[redacted]

A Sysmex Group Company

OGT Handbook

Interpret Cloud User Guide

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Accessing Interpret Cloud

Following deployment of an installation of the Interpret software, OGT support will provide users with:

1. The URL of the Interpret deployment (**https://web-app.*.interpret-ogt.com,** where "*" is a name specific to the deployment. E.g. **https://web-app.mylab.interpret-ogt.com**).
2. A user name (e.g. "admin")
3. A password for the user name.

To access Interpret:

1. Open a web browser and navigate to the URL provided. A screen like the following may appear, indicating that the software is loading. This may take a few minutes.

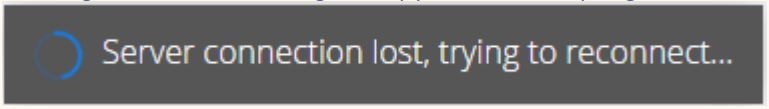
Figure 1: The Interpret start page, indicating that the software is loading

2. Once the software has loaded, a login screen like the following will be displayed. Enter the user name and password provided, and click **Log In**.

Figure 2: The Interpret login page, displayed when the software has loaded

Access Restrictions
Please note that it is possible to configure Interpret such that it is only accessible from specific IP addresses. If the message "You do not have permission to access this resource" is displayed instead of the loading or login screens, please contact OGT for support.

Automatic shutdown
In order to optimise use of computing resources, the Interpret web interface will shut down automatically after a period of user inactivity (between 10 and 20 minutes). When this occurs, a message like the following will appear at the top-right corner of the screen:



To access Interpret, simply refresh the page and login again when prompted. Please note that processing of samples by the analysis pipeline is unaffected by this shutdown.

Uploading FASTQ Files

There are currently 2 methods for providing FASTQ files to the system:

1. Via the AWS web "console".
2. Via the "Upload FASTQs" page in the Interpret software.

UMIs
If the intention is to run UMI processing on the FASTQ files, they must have beeen generated with UMIs included. If UMIs are not included then the analysis will not complete.

Uploading via the AWS web console

To access the AWS web console, which provides the most reliable upload mechanism, the following information will be provided:

1. A URL to the upload page (similar to **https://s3.console.aws.amazon.com/s3/upload/ogt-data-mylab?region=eu-west-2&prefix=incoming/**)
2. An AWS account ID (a 12-digit number)
3. A user name
4. A password

To upload FASTQ files to the system:

1. Navigate to the upload page URL in a web browser.
2. Select **IAM user**.
3. Enter the Account ID provided and click **Next**.
4. Enter the user name and password provided and click **Sign in**.
5. Click either **Add Files** (to select FASTQ files) or **Add Folder** (to select a folder containing FASTQ files), and select the appropriate file/folder from the file system.
6. Scroll down to the bottom of the page and click **Upload**.
7. Upload progress will be displayed in the next page. Do not navigate away from this page until the upload is complete, otherwise it may fail.
8. When the upload is complete, confirmation will be displayed on the page.
9. To verify that all FASTQ files have been added to the system, in Interpret, select **Batches -> Run Batch**, and check that they have appeared in the samples table.

Automatic Sample ID
In order to monitor changes in Variant Allele Frequency between samples from the same source in different batches, all samples must be assigned the same Sample ID when uploaded into the system. When FASTQ files are uploaded via the AWS console, the system will automatically extract a Sample ID from the name of the FASTQ file using the **standard naming convention**. If necessary, rename the FASTQ files before upload to ensure they are assigned the correct Sample ID.

Uploading via the Interpret software

Once logged in to Interpret, to upload FASTQ files to be processed by the system:

1. Click on the **Batches** button in the toolbar and select **Upload FASTQs**.
2. Click **Select FASTQ Files**, select the FASTQ files from your file system and click **Open**. The FASTQ files should be automatically paired and listed in the **Paired FASTQs** table.
3. If necessary, modify the Sample ID assigned to each pair of FASTQs by clicking on the button in the **Sample ID** column, typing the correct name of the sample in the input field, pressing Enter and clicking **Done**.

Note that, in order to monitor changes in Variant Allele Frequency between samples from the same source in different batches, all samples must be assigned the same Sample ID when uploaded into the system

4. Click **Upload Paired FASTQ Files**.
5. Click **Ok**.
6. Click **No**.

Progress of the upload can be monitored in the **Upload FASTQs** page, and an **Estimated Time Remaining** for all selected files to be uploaded is provided, which will be dependent on the total size of the FASTQ files and the upload speed.

While the upload is in progress, please note the following:

1. It is essential that the user does not navigate away from the page before upload is complete.
2. If using the Chrome web browser, ensure that Upload FASTQs tab is the one selected in the browser window, as selecting another tab will result in the upload being paused.

User Interface

The Dashboard View

The dashboard view displayed below comprises 3 sections:

- Menu Bar
- Dashboard buttons to provide function shortcuts
- User account options

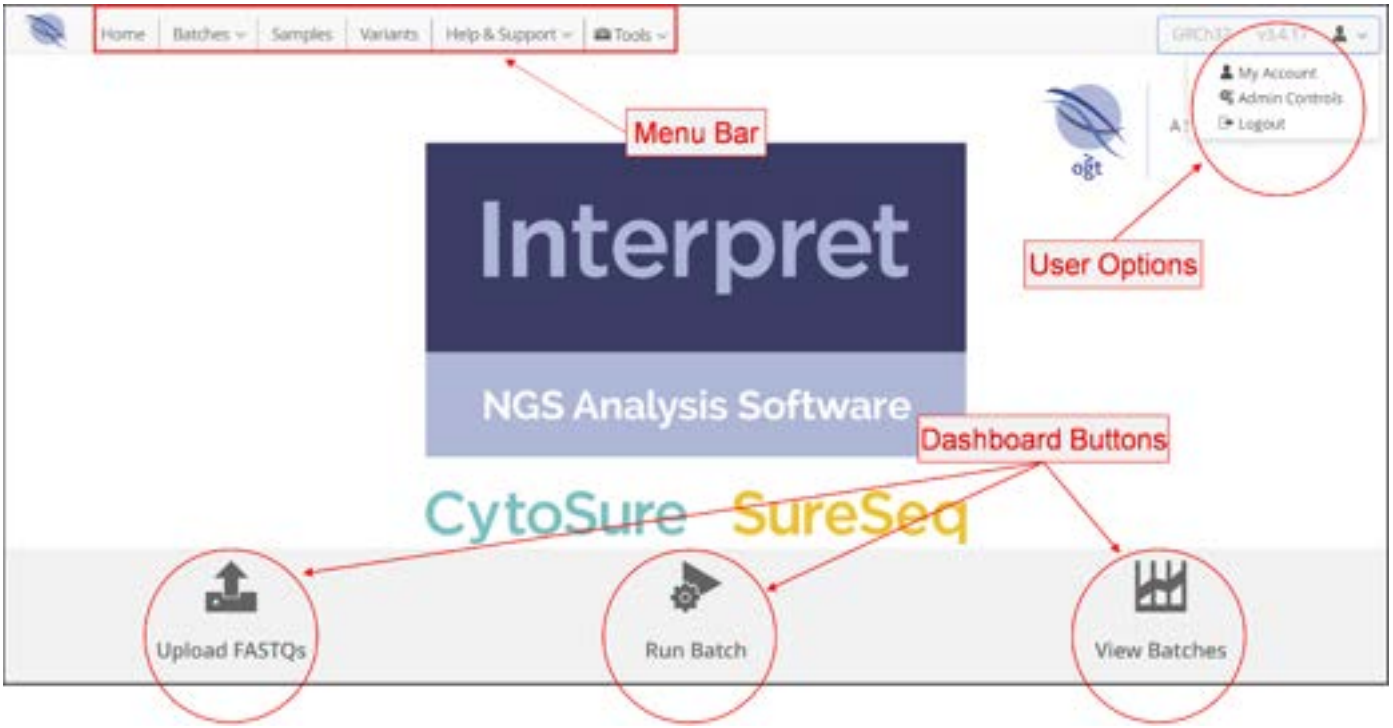


Figure 3: Annotated view of the dashboard

Menu Bar

The menu bar provides access to the functionality:

- **Home**- Link back to the Dashboard View
- **Batches** -Setting up and reviewing analysis batches
- **Samples** - Sample related functions
- **Variants**- Provides a means to view all data from a variant centric view
- **Help & Support** -A means to provide feedback as well request support
- **Tools** - Access to any additional tools

Figure 4: The menu bar from the dashboard

Dashboard Buttons

These provide shortcuts to the common actions required by users.

- **Upload FASTQs** - Select and upload FASTQ files.
- **Run Batch** - Run an analysis of a batch of loaded sample files
- **View Batches** - View the results of the batch analyses

Figure 5: Shortcut icons on the dashboard view

User Options

The User Options drop down menu gives the user access to a range of administration tools. Additionally this section of the dashboard displays the build of the genome being used as well as the version of the software. In this case it is GRCh37 and v3.3.61.

The drop down options are as follows:

- **My Account** - Your account details
- **Admin Controls** - Additional options described in detail in the Admin Options section of the guide
- **Logout** - Return to the Login page

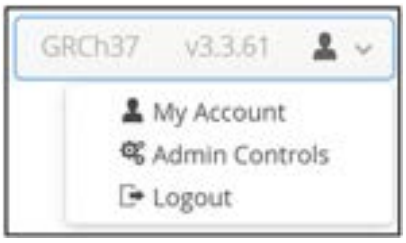


Figure 6: User account options

Viewing Samples

Once the FASTQ files have been uploaded the samples will be available to view in the Samples page

Accessing this is via the Samples button on the dashboard menu bar shown in the figure below.

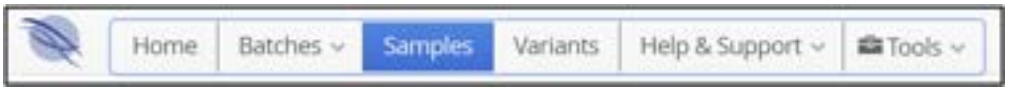


Figure 7: Selection of Samples from the Dashboard menu bar

Samples and status are displayed on the left hand side of the window and when a sample is selected further information is displayed on the right hand side.

When a sample is first loaded there is no additional information present.

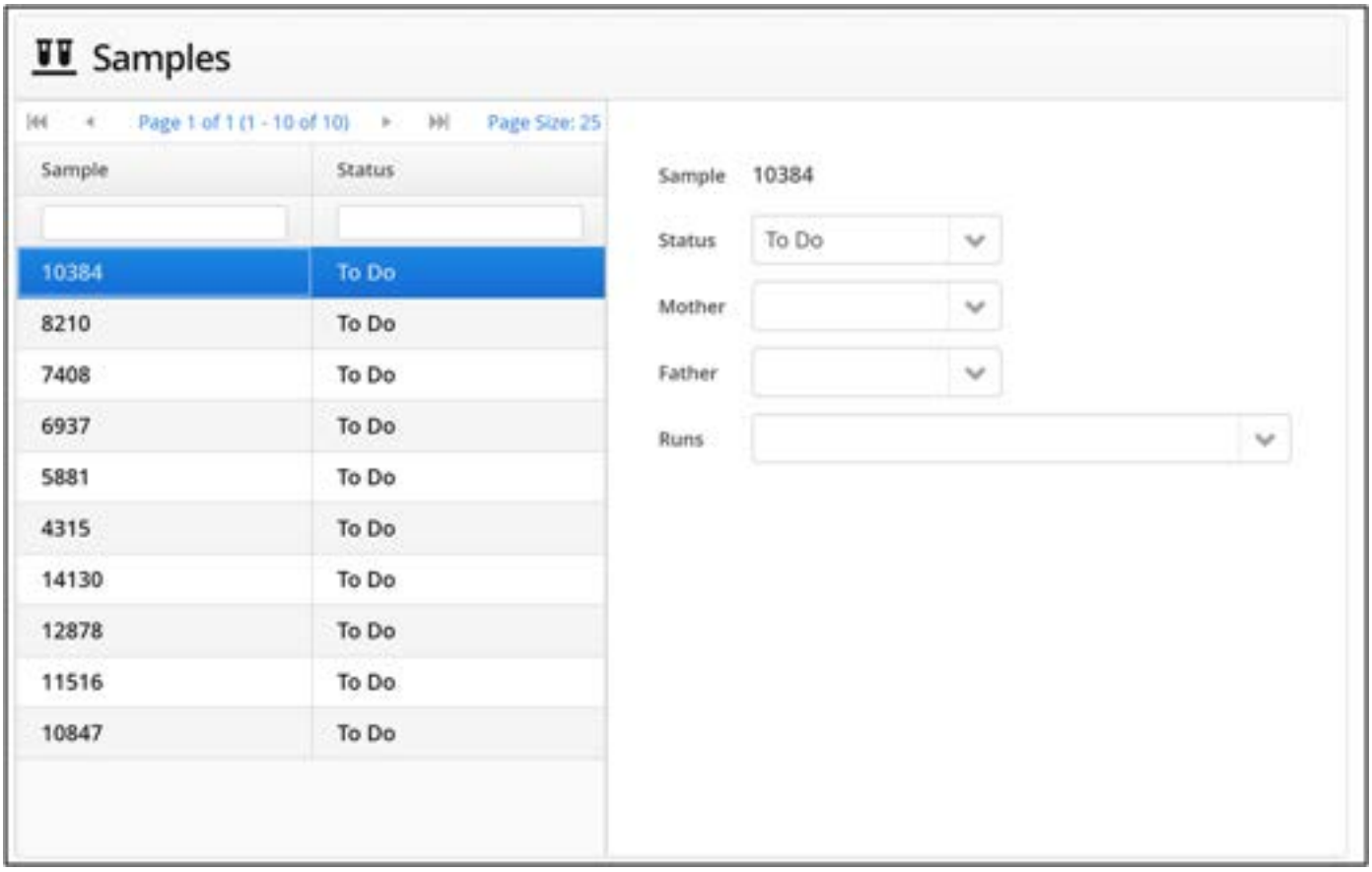


Figure 8: The initial view of samples in the Sample page

Samples can be searched by entering a part of the sample name in the search box. In the example below all samples containing "10" are displayed.

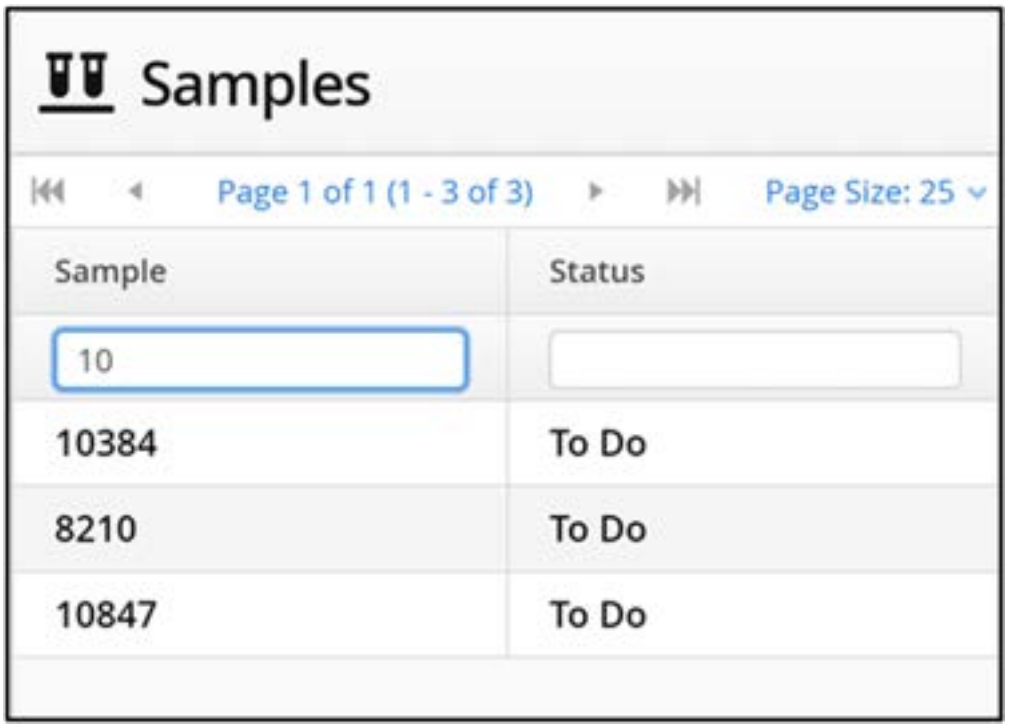


Figure 9: Searching for samples containing 10

The status of a sample can be updated. When first loaded, the status will be set to "To Do" and will be updated to "Running Pipeline" once processing has begun. Once sample processing is complete, the status will be further updated to "In Review". Users can assess the results if the analysis and manually update the sample status to "Completed" as required.

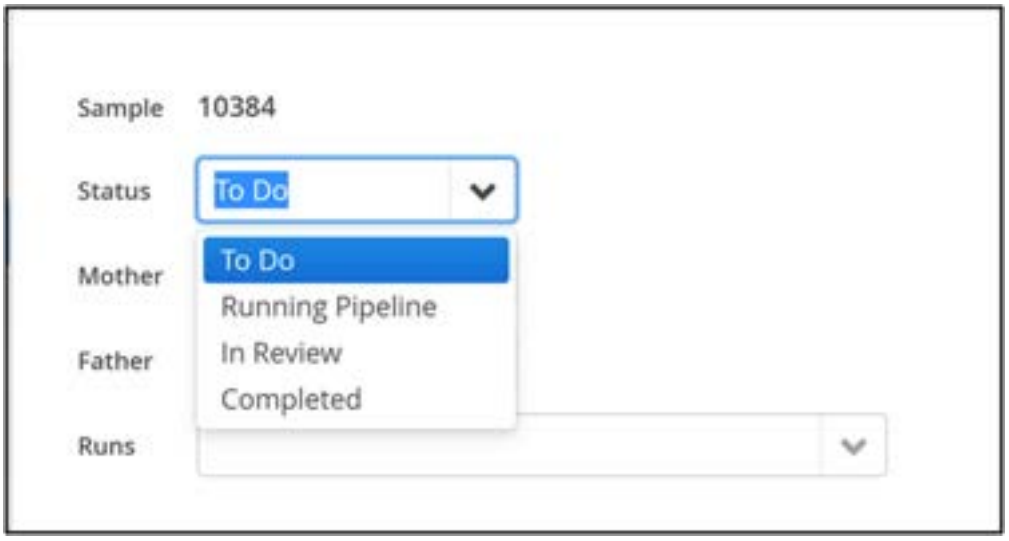


Figure 10: Modifying the status of a sample in the Sample view

It is also possible to specify the mother and father of a sample if they are also loaded in Interpret.

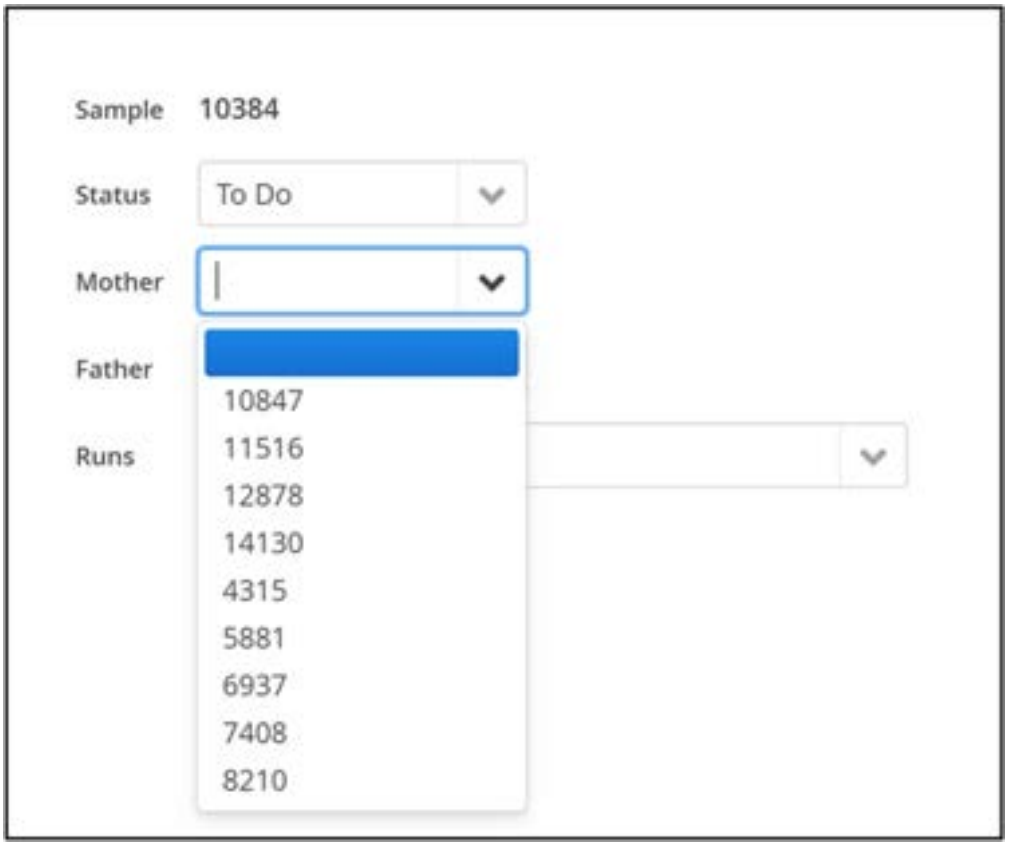


Figure 11: Specifying the mother of a sample

Initially, before any analysis, the run drop-down list will be empty.

Figure 12: A sample that has not been processed yet having no run data listed

When a sample has been analysed, each run can be accessed from the drop-down menu. Each run will have a set of data which is displayed by 3 tabs. These are for general run information, QC metrics and results of the analysis.

The General tab displays basic information about the analysis and provides a link to batch view.

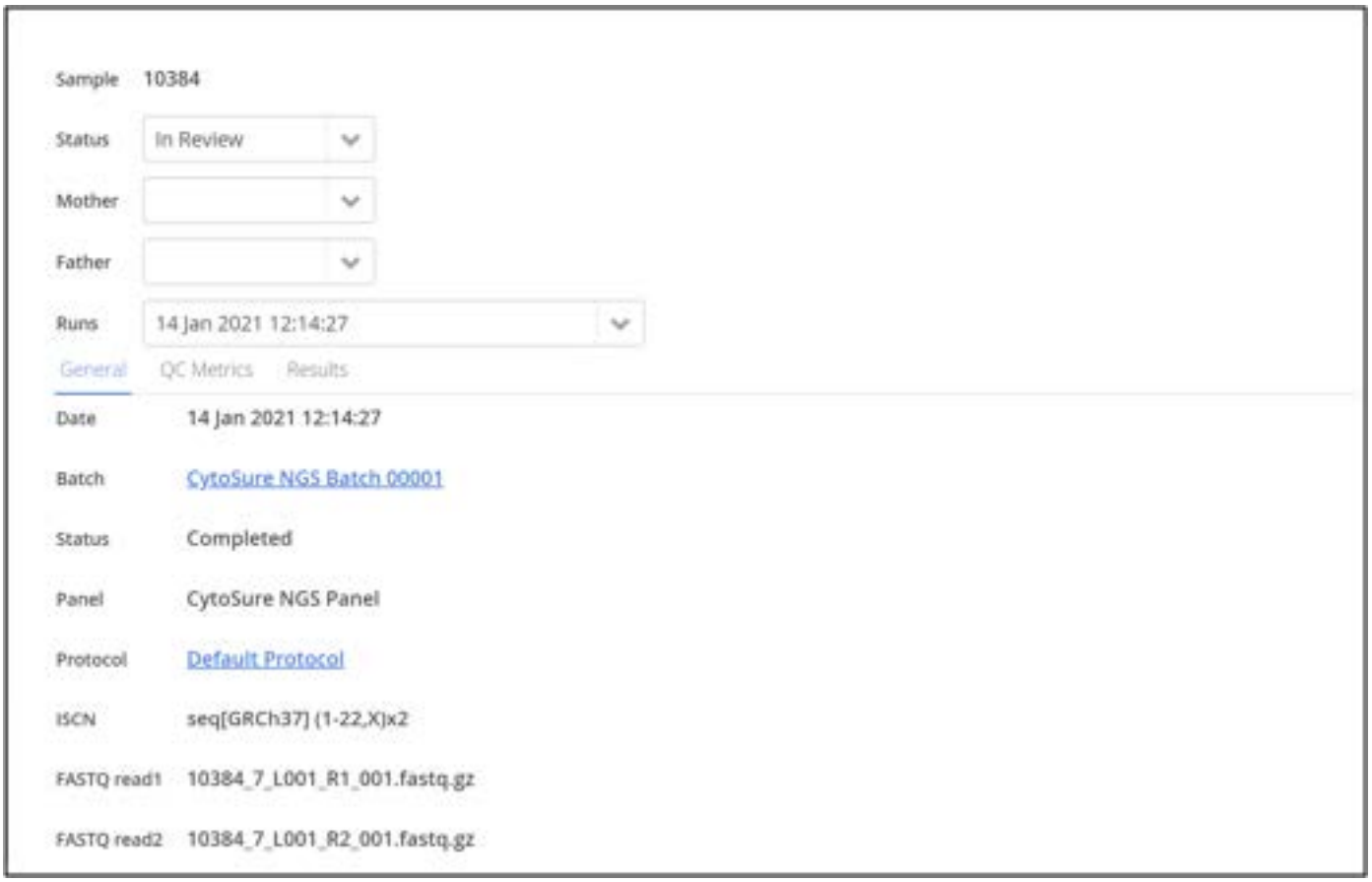


Figure 13: Viewing the General tab for a sample run

The QC Metrics tab gives an overview of the metrics of the sample. The data will be colour-coded according to the metric set that was defined for the analysis protocol.

There is further information on metric sets in the section of the guide that covers the admin options.

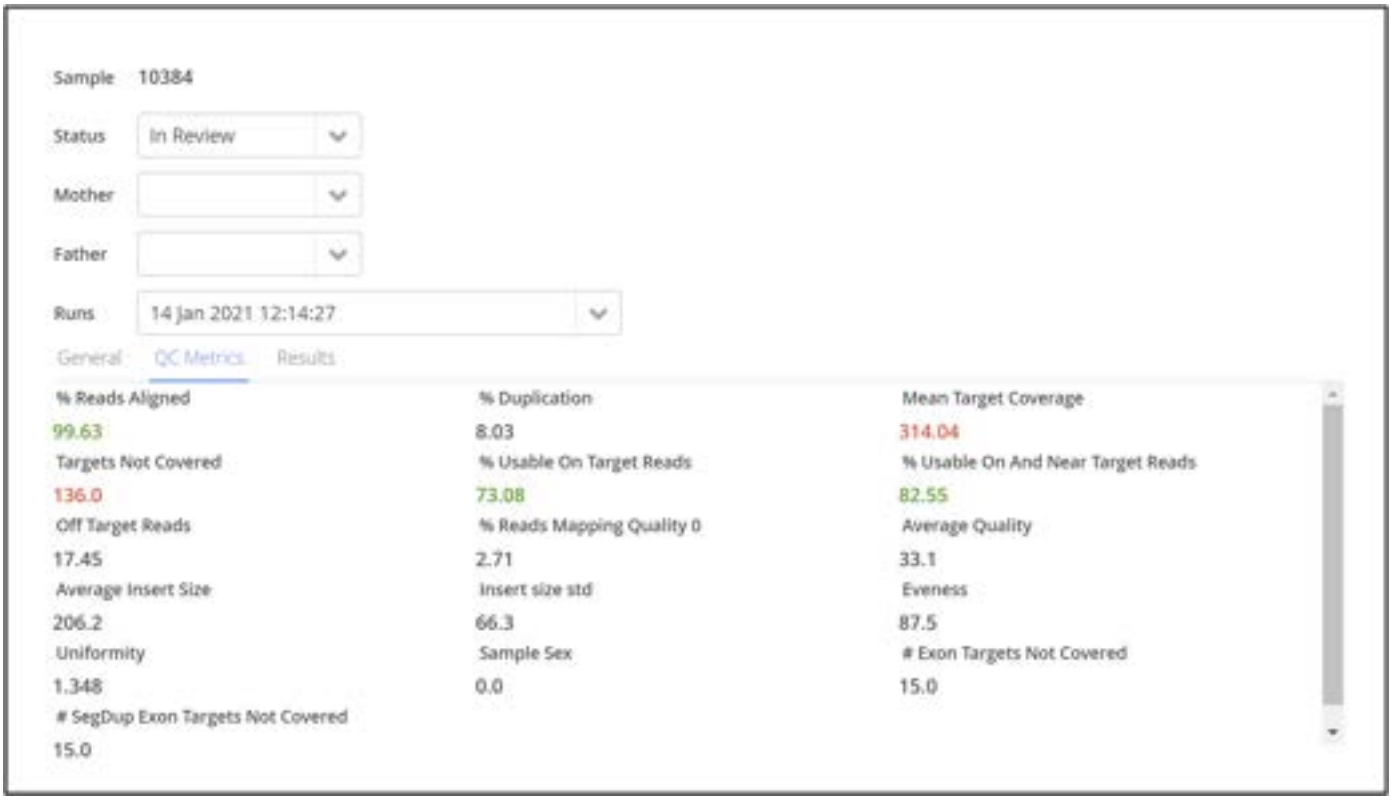


Figure 14: Viewing the QC Metrics tab for a sample run

Finally, the Results tab provides links (in green) to download files from the analysis as well to view (in blue) the different variants that have been detected.

Figure 15: Viewing the Results tab for a sample run

Adding User-defined Variables

In order to enable the user to capture and report custom information related to samples processed in Interpret, the admin controls section provides a means to create variables of different data types via Admin Controls > Analysis > Manage Samples > Variables.

Figure 16: The manage samples page in the Admin Controls

Selecting **Add New Variable Category** provides a text box to name the new variable and clicking **Add** adds a new sub-tab to the **Variables** tab.

Figure 17: Creating a new variable category named Additional

With the new category called "Additional" generated, users can create variables associated with the category by selecting **Add New Variable**.

Figure 18: An empty custom category named "Additional"

To delete an empty category, click the **Remove Empty Category** button. To create new variables in the category, click the **Add New Variable** button, assign a **Name** to the variable, confirm the **Category** with which it should be associated, and select the appropriate data type from the **DataType** drop-down box, then click the **Add** button.

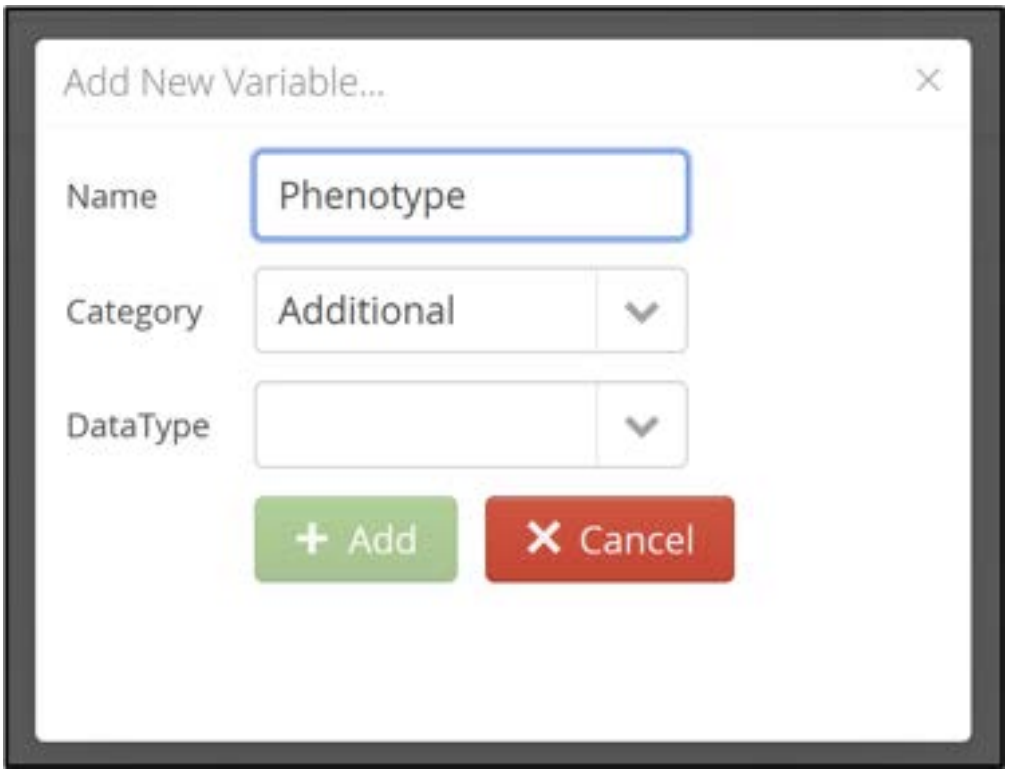


Figure 19: Creating a new variable named "Phenotype" in a category named "Additional".

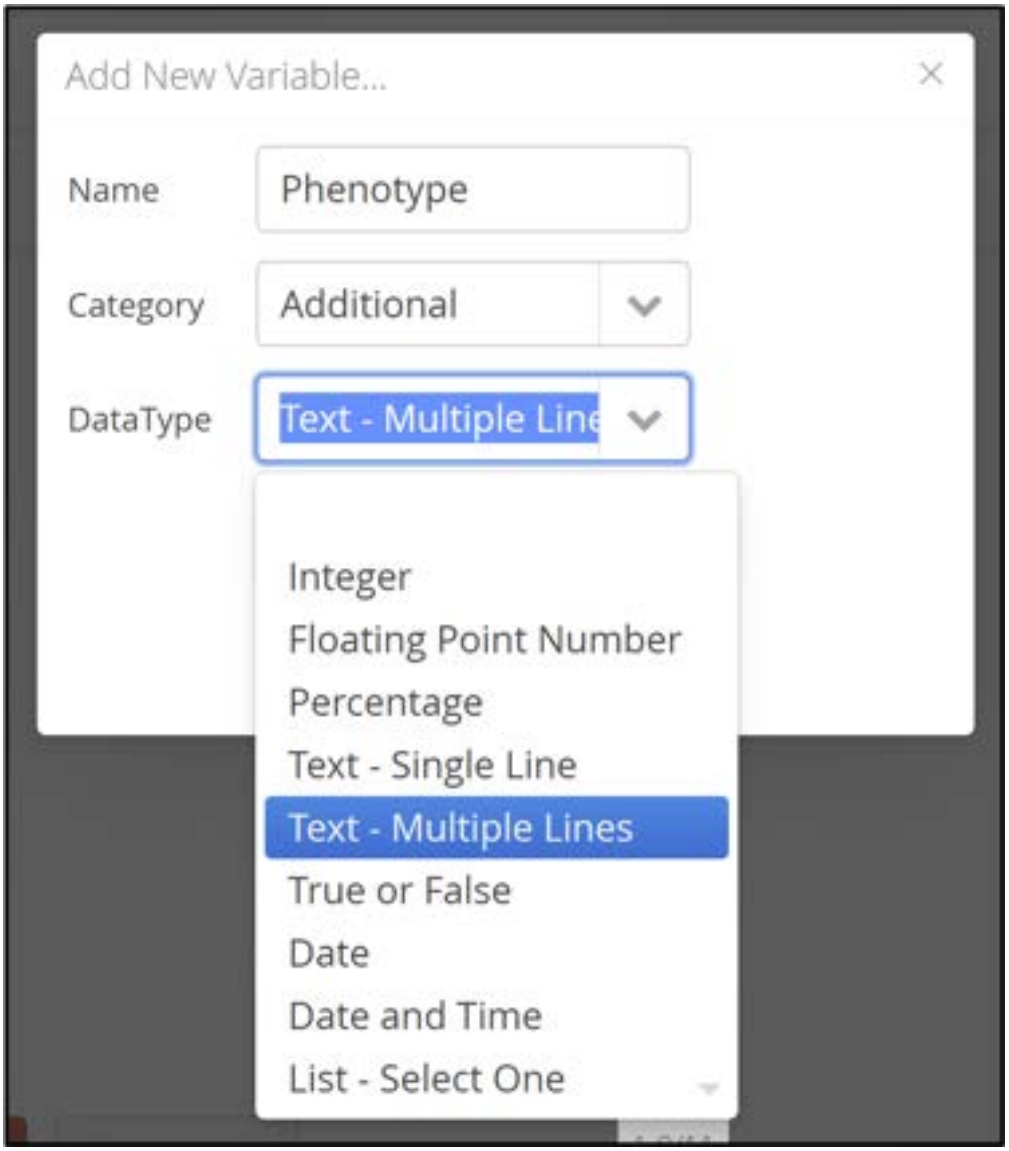


Figure 20: Selecting the appropriate data type for the new variable - in this case, "Text - Multiple Lines"

Once a variable has been created, it will be listed, along with its data type, in the appropriate category in the **Manage Sample Variables** section. To delete a variable, click on the cross next to the variable name.

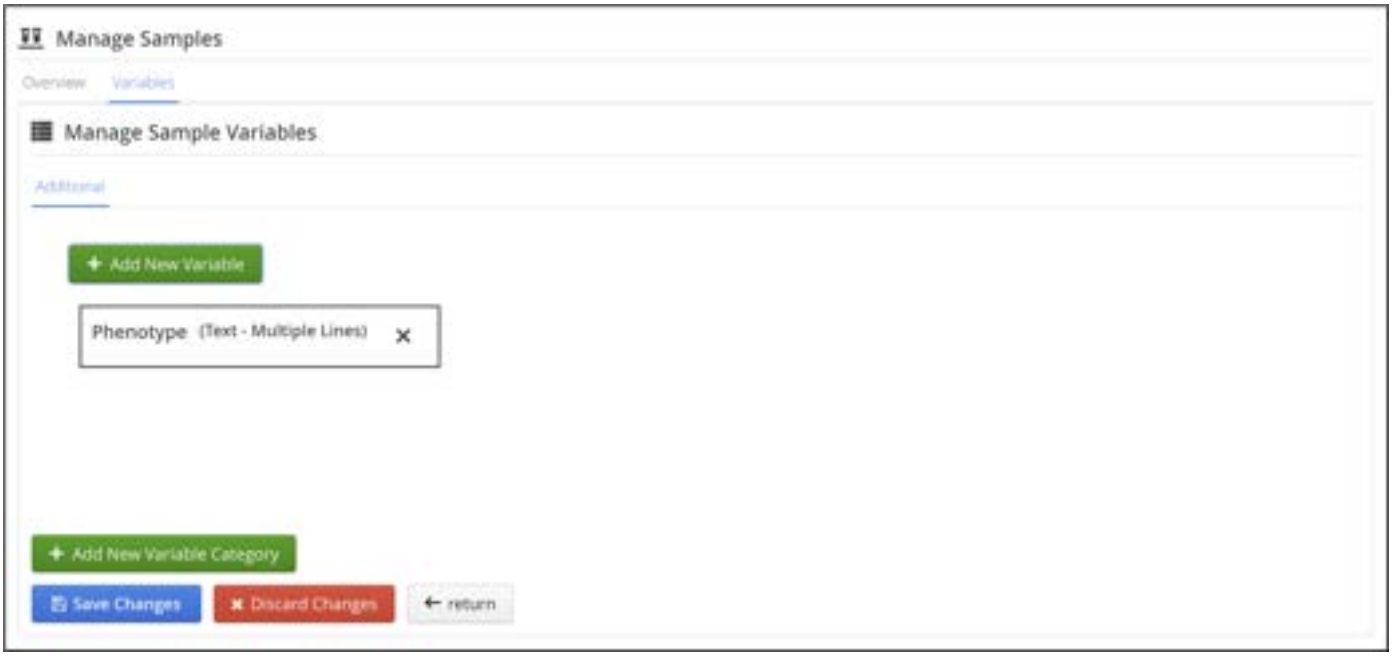


Figure 21: A custom field named "Phenotype" listed under the "Additional" category

Having been created in the system, custom fields may be populated for each sample in the **Samples** view, and will also be displayed in the sample run page whenever the sample has been processed in a batch (accessible by clicking on the sample row in the **Completed Samples** table in the **Batch Overview** page).

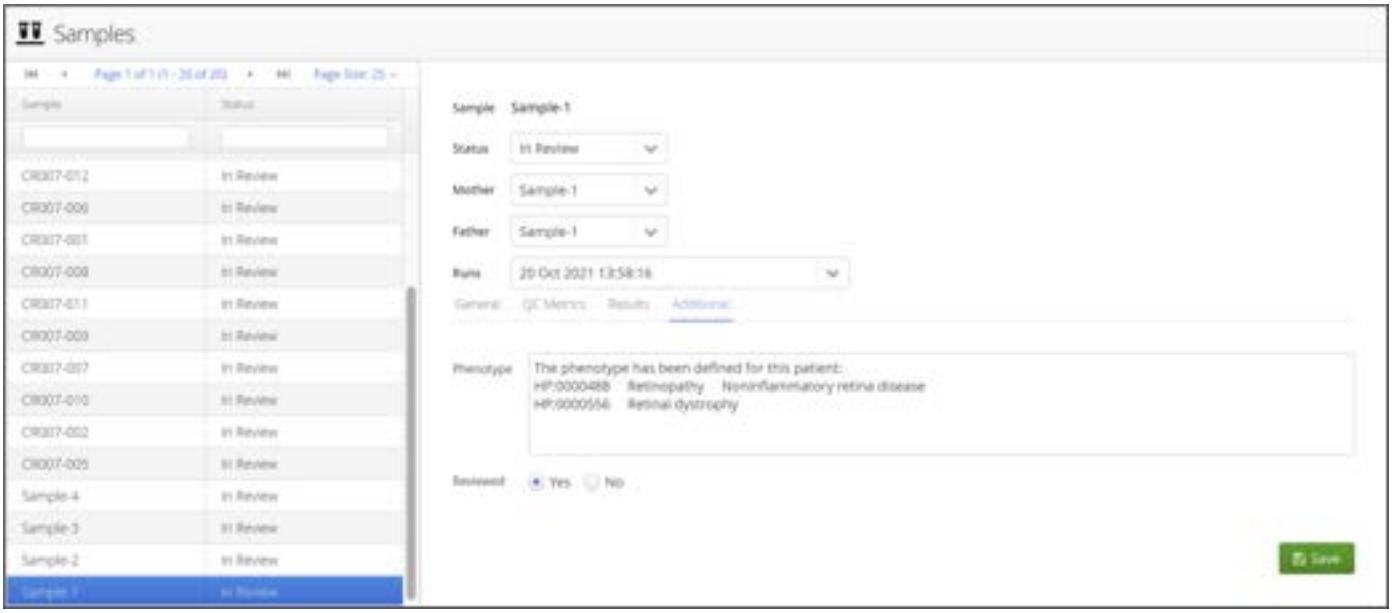


Figure 22: Editing the content of the "Phenotype" field in the Samples page

Interpret also provides a framework enabling the development of plug-ins to import sample data in bulk from other sources, such as spreadsheets, text files or a LIMS. If you are interested in importing data in bulk, contact OGT - a suitable plug-in may be available, or it may be possible to develop a plug-in to satisfy your requirements.

Running an Analysis

On the dashboard either select "Run Batch" in the drop down from the 'Batches' menu item.

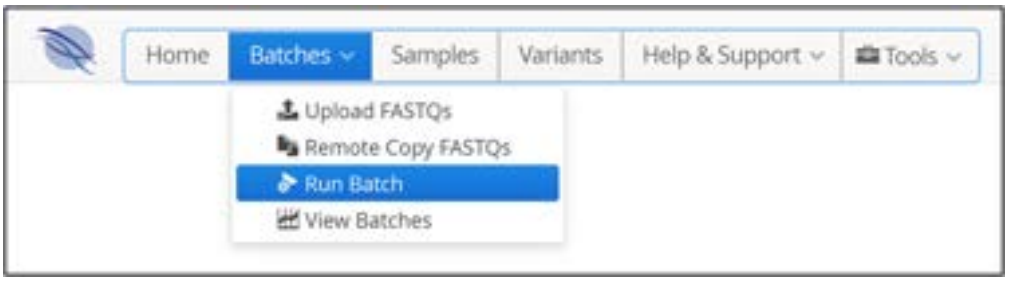


Figure 23: Selection of Run Batch from the Dashboard menu bar Batches drop down menu

Or, click on the 'Run Batch' icon on the dashboard page

Figure 24: Selection of Run Batch from the dashboard short-cut buttons

Either choice leads to the initial Run Batch page is as follows:

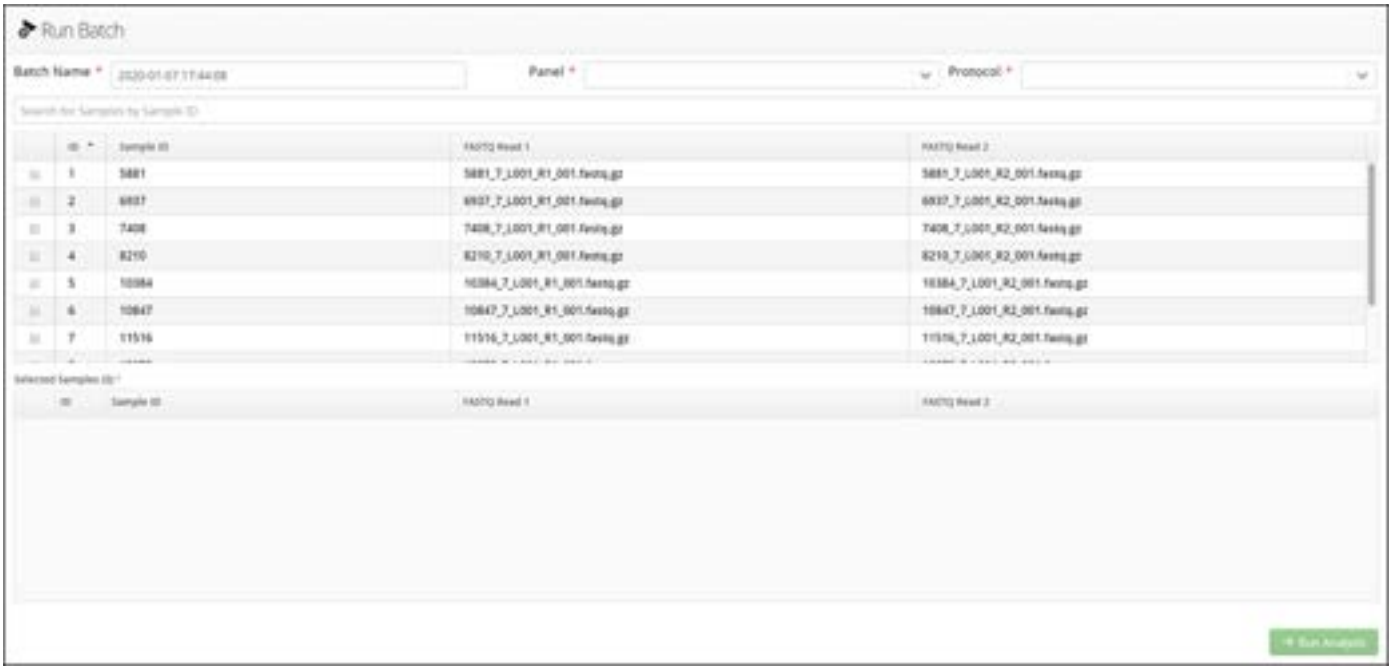


Figure 25: Initial view of the Run Batch window

Besides showing the list of available samples there are additional text fields and drop-down menus.

In order to run an analysis the user needs to

1. Select samples for the analysis
2. Select the correct panel for the samples
3. Select the analysis protocol

Figure 26: Input fields for the Run Batch window

Optionally users can specify a name for the batch analysis. A default batch name is provided with the date followed by the time in the format YYYY-MM-DD HH:MM:SS.

In the example below the user has created the batch name CytoSure NGS Batch 00001

Figure 27: Entering a batch name

The samples have been processed with OGTs CytoSure NGS panel so that is the selection to make from the Panel dropdown menu.

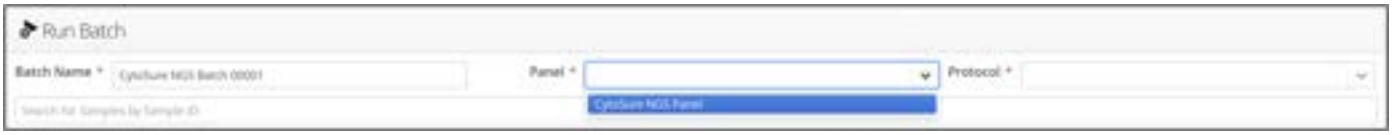


Figure 28: Selecting the appropriate Panel

The user now specifies the protocol that will be used, in this case the Default Protocol.

Figure 29: Selecting the protocol to use for processing the batch

Panel-Protocol Compatibility
Only protocols whose **Pipeline Type** are included in the list of pipeline types supported by the selected **Panel** will be listed in the **Protocol** drop-down list. Additionally, if any **Pipeline Capabilities** supported by the protocol are not supported by the selected panel, a warning will be displayed indicating which processes will not be run.

Lastly, the user specifies the samples to be analysed.

There may be a large number of samples loaded into the system, so to enable easier sample selection it is possible to add a search term. In this case the user is looking for all samples containing the number 5. Additionally, search terms are independent of the case used.

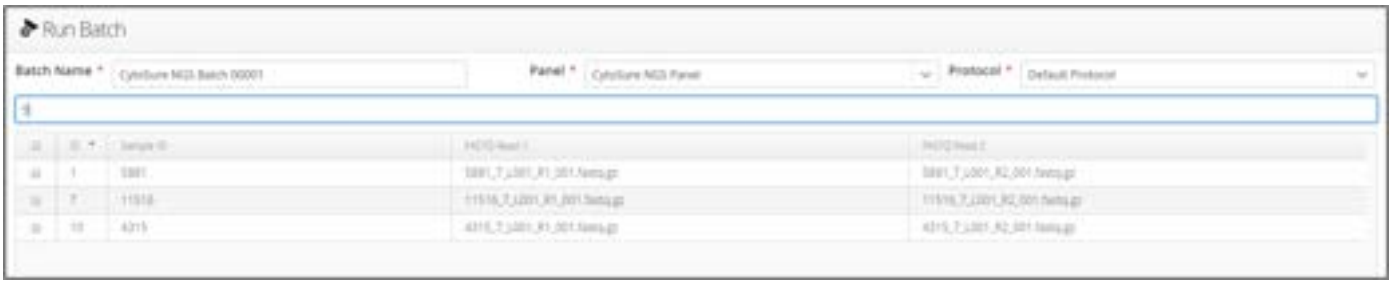


Figure 30: Filtering loaded samples with a search term

Selecting the checkbox next to a sample moves a loaded sample into the Selected Samples table.

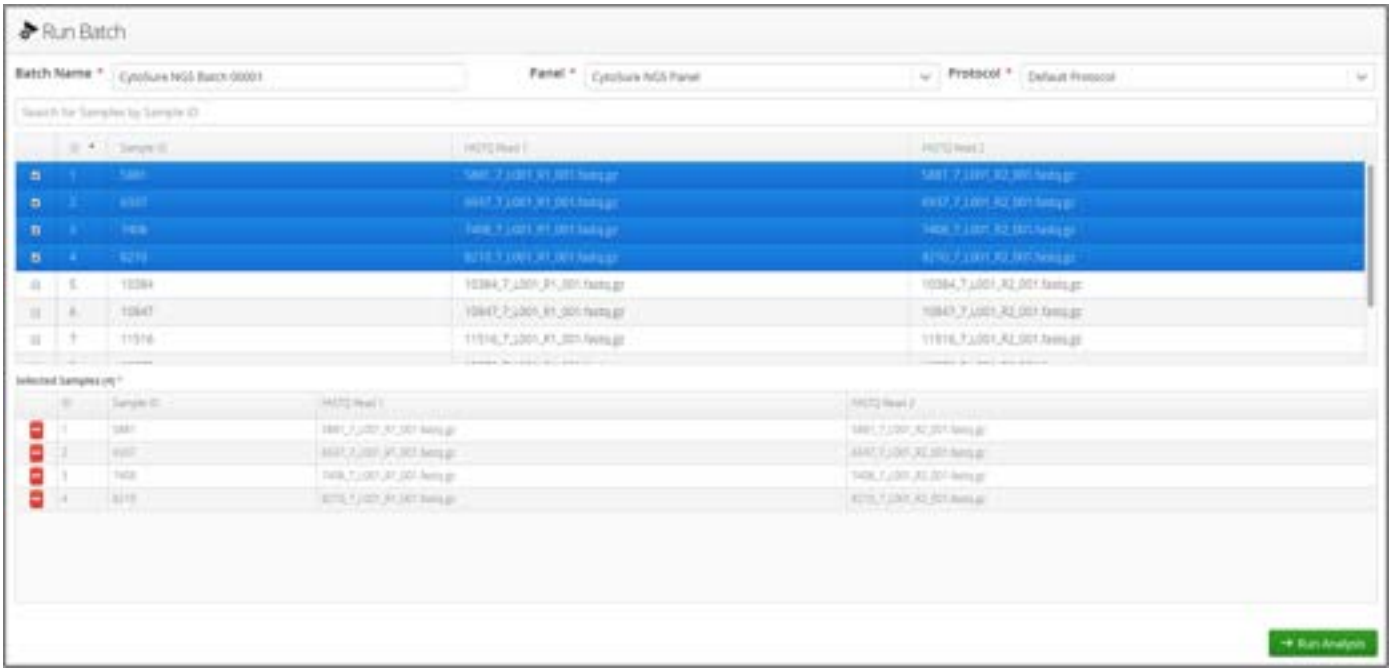


Figure 31: Adding a sample to an analysis batch

Clicking on the minus icon ⊟ will remove the sample from the Selected Samples tables.

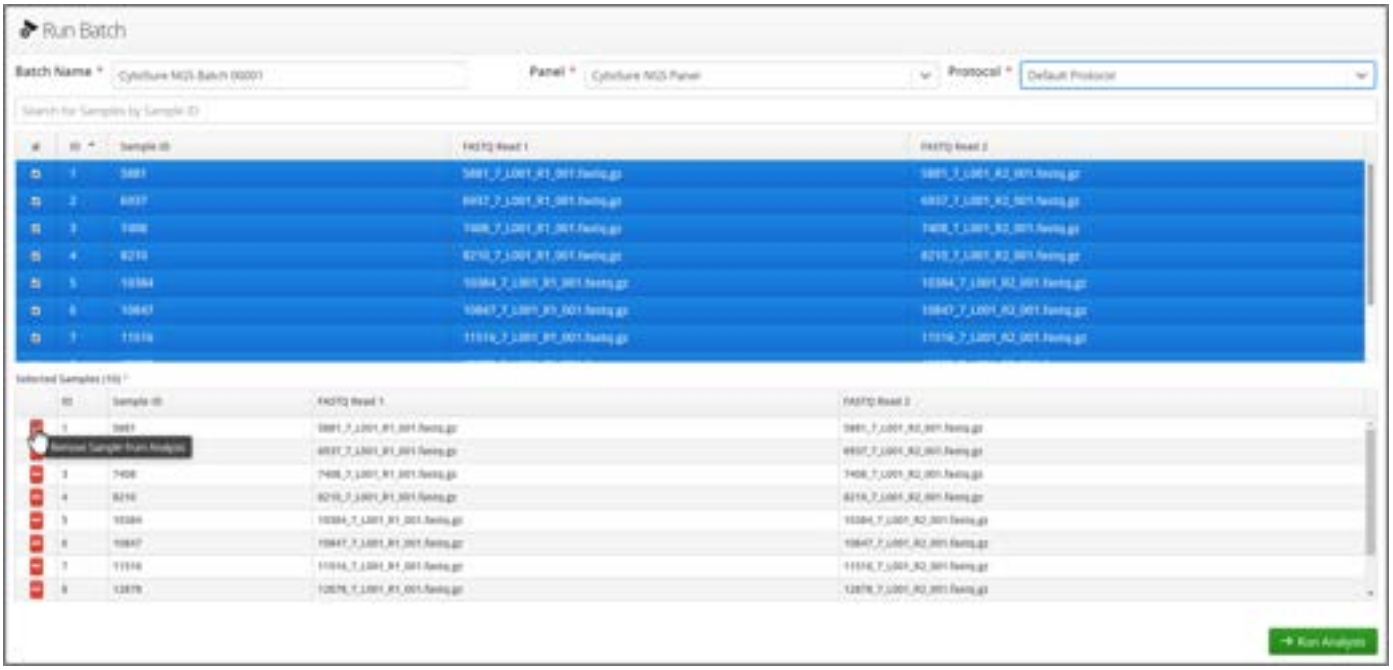


Figure 32: Removing a sample from an analysis batch

When all selections have been made the run can be started by selecting → Run Analysis

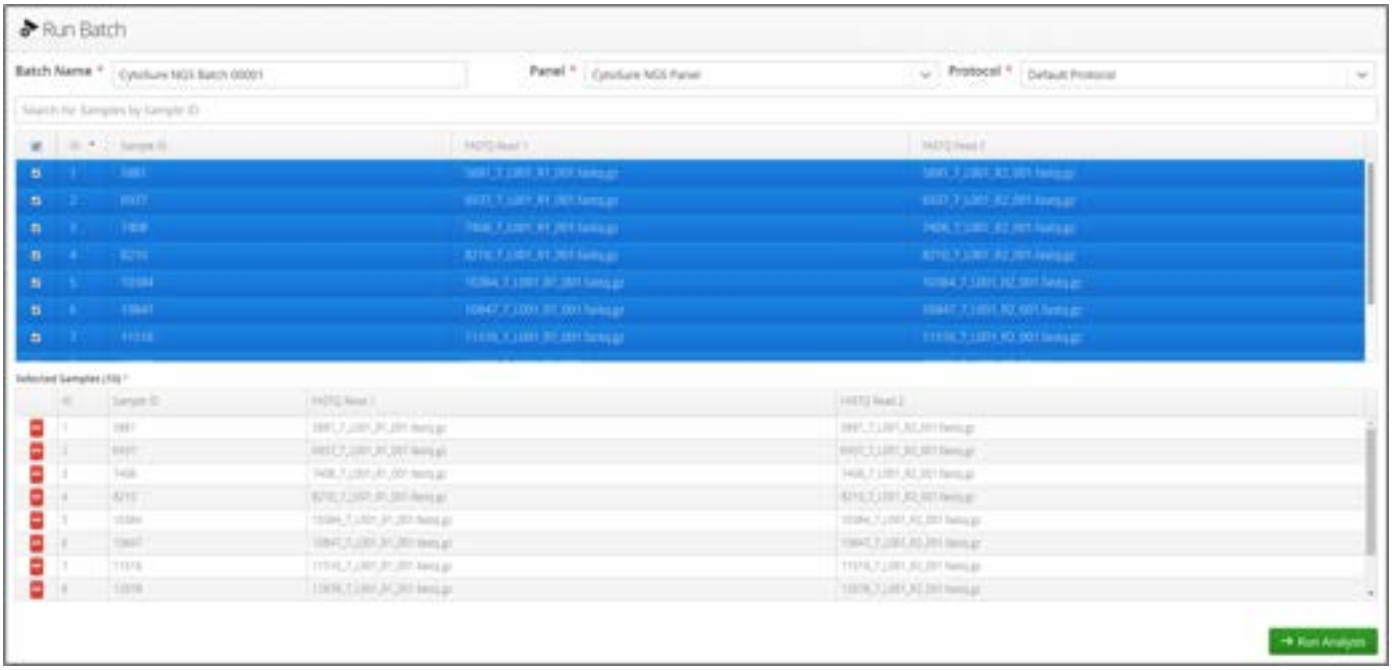


Figure 33: Starting an analysis

If the selected protocol has "Enable CNV and LOH Calling" set to "Yes", CNVs will be detected by comparison with a set of reference samples which need to be defined in the protocol as either "All Batch Samples" or a specific set of reference samples whose FASTQ files have already been uploaded and designated to the system by the user.

In the latter case, OGT may provide a set of data files that can be used as a reference set for CNV analysis. As more samples are processed users may extend the reference pool by adding any samples they believe are suitable as controls for CNV calling. A user can modify samples designated as reference pool in the protocol in Admin Controls-Manage Samples-Protocols.

If CNV calling is enabled without a reference data set being defined, then, on selecting → Run Analysis, the following error will be displayed.

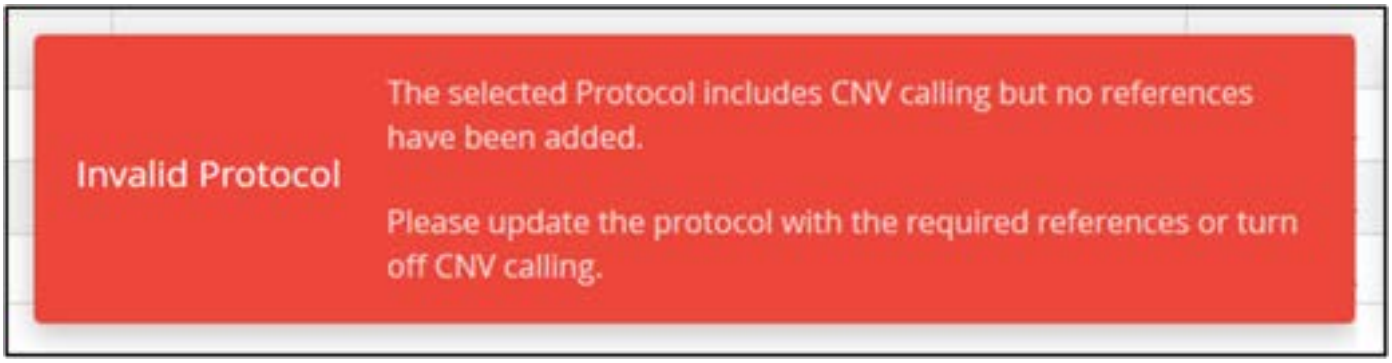


Figure 34: Error message for using an invalid protocol

Click on the message to remove the warning and select Admin Controls > Analysis > Protocols to set reference samples. More details are in the Protocols section of this User Guide.

Otherwise, a popup presents the chosen files and selected parameters. Following this there is a request for confirmation and upon confirmation the analysis run will be initiated.

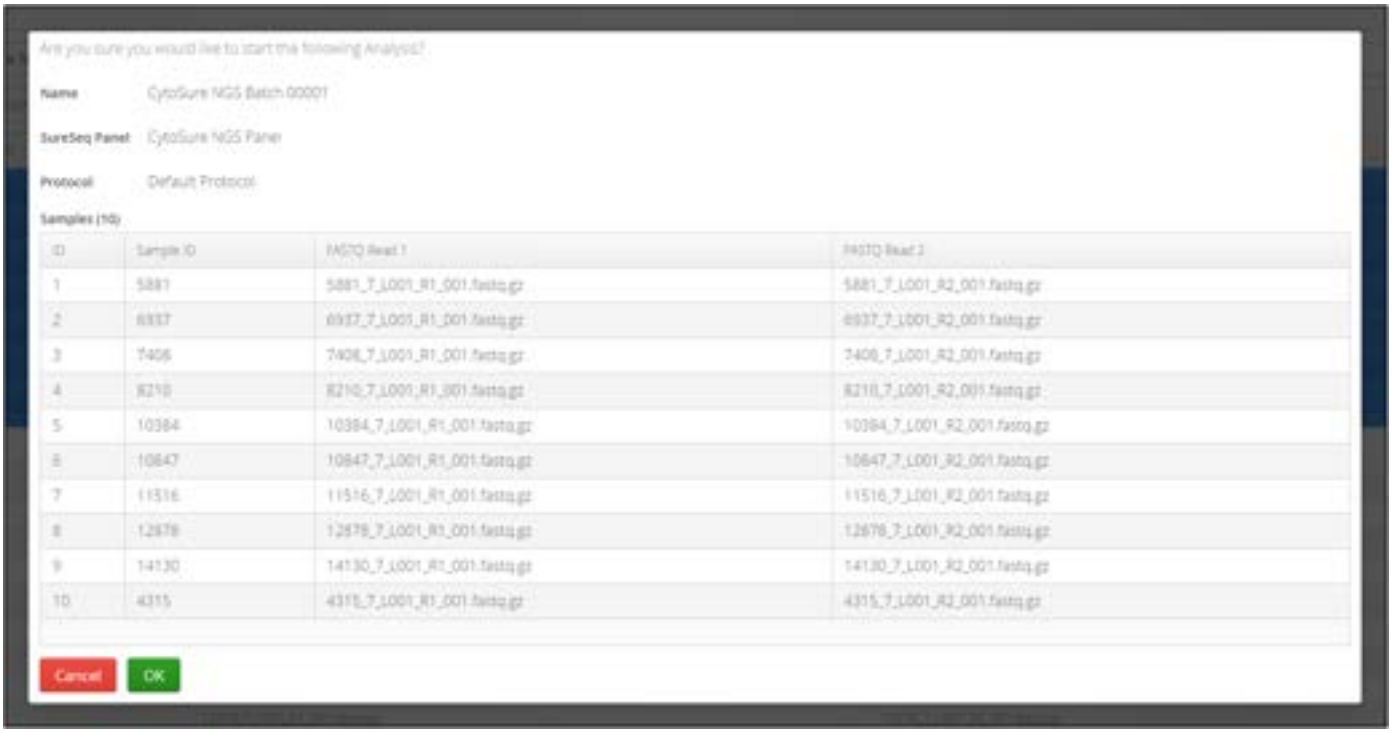


Figure 35: Window requesting confirmation to run an analysis

Selecting 

will start the analysis and the display will change to show information about batch being analysed.

Within this there is an overview window providing an overview of the analysis and a sample window giving information about the status of each sample.

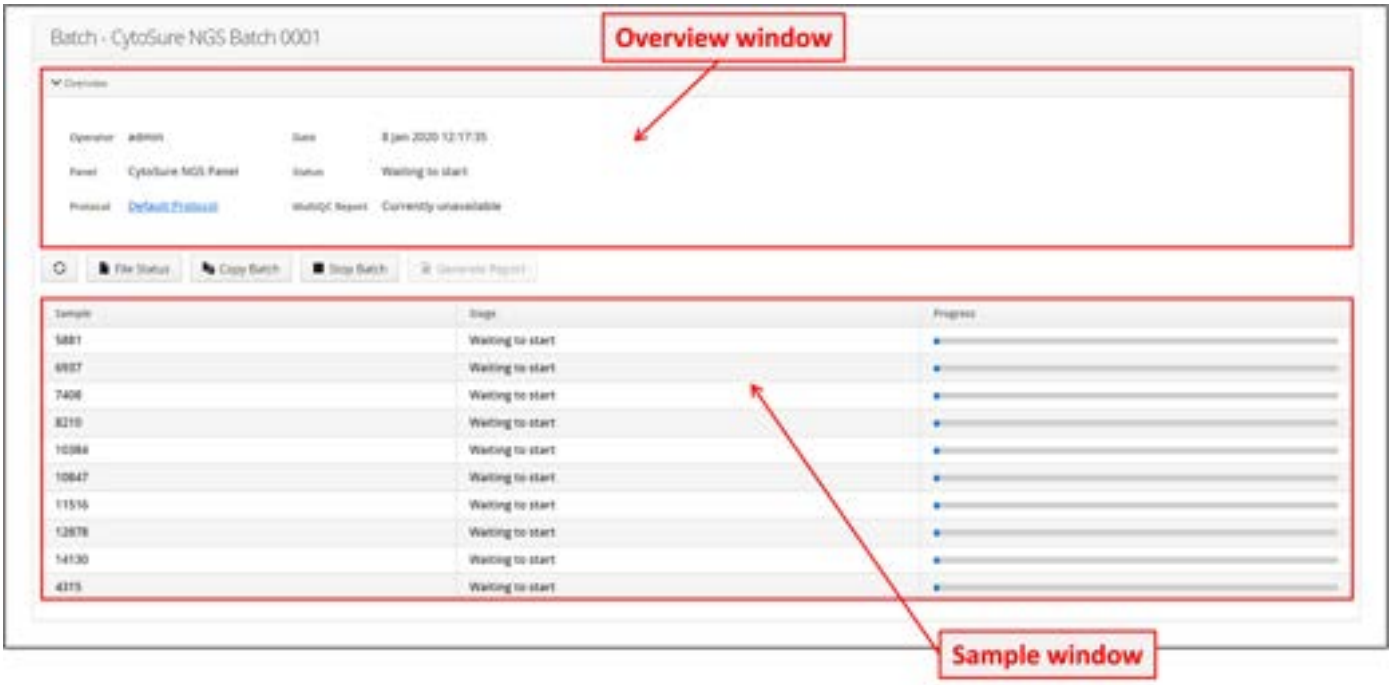


Figure 36: The batch processing view with the overview window and sample window highlighted

Initially the status of the samples will be listed in the overview window as "Waiting to start"

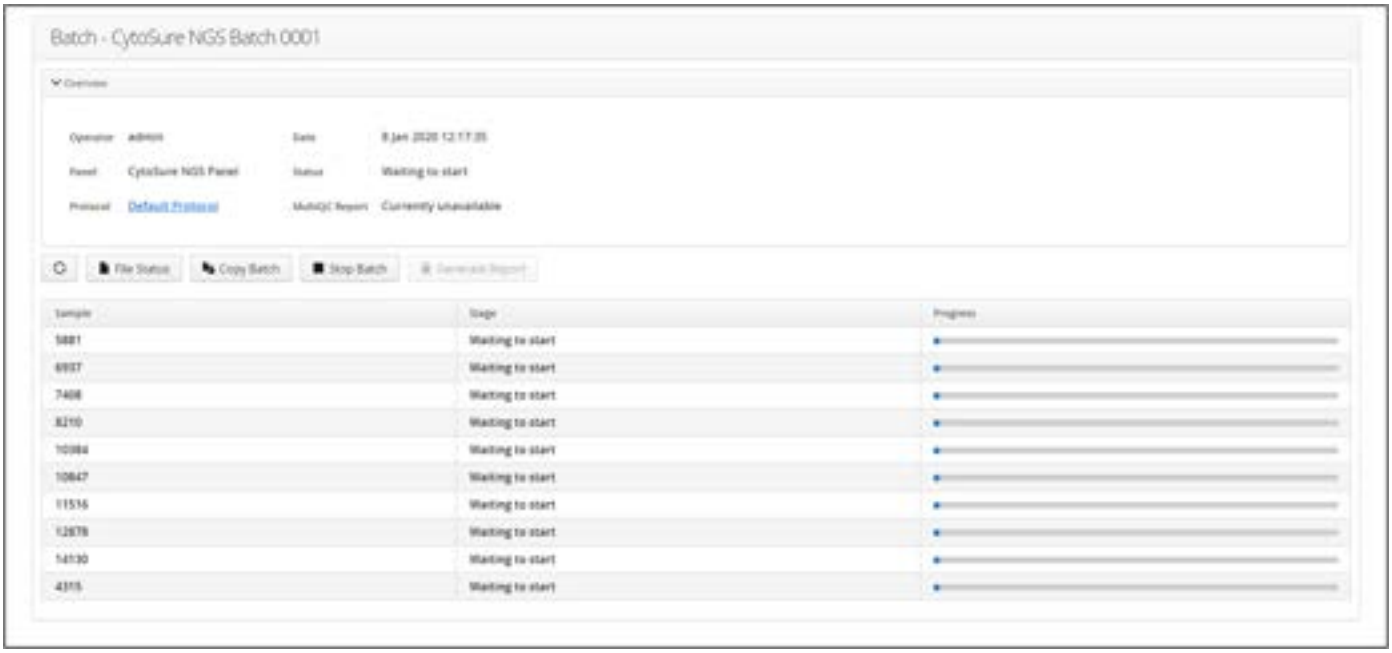


Figure 37: Initial batch status before analysis starts

Waiting for a reference to be generated

If a reference pool needs to be generated the status shown in the batch overview will report this and provide a means to track progress of the reference pool creation.

Figure 38: The analysis status in the overview window, highlighted, showing reporting the status of pre-processing of the reference samples

The status of reference building can also be tracked in the View Batches window which is discussed in the View Batches section of this User Guide.

Figure 39: Reference building status being shown in the View Batches window

If the protocol performs CNV analysis and samples in the analysis are to be used to generate the reference pool against which to make CNV calls then the overview will report the combining of the reference samples.

Once the reference samples have been aligned and counted, they are combined into a pool for the CNV analysis

Figure 40: The analysis in the over window, highlighted, reporting the combining of the reference samples into a pool

Samples will be queued until there is capacity available in the pipeline. Once this is available the software will start processing the samples sequentially. The stage of the process is updated and the overall progress can be monitored in the progress bar.

Figure 41: The batch view showing progress of analysis

Once analysis started the stage of each sample is displayed and can be followed

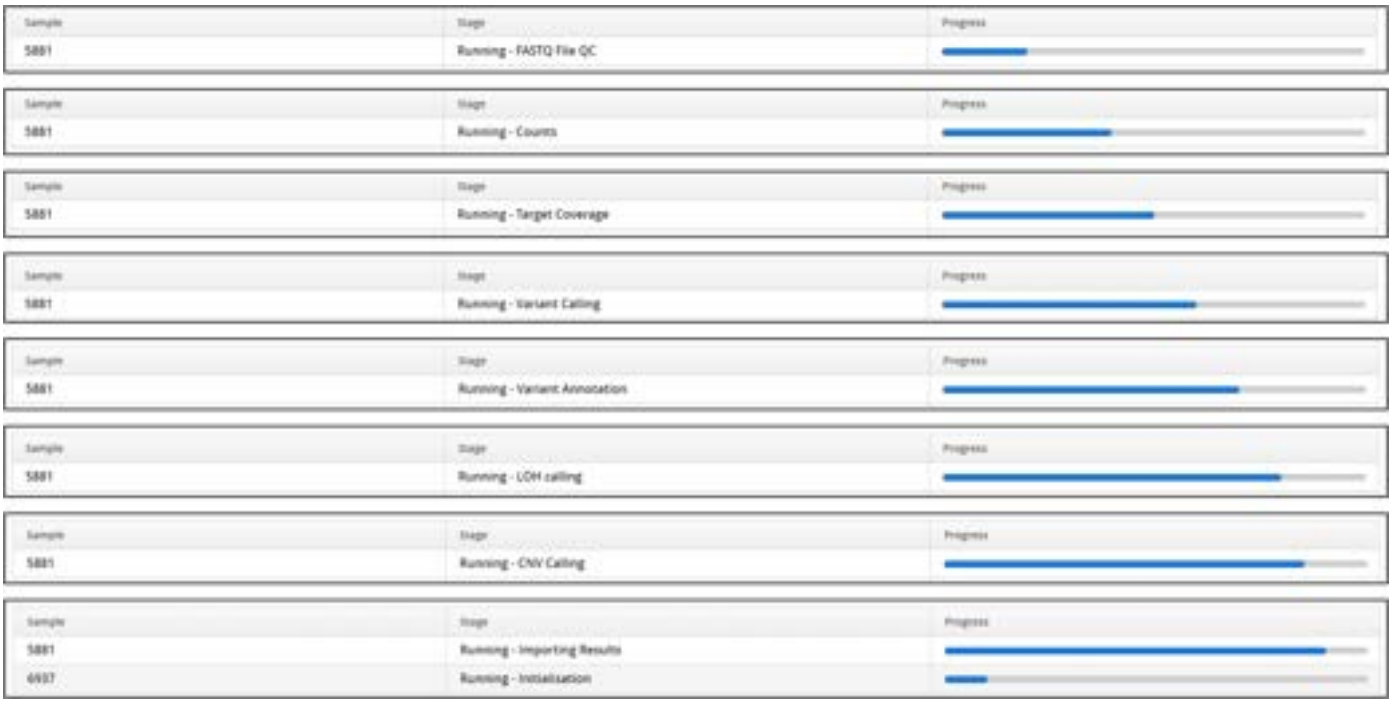


Figure 42: Tracking progress of a sample processing

Once a sample has been analysed the overview updates the count and a summary of the analysis is displayed in a Completed Samples table.

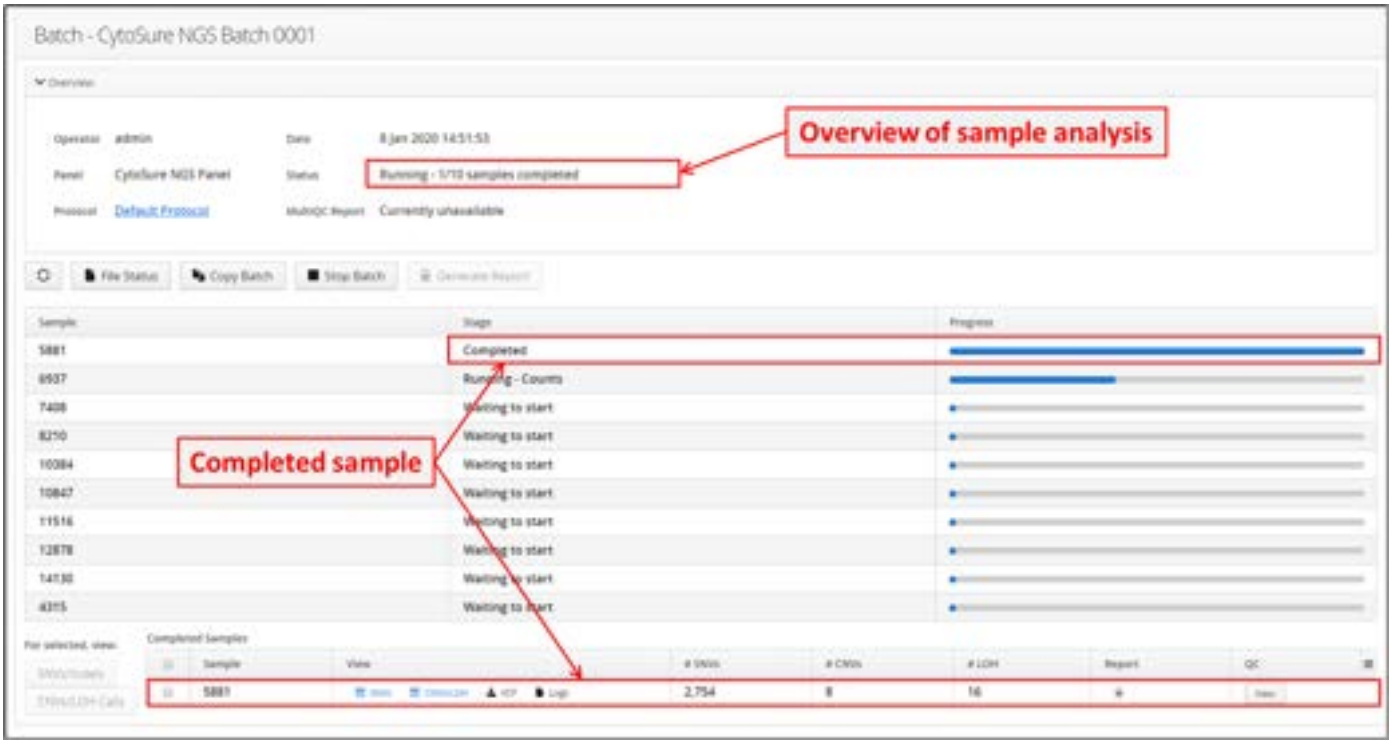


Figure 43: The first completed sample is displayed below the samples to be processed

When all samples are completed

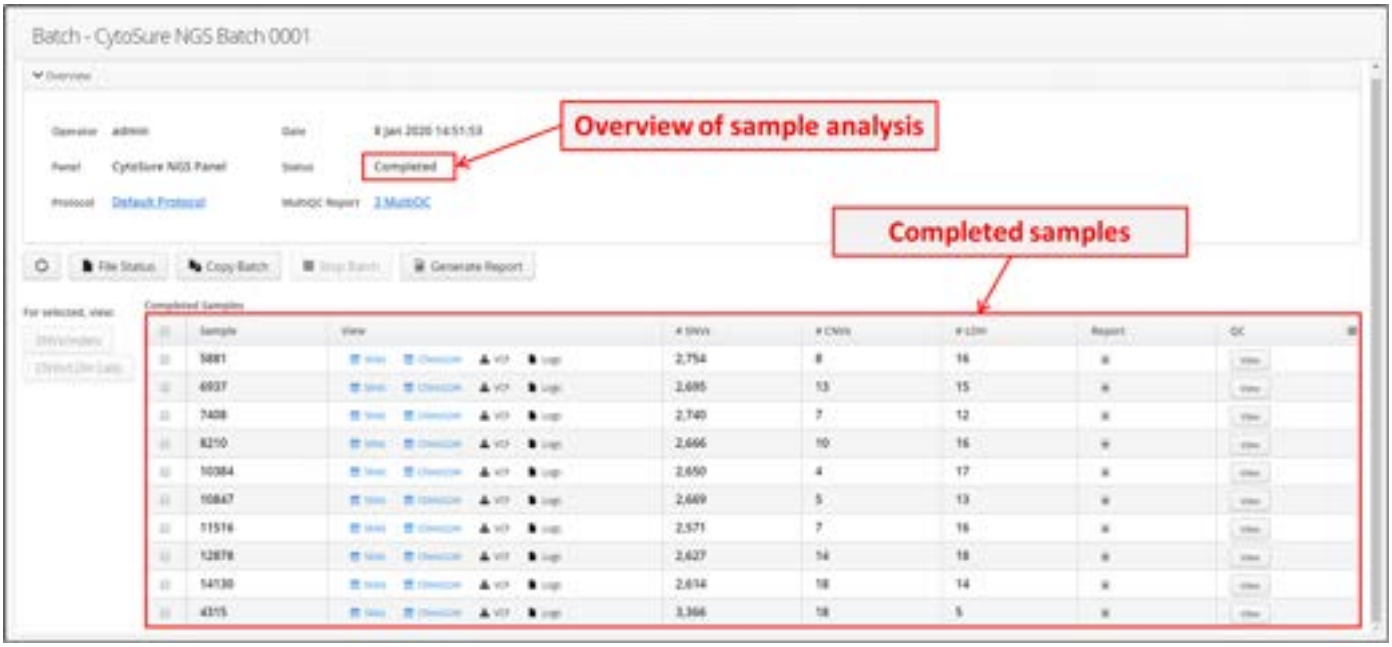


Figure 44: An analysis with all samples analysed

There is no need to wait until all samples have been processed to view the results for a completed sample.

This will be discussed in the Viewing Analysis Results section of the manual.

Viewing Analysis Batches

On the dashboard either select "View Batches" in the drop down from the 'Batches' menu item.

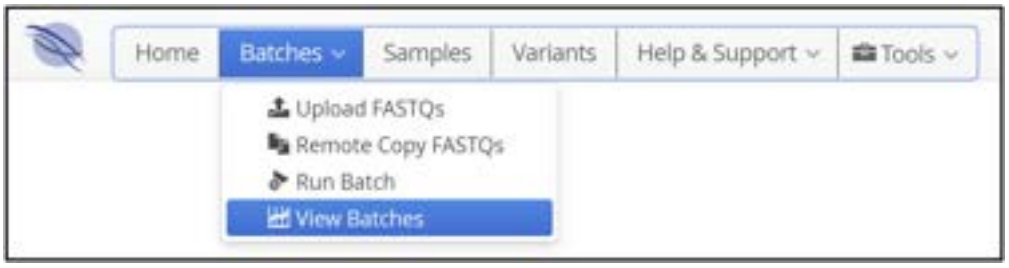


Figure 45: Selecting View Batches from the menu bar drop down menu

Or, click on the 'View Batches' icon on the dashboard page

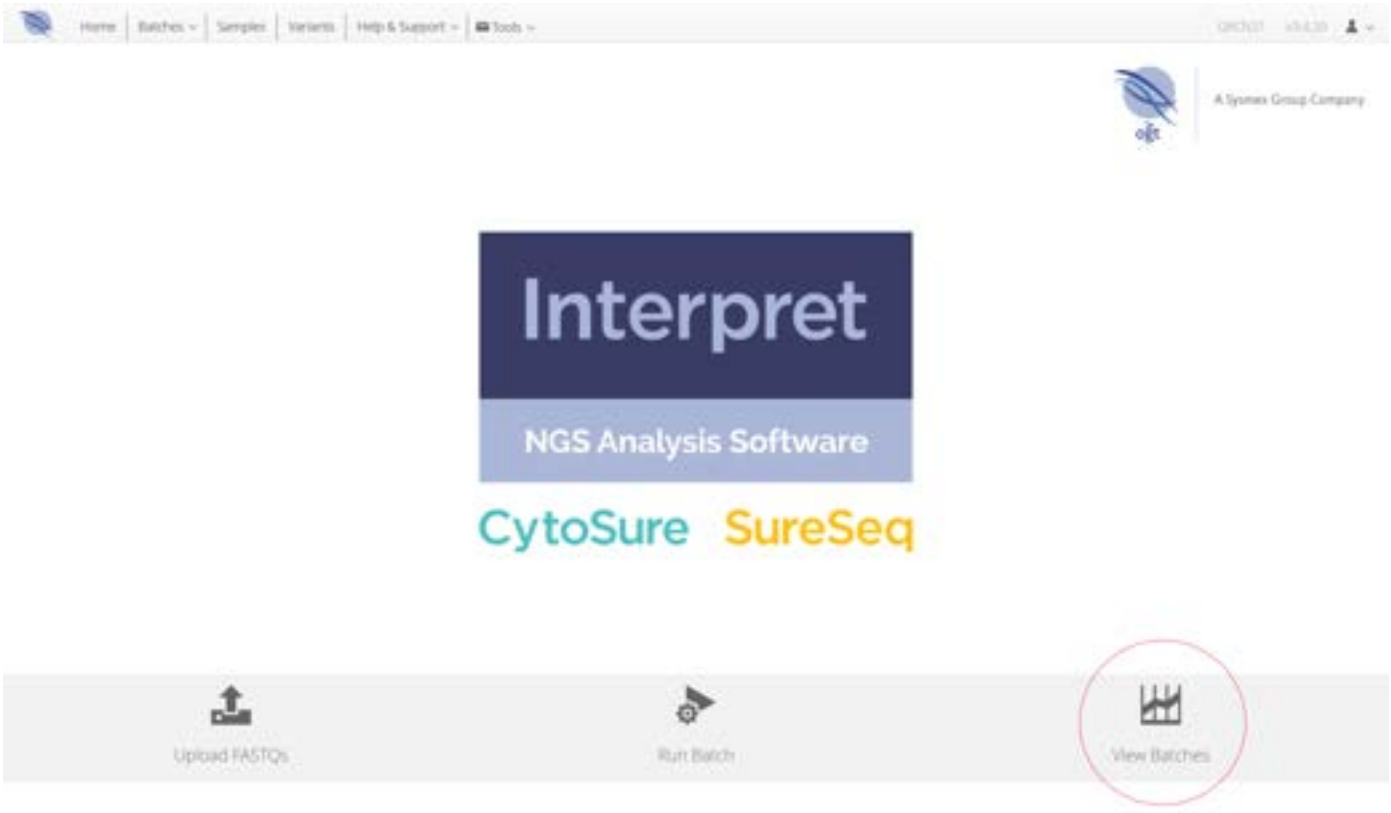


Figure 46: Selecting View Batches from the dashboard shortcut buttons

The Batches are presented in a table as below.

Figure 47: Initial view of the Batches window

As with other tables in Interpret where there is a column selector icon a user can add or remove columns from the display

Figure 48: Column selection options for the Batches window

Column names annotated with a tick are in the current display and changes can easily be made to add or remove columns

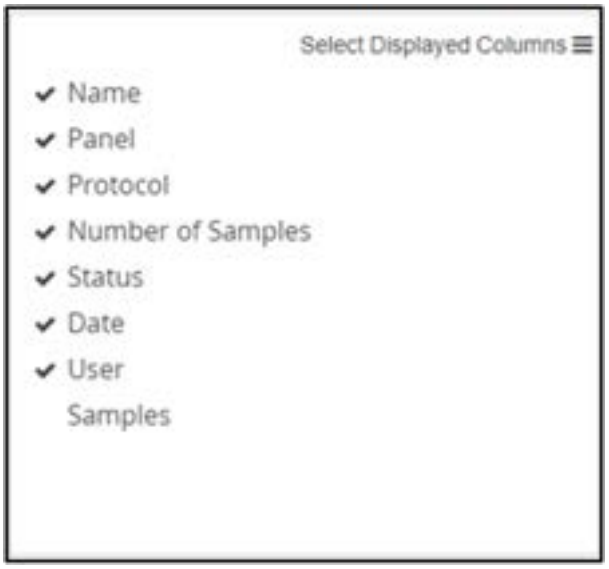


Figure 49: Selection of columns to display in the Batches window

By default, all batches are presented in the first instance but these can easily be filtered.

Where the column header has a text field, users can type in a search term and all batches with that text contained somewhere in the name, will be retained. The text search is independent of lower- or upper-case letters, "Demo" will return the same samples as "demo".

Alternatively, where there is a drop-down menu selecting one of the values in the menu will lead only to the batches matching the selection being displayed, for example, below only batches that have completed will be displayed.

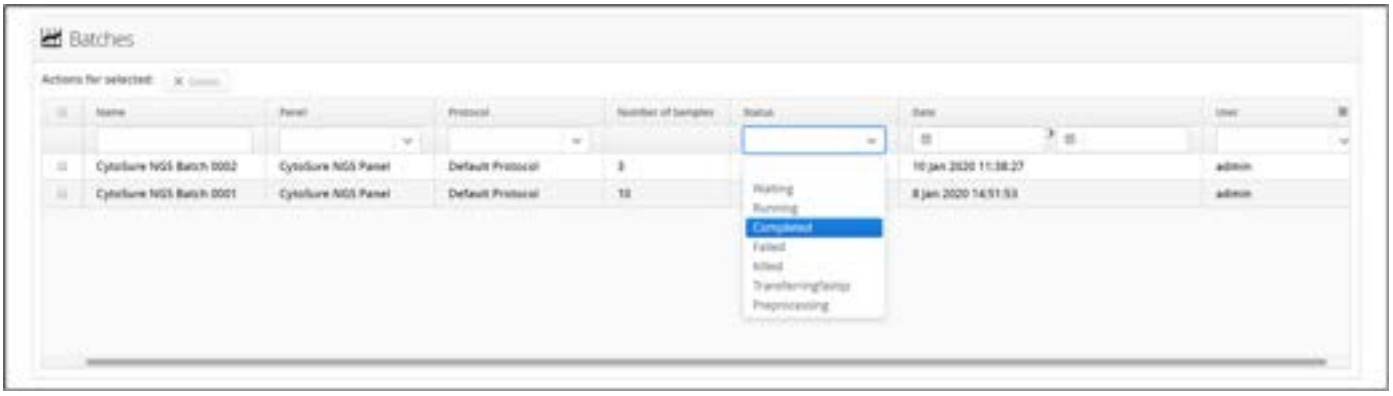


Figure 50: Filtering batches on status

Lastly, there are date fields, allowing selection of batches run within a set time frame.

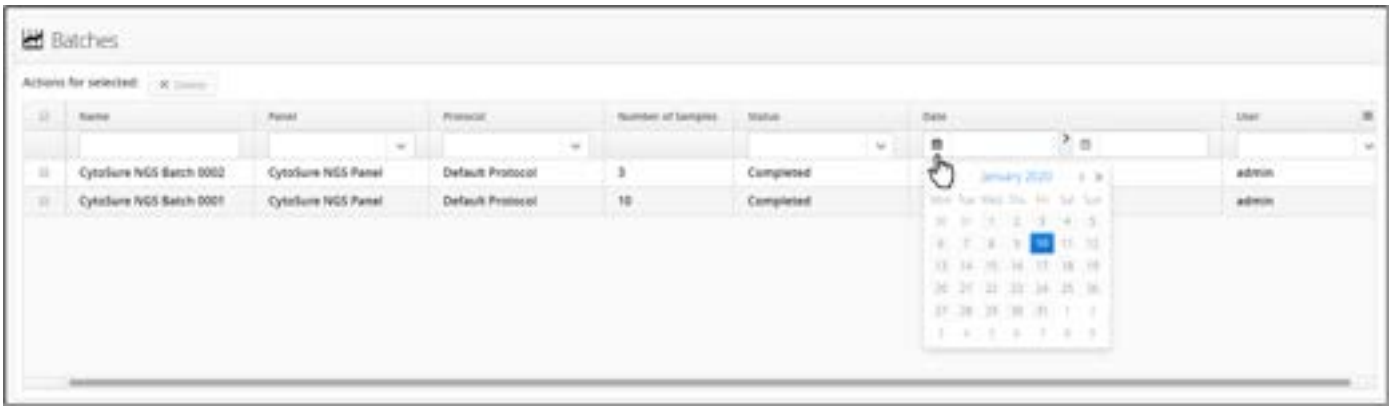


Figure 51: Filtering batches on date of processing

Deleting Batches

In the batch view it is possible to delete batches. When first opened there is a greyed out Delete button in the display.

If a batch is selected it is highlighted in blue and Delete button is now active, Clicking the delete button will delete the batch from the software.

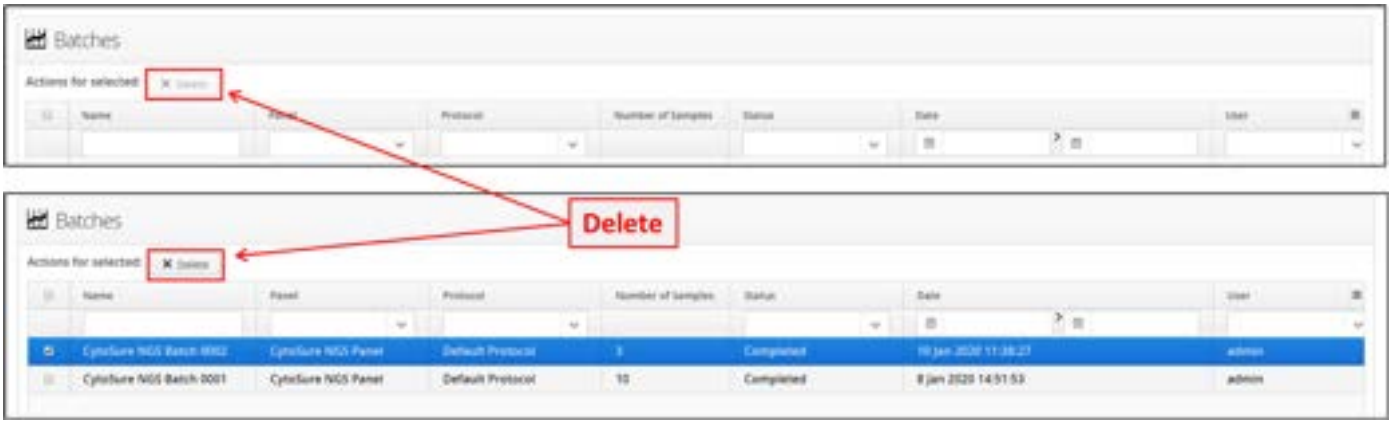


Figure 52: Selection of a batch to delete highlights the delete button

If the delete button is selected there will be a popup box requesting confirmation of the deletion.

Selecting OK will lead to the batch being deleted.

Once a batch is deleted it **CANNOT** be recovered.

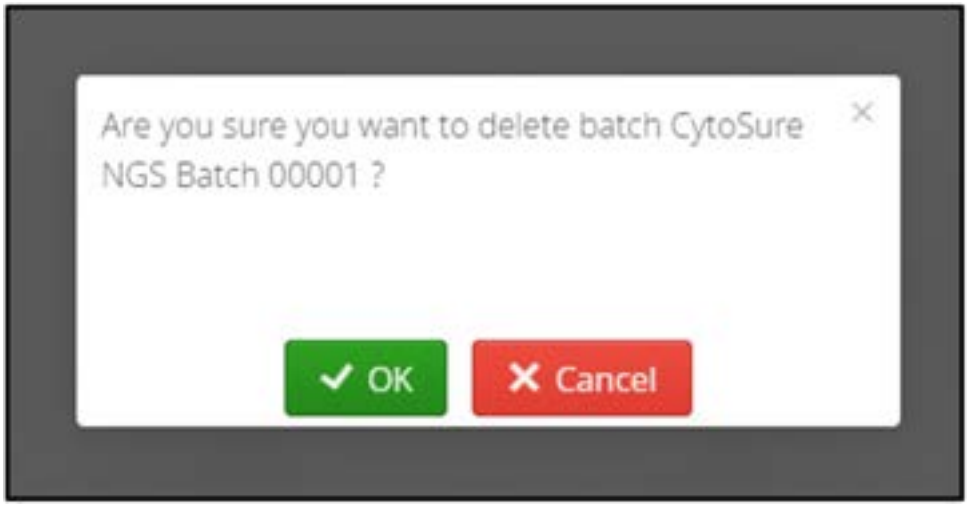


Figure 53: Popup box requesting confirmation of batch deletion

Individual Batches

Clicking on a row in the View Batches page will open a new page showing the selected batch in more detail.

There are 3 parts to the information provided,

1. Overview

   The overview provides information about the analysis

   2. Batch Functions

The batch functions allow users to download files form the analysis, repeat the analysis or generate a report

3. Sample Details

In this part there is the headline information form the run about each sample such as the number of SNVs and CNVs called.

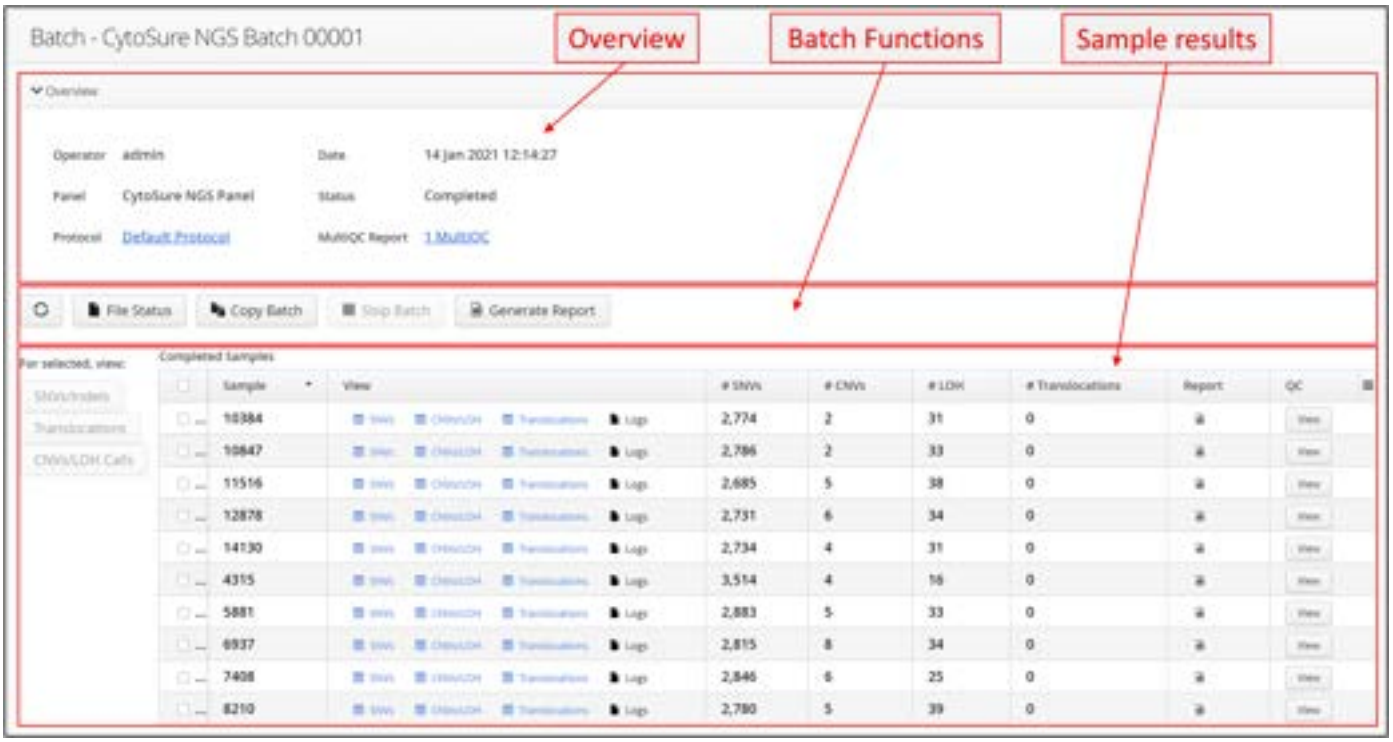


Figure 54: The sections of the batch analysis window

Batch QC

Included in the Batch page are two QC reports.

In the batch overview there is a link to a MultiQC report which gives an overview of all the samples that were in the batch.

Additionally, each sample in the completed table has a FastQC report for each read file.

Examples of both of these QC reports are shown below.

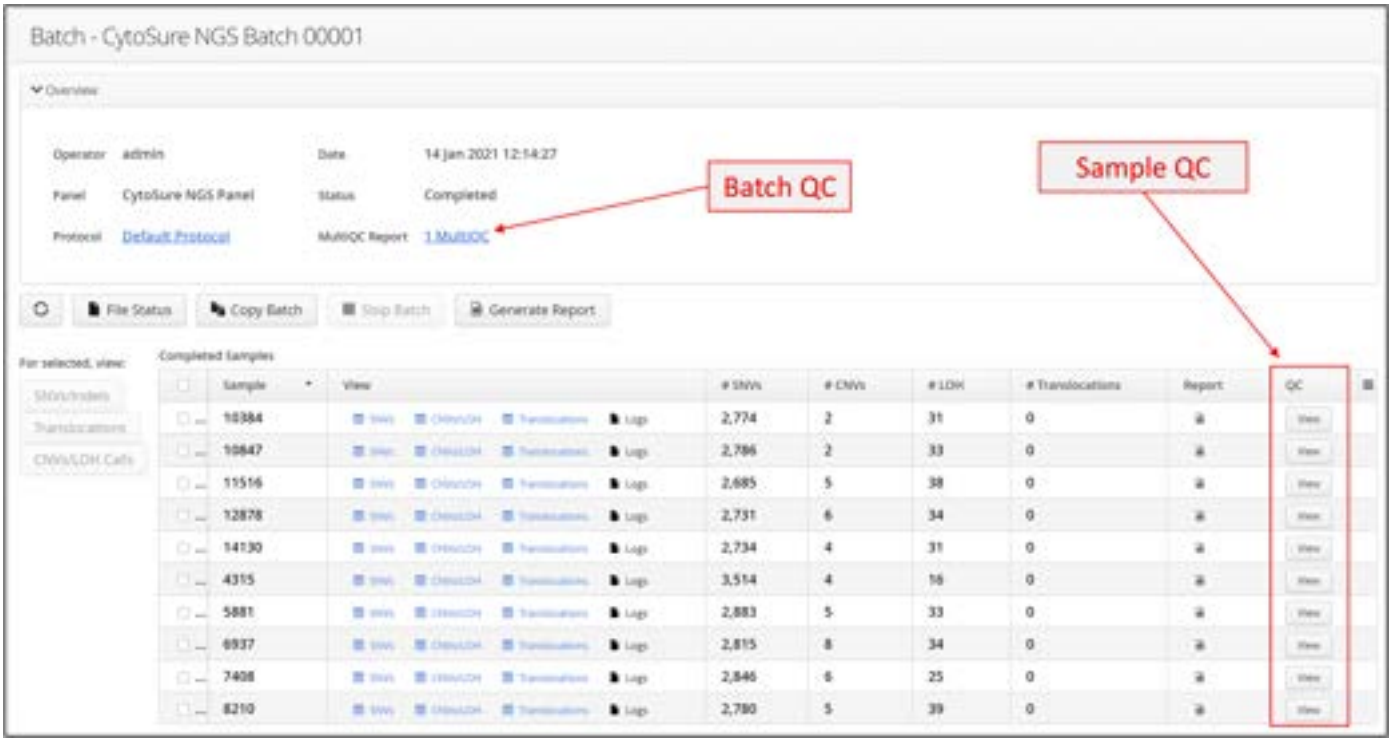


Figure 55: Links to QC reports for a batch and a sample

MultiQC

MultiQC is a reporting tool for the whole batch of samples. It parses summary statistics from results and log files generated by other bioinformatics tools.

When you launch MultiQC, it recursively searches through any provided file paths for specific files. These files are parsed for relevant information and used to generates a single stand-alone HTML report file. It also saves a directory of data files with all parsed data for further use downstream. To save MultiQC report to user's computer, right click on the page, and choose "Save as...".

Additional information about MultiQC can be found in the next section of this guide.

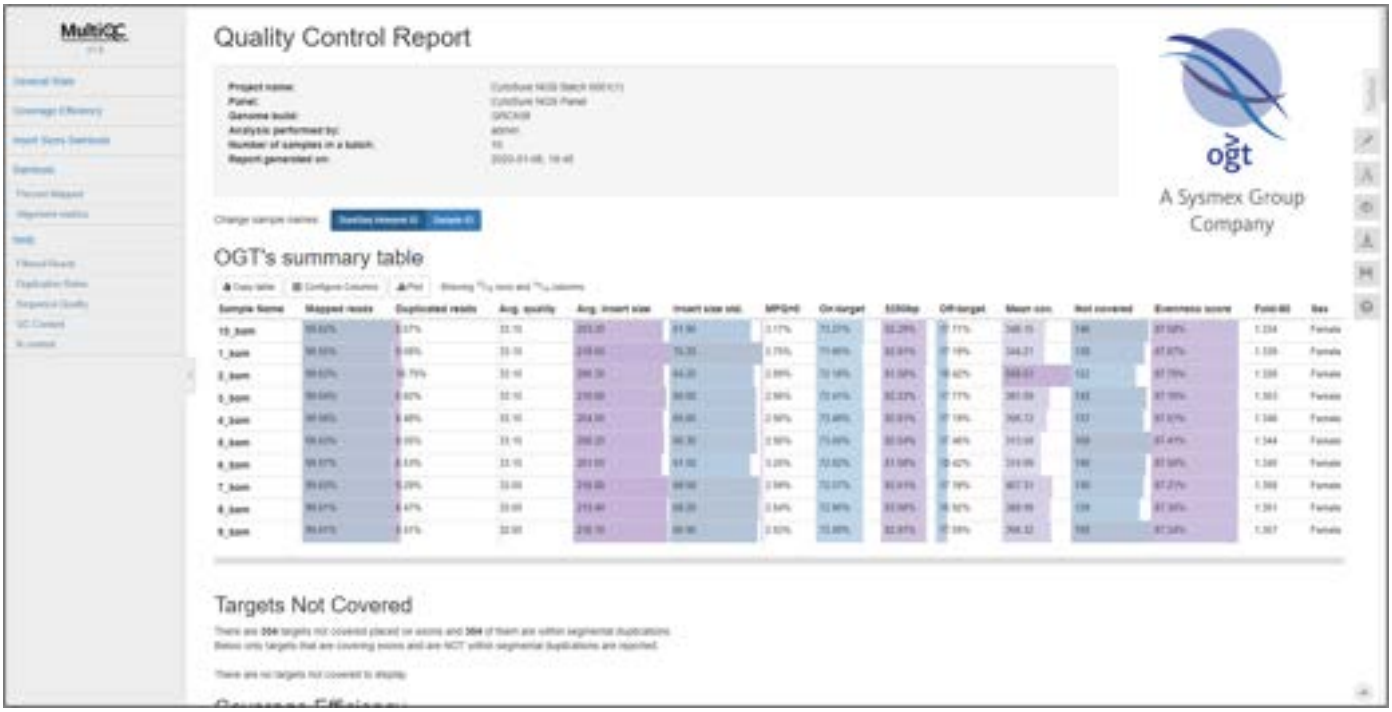
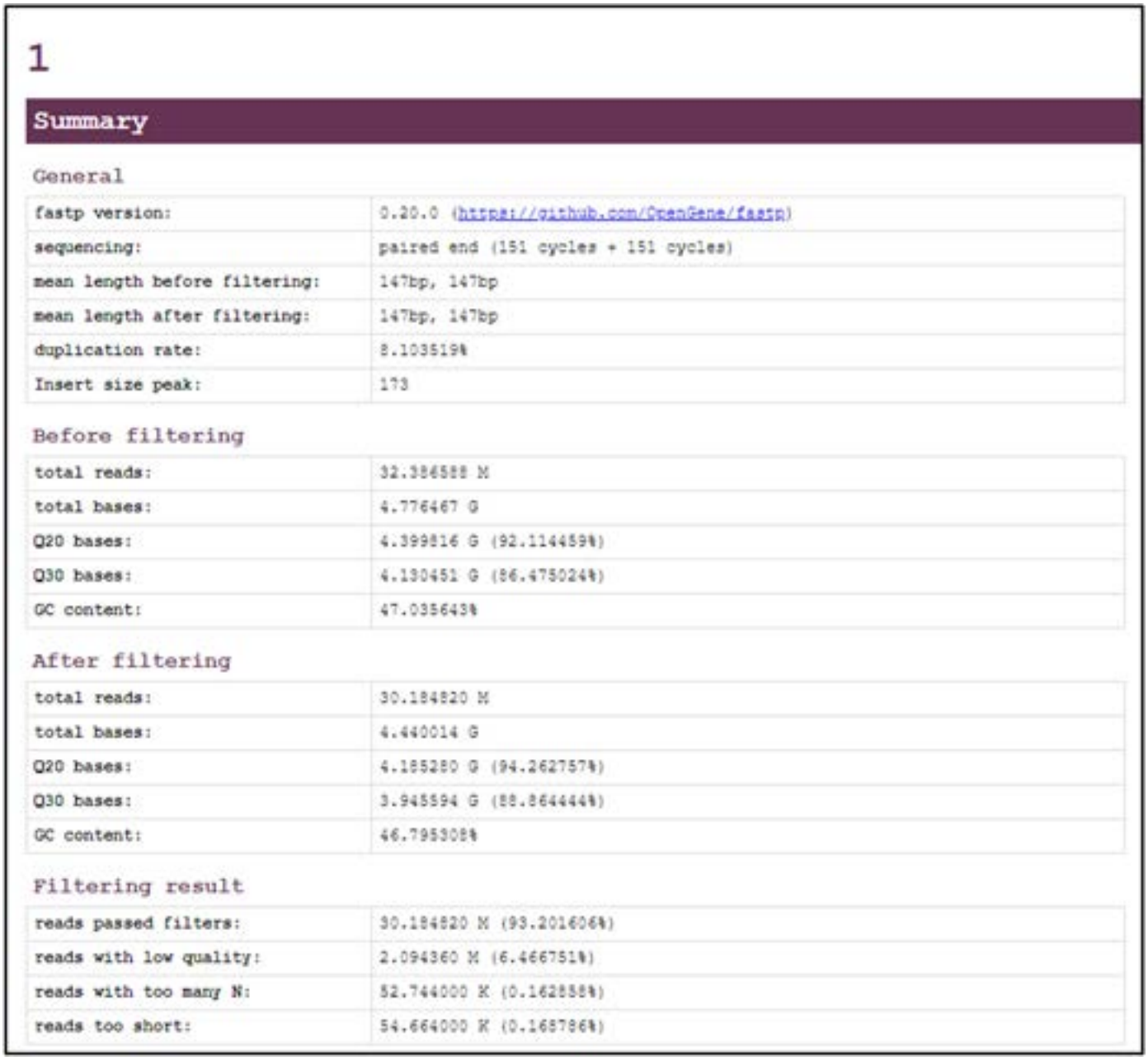


Figure 56: Example of a MultiQC report

Sample QC

FastP is used for sample QC data generation. Clicking on the View button on the sample view will open up a new tab in the web browser with the sample QC details.



1

Summary

General

fastp version:	0.20.0 (https://github.com/OpenGene/fastp)
sequencing:	paired end (151 cycles + 151 cycles)
mean length before filtering:	147bp, 147bp
mean length after filtering:	147bp, 147bp
duplication rate:	8.103519%
Insert size peak:	173

Before filtering

total reads:	32.386588 M
total bases:	4.776467 G
Q20 bases:	4.399816 G (92.114459%)
Q30 bases:	4.130451 G (86.475024%)
GC content:	47.035643%

After filtering

total reads:	30.184820 M
total bases:	4.440014 G
Q20 bases:	4.185280 G (94.262757%)
Q30 bases:	3.945594 G (88.864444%)
GC content:	46.795308%

Filtering result

reads passed filters:	30.184820 M (93.201606%)
reads with low quality:	2.094360 M (6.466751%)
reads with too many N:	52.744000 K (0.162858%)
reads too short:	54.664000 K (0.168786%)

Figure 57: Example of a FastP report

Batch Functions

Below the overview section there are a set of buttons providing a set of option - when the batch has finished processing the Stop Batch button is disabled.

Figure 58: Batch options

File Status

File status provides shows the files that have been generated for each sample during the analysis.

Files provided are:

1. Alignment files
2. QC files
3. VCF files
4. CGH files for loading into CytoSure Interpret
5. Log files

Where a green tick is displayed, that file is available for download and this can be achieved by clicking on the Download button.

Figure 59: Status of files generated by the pipeline for each sample

It is possible to download all files, or selected files, simultaneously via the bulk download button Bulk Download

Figure 60: Bulk download file selector

Specific files can be selected as below

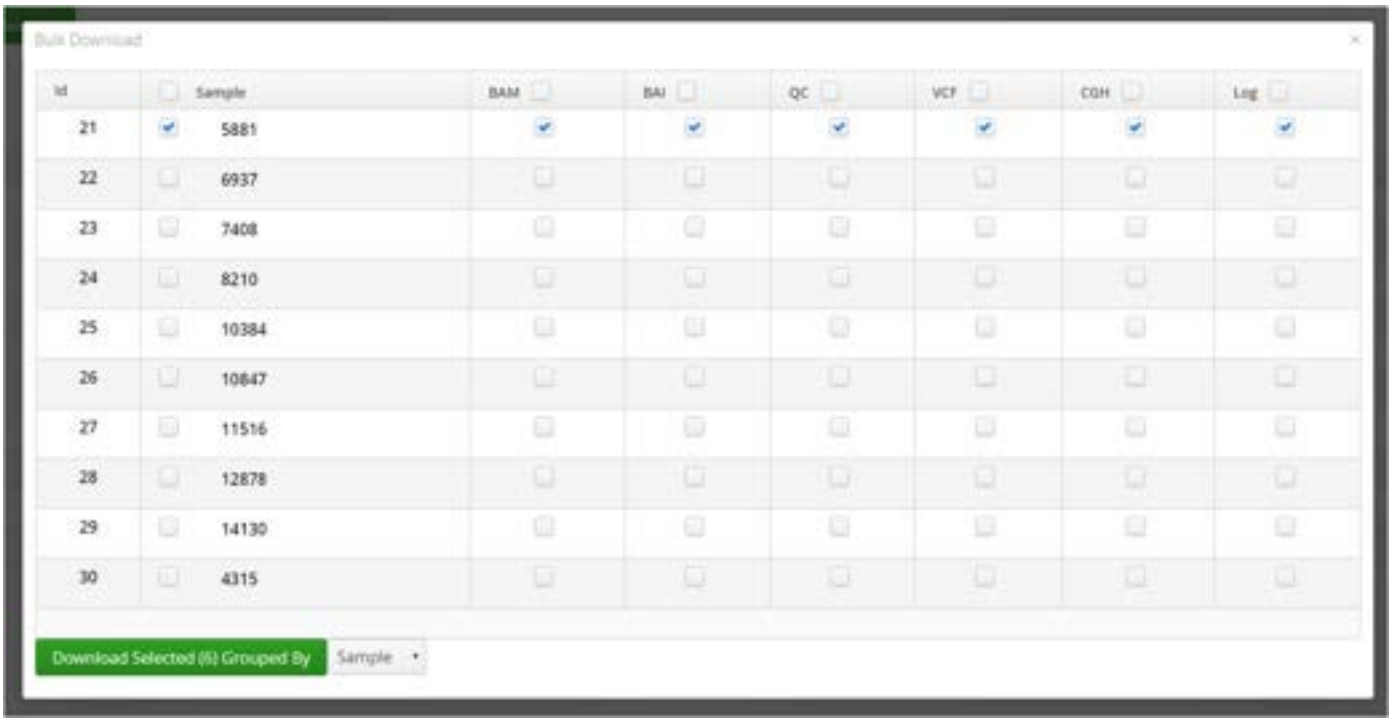


Figure 61: Bulk download with single sample selected

Alternatively, all files can be selected for download

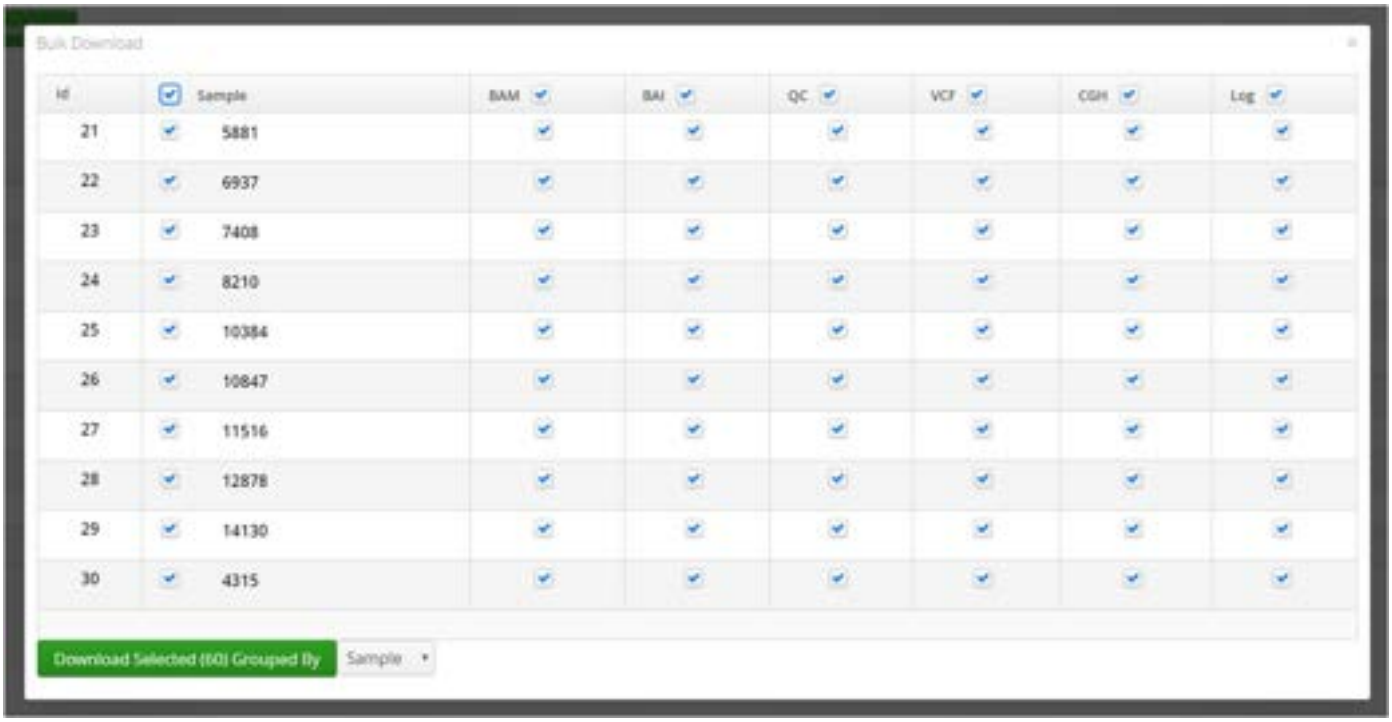


Figure 62: Bulk download with all files selected

Files downloaded in bulk can be grouped by sample or file type

Figure 63: Bulk download selecting grouping by Sample of downloaded files

Copy Batch

This function allows the user to repeat the batch analysis with the same settings. When selected a Run Batch window opens and if the user selects to Run Analysis the processing will be repeated.

The software will automatically update the Batch Name but otherwise nothing is changed including the time stamp.

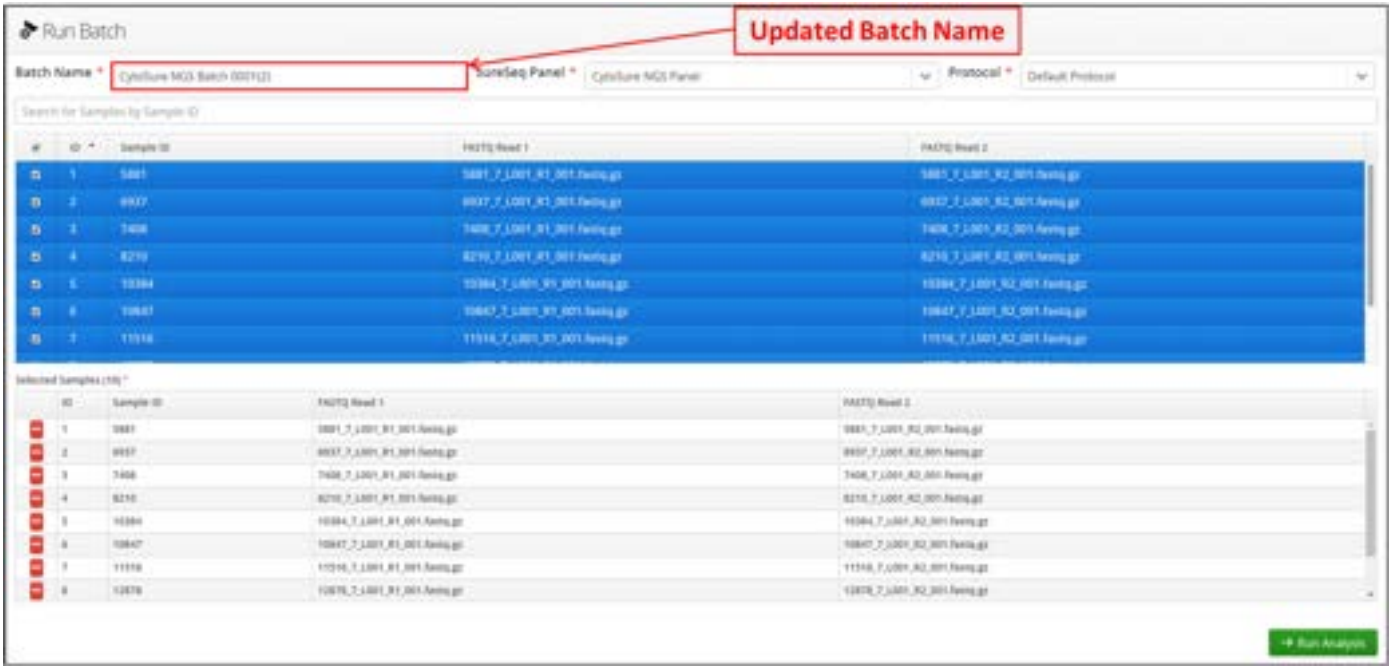


Figure 64: A batch analysis being repeated using the Copy Batch option showing the updated batch name

Report Generation

Report Generation shows a drop down in which the user can select the report to be generated

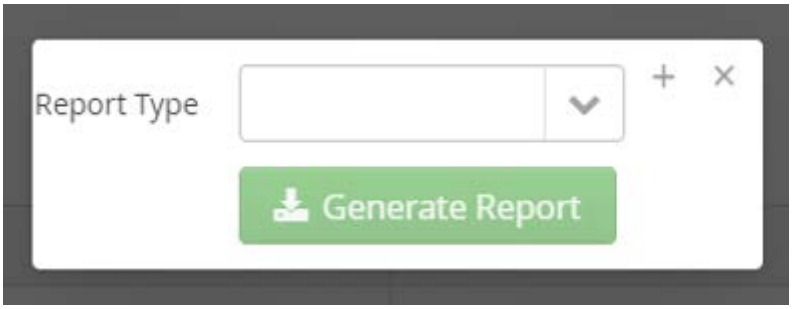


Figure 65: Initial view of the report options

Currently, the only template loaded is the Batch Report

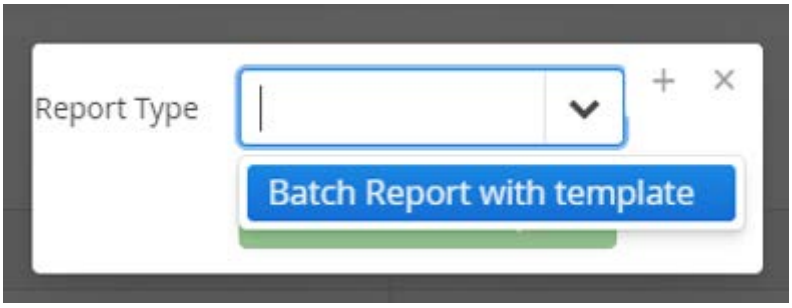


Figure 66: Selecting a report type for the QC of the run

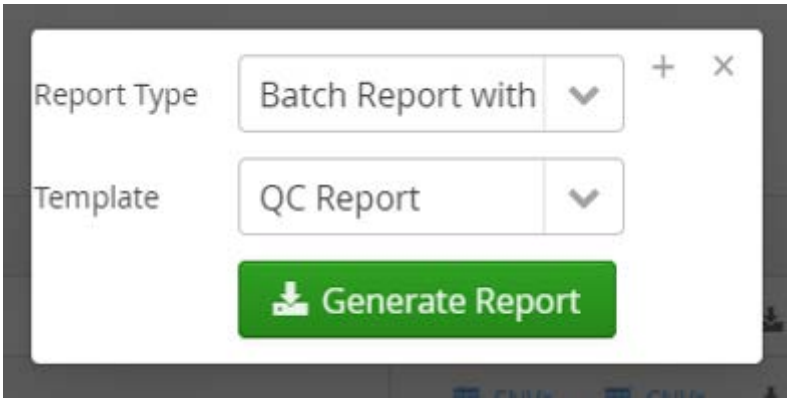


Figure 67: Selection of a template for the report

When the report is generated the output is a table with a set of metrics for each sample in the batch.

Sample	Percent Reads Aligned	Percent Duplication	Mean Target Coverage	Targets Not Covered	Aligned Reads GC	Aligned Reads Per Base Quality	Usable On Target Reads	Usable On Target Bases
5881	99.4	43.3	536	0	40	37.4	53.9219	35.5563
6937	99.4	43.1	627	0	40	37.4	55.4457	36.6627
7408	99.1	50.2	440	0	40	37.3	39.6108	25.9758
8210	99.1	50.7	418	0	41	37.3	40.9108	26.9532

Table 1: Example output of the QC report for a batch

Selecting a completed sample or samples allows viewing of the variant information and this is described in Viewing Analysis Results section.

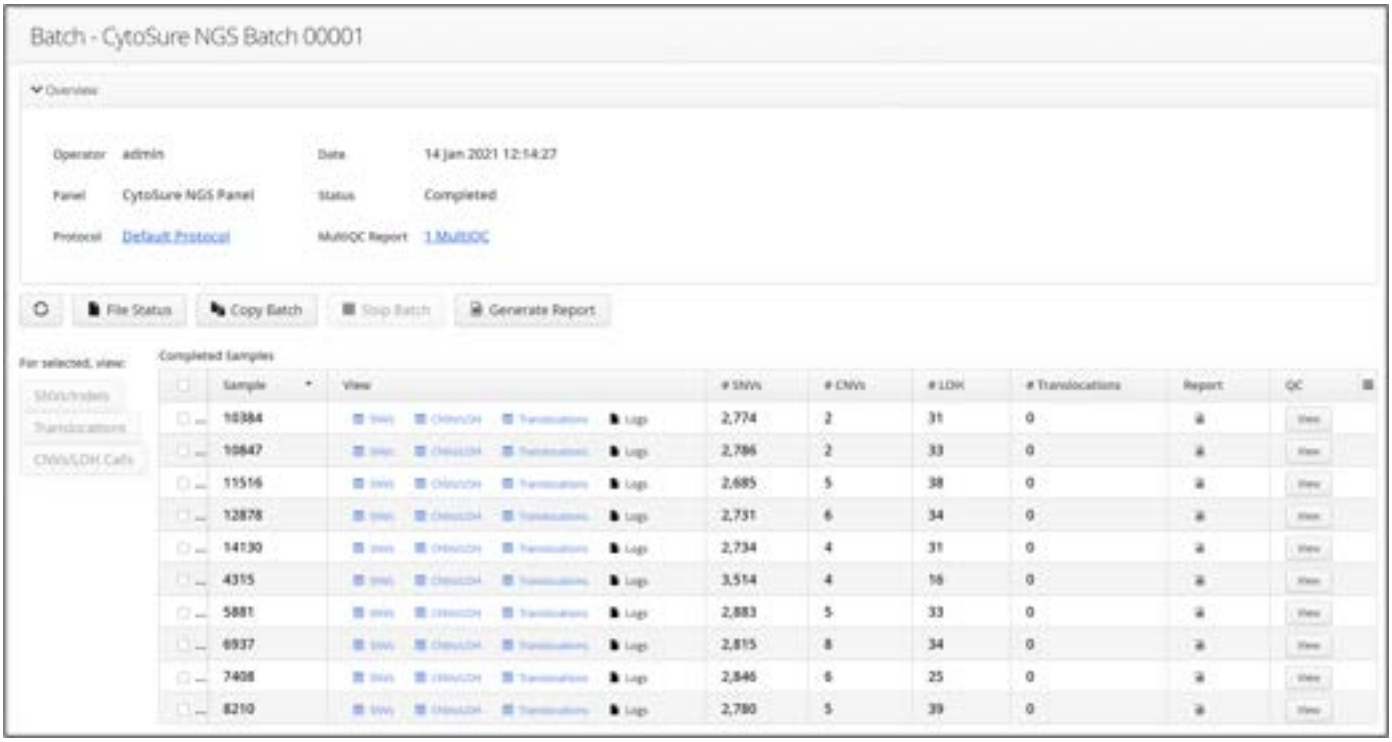


Figure 68: Selecting a batch to view

Viewing Analysis QC

When a batch of samples is processed, besides individual sample metrics that were discussed in the previous section, there is a batch QC report generated. This uses MultiQC and fastp to collate a set of metrics for each sample and merge into a set of graphs and tables.

The report can be accessed from in the batch overview displayed once a batch has completed analysis.

Figure 69: Accessing the Batch QC report

When the user clicks on the MultiQC Report link a new tab opens up in the browser displaying the QC report. The view is divided into 3 parts - the quality control report for the batch, which comprises the bulk of the display, and 2 tabs that come into the

view from the left and the right of the page. These tabs can be viewed and hidden by clicking on their respective buttons. The second tab provides the MultiQC toolbox for:

1. The Quality Control report for the batch
2. The report short cut tab
3. The tool box tab

At the head is the quality control report; this provides general information about the analysis such as the date of the analysis and which user performed it.

Figure 70: Example batch overview details

OGT's Summary Table

Each sample has a row in the table with some key metrics.

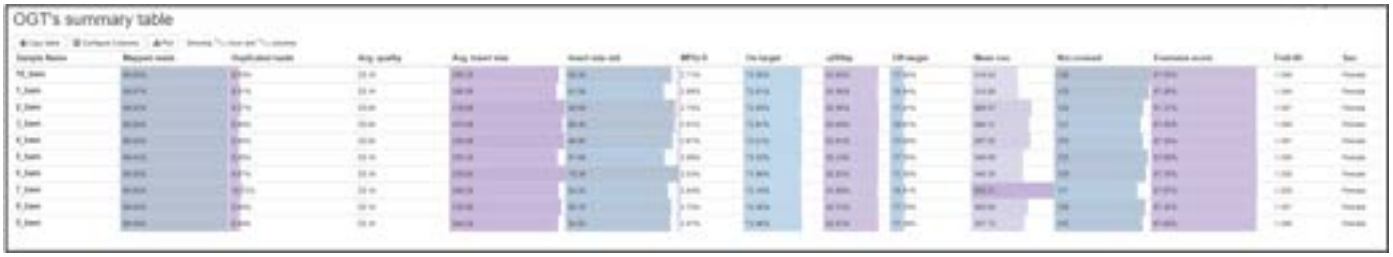


Figure 71: Example sample QC summary table

The column names from the summary table are listed in the table below with some additional detail as to their meaning.

Column Name	Description
Mapped reads	The percentage of reads that mapped to the reference genome
Duplicate reads	The percentage of reads that were duplicated
Avg. quality	The average read quality reported by Samtools stats
Avg. insert size	The average insert size reported by Samtools stats
Insert size std	The standard deviation of the insert size reported by Samtools stats
MPQ = 0	The percentage of reads that were mapped that have a mapping quality of 0
On-target	The percentage of reads that map on target that are not duplicate reads
± 250bp	The percentage of reads that overlap target regions extended by 250bp

Column Name	Description
Off-target	The percentage of reads that are neither on target nor within the specified flanking region
Mean cov.	The mean target coverage
Not covered	The number of targets with a coverage of less than 1
Evenness score	The fraction of the whole sequencing output that is correctly distributed
Fold-80	The fold of additional sequencing that would be required to ensure that 80% of targeted bases achieve the mean target coverage.
Sex	The chromosomal sex of the sample predicted from the distribution of reads that map to the sex chromosomes

Table 2: Column names and their description from the QC summary table

Targets Not Covered

Any targets not covered are detailed, providing that they are not within a segmental duplication.

Figure 72: Example targets not covered summary

Coverage Efficiency

The efficiency of coverage as a measure of depth are displayed.

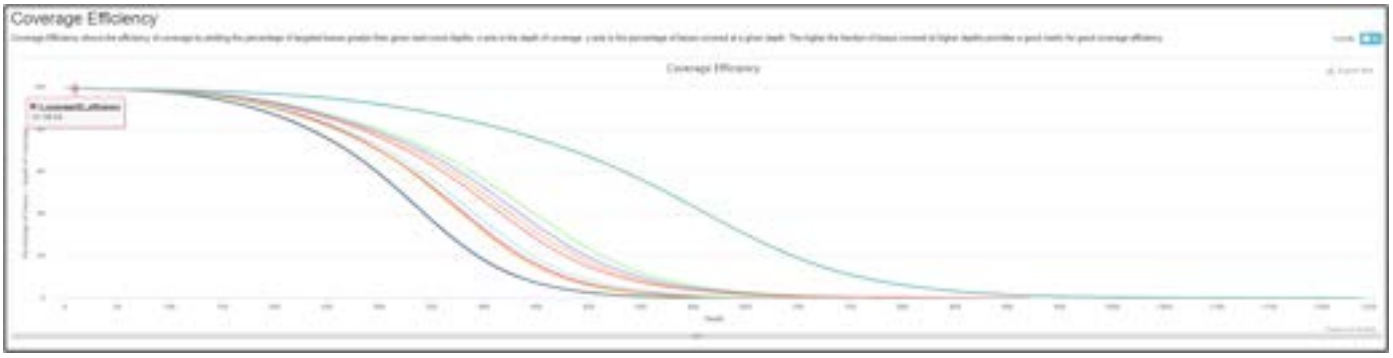


Figure 73: Example QC report summary

Insert Sizes Samtools

The distribution of insert sizes for each sample is displayed.

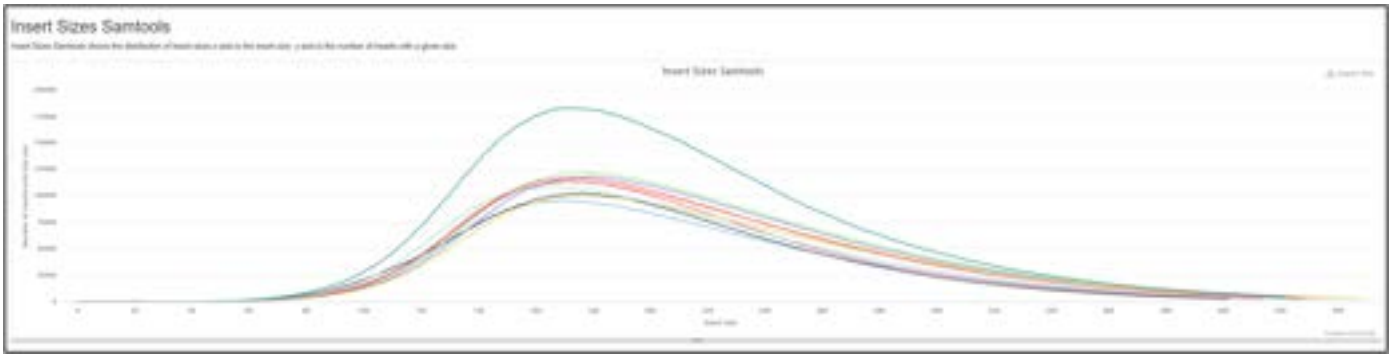


Figure 74: Example QC report summary

Percent Mapped

The percentage of base calls at each position for which an N was called.

Figure 75: Example QC report summary

Alignment Metrics

The alignment metrics for all the samples in the batch are plotted.

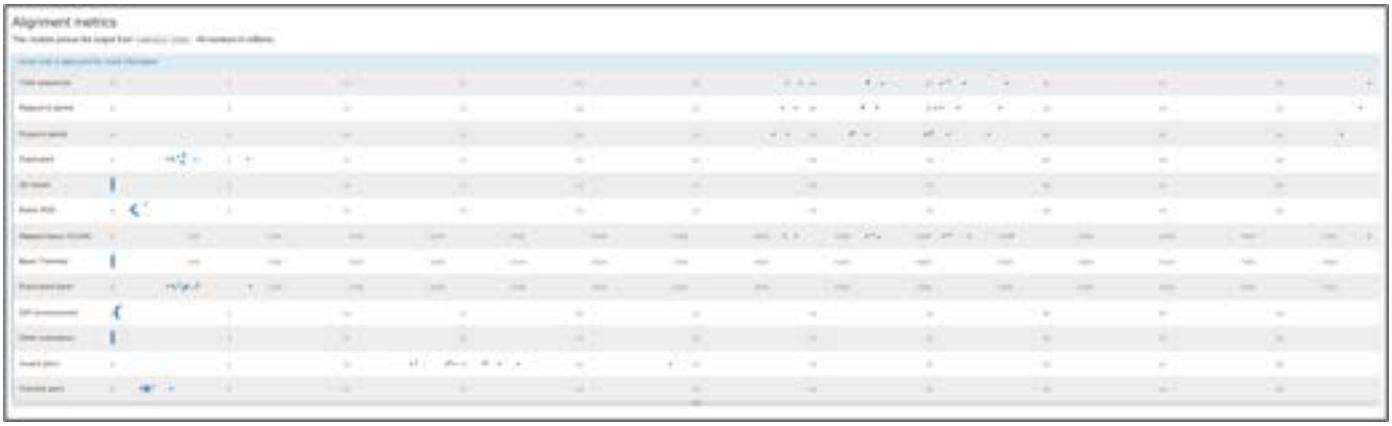


Figure 76: Example of the alignment metrics

Filtered Reads

The filtered reads graph shows the number or percentage of reads that have been removed by the filter.

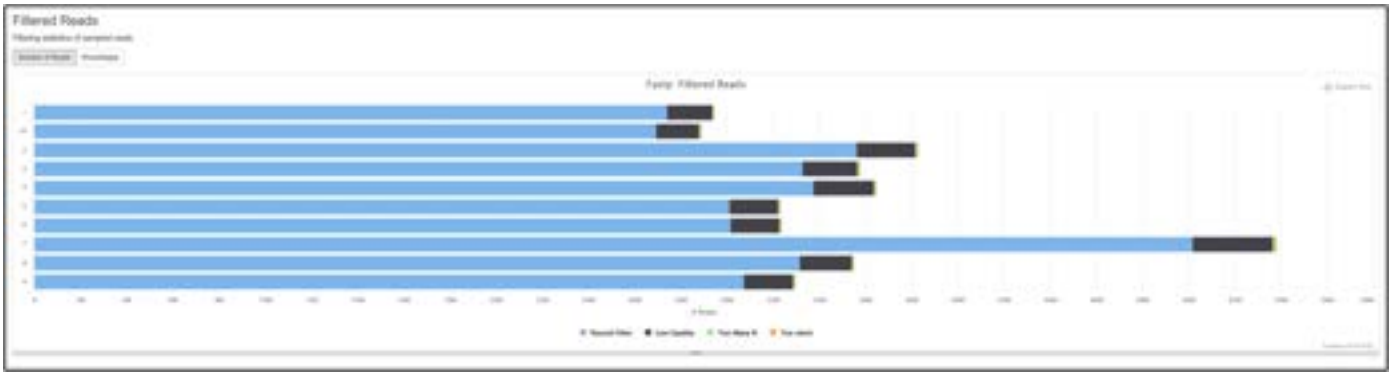


Figure 77: Example QC report summary

Duplication Rates

The relative level of duplication found for each sample as a percentage.

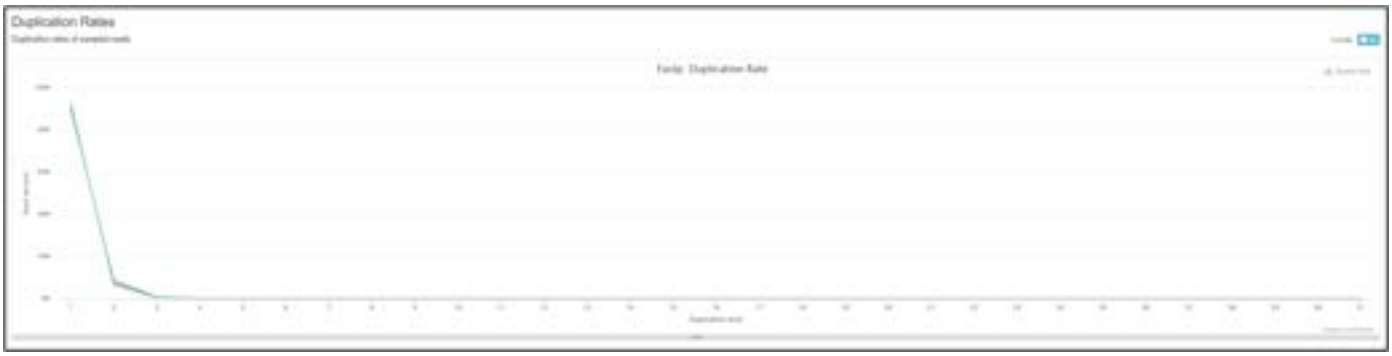


Figure 78: Example QC report summary

Sequence Quality

The mean sequence quality or Phred score of each base in the insert for each sample.

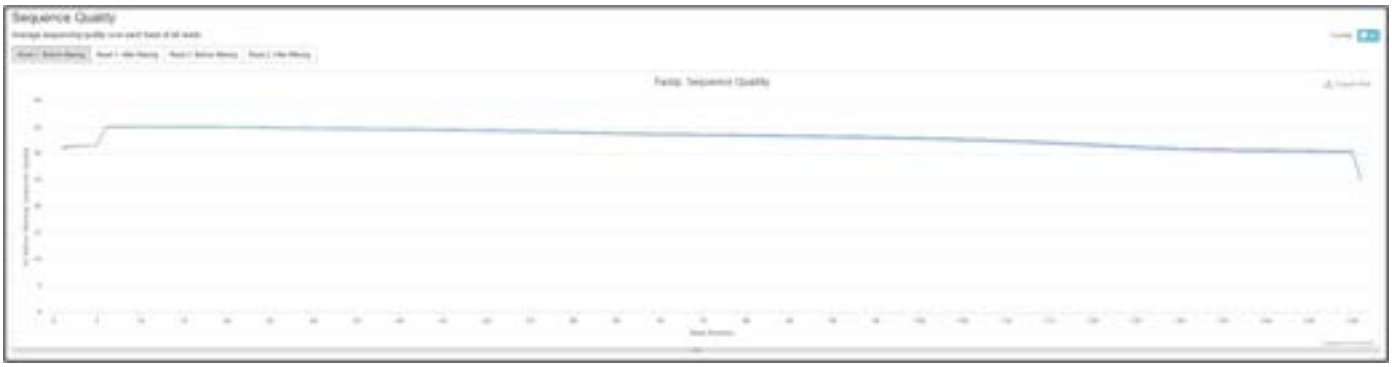


Figure 79: Example QC report summary

GC Content

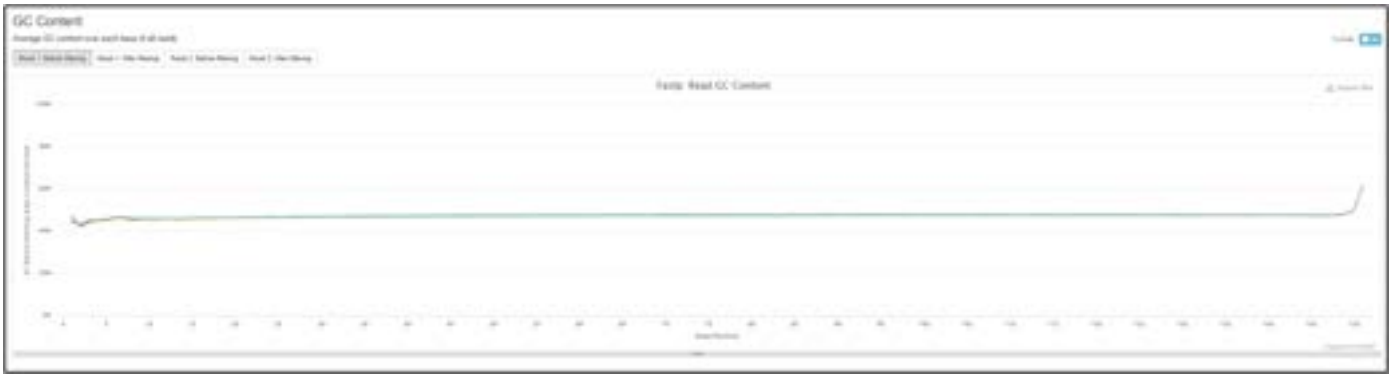


Figure 80: Example QC report summary

N Content

Figure 81: Example QC report summary

Sample QC

Sample QC information can also be access via the Sample page. For a particular run selecting the QC metrics tab will provide the relevant information. Colours are defined by the metric set used in the analysis protocol.

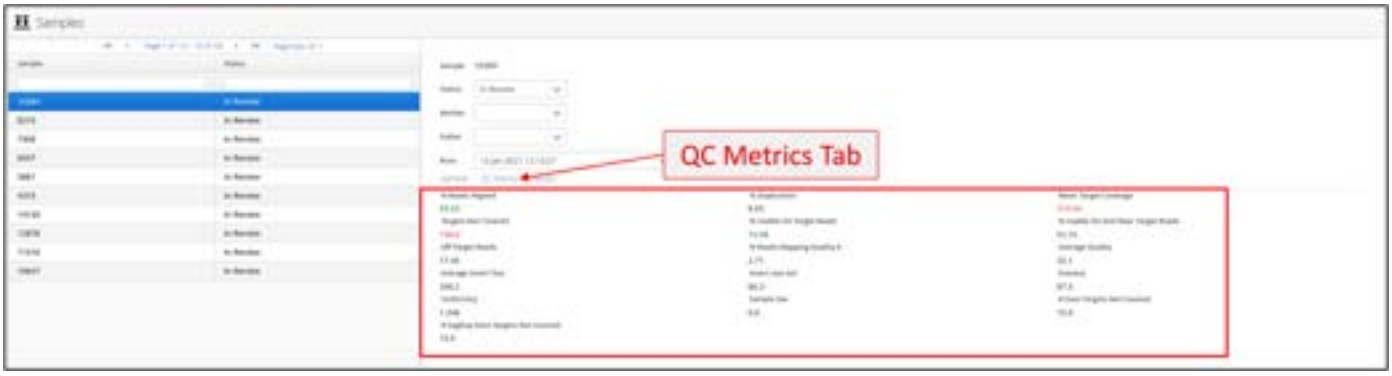
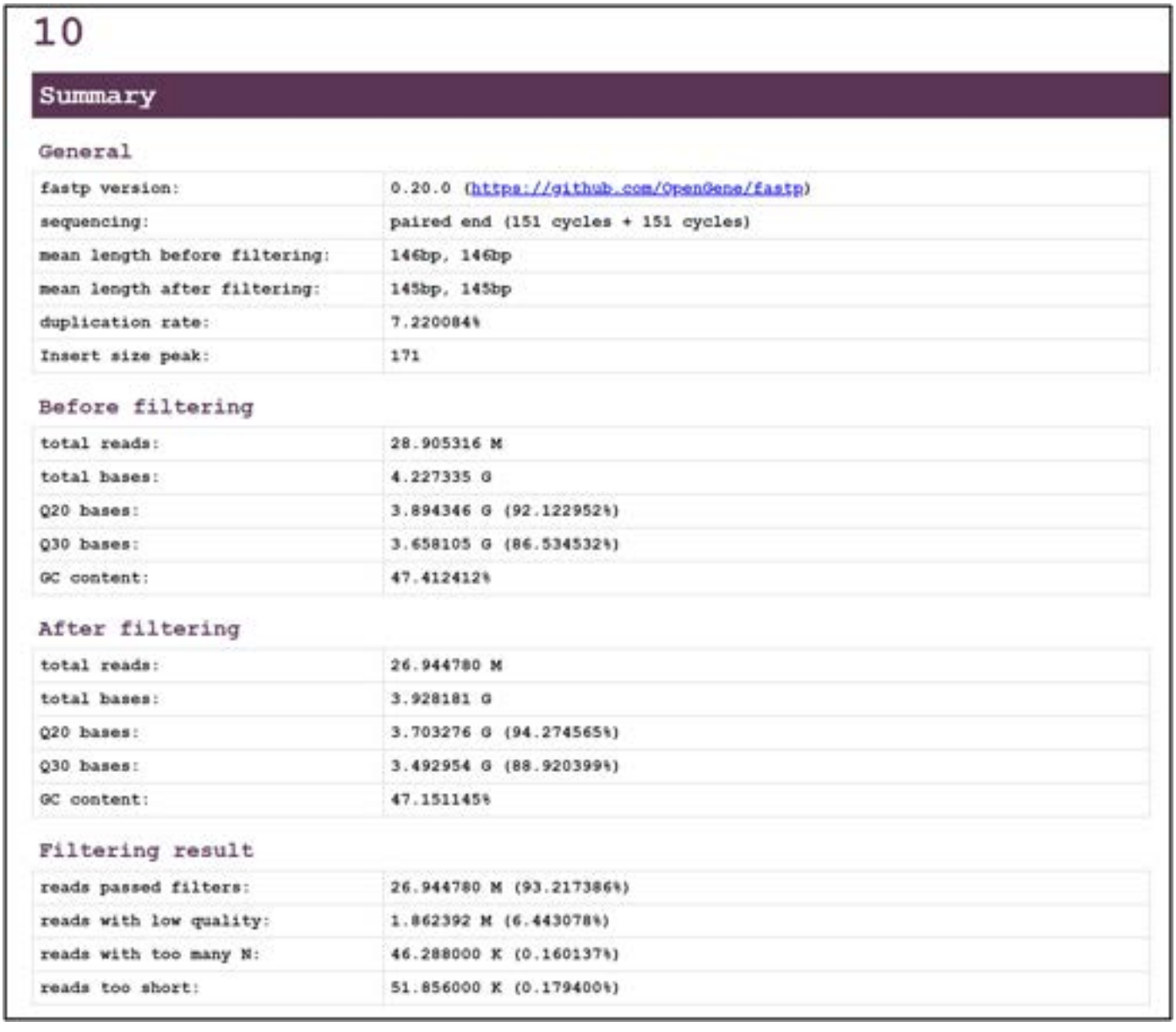


Figure 82: Viewing the metrics for an individual sample

Figure 83: Accessing the Sample QC report from the sample results tab



10

Summary

General

fastp version:	0.20.0 (https://github.com/OpenGene/fastp)
sequencing:	paired end (151 cycles + 151 cycles)
mean length before filtering:	146bp, 146bp
mean length after filtering:	145bp, 145bp
duplication rate:	7.220084%
Insert size peak:	171

Before filtering

total reads:	28.905316 M
total bases:	4.227335 G
Q20 bases:	3.894346 G (92.122952%)
Q30 bases:	3.658105 G (86.534532%)
GC content:	47.412412%

After filtering

total reads:	26.944780 M
total bases:	3.928181 G
Q20 bases:	3.703276 G (94.274565%)
Q30 bases:	3.492954 G (88.920399%)
GC content:	47.151145%

Filtering result

reads passed filters:	26.944780 M (93.217386%)
reads with low quality:	1.862392 M (6.443078%)
reads with too many N:	46.288000 K (0.160137%)
reads too short:	51.856000 K (0.179406%)

Figure 84: Start of a FastP report for an individual sample

Viewing Analysis Results by Sample

Viewing a Sample

Access to the results from running the pipeline are described in the previous section "View Analysis Batches".

Within each batch are the samples processed in that batch comprising analysed variants and QC metrics.

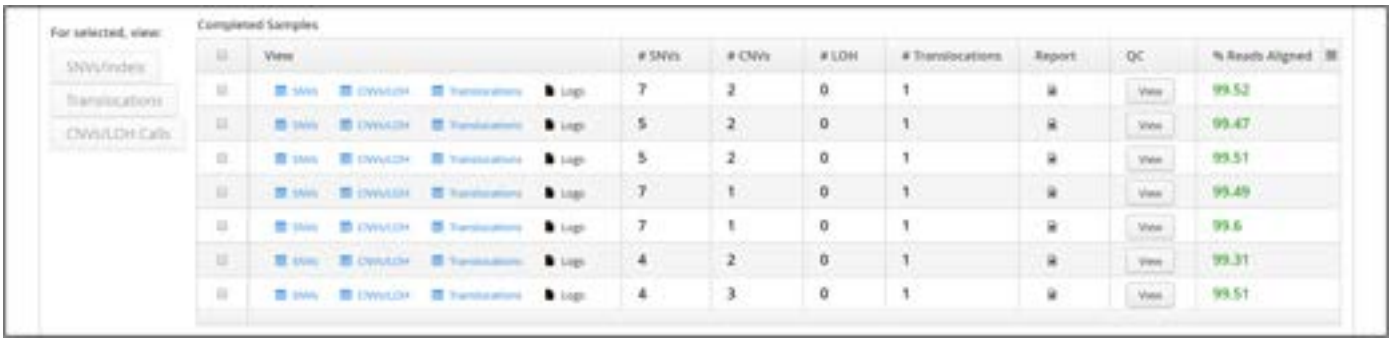


Figure 85: View of a set of processed samples in the batch view

As with other tables in Interpret, where there is a column selection icon  users can use it to configure which columns are being displayed.

Figure 86: Column selector button for configuring columns to view in display

The column options for this view are shown in the Figure.

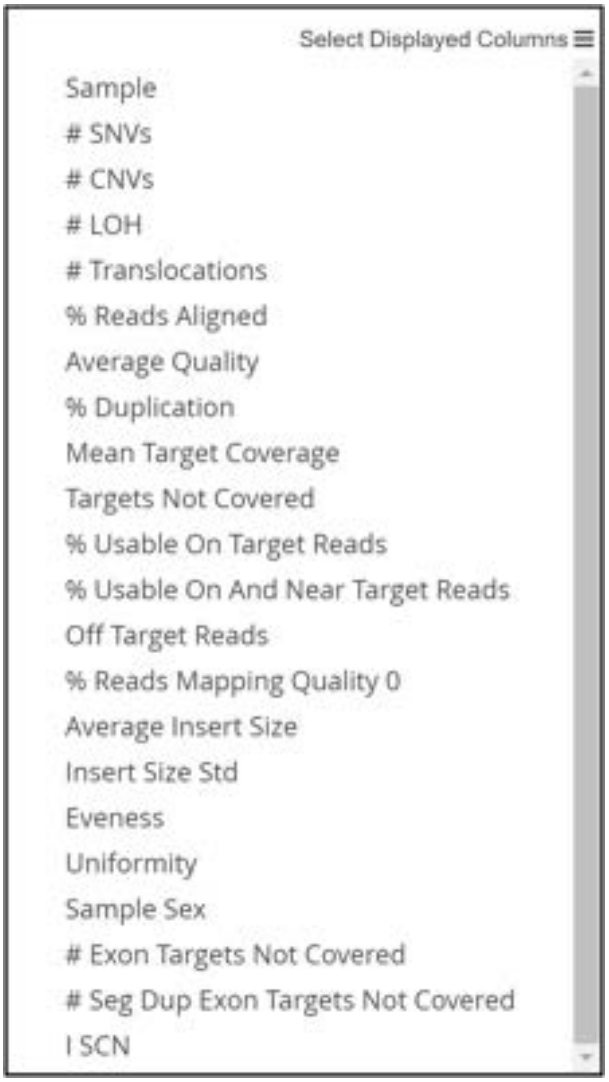


Figure 87: Columns available for selection to display

There is one completed sample per row and for each sample there is a range of information available to view.

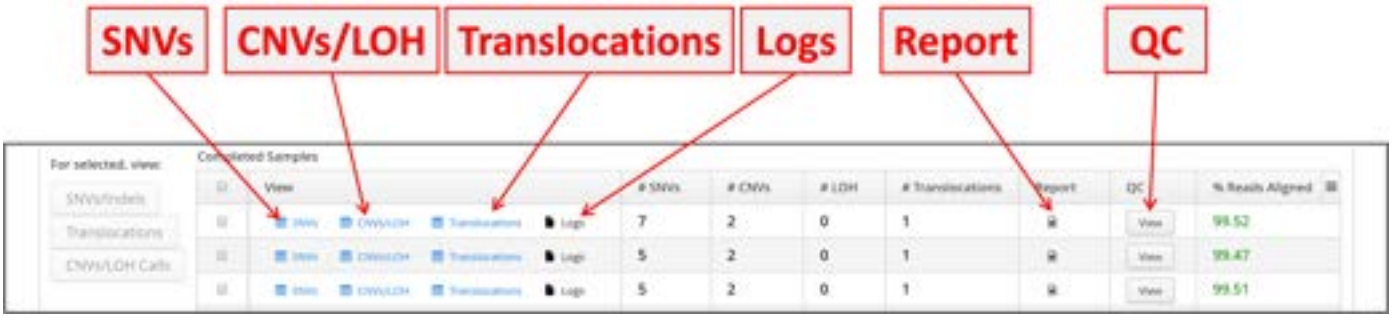


Figure 88: Information available for each sample

Variants for a sample can be viewed by selecting the SNVs or CNVs/LOH links present in each row.

Multiple samples can be viewed simultaneously by selecting the check boxes of the required samples which will then activate the SNVs and CNVs buttons on the left hand of the view.

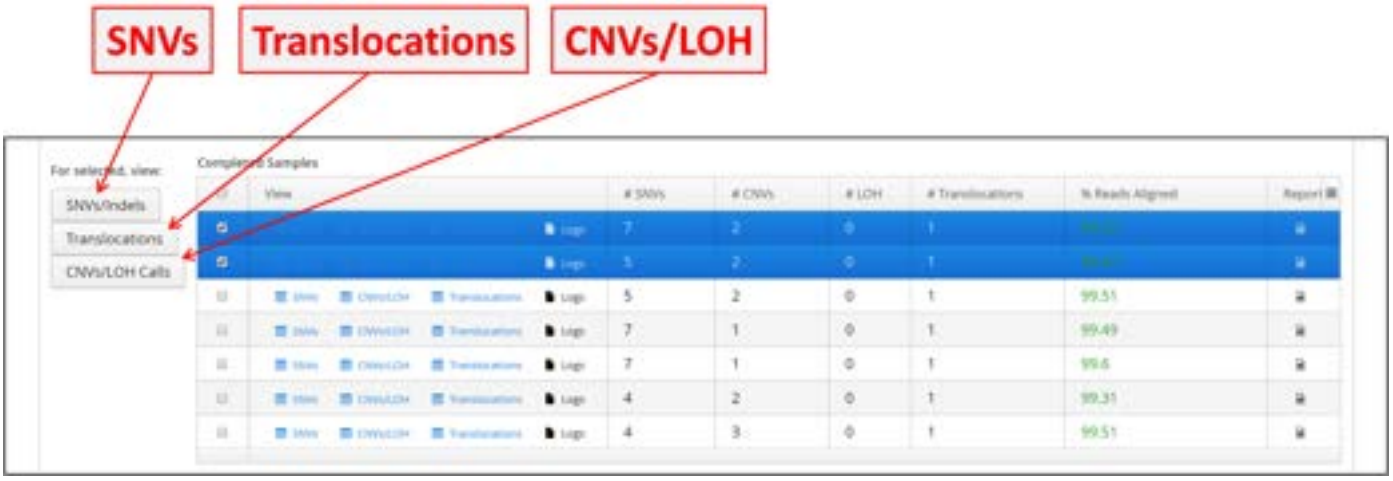


Figure 89: Selecting multiple samples to view simultaneously

Once selected, the variants will be displayed on a Variants page.

Figure 90: The initial SNV/Indel display page

At the top of the page are buttons that allow the user to toggle between the CNV and SNV views.

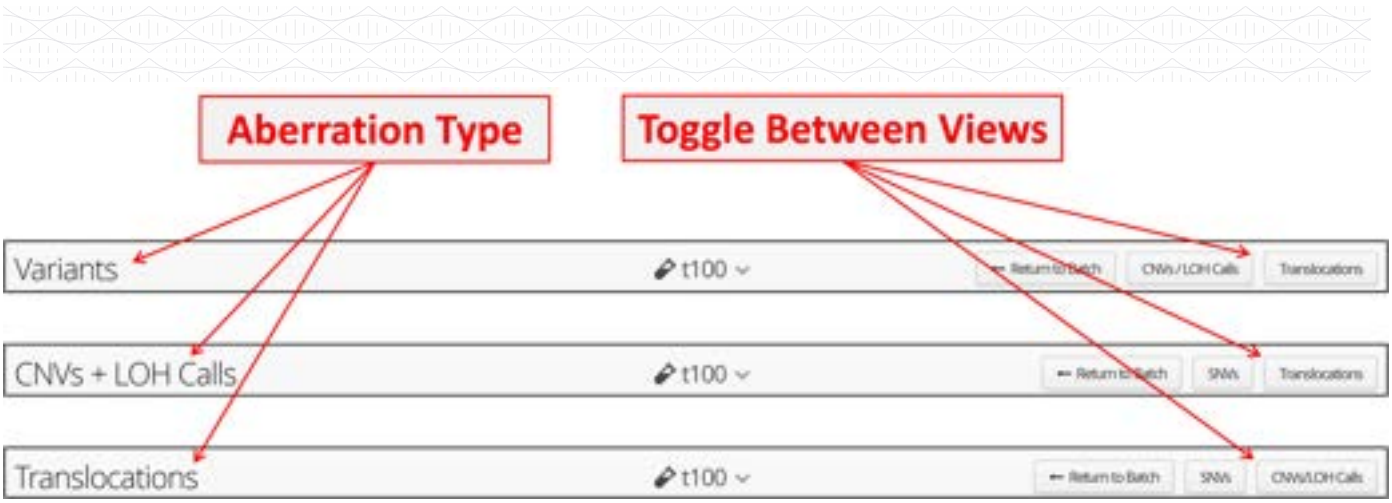


Figure 91: Toggling between SNV, CNV and Translocation reports

The Variants viewer is divided into three parts:

1. The Protocol Filter, initially this will be showing the Protocol Filter used in the analysis. The filter is modifiable in the Admin Controls (Admin Controls > Analysis > Protocols)
2. The Variant Table, showing the variants, one on each row. In the header there is a drop down "Actions" menu options; these are discussed below.
3. The Integrated Genome Viewer (IGV) that has been embedded in the software. Further details on using IGV are below.

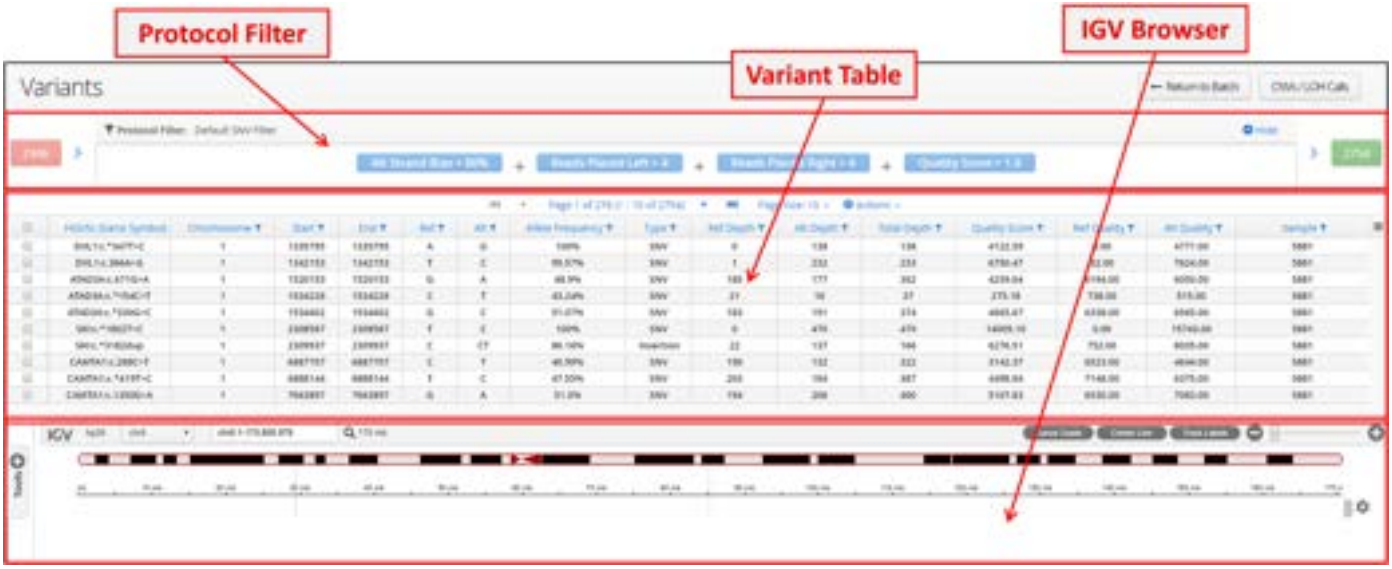


Figure 92: The sections of a sample results view page

Viewing SNV and Indel Events

The variant table has a column selector icon allowing user to configure which columns are displayed.

There are different columns available depending on whether you are viewing the SNV variants page or the CNV/LOH variant page. The options for SNVs are displayed below.

Figure 93: Columns available to display in the SNVs variant page

Selection of a variant will load the alignment file in IGV allowing review of the alignment.

A range of variants can be displayed and examples of each of these are:

1. SNV

Figure 94: Example of a SNV being displayed in the IGV browser

2. Deletion

Figure 95: Example of a deletion being displayed in the IGV browser

3. Insertion

Figure 96: Example of an insertion being displayed in the IGV browser

4. Complex

Figure 97: Example of a complex event being displayed in the IGV browser

5. Multi Nucleotide Polymorphism (MNP)

Figure 98: Example of a MNP variant being displayed in the IGV browser

6. Partial Tandem Duplication (PTD)

Figure 99: Example of a PTD being displayed in the IGV browser; the duplication event is highlighted by the transparent red box

SNV Options

Right clicking on a row will generate a popup menu with a range of options.

Figure 100: Options available for each SNV or Indel variant

Add to a Shortlist

Figure 101: Adding a variant to shortlist

Once a variant has been added to the shortlist it will be annotated with a tick.

Figure 102: Annotation of a selected variant

A variant can be removed from the shortlist using the Remove from Shortlist command.

Figure 103: Removing a variant from a shortlist

Subsequently the tick annotation will be removed.

Figure 104: Annotation denoting shortlisting has been removed

Add to New List

Variants can be added to lists that can be used in software; for example a list of variants can be used in a filter as a means to specifically search for a data set.

Figure 105: Adding a variant to New List

Initially users will be prompted to create a new variant list by setting the name of the list.

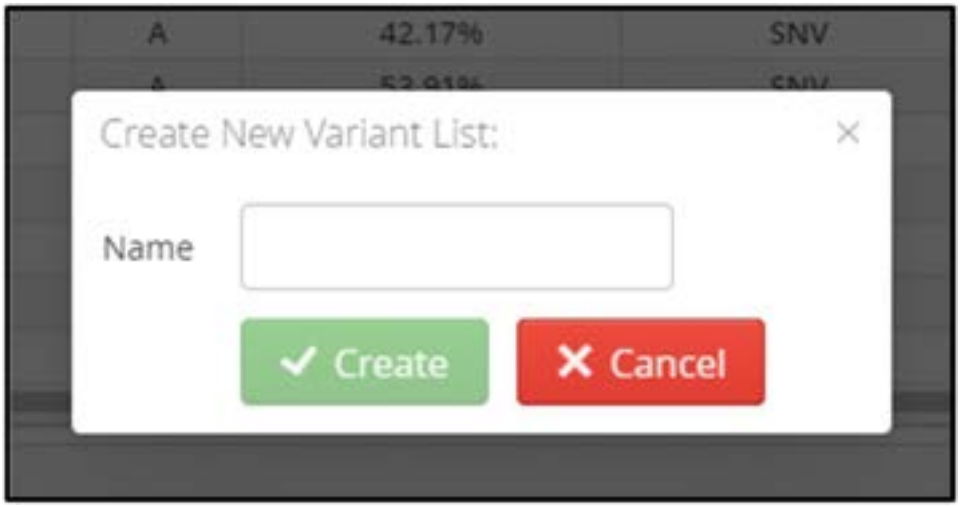


Figure 106: Creating a new variant list

In the example below a list called New List has been created.

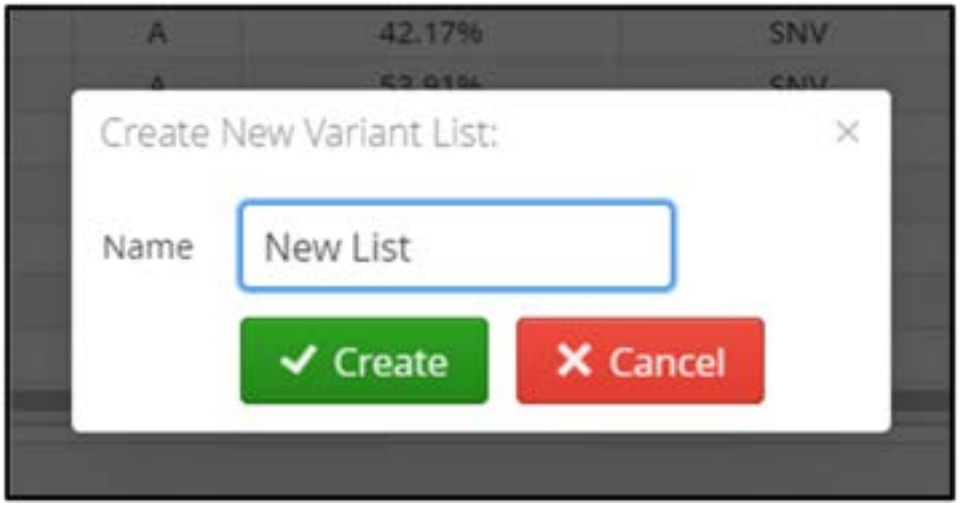


Figure 107: Setting the name of a new variant list

The list New List is now available and variants can be added to it.

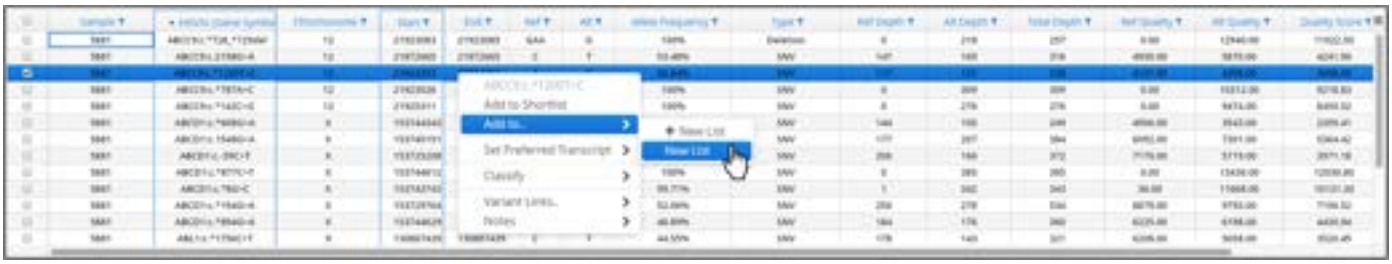


Figure 108: Adding a variant to the newly created list

Select Transcript

By default the a gene will have the largest canonical transcript set as the preferred transcript.

To change this, users can use the Set Preferred Transcript option to select an alternative transcript from the list available in the database.

Figure 109: Setting a preferred transcript for a gene

Variant Classification

Users are able to classify variants in two ways; firstly, a variant can be directly assigned one of the defined classifications.

Additional classifications can be added via the Admin Controls (Admin Controls > Analysis > Classifications).

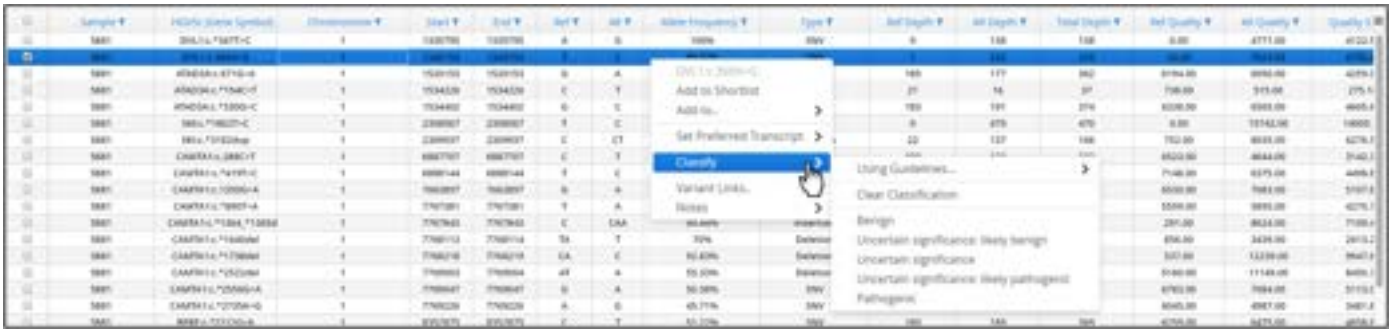


Figure 110: Variant classification options

A variant classification is selected form the list that is included by default. These are:

- Benign
- Uncertain significance, likely benign
- Uncertain significance
- Uncertain significance, likely pathogenic
- Pathogenic

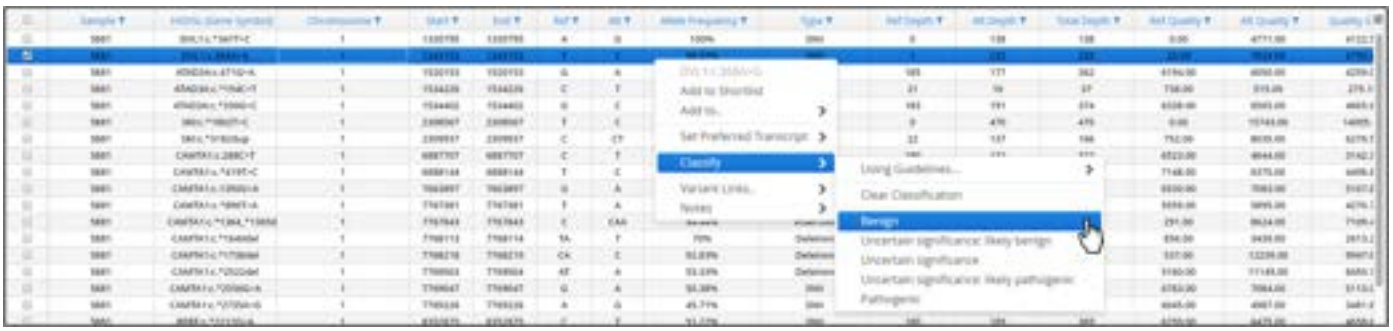


Figure 111: Annotating a variant as benign

Once this classification has been made the variant will be annotated with the corresponding colour classification. This colour can be changed in the Admin Controls section of the software (Admin Controls > Analysis > Classifications)

This update will be applied to the variant annotation. As a result where the same variant appears in other samples it will have the same colour coding in the table.

Figure 112: Update of the annotation to show a variant as benign

A variant classification can be removed using the clear classification method

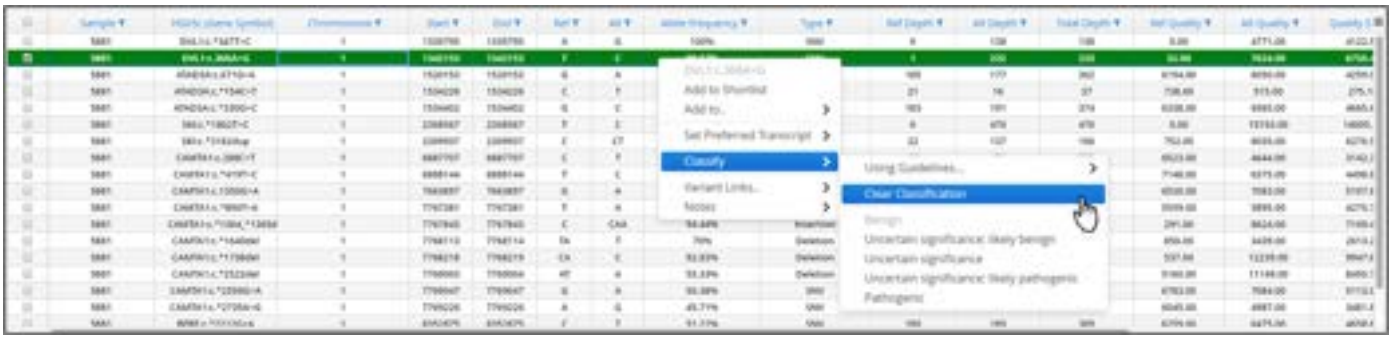


Figure 113: Removing a variant classification annotation

The classification will be removed for the variant in the table and all other samples with the same variant will be similarly updated.

Figure 114: Update of the annotation to show variant without any classification

Using the American College of Medical Genetics and Genomics (ACMG) Guidelines

An alternative means to derive a classification for a variant is via guidelines described by the ACMG. These guidelines are included with Interpret.

To follow the ACMG guidelines the user provides answers to a specific set of questions. Each answer will navigate the user through the conditions of the guidelines until a classification of the variant can be made.

Selecting to use the guidelines option leads to a new window opening.

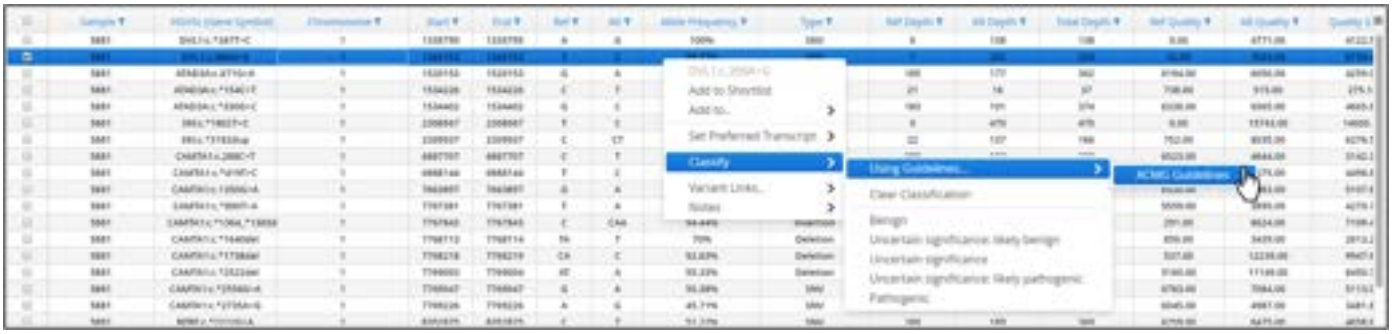


Figure 115: Selection of the inbuilt ACMG classification guidelines

The initial ACMG window, shown below, consists of a progress bar that will report how close to a classification

- Progress Bar - Showing the progress of the classification.
- Questions - These are the questions to be answered.
- Toggle - Allowing the display to be a graph view or a table view.

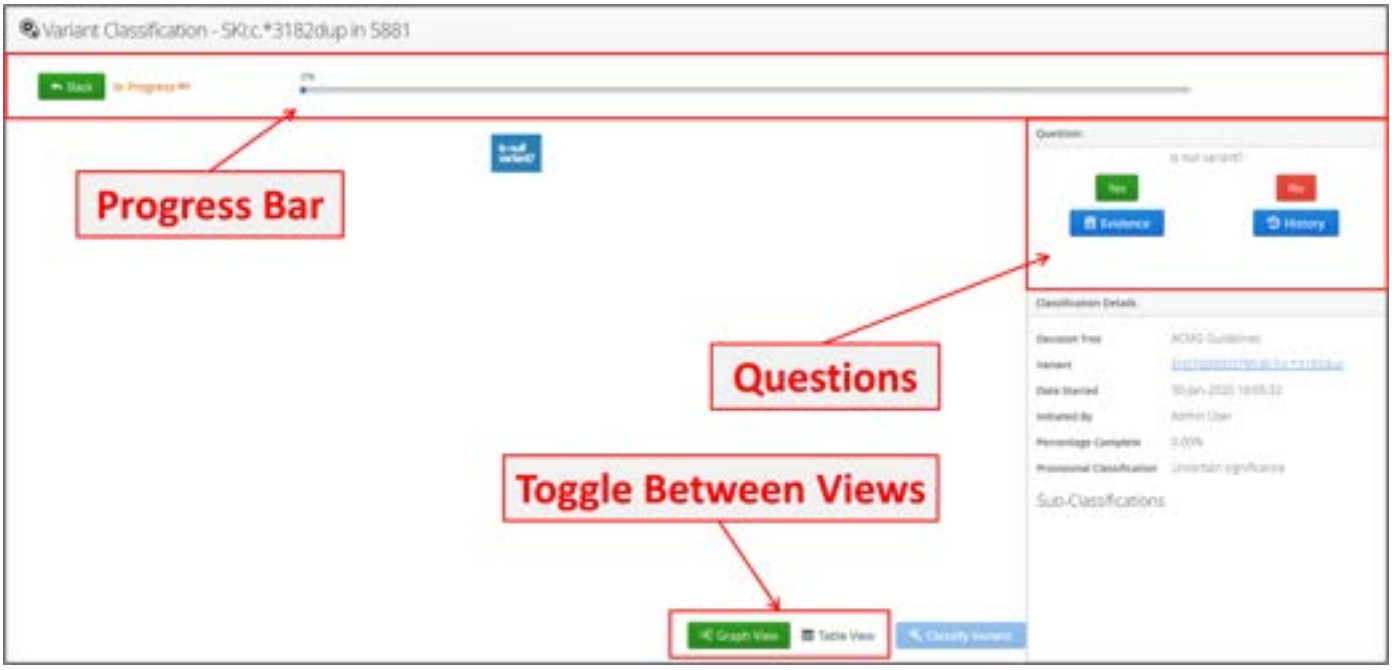


Figure 116: The initial ACMG classification window

As the user answers questions the progress bar will update.

Figure 117: A classification at the start of the guidelines, with 4% progress

Figure 118: A classification with 67% progress

When sufficient questions have been answered to allow a classification the progress bar will update to show 100% and say Ready to be classified.

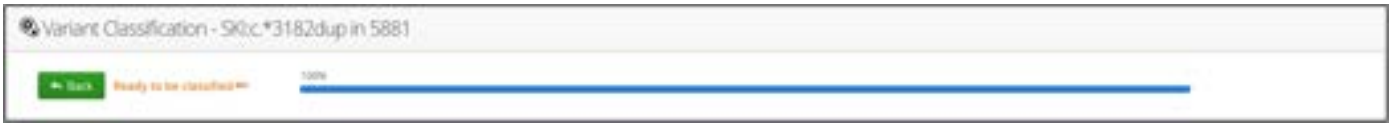


Figure 119: A completed ACMG classification

A window will appear showing the classification and give the user the option of making the classification or cancelling.

Figure 120: A completed classification showing a Pathogenic all has been made

Selecting the classification will update the variant's annotation accordingly.

Figure 121: Updated annotation for the variant to show its status as pathogenic

It is possible to review the choices made in the guidelines; using the table view, users can see which questions were asked and how they were answered by whom and when.

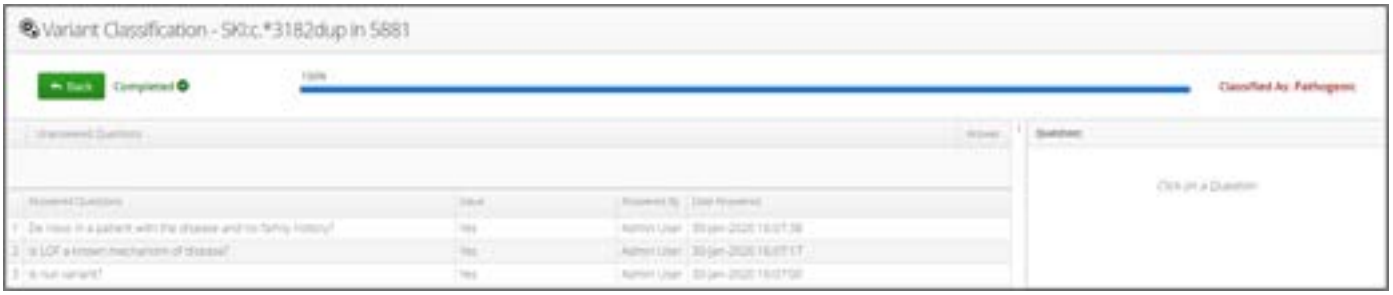


Figure 122: Table view of a completed classification

Selecting a row from the table view allows a result to be modified if that is required. Alternatively, evidence can be added to support the answer to the question

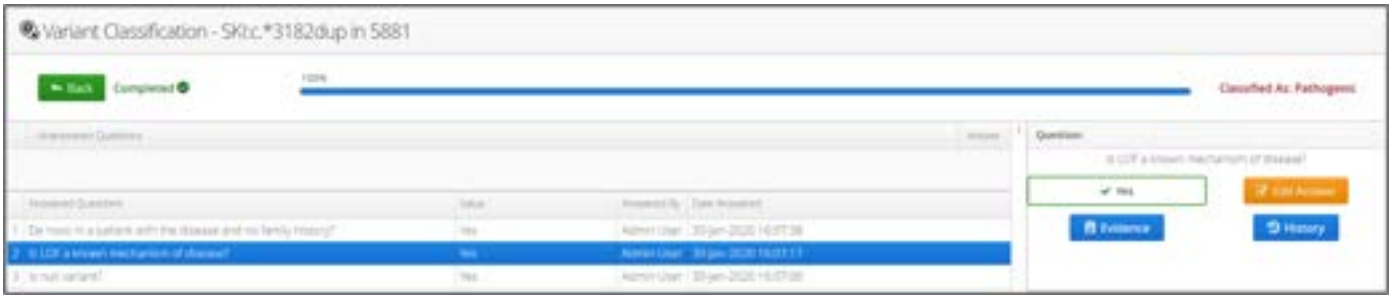


Figure 123: Reviewing an answer in the table view

Variant Links

The software allows users to link out to external sources of documentation. Currently included are:

- EnsEMBL
- ClinView
- ExAC

Additional resources can be added in the Admin Controls (Admin Controls > Analysis > Manage Links).

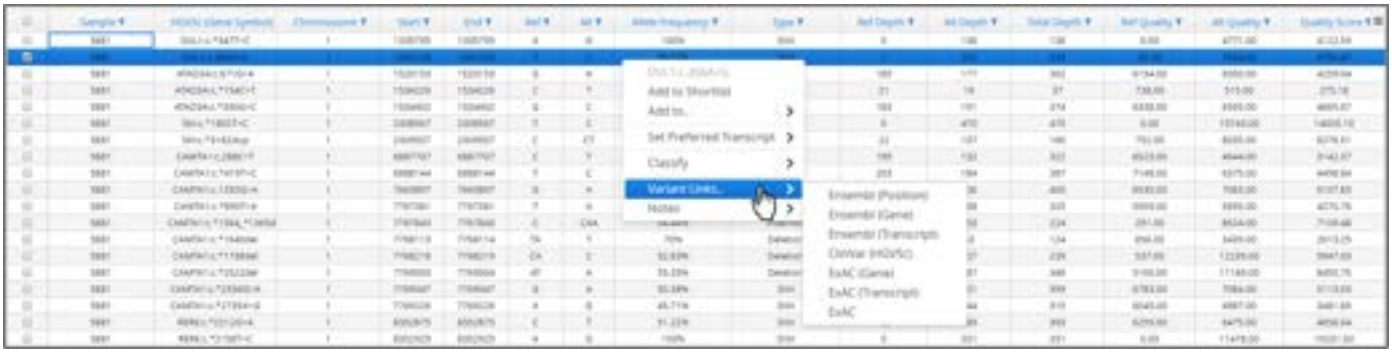


Figure 124: Variant links available in the software

If a source is selected Interpret will show the information in a separate tab in the web browser.

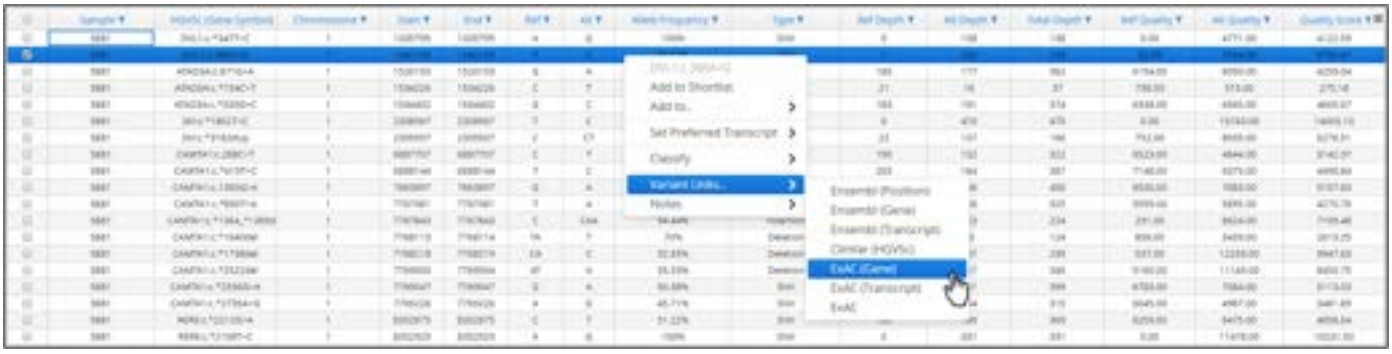


Figure 125: Selection of ExAC as an external resource for the gene selected

Figure 126: Example of the software linking out to an external data source, in this case the GnomAD for the gene containing the variant in Interpret

Add Notes

Interpret allows users to add notes for a variant and to also edit notes on the system. This is accessed through the Notes menu item.

Figure 127: Adding a note to a variant

Selecting the Add Note will generate a popup window

Figure 128: The note template window for the selected variant

Users can add the required text, up to 250 characters, in the text box

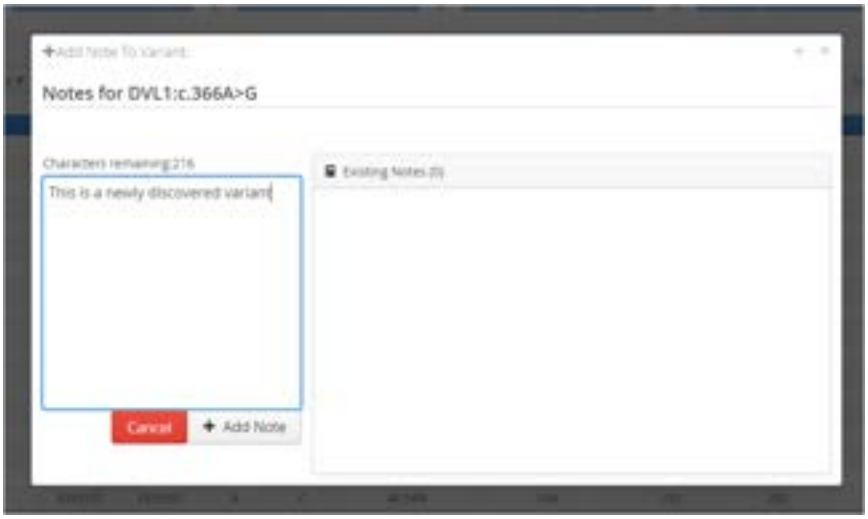


Figure 129: Addition of a note to a variant

Selecting Add Now will append the note to the variant.

Figure 130: An example of a note on the system

The additional text will now be displayed

Figure 131: Appending text to an existing notation

Notes can be modified by clicking on the pen icon. This makes the text box editable

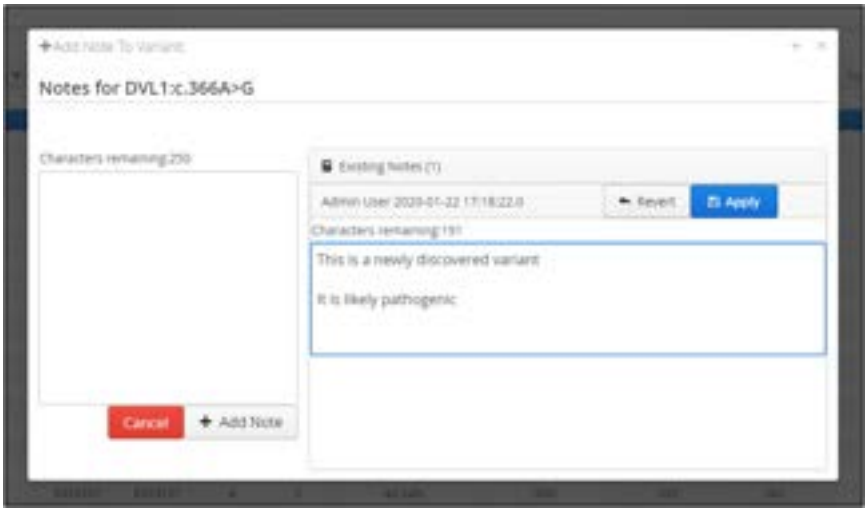


Figure 132: Adding an update to a note

Once any update has been made, selecting Apply will incorporate the changes.

Figure 133: A note showing the updated annotation

Similarly, a note can be deleted through the red bin icon.

Users are asked to confirm the delete request after which the note will be removed.

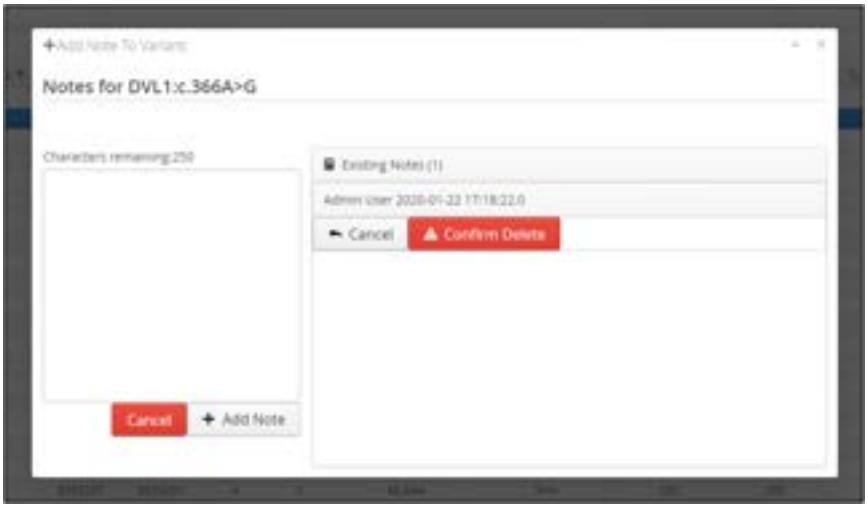


Figure 134: Deleting a variant note

Where there is a note for a variant the note can be viewed through the Notes options seen when right clicking on the variant.

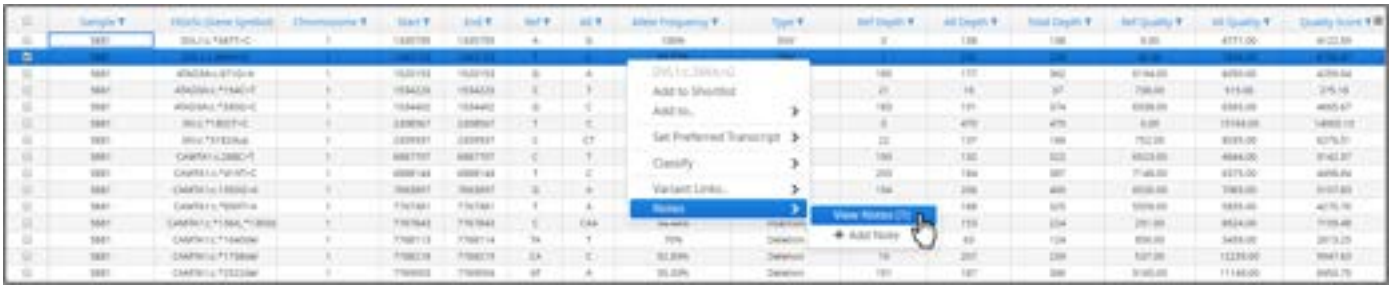


Figure 135: Selecting a Note to view

The note is displayed on the screen.

Viewing CNV and LOH Events

The variant table has a column selector icon  allowing user to configure which columns are displayed. The figure below shows the columns available for display.

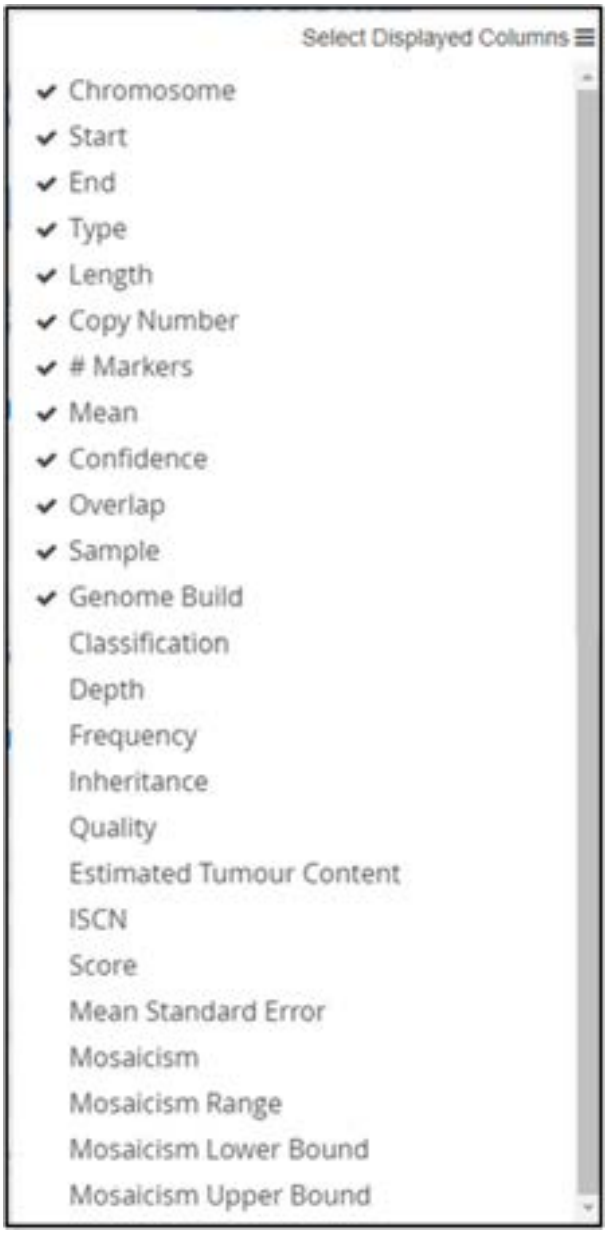


Figure 136: Columns available to select for display in the CNV/LOH variants page

Selecting a variant will show it in IGV, a track for both CNV and LOH will displayed. Sometimes there will only be a CNV call as in the example below.

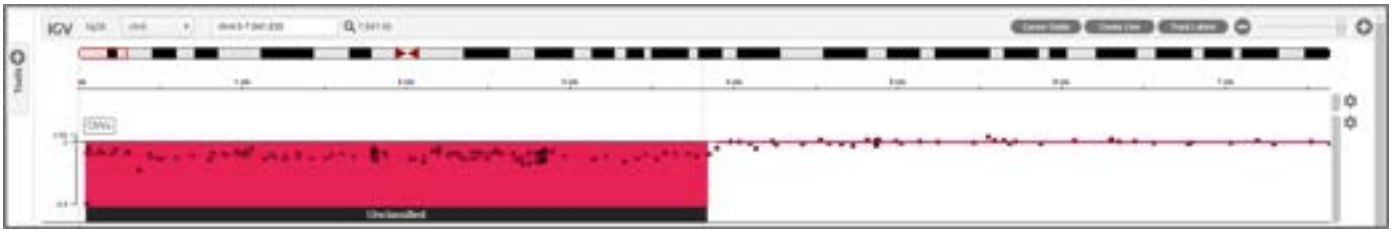


Figure 137: Example of a CNV call only

Sometimes there will only be a LOH call

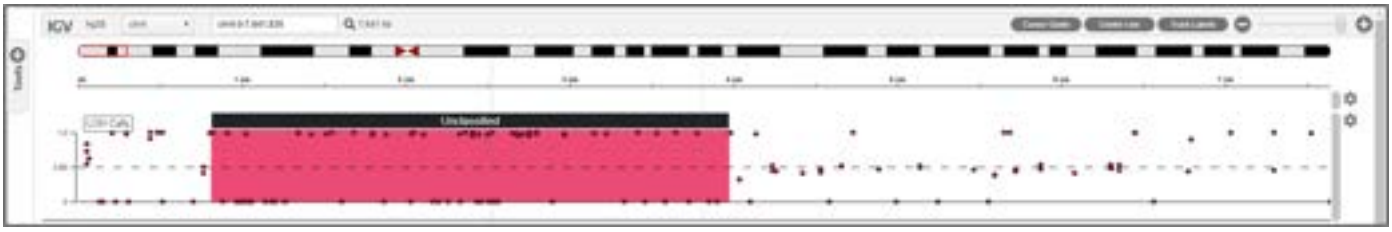


Figure 138: Example of a LOH call only

Sometimes there will be CNV and LOH calls

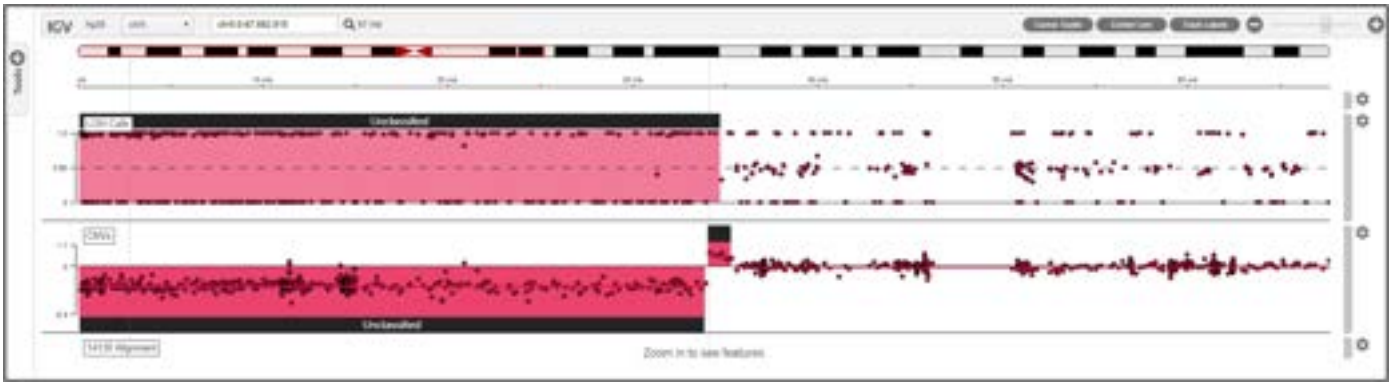


Figure 139: Example of a sample with a CNV call and a LOH call in the same genomic location

CNV and LOH Options

As with the page displaying SNV and Indel calls there are options available for each variant called by the CNV/LOH pipeline,

Right clicking on a variant will provide a menu of the possible options.

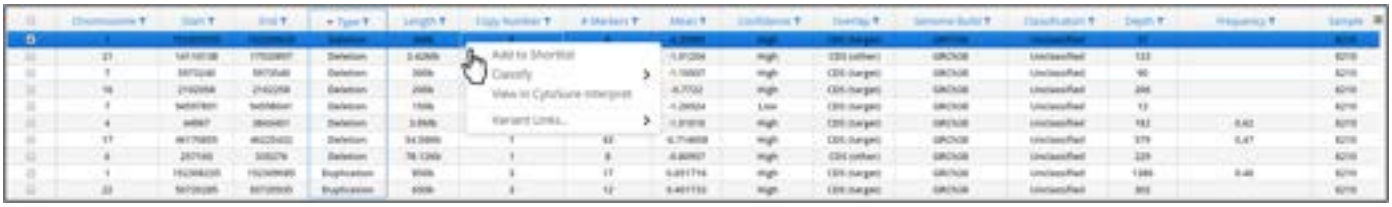


Figure 140: Available options for a selected CNV or LOH call

Adding to a shortlist

Variants added to a shortlist are annotated with a tick

Figure 141: Variants added to the shortlist displayed

Shortlisted variants can be viewed.

Figure 142: Accessing the shortlist of selected variants

The shortlist opens in a separate view. A variant can be removed from the shortlist be clicking on the red bin icon in the shortlist view.

Figure 143: Viewing the shortlist of CNV or LOH variants

Alternatively, a variant can be removed from the shortlist using the CNV options menu.

Figure 144: Deleting a variant from the shortlist

The shortlist will be updated to reflect the removal of a variant.

Figure 145: The shortlist showing that the variant has been removed

Variant Classification

A variant can be classified from the list that is included by default. These are:

- Benign
- Uncertain significance, likely benign
- Uncertain significance
- Uncertain significance, likely pathogenic
- Pathogenic

Additional classifications can be added in the Admin Controls section of the software (Admin Controls > Analysis > Classifications)

Figure 146: Default classifications

Figure 147: Classifying a CNV deletion as pathogenic

Figure 148: Updating of the variant to show the new classification

Figure 149: Removing a variant classification

View Classification History

User can review the classification of a variant by selecting that option in the menu.

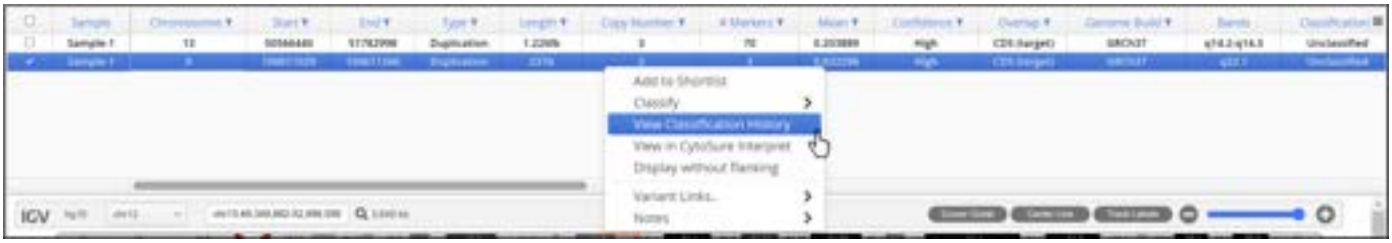


Figure 150: Selecting view classification history option

When chosen a table appears displaying how a variant has been classified, who made the classification and when any changes were made

Classification History

Classification history for seq[GRCh37] Xq22.1(100611029_100611266)x3

Classification	User	Date
Unclassified	admin	Oct 21, 2021 4:13:44 PM
Pathogenic	admin	Oct 21, 2021 3:46:36 PM
Unclassified	admin	Oct 15, 2021 9:18:38 AM
Pathogenic	admin	Sep 21, 2021 2:27:30 PM

Figure 151: An example of a variant's classification history

View in CytoSure Interpret

CytoSure Interpret is OGT's class-leading microarray software analysis platform. For existing microarray customers, CNV and LOH events can be loaded into CytoSure Interpret.

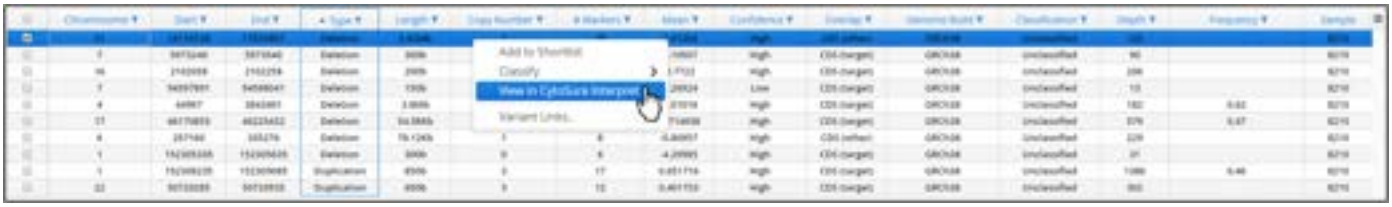


Figure 152: Selecting to view a CNV deletion in CytoSure Interpret microarray software

It is necessary to have CytoSure Interpret open prior to selecting this option.

If it is not yet running Interpret will issue a prompt to the user.

Figure 153: Prompt from Interpret if trying to load data in CytoSure Interpret when it is not running

Variant Links

The software allows users to link out to external sources of documentation. Currently included are:

- EnsEMBL

Additional resources can be added in the Admin Controls (Admin Controls > Analysis > Manage Links).

Figure 154: Accessing variant links

Figure 155: Accessing EnsEMBL as an external source for data annotation

Adding Notes to CNVs

Users can add notes to CNVs

Figure 156: Selecting the option to add notes to a CNV

When chosen a text editor is displayed as well any pre-existing notes. At the top is an option to choose a file and to then upload it. Below is the text box where details can be entered.

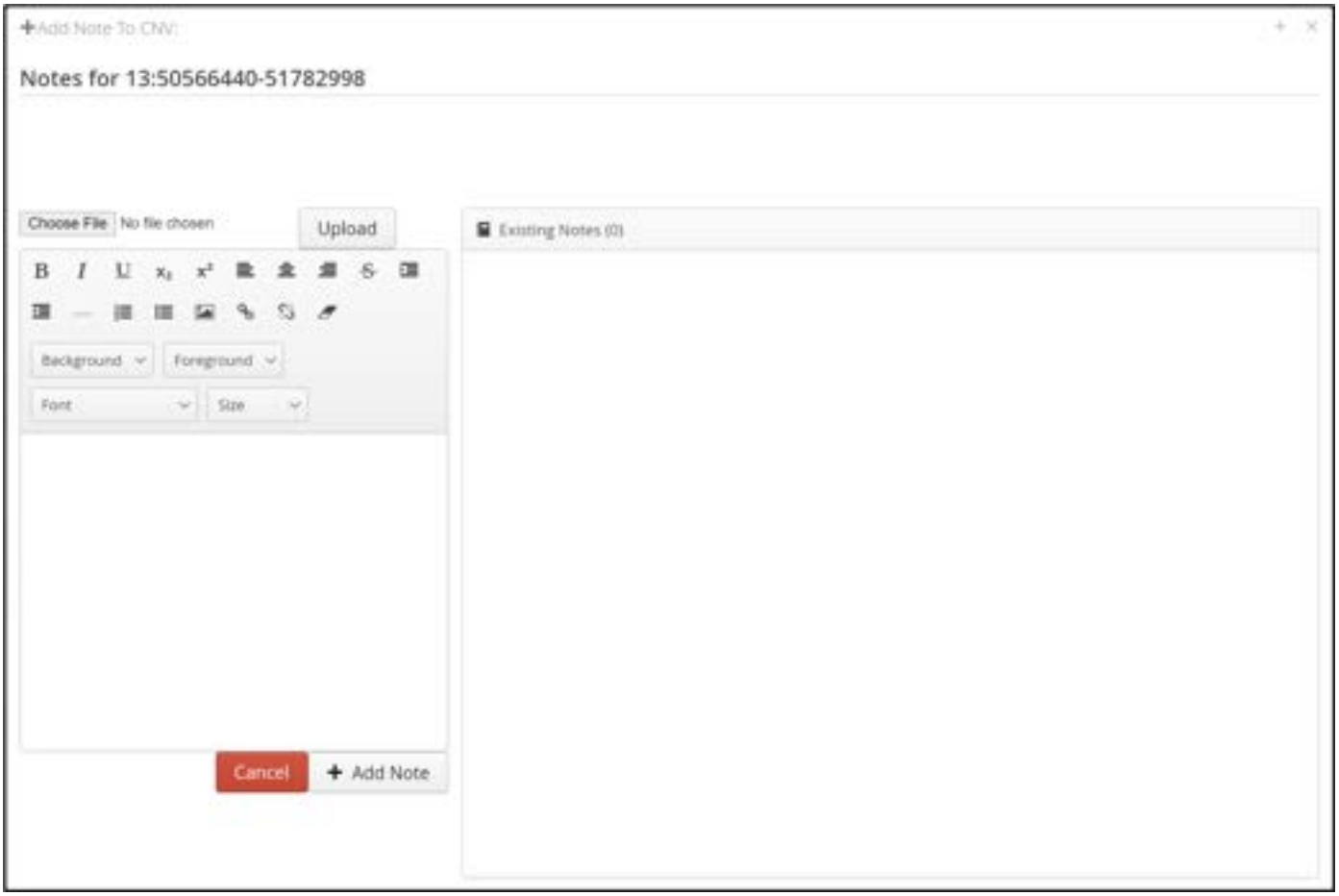


Figure 157: A blank template for creating a note

In the example below a file has been uploaded and text entered.

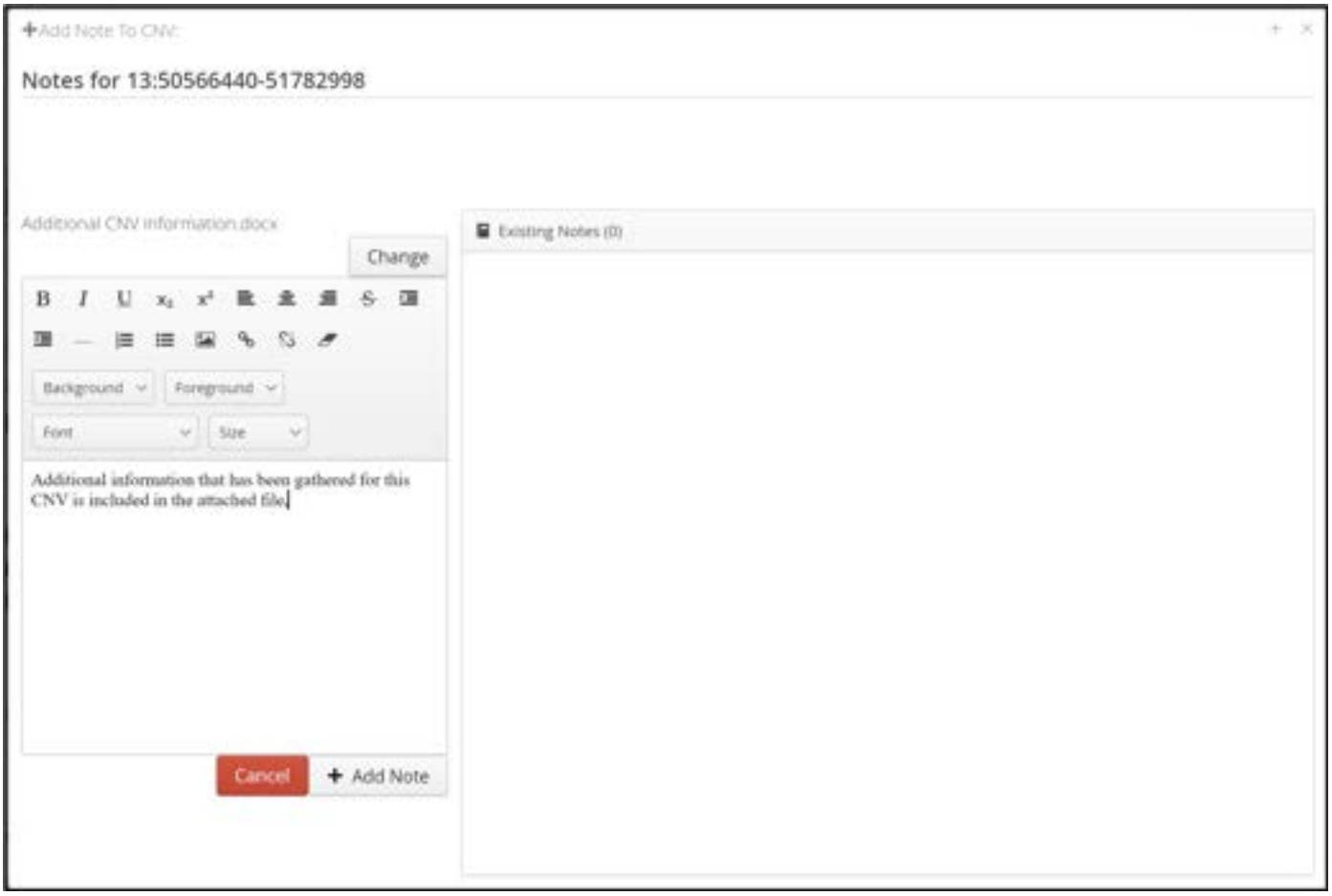


Figure 158: Addition of text and a file to a note

Selecting + Add Note completes creation of the note and it is added to the existing notes section. Any file that has been uploaded with the note is shown and can be downloaded if required.

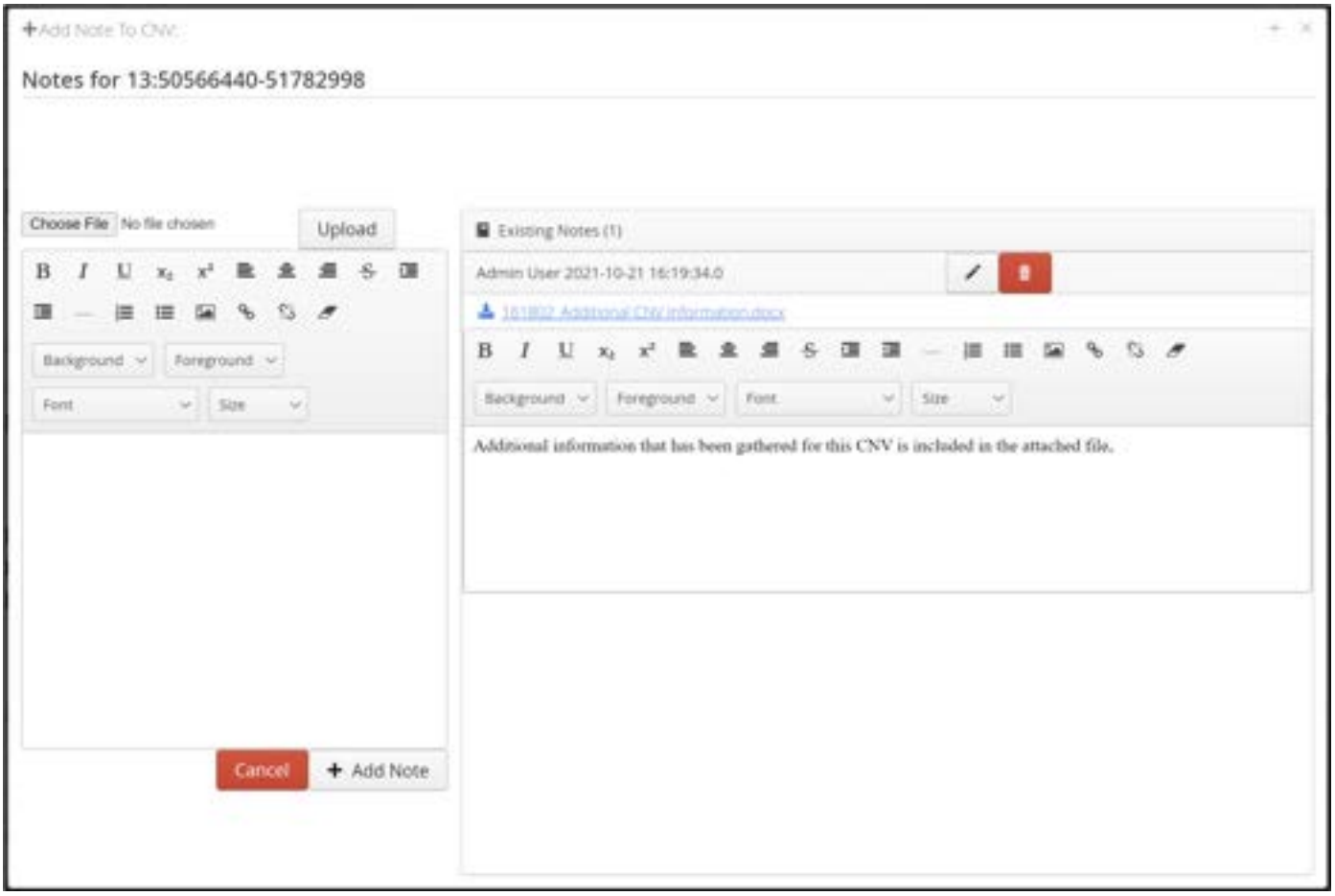


Figure 159: A new note is shown

Now when the user accesses the menu for the variant there is an additional option providing the display of any notes.

Figure 160: Existing notes are available to view

If selected, the note(s) are shown in a separate box.

Figure 161: Viewing an existing note

Additional notes can be added as shown below.

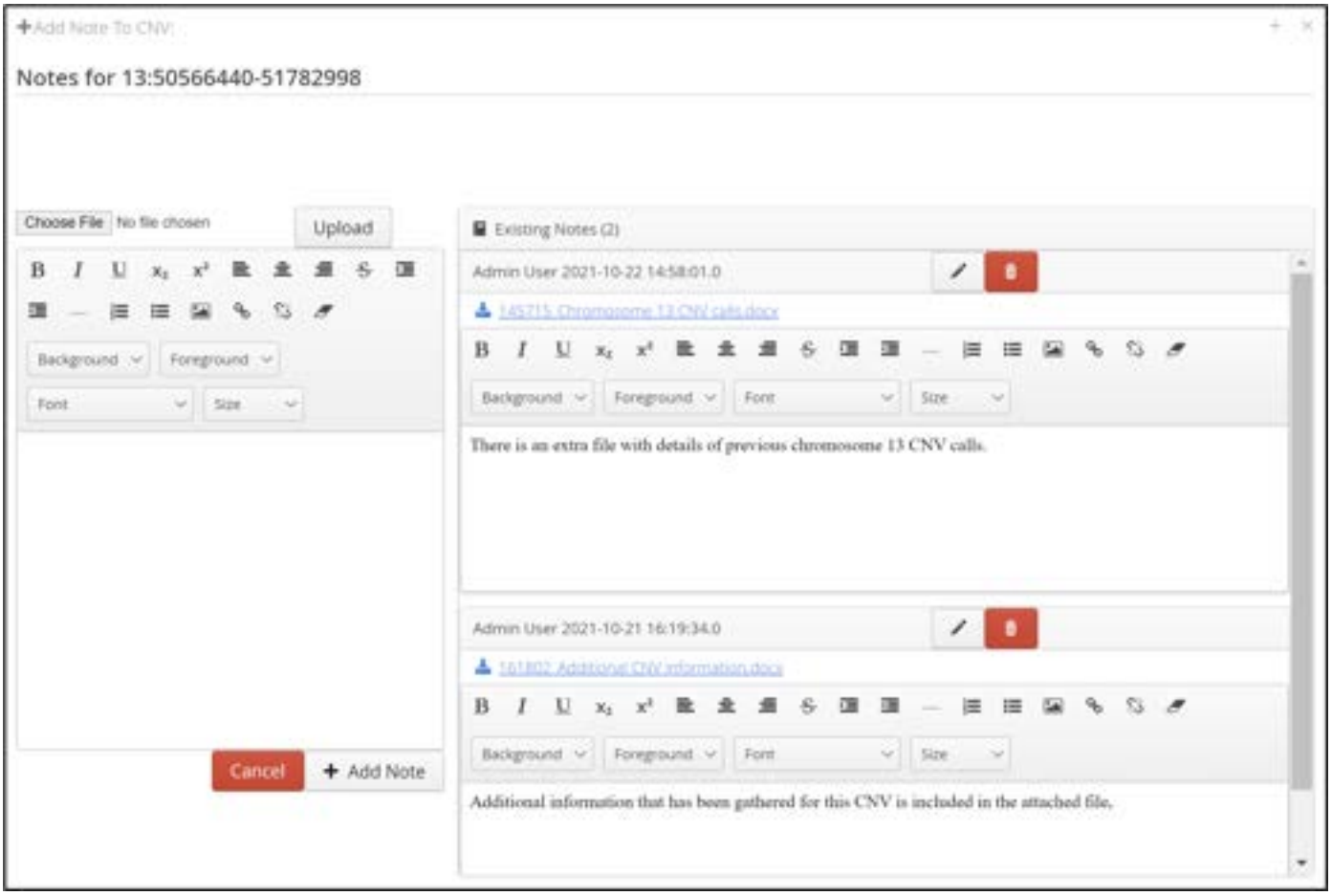


Figure 162: A blank note template with two existing notes

Manual Creation of CNVs

It may be that the user believes, based on the visual representation of the CNV data, that the software has missed a CNV call and would like to manually generate it. For example, a user may believe that the region highlighted in the screenshot below represents a CNV, but it has not been automatically detected by the software.

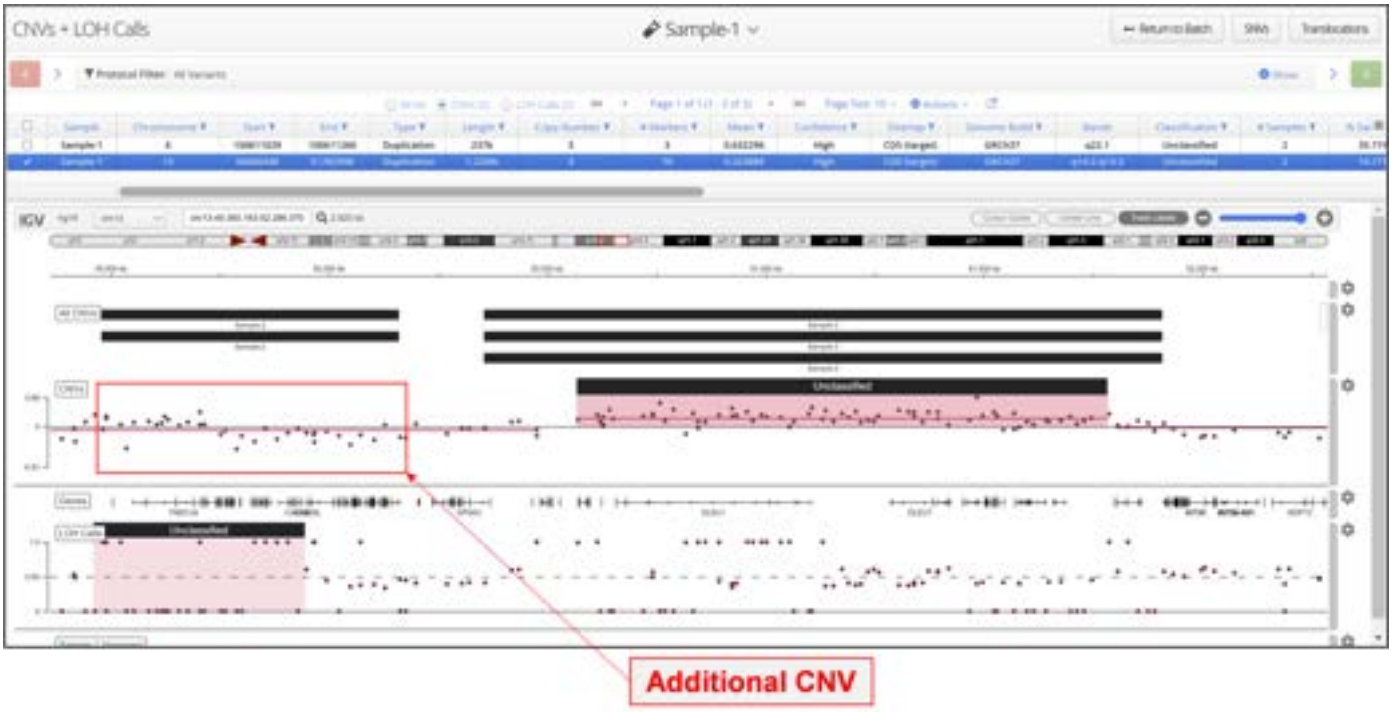


Figure 163: A region, not called by the software as a CNV, that the user wants to manually define as a CNV

In order to manually create the CNV call, the user defines the CNV region by using the mouse to select the region in the chromosome ruler track. Alternatively, the coordinates can be provided in the text box in the menu bar of IGV.

Figure 164: Using the ruler region in IGV to select the region to display

The IGV window resets to the size of the required region

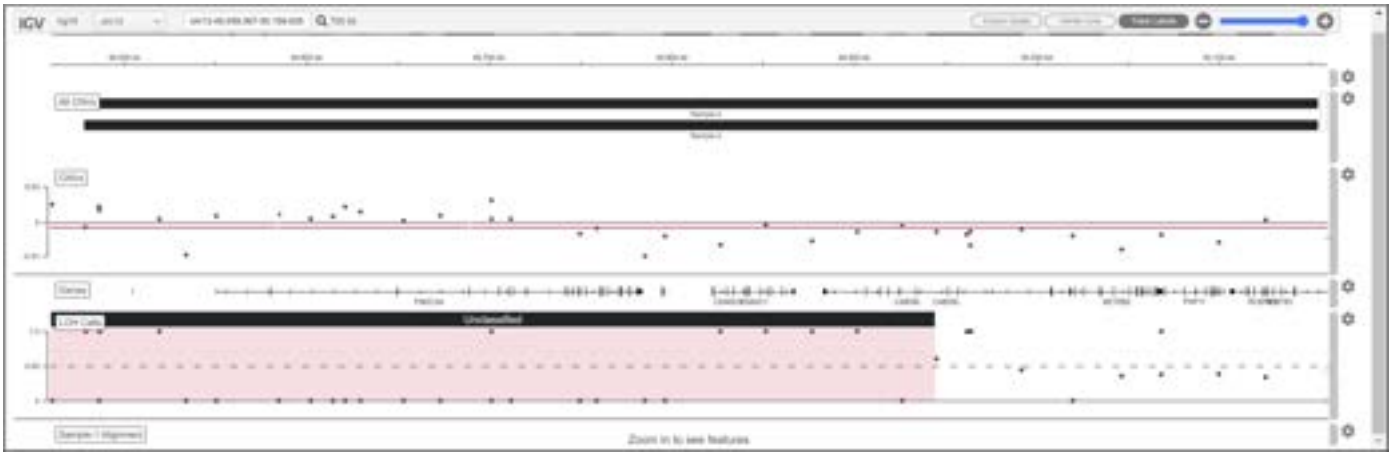


Figure 165: IGV set to the boundaries of the region to be defined as CNV

Select the Add CNV option in the Actions menu.

Figure 166: Selection of the Add CNV option from the Actions menu in the variant table header

Users have the option to define the entire region in IGV as the CNV or the software will snap to the nearest probe at each end.

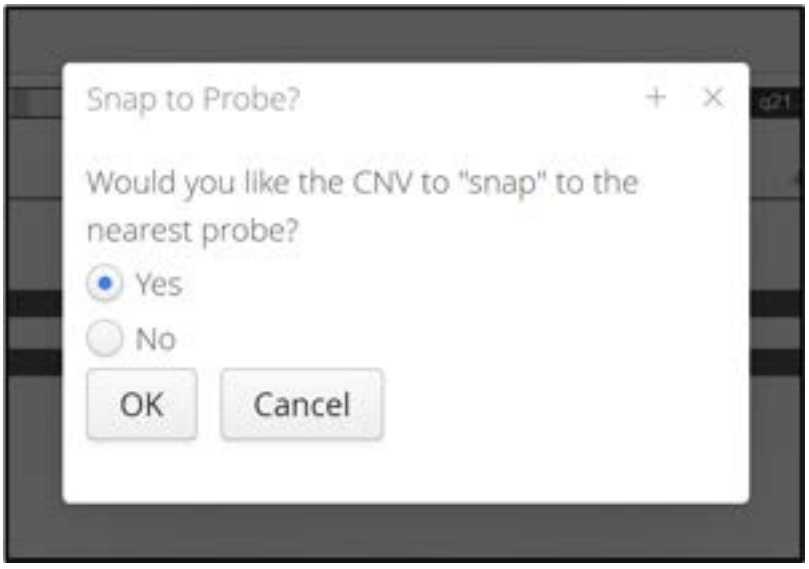


Figure 167: The option to snap to the nearest probe or to keep the region as that displayed in IGV

Once a CNV has been created manually it is **NOT** automatically displayed in the software. The creation of the CNV can be confirmed by the number of CNVs detected that feed into the protocol filter; in the figure below the number has incremented by one to five.

Figure 168: The number of CNVs has been incremented by one

However, in order to display it in the variant table additional steps need to be taken. In the original analysis the protocol and filters did not select the manually defined region as being a CNV and so in order to include it the default protocol CNV filter need to be updated. This can be done in the Admin Controls > Analysis > Protocols section.

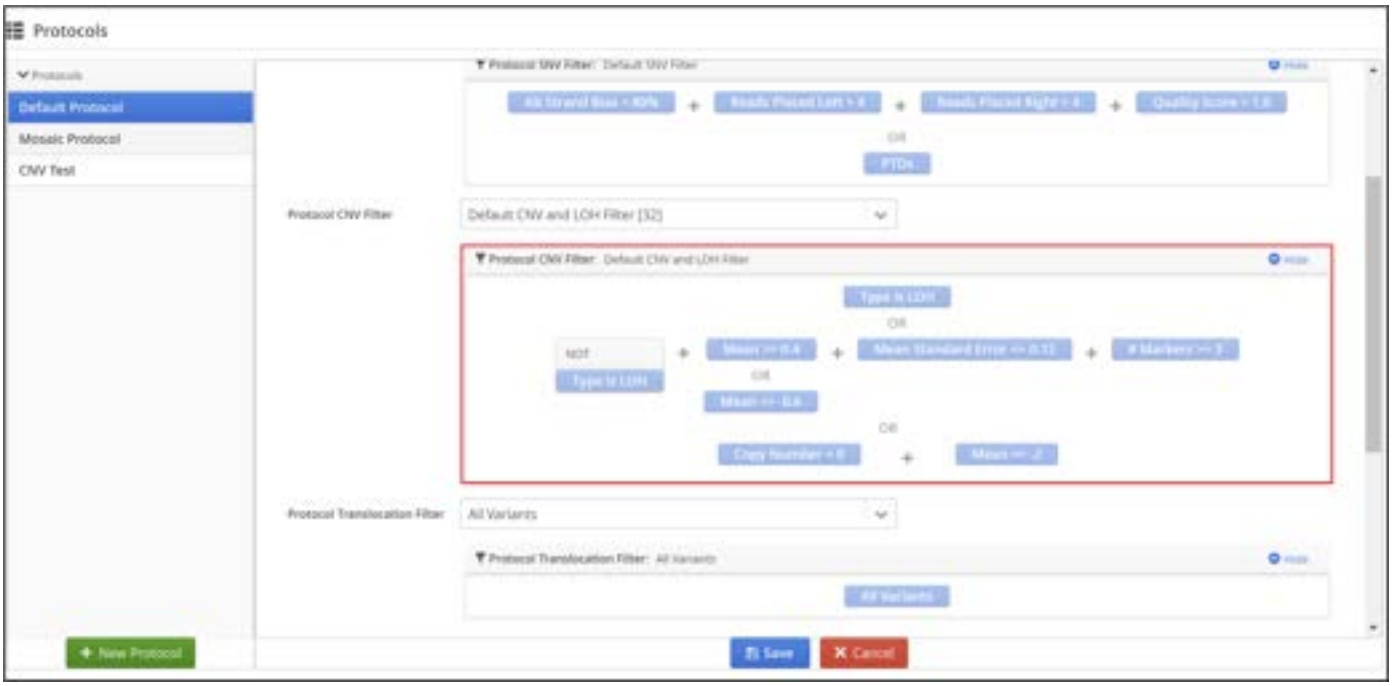


Figure 169: The default CNV filter in the default analysis protocol

Users need to create a filter that allows manually created CNVs to be included and this can be added to the default CNV filter. This is shown in the figure below.

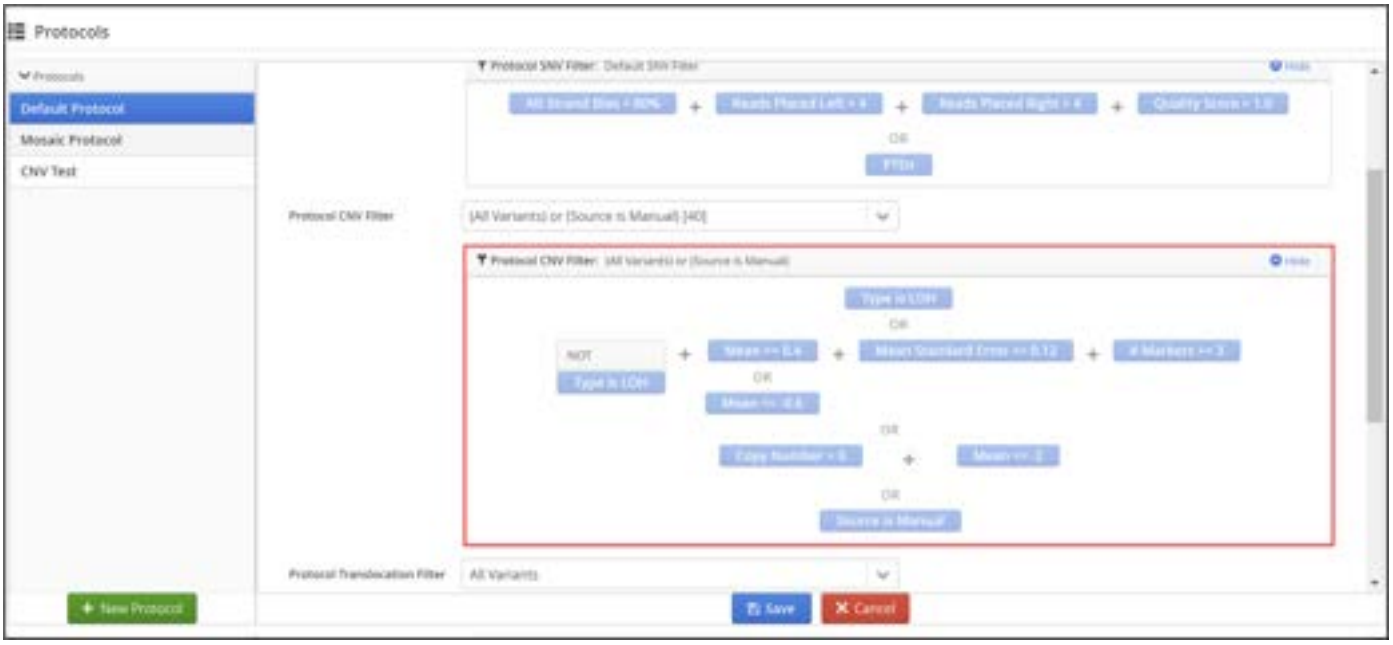


Figure 170: The CNV filter in the Default Protocol has been edited to include OR Source is Manual

Repeating the analysis with the updated filter will result in any manual CNVs being added to the sample variant list.

Merging CNV calls

There are occasions when CNVs are called with small regions in between that the user would like to combine into a single larger CNV.

In order to do this, adjust the scaling in the IGV window such that both CNVs are visible, then right clicking between them in the track will generate a popup menu with the option to Merge Displayed provided.

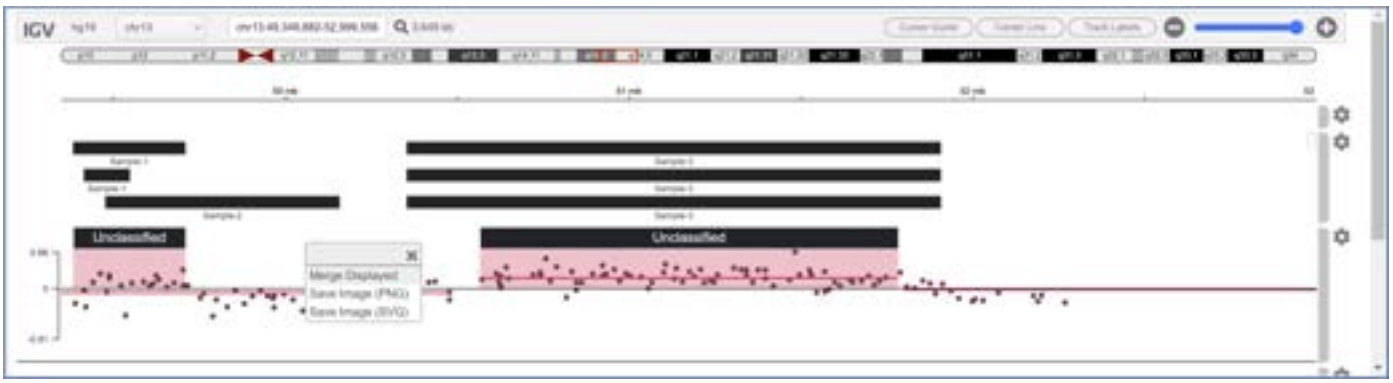


Figure 171: Selecting the option to merge displayed CNVs

If selected the software will request confirmation of the merge option.

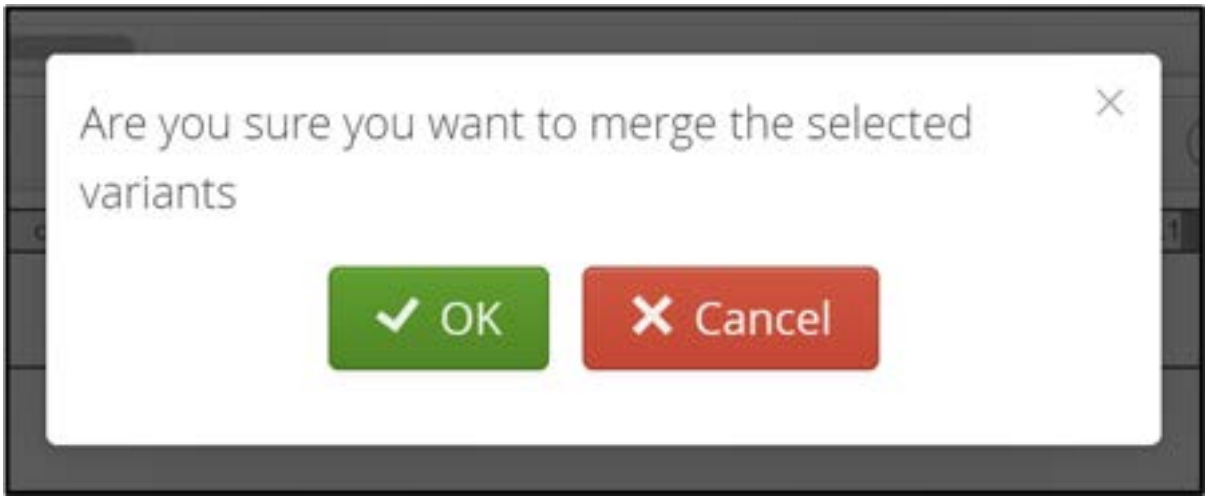


Figure 172: Confirmation of merge option

Following confirmation of the merge option the variant table will be updated. There will be a single row containing the new merged CNV that spans the two previously separate calls. Additionally, the variant counts above the table will be updated.

Figure 173: An updated variant table showing the merging of 2 CNVs to a single row in the table as well as the decrease in the number of All variants and CNV variants

Likewise, in the IGV window the two calls are now combined.

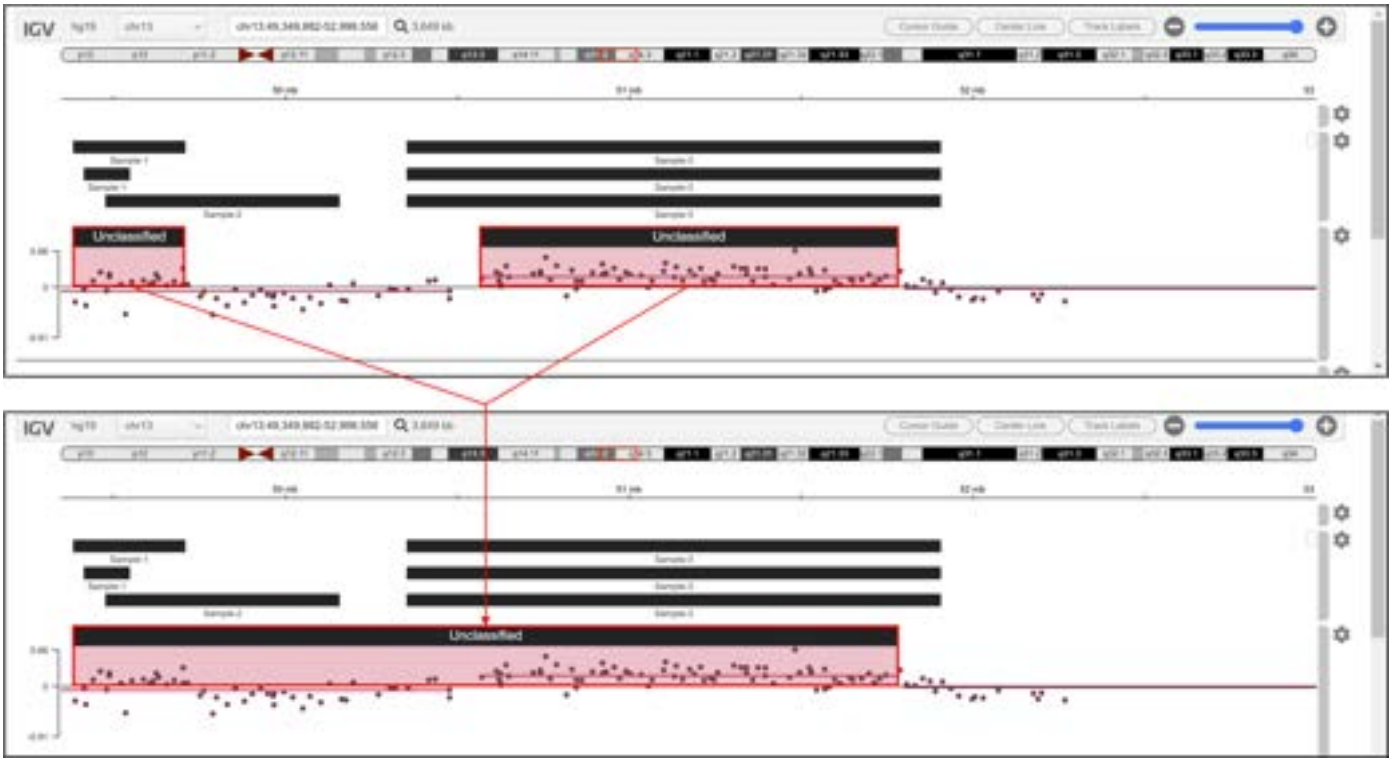


Figure 174: Following the merger of 2 CNVs a single CNV is now displayed

Separating Merged CNV calls

Having been created, users are able to dissolve a merged CNV. Right clicking on the CNV row in the variant table will display the standard popup menu but now with an additional option of Dissolve which will split the merged CNV back into the original separate calls.

Figure 175: Selecting the dissolve option in CNV variant table

Aneuploidy Plots

Interpret is able to provide aneuploidy plots in order for the user to assess whether there is a difference in chromosome number in a set of patients.

This functionality is accessed through the Tools sub-menu in the software dashboard as shown below.

Figure 176: The create aneuploidy plot option

The aneuploidy plot option can only be used when the user is viewing CNV data. If this is not the case then the following error message will be displayed. As with all error messages in the software it can be removed by clicking on it.

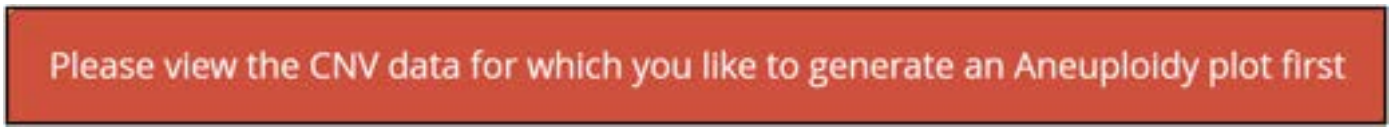


Figure 177: Error message from aneuploidy plot option

The figure below illustrates the correct view from which to launch an aneuploidy plot.

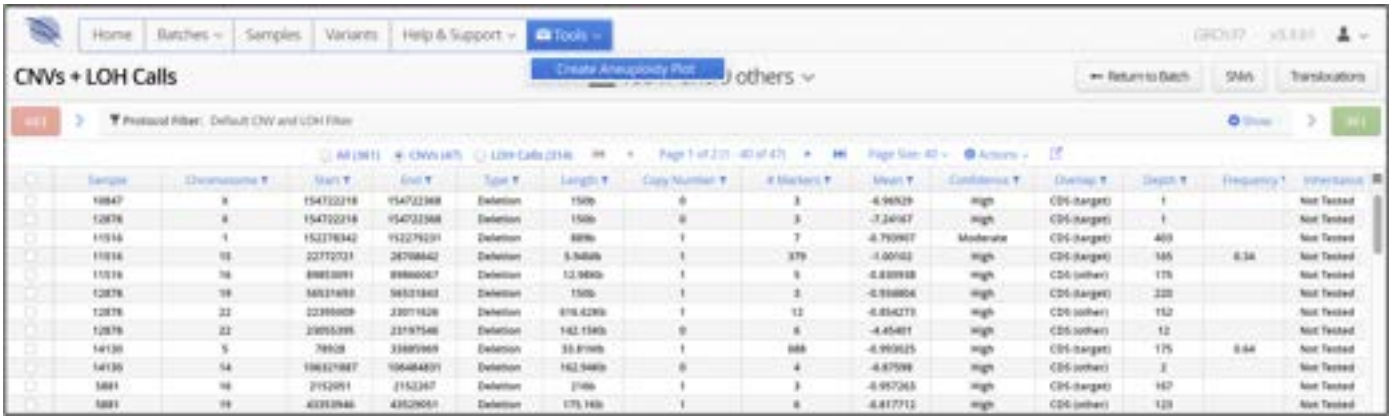


Figure 178: Selection of aneuploidy plot from menu bar Tools

The user will then be asked to provide the region list that needs to evaluated for plotting. Information on creating a region list is documented in this manual in the Administration Controls > Analysis > Region lists.

Figure 179: Select a region list pop-up

The available regions lists will be displayed in a drop-down menu; in this example there is a single region list created in the software.

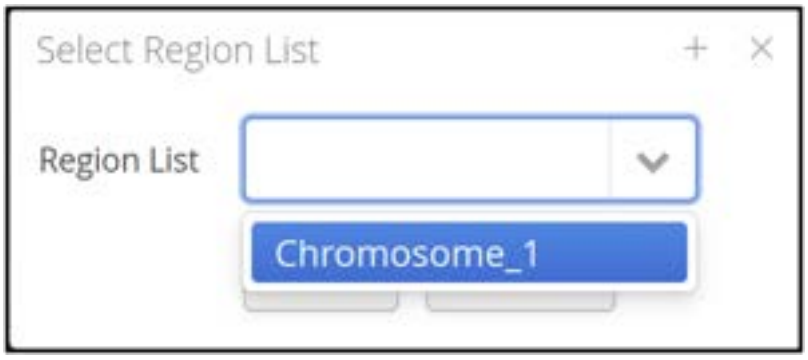


Figure 180: Selection of a region from the drop-down list

Following selection of the required region list the user clicks on the View button.

Figure 181: Selection of the region Chromosome_1

An example aneuploidy plot is displayed below.

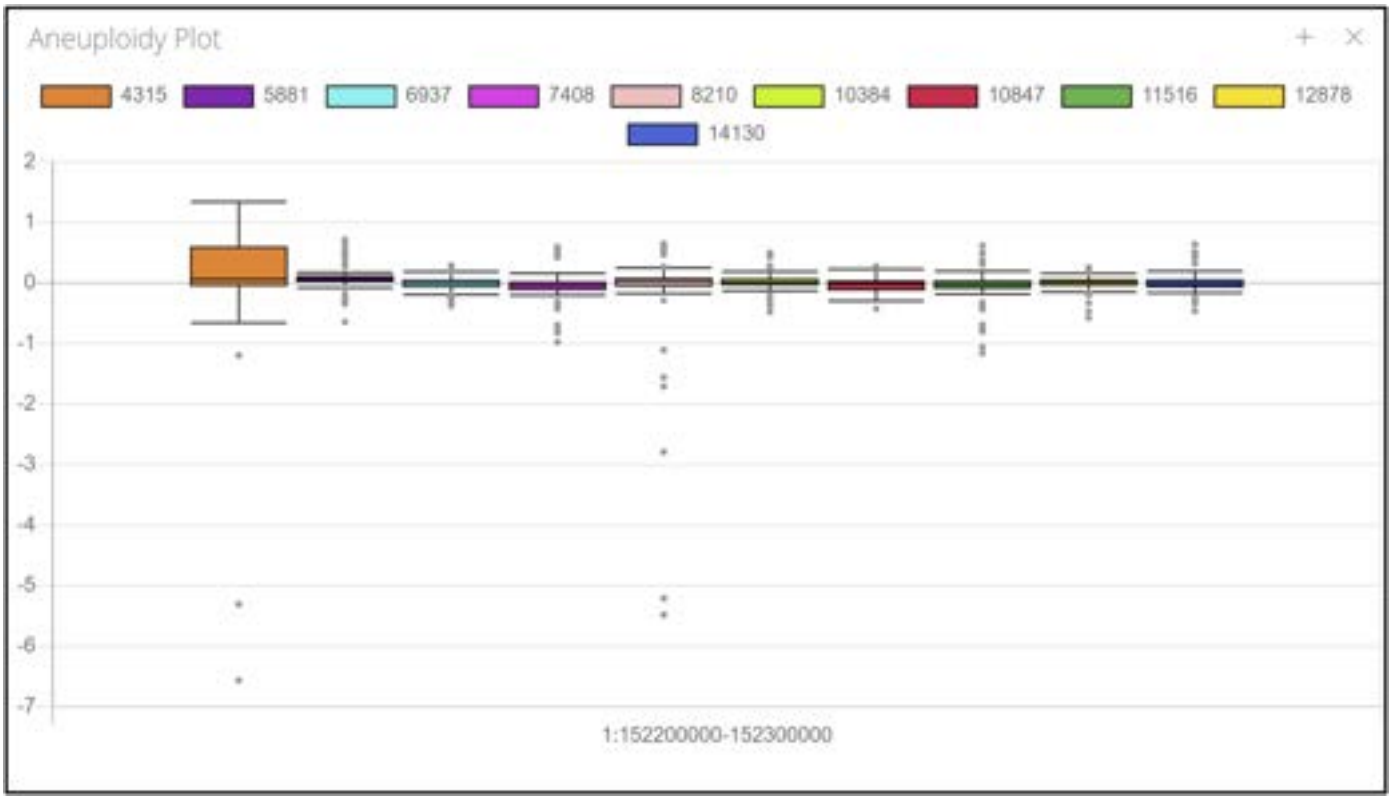


Figure 182: An example of an aneuploidy plot for the chosen region across the samples in the batch

Viewing Translocation Events

The variant table has a column selector icon allowing user to configure which columns are displayed. The figure below shows the columns available for display.

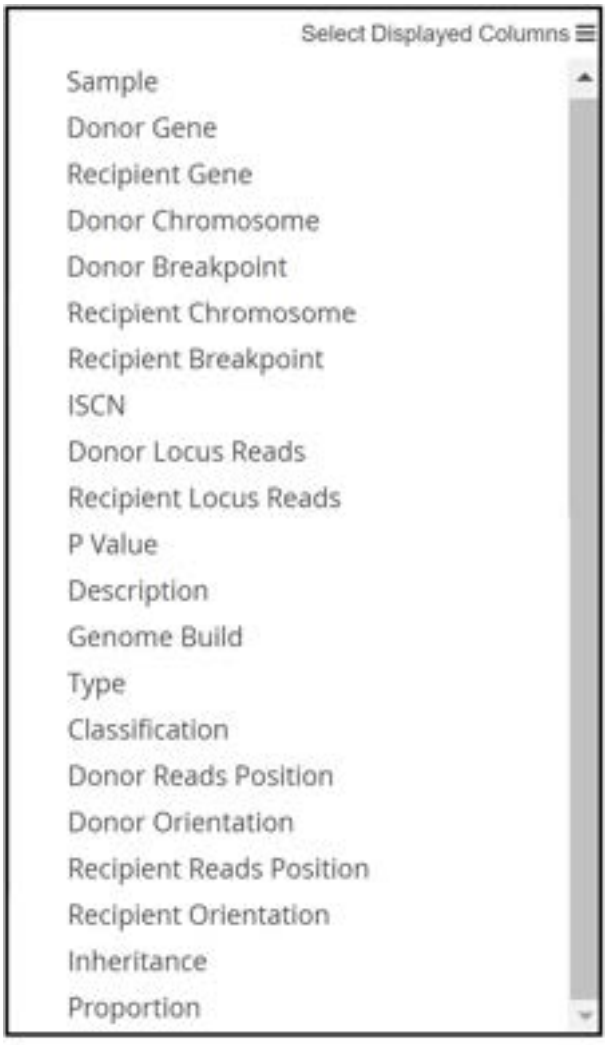


Figure 183: Columns available to select for display in the translocations variant page

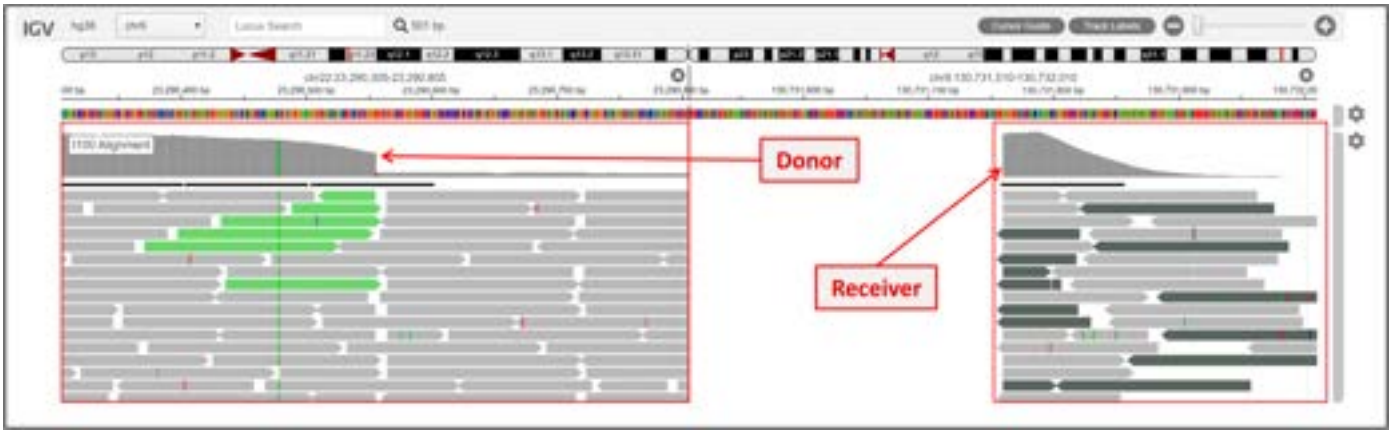


Figure 184: Example of a translocation

Translocation Options

As with the page displaying SNV and Indel calls there are options available for each translocation variant called by the software,

Right clicking on a variant will provide a menu of the possible options.

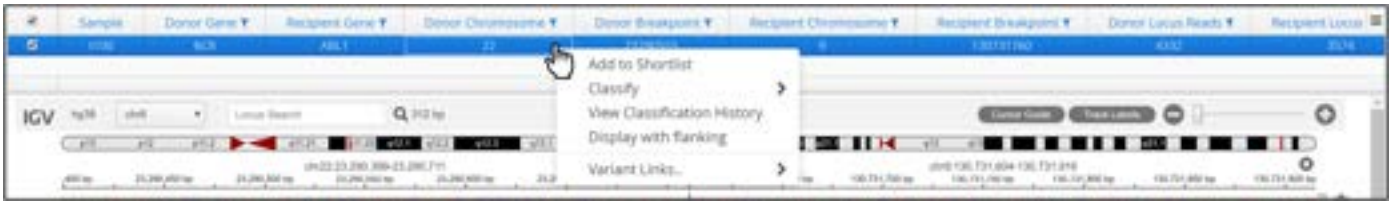


Figure 185: Translocation options

Adding to Shortlist

Figure 186: Adding a translocation to the shortlist

Once a variant has been added to a shortlist the available option is updated to now allow that variant to be deleted from the shortlist.

Figure 187: Selecting to delete a variant from the shortlist

Variant Classification

A variant can be classified from the list that is included by default. These are:

- Benign
- Uncertain significance, likely benign
- Uncertain significance
- Uncertain significance, likely pathogenic
- Pathogenic

Additional classifications can be added in the Admin Controls section of the software (Admin Controls > Analysis > Classifications)

Figure 188: Classify the translocation

A variant classification may change over time and it is possible to track the changes and view the classification history.

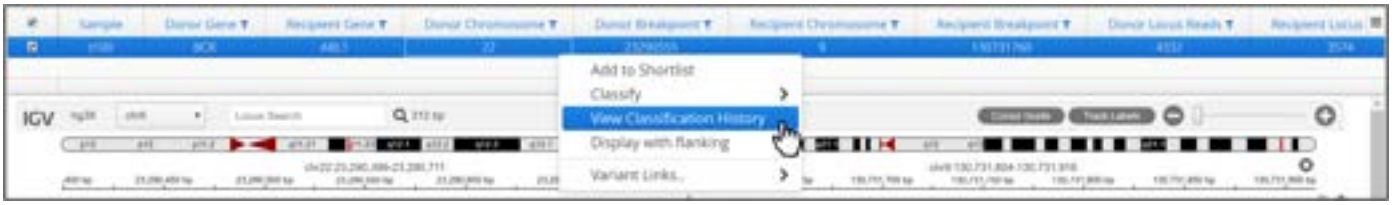


Figure 189: Viewing a variant's classification history

Initially, the classification will be blank.

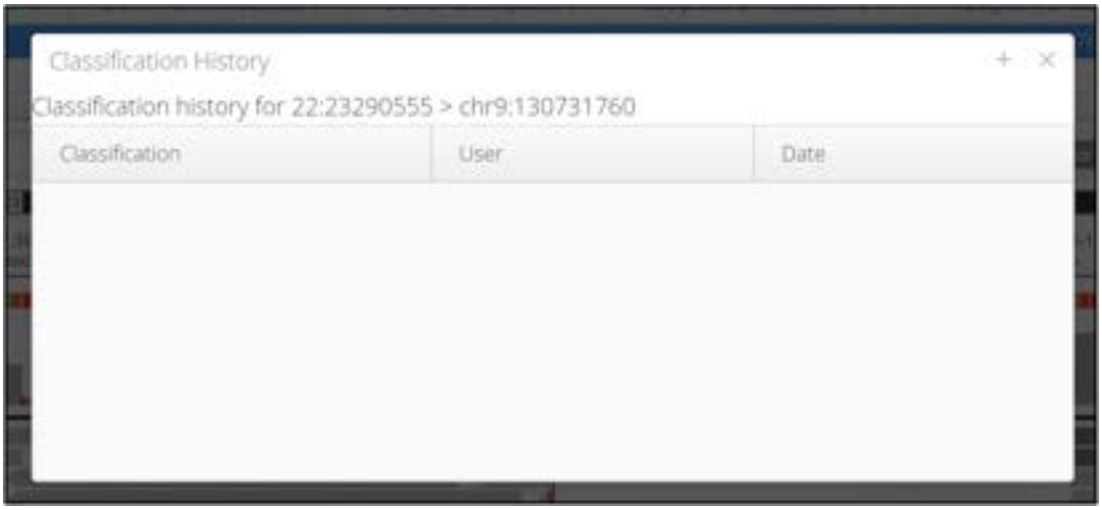


Figure 190: A variant with no classification history

When a classification is made the history table will show the classification type, who made it and when it was made.

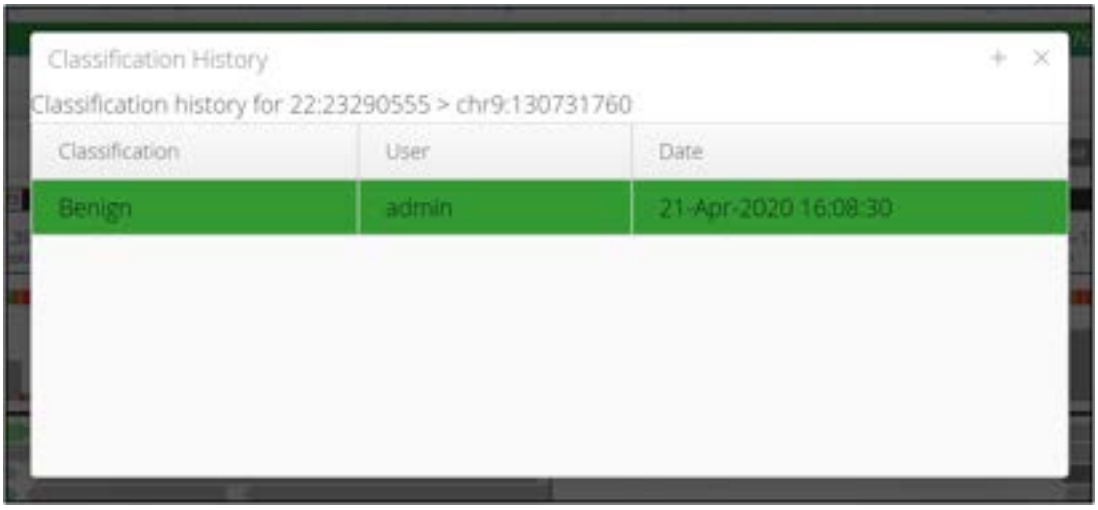


Figure 191: Example of a benign classification

Any updates to the classification will be recorded with previous designations retained.

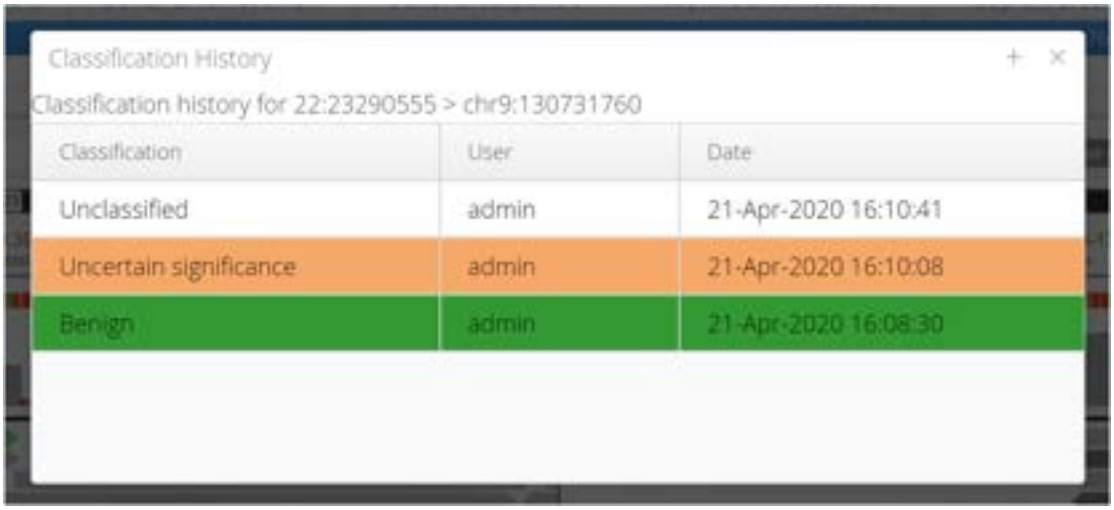


Figure 192: Example of a tracking a translocation classification change

Display with Flanking

Users can select to view translocations with flanking sequence

Figure 193: Selecting to show a translocation with flanking sequence

Variant Links

Links to external data sources are available; these are managed in Admin Controls > Analysis > Manage Links

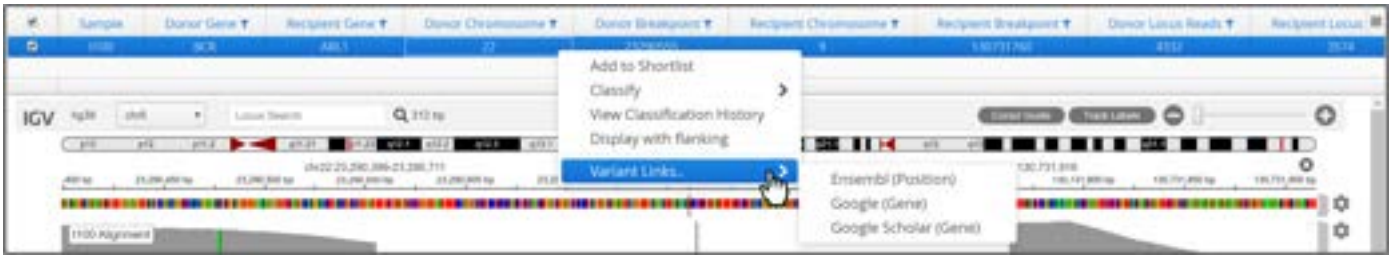


Figure 194: Linking out to external data sources

Variant Table Options

Column Sorting

Rows in the variant table can be sorted using the column header. In the example below the results have been sorted by decreasing and increasing allele frequency.

Currently, data can only be sorted by one column.

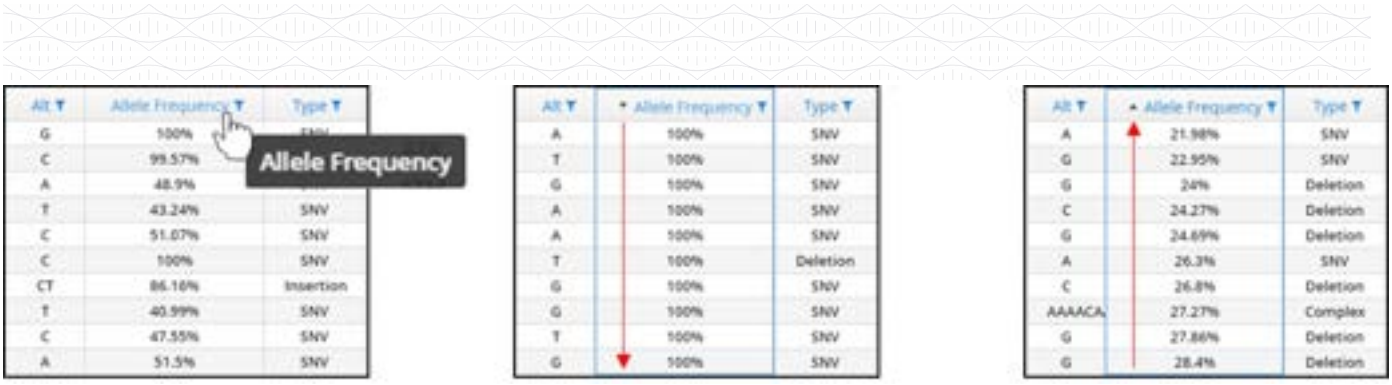


Figure 195: Sorting by Allele Frequency

Dynamic Filtering

As shown previously the variants page displays the Protocol Filter, the number of variants detected by the pipeline and presented to the filter is depicted in a red box and the number remaining in a green box.

In the image below you can see that there are 2946 variants (in the red box) detected by the pipeline that are to be filtered based on the settings in the protocol. Subsequently there are 2754 remaining (as shown in the green box).

Figure 196: The filter used by the protocol in the analysis of the sample displayed in the Variants page

However, the user is able to implement additional filtering dynamically. Any column header with the funnel icon can be used as a filter. For example, a user may want to filter on Gene Symbol

Figure 197: Selection of the funnel icon for the Gene Symbol column

In this case they want to see only variants found when the total depth is greater than 200.

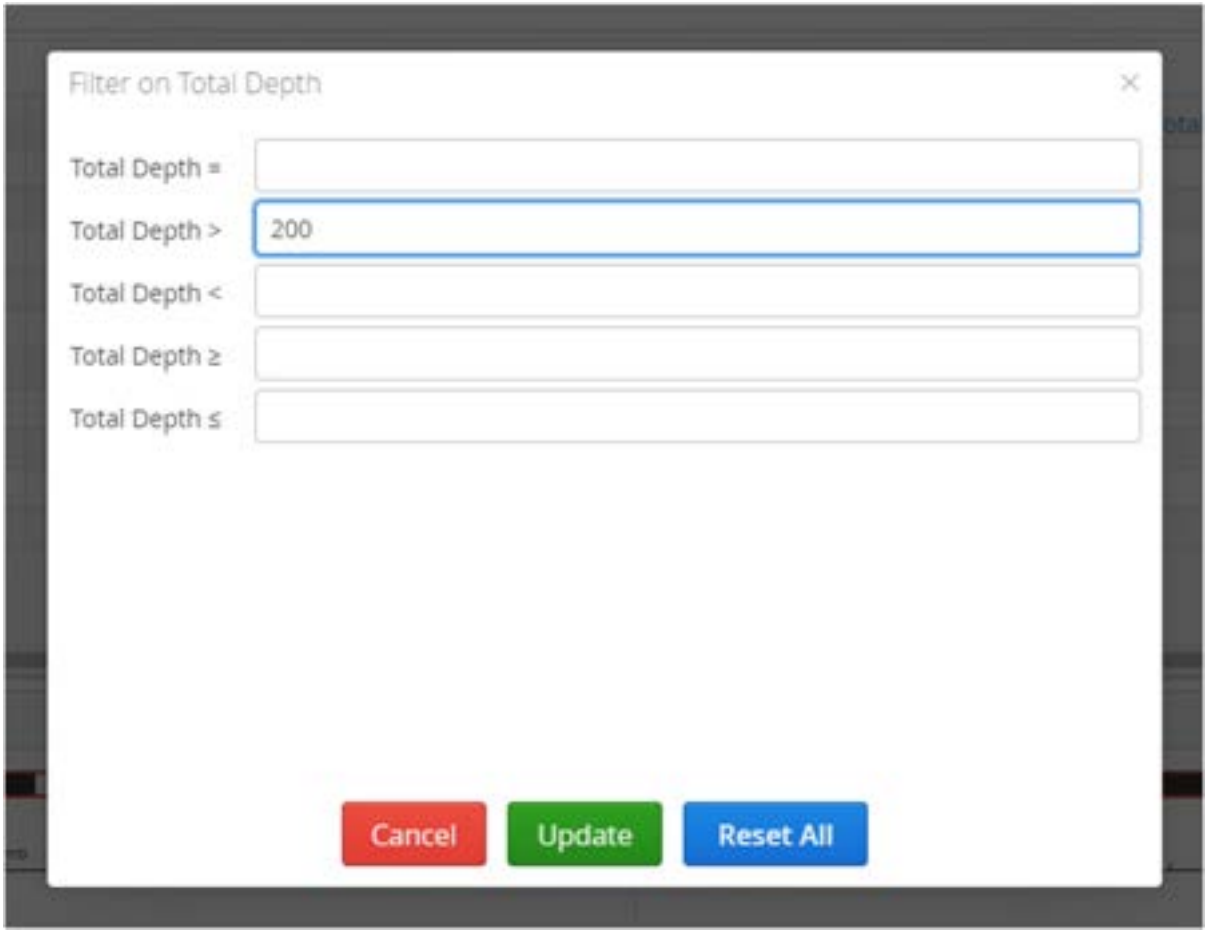


Figure 198: Dynamic filtering of the variants using the Total Depth column

After updating the Variants view now shows a Dynamic Filter window and within it is the "Total Depth > 200" filter.

From the 2754 variants generated by the protocol using the default filter, it can be seen that a further 265 have been removed filtered with 2489 remaining.

Figure 199: Example of Variants filtered by the protocol filter and a dynamic filter

Dynamic filters can be chained together so additional filters can be added for instance an Allele Frequency greater than 80%

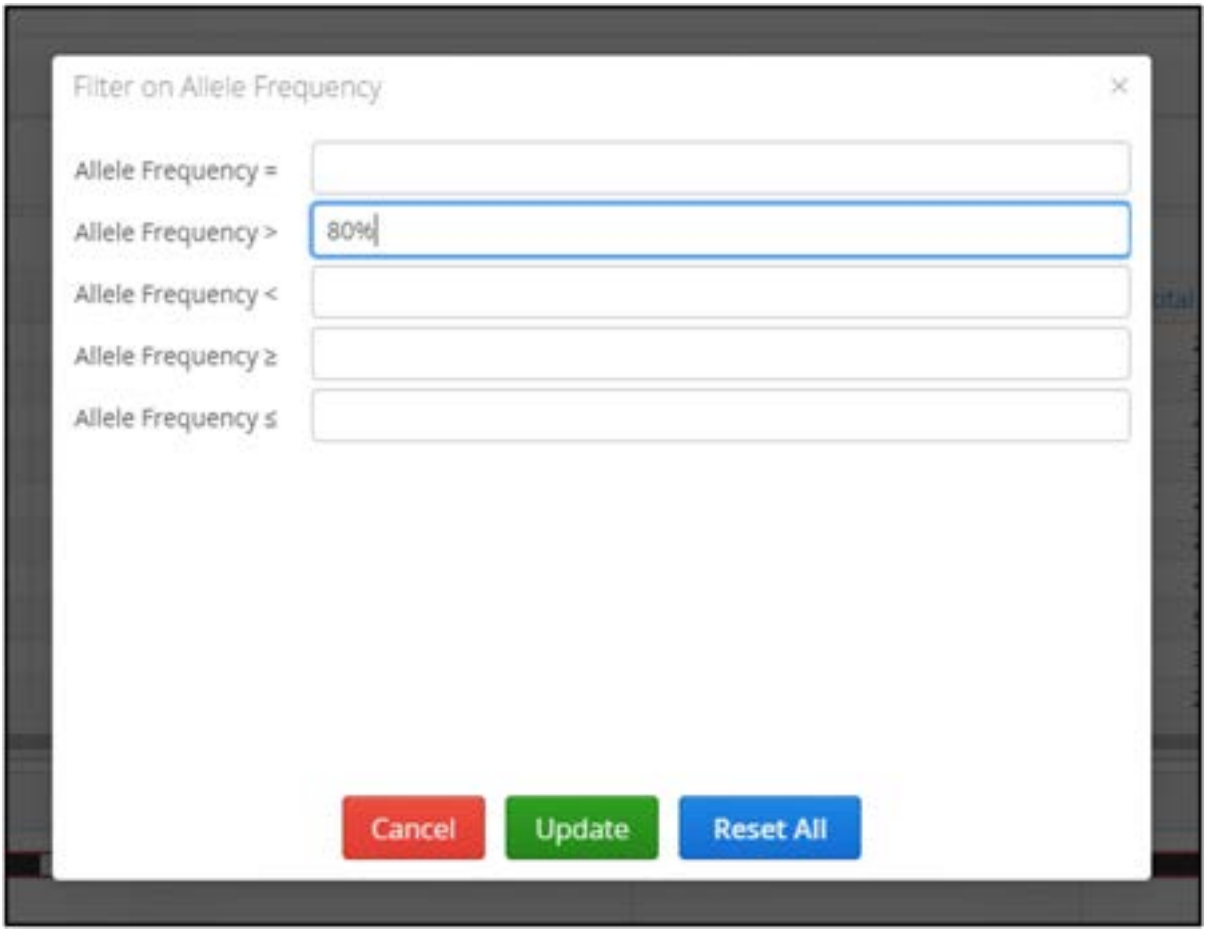
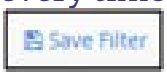


Figure 200: Selection of another dynamic filter to start creating combinations

Now the Dynamic Filter shows "Total Depth > 200 and Allele Frequency > 0.8" and there are now 1457 variants remaining from the input of 2754.

Figure 201: Variants being filtered by a compound dynamic filter

There is no requirement for the user to have to repeat the setting of dynamic filters every time they use the software, there is the option to name the filter and pressing 

to retain for re-use.

Alternatively, all dynamic filters can be removed from the display by selecting to clear the filter 

Viewing a Sample in IGV

Selection of a variant in the Variants Table causes it to be displayed in the embedded IGV.

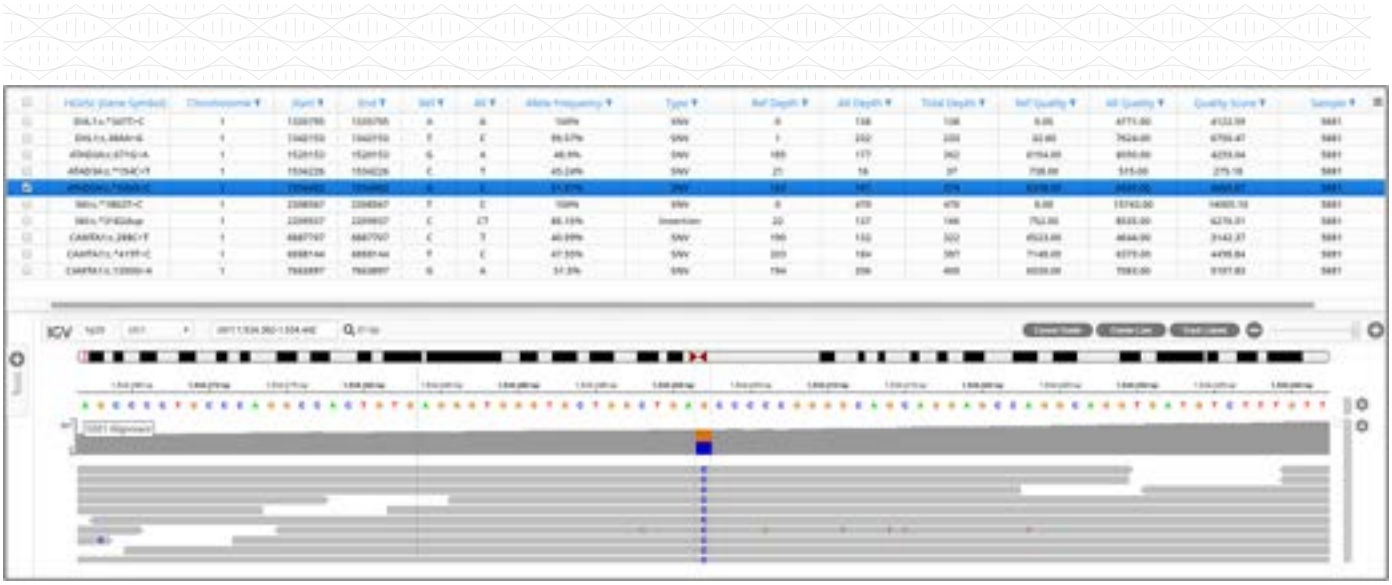


Figure 202: A variant selected and the aligned displayed in IGV

Within the IGV window there are several options for modifying the data being displayed.

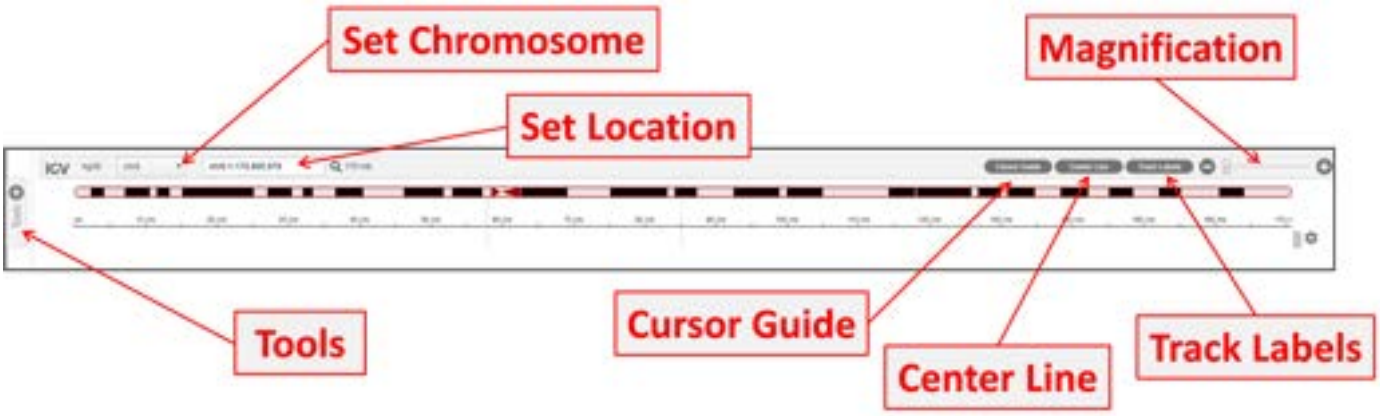


Figure 203: Display options for IGV

By default, the sequence viewer is centered upon the selected variant but users can drag the display upstream and downstream of the variant position. Also, it possible to zoom in and out via the magnification slider at the top of the window.

Additionally, the tracks displayed can also be modified via the setting options available on right hand side of the viewer  .

For example:

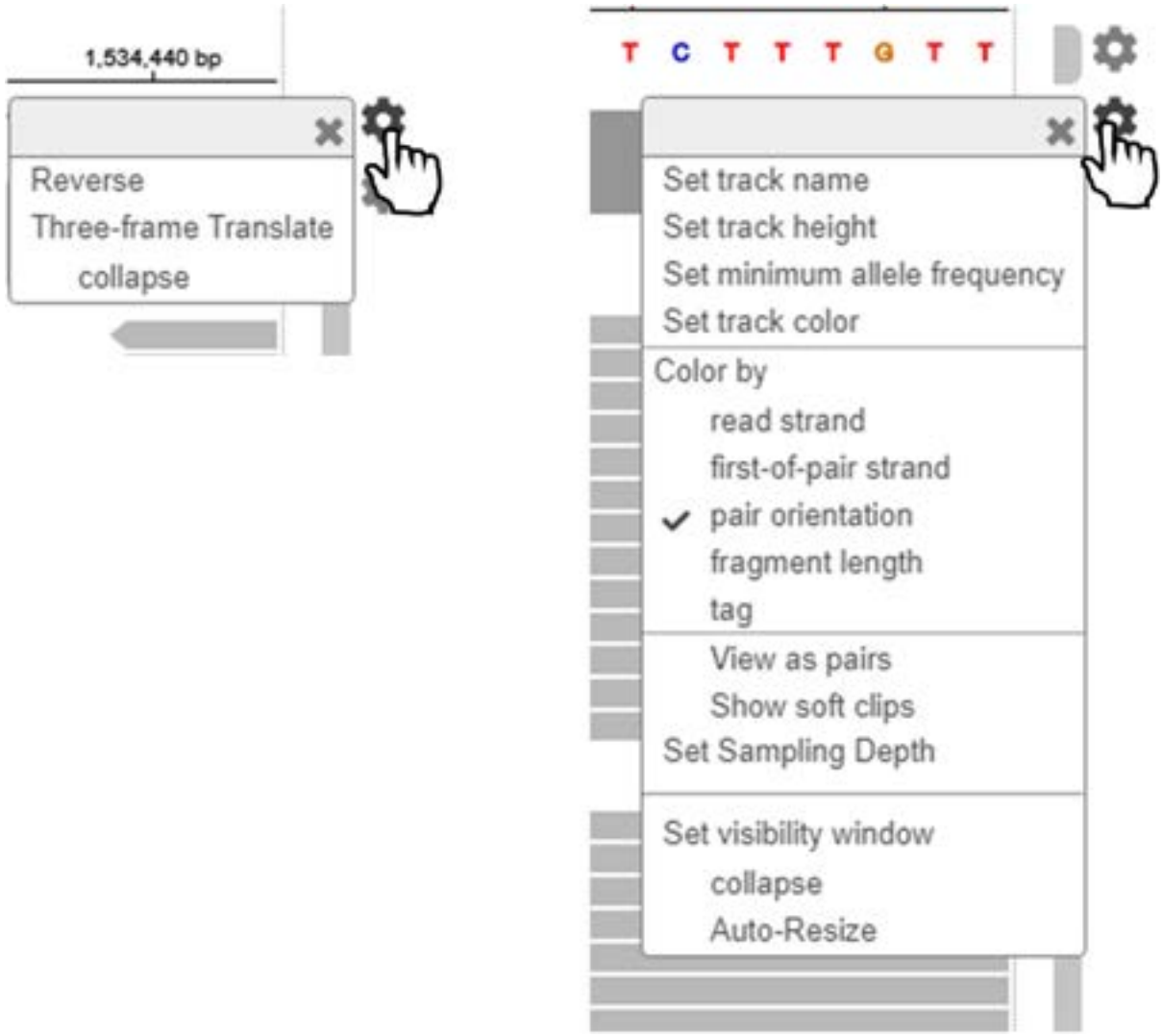


Figure 204: Display options for the integrated IGV browser, firstly using a left click on the mouse and secondly using a right click

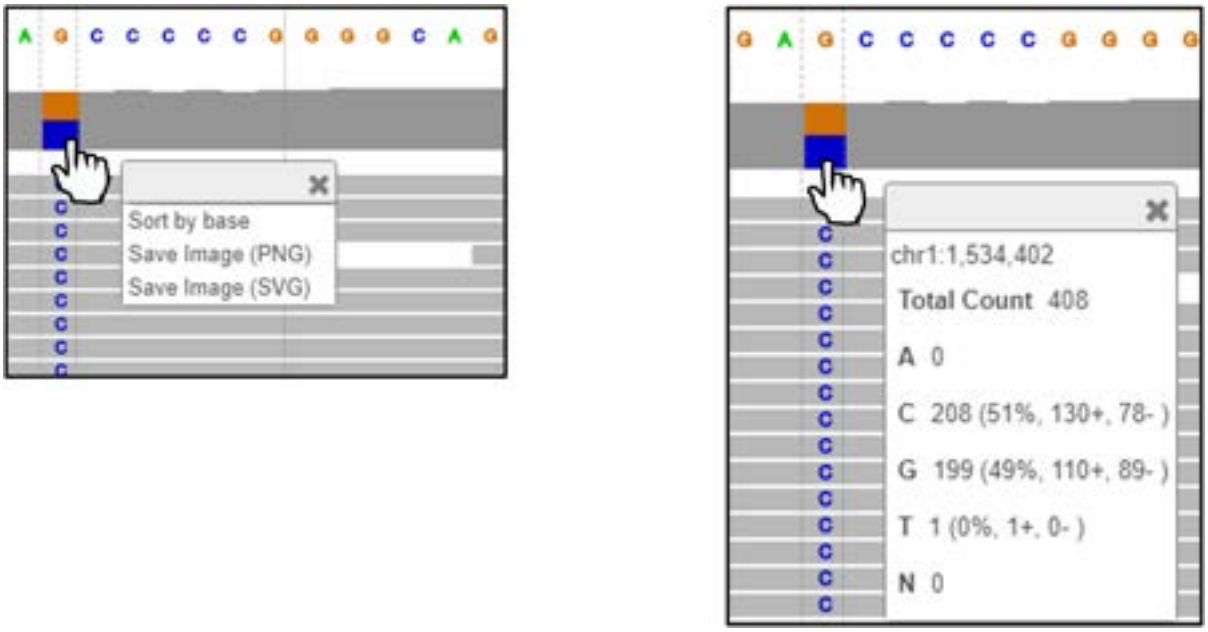


Figure 205: Display options available for sample reads, firstly with a left click and secondly with a right click

Using Tracks

Users can add or remove data tracks to the IGV view. This can be from publicly available sources or from proprietary internal or subscription-based sources.

Tracks can be added in the Software section of the Admin Controls (Admin Controls > Software > Annotation) and documentation of how to do this is in this section of the user guide.

To use this functionality, users need to access the Tools tab of IGV

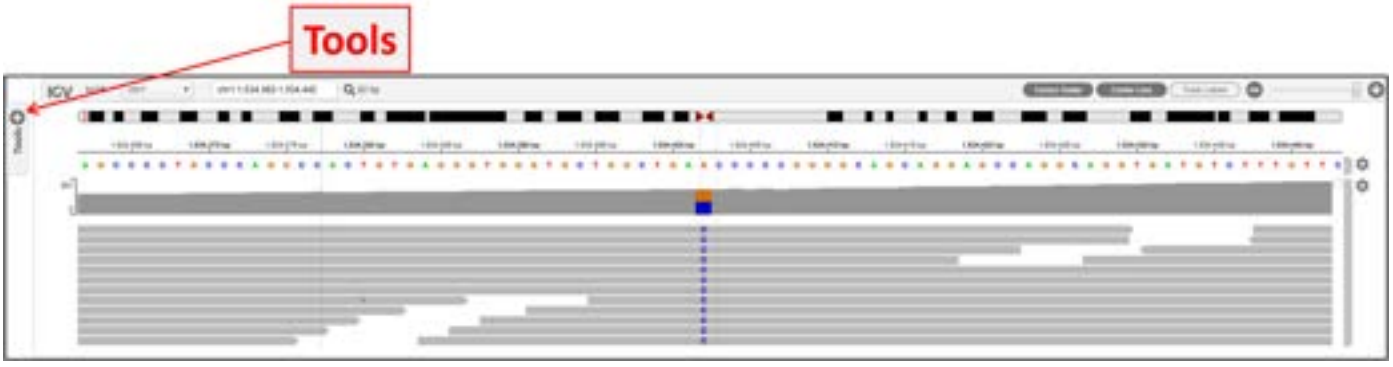


Figure 206: The Tools tab for adding data tracks to annotate an alignment displayed in IGV

Once accessed selecting the drop-down arrow will list the available tracks.

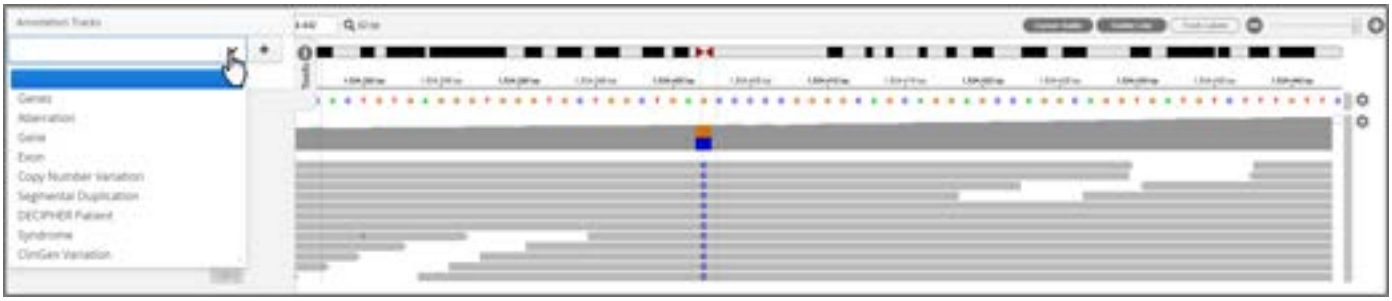


Figure 207: The drop-down list of data tracks available

Select the data track to be added.

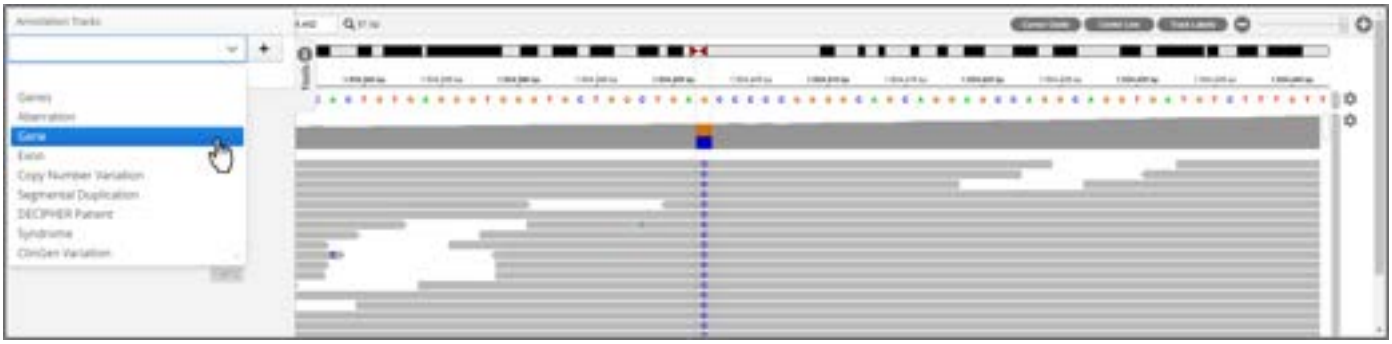


Figure 208: Selection of a track

And then click on the + icon to add it to the set of tracks for the software to display

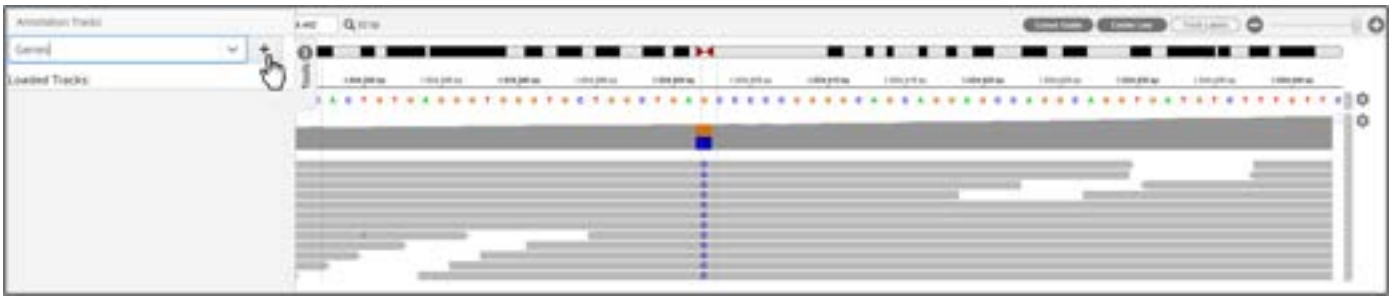


Figure 209: Click on the + icon to add the data track to the display

The selected track will be displayed. It can be removed by clicking on the minus icon −

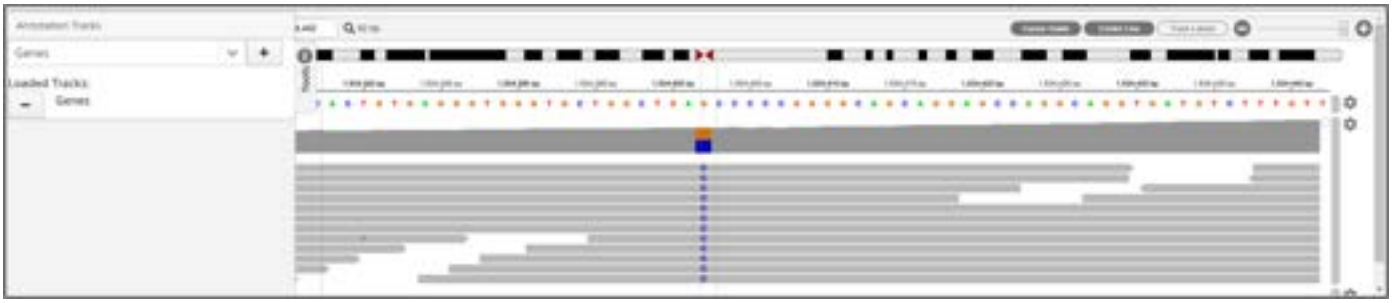


Figure 210: The new track is now loaded

Select any further tracks to add to the view

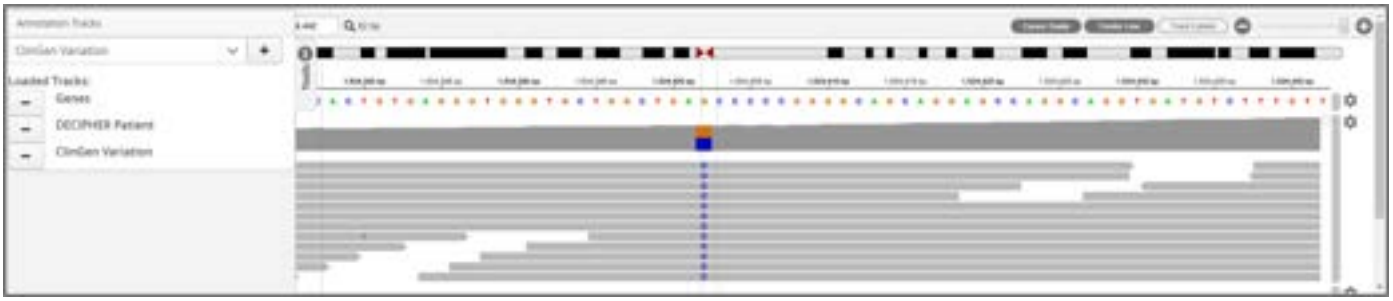


Figure 211: Addition of the required tracks

Finally, close the Tools tab and the data tracks will be displayed.

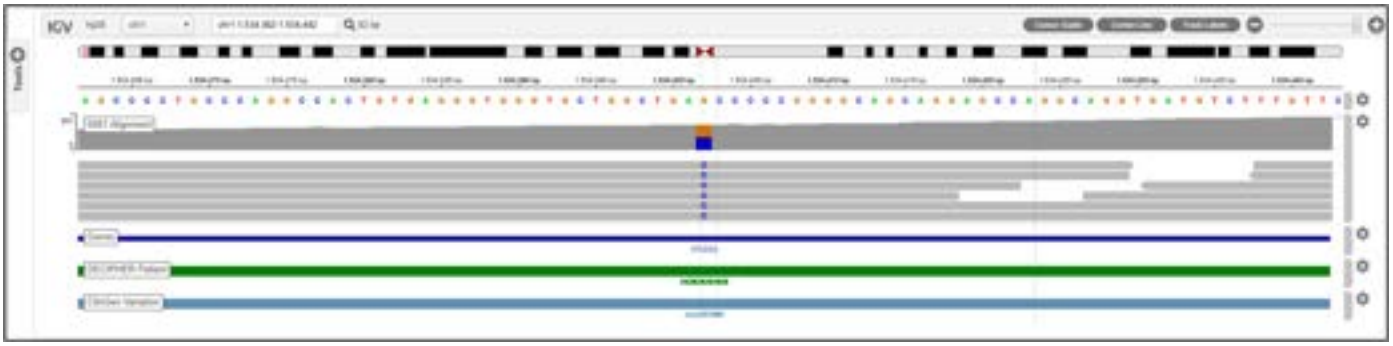


Figure 212: Display of the selected data tracks following closure of the tools tab

'Popping out' of the IGV display

There is, potentially, a substantial amount of information that can be displayed in the IGV view. To accommodate the information and make it easier for the user it is possible to 'pop out' the IGV view into a new browser tab.

This is accomplished using the button in the display

Figure 213: Button to allow display of IGV into a new tab in the browser

Selecting Multiple Samples

As discussed above users can opt to view multiple samples simultaneously by selecting them in the batch view.

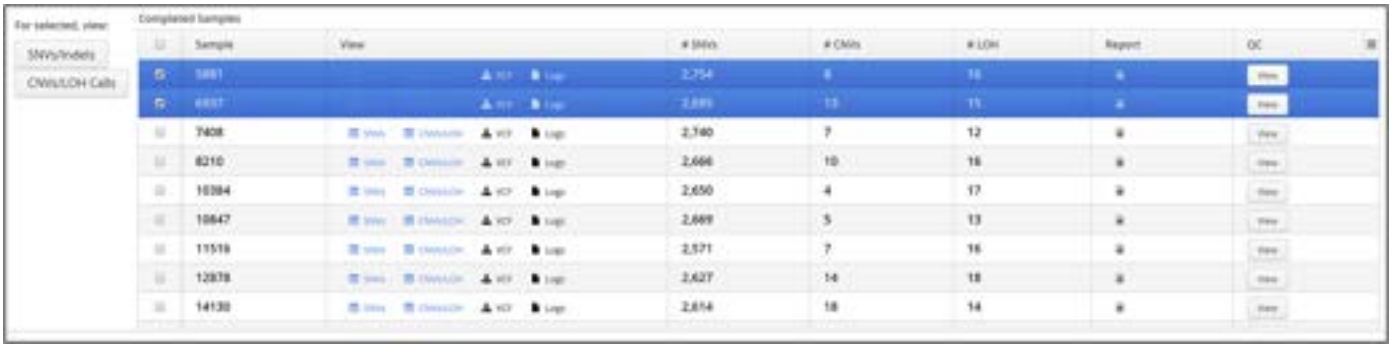


Figure 214: Selecting multiple samples to view in the Variants page

When multiple samples are selected there will be separate tracks for each sample in IGV. This makes it possible to compare the same variant in different samples.

Figure 215: An example of two samples sharing a variant as displayed in the integrated IGV browser

Variant Table Options

There are options within the Variant table accessed using the Actions drop down menu.

Figure 216: Accessing the options in the variant table

Reporting

Results can be exported by clicking on the Generate Report button below the variant table.

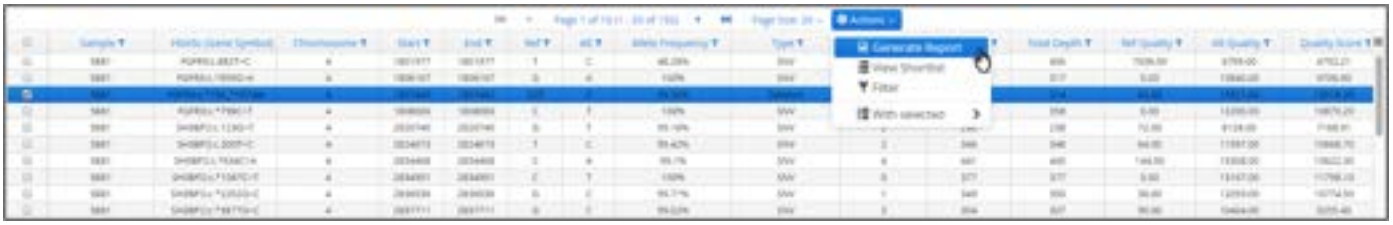


Figure 217: Selecting the Generate Report option from the variant table header menu

Interpret provides multiple types of report and for each of these types there are templates. These are highly customisable and updates can be easily applied in Admin Controls-Analysis-Reports.

When selecting to generate a report, the initial window allows users to select the type of report to generate.

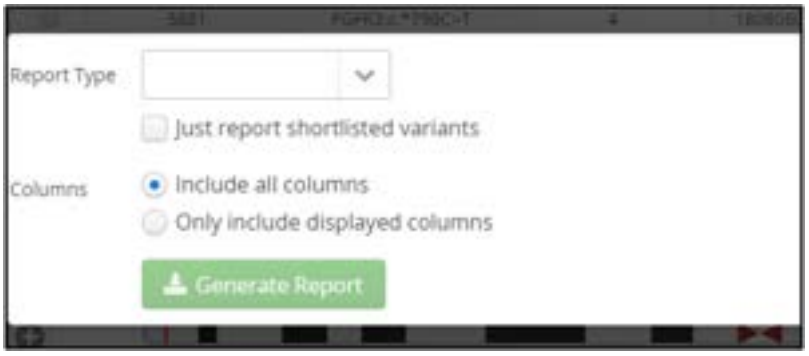


Figure 218: Initial report option

Default reports supplied with the software are listed.

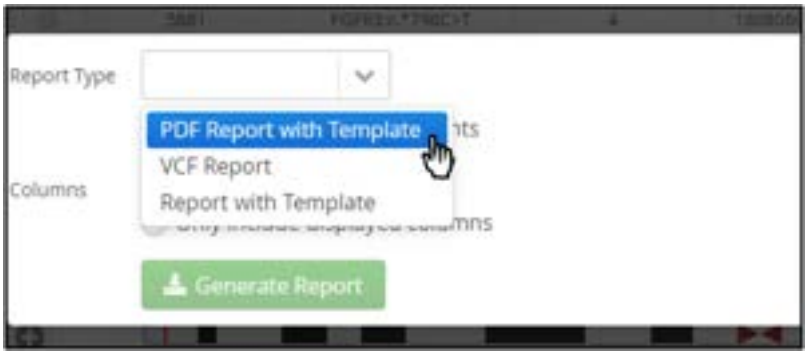


Figure 219: Selection of PDF report type

Once the report type has been selected the user needs to specify the template to use.

Figure 220: Selection of the template to use with the PDF report type

Once all options are chosen, pressing Generate Report will create the PDF file and the web browser will download it.

Sample	Chromosome	Start	End	Length	Genome Build	Ref	Alt	Type
5881	4	1801977	1801977	0b	GRCh38	T	C	SNV
5881	4	1806167	1806167	0b	GRCh38	G	A	SNV
5881	4	1807400	1807402	2b	GRCh38	CGT	C	Deletion
5881	4	1808060	1808060	0b	GRCh38	C	T	SNV
5881	4	2820740	2820740	0b	GRCh38	G	T	SNV
5881	4	2824673	2824673	0b	GRCh38	T	C	SNV
5881	4	2834468	2834468	0b	GRCh38	C	A	SNV

Figure 221: An example of the PDF report generated

Other options are included, for instance an HTML based report.

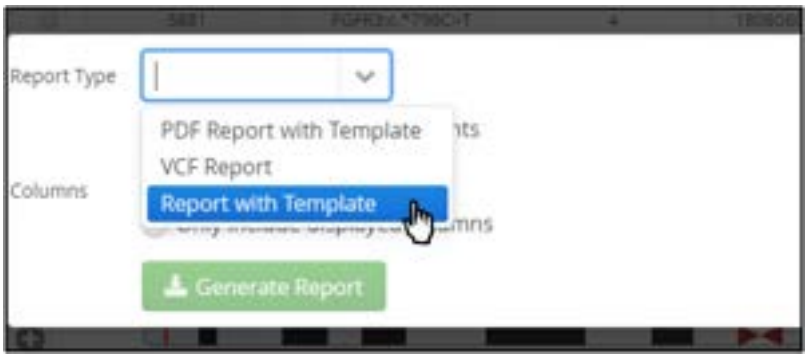


Figure 222: Selecting a template type report

Again, a template needs to be chosen

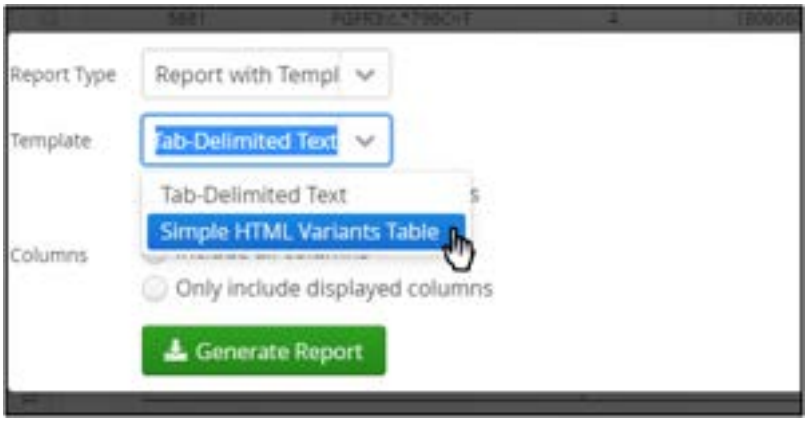


Figure 223: Selection of a HTML format report

The HTML formatted report is then generated and available,

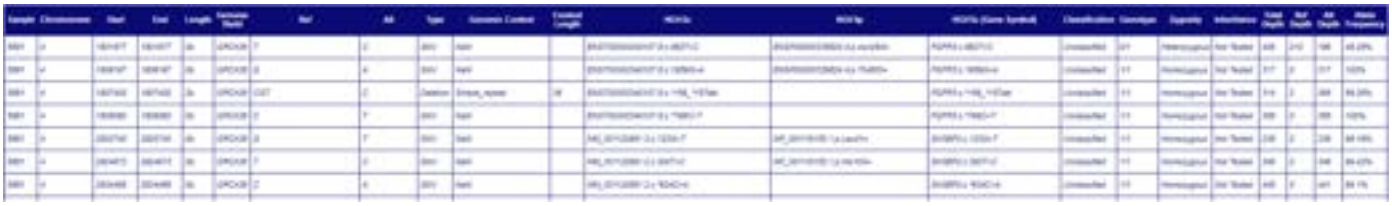


Figure 224: An example of the HTML report

Actions

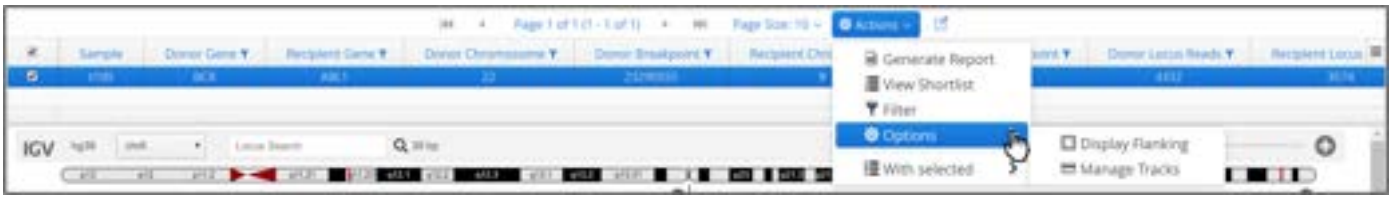


Figure 225: Options available for configuring the view in IGV

Display Flanking

Users can choose whether or not to display flanking sequence in the IGV display.

Figure 226: Selecting the Display flanking option in the Actions menu

Manage Tracks

Users can add or remove data tracks to the IGV view. This can be from publicly available sources or from proprietary internal or subscription-based sources.

Tracks can be added in the Software section of the Admin Controls (Admin Controls > Software > Annotation) and documentation of how to do this is in this section of the user guide.

Figure 227: Selecting the manage tracks options

The available tracks will be displayed in a pop-up window and users can select the tracks that they want to add to the display.

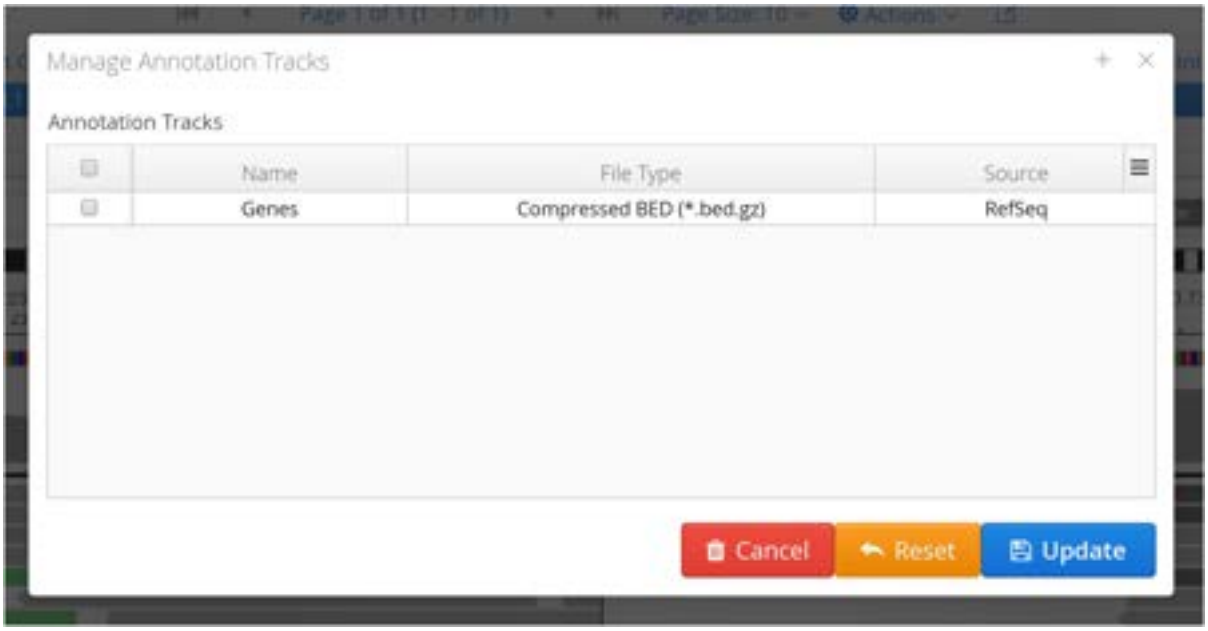


Figure 228: Tracks available to display

Once the required tracks are selected, users can press Update to update the IGV display.

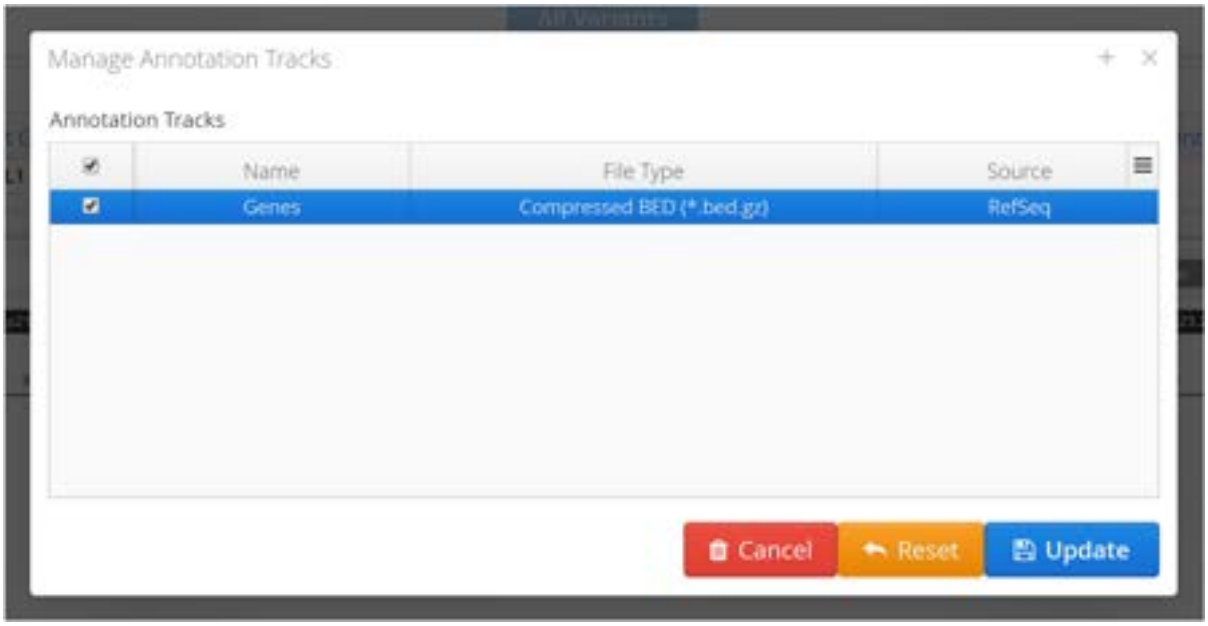


Figure 229: Selecting tracks to add to the IGV display

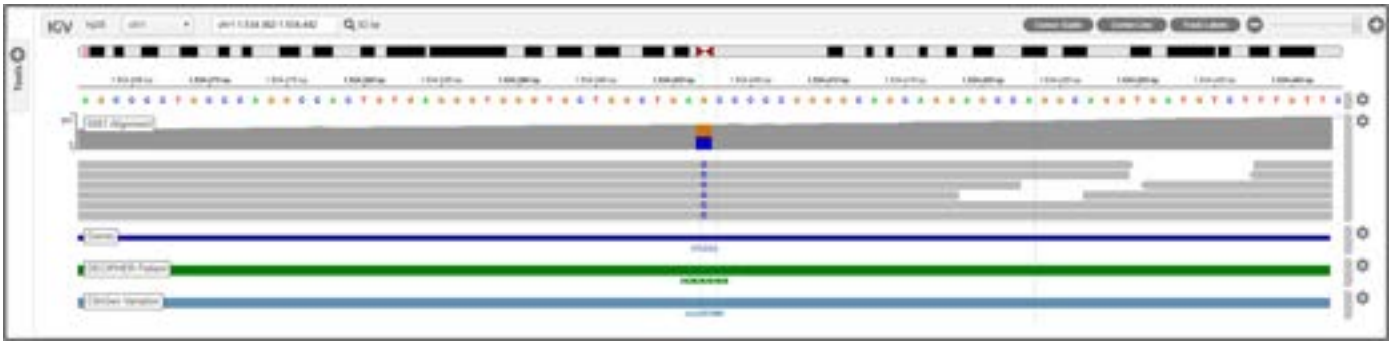


Figure 230: Displaying of tracks in the IGV display

Viewing Analysis Results by Variant

As results of samples are generated, they are stored in the Interpret database and can be analysed from a variant-centric point of view

Accessing of this viewpoint is via the Variants button on the dashboard menu bar shown in the figure below.

Figure 231: Selection of Variants from the Dashboard menu bar

Selecting the Variants tab in the menu bar opens up a new page to display all the variants recorded in the database.

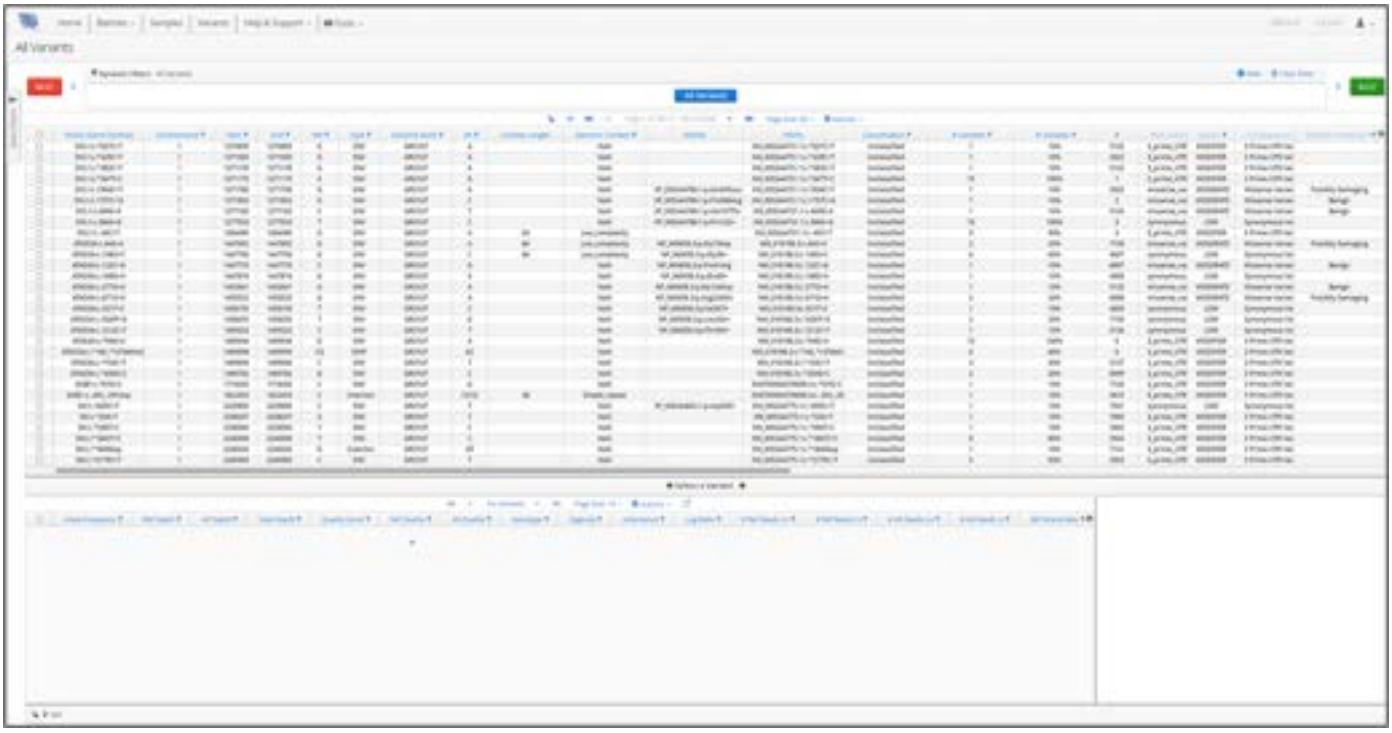


Figure 232: The start page for viewing variants

There is a substantial amount of information available in the variants page and the different sections are highlighted in the figure below.

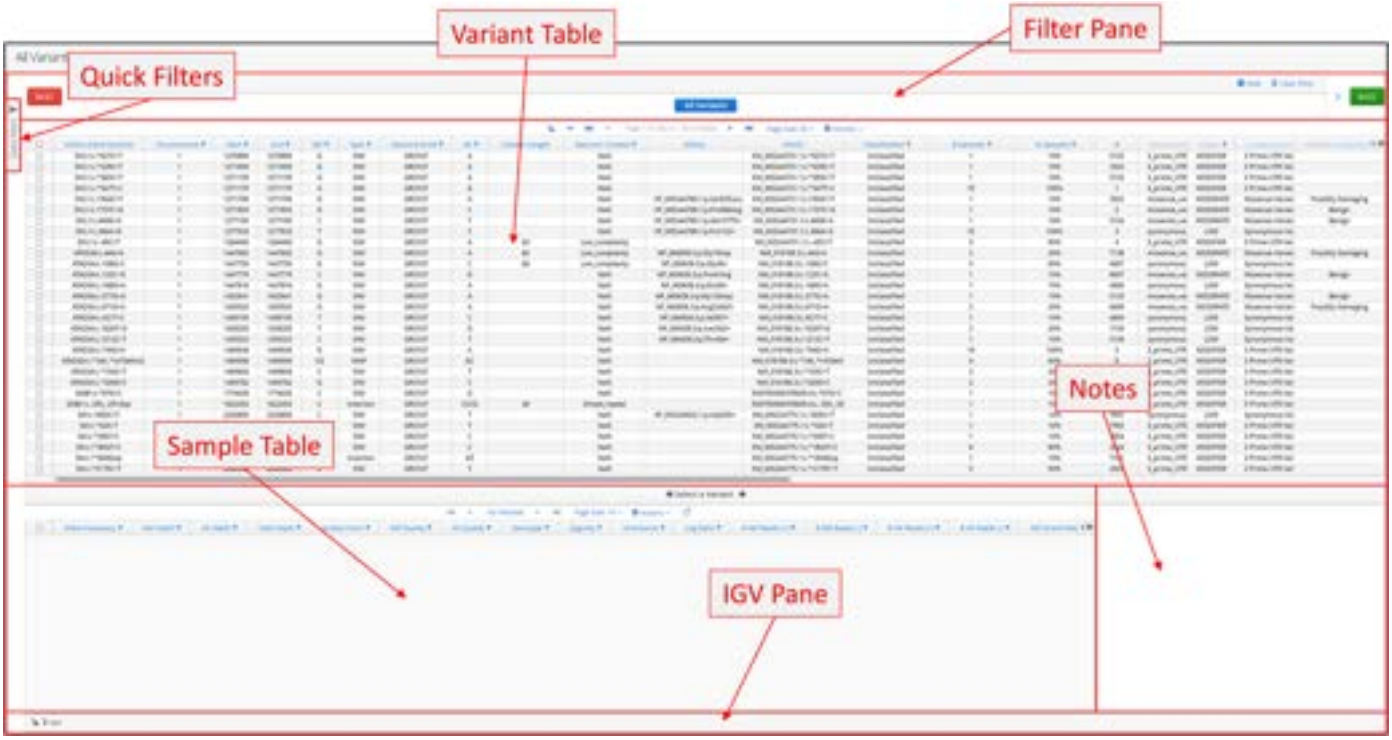


Figure 233: The different sections of the variant page

There a number of active regions

- **Filter Pane**

The filter pane allows for dynamic filtering of the variants. By default the filter is set to All Variants, so all variants are displayed in the variants table, however these can be refined according to your specific requirements.

- **Variant Table**

This displays all variants in the database that meet the filtering requirements of the dynamic filter. By default this is for displaying all variants.

- **Sample Table**

When a row in the variant table is selected all samples that contain the selected variant will be displayed in this table.

- **IGV Pane**

Selection of samples in the

- **Notes**

Users can add notes to variants.

- **Quick Filters**

These are selection of options that allow users to rapidly filter variants on the basis of some general conditions.

Clicking on the  icon on far right of the column headers in the variant table will display all the columns that can be selected for display in the variant table.

Figure 234: Columns available to select for display in the variant table

Similarly clicking on the same icon on the sample table provides a series of column options.

Figure 235: Columns available to select for display in the sample table

Dynamic Filtering

The dynamic filters are the same set of options discussed in the previous section. They can be accessed from the Actions drop down menu shown below.

Figure 236: Accessing the dynamic filtering options

The dynamic filtering window provides a detailed set of options for investigating the variants stored within the Interpret database.

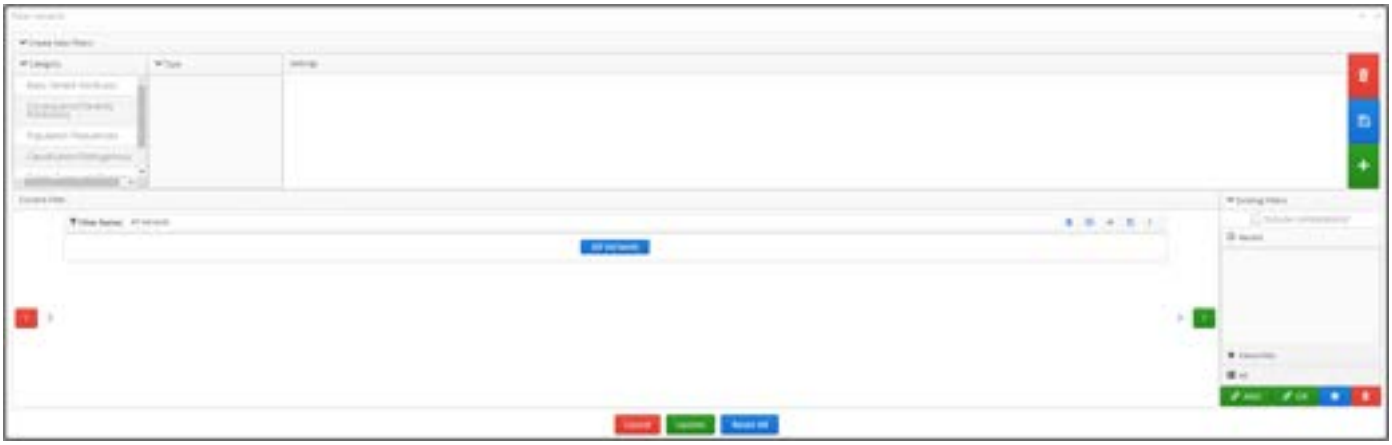


Figure 237: Full filtering options available to filter variants

Filtering by Quick Filters

Selecting the Quick Filters tab on the side of the variants page opens a tab that provides some options for quickly drilling down into variants of interest. The options currently available are to select based on a classification type, the NGS panel or gene.

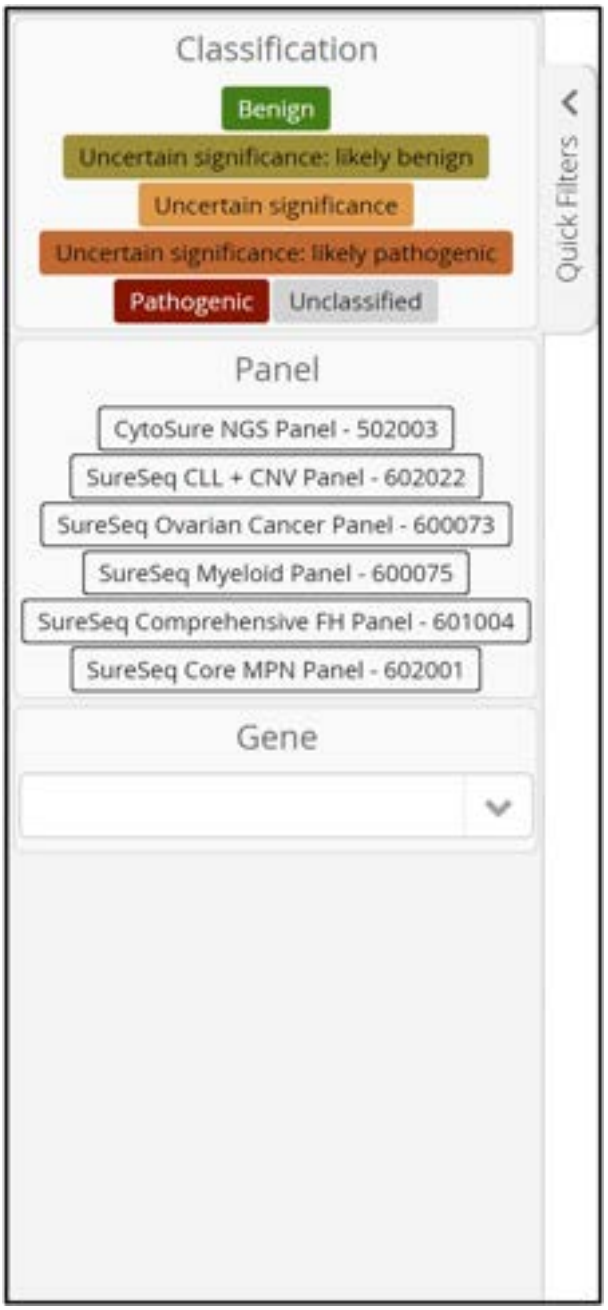


Figure 238: Quick filter options

Classification or Panel can be selected by pressing the corresponding buttons with multiple selections allowed. To filter by genes start typing the gene name in the text box matching values will be displayed.

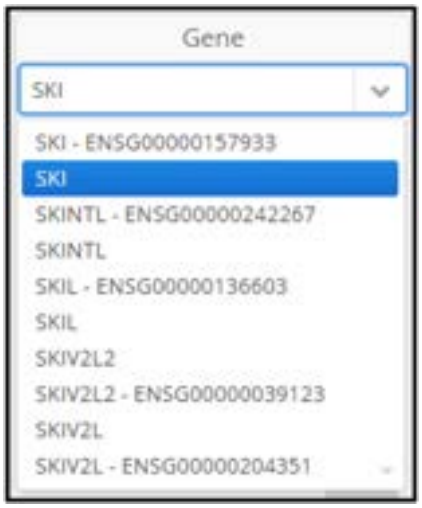


Figure 239: Using quick filters to select for the gene SKI

Once a gene is selected it will be displayed as below and can be removed by clicking on the x next to the gene name.

Figure 240: Using quick filters to filter by the SKI gene

The dynamic filter is now updated and shows that, from the input of 8432 variants, there are only 10 found within the SKI gene.

Figure 241: Displaying only variants in the SKI gene

Additional genes can be selected and these will be displayed in the same way.

Figure 242: Using quick filters to select variants in the SKI or DVL1 genes

When the 2-gene filter is applied the output now increases to 19 variants being displayed.

Figure 243: Displaying only variants in the SKI or DVL1 genes

Displaying Variants

Each row of the variant table represents a variant that has been detected in at least one sample. Selecting a variant displays all the samples in which the variant is present. In the figure below, the variant DVL1:c.*347T>C is present in 10 samples.

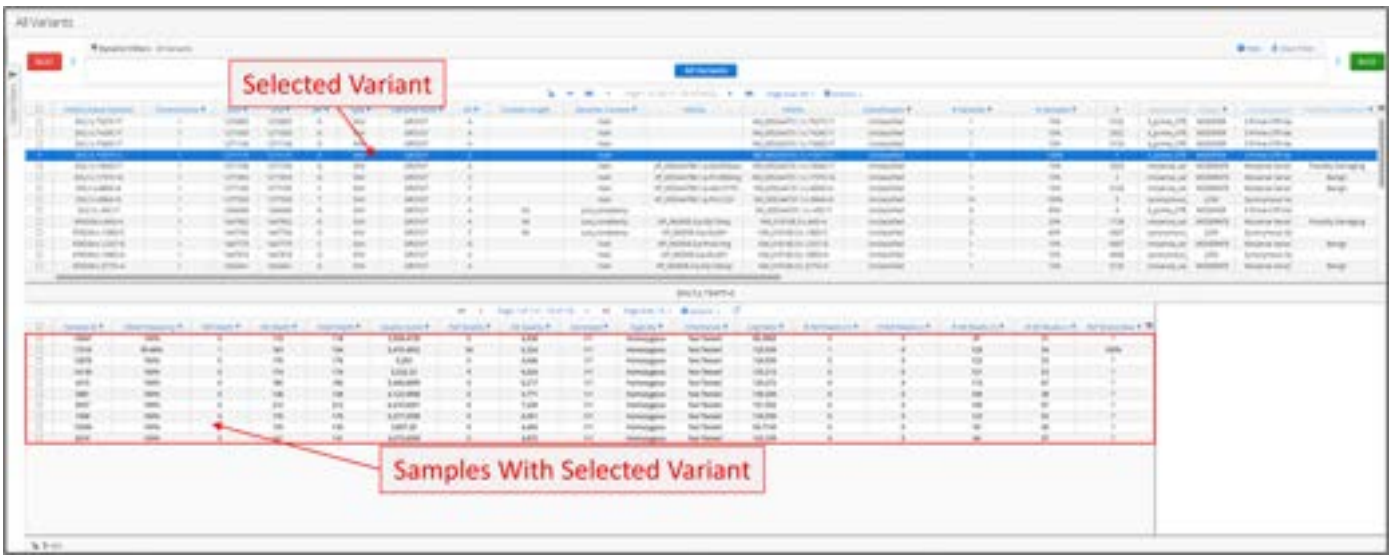


Figure 244: Selecting a variant displays all samples in which it is present

Subsequently, selecting any of the sample or samples rows will display the alignment for the variant in the corresponding sample.

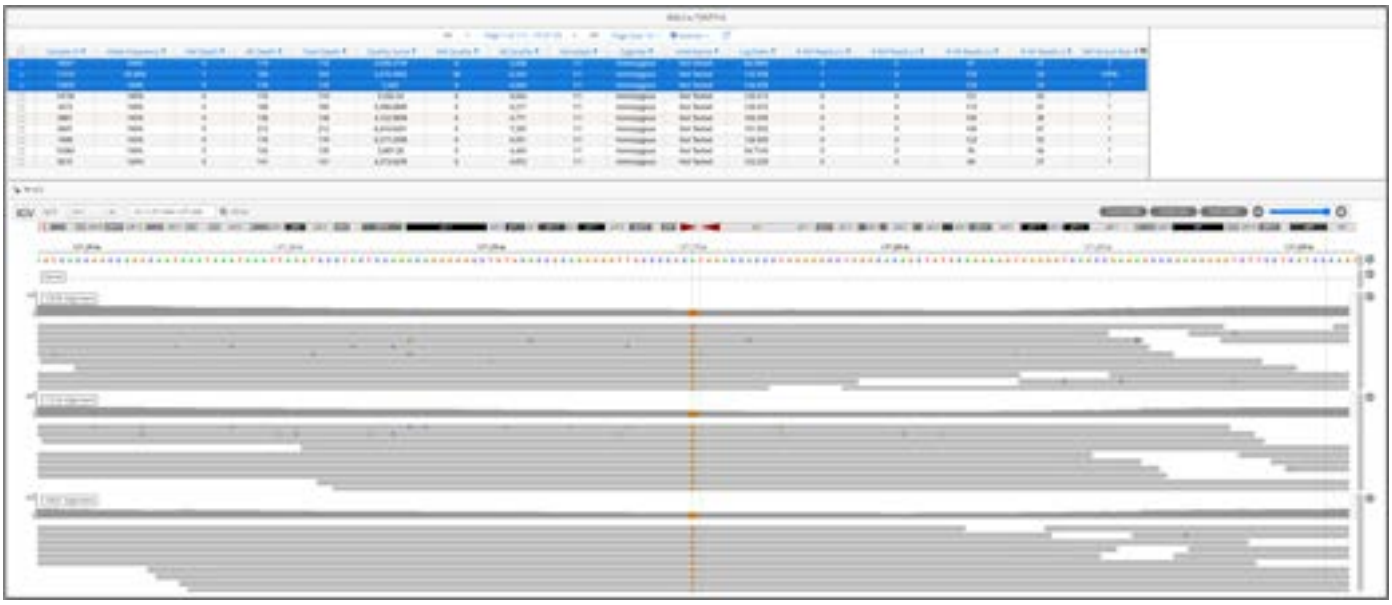


Figure 245: Display of the alignment for 3 samples in IGV

Adding Notes to Variants

It is possible to for users to add annotations to variants through the notes function. When a variant has been selected and there are rows populated in the sample table, the user can make a right click on one of these. From the popup menu select the Notes > Add Note options

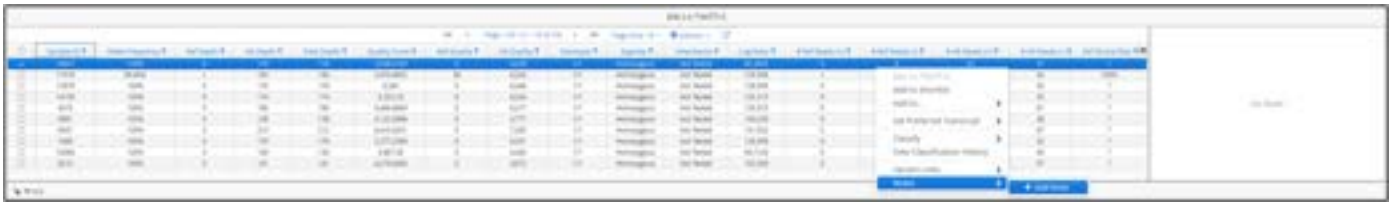


Figure 246: Selecting the Add Note option

A window is displayed with a text box where up to 250 characters can be used. Any other pre-existing notes will also be shown.

Figure 247: Note creation template

The user can enter the required notation.

Figure 248: Note creation

Then selecting + Add Note completes the process and the existing notes section is updated to include the newly created note.

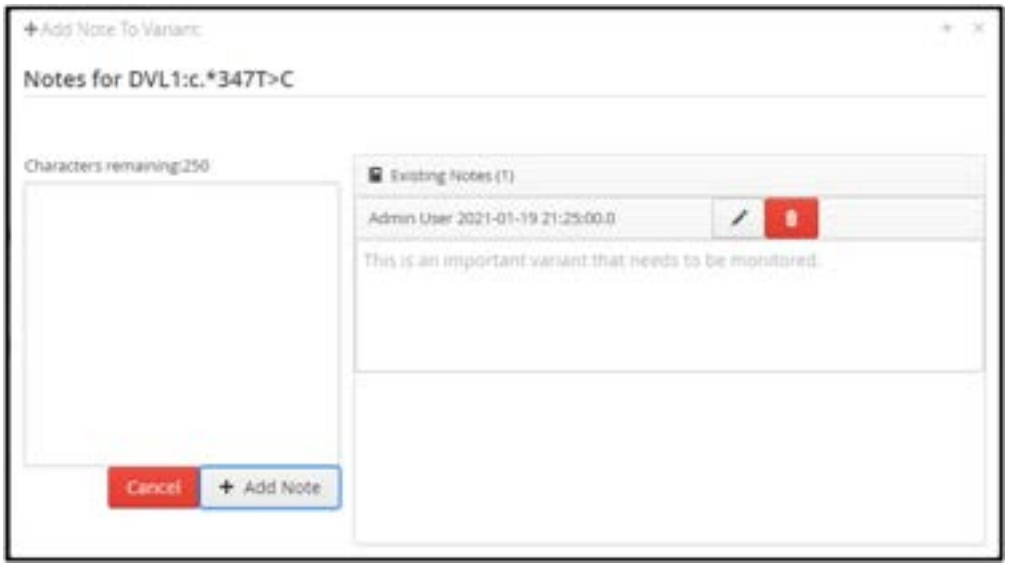


Figure 249: Note generation

Once all changes have been made, the notes window can be closed and the view will return the normal variant display with the note now being displayed in the Notes panel.

Figure 250: Displaying a note in the note panel

A note can be deleted by pressing the red rubbish bin icon. If the Confirm Delete option is then selected the note will be removed.

Figure 251: Deleting a note

Users can also edit a note by clicking on the pen icon; which will show the note in a text box where changes can be made. The update is confirmed by the pressing the Apply button.

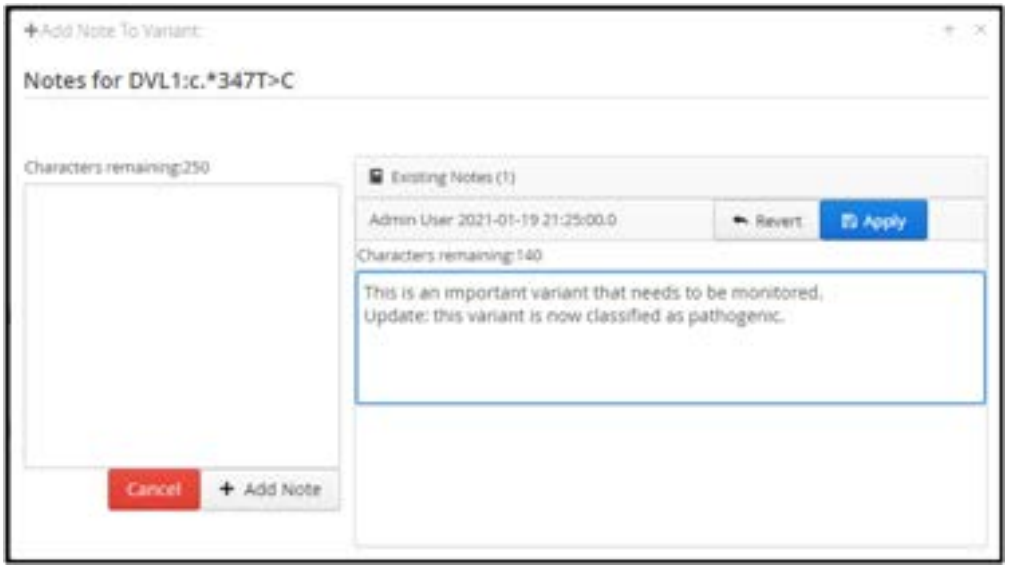


Figure 252: Update an existing note

The notes panel is subsequently updated to show the revised note.

Figure 253: Display of an updated note

Administration Controls

These are accessed by selecting the Admin Controls options in the user drop down menu.

Figure 254: Accessing the Admin Controls section

There are 4 main parts to the admin controls:

1. Overview
2. User Controls
    - Current Users
    - Add Users
3. Analysis
    - Manage Samples
    - Current Analyses
    - Protocols
    - Panels
    - Region Lists
    - Variant Lists
    - Classifications
    - Metric Sets
    - Manage Links
    - Filters
    - Preferred Transcripts
    - Reports

Attribute Definitions

Attribute	Variant Type	Description
#	SNV/Indel	Variant Database Identifier
# Alt Reads (-)	SNV/Indel	Number of alternative alleles on negative strand
# Alt Reads (+)	SNV/Indel	Number of alternative alleles on positive strand
# Markers	CNV/LOH	Number of bins (markers) used to identify CNVs
# Ref Reads (-)	SNV/Indel	Number of reference alleles on negative strand
# Ref Reads (+)	SNV/Indel	Number of reference alleles on positive strand
# Samples	SNV/Indel	Number of samples the variant is present in the database
% Samples	CNV/LOH	Percentage of samples the variant is present in the database
% Samples (Similar CNVs)	CNV/LOH	Number of samples in the database with an overlapping CNV
Allele Frequency	SNV/Indel	Allele Frequency
Alt	SNV/Indel	Alternate allele
Alt Depth	SNV/Indel	Number of reads supporting the alternative allele at the position
Alt Quality	SNV/Indel	Sum of alternative base qualities at the position
Alt Strand Bias	SNV/Indel	Sequencing bias in which one DNA strand is favoured over the other in the reads containing the alternative allele (Percentage)
Bands	CNV/LOH	Location of the variant on the chromosome
Batch Date	SNV/Indel	Date the batch was performed
Batch Name	SNV/Indel	Name of the batch containing the run

Attribute	Variant Type	Description
Canonical?	SNV/Indel	A flag indicating if the transcript is denoted as the canonical transcript for this gene
Chromosome	CNV/LOH	Chromosome of the CNV/LOH
Chromosome	SNV/Indel	Chromosome of the variant
Classification	CNV/LOH	User-assigned classification of the variant
ClinVar Significance	SNV/Indel	Clinical significance of variant according to ClinVar (e.g. benign, pathogenic, uncertain significance etc.)
Confidence	CNV/LOH	Confidence that the call is correct (e.g. High, Low) dependant on the Standard Error of Mean
Consequence Terms	SNV/Indel	Most severe outcome caused by the specific variant (e.g Frameshift variant, Stop gained, Synonymous variant etc.)
Context Length	SNV/Indel	Length of the genomic context overlapping the variant
Copy Number	CNV/LOH	Number of copies of the CNV event
Depth	CNV/LOH	Depth of coverage of the sample at position of the CNV
Description	Translocation	Donor and recipient gene symbol pair
Donor Breakpoint	Translocation	Position on the donor chromosome of the translocation
Donor Chromosome	Translocation	The chromosome number of the donor gene
Donor Gene	Translocation	Donor gene symbol where the translocation originated
Donor Locus Reads	Translocation	Read depth at the donor breakpoint position
Donor Orientation	Translocation	Orientation (strand) of the donor gene
Donor Reads Position	Translocation	Which side of the breakpoint the donor read lies (left or right)
End	CNV/LOH	Genomic position of end of CNV
End	SNV/Indel	Genomic position of end of variant
Estimated Tumour Content	CNV/LOH	Estimated tumour content (only used in cancer panels)
Exon ID	SNV/Indel	Unique ID for the exon

Attribute	Variant Type	Description
Exon Number	SNV/Indel	The number of the exon in the gene the variant is present
Frequency	CNV/LOH	Average Allele Frequency of common SNP's overlapping the CNV
Fusion Type	Translocation	The method of detection that highlighted the fusion, either: Over-expression of the gene (Expression), detection of a known fusion by sufficient supporting reads (Canonical), detection of an unknown fusion by sufficient supporting reads (Non-canonical)
Gene ID	SNV/Indel	Unique ID of the gene where the variant is
Gene Symbol	SNV/Indel	Gene symbol where the variant is
Genes	CNV/LOH	List of genes overlapping the CNV
Genome Build	CNV/LOH	Genome assembly version
Genomic Context	SNV/Indel	Genomic context the variant is overlapping (Low Complexity, Homopolymer, Simple Repeat)
Genotype	SNV/Indel	Genotype (Heterozygous/Homozygous)
gnomAD - African	SNV/Indel	The frequency variant appears on the Genome Aggregation Database from people of African decent
gnomAD - Ashkenazi Jewish	SNV/Indel	The frequency variant appears on the Genome Aggregation Database from people of Ashkenazi Jewish decent
gnomAD - East Asian	SNV/Indel	The frequency the variant appears on the Genome Aggregation Database from people of East Asian decent
gnomAD - European (Finnish)	SNV/Indel	The frequency the variant appears on the Genome Aggregation Database from people of Finnish decent
gnomAD - European (non-Finnish)	SNV/Indel	The frequency the variant appears on the Genome Aggregation Database from people of European (Non-Finnish) decent
gnomAD - Latino	SNV/Indel	The frequency variant appears on the Genome Aggregation Database from people of Latino decent

Attribute	Variant Type	Description
gnomAD - Other	SNV/Indel	The frequency the variant appears on the Genome Aggregation Database from people of another decent
gnomAD - South Asian	SNV/Indel	The frequency the variant appears on the Genome Aggregation Database from people of South Asian decent
gnomAD - Total	SNV/Indel	The frequency variant appears on the Genome Aggregation Database from all reference genomes
HGVSc	SNV/Indel	The HGVS coding sequence name
HGVSc (Gene Symbol)	SNV/Indel	The HGVS coding sequence name with the Transcript identifier replaced with its Gene Symbol
HGVSp	SNV/Indel	The HGVS protein sequence name
Homozygosity	SNV/Indel	Proportion of the genome covered by LOH regions larger than 5Mb
Impact	SNV/Indel	The Impact score according to Ensembl VEP of the genetic variation in the genetic sequence (e.g. LOW, MODERATE, HIGH etc.)
Inheritance	CNV/LOH	Estimated inheritance of the variant based on the presence of the variant in parental results, if available.
Inheritance	SNV/Indel	Estimated inheritance of the variant based on the presence of the variant in parental results, if available.
Inheritance	Translocation	Estimated inheritance of the variant based on the presence of the variant in parental results, if available.
ISCN	CNV/LOH	CNV/LOH variant encoded according to ISCN (International System for Human Cytogenomic Nomenclature)
ISCN	Translocation	Translocation variant encoded according to ISCN (International System for Human Cytogenomic Nomenclature)
Length	CNV/LOH	Length of CNV
Log Ratio	CNV/LOH	Mean log2 ratio of sample/reference of the CNV
Mean	CNV/LOH	Rescaled mean log2 of sample/reference of the CNV (only used in cancer panels)

Attribute	Variant Type	Description
Mean Standard Error	CNV/LOH	Standard Error of the Mean
Minor Allele	SNV/Indel	Base of the minor allele
Minor Allele Frequency	SNV/Indel	Rate at which the second most common allele occurs
Mosaicism	CNV/LOH	Estimate of the percentage of mosaicism observed in CNV region
Mosaicism Lower Bound	CNV/LOH	Estimate of the lower bound of mosaicism observed in the CNV region
Mosaicism Range	CNV/LOH	Estimate of the range of mosaicism observed in the sample
Mosaicism Upper Bound	CNV/LOH	Estimate of the upper bound of mosaicism observed in the CNV region
Most Severe Consequence	SNV/Indel	Most severe outcome caused by the specific variant (e.g Frameshift variant, Stop gained, Synonymous variant etc.)
Normalised Expression	Translocation	The expression of the baited gene relative to the housekeeping genes and normalised by total read count
Overlap	CNV/LOH	Genomic context of the CNV
P Value	Translocation	Probability of observing the translocation
Panel	SNV/Indel	Panel used for the analysis
PolyPhen Prediction	SNV/Indel	The prediction of how damaging a variant will be, based off the PolyPhen Score
PolyPhen Score	SNV/Indel	The probability that a substitution is damaging (e.g. 0.25 benign, 0.5 possibly damaging, 0.95 probably damaging)
Proportion	Translocation	Proportion of split reads over total reads at the donor breakpoint
Protein ID	SNV/Indel	Unique ID for the protein
Protocol	SNV/Indel	OGT Interpret software protocol used to analyse the run
Quality	CNV/LOH	(Not implemented)
Quality Score	SNV/Indel	Phred Quality score of the variant

Attribute	Variant Type	Description
Ratio	SNV/Indel	Ratio of depth observed in duplicated PTD exons compared to the exons in the rest of the gene
Read 1	SNV/Indel	File name of the FASTQ from R1 reads
Read 1 Size	SNV/Indel	Size of the FASTQ file from R1 reads
Read 2	SNV/Indel	File name of the FASTQ from R2 reads
Read 2 Size	SNV/Indel	Size of the FASTQ file from R2 reads
Reads Placed Left	SNV/Indel	Number of reads with supporting evidence to the left of the variant
Reads Placed Right	SNV/Indel	Number of reads with supporting evidence to the right of the variant
Recipient Breakpoint	Translocation	Position on the recipient chromosome of the translocation
Recipient Chromosome	Translocation	The chromosome of the recipient gene
Recipient Gene	Translocation	Recipient gene symbol where the translocation ended up
Recipient Locus Reads	Translocation	Read depth at the recipient breakpoint position
Recipient Orientation	Translocation	Orientation (strand) of the recipient gene
Recipient Reads Position	Translocation	Which side of the variant the donor read lies (left or right)
Ref	SNV/Indel	Reference nucleotide base
Ref Depth	SNV/Indel	Number of reads supporting the alternative allele at the position
Ref Quality	SNV/Indel	Sum of alternative reference qualities at the position
Ref Strand Bias	SNV/Indel	Sequencing bias in which one DNA strand is favoured over the other in the reads containing the reference allele (Percentage)
rsID	SNV/Indel	SNP id from NCBI dbSNP
Sample	CNV/LOH	ID of the sample containing the CNV
Sample	SNV/Indel	ID of the sample containing this variant
Sample	Translocation	ID of the sample containing this variant
Sample ID	SNV/Indel	ID of the sample containing this variant

Attribute	Variant Type	Description
Score	CNV/LOH	LOH score (Higher scores >30 indicate a higher confidence in the call)
Sex	SNV/Indel	Inferred chromosomal sex of the sample (Male, Female, Unknown)
SIFT Prediction	SNV/Indel	Prediction of how detrimental a variant will be to protein function (The opposite of Polyphen in terms of numbering)
SIFT Score	SNV/Indel	A score that predicts whether a variant will affect protein function (0 = deleterious , 1 = tolerated)
Source	CNV/LOH	Tool used for CNV identification
Start	CNV/LOH	Genomic position of start of CNV
Start	SNV/Indel	Genomic start position of variant
Supporting Reads	Translocation	The sum of split and discordant reads in support of the fusion call
Total Depth	SNV/Indel	Depth of coverage at the position
Transcript ID	SNV/Indel	Unique ID of the specific selected transcript
Transcript Resolution Method	SNV/Indel	Method used to determine which transcript to use
Type	CNV/LOH	Variant type (e.g. CNV, LOH)
Type	SNV/Indel	Variant type (e.g. SNV, ITD, PTD, etc.)
Type	Translocation	Variant type (e.g. Translocation)
User	SNV/Indel	Login name of user which ran the batch
VEP Version	SNV/Indel	Version of Ensembl Variant Effect Predictor
Zygosity	SNV/Indel	The degree at which both copies of the chromosome have the same genetic sequence (e.g. Homozygous or Heterozygous)

Table 3: Definitions of attributes displayed in various tables in Interpret

Product-specific Guidance

Minimal Residual Disease

Overview

Detection and monitoring of Minimal Residual Disease (MRD) with the SureSeq Myeloid MRD Panel is made possible in Interpret through:

1. The ability to specify "Hotspots" (variants) which should be specifically interrogated by the pipeline for their presence at very low frequency.
2. The ability to visualise the change in allele frequency of these hotspots in multiple sequencing runs over time.

Discovery Mode

In order to identify candidate variants for use in "Monitoring Mode", where specific variants are interrogated by the pipeline in order to determine their allele frequency at very low depth, it may be necessary to process samples in "Discovery Mode". Discovery Mode uses the standard SNV, Indel and ITD detection algorithms built into OGT's NGS analysis pipeline to report all variants present in a sample above a specific allele frequency and according to other quality-related criteria. To process samples in Discovery Mode:

1. Click on the **Batches** button in the toolbar and select **Run Batch**.
2. Enter a name for the batch in the **Batch Name** field.
3. Select the **SureSeq Myeloid MRD Panel** from the **Panel** drop-down list.
4. Select **Discovery Mode** from the **Protocol** drop-down list.
5. Select the samples to be processed from the list of available samples such that they are displayed in the **Selected Samples** table.
6. Click **Run Analysis**.
7. Click **OK**.

Once the batch has been started, the **Batch** page will be displayed showing the current status of the processing of the samples in the batch. The status of each sample will be updated automatically (unless the web interface is shut down automatically due to inactivity – see **Automatic Shutdown** above), and, on completion, the **Completed Samples** table, displaying a summary of the results and relevant QC metrics, will appear.

Minimum Allele Frequency
By default, Discovery Mode is configured to detect variants at a minimum allele frequency of 1%. To reduce this value in order to increase the sensitivity, modify the Discovery Mode protocol as follows:

1. In the top-right corner of the screen, click on the user icon and select **Admin Controls**.
2. In the menu on the left-hand side, select **Analysis -> Protocols**.
3. In the **Protocols** list, select **Discovery Mode**.
4. Click the **Edit** button at the bottom of the screen.
5. Scroll down and select the **Advance Pipeline Configuration** tab.
6. In the **SNV Detection** section, modify the value of **Minimum Alt Fraction** as required.
7. Click **Save**.

Hotspot Monitoring

In order to visualise the results of hotspot monitoring:

1. Select**Tools -> Hotspot Monitoring Report**, and select the sample/source to be reported.

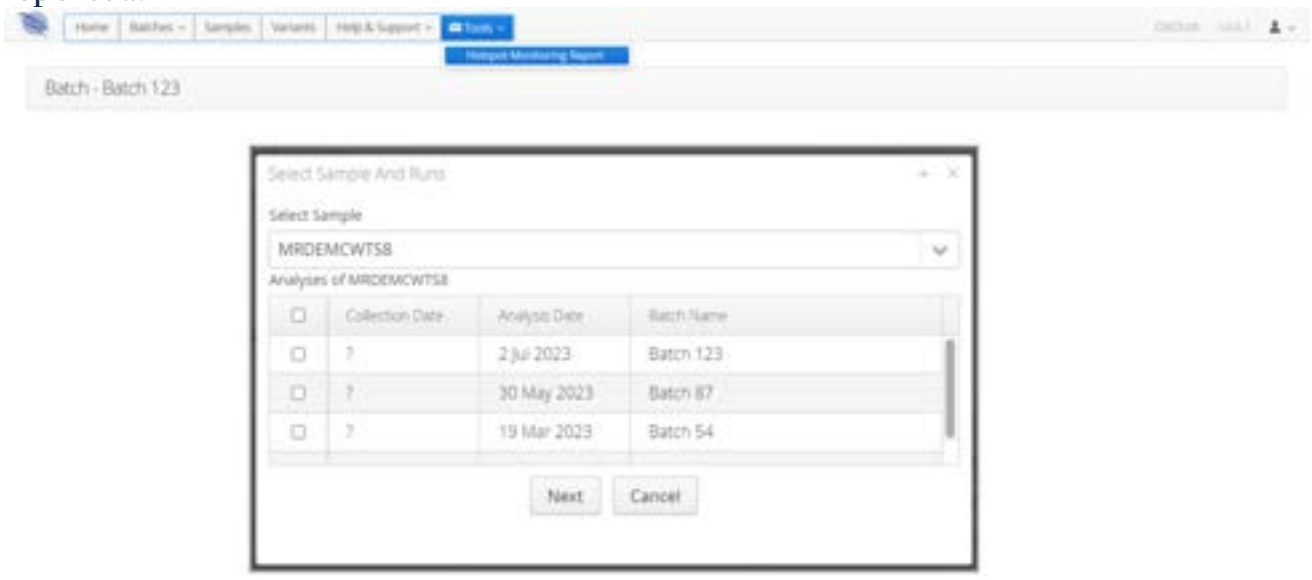


Figure 255: Selecting the sample(s) to be reported

2. If necessary, enter the **Collection Date** of the sample(s). This only needs to be carried out once for each sample and will be remembered for future reports. Click **Next**.

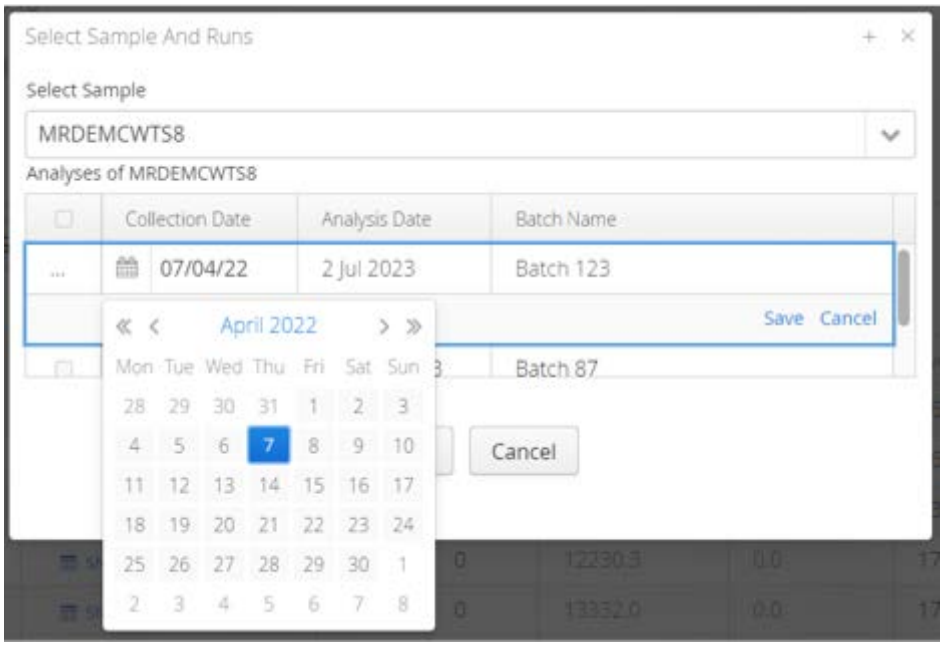


Figure 256: Entering the collection date of the sample

3. Select the hotspots to be reported using the same method described in **step 4e-f in the Selecting Hotspots** section and click **View**.

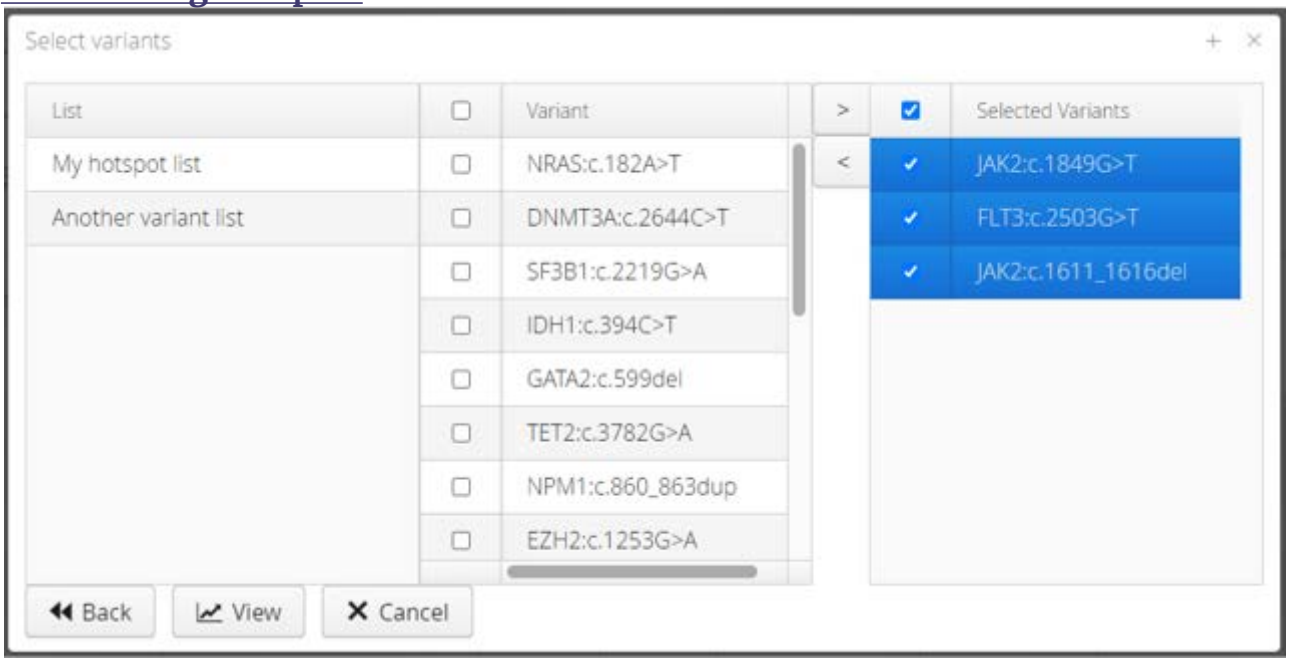


Figure 257: Selecting Hotspots to include in the report

4. A graph containing the allele frequencies of all selected hotspots in all selected sample runs will be displayed, along with tabs allowing the user to view the results for individual hotspots. Graph images may be exported by right-clicking of the graph and selecting "Save Image". The table containing the data underlying the graph may be exported as a CSV via the**Export Data**button.

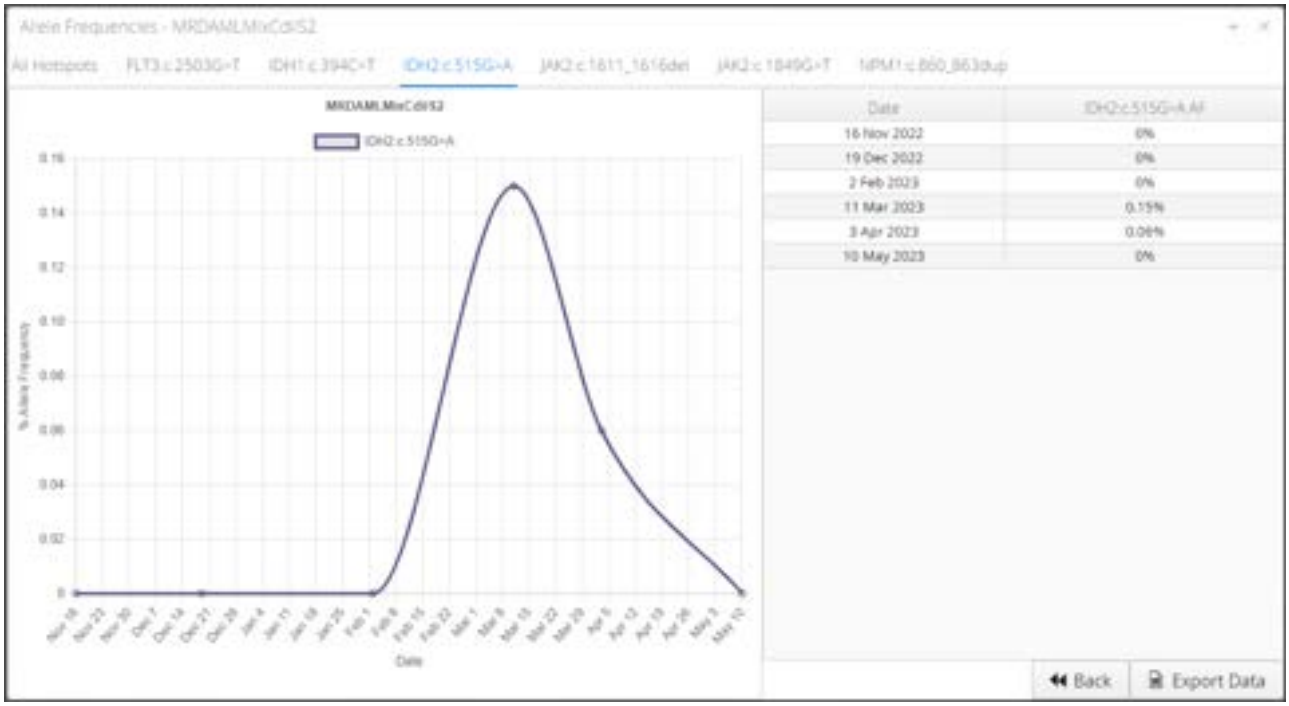


Figure 258: An example of a report

Monitoring Mode

To determine the allele frequency of hotspots in a batch of samples at very low depth, the samples should be processed using the "Monitoring Mode" protocol:

1. Upload the FASTQ files for the batch using the method described in the **Uploading FASTQ Files** section above.
2. Click on the **Batches** button in the toolbar and select **Run Batch**.
3. Enter a name for the batch in the **Batch Name** field.
4. Select the **SureSeq Myeloid MRD Panel** from the **Panel** drop-down list.
5. Select **Monitoring Mode** from the **Protocol** drop-down list.
6. Select the samples to be processed from the list of available samples such that they are displayed in the **Selected Samples** table.
7. Click **Run Analysis**.
8. Click **OK**.

Once the batch is complete, allele frequencies of hotspots selected in the Monitoring Mode protocol for a specific sample may be viewed by clicking on the **SNVs** button in the **Completed Samples.**

Selecting Hotspots

To select hotspots for use in Monitoring Mode, they must first be selected from variants identified in Discovery Mode:

1. In the **Batch** page for the Discovery Mode batch, click on the **SNVs** button in the **Completed Samples** table for a sample that may contain potential hotspots.
2. If necessary, filter the list of variants in the **Variants** page in order to identify potential hotspots more quickly.
3. For each variant to be monitored in Monitoring Mode:
    a. Right-click on the variant in the table.
    b. Select **Add to...**
        i. If no Variant List has been created yet:
            1. Click **New List**
            2. Enter a **Name** for the list (e.g. "Hotspots")
            3. Click **Create**
        ii. Otherwise, click on the name of the Variant List.
4. Once all required hotspots have been added to the Variant List, modify the "Monitoring Mode" protocol to use those hotspots:
    a. In the top-right corner of the screen, click on the user icon and select **Admin Controls**.
    b. In the menu on the left-hand side, select **Analysis -> Protocols**.
    c. In the **Protocols** list, select **Monitoring Mode**.
    d. Click the **Edit** button at the bottom of the screen.
    e. Scroll down until the **Hotspots** table is displayed, and click on the name of the Variant List created in step 3 in the **List** column.
    f. In the **Variant** column, select all variants whose allele frequencies should be monitored, and click on the > button to add them to the **Selected Variants** table.
    g. When all variants have been added to the **Selected Variants** table, click the **Save** button.

Batch hotspot selection
The list of variants included in the Hotspots list for the Monitoring Mode protocol should cover all variants to be monitored in all samples in a batch. If different variants are relevant to different samples, (preferably) create the super-set of these variants in the protocol, or create separate protocols for each set of variants, and run the samples in different batches using the appropriate protocol.

Hotspots not detected in Discovery Mode
If the variant required for monitoring has not been detected in Discovery Mode, contact OGT for assistance to add the variant to a variant list.

Legal Information

Interpret Software

Interpret software was developed by Oxford Gene Technology. The purchaser has the non-transferable right to use the product for RESEARCH USE ONLY AND NOT FOR DIAGNOSTICS PROCEDURES. It is not intended for use, and should not be used, for the diagnosis, prevention, monitoring, treatment or alleviation of any disease or condition, or for the investigation of any physiological process, in any identifiable human, or for any other medical purpose.

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What binds us, makes us.