functional scores based on SIFT, PolyPhen, and Grantham scores. SIFT and PolyPhen scores are predictions of the functional effect of a variant on a protein. A Grantham score attempts to predict the distance between two amino acids, in an evolutionary sense (lower is less distance, higher is greater distance).

Filter conditions include the ability to include or exclude SIFT, PolyPhen, and Gratham scores, and to set ranges for those scores. SIFT and PolyPhen ranges can be set from 0.0 to 1.0, and Grantham can be set from 0.0 to 215.0. In addition, the range boundary values can be included or excluded, and unannotated variants can be included or excluded.

The filter returns a functional score based on the three (or fewer) scores.

Fusion Read Counts filter

This filter can be added to filter chains in Ion Reporter™ Software to produce counts for fusion reads.

Filter conditions include the ability to set a minimum count of 0 or higher, to include the boundary value for the count, and to include or exclude unannotated variants.

This filter returns results that meet the fusion read minimum count value.

Genetic Category Type filter

This filter can be included in a filter chain in Ion Reporter™ Software to detect variants of specific genetic category types.

Filter conditions include the ability to select all, none, or specific genetic category type annotations, and to include or exclude unannotated variants. Genetic category types include:

- HasDeNovoNonRefAllele
- HasDeNovoRefAllele
- IsNewlyHomzygousNonRef
- IsNewlyHomozygousRef
- InCompoundHeterozygote
- InTransPhaseCompoundHeterozygote
- HasMaleMaternalX
- HasUnknownX
- InconsistentWithFather
- InconsistentWithMother
- InconsistentWithParents

This filter returns genetic category type annotations for the variants included in the samples.

Genomic Coordinates filter

This filter can be added to filter chains in Ion Reporter™ Software to narrow analysis results to specific 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.

This filter returns variants in samples that fall between the genomic coordinates you set.

Homopolymer Length filter

This filter can be used in a filter chain in Ion Reporter™ Software to detect INDELs of specific homopolymer lengths.

Filter conditions include the ability to set a range from 0 to 1,000 for homopolymer length, to include or exclude range boundary values, and to include or exclude unannotated variants.

This filter returns INDEL variants in samples that meet the specified homopolymer length.

Hotspot filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants that overlap specific hotspots.

Filter conditions include the ability to include (In) or exclude (Not In) hotspots as defined by a hotspots file.

The filter returns variants that overlap hotspots included in an uploaded hotspots file.

Location filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants in specific positions, such as exonic.

Filter conditions include the ability to select all locations or specific locations, such as:

- unknown
- intergenic
- intronic
- exonic
- utr_5
- utr_3
- splicesite_5
- splicesite_3
- upstream
- downstream
- exonic_nc
- intronic_nc
- ncRNA
- nonCoding

The filter returns variants in samples that match the selected filter locations.

MyVariants filter

This filter can be included in a filter chain in Ion Reporter™ Software to report on MyVariants annotations as set by users.

Filter conditions include the ability to select Important, Ignore, or None for specific MyVariants annotations, and to include or exclude variants that are not flagged. Possible combinations include:

Option	Description
Important	Select **Important** to filter for MyVariants flagged "Important".
Ignore	Select **Ignore** to filter for MyVariants flagged "Ignore".
Important and Ignore	In the **Filter Option** dropdown list, select **In** to include all flagged MyVariants.
Ignore and None	1. Select **Include unannotated variants** to include all unflagged variants. 2. Select **Ignore** to include MyVariants flagged "Ignore".
Important and None	1. Select **Include unannotated variants** to include all unflagged variants. 2. Select **Important** to include MyVariants flagged "Important".
None	1. In the **Filter Option** dropdown list, select **Not In** to not include any flagged MyVariants. 2. Select **Include unannotated variants** to include all unflagged variants.

The filter returns variants in samples that have the specified MyVariants annotations.

Named Variants filter

This filter can be added to filter chains in Ion Reporter™ Software to detect known variants in the Ion AmpliSeq™ Community Panel for the cystic fibrosis transmembrane regulator (CFTR) gene, for example: c.2054delA.

Filter conditions include the ability to select all named variants, to select specific named variants, and to include or exclude unannotated variants.

The filter returns variants in samples that match the CFTR variants that are specified in the filter.

Oncomine filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants that match those in specific Oncomine™ panels, including:

- Oncomine BRCA DNA Regions
- Oncomine Breast cfDNA Regions
- Oncomine Colon cfDNA Regions
- Oncomine Comprehensive DNA Regions
- Oncomine Focus DNA Regions

- Oncomine Lung cfDNA Regions
- Oncomine Myeloid DNA Regions
- Oncomine Pan-Cancer DNA Regions
- Oncomine TagSeq Breast DNA Regions
- Oncomine TagSeq Lung DNA Regions
- Oncomine Tumor Mutation Load Regions

Filter conditions include the ability include (In) or exclude (Not In) Oncomine™ variants.

The filter returns variants in samples that match those in the selected Oncomine™ panel.

PValue filter

This filter can be added to filter chains in Ion Reporter™ Software to determine the *p*-value of variants. *p*-value is a statistical method for the detection of variant calls from next-generation sequencers.

Filter conditions include the ability to set a range from 0.0 to 1.0, to include the range boundary values, and to include or exclude the unannotated variants.

The filter returns *p*-values for variants calls in the samples.

Variant Classification filter

This filter can be added to filter chains in Ion Reporter™ Software to narrows results to the following user-set variant classifications:

- Unknown
- Benign
- Suspected Benign
- Suspected Deleterious
- Deleterious

Filter conditions include the ability to include all (set Filter Option to In), none (set Filter Option to Not In), or to select specific classifications.

The filter returns variants that are classified by the Ion Reporter™ Software users.

Variant Effect filter

This filter can be added to filter chains in Ion Reporter™ Software to detect the effect of variants on coding sequences. Specific values include:

- refAllele
- unknown
- synonymous
- missense
- nonframeshiftInsertion
- nonframeshiftDeletion
- nonframshiftBlockSubstitution
- nonsense
- stoploss
- frameshiftInsertion
- frameshiftDeletion
- frameshiftBlockSubstitution

Filter conditions include the ability to select all (set Filter Option to In), none (set Filter Option to Not In), or to select specific values, and to include or exclude unannotated variants.

The filter returns variants in samples that match the selected variant effects.

Variant Subtype filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variant subtypes, including:

- BigDel
- BigDup
- GeneCNV
- REF
- NOCALL

Filter conditions include the ability to select all or specific variant subtypes.

The filter returns variants that match the selected specific variant subtypes.

Variant Type filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants that match the following variant types:

- SNV
- INDEL
- MNV
- REF
- NOCALL
- CNV
- LONGDEL
- FUSION
- EXPR_CONTROL
- ASSAYS_5P_3P
- RNA_HOTSPOT
- GENE_EXPRESSION
- RNAExonVariant
- ProcControl
- FLT3ITD

  Note: The FLT3ITD variant type is detected only in Oncomine™ Myeloid Assay workflows.

Filter conditions include the ability to select all variant types or specific variant types.

The filter returns variants in analyses that match the selected specific variant types.

Zygosity filter

This filter can be added to filter chains in Ion Reporter™ Software to detect variants that are homozygous or heterozygous.

Filter conditions include the ability to search for both homozygous and heterozygous or either of them.

The filter returns variants in analyses that are homozygous or heterozygous.

VCIB CNV baseline creation

In AmpliSeq™, Ion AmpliSeq™ HD, and Oncomine™ assays, somatic 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 adjustment.

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 method 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.

The following instructions walk you through using the new Variability Correction Information Baseline (VCIB) CNV baseline, creating a new VCIB CNV baseline, or augmenting an existing VCIB CNV baseline for Oncomine™ Comprehensive Assay panels.

Use VCIB CNV baseline

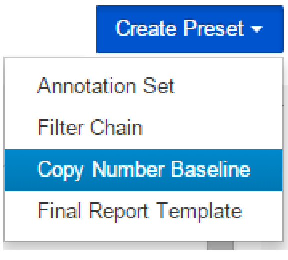
If you want to use the VCIB CNV baseline included in Ion Reporter™ Software, simply select it when creating your workflow.

Note: The VCIB CNV baseline is currently noncompatible with the Ion GRCh38 human reference.

Create a CNV baseline

Ion Reporter™ Software provides a wizard to guide you through Copy Number Variation (CNV) Baseline creation.

1. In the **Workflows** tab, in the **Presets** screen, click **Create Preset**, then select **Copy Number Baseline** from the dropdown list.



2. Click **AmpliSeq** or **AmpliSeq HD**, then select your Target Regions file, then click **Next**.

3. Select at least 48 samples and flag at least 6 of these "Normal", then click **Next**. Male or Female gender must be specified for Normal samples, but samples that are not flagged as normal can be male, female, or unknown. You can use the Summary panel to see your totals.

4. Enter a name for your baseline, then click **Create Baseline**.

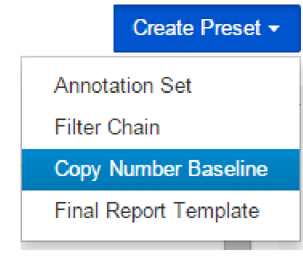
   Note: Log files for both successful and failed analyses include the "BaselineCreation.log" file, which has the BAM files named that were rejected due to similarity to other files in the baseline, as well as the "map.TmapMergeActor-00.err" file that has the BAM files named that were rejected due to QC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

Proceed to “Create an Ion Reporter™ Software analysis workflow“ on page 455 to add this new baseline to your workflow.

Augment (add Samples to) an existing VCIB CNV baseline

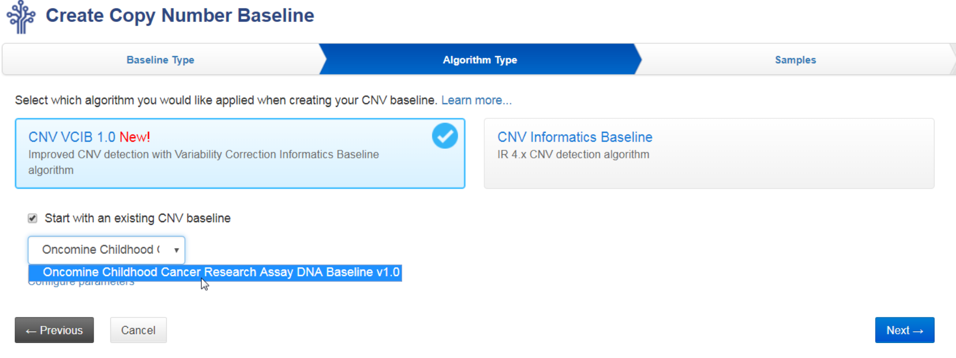
Ion Reporter™ Software provides a wizard to guide you through Copy Number Variation (CNV) baseline creation. This example describes how to add additional samples to an existing CNV baseline.

1. Under the **Workflows** tab, in the **Presets** screen, click **Create Preset**, then select **Copy Number Baseline** from the dropdown list.



2. Click **AmpliSeq**, select your **Targets Regions** file, then click **Next**.

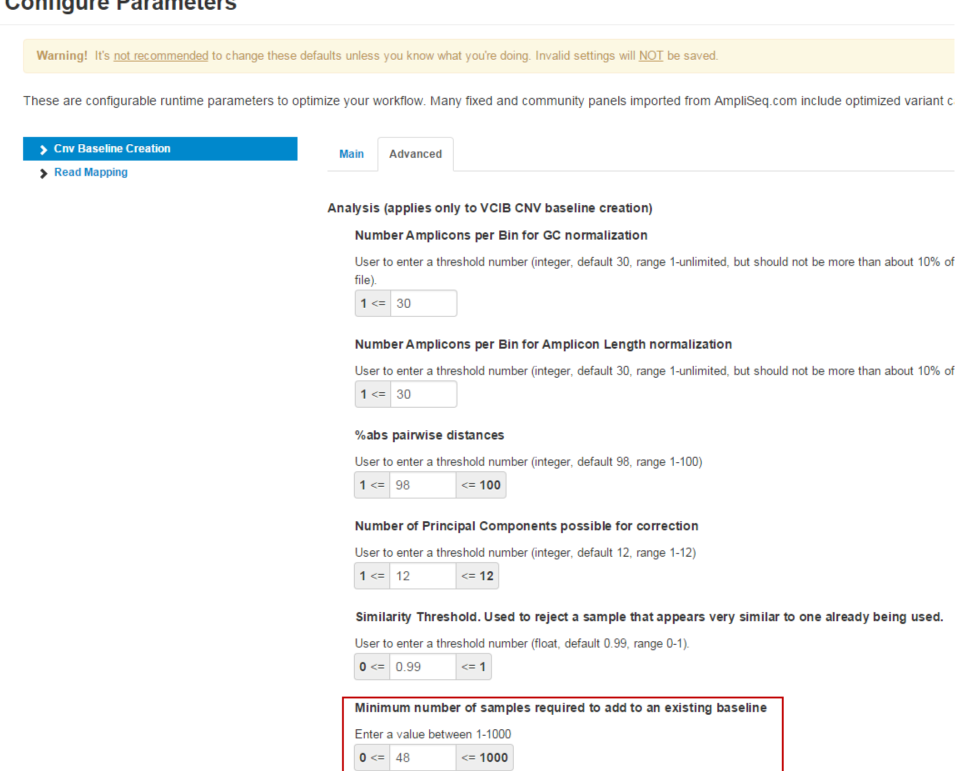
3. Select the **Start with an existing CNV Baseline** checkbox, then select a baseline from the dropdown list.



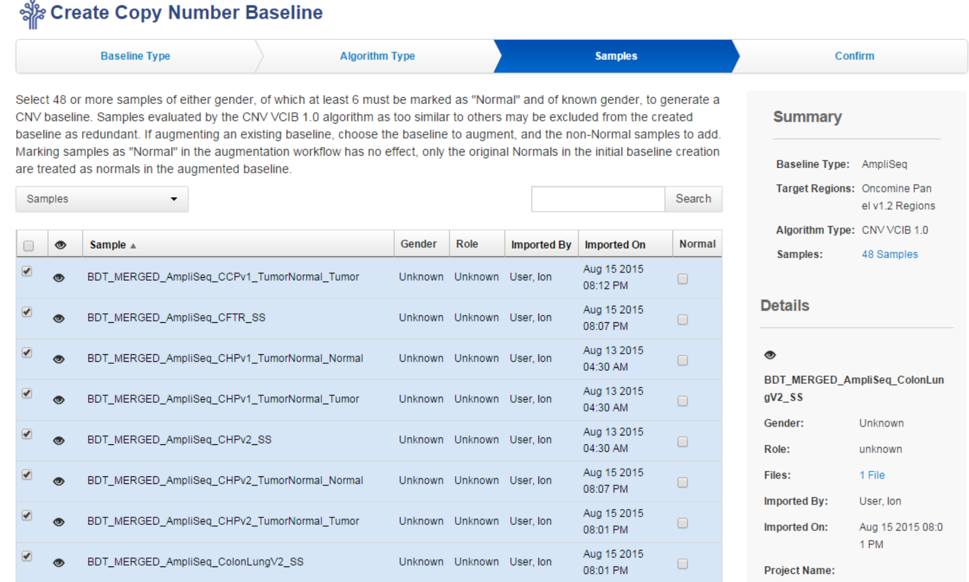
   Note: By default, the software prompts you to add another 48 samples. However, you can set the number to 1 or more. Add non-Normal samples. Marking samples as "Normal" in the augmentation workflow has no effect, only the original Normals in the first baseline creation are treated as Normals in the augmented baseline.

4. Click the **Configure Parameters** link.

5. In the **Configure Parameters** dialog, click **Cnv Baseline Creation**, then **Advanced**. Set the **Minimum number of samples required to add to an existing baseline** to the number you are adding, click **Done**, then click **Next**.



6. Select additional samples, then click **Next**.

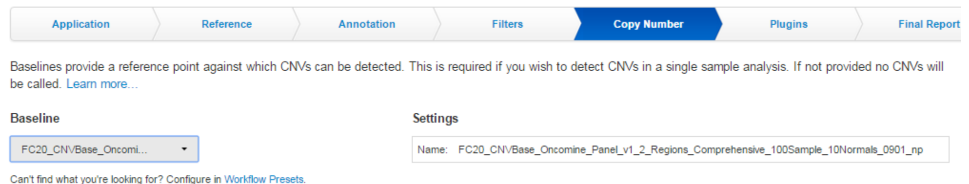


7. Enter a name for your baseline, then click **Create Baseline** to save.

Create an Ion Reporter™ Software analysis workflow

Ion Reporter™ Software provides a wizard that you can use for guidance to create a workflow. However, it can be easier to copy an existing Ion Reporter™ predefined workflow, then add the your newly created baseline.

1. Under the **Workflows** tab, in the **Overview** screen, select an appropriate Oncomine™ workflow.
2. Click **Actions**, then select **Copy** from the dropdown list to launch the workflow wizard. Click **Next** to advance to the **Reference** tab.
3. Ensure that **hg19** is the selected **Reference**, select a **Target Regions**, **Hotspot Regions**, and **Fusions** BED file from the respective dropdown lists, then click **Next**.
4. Select an **Annotation Set** from the dropdown list, then click **Next**.
5. Select a **Filter Chain** from the dropdown list, then click **Next**.
6. In the **Copy Number** tab, select the baseline that you want to use from the **Baseline** dropdown list, then click **Next**.

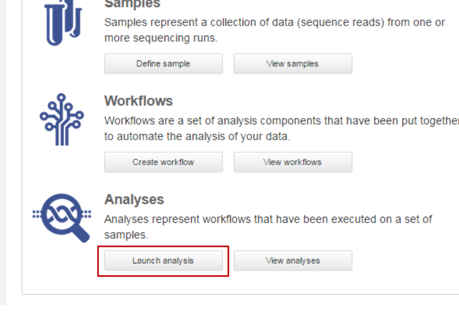


7. In the **Plugins** tab, ensure that all **In-Analysis** plugins are deselected, then click **Next**.
8. Select a **Final Report Template** from the dropdown list, then click **Next**.
9. In the **Parameters** tab, review the default settings, then click **Next**.

   Note: Although Read Mapping parameters are exposed in workflow creation, it is not necessary to change any settings.
10. In the **Confirm** tab, enter a **Workflow Name** and description, then click **Save Workflow**.

Launch an analysis

1. Under the **Home** tab, in the **Dashboard** screen, click **Launch Analysis**.



2. In the **Launch Analysis** wizard **Workflow** tab, select your custom workflow or one of the pre-installed Oncomine™ workflows, then click **Next**.

3. Select the sample(s) to include in the analysis:

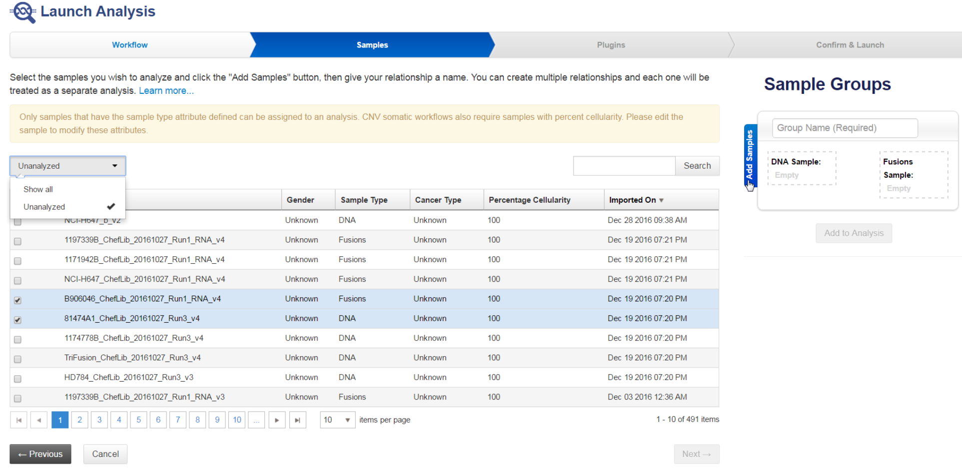
   a. Use the **Samples** dropdown list to filter the available samples.

   b. Click within a sample row to select each sample to include in a sample group, then click **Add Samples** in the **Sample Groups** pane.

   c. Enter a **Group Name**, then click **Add to Analysis**.

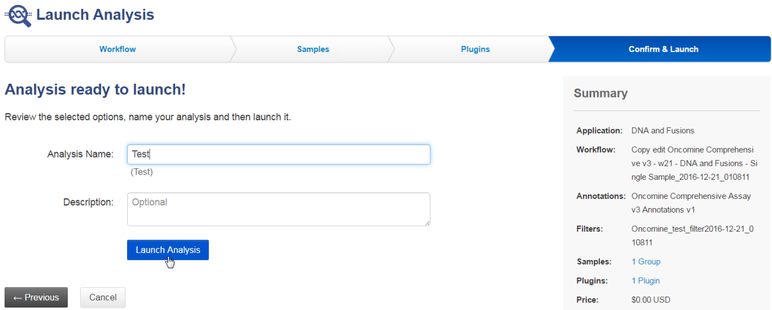
   d. Repeat substeps **b.** and **c.** to add additional **Sample Groups**.

   e. Click **Next** 2 times.



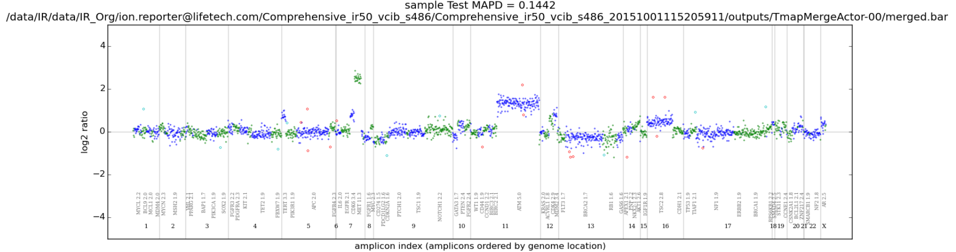
Note: The **Percentage Cellularity** sample attribute is required for DNA samples.

4. In the **Confirm & Launch** tab, enter an **Analysis Name** and description for the analysis, then click **Launch Analysis**.



5. Review your results in the **Analyses** tab. Confirm the CNV workflow and baseline used in the **Details** pane.

6. *(Optional)* Download the analysis results and view them visually with the cn_results.png.



Interpretation example: This plot shows log2 ratios across the genome and highlights panel CNV IDs. The alternating blue and green color is used to distinguish between adjacent CNV IDs. The outliers data are the small pink circles. The numbers on the X axis are the chromosomes. Above this are the CNV ID names and the mean CN call for each CNV ID. You can see copy number gains on chromosome 7 and chromosome 11. The MAPD number at the top of the plot is a QC metric measuring the noisiness of the sample. A low MAPD is good. MAPD >0.5 is considered to fail QC. Below the MAPD is the BAM file name. Log2 ratios of 0 are equivalent to a copy number call of 2 (normal). If the sample was a male, you would expect to see a copy number of 1 on chromosome X.

Documentation and support

Related documentation

Document	Publication number	Description
Torrent Suite™ Software 5.10 Help	MAN0017597	Describes the Torrent Suite™ Software and provides procedures for common tasks.
Oncomine™ Childhood Cancer Research Assay User Guide	Pub. No. MAN0017117	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Childhood Cancer Research Assay.
Oncomine™ TCR Beta Assay User Guide	Pub. No. MAN0017438	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ TCR Beta Assay.
Oncomine™ Comprehensive Assay v3 User Guide	MAN0015885	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Comprehensive Assay.
Oncomine™ Focus Assay, Part III: Variant Analysis User Guide	MAN0015821	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ Focus Assay.
Oncomine™ cfDNA Lung Assays, Part III: Variant Analysis User Guide (Pub. No. MAN0015874)	MAN0015874	Describes how to use Ion Reporter™ Software for data analysis of the Oncomine™ cfDNA Assay.
Ion ReproSeq™ PGS Kits – Ion S5™/Ion GeneStudio™ S5 Systems User Guide	MAN0016158	Describes how to use Ion Reporter™ Software for data analysis of the Ion ReproSeq™ assay.
Oncomine™ Knowledgebase Reporter 3.0 User Guide	MAN0017300	Describes how to generate reports in Oncomine™ Knowledgebase Reporter Software.

Library preparation documentation

- *Ion AmpliSeq™ Library Kit 2.0 User Guide*, (Pub. No. MAN0006735)
- *Ion AmpliSeq™ HD Library Kit User Guide*, (Pub. No. MAN0017392)

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**.
- *Cancer genomics and transcriptomics research*, available at **https://www.thermofisher.com/us/en/home/global/forms/life-science/request-NGS-application-note.html?appnote=5**.
- *Low-frequency mutations from cell-free DNA*, available at **https://www.thermofisher.com/us/en/home/global/forms/life-science/request-NGS-application-note.html?appnote=2**.
- *Reproductive genomics and inherited disease research*, available at **https://www.thermofisher.com/us/en/home/global/forms/life-science/request-NGS-application-note.html?appnote=3**.
- *Rapid sequencing of microorganisms*, available at **https://www.thermofisher.com/us/en/home/global/forms/life-science/request-NGS-application-note.html?appnote=4**.

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

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23 July 2018