User Manual

Alamut™ Visual Plus

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

Summary Information

Product Name	Alamut™ Visual Plus
Product Type	Analytical/Visualization software
Product Family	Software
Reference (Product Code)	LAVP
Product Version	1.6.1
Document ID	SG-00153
Document version	3.1
Revision date	July.2022

Please read this User Manual thoroughly before you use this product.

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LAVP

Table of Contents

1. Product Description

Alamut™ Visual Plus is a comprehensive genome browser compliant with HGVS nomenclature and powered by multiple genomic sources and prediction tools displayed in an interactive interface to ease variant interpretation.
Alamut™ Visual Plus is an upgrade of Alamut™ Visual.

2. Intended Use/Purpose

2.1 Intended Use

Alamut™ Visual Plus is a Research Use Only (RUO) software that assists in routine genomic analysis. Alamut™ Visual Plus allows the visualisation of variants, transcripts, genomic sequences, and genomic data to simplify variant interpretation and pathogenicity assessment.

2.2 Intended User Profile

The application is intended to be used by trained medical professionals (clinicians, researchers, research technicians, etc.) working in the field of Genomics.

2.3 Intended Use Environment

The place of use is determined to be in a hospital, laboratory, or clinical setting, on a regular computer.

3. General Statement of the Test Principle(s) / Procedure

Alamut™ Visual Plus general procedure is as follows:

- Explore genomic regions
- Search for specific genomic data (genes, transcripts, variants)
- Visualise sequence files and variants from BAM, VCF or Sanger
- Access to multiple genomic databases and prediction tools
- Manual creation of genomic variants
- Curation of variant database, occurrences and history
- Import and export of private variant annotations
- Reporting of variant data
- Connect to external resources (API)

CONNECT

Alamut Visual Plus API allows to:

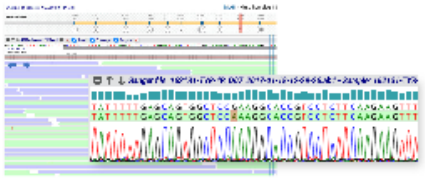
- Load/Explore a specific genomic region
- Load BAM/VCF and Sanger files
- Get variants and their annotations

EXPLORE

- GoTo feature: Look for gene/transcript/variant
- Full genomic and mitochondrial view
- Display a gene/transcript regions

VISUALIZE

- BAM and VCF Visualization
- Sanger alignement and visualization capability

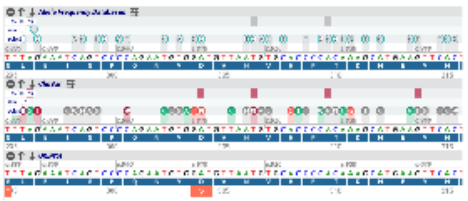


DATABASE IMPORT

VCF XLS

EVALUATE

- Display and configure specific tracks
- Visualize Alamut variant sources and databases
- Create variants and Private annotations



ANNOTATE

- Local Variant Databases
- Private Annotation Databases

CURATE

- Variant flagging
- Variant occurrence management
- Variant history

INTERPRET

- External Databases: ClinVar, gnomAD, ...
- Prediction Algorithms: splicing, missense
- ACMG Guidelines

EXPORT

XLS VCF

REPORT

Customizable Report

PDF

4. Product Components

The product is only composed of the Alamut™ Visual Plus software.

Alamut™ Visual Plus is installed with a floating license. Floating licenses can be used on multiple computers, with a limited number of concurrent users and are managed via a web page (extranet). Alamut™ Visual Plus extranet page: http://extranet.interactive-biosoftware.com.

Equipment and Materials Required, Not Provided:

The user needs to provide a computer, a keyboard, a mouse, and an internet connection according to section 5.1 of this user guide.

5. Specifications and Installation

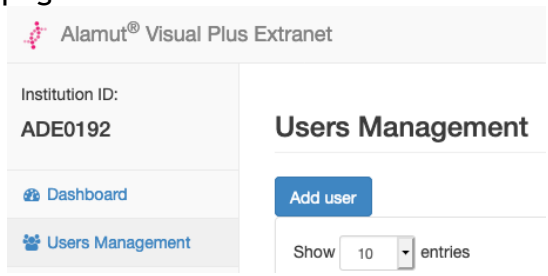
5.1 Specifications

Alamut™ Visual Plus requires the following technical specifications:

System Component	Minimum Requirement
Operating system	Microsoft Windows 7 or above, 64-bit version The program is available as an installer program (.exe) or self-extractable archive (.exe) or compressed file (.zip) Mac OS X- starting from Mac OS 10.14 (Mojave)
Internet Connection	Connection to the following IP addresses are required: • 20.224.43.76 • 212.83.147.70 The software handles connections through HTTP/HTTPS on port 80 and 443, optionally through a proxy server.
Hardware Requirements	Computer: 1.5GHz+ – 8GB RAM – 500MB free disk space. Display screen resolution: 1024x768 pixels The software program does not alter system directories or the registry. Write permissions are required on the software directory to ensure continued functioning of the application and to save user parameters.
Installation Instructions	Alamut Visual Plus should be installed locally. We do not recommend sharing a same settings folder between multiple users. Furthermore, having different versions installed and used alternatively is not a good practice, since the databases schema can evolve from one version to another.

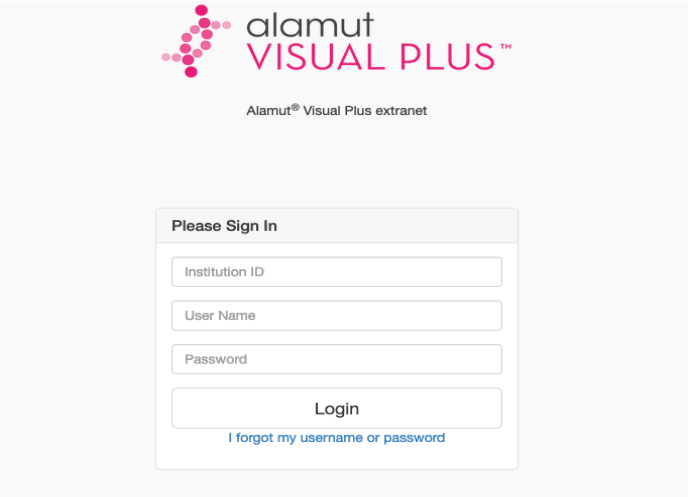
5.2 Installation

Once the Administrator's credentials have been created, the Administrator must log-on to the Alamut™ Visual Plus extranet page and create a new user account through the "Add user" button.

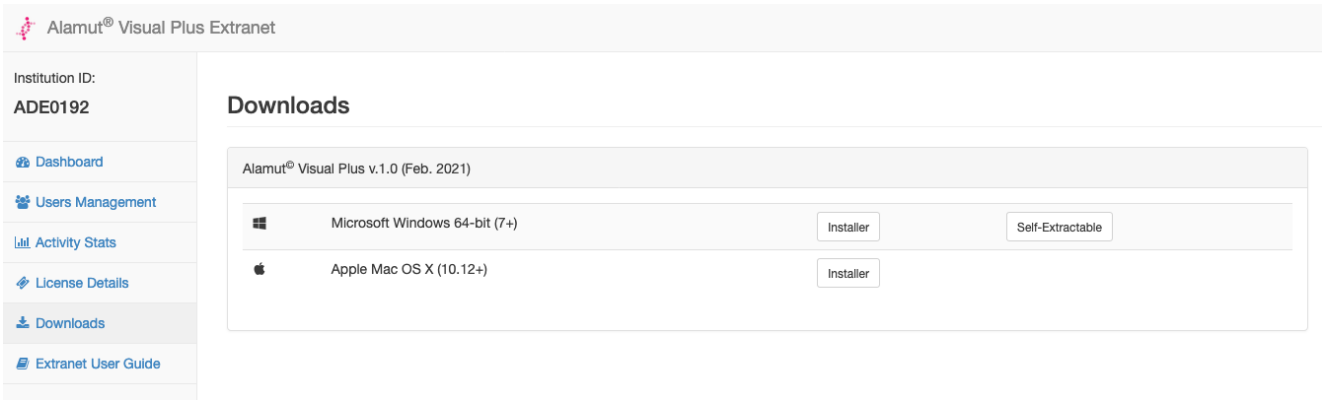


The new user will receive the Institution ID, Username and Password via e-mail.
If the recipient does not receive the e-mail, the administrator can retrieve a unique link allowing them to complete their user account. In the "Users Management" page of the extranet, the administrator can copy into their clipboard the URL by clicking on button and paste it in a direct e-mail to the concerned user.

To install the program, the new user must open the Alamut™ Visual Plus extranet page and sign in with the Institution ID, Username and Password provided.



Alamut™ Visual Plus binaries can be downloaded from the "Downloads" section.



5.2.1. Install on Microsoft Windows

Download the Alamut™ Visual Plus Installer or Self-Extractable executable (.exe) from our extranet page: http://extranet.interactive-biosoftware.com.

- From the Installer: execute it and choose an installation folder where you have "write" permissions
- From the Self-Extractable executable (.exe) file: double-click on the installer to launch the installation program and follow the instructions presented to you. Select a folder where you have "write" permissions.

Once the program is installed, the files used for the installation can be removed.

5.2.2. Install on Mac OS X

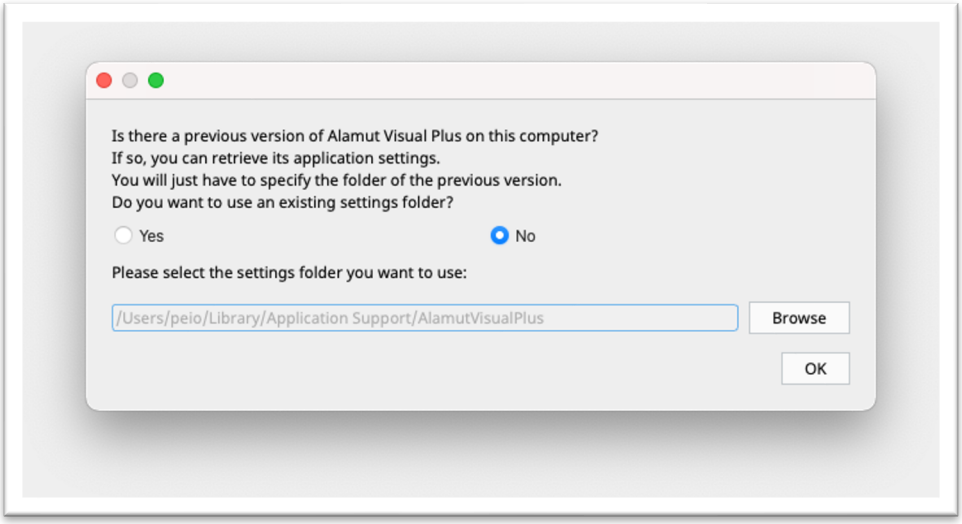
Download the Alamut® Visual Plus (**.dmg**) file from the extranet page: http://extranet.interactive-biosoftware.com.

1. double click the **.dmg** file to make its contents available
2. drag the application from the **.dmg** window into the /Applications folder to install (may need an administrator password)
3. You will also have to authorize the installation of this software by clicking on apple sign > system preferences > security and privacy > lock sign > allow alamut visual plus software
4. wait for the copy process to finish
5. Close the .dmg window
6. delete the **.dmg** from Downloads
7. Now start using the Alamut from the applications folder for the first launch as described in 5.3

5.3 Post installation: First launch

To set up Alamut™ Visual Plus, launch the software and:

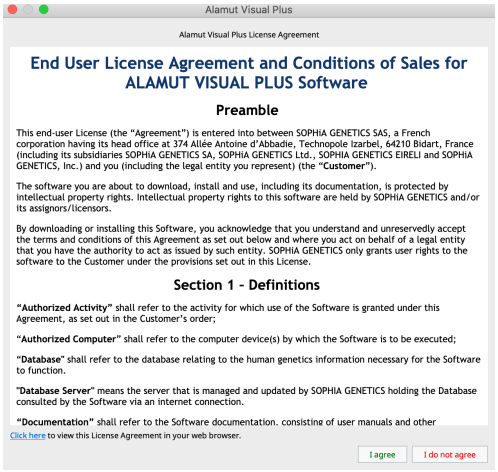
- Define the application's data folder, where Alamut™ Visual Plus will store its settings. To retrieve your current settings and databases, you may decide to reuse an existing folder created by an older version of Alamut™ Visual Plus. By selecting 'No', settings will be restored.



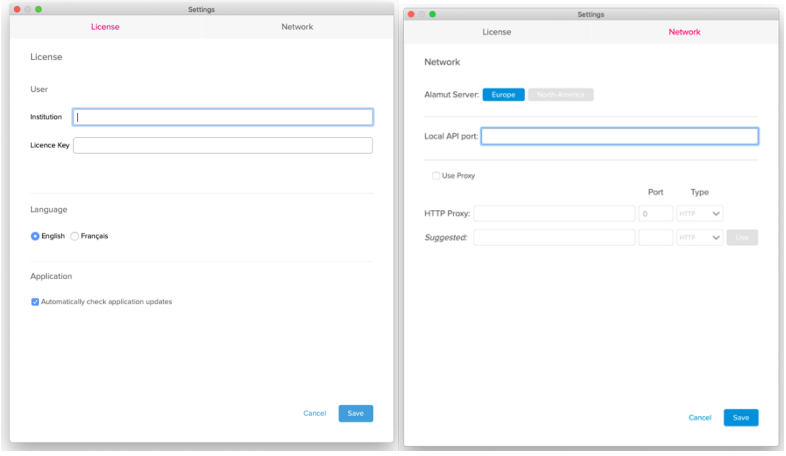
Note: If, for quality purpose, you do need to validate a new version of Alamut™ Visual Plus before distributing it in your institution and that you use the shared database feature, we recommend you follow these steps (this will require you to install Alamut Visual Plus in a different location):

- **Copy the .db files of your current shared databases and store them into a new folder**
- Install the Alamut™ Visual Plus version you wish to validate, when asked for, choose to **use a new settings directory**
- From the Local Variant Databases menu, use the "Add existing database" feature and add the databases you have previously copied
  If an upgrade of the database is needed, it will be automatically applied at this point.
- Validate Alamut™ Visual Plus
- Uninstall Alamut™ Visual Plus
- Install Alamut™ Visual Plus and **choose the settings directory used by the previous version of Alamut Visual Plus**

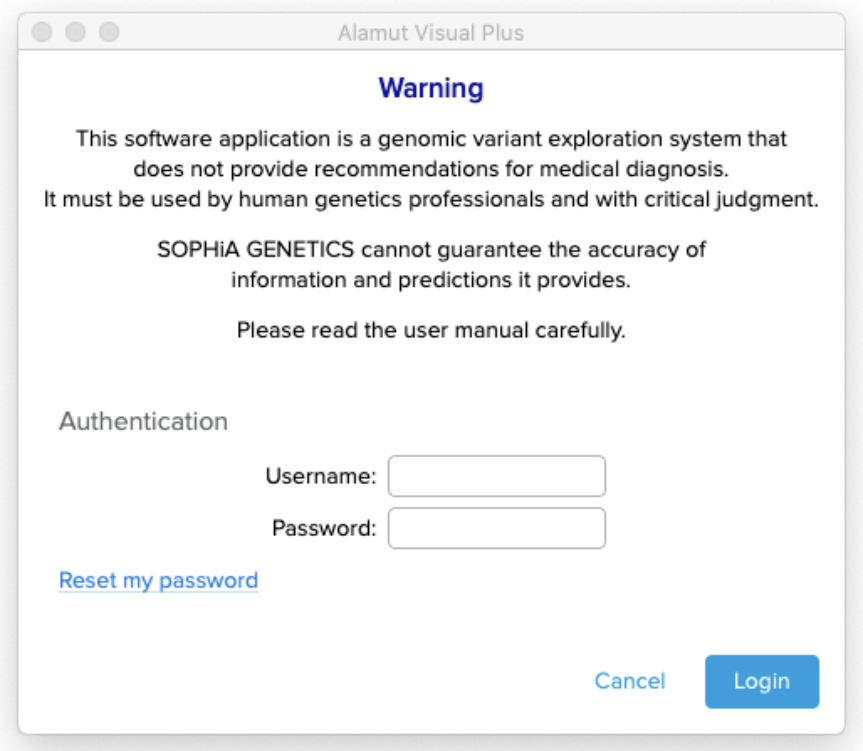
- Accept the End User License Agreement.



- Provide
  - Your institution code and your license key
  - If any, information relating to your proxy server



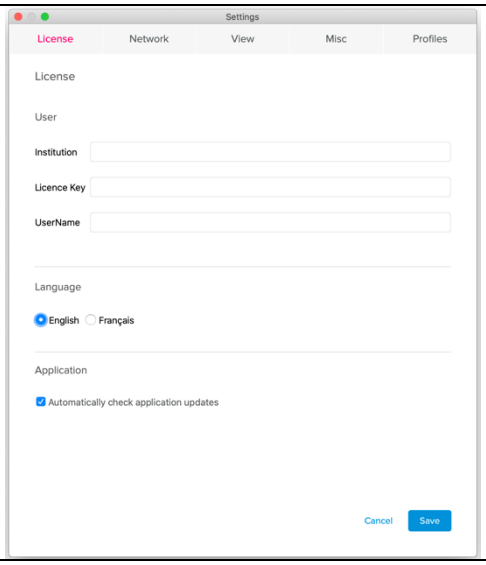
Once completed, users will only provide their username and password to connect to the application. The password will be renewed by the user every 90 days.



5.4 Getting started: settings

The Application Settings window includes the following tabs: License, Network, View, Misc. (Miscellaneous)

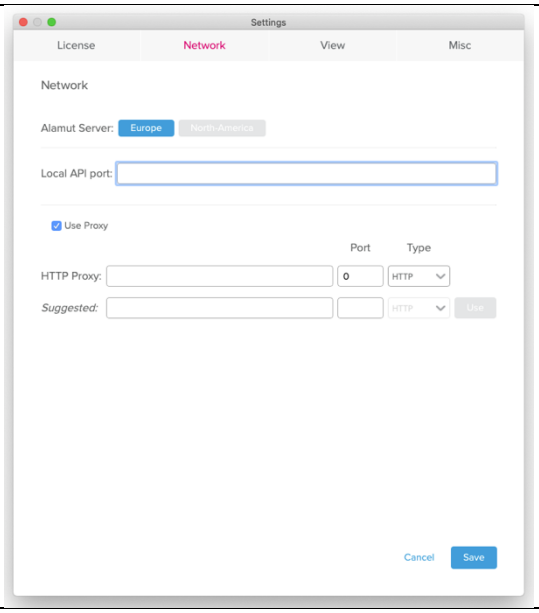
License

Institution: the code of the institution that purchased the license **License Key:** the license key of the institution **User Name:** the name of the connected user **Language:** Select your preferred language (English or French). **Application:** Tick the box to automatically check for updates.	

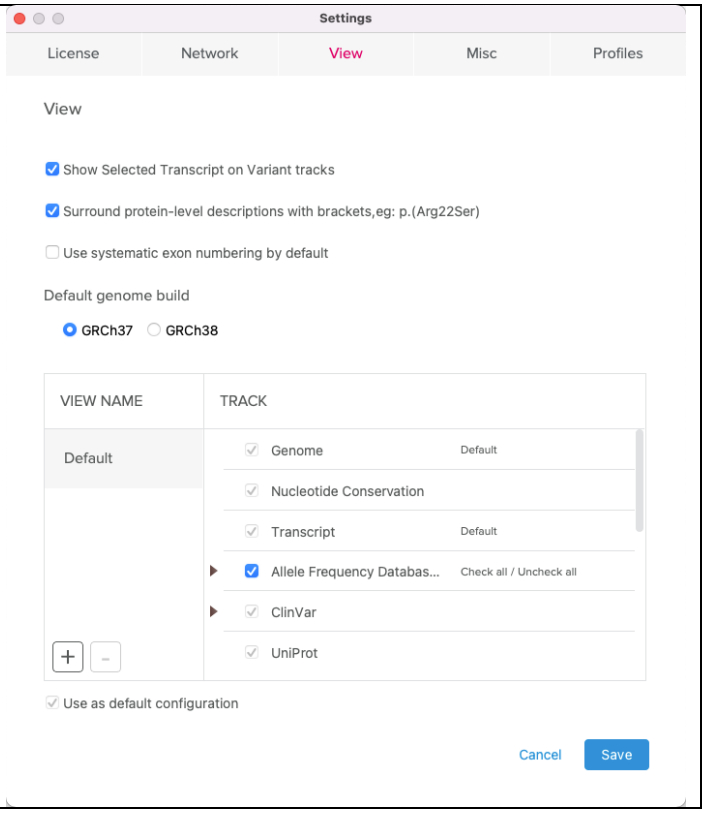
Click Save before leaving the tab. These details will be saved for future log-ins.

Network

To connect via proxy complete the necessary information in the Network tab (this may require the input of your IT administrator).

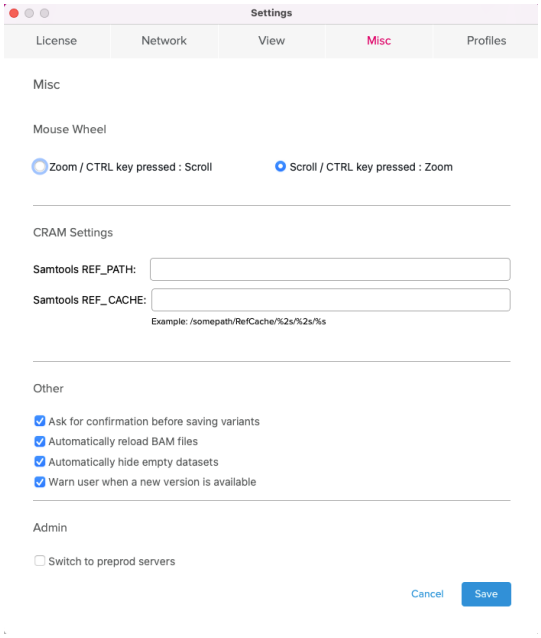
Use Proxy: To use a proxy server, tick the Use Proxy box and provide the required information to connect to it.	

View

Show Selected Transcript on Variant Tracks: select to view the selected transcript on the tracks. **Surround protein-level descriptions with brackets:** select to use brackets in pNomen. **Use systematic exon numbering by default:** select to use systematic exon numbering by default for transcripts. **Default Genome Build (GRCh37 or GRCh38):** select which genome build you would like to use by default. **View table:** See part allows you to create custom views. For more information, see Section 10.2.	

Miscellaneous

Mouse Wheel: two mouse scroll options are available to define the behaviour of the application when using only the mouse wheel or the mouse wheel in combination with the CTRL key.



CRAM Settings: CRAM handling in Alamut is based on Samtools. Samtools needs the reference genome sequence to decode a CRAM file. Samtools can use either the REF_PATH or REF_CACHE environment variables to find reference sequences.
It uses the MD5 sum of each reference sequence as the key to link a CRAM file to the reference genome used to generate it (see also the Samtools man page). You will need to provide the path to MD5 reference sequences in the REF_PATH or REF_CACHE field unless you use CRAM files with embedded reference sequences.
For your convenience we have prepared a package of reference MD5 files for GRCh37 and GRCh38 primary sequences. It is available at:
http://downloads.interactive-biosoftware.com/CRAM/RefCache.tgz

For instance, if you uncompressed this file to the D:\SAM folder under Windows, the REF_CACHE should be: D:\SAM\RefCache\%2s\%2s\%s

Ask for confirmation before saving variants: select to confirm each time you save a variant into a Local Variant Database.

Automatically reload BAM files: select to automatically reload BAM files when opening a new tab from a tab with BAM track.

Automatically hide empty datasets: select to automatically hide empty datasets in Private Annotation Track when opening a new tab.

Profiles

In this tab, you can activate and manage the Generic Profile feature. This feature allows to share configurations among users using a same License Key.

The elements shared through a profile are:
- the gene shortcuts
- the preferred transcripts
- the default assembly
- the genome area view configuration
- the Local Variant Databases
- the Private Annotation Databases
- the default configuration for BAM visualization
- the options for splicing visualization

By default, this generic profile feature is disabled, and the configuration of the local installation is used.

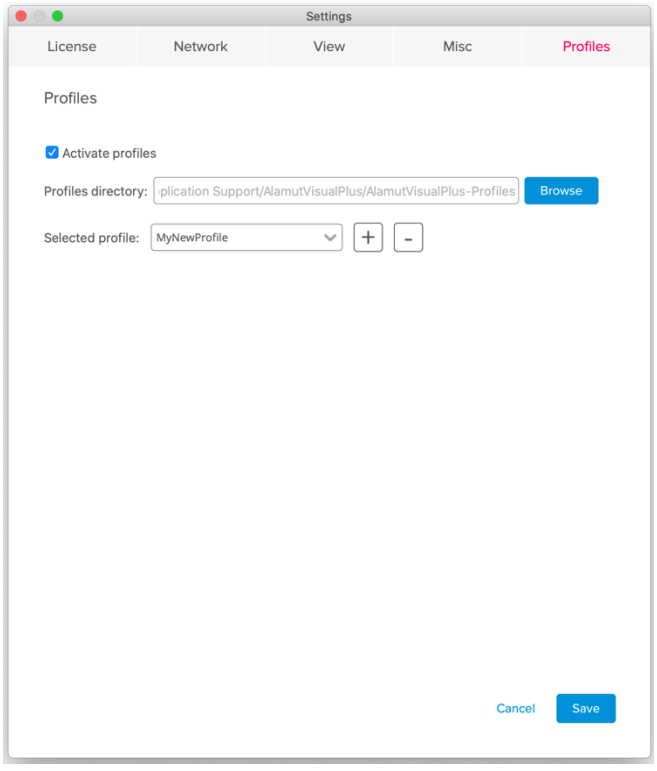
Note: When a profile is activated, any changes on the elements mentioned above update the profile.

Activate Profiles:
Use this checkbox to enable/ disable the feature

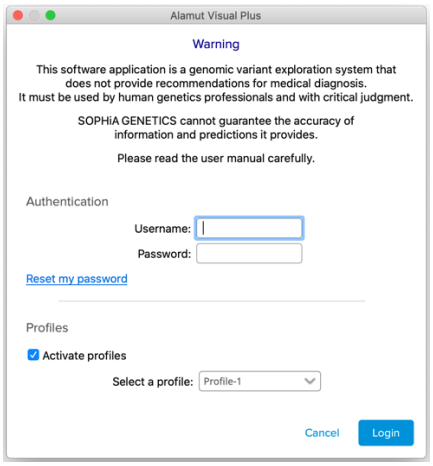
Profile Directory:

- When activating the feature, you must choose a Profile Directory. This directory is used by the application to store the profiles relative files.
- If the directory does not contain any profile linked to your license key, you must first create a profile. Profile names must be unique within a profile directory, regardless of the license key of the profile.
- You can update your profile directory at any time by clicking on the Browse button.

Selected Profile:
You can use the combo-box to choose your current profile. Use the + and - buttons to create or delete profiles.



When the profile feature is enabled in the settings tab, you can select the profile you wish to use directly from the login dialog.

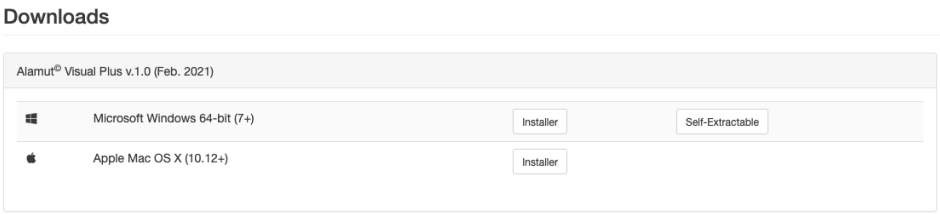


Since profile directories are shared by several users, caution must be taken so that multiple people don’t edit it simultaneously.

5.5 Manual update

If you wish to update the application manually, please follow these steps:

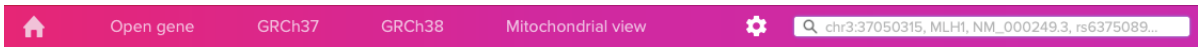
- Connect to our extranet page (http://extranet.interactive-biosoftware.com)
- select the downloads section
- if available download the latest version.



Or click on the “Update” button in the left part of the Alamut™ Visual Plus homepage

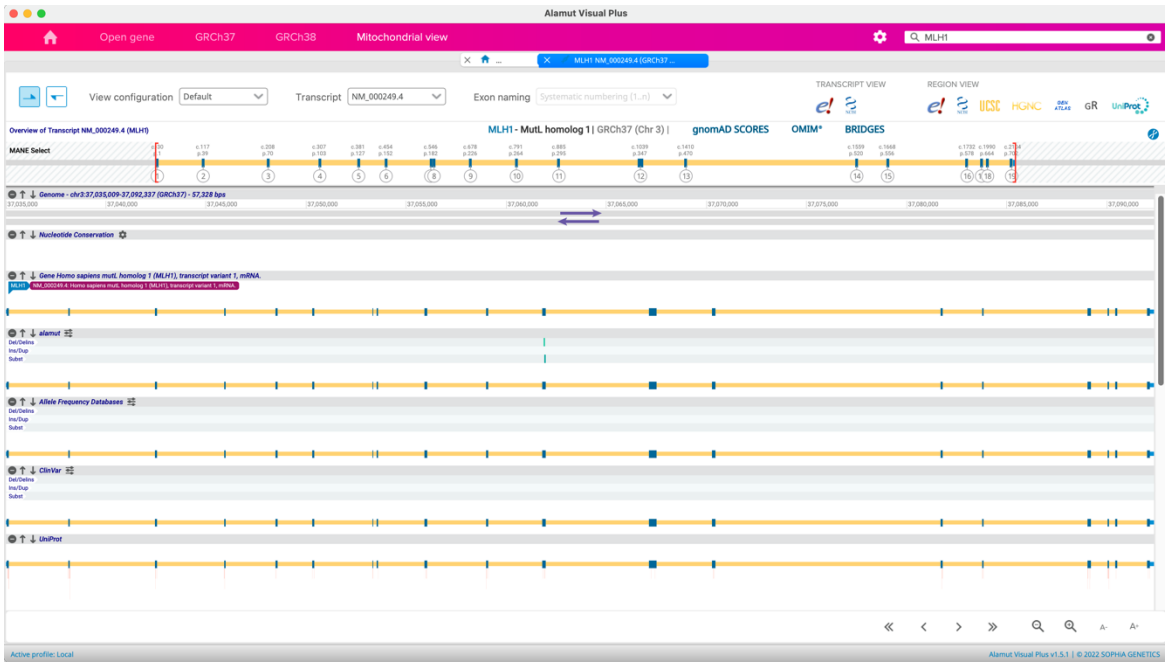
6. Explore

6.1 Navigation

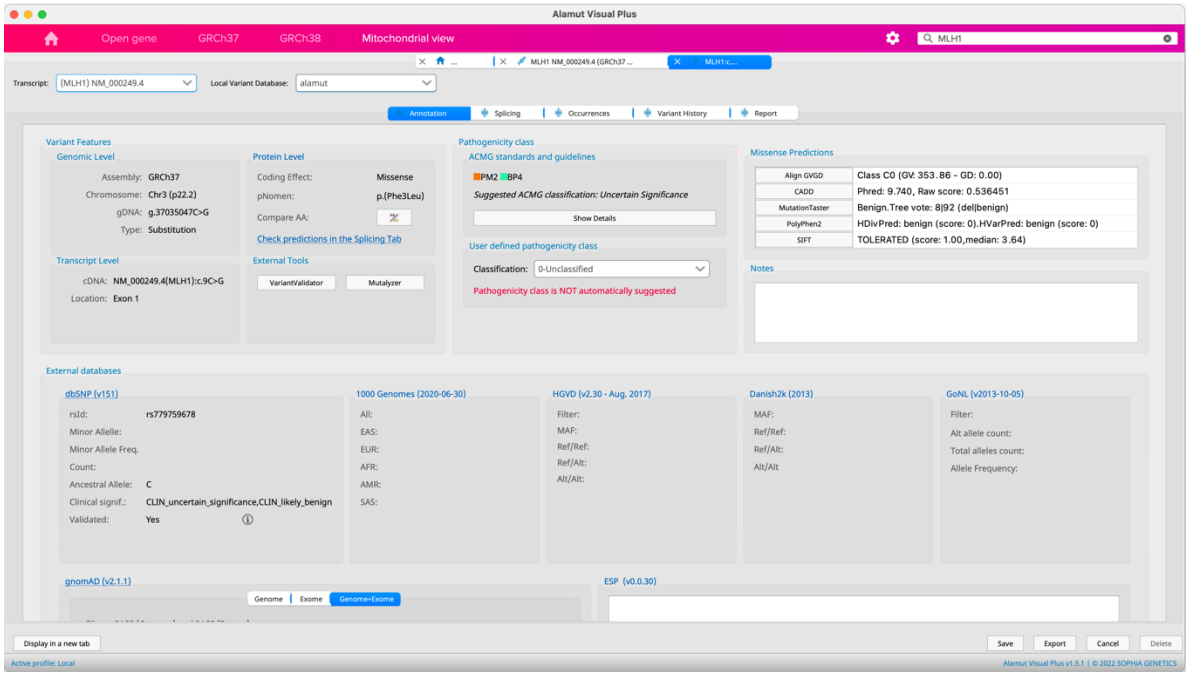


From the top navigation bar, you can open a gene, a genome assembly (GRCh37, GRCh38 or the mitochondrial genome) or directly perform a search. This allows you to study either a genomic region or a specific variant.

The exploration of a genomic region is done through the genomic view in which all internal and external data are gathered and organized in tracks.

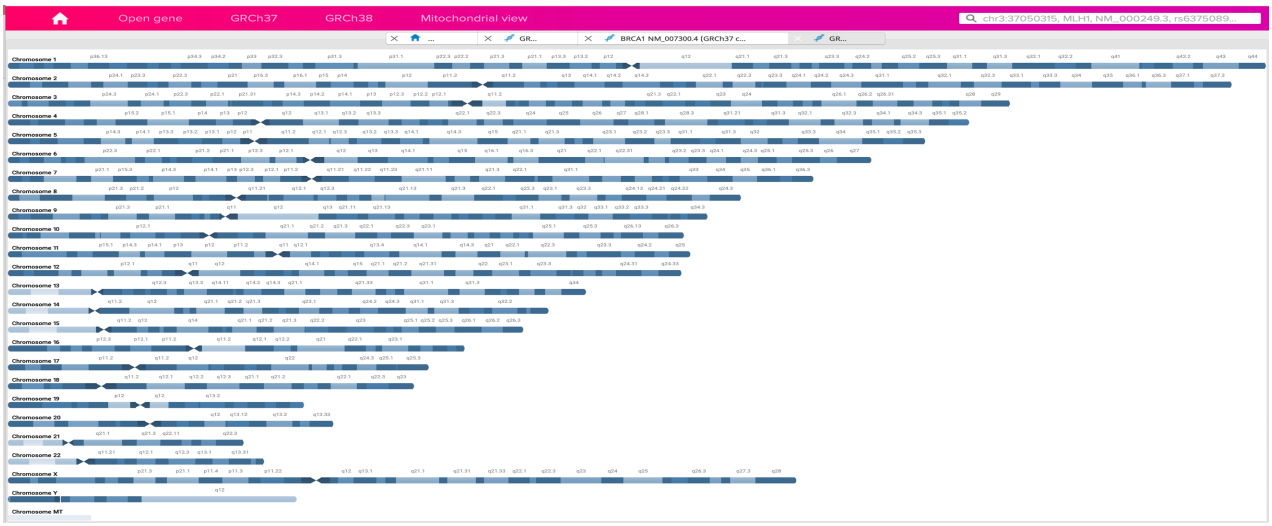


Information about a specific variant will be displayed into the Variant Panel interface. It gathers all external source data () and give access to some other variant oriented features (see section 11 for more information).

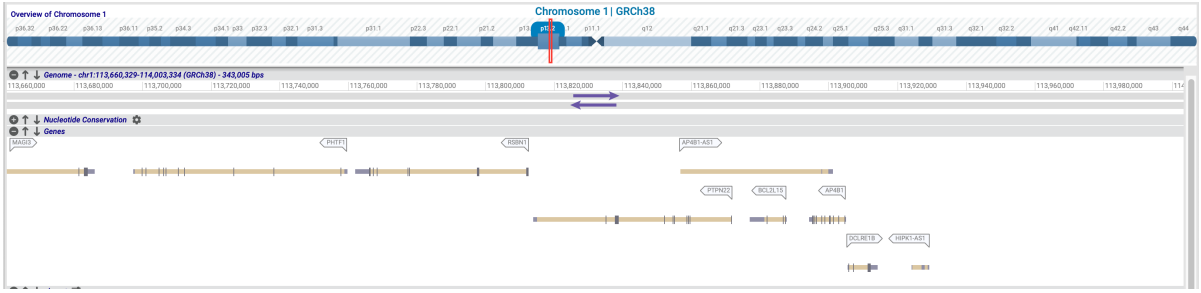


6.2 Full Genomic Views

The Genomic Views allows visualisation of all chromosomes and mitochondrial DNA. You can visualize structural variants, intergenic and regulatory regions. You can access to a Genomic Views by using the ‘GRCh37’, ‘GRCh38’ and ‘Mitochondrial view’ buttons of the navigation bar.



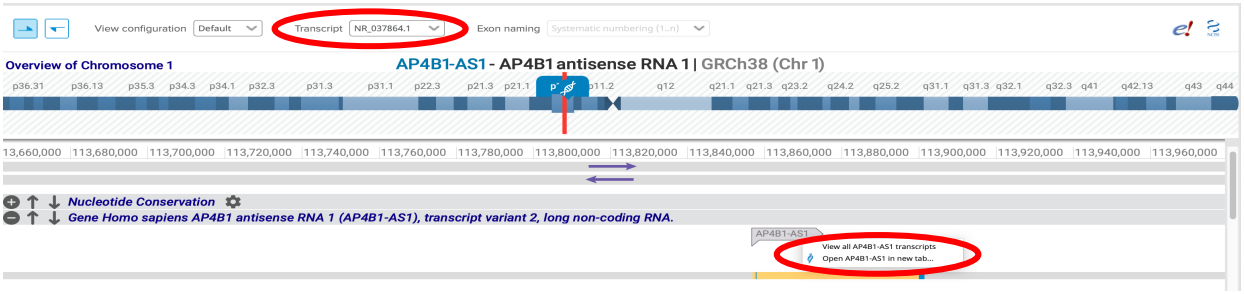
Select the chromosome you wish to study, by clicking on it. The overview of the selected chromosome will open. Select a specific chromosomal region and zoom in to view the genes available in the selected band. Genes are displayed with name tags along the strand.



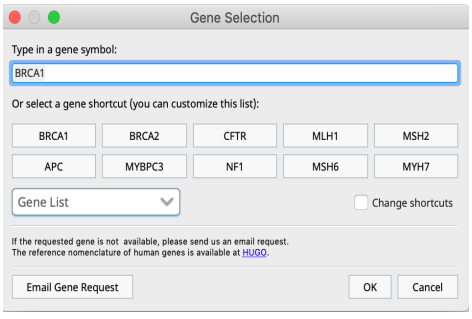
6.3 Transcript overview

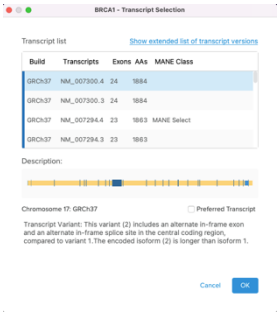
You can have access to the transcript view from:

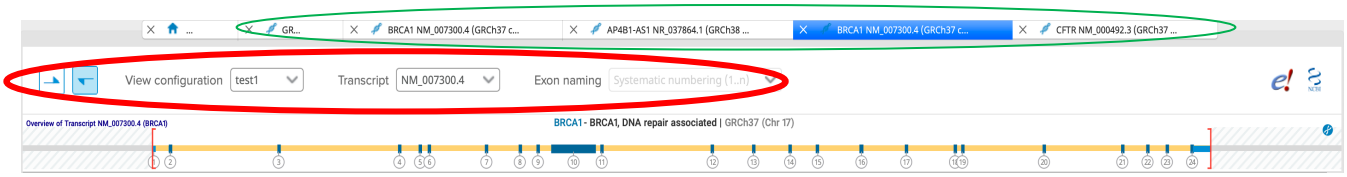
- the genomic views, by clicking on your gene of interest. You can display all transcripts related to one gene. The name of the transcript will be displayed in the transcript box.



- the ‘Open gene’ button in the homepage. In the Gene Selection window, to select a gene, you can either type a gene symbol or use shortcuts. Shortcuts are configurable. Once your gene selected, you will have to select your transcript of interest via the Transcript Selection window. The transcript of your selected gene is displayed in the overview, with the exons in blue and introns in yellow.



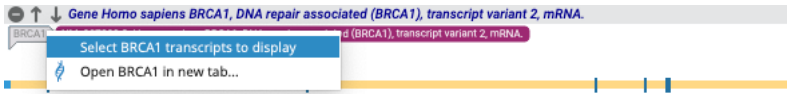




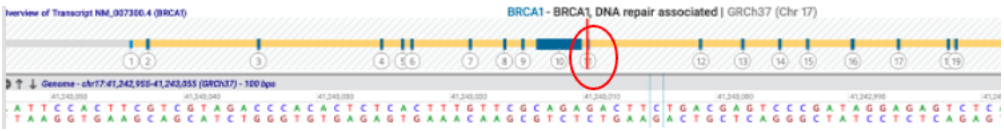
Notes

- The reading direction of the gene (i.e., the DNA strand) can be changed by using the forward and reverse direction icons on the toolbar.
- The transcript and exon nomenclature (if both Systematic and Custom options are available for this transcript) can be modified interactively.
- The view configuration can be modified. You can use different view configurations in different tabs.
- Multiple tabs can be opened showing different genes/transcripts.
- It is also possible to display all available transcripts simultaneously.
- When available, the "Transcript selection" tab allows the display of up to four versions of one transcript.

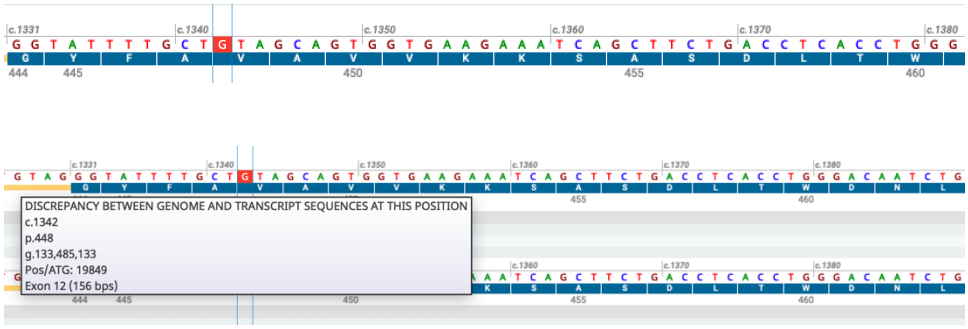
Several transcripts can be shown in the interface by exploring options in the transcript track by right clicking on the gene name.



Click on an exon number in the gene overview to zoom in directly and see the nucleotide sequence.



Note: Mismatches between transcript and reference genome sequences can be found. Transcript nucleotides are highlighted in **red** where they differ from the reference genome sequence and the tooltip gives a warning message.



6.4 GoTo Feature / search bar

Alamut™ Visual Plus provides a search bar to access the following:

Standard Genetic References

Reference	Example
Official HGNC gene symbol	MLH1
HGNC Id	HGNC:7127
cDNA RefSeq Id	NM_000249.3
Ensembl Transcript Id (mapped to a NCBI RefSeq)	ENST00000231790
LRG Id	LRG_1
Protein RefSeq Id	NP_000240.1
UniProt Id	P40692
Reference SNP Id	rs63750891
OMIM Id	OMIM:120436

Genomic, cDNA and protein positions

Reference	Example
Standard gDNA query	chr3:g.37050315
Short gDNA query	3:37050315
Interval gDNA query	3:36000000-38000000
gDNA query inside current gene	g.37050315
gDNA query with assembly	chr3(GRCh37):g.37059038
cDNA position query	NM_000249.3:c.464
Short cDNA position query	NM_000249.3:464
cDNA position query on Ensembl Trancript Id (mapped to a NCBI RefSeq	ENST00000231790:c.464
NM_000249.3:p.155	NM_000249.3:p.155
Protein substitution	NM_000249.3:p.Leu155Arg

Genomic and cDNA variants

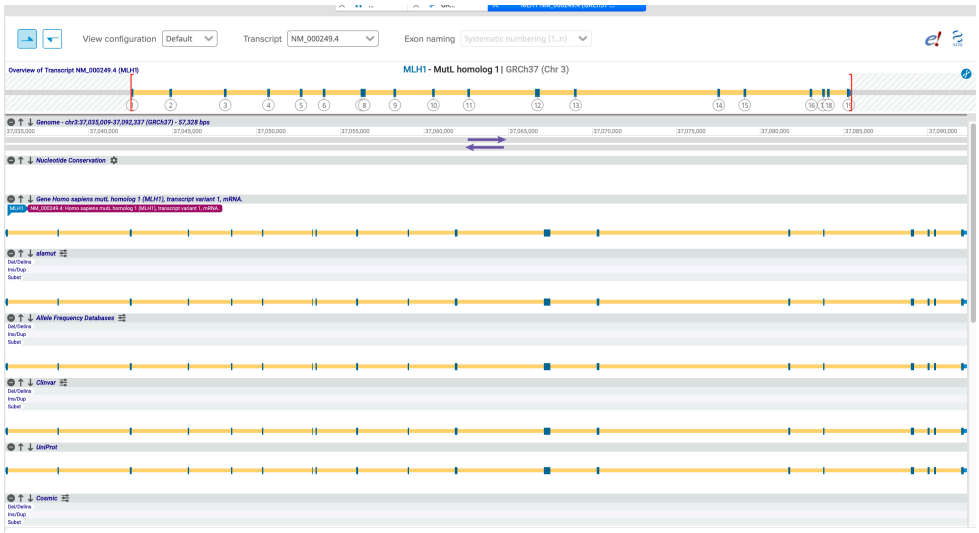
Reference	Example
Query with genomic variation - **Substitution**	chr3:g.37050315T>G
Query with genomic variation - **Insertion**	chr3:g.37050315_37050316insTG
Query with genomic variation - **Deletion**	chr3:g.37050315del
Query with genomic variation - **Delins**	chr3:g.37050315_7050320delinsAA

Query with genomic variation - **Duplication**	chr3:g.37050315dup
Short query with genomic variation	3:37050315T>G
Use given assembly	Chr3(GRCh37):g.37067317G>A
cDNA variant	NM_000249.3:c.464T>G
cDNA variant on Ensembl Trancript Id (mapped to a NCBI RefSeq)	ENST00000231790:c.464T>G
A given position from the transcript view	4421

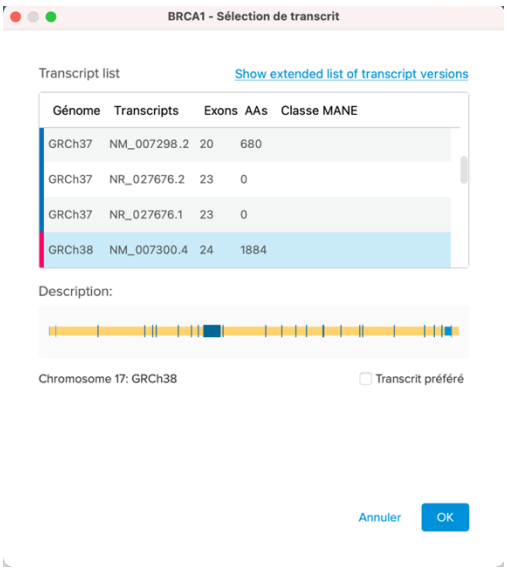
- **Note:** The "GoTo" bar also enables to search for the gene and the codon position copy-pasted from an excel file (e.g. MLH1:c.12 or MLH1:12).

Depending on the query type, Alamut™ Visual Plus may:

- Open a gene and one of its transcripts

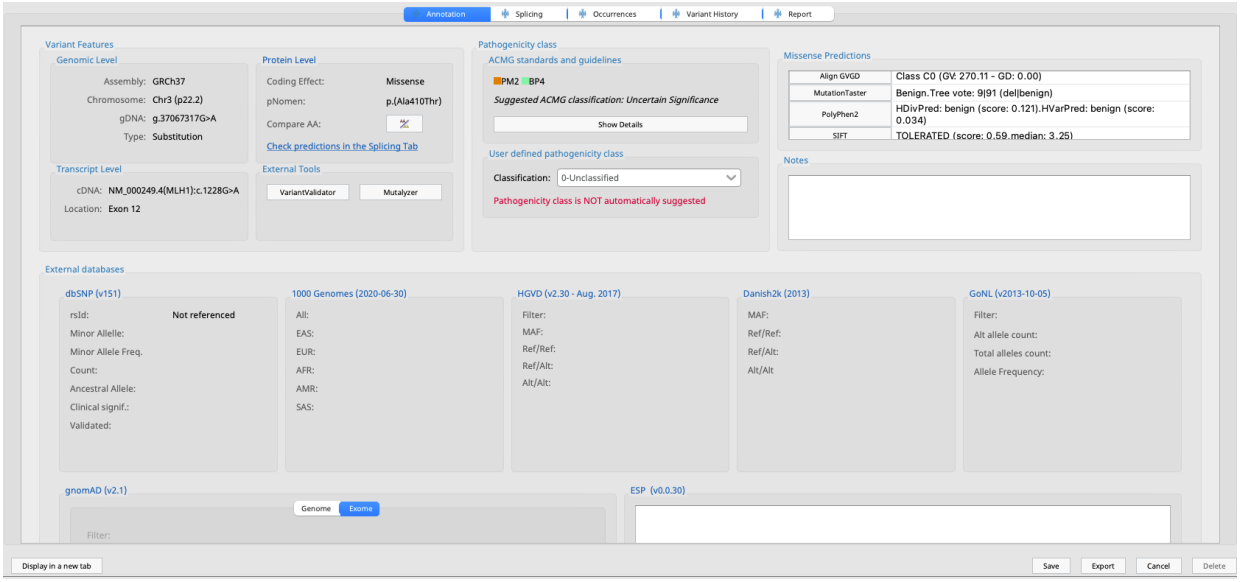


- Open a transcript selection dialog



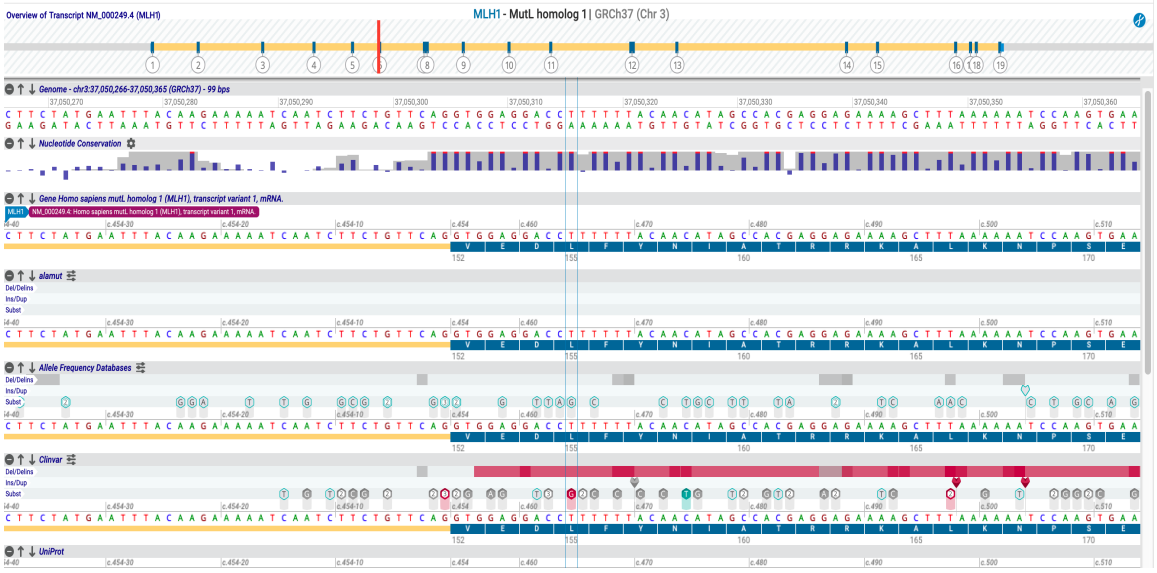
The current transcript selection dialog shows a short list of transcript versions (up to 2 versions). Clicking to the link “show extended list of transcript versions” will display the full available transcript versions for this gene.

- Open the variant panel

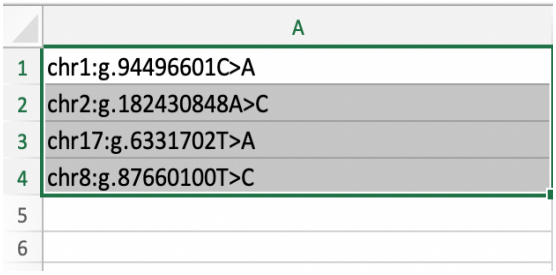


The variant panel windows allow to display all variant related data into one single interface. For more information, see section 11.

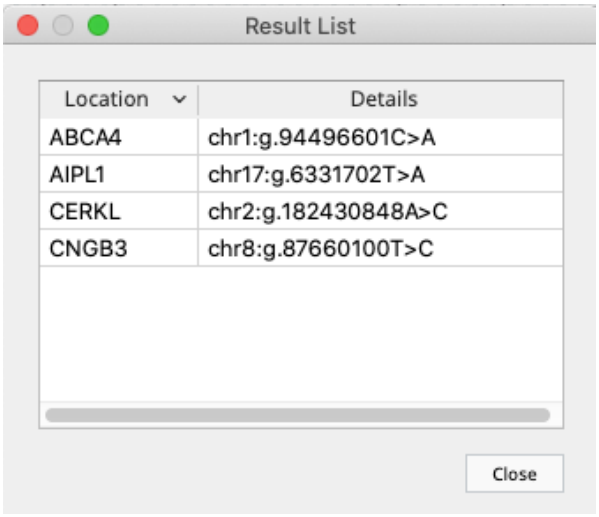
- Directly focus on a genomic location



Moreover, several lines from NGS output file can be copied and pasted into the search bar.

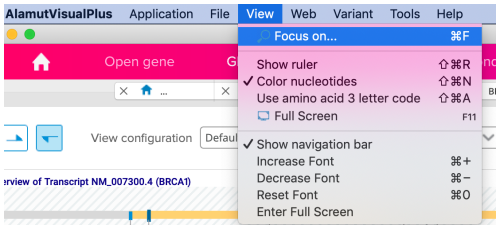


The pasted data then shows up in a variant list. Double clicking on a row will open the selected variant in a new window.



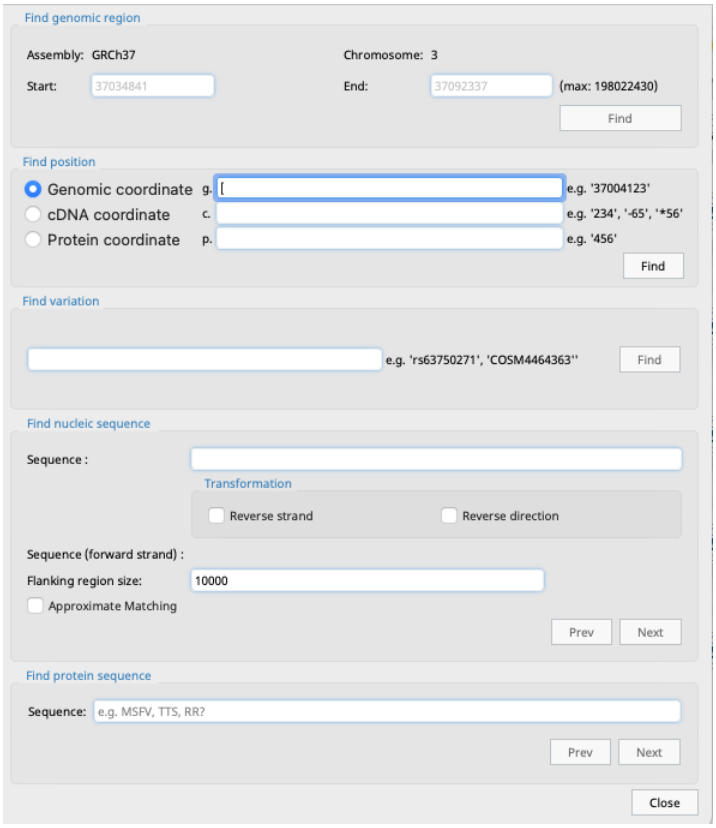
6.5 "Focus On" Feature

The feature 'Focus on' allows you to search for genomic coordinates and more. This feature is available from the **"View > Focus on"** menu.



This functionality allows the user to find:

- genomic region
- genomic position
- cDNA position
- protein position
- variation
- nucleic sequence
- protein sequence



On the “Find nucleic sequence” box, two options are available to look for nucleic sequences:

- The reverse strand checkbox will allow to look for a nucleic sequence onto the reverse strand.
- The reverse direction checkbox will look for a nucleic sequence following the reverse strand direction.

The “Flanking region size” field delimits the region adjacent to the given gene when searching for a sequence.

The approximate matching checkbox can be used to find a sequence that are similar to the reference sequence allowing to check for any errors, such as mismatches or indels (for more information).

7. Visualize

7.1 Viewing BAM/CRAM alignment

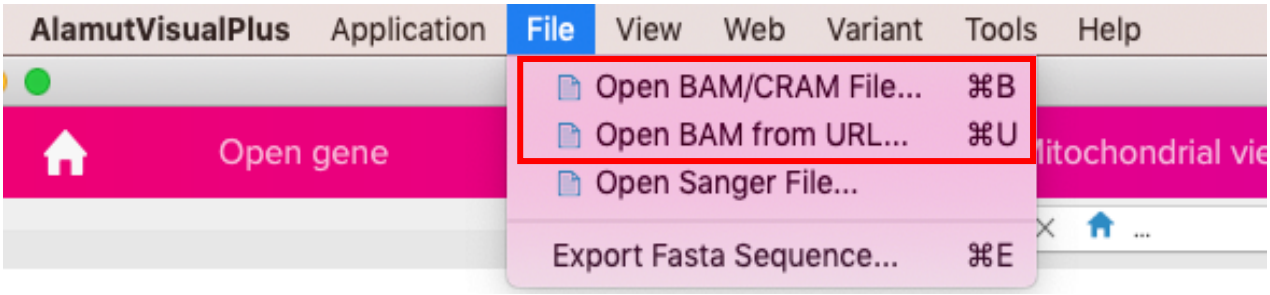
Alamut™ Visual Plus allows to visualize alignment from BAM and CRAM files.

Loading BAM and CRAM files

Two options are available to load alignment files once your gene of interest is opened:

1. Select menu "File > Open BAM/CRAM File" to load one or more BAM or CRAM files from your computer or local network.
2. Select menu "File > Open BAM from URL" to load a BAM file from a web server. HTTP and HTTPS protocols are supported.

Using CRAM files usually requires defining the location of reference sequences. (See Miscellaneous)

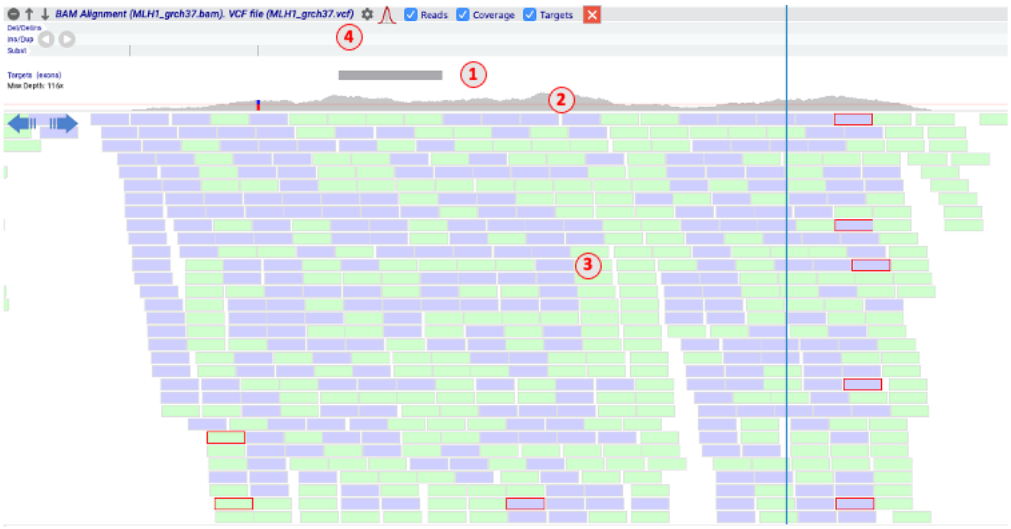


Notes:

- One or multiple alignments can be uploaded for each gene.
- When opening a new gene in a new tab, the BAM file will be automatically uploaded if the option “Automatically reload BAM files” is enabled in the “Settings” > “Misc” options.

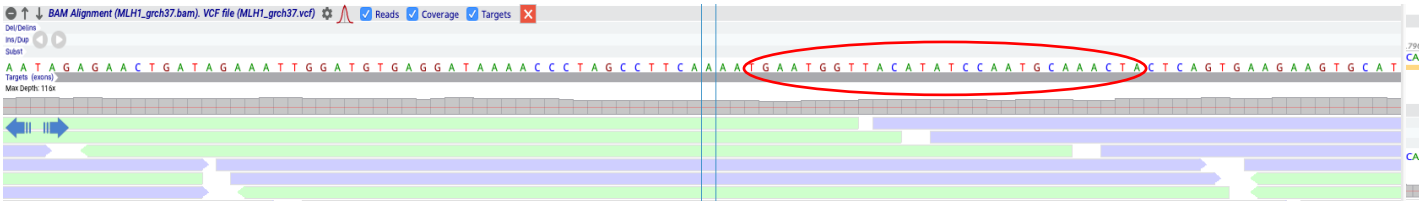
BAM Viewer components

The BAM track includes different components

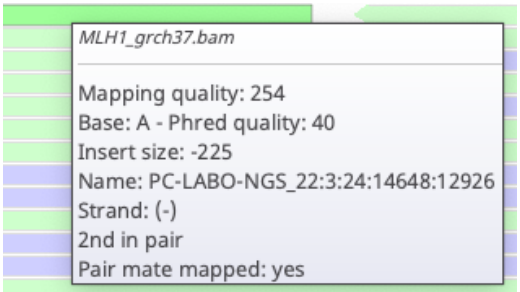


- Sequencing targets
- Depth of coverage
- Reads
- Variants

When zooming to nucleotide level visualization, the genomic reference sequence is displayed above the targets sub-track:



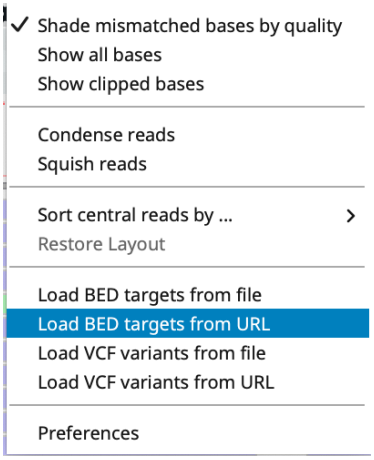
Hovering over the read will display information related to reads as indicated in the BAM file:



Viewing sequencing targets

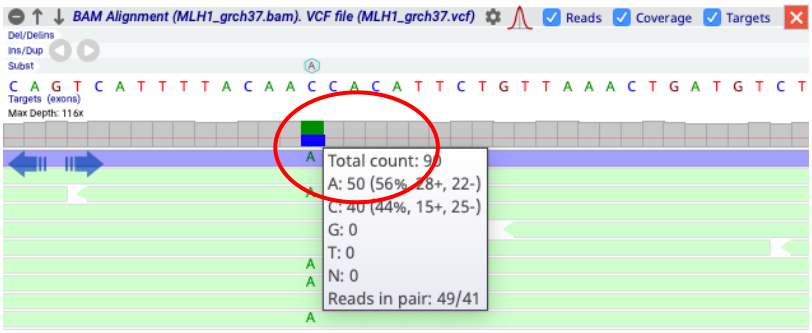
In Alamut™ Visual Plus, sequencing targets are supposed to cover current gene's exons with some exon-flanking intronic bases (20bp, by default):

You can also load targeted regions from BED files in the BAM track. From the BAM track ‘Options’  or by right clicking on the BAM track. Select "Load BED targets from file" to load a BED file from your computer or select "Load BED targets from URL" to load a BED file from a web server. HTTP and HTTPS protocols are supported.



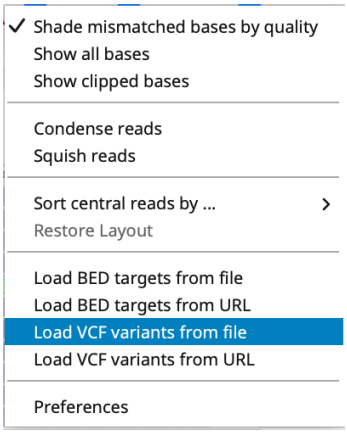
Viewing variants

Single nucleotide variants (SNVs) are automatically detected where non-reference bases are called at a frequency above the 'Allele frequency threshold' defined in the Preferences panel. Jump from one SNV to the other by clicking the arrow buttons on the left of the coverage sub-track.



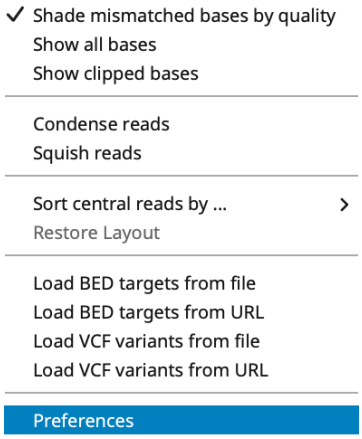
By default, the VCF is loaded when importing the BAM file if it is located in the same folder with same file name.

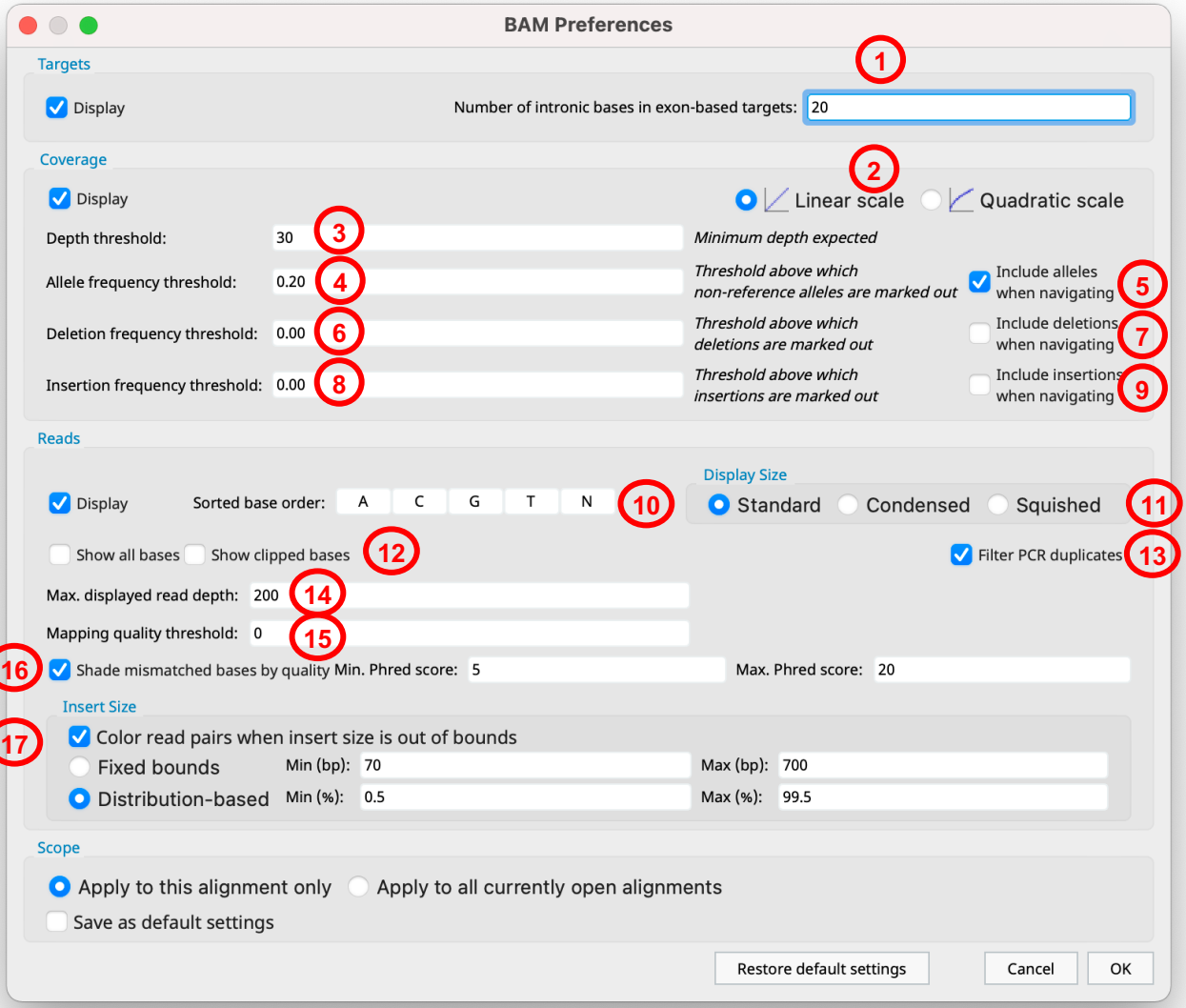
From the BAM track ‘Options’  or by right clicking on the BAM track. Select "Load VCF variants from file" to load a BED file from your computer or select "Load VCF variants from URL" to load a BED file from a web server. HTTP and HTTPS protocols are supported.



Visualization options

BAM preferences are accessible through the BAM track 'Options'  or by right clicking on the BAM track.





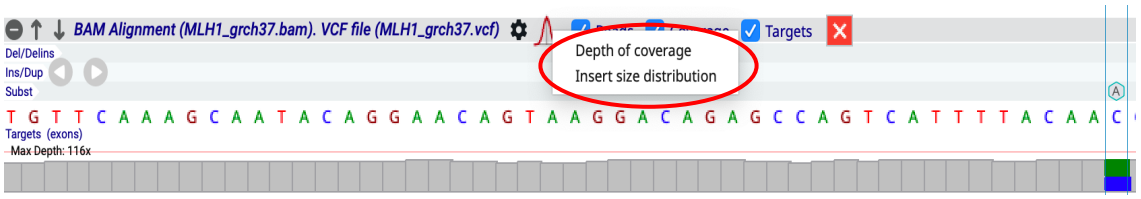
1. By default, sequencing targets are supposed to cover current gene's exons. This setting specifies the number of exon-flanking intronic bases to add to exon-defined targets.
2. The coverage histogram can either be displayed using a linear scale where the height of each bar is directly proportionate to the depth value or using a quadratic scale where low depth values are increased, and high depth values are decreased.
3. Targets are highlighted with red color where coverage depth is below this threshold. Besides, detected SNVs are only reported at positions where coverage is above this threshold.
4. Single nucleotide variants (SNVs) are detected where non-reference bases are called at a frequency above this threshold. Value given in % (0.2 means 20%).
5. The navigation arrows of the BAM track will consider single nucleotide variants (SNVs) when navigating through variants, when this checkbox is ticked.
6. Deletions are detected where they appear in reads at a frequency above this threshold.
7. The navigation arrows of the BAM track will consider deletions when navigating through variants, when this checkbox is ticked.
8. Insertions are detected where they appear in reads at a frequency above this threshold.

9. The navigation arrows of the BAM track will consider insertions when navigating through variants, when this checkbox is ticked.
10. When sorted through the “Sort central reads by base” contextual menu option, reads will be sorted according to the order defined here (A first, C second, etc in this example).
11. These settings define the graphical height of reads.
12. If 'Show all bases' is not checked, then read bases are displayed only if they differ from the reference sequence. Some NGS data processing tools "soft-clip" bases at either end of reads if appropriate. Checking the 'Show clipped bases' option reveals these bases. This option should usually remain unchecked.
13. Alamut™ Visual Plus does not itself detect PCR duplicates. Reads marked as PCR duplicates in the BAM file are removed if this option is checked.
14. This setting only affects the graphical display of reads, not computations.
15. Reads with a mapping quality under this threshold are removed.
16. If this option is checked, then called bases with a Phred score under the specified minimum threshold are not displayed. Bases with a Phred score between the specified minimum and maximum thresholds are shaded. Bases above the maximum threshold are displayed in full color.
17. Based on insert size values provided in the BAM file, paired reads too distant or too close from each other are highlighted if this option is checked. Expected normal insert sizes can be expressed as fixed values or as a percentage over the distribution. If the insert size is large, denoting a deletion, reads are colored red. If the insert size is small, denoting an insertion, reads are colored blue.

Viewing BAM alignment statistics

Alamut™ Visual Plus computes descriptive statistics from BAM file (depth of coverage and insert size). These statistics are computed for the current displayed gene locus.

Click on the picture (red histogram near options in the BAM track) and, then select either "Depth of Coverage" or "Insert Size Distribution" from the menu.



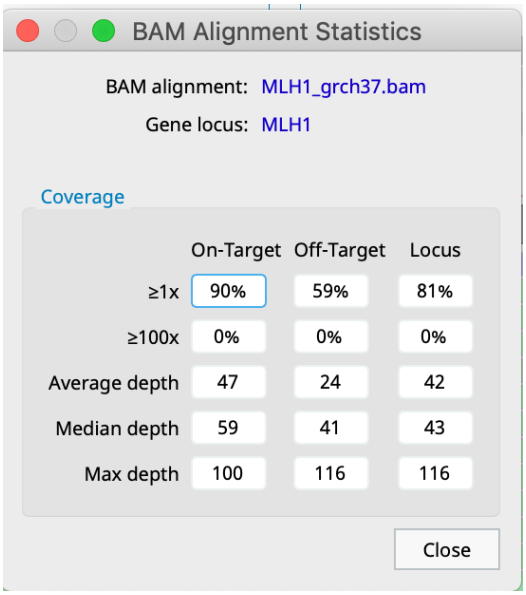
To display the depth of coverage statistics, select "Depth of Coverage" from the menu. A table summarizes for the current gene locus, the following statistics:

- **Depth of coverage 1X**: the percentage of a region that is covered by at least one read.
- **Depth of coverage based on a user defined coverage threshold**: the percentage of a region that is covered by at least this coverage threshold.
- **Average, Median and Maximum of the depth of coverage.**

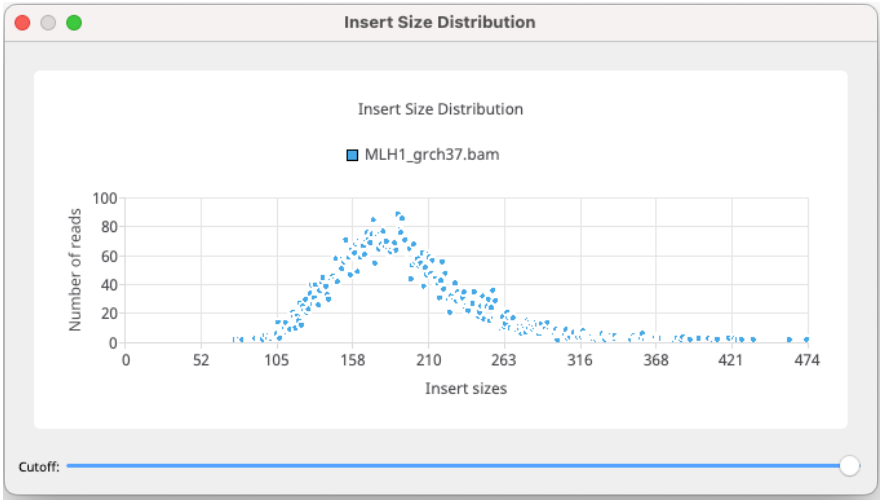
These statistics are computed for the following regions:

- **On-Target**: targeted region (the region of interest that needs to be investigated by the sequence analysis)
  The region is based on the region defined by a user BED file or by the Alamut™ Visual Plus targeted region (see ‘Viewing sequencing targets’ section for more details)
- **Off-Target**: region outside the targeted region (not investigated by the sequence analysis)

- **Locus**: current gene locus

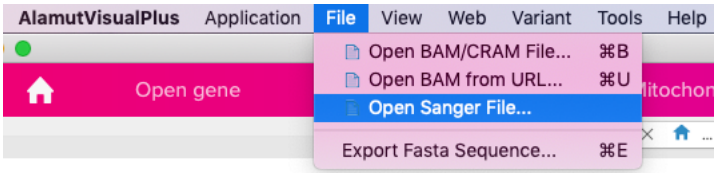


To display “Insert Size Distribution" (only for paired-end sequencing data), select "Insert Size Distribution" from the menu.
The insert sizes are computed from the reads that are in the current gene locus (displayed in Alamut™ Visual Plus) and, then they are plotted as follows.
The cutoff option allows to choose an insert size interactively by displaying in a graphical format.

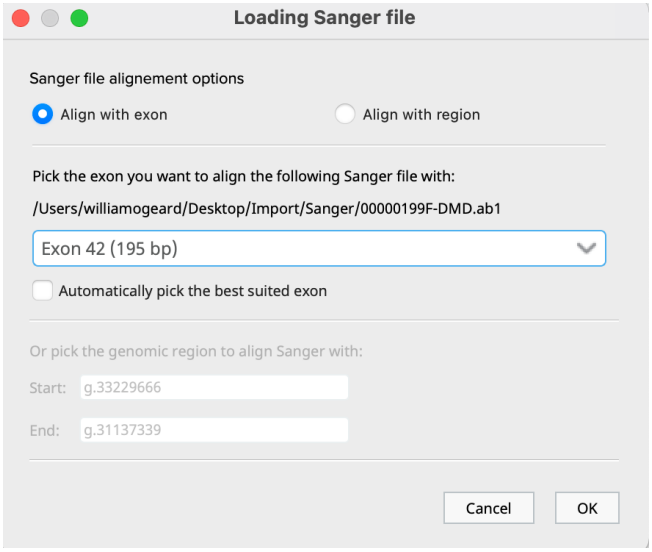


7.2 Viewing Sanger Electropherogram

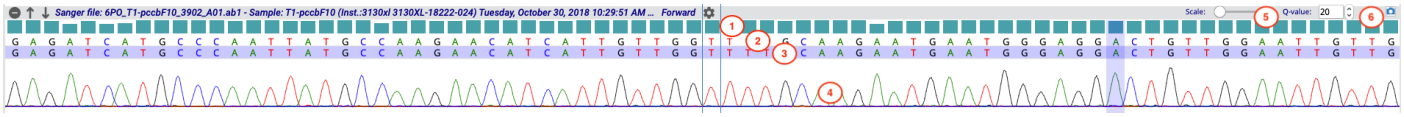
Alamut™ Visual Plus allows you to display Sanger Electropherogram (Sequencing) data by opening a new track through the “File > open Sanger file” menu.



A pop-up will appear where you can select the exon that matches the Sanger sequence you wish to align. Alamut™ Visual Plus is also able to select the best suited exon automatically. If the Sanger file you wish to load, covers multiple exons or intronic regions, you can manually select the genomic region to be considered. Alamut Visual Plus will then run its alignment algorithms considering the nucleotides located inside the specific area.



The Sanger track shows different components

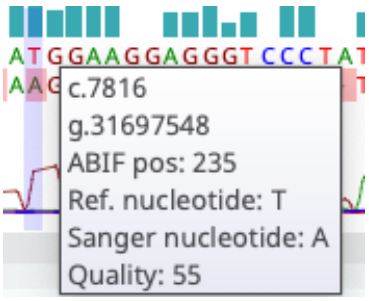


1- Quality scores are displayed in bars.
2- Reference sequence. The reference sequence displayed is always the forward sequence. It can be hidden via the ‘Options’ menu
3- Sanger reference. If the sanger sequence has been aligned with the forward sequence, it will be displayed with a blue background; if it has been aligned with the reverse sequence the background will be green. When a substitution is detected, it is highlighted in red. A deletion is indicated by a ‘-‘ and highlighted in red. An insertion is indicated by an arrow.
4- Electropherogram. The display can be scaled with the ‘Scale’ button.

5-The quality threshold. By default, the threshold is set at 20. The user can customize the threshold. When it shows a quality score lower or equal to the indicated 'Q-value' in the track, the bar in the quality scores sub-track is displayed in orange.
6- Print Sanger track.

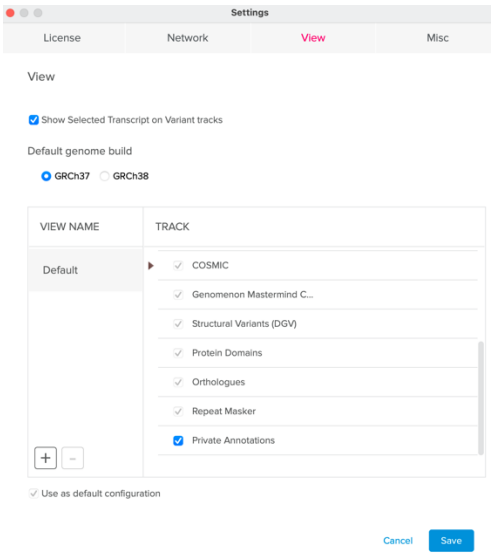
A Sanger variant report can be exported form the Sanger track. 'Options' menu ⚙ > Export variants

When hovering the nucleotide in the Sanger track, you can see the nucleotide and its coordinates as following:



7.3 Viewing sequence-based annotation files (BED, GFF)

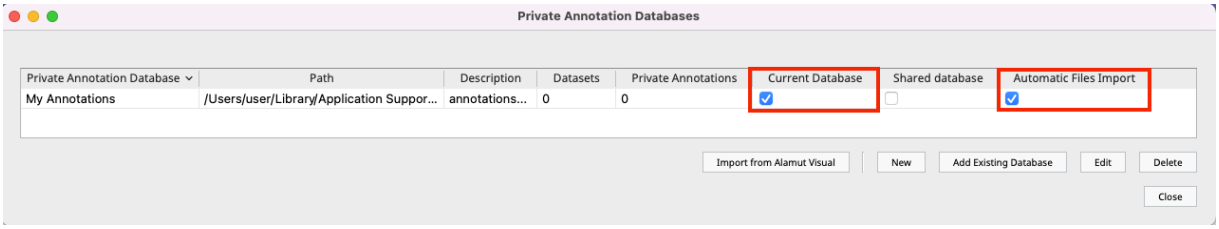
Alamut™ Visual Plus supports the visualization of annotations in BED, GFF version 2/GTF, GFF version 3 in the Private Annotations Track. By default, this track is not visible. To display it, you need to enable it in the View tab of the Application Settings window.



Automatic Import

To automatically import sequence-based annotation files into a private annotation track, you will need to create a Private Annotation Database. The workflow to create a Private Annotation database is described in Section 8.2..

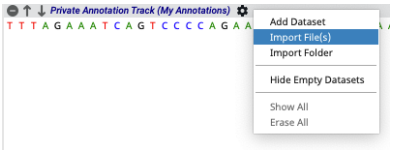
Once the database created, you need to store all the files you want to import in the private annotation database folder. Then, open Alamut Visual Plus and open the Private Annotation Database window via Menu > 'Private Annotations' > 'Private Annotation Databases'. Check the "Current Database" checkbox to use this database in the Private Annotation Track and the "Automatic Files Import" checkbox to trigger the automatic load of files.



Your sequence-based annotation files will now be automatically loaded each time you open a new tab in Alamut Visual Plus.

Manual import

To import sequence-based annotation files, open the 'Options' menu ⚙ of the Private Annotation Track. You can click on 'Import Files', choose the files you want to import and validate. You can import several files at once. If you want to import all the sequence-based annotation files of a folder, you can click on 'Import Folder', choose the folder, and validate.



Each file will generate at least one dataset and the sub-track will appear in the track. As for manually created datasets, right-clicking on a dataset label will open a context menu.

This menu contains two items to manage how the private annotations are displayed in the associated sub-track:

- **Full:** change the display to view all segments in full mode
- **Squish:** change the display to view all segments in squish mode

Data imported from sequence-based annotation files cannot be edited so a dataset created by an import cannot be renamed or erased.

You can visualize the information of the imported private annotations by double-clicking on it or by using its context-menu. The information of imported private annotations cannot be edited.

Note: Data from sequence-based annotation files are loaded into Alamut Visual Plus in read only mode. If you need to update them, update the source file and load it again into Alamut Visual Plus.

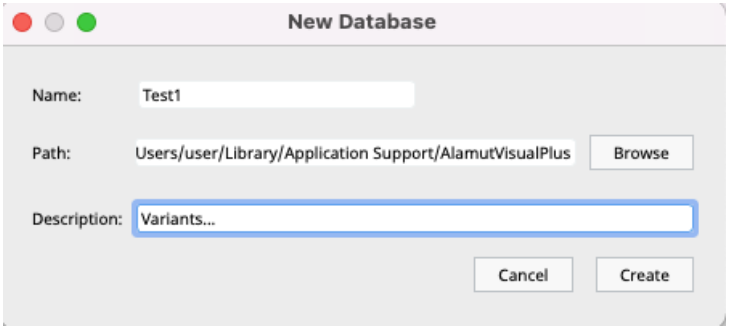
8. Annotate

8.1 Variant Databases

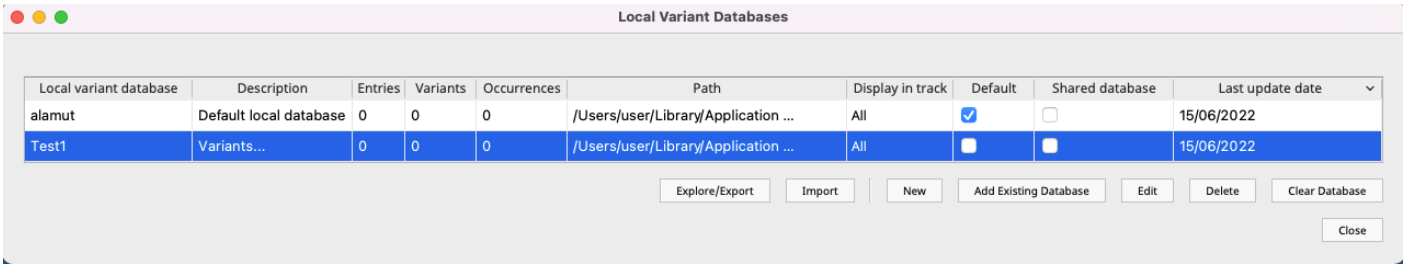
Alamut™ Visual Plus allows you to annotate and store variants. Click on menu > Variant > Local Variant Databases.

By default, a local database called “Alamut” is available, you can use it to store your internal variants.

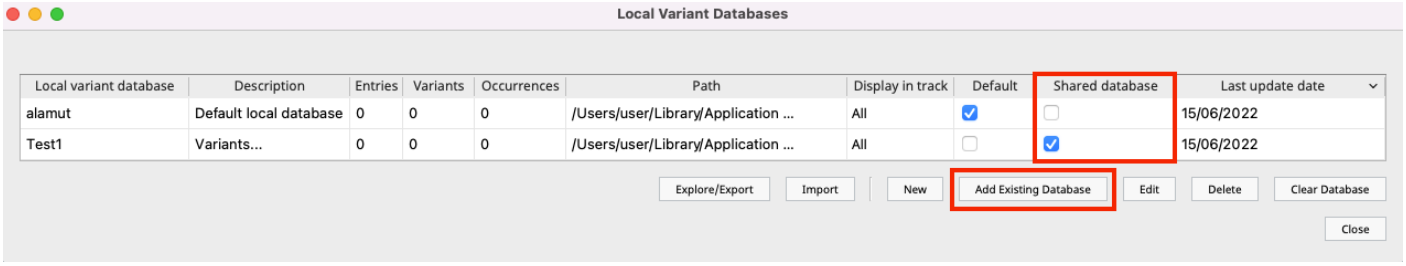
By clicking on ‘New’ in the Local Variant Databases, a new tab will appear ‘New Database’. You can define a new database by selecting a path, adding a name and a description. You can easily switch from one database to another.



The basic variant database functionality implementation allows to store databases on each user's computer, or on a shared folder. When variants are stored in a shared file system, caution must be taken so that two people don't edit simultaneously.

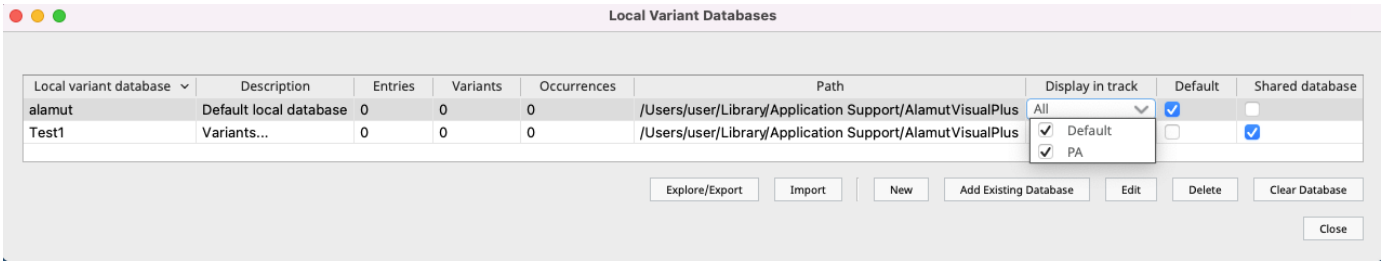


If you want to work on an already existing database, you can add it to your workspace by clicking on ‘Add Existing Database’. If this database is shared between users, tick the ‘Shared Database’ checkbox.



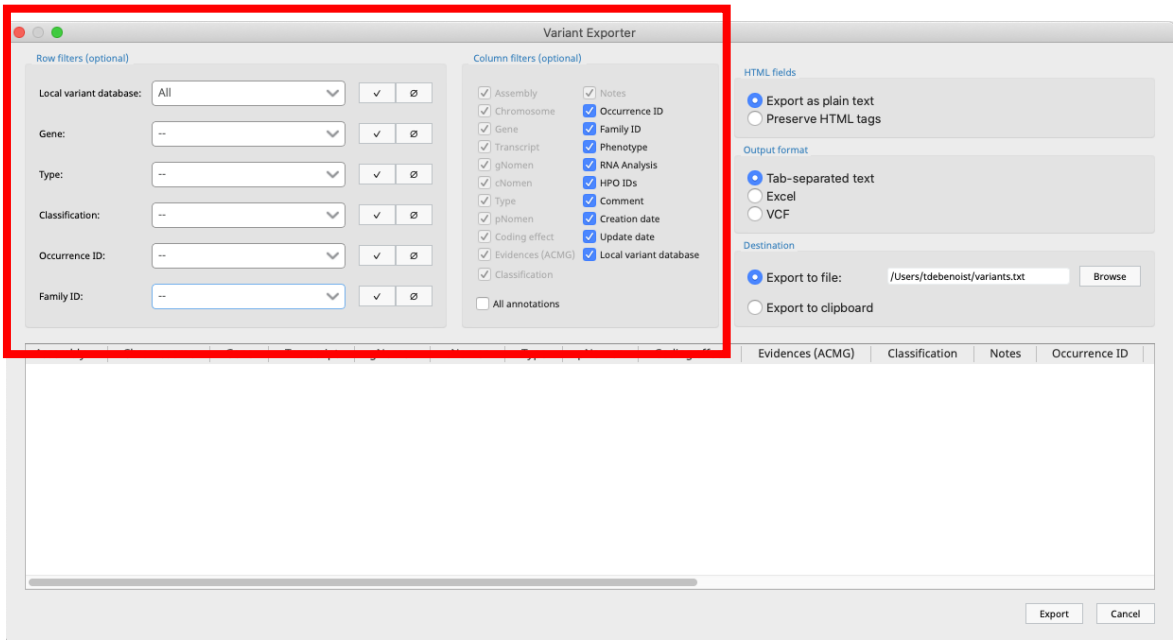
Note: We do not recommend sharing databases between different versions of Alamut Visual Plus. If you do use the shared database feature, all users should switch to a new version of Alamut Visual Plus at a same time.

When exploring a genomic region, your internal variants will be displayed in a specific track (one for each Local Variant Database). For each Local Variant Database, you can use the 'Display in track' column to choose in which 'View configuration' the associated track should appear (see section 10.2 to create or edit a view configuration).

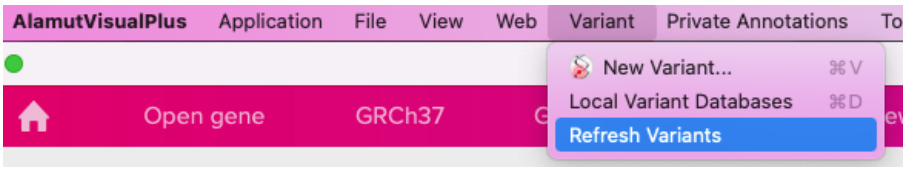


Alamut™ Visual Plus also enlists and navigates through all internal variants for the current gene by selecting a database and clicking on 'Explore/Export' button in the 'Local Variant Databases' tab.

Variants will be displayed in a new tab 'Variant exporter'. You can display different options as showed in the 'Row filters' and 'Column filters' fields. You can scroll down/up and right/left to explore your internal variants.



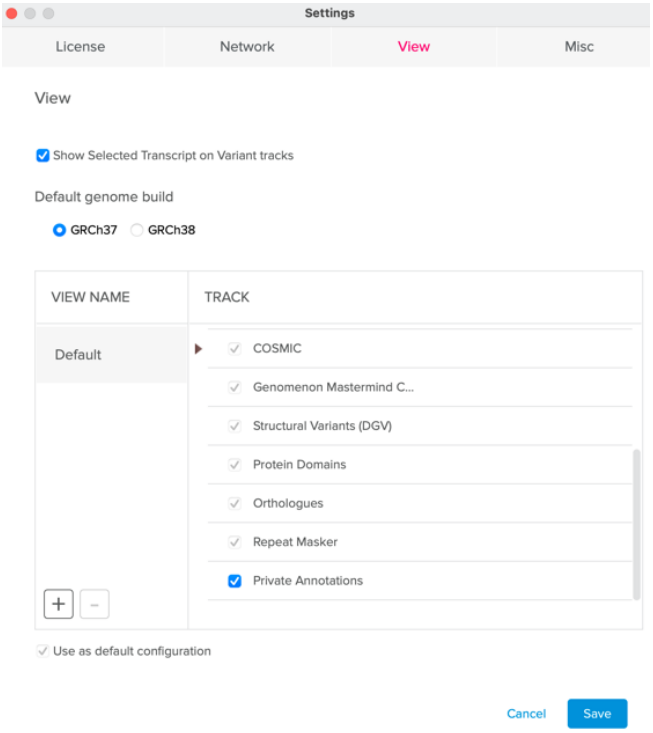
When working on a shared database, you may need to manually refresh your variants to retrieve changes made by your coworkers. To do so, use the ‘Refresh Variants’ item of the ‘Variant’ menu.



The manual refresh may be needed when you want to retrieve changes on a region for which Alamut Visual Plus has already loaded database data (because you are working or have worked on it).

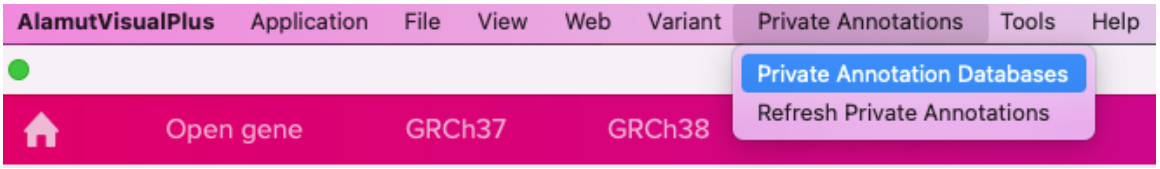
8.2 Private Annotation Databases

Private annotations functionality enables you to manually define sequence-based annotations. This feature is available on a dedicated track: the “Private Annotation Track”. By default, this track is not visible. To display it, you need to enable it in the View tab of the Application Settings window.

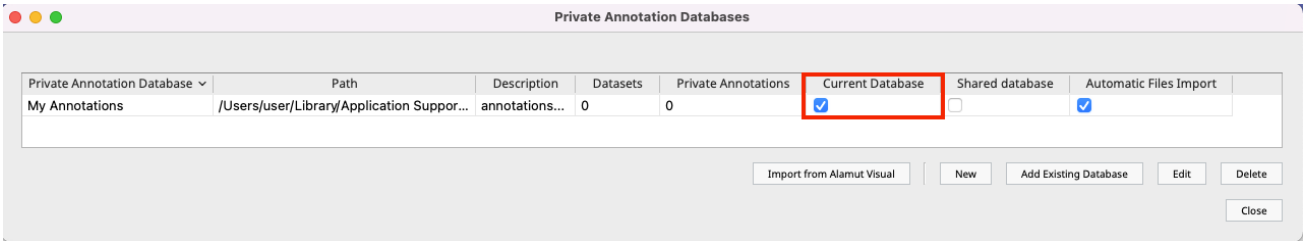


Creating and managing private annotation databases

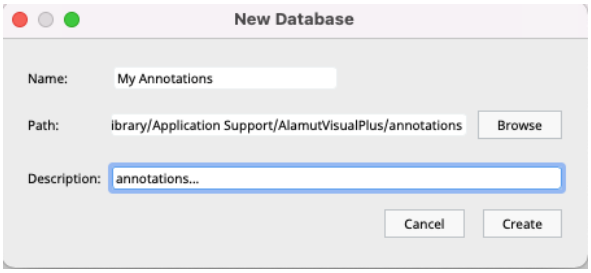
Private annotations are stored in a dedicated database. To manage your private annotation databases, click on menu > Private Annotations > Private annotation Databases.



The Private Annotation Databases window lists the existing databases available for visualization and annotation.
In the Private Annotations Track, you will be able to see the content of **one of your private annotation databases at a time**. You can choose which database you wish to display by using the ‘Current Database’ checkbox.

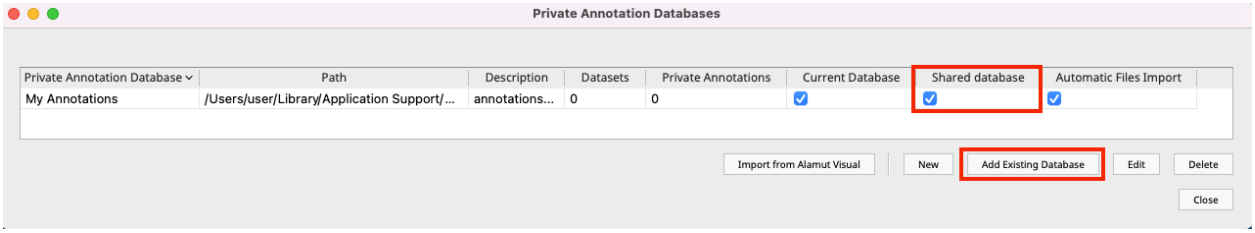


To create a new database, click on ‘New’ button. A new pop-up will appear. You can define a new database by selecting the path of the newly created database, a name, and a description.



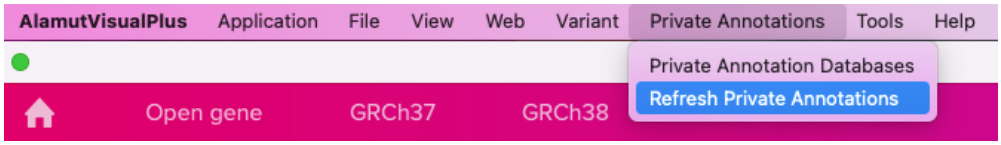
The basic private annotation database functionality allows to store databases on each user’s computer, or into a shared folder. When private annotations are stored in a shared file system, caution must be taken so that two people don’t edit the private annotations, simultaneously.

If you want to work on a database created by another user, you can add it to your workspace by clicking on ‘Add Existing Database’. Then, tick the ‘Shared Database’ checkbox.



Note: We do not recommend sharing private annotations databases between different versions of Alamut Visual Plus. If you do use the shared database feature, all users should switch to a new version of Alamut Visual Plus at a same time.

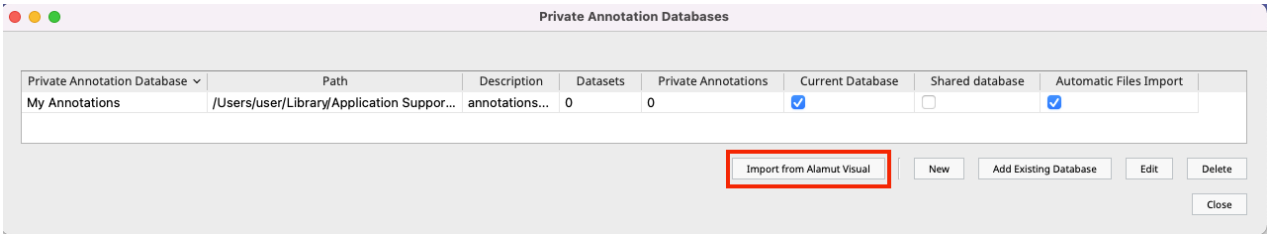
When working on a shared database, you may need to manually refresh your variants to retrieve changes made by your coworkers. To do so, use the 'Refresh Private Annotations' item of the 'Private Annotations' menu.



The manual refresh may be needed when you want to retrieve changes on a region for which Alamut Visual Plus has already loaded database data (because you are working or have worked on it).

Import Private Annotations from Alamut™ Visual

Private Annotation files from Alamut™ Visual (.apa) can be imported into the Private Annotation Databases of Alamut™ Visual Plus. To do so, open the Private Annotation Database window via Menu > ‘Private Annotations’ > ‘Private Annotation Databases’. Select the database you want to import the private annotations into. Click on ‘Import’ and select the file you want to import.



9. Import Variants

Supported file format

Alamut™ Visual Plus can import variants from external sources into local variant databases. The imported variants will be stored into the selected Local Variant Database (LVD).

Successfully imported variants are saved as standard Alamut™ Visual Plus internal variants and can then be handled like other internal variants.
Alamut™ Visual Plus can import variant annotations from TSV (Tab-separated values) formatted files as Variant Calling Format files (VCF).

Note that for Alamut™ Visual users, the same workflow can be used to import .mut files and .txt variant files that may have been exported from Alamut™ Visual.

9.1 Import variant from an excel file or TSV format

Alamut™ Visual Plus accepts a tabular import file containing variant descriptions and annotations. Import files must follow a precise format that is most easily created using a spreadsheet application like Excel. Here is an example. The column order must be strictly observed.

The header line (with column labels) is mandatory. Columns must be named as in the example in below:

Assembly	Chromosome	Gene	Transcript	gNomen	cNomen	Type	pNomen	Coding effect	Evidences (ACMG)	Classification	Notes	Occurrence ID	Family ID	Phenotype	RNA Analysis	HPO IDs	Comment
GRCh37	2	MSH2	NM_000251.2	g.47630335C>A	c.5C>A	Substitution	p.(Ala2Glu)	Missense	PM1,PM2,PP3	Likely Benign	This is a note relating to this variant	1	123	Adam+			
GRCh37	2	MSH2	NM_000251.2	g.47630347_47630350del	c.17_20del	Deletion	p.(Lys6Argfs*57)	Frameshift		Likely Pathogenic		2	456				
GRCh37	2	MSH2	NM_000251.2	g.47657084A>C	c.1276+4A>C	Substitution	p.?			Undefined Significance							

The following sets of columns must be populated to define the variants:

- Assembly
- Chromosome
- gNomen

or

- Gene
- Transcript
- cNomen

Assembly — GRCh37 or GRCh38.

Chromosome — chr**N** or **N,** where N is the number of the chromosome (or X or Y, or MT)

Gene — The official symbol of the gene carrying the variant.

Transcript — The RefSeqDna of the transcript used to describe the variant.

gNomen — The gDNA-level variant description, using the HGVS nomenclature.

cNomen — The cDNA-level variant description, using the HGVS nomenclature.

Type — The variant type: Substitution, Deletion, Insertion or Duplication.

pNomen — Consequence of the variant at the protein level.

Coding Effect — Missense, Start loss, Nonsense, Stop loss, Frameshift, In-frame or Synonymous.

ACMG evidences — ACMG rules, separated by coma.

Classification — Variant classification as per the ACMG guidelines :

- Benign
- Likely Benign
- Uncertain Significance
- Likely Pathogenic
- Pathogenic

If no value is supplied here, " Undefined Significance" is assumed

Notes — Comments associated to the variant. Free content field.

Occurrence ID — Free content field.

Family ID — Free content field.

Phenotype — Free content field.

RNA Analysis — Free content field.

HPO IDs — Human phenotype ontology IDs

Comment - Comment associated to the occurrence. Free content field.

9.2 Import variant from a vcf file

Standard VCF files can be imported. Fields are #CHROM POS ID REF ALT QUAL FILTER INFO FORMAT Only CHROM, POS, REF and ALT are used to set up the variant.

9.3 Important variant from an existing Alamut database

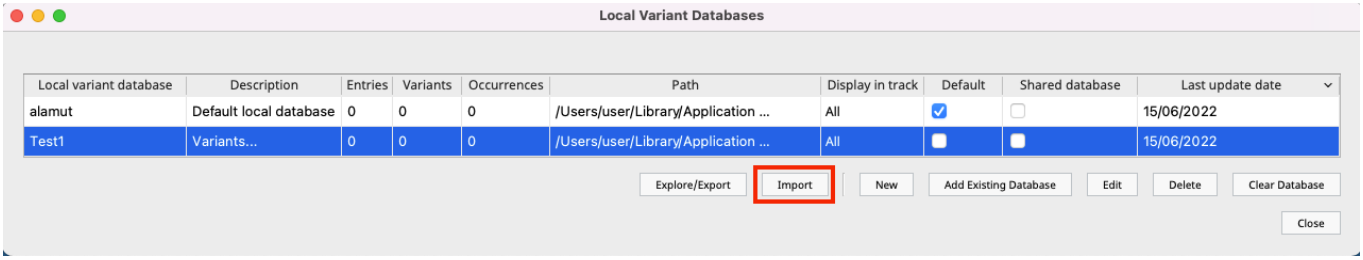
Variant files from Alamut™ Visual (.mut) can be imported into the Local Variant Databases of Alamut™ Visual Plus.

9.4 Import process

To import variants from an import file as described above, in Alamut™ Visual Plus:

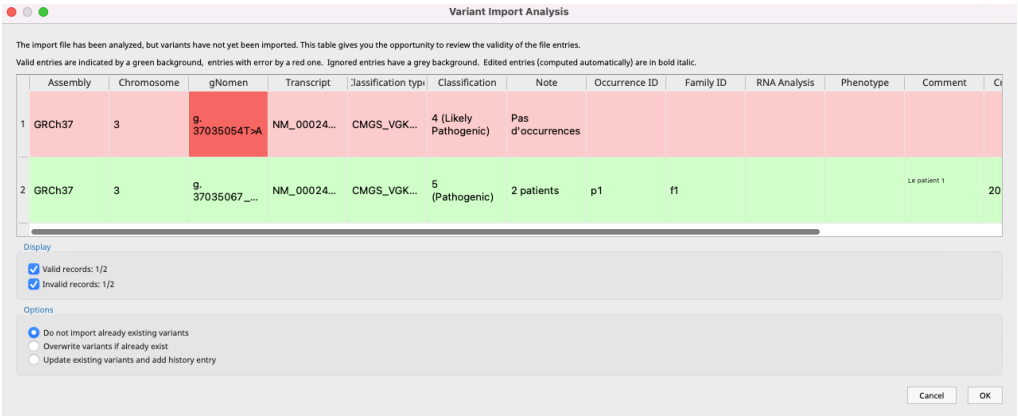
Menu > 'Variants' > ‘Local Variants Databases'

Select a line (Local Variant Database) where the variants will be stored and specify the import file you have by clicking on ‘Import’.



Alamut™ Visual Plus will analyse the imported file and report valid and invalid entries based on the format.

Only validated variants (shown in green) will be imported to the Local Variant Database.



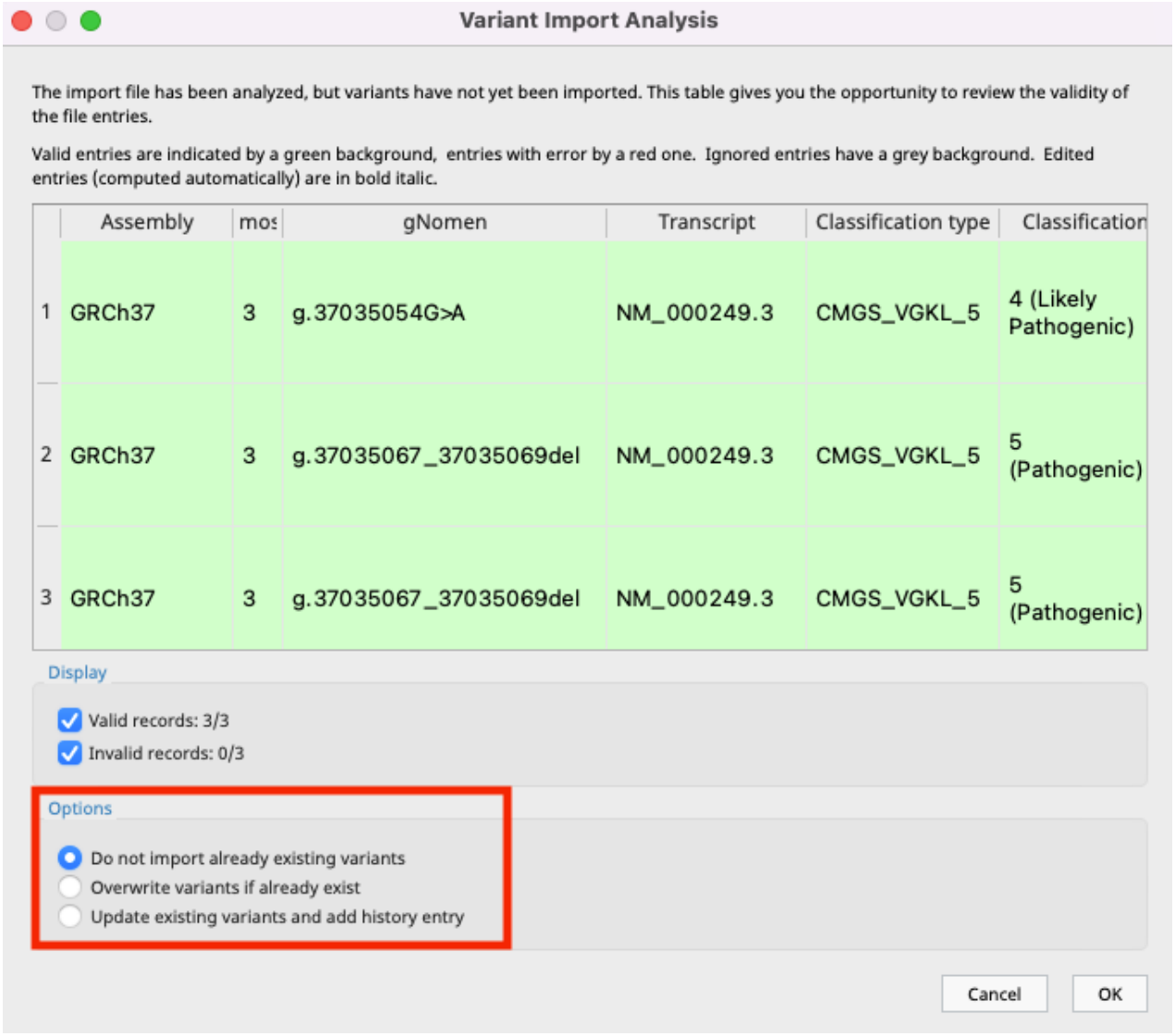
Notes:

- The import process can be managed without opening a specific gene or transcript from the homepage of Alamut™ Visual Plus
- The import process can also be managed when a gene and a transcript are already selected and opened.
- For VCF and TSV (with gNomen) import files, variants outside of the gene locus will also be processed.

Handling conflicts:

When importing variants into a Local Variant Database, some of the variants to import might already exist in the database.

To handle possible conflicts, if the variants to import already exist into the database, three options are available:



- Do not import already existing variants – conflicting variants will be ignored during the import process
- Overwrite variants if already exist – in case of conflicts, the variants to import will replace the existing ones
- Update existing variants and add history entry – variants get normally imported, a new entry will be added to the variant history to track the updates

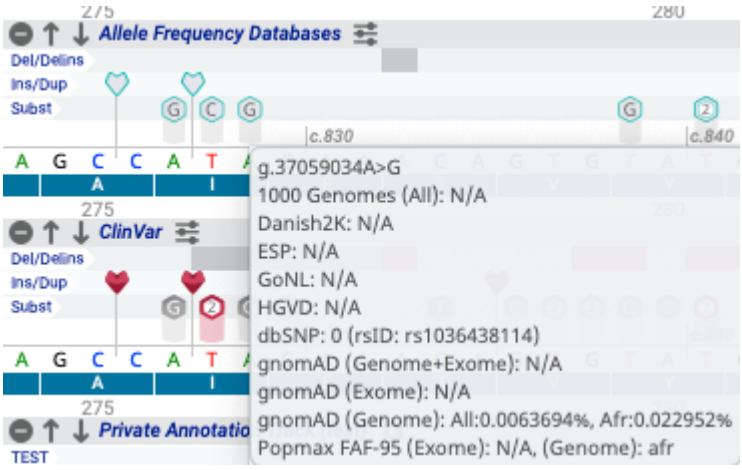
10. Evaluate

10.1 Visualize Alamut variant sources and databases

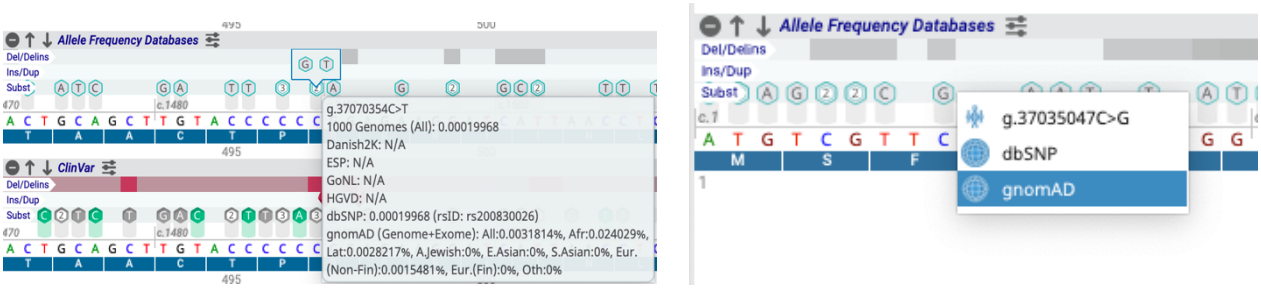
Alamut™ Visual provides a convenient access to several databases of known variants and public databases.

Population Frequencies Database

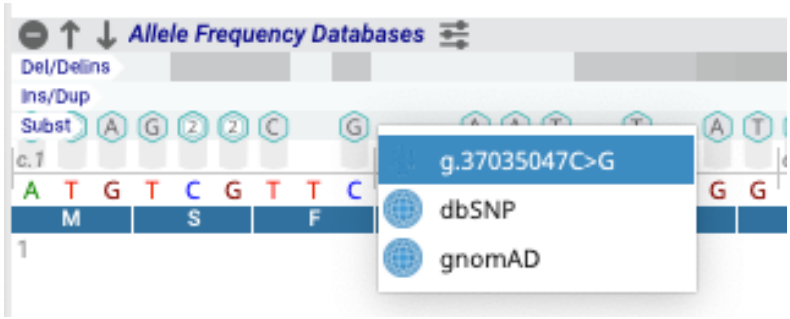
Population frequencies data are displayed in the 'Allele Frequency Databases' track.



Hovering one variant will show information from different databases when available. Right clicking on a variant opens the official website of the selected source.



More information from external databases is available in Alamut™ Visual Plus by right clicking and selecting the variant's gnomen:



The variant panel will pop-up with more information in the 'External databases' section. For more information see section 11.1.

You can configure this track to filter its variants, see section 10.3 for more information.

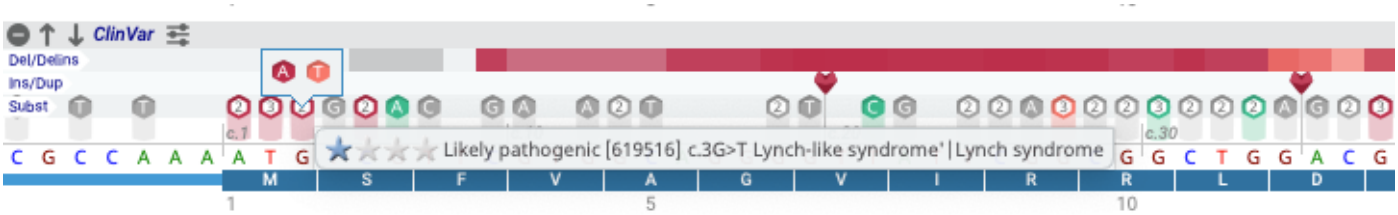
ClinVar

Alamut™ Visual Plus displays ClinVar variants in a dedicated track
Variant background colors are based on the clinical significance provided by ClinVar submitters.

a. **Red** for pathogenic variants

b. **Orange** for likely pathogenic variants
c. **Light Green** for likely benign variants
d. **Green** for benign variants
e. **Gray** for variants of an uncertain significance
f. **White** for Unclassified variants

If multiple SNVs are reported at the same position, a tooltip pops-up to show significance for both variants.

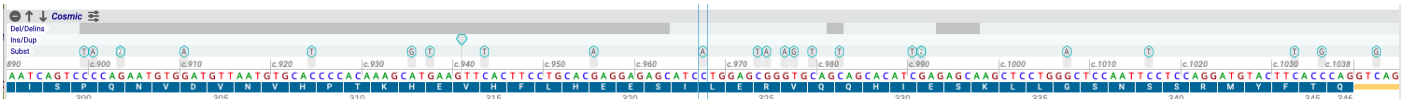


You can configure this track to filter its variants, see section 10.3 for more information.

Right-clicking will show the link to the ClinVar website and to the Variant panel where information from ClinVar is displayed.

COSMIC

Alamut™ Visual provides access to COSMIC variant in a separate track.

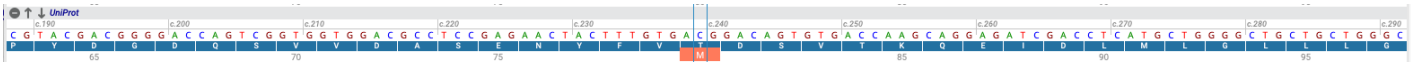


You can configure this track to filter its variants, see section 10.3 for more information.

Right clicking on a mutation opens the variant panel with information and directs you to the COSMIC website.

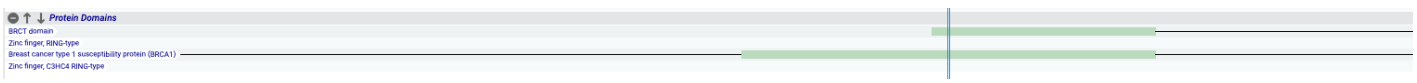
UniPROT

The “UniProt” track displays variants set at protein level from UniProt. You can see the amino acid information and the disease involvement by hovering over a variant on the track. This information is also available in the variant panel.



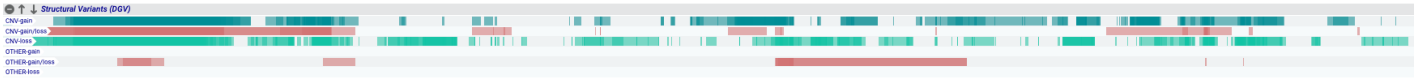
Protein Domain

The “Protein Domains” track displays the domain information from InterPro (via Ensembl).



Database of Genomic Variants

The track displays the structural variations in the genome via the DGV database.



Repeat Masker

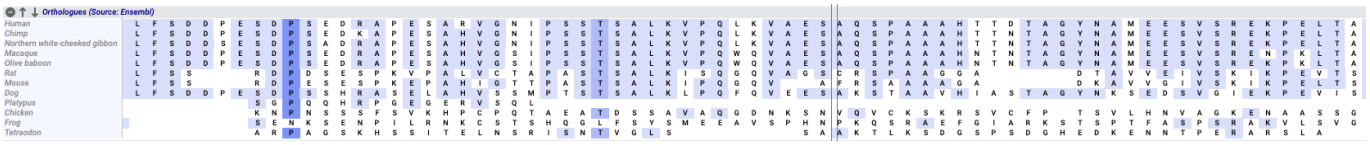
The Repeat Masker track displays the repeat DNA regions identified by the Repeat Masker tool.

The regions are displayed by type:

- SINEs
- LINEs
- DNA elements (DNA)
- Simple Repetitions (Simple Repeat)
- Low Complexity
- Satellite Elements (Satellite)
- Small RNAs (RNA)
- Not Classified (Unknown)
- Other

Orthologues alignment

The Orthologues track displays alignments of protein orthologue from the Ensembl database, ICAR or IBC (In-house). These alignments show the conservation of amino acids across different species.



By default, orthologues aligned and displayed in Alamut™ Visual Plus are taken from the Ensembl Compara database.

References:

- We would like to express our thanks to the Genetic Cancer Susceptibility Group at IARC for their kind help in defining our alignment protocol.
- Tavtigian, SV., Greenblatt, MS., Lesueur, F., Byrnes, GB. (2008). In silico analysis of missense substitutions using sequence-alignment based methods. Hum Mutat.11 : 1327-36
- Tavtigian, SV., Oefner, PJ., Babikyan, D. et al (2009). Rare, evolutionarily unlikely missense substitutions in ATM confer increased risk of breast cancer. Am J Hum Genet. 85 : 427-46.

- Deforche A., Blavier A. (2010). Systematic Building of Multiple Protein Alignments for Variant Interpretation Human Genome Meeting poster.

Genomenom Mastermind

Mastermind® by Genomenon® is a search engine with a comprehensive collection of genomic evidence and a user-friendly database. More information about Genomenon® is available at https://www.genomenon.com/tutorials/.

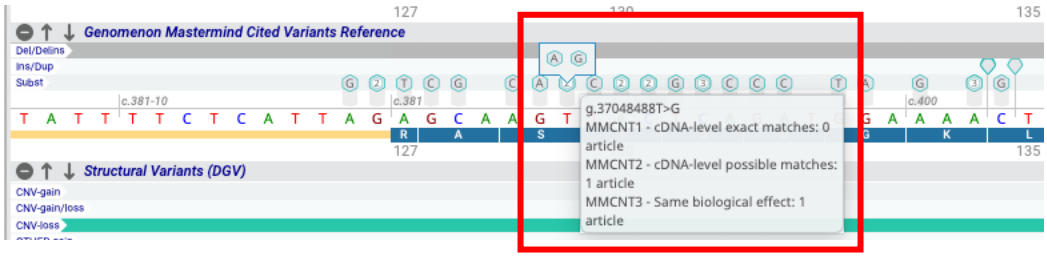
Mastermind® visualization can be activated in the "Settings > View" tab for each view configuration.

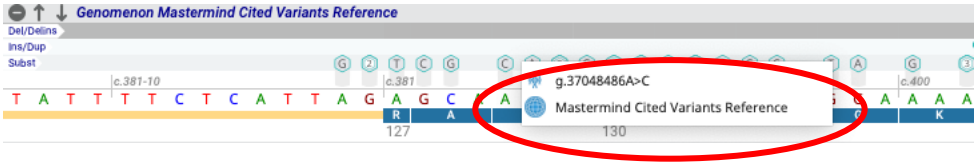
Alamut™ Visual Plus displays Mastermind variants in the dedicated "Genomenon Mastermind Cited Variants Reference" track. Variants are reported from Cited Variants Reference (CVR), equivalent to a Variant Call Format (VCF) file. The CVR contains a count of articles associated with each variant, along with a deep link into the related Mastermind UI. More information on CVR at https://www.genomenon.com/cvr/.

If multiple SNVs are reported at the same position, a number indicating the substitutions occurring at this position. A pop-pup at the top of the position will display the different variations. Right-click on a variant to display links to the variant panel and to the Mastermind® website. A field in the variant panel displays the number of articles, including the three MMCNT values.

Mouse over on a CVR variant to pop-up a tooltip displaying the gnomen and the three MMCNT values:

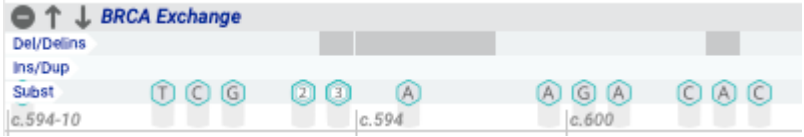
- **MMCNT1: cDNA-level exact matches**
  The number of articles mentioning the variant at the nucleotide level in title/abstract or full-text.
- **MMCNT2: cDNA-level possible matches**
  The number of articles with nucleotide-level (from 1) and protein-level matching with not specified cDNA-level change (articles could refer to this nucleotide-level variant but there is insufficient data to determine conclusively).
- **MMCNT3: same biological effect matches**
  The number of articles citing any variant with the same biological effect as the considered variant. This includes MMCNT1 and MMCNT2 articles plus those with alternative cDNA-level variants that result in the same protein effect.





BRCA Exchange

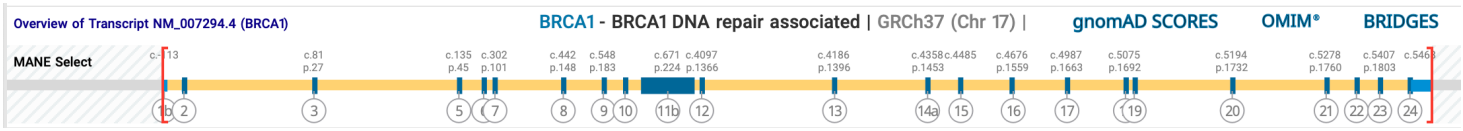
The BRCA Exchange database is available in a specific track while opening BRCA1 and BRCA2 (https://brcaexchange.org/help).



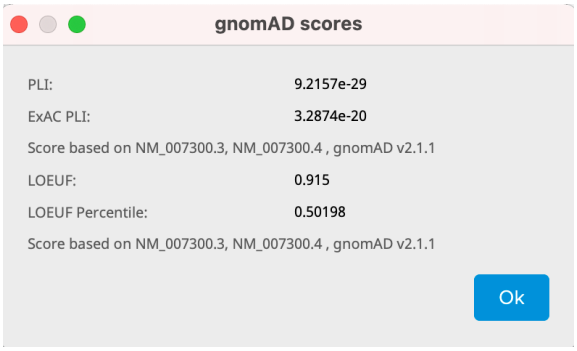
Hovering on the variant will give you some insights on the pathology stored in BRCA Exchange database. This information is also available in the variant panel through the BRCA Exchange section and in the report.

Gene External Features

OMIM®, gnomAD scores (pLI, LOEUF), BRIDGES and MANE information are also reported in Alamut™ Visual Plus in the genomic view.

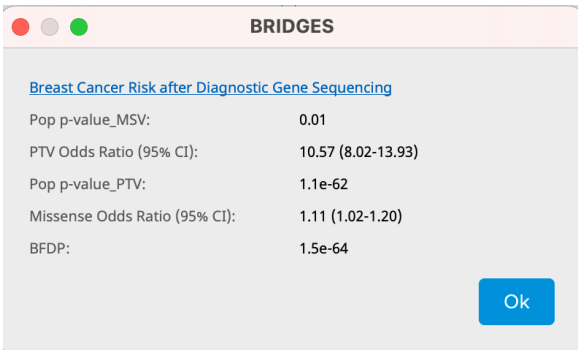


Clicking on one of the three blue buttons at the top right of the genomic view (gnomAD SCORES, OMIM® or BRIDGES) will display a dialog window:

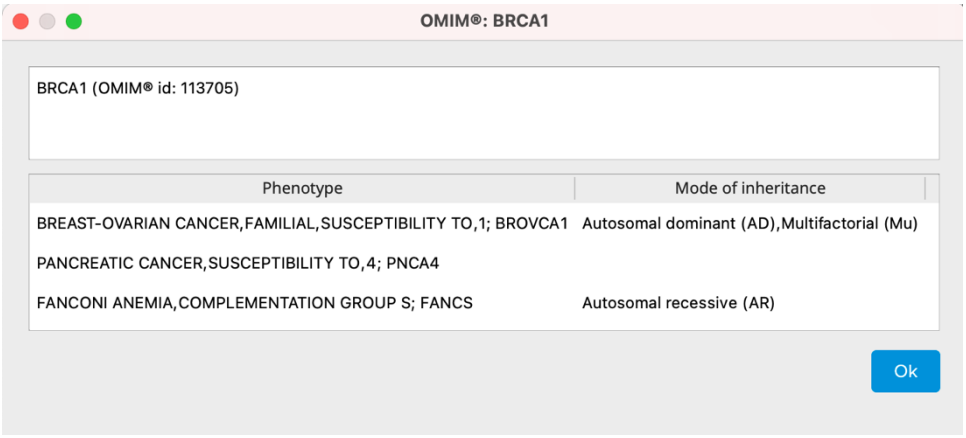


BRIDGES dialog window displays the following scores:

- Pop p-value_MSV: P-value for Missense Variant and breast cancer
- PTV Odds Ratio (95% CI): Odds ratio for Protein-truncating variant and breast cancer. Please note that for PTVs, the risks apply to every variant that ClinVar calls “pathogenic”
- Pop p-value_PTV: P-value for Protein-truncating variant and breast cancer.
- Missense Odds Ratio (95% CI): Odds ratio for Missense Variant and breast cancer. Please note that for missense variants ClinVar assertions were not used for most genes, except BRCA1, BRCA2, TP53
- BFDP: Bayesian false-discovery probability



Double clicking on OMIM-Id or on a phenotype row will open the corresponding gene or phenotype in the OMIM® web page:

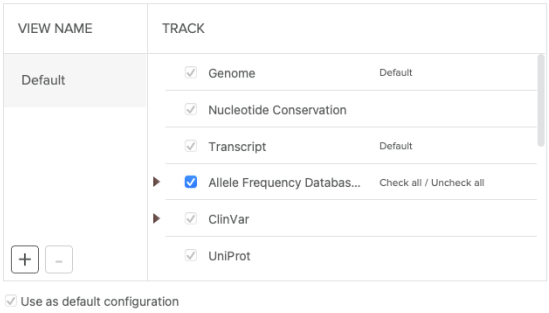


The MANE categorisation is also available in the transcript selection tab.

10.2 Display and Configure specific tracks

View Configuration

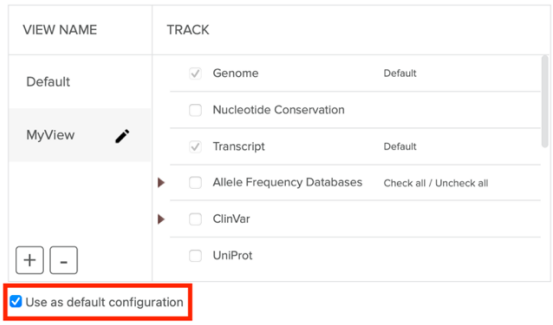
The View tab in the Application Settings window allows to edit the default view configuration or to create a custom one.



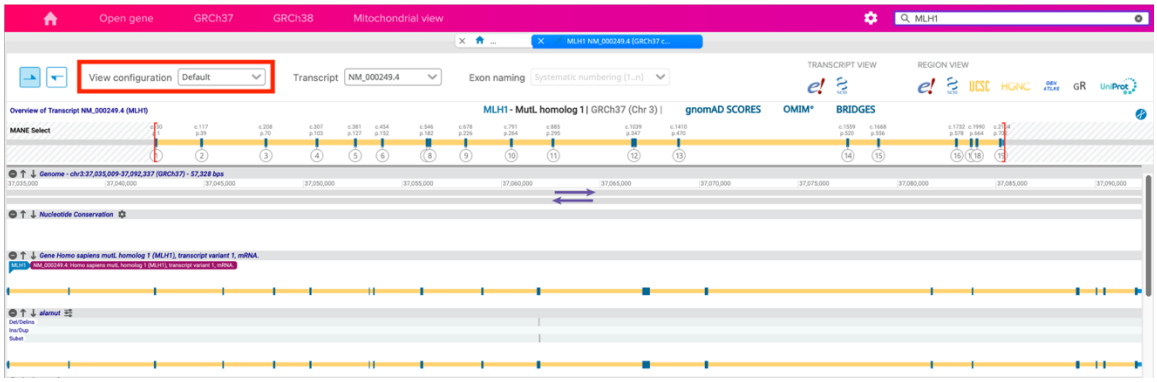
In the Default view configuration, all tracks are displayed. By creating custom views, you can check/uncheck the tracks you want to see/hide. You may also change the tracks display order via drag and drop.

Note: The Local Variant Database track does not appear in the list of tracks. You can configure in which view configuration a Local Variant Database track appears via the Local Variant Databases window, see section 8.1 for more information.

You can define a view as default configuration by selecting it and by ticking the 'Use as default configuration' checkbox. The view will then be used by default when a new genomic view is opened.



You can change the view configuration used by a genomic view at any time via the 'View configuration' combo box.



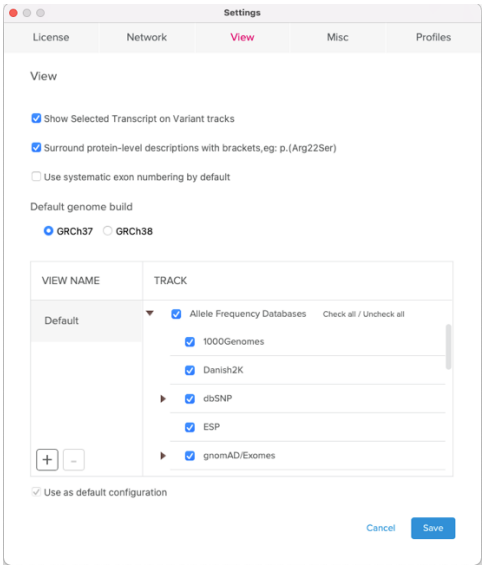
Specific Track Configuration

Applying filters to a specific track is a convenient way to filter the variants displayed by a given data source.
It can be done while editing a view configuration or you may open the view configuration window by clicking at any time on the 'Options' button of the track to filter.

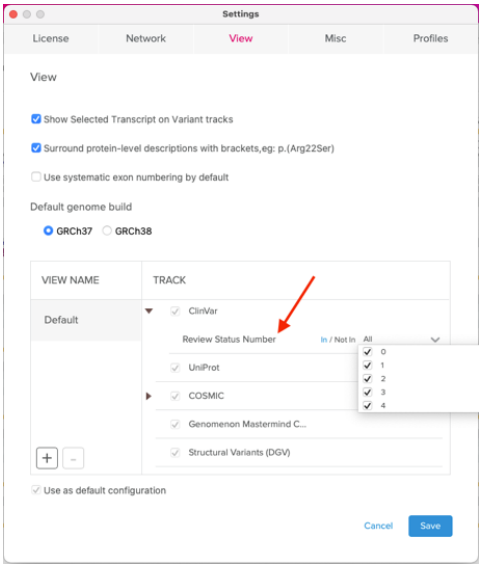
Options

Filters can be set for 3 tracks:

1. **Population Frequencies Database Track**: you can select or deselect data sources and choose filters.



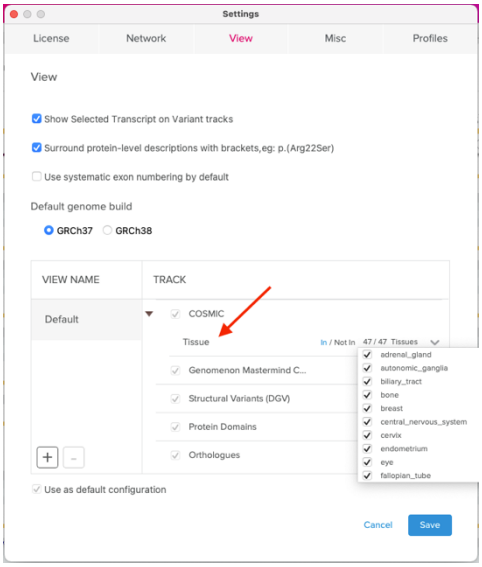
2. **Clinvar Track**: you can filter variants based on 'Review Status'.



The Review status numbers are defined as follows:

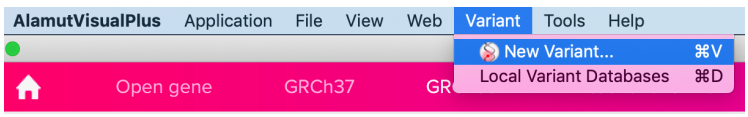
- 0: No submitter provided an interpretation with assertion criteria (no assertion criteria provided), or no interpretation was provided (no assertion provided).
- **1:** At least one submitter provided an interpretation with assertion criteria (criteria provided, single submitter) or multiple submitters provided assertion criteria but there are conflicting interpretations in which case the independent values are enumerated for clinical significance (criteria provided, conflicting interpretations).
- 2: Two or more submitters provided the same interpretation (criteria provided, multiple submitters, no conflicts)
- 3: Reviewed by expert panel.
- 4: Practice guideline.

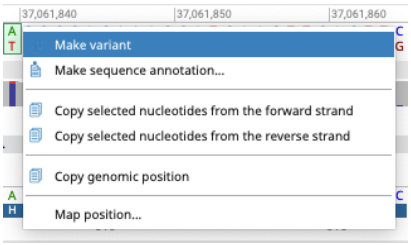
3. **COSMIC Track**: you can filter variants based on the 'Tissue' criteria.



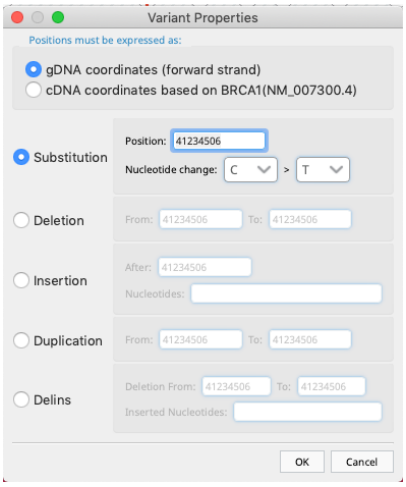
10.3 Create variants

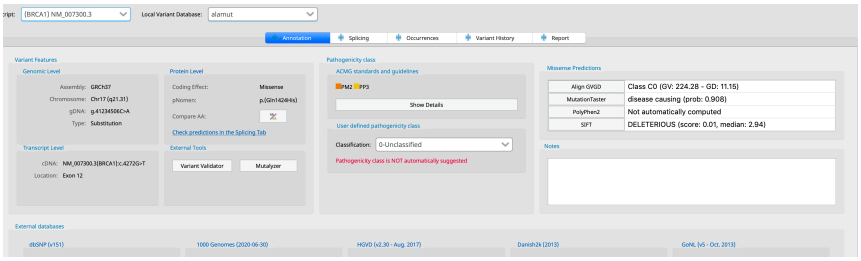
You can manually create variants either from the 'Variants' menu, or from a nucleotide selection in the genome or transcript track and its associated context menu (by right-clicking inside the selection).





Once you have specified variant basic properties (position and type of change), the 'Variant Panel' (annotation window) opens in a new tab. In the 'Variant properties' you can choose to apply your variant at the genome or transcript level.

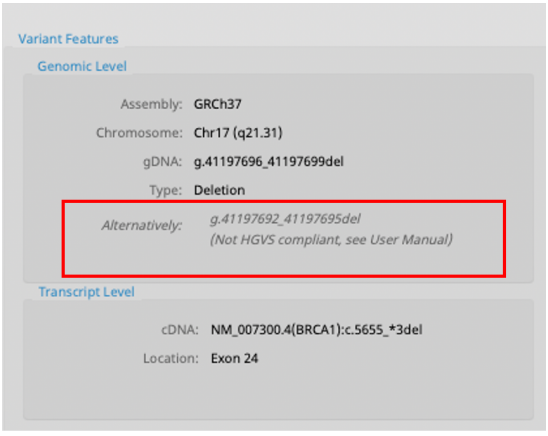




This will open a Variant Panel where you can study your variant and save it in your Local Variant Database. For more information on the Variant Panel, see section 11.

Notes : **Application of HGVS recommendations**

- Alamut™ Visual Plus enables the user to check the variant with Mutalyzer Name Checker or VariantValidator from the annotation window
- The HGVS recommendations for the description of sequence variants implies that for all descriptions the most 3' position possible is arbitrarily assigned to have been changed. Application of this recommendation can make variant entry in Alamut™ Visual Plus ambiguous, since the new variant entry may result in a variant located at a different position. Thus, both mutated sequences – the one entered and the resultant – should be identical.
- For gNomen, the 3' rule is always applied for a gene on the forward strand of the DNA and for cNomen, the 3' rule is applied at the cDNA level. Thus, for a gene encoded on the reverse strand, the cDNA-level variant positions will not always map to the same genome position as sown in gNomen (this is not compliant with HGVS (*For Alamut Visual users: this is handled differently as compared to Visual. In the Variant Panel > Variant Features > Genomic Level > alternatively; it is indicated the HGVS nomenclature as referred to Visual*)



You can change at any time the ‘Transcript’ and the ‘Local Variant Database’ of your variant by using the menu at the Top of the ‘Variant Panel’:

Note: In a same Local Variant Database, you cannot save a same variant with different transcripts.

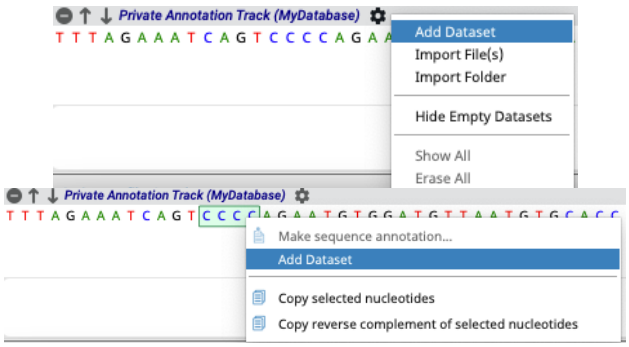
10.4 Create Private Annotations

To be able to create private annotations, you first need to create a Private Annotation Database and to select it as the current Private Annotation Database. For more information, see "Creating and managing private annotation databases" part of Section 8.2.

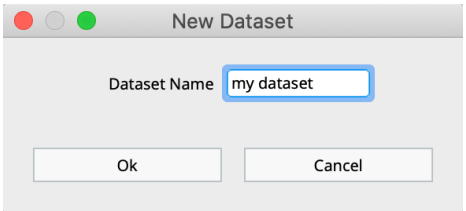
Creating and managing datasets

Private annotations are organized in sub-tracks called datasets. To create custom datasets, you can use the ‘Options’ menu ⚙ of the Private Annotation Track or the context menu of a nucleotide selection in the Private Annotation Track (by right-clicking inside the selection).

You need to have a database selected as your current private annotation database to be allowed to create custom datasets.

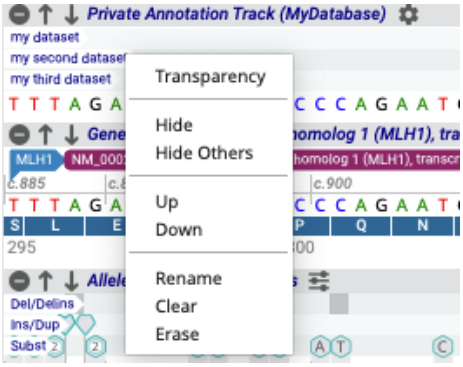


Clicking on ‘Add Dataset’ will open a new pop-up where you can specify the name of your new dataset. Dataset names must be unique among a database. Clicking on ‘Ok’ will create the dataset and the new sub-track will appear.



Right-clicking on a dataset label will open a context menu. This context menu allows the following actions:

- **Transparency:** the private annotations are displayed with semi-transparent colours when this option is checked and with plain colours otherwise
- **Hide:** hide the dataset from the track
- **Hide Others:** hide the other datasets from the track
- **Up:** move up the dataset
- **Down:** move down the dataset
- **Rename:** rename the dataset
- **Clear:** delete all annotations of the dataset
- **Erase:** delete the dataset (from the track and from the database)

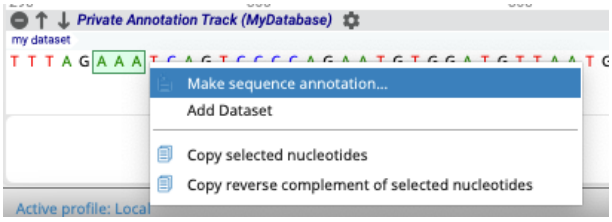


The 'Options' menu ⚙ of the Private Annotation Track contains items to manage the group of datasets: **Show All** to show all datasets when some have been hidden previously and **Erase All** to erase all the datasets.

Creating and managing Private Annotations

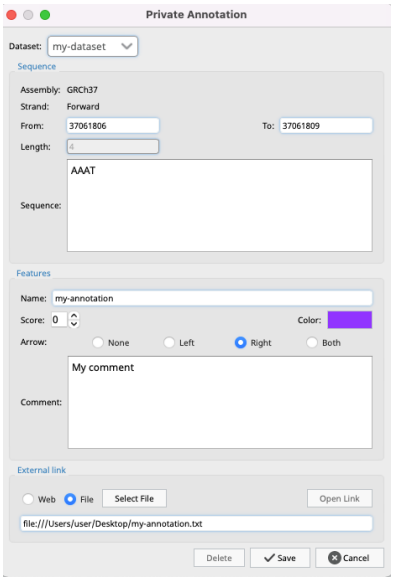
A new private annotation can be manually created from the context menu associated to a selection of nucleotides. The nucleotide selection can be done in the Private Annotation, the Genome or the Gene tracks.

⚠ **You need to have a database selected as your current private annotation database and to have at least one dataset in your current database to be allowed to create private annotations.**



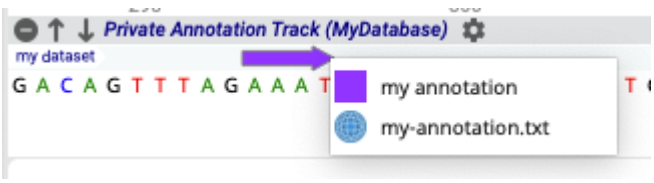
Clicking on ‘Make sequence annotation…’ will open a new pop-up where you can specify the properties of your private annotation:

- **Dataset**: the dataset/sub-track
- **Assembly:** the assembly of the annotated sequence. A private annotation is visible only on the assembly to which the sequence has been mapped. When updating the assembly, the positions ‘From’ and ‘To’ will be automatically mapped to the new assembly.
- **From/To:** origin and end of the annotation. The strand is automatically set according to the strand that has been used to select the nucleotides in the track. The length and the sequence are automatically computed.
- **Name:** name of the private annotation. Private annotation names have to be unique among a dataset
- **Arrow:** the arrow initialized according to the strand of the private annotation
- **Score**
- **Color**
- **Comment**
- **External link:** web link or link to an internal document



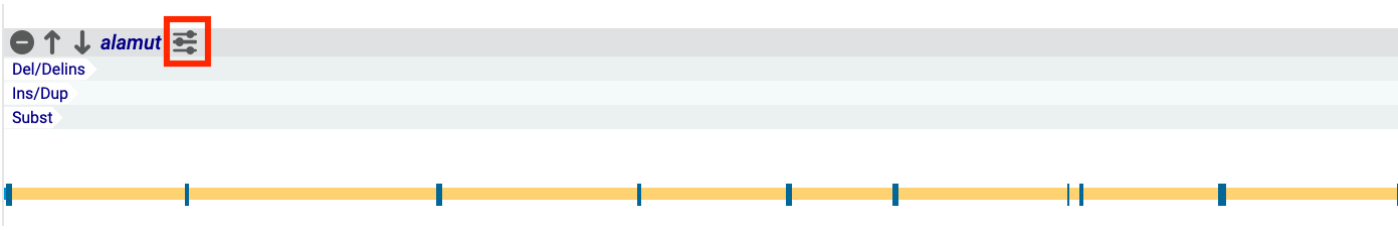
Clicking on ‘Save’ will create the private annotation and it will be visible in the sub-track to which it belongs.

Then, to edit the private annotation information, you can either double-click on the private annotation track or use its context-menu.

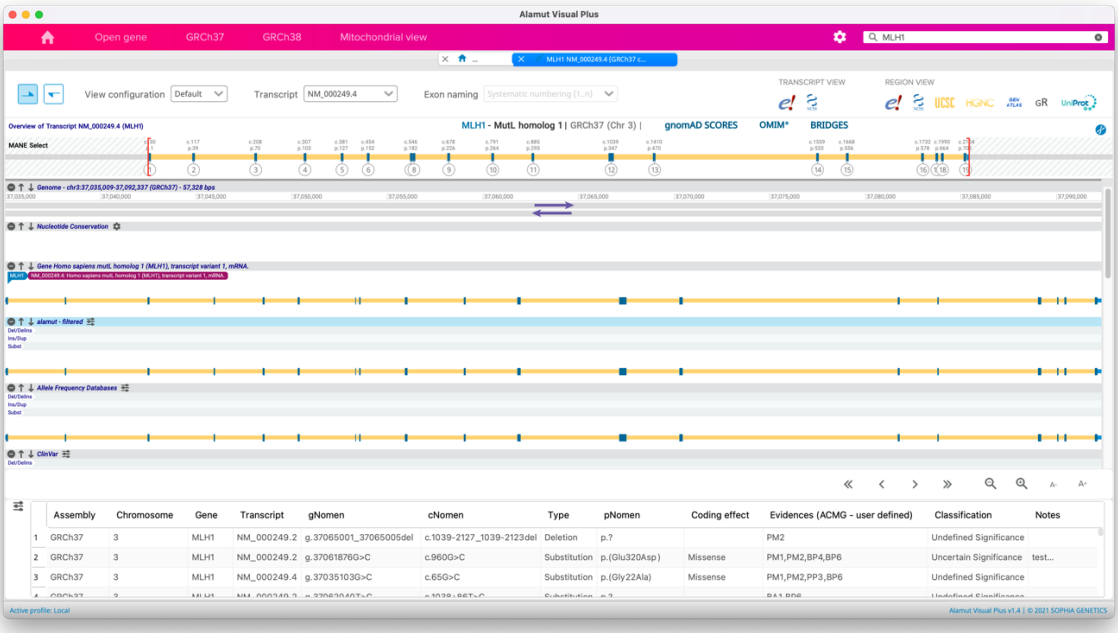


10.5 Variant Interactive Filtering

The Variant Interactive Filtering feature allows the user to visualize information on variants visible in a Local Variant Database track and to create filters on them.



To activate the Variant Interactive Filtering feature, click on ⚙ icon in the Local Variant Database track. The feature can be activated on a single database at a time.



Once the Interactive Filtering feature is enabled, a table is displayed at the bottom of the screen. This table displays the information of the variants currently visible in the Local Variant Database track. The title of the filtered track is suffixed by " - filtered" and its background is blue.

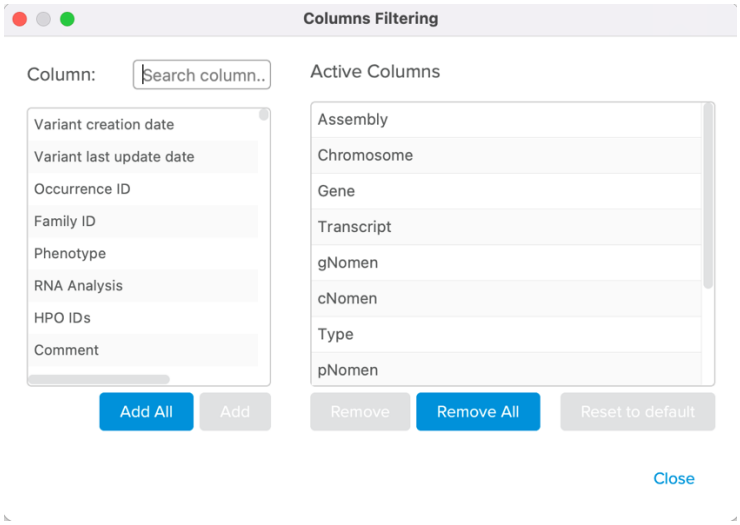
By default, the following information are displayed in the table: Assembly, Chromosome, Gene, Transcript, gNomen, cNomen, Type, pNomen, Coding effect, Evidences (ACMG - user defined), Classification, Notes.

The variants displayed in the table are automatically refreshed while you navigate through the genome. You can focus on a variant by clicking on a line of the table.

You can configure the table by using the ⚙ icon at the top left of the table. Two actions are available to either filter columns or rows of the table.

Filter Columns

When clicking on "Filter columns", a dialog pops-up where you can select which information you want to display in the table.



To add columns via the "Column" list:
- select columns and clicking on "Add" button.
- click on "Add All" button.
- double-click on a column.

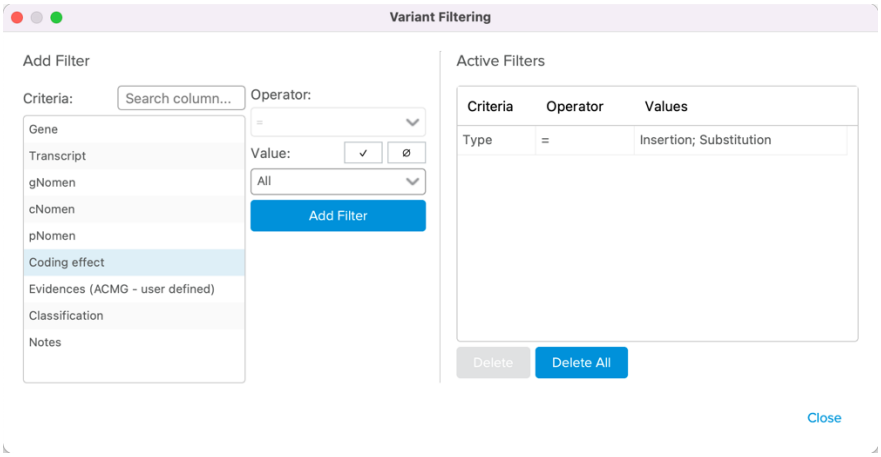
To remove columns via the "Active Columns" list:
- select columns and clicking on "Remove" button.
- click on "Remove All" button.
- double-click on a column.

The "Reset to default" button resets the table to its defaults state (containing the default columns only).

Filter Variants

When clicking on "Filter variants", a dialog pops-up where you can create filters on variants displayed in the table and in the track.

You can create filters only for information/columns currently displayed in the table.
You can create at most 1 filter by column.



You can create new filters via the left side of the dialog. A filter is defined by the following information:

- the column to apply the filter on.
- a value or a list of values.
- an operator used to compare the variant value(s) with the filter value(s).

The way to define values depends on the type of data of the column. For numeric column, you must manually fill values whereas for other columns you have to choose the values among a list.

The available operators depend on the type of the data of the column. For numeric columns the available operators are: =, <=, >=, ∈, ∉. For other columns, the only valid operator is =.

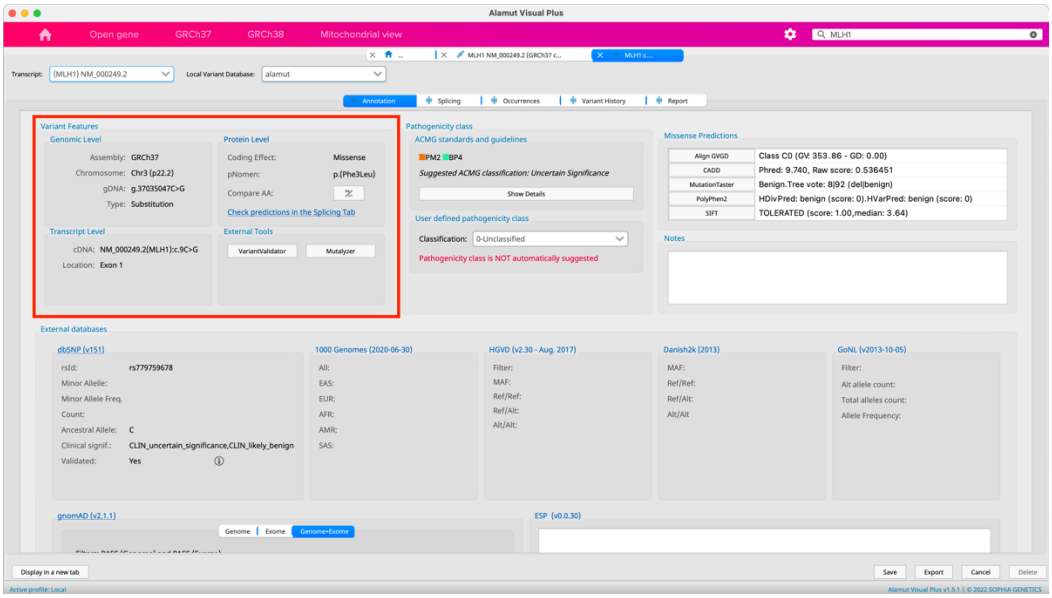
Once all the filter information filled, you can click on "Add Filter" button to add the filter to the table.

The list of "Active Filters" is displayed on the right side of the dialog. You can remove filters from the table by:
- selecting filters and using "Delete" button.
- clicking on "Delete All" button.

11.Interpret

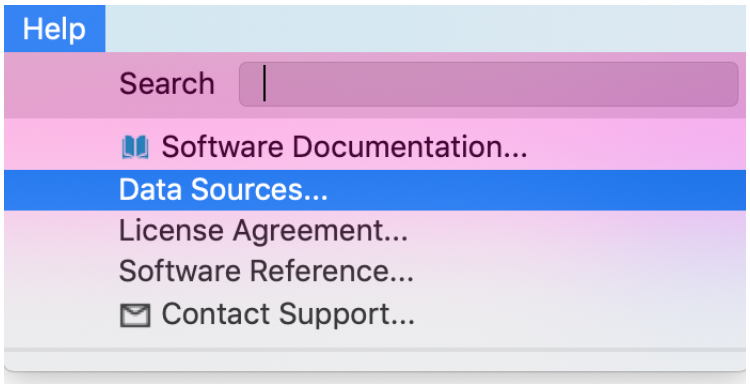
The Variant Panel gathers all information needed for variant interpretation in the **'Annotation'** and **'Splicing'** tabs.

The **'Annotation'** tab first contains a 'Variant Features' section where all variant nomenclature information (gDNA, cDNA, protein consequence, etc…) are displayed.

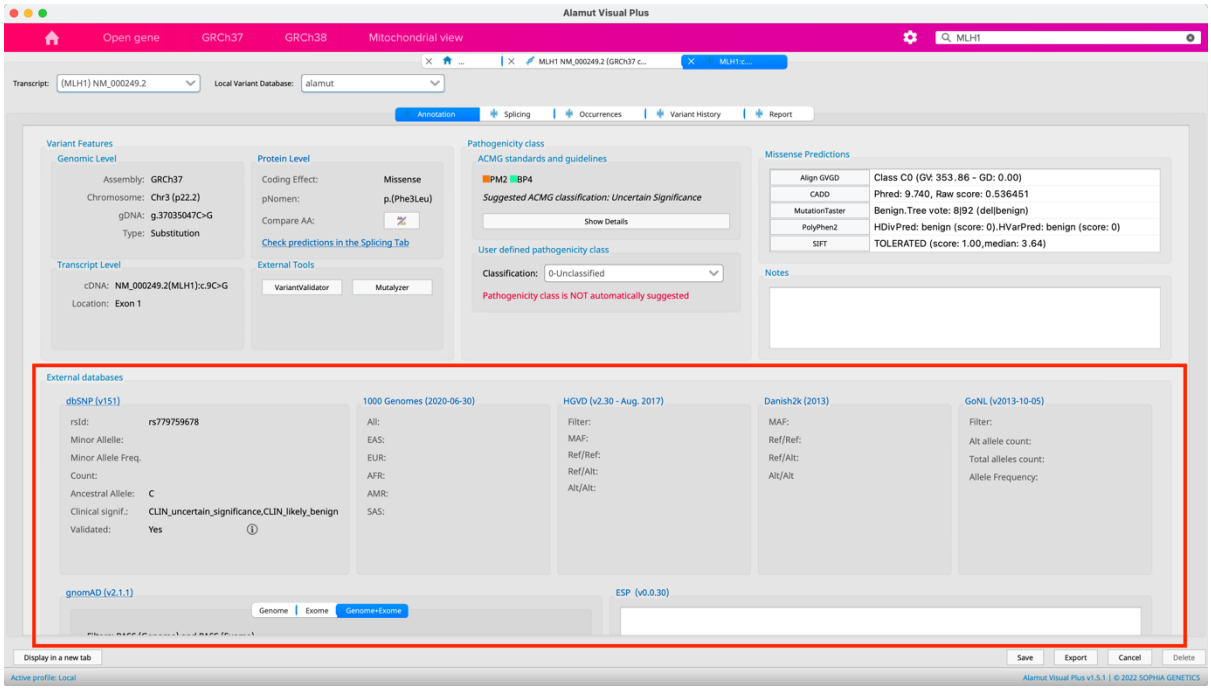


11.1 External Sources

To view the up-to-date list of data sources integrated into Alamut™ Visual Plus, go to **"Help > Data Sources"**. Database versions of each source is also specified in the Variant Panel.



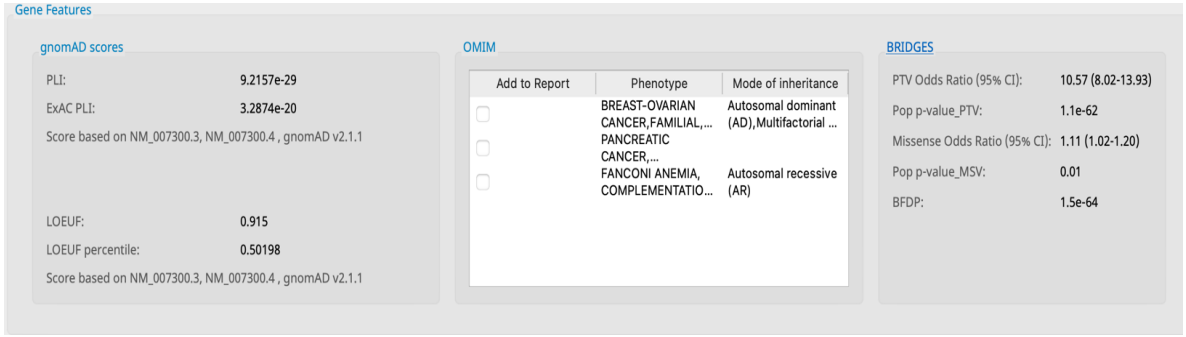
The '**Annotation**' tab of the Variant Panel contains an '**External Databases**' section which gathers all the data sources available for the given variant.



Note: The review status displayed in the variant panel are reported from COSMIC as following:

- The status "NA": “Not specified”, “Variant of unknown origin”, “Previously observed”
- The status “No”: "Reported in another sample as germline", "Confirmed germline variant"
  The status “Yes”: "Reported in another cancer sample as somatic", "Confirmed somatic variant"

The ‘**Annotation**’ tab also contains a ‘**Gene Features**’ section which contains some extra scores computed at the gene level (gnomAD scores, OMIM, BRIDGES).



Notes:

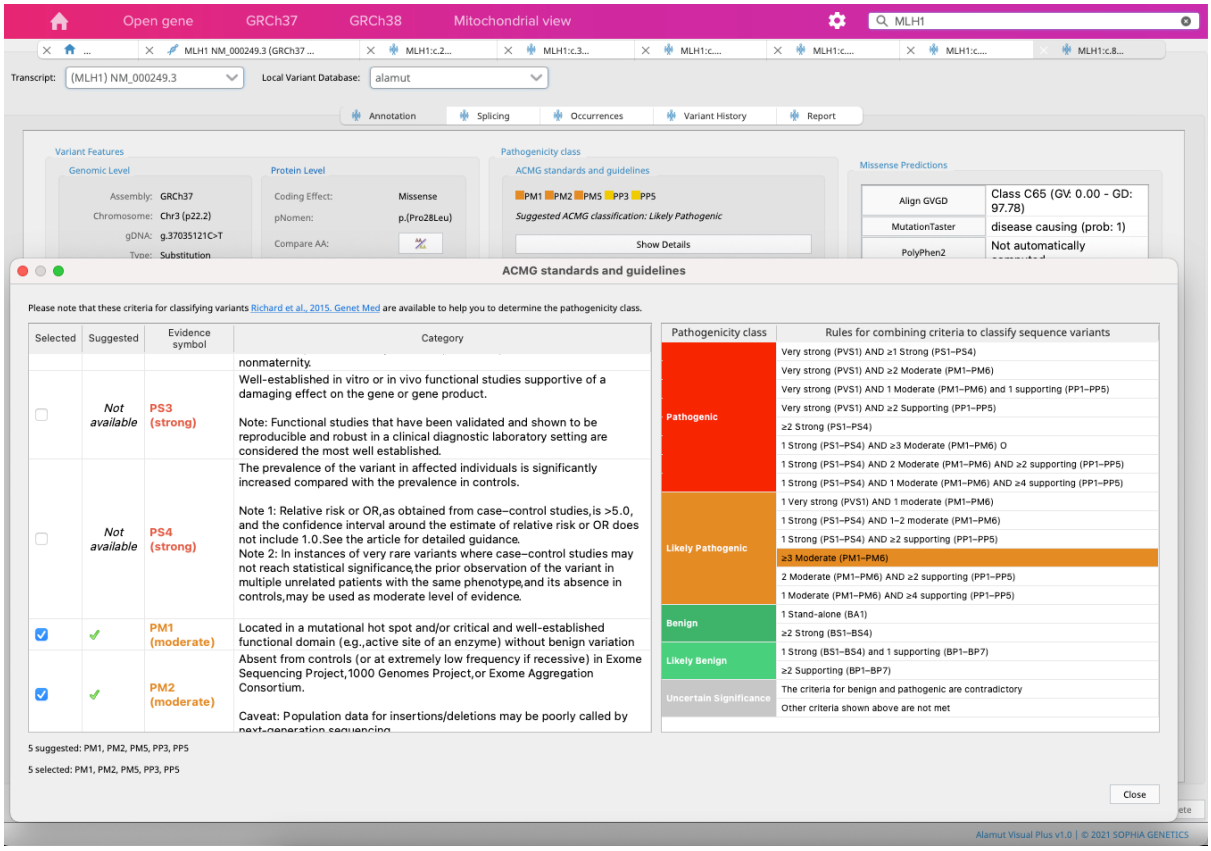
- To include OMIM® phenotypes in the report, you may tick the “Add to Report” checkbox
- Double clicking on an OMIM® row will open the corresponding OMIM® web page.

11.2 ACMG-AMP standards and guidelines

ACMG-AMP Variant Interpretation Standards and Guidelines Support (Richards et *al.*, 2015. *Genet Med* 17:405–424) are available in Alamut™ Visual Plus.

The ACMG guidelines support is available in the Variant panel > **'Annotation'** tab > **'Pathogenicity Class'** section. The suggested classification is displayed under **'ACMG standards and guidelines'**.

By clicking in 'Show details', the user has access to definitions of categories as reported in Richards et al., 2015.



When data are not available in Alamut database to support evidence (such as experimental results):

- 'Not available' is displayed in the 'Suggested' field
- A blank/empty evidence field indicates that Alamut Visual Plus does not support these ACMG criteria

11.3 Splicing Prediction Tools

Alamut™ Visual Plus includes a splicing module integrating several prediction algorithms and splicing prediction data:

a. Splicing signals:

- SpliceSiteFinder-like (donor, acceptor, branchpoint): Alamut™ Visual Plus uses the matrix described by Zhang et al. (1998) for branch points and the algorithms described in Shapiro et al. (1987).
- MaxEntScan (donor, acceptor): MaxEntScan splice site datasets and algorithms are fully integrated in Alamut™ Visual Plus, and reports scores from the Maximum Entropy Model.
- GeneSplicer (donor, acceptor): University of Maryland CBCB
- NNSPLICE (donor, acceptor): (the Berkeley Drosophila Genome Project) Alamut™ Visual Plus reports scores from NNSPLICE 0.9.
- Known constitutive signals (donor, acceptor): Alamut™ Visual Plus reports in the splicing module each occurrence of the 9-mers (3 exonic + 6 intronic nucleotides) found in the donor subset of human constitutive exon/intron junctions, and each occurrence of the 6-mers (4 intronic + 2 exonic) found in the acceptor subset. Acceptor 6-mers are reported only where at least 6 of the 8 upstream nucleotides are pyrimidines.
- Mercer high confidence branchpoints:

b. Exonic Splicing Enhancers (ESE) binding site detection:

- ESEFinder: ESEFinder matrices are embedded in Alamut™ Visual Plus to perform the computation provided on the ESEFinder web site.
- RESCUE-ESE: The set of human hexamers available from the RESCUE-ESE web site is embedded inside Alamut™ Visual Plus
- EX-SKIP: The EX-SKIP tool is available, through the Alamut™ Visual pre-filled form functionality, computes the number of RESCUE-ESEs, FAS-ESSs, PESEs/PESSs, neighbourhood inference and EIE/IIEs for each segment.

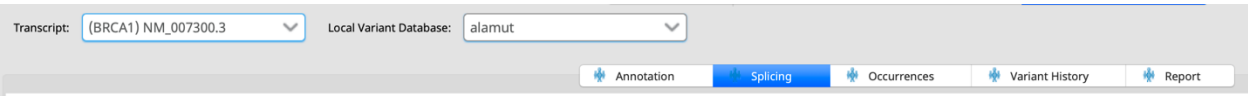
c. Splicing module:

The splicing module includes:

- The variant annotation window with automatically computed splicing predictions at the nearest junction for MaxEntScan and SSF predictors.
- The splicing prediction algorithm NNSPLICE from fruitfly.org partially integrated: it can be interrogated from Alamut™ Visual Plus through the Internet and its results are displayed seamlessly in the graphical interface.
- The splicing report that provides scores for each predictor in a tabular format.

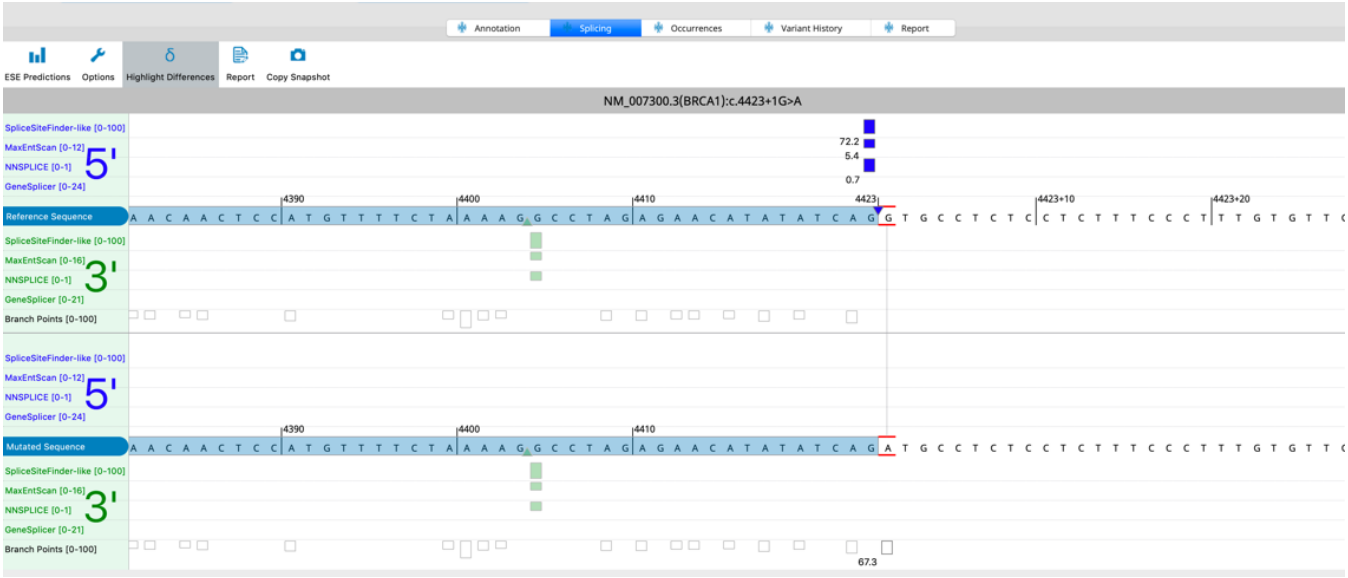
Using the splicing window:
To open the splicing window:

- click on the 'Splicing' tab in the variant panel



- The window displays the reference (wild-type) and mutated sequences and predictions are reported above and under each one.
- Exons are represented with blue boxes.
- Hits from SpliceSiteFinder-like, MaxEntScan, NNSPLICE and GeneSplicer are displayed as blue vertical bars for 5' (donor) sites, and as green vertical bars for 3' (acceptor) sites. The height of each bar is proportional to the maximum possible score computed by the corresponding algorithm.
- Known constitutive signals are displayed as small blue (5') or green (3') triangles, close to the sequence letters.
- Mercer et al. high confidence branchpoints are displayed as red triangles in the Branch Points sub-track of the Reference Sequence.

When moving the mouse over each vertical bar or triangle, a tooltip appears with the corresponding score. You can display score numbers for each hit bar by just clicking the bar itself.

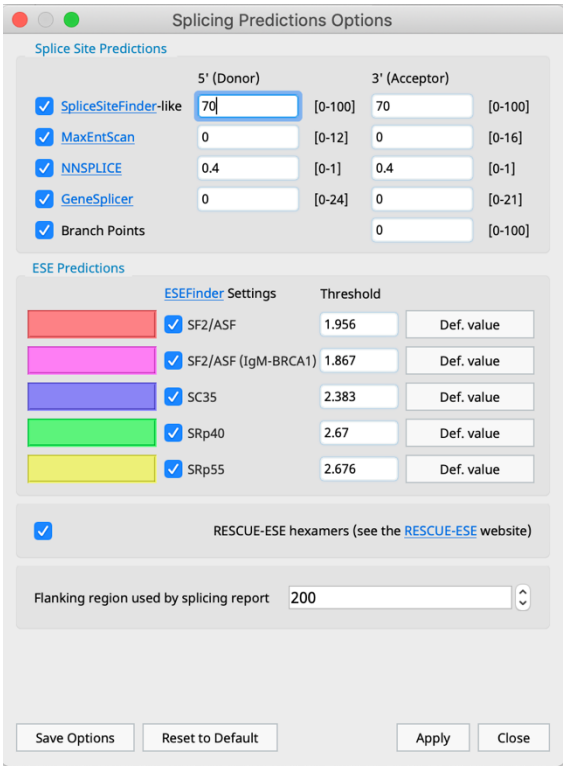


The region for which predictions are computed is centered on the position of the variant. You can navigate before and after this region by clicking and dragging the mouse.
Use the “Options” menu to select which predictions to display and to modify thresholds.

- To reveal differences between wild-type and mutated scores, click on the '**Highlight Differences**' button. Unchanged hits get dimmed, while scores are displayed beside those that differ.
- To reveal differences between wild-type and mutated scores, click on the '**Highlight Differences**' button. Unchanged scores get dimmed, while score numbers are displayed beside those that differ.

- To display ESE predictions, click the "ESE Predictions" button. ESE hits from ESEfinder are displayed above each sequence, and RESCUE-ESE hexamers are drawn under them:

- To launch the EX-SKIP tool: go to "ESE predictions" and click the "EX-SKIP" button. The pre-filled web form of the EX-SKIP tool is displayed in a new window. Input sequences are created as follows by Alamut Visual Plus: only exonic sequences are considered with up to 30 exonic nucleotides before or after the variant position within the exon.
- To generate a tabular report of splicing signals predictions, click the 'Report' button. By right clicking on the report, you can save it in your computer. The flanking region threshold can be set by the user via the “Splicing predictions options” tab. The default is fixed at 200.
- The user can select displayed splicing methods from the “Options” menu. Thresholds for splice site predictors can be saved as user-defined parameters.



References:

- Cartegni et al. ESEfinder: A web resource to identify exonic splicing enhancers.
  Nucleic Acids Res (2003) vol. 31 (13) pp. 3568-71
- Fairbrother et al. Predictive identification of exonic splicing enhancers in human genes.
  Science (2002) vol. 297 (5583) pp. 1007-13
- Hellen Splice Site Tools: A Comparative Analysis Report.
  NGRL Manchester Report 2009.
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  Hum Mutat. 2012 Aug;33(8):1228-38.
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  Genome Res (2015) 25(2): 290-303.
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  Nucleic Acids Res (2001) vol. 29 (5) pp. 1185-90
- Raponi, M., Kralovicova, J., Copson, E., et al. Prediction of single-nucleotide substitutions that result in exon skipping: identification of a splicing silencer in BRCA1 exon 6.
  Hum Mutat. (2011), 32, 436-444.
- Reese et al. Improved Splice Site Detection in Genie.
  J Comp Biol (1997) vol. 4 (3), pp. 311-23
- Shapiro, M. B. and P. Senapathy (1987). RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression.
  Nucleic Acids Res 15(17): 7155-7174.

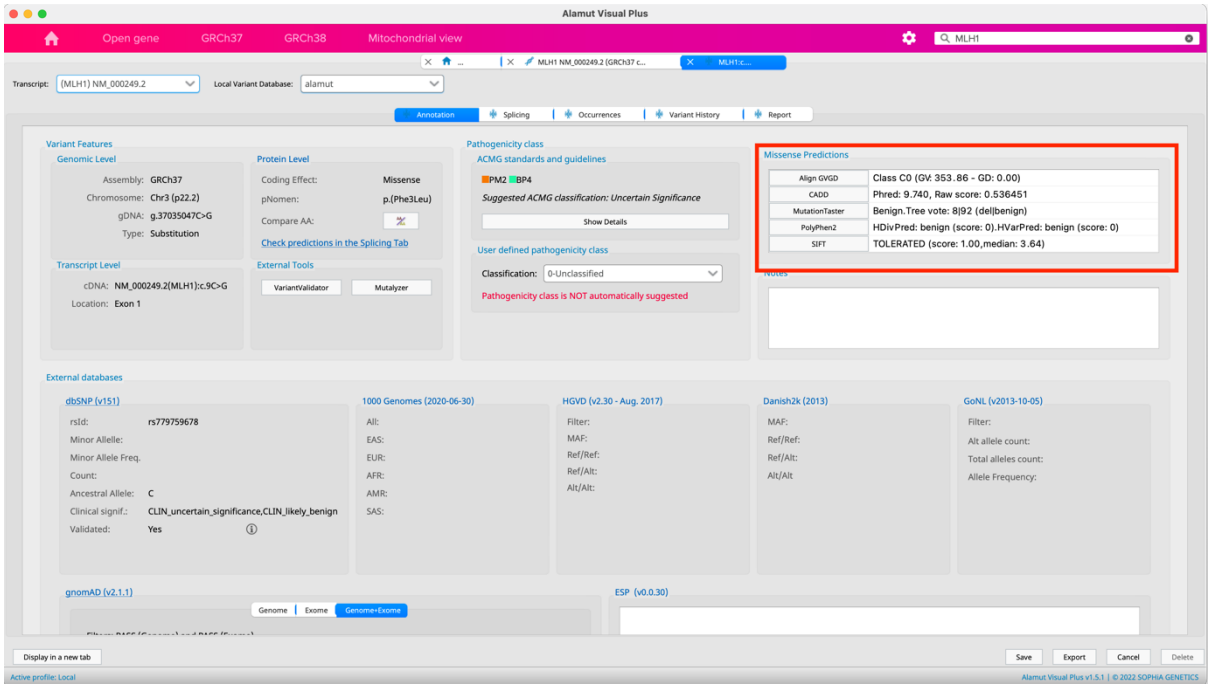
- Yeo et al. Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals.
  J Comput Biol (2004) vol. 11 (2-3) pp. 377-94
- Zhang et al. Statistical features of human exons and their flanking regions.
  Hum Mol Genet (1998) vol. 7 (5) pp. 919-32

11.4 Missence Prediction

Alamut™ Visual Plus provides automatically computed missense predictions done by Align GVGD and SIFT, based on orthologues alignments (see section 10.1.8).
With the genome build GRCh38 available in Alamut™ Visual Plus, some orthologues alignments coming from ENSEMBL Compara have been updated and may lead to different predictions that those described for the same genes/transcripts for GRCh37.

Currently, automatically computed predictions are provided for Align GVGD, SIFT, MutationTaster, Polyphen 2 and CADD.

Automatically computed predictions are available in the variant panel. The **'Annotation'** tab of the Variant Panel has a **'Missense Predictions'** section which contains the *in-silico* missense prediction scores from multiple tools. Clicking on the predictor name will bring you to the missense predictor website.



This section describes how automatically computed missense predictions are provided in Alamut™ Visual Plus:

Align GVGD
Align GVGD predictions are computed using the orthologue alignment provided by Alamut™ Visual Plus for the gene under study (See section 10.1.8).
Align GVGD scores interpretation are available here.
Current version for precomputed predictions: Align GVGD Tavtigian et al. (2007)

CADD
CADD prediction is a tool for scoring the deleteriousness of single nucleotide variants as well as insertion/deletions variants in the human genome.
CADD scores interpretation is available here.
Current version for precomputed predictions: CADD from CADD website

MutationTaster
MutationTaster predictions are computed using the mapping between NCBI RefSeq and Ensembl Transcript Ids available in Alamut™ Visual plus.
MutationTaster scores interpretation are available here.
Current version for precomputed predictions: MutationTaster2 from MutationTaster website

PolyPhen-2
Alamut™ Visual Plus displays precomputed scores from WHESS.db database. You may observe differences between the precomputed scores and the scores obtained via Polyphen-2 main query page between two updates of WHESS.db.
Current version for precomputed scores: WHESS.db for PolyPhen-2 website.

SIFT
SIFT predictions are computed using the orthologue alignment provided by Alamut™ Visual Plus for the gene under study. See SIFT Aligned Sequences.
SIFT scores interpretation are available here.
Current version for precomputed predictions: SIFT 6.2.0

References :

- NGRL Manchester Multiple sequence alignment use in missense prediction tools.
- Tavtigian SV, Deffenbaugh AM, Yin L, Judkins T, Scholl T, Samollow PB, de Silva D, Zharkikh A, Thomas A. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J Med Genet. 2005 Jul 13
- Ng PC, Henikoff S. Predicting Deleterious Amino Acid Substitutions. Genome Res. 2001 May;11(5):863-74. PubMed
- Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense variants. Nat Methods 7(4):248-249 (2010)
- Schwarz JM, R¤delsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010 Aug;7(8):575-6.
- Special thanks to the Genetic Cancer Susceptibility Group at IARC for their kind help in implementing the Align GVGD algorithm.
- Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. (29 October 2018), CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Research, 47, D886–D894Kircher M
- Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J (2014 Feb 2), A general framework for estimating the relative pathogenicity of human genetic variants, Nature Genetics, 46, pages 310–315

12. Curate

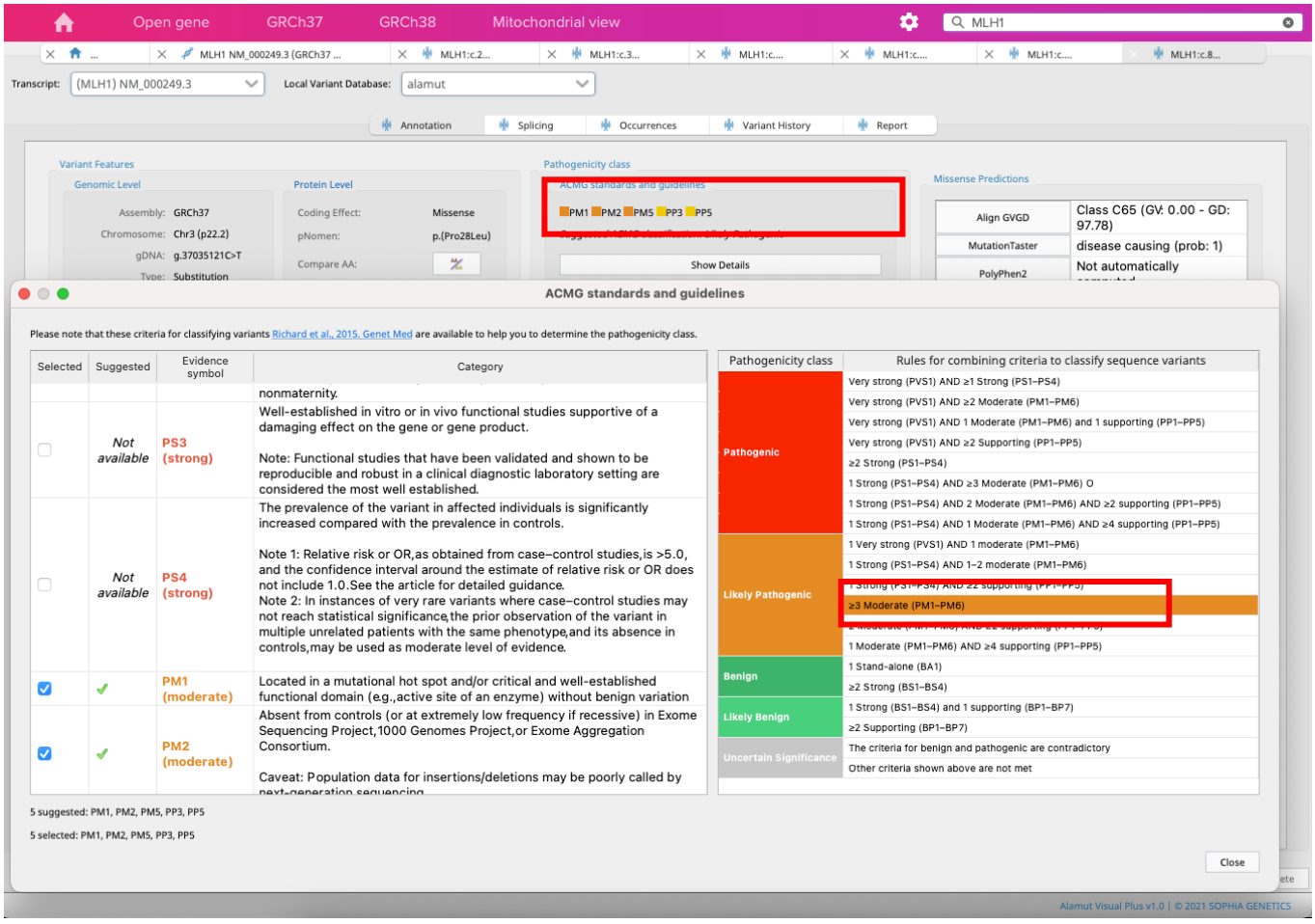
The Variant Panel can also be used to curate information on variants which are stored in the Local Variant Databases.

12.1 Variant Flagging

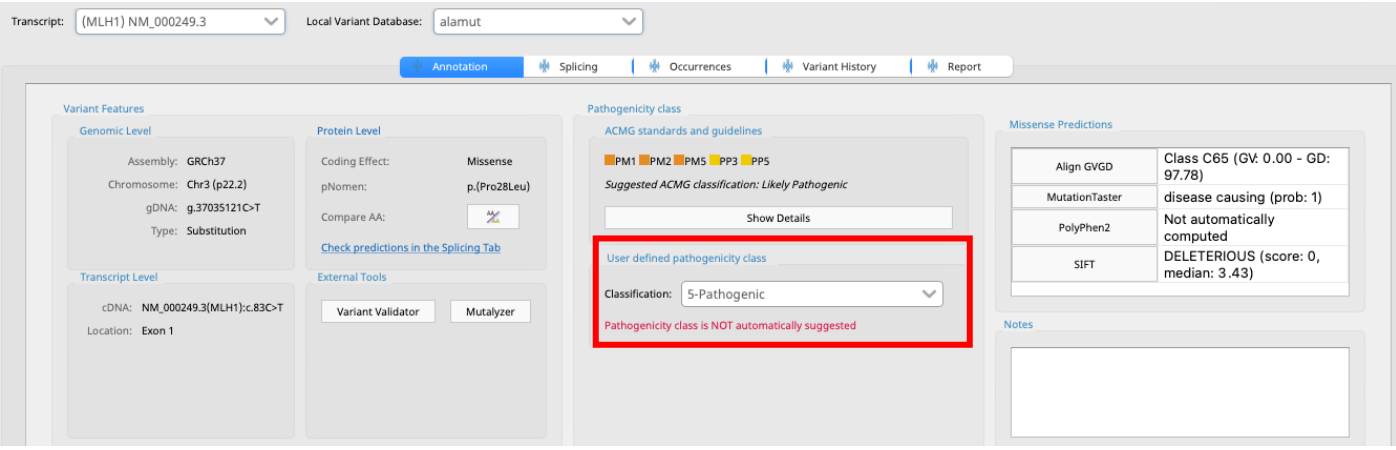
In the '**Annotation**' tab of the Variant Panel, you can flag your variant.

You can set pathogenicity information via the '**Pathogenicity class**' section.

You can select ACMG evidence for your variant based on the scientific context. To do so, click on 'Show Details' button and use the 'Selected' column checkboxes to select ACMG evidence. The selected evidence will be displayed in the variant panel. The suggested ACMG pathogenicity class is then assessed based on selected evidence.



The final pathogenicity classification is defined by the user in the '**User defined pathogenicity class**' section in the Variant panel.



The color of variant graphic items in the Local Variant Database track depends on its classification. By default:

1. Unclassified (light gray),
2. Benign (dark green),
3. Likely benign (light green),
4. Uncertain significance (dark gray),
5. Likely pathogenic (Orange)
6. Pathogenic (red).

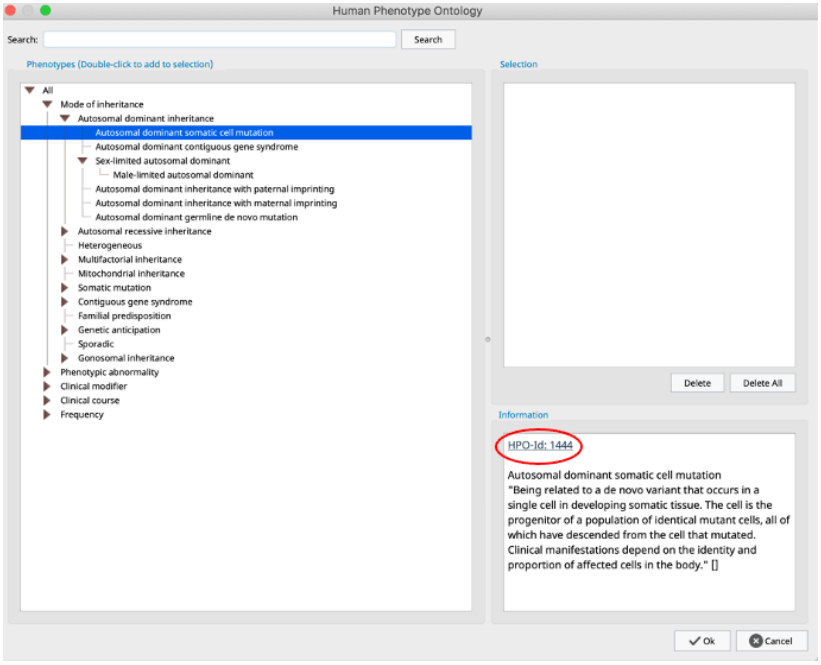
You can also add a comment on your variant via a free text field in the '**Note**' section.

12.2 Variant Occurence Management

In the '**Occurrences**' tab of the Variant Panel, you can record different occurrences of the same variant.

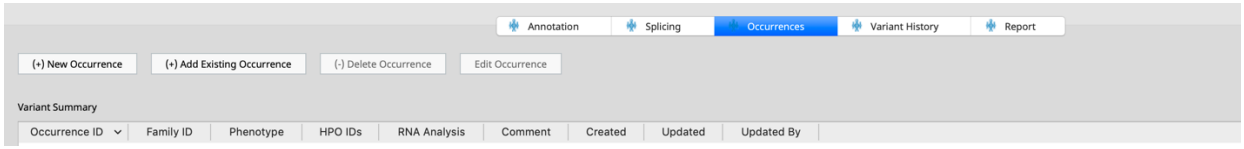
To create an occurrence, in the '**Occurrences**' sub-tab, click on the 'New Occurrence' button. You can then enter specific information about the Occurrence. Fields RNA Analysis, Phenotype and Comment are enabled.

Clicking on the ‘see HPO’ button, you can select a phenotype for your occurrence as referred to the HPO. A link to the HPO website is also provided. By double clicking on a phenotype, the selection field will be filled in. The HPO-IDs will be reported in the final ‘Occurrences’ sub-tab.



The entered information related to the occurrence are shown in a table in the ‘Occurrences’ sub-tab.

You can then manage your occurrences by deleting, editing, or adding existing occurrences:



12.3 Variant History

The Variant Panel has a ‘**Variant History**’ sub-tab which provides a history related to a same variant when updates are provided.

The variant history includes the ‘Date’, the ‘User’, the final pathogenicity ‘Classification’, the ‘Notes’ as written in the ‘Annotation’ sub-tab and the ‘ACMG Evidence’.

13.Reporting

You can create a final report including all information related to one specific variant. This functionality is available via the 'Variant Panel' > 'Report' sub-tab.

In the menu on the right, you can select features to display in the final report. By right clicking in the report, you can save the report in a PDF or HTML format. When the report is saved in HTML, it can be opened in Microsoft Word.

Note:
Splicing section: Only the impact on the nearest reference splice site (±2bp at the donor and acceptor site of an exon) is included in the report. For each splice site predictor (SpliceSiteFilder, MaxEntScan, NNSplice), Alamut Visual Plus evaluates how much the site score changed by computing the following ratio:

$$\frac{(score\ on\ the\ mutated\ sequence\ -\ score\ on\ the\ reference\ sequence)}{score\ on\ the\ reference\ sequence}$$

An impact of the splicing is detected if the splice site scores have changed from more than 1% on average or if the variant is at less than 5 bps of the splice site.

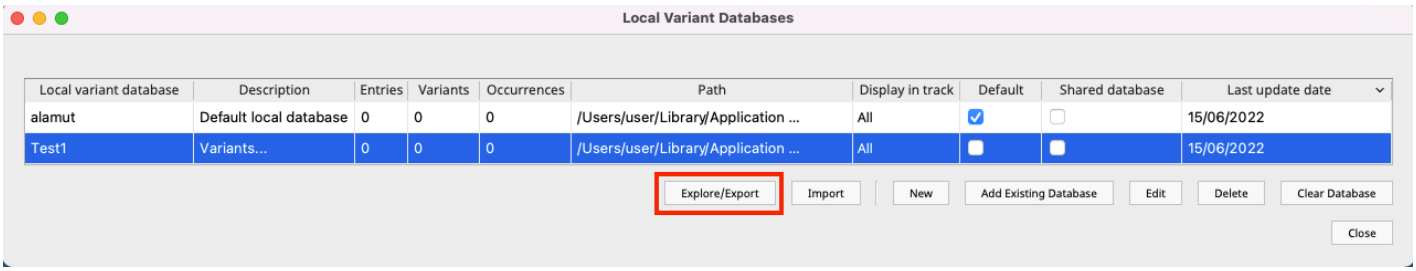
14.Exporting (batch-like feature)

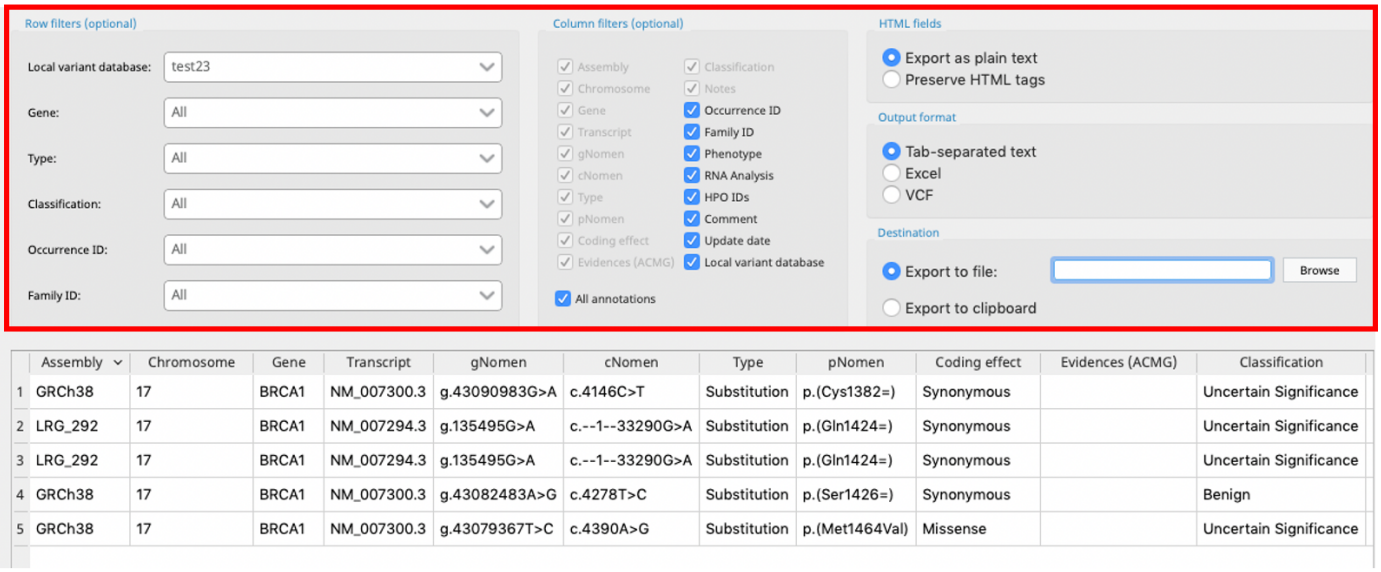
Export internal Variants

To export variants already entered in Alamut™ Visual Plus to Excel, to tab-delimited text files or to VCF, go to:

- menu 'Variants' > 'Local Variants Databases'
- Select the source Local Variant Database
- Click on 'Explore/Export' button

The 'Variant Exporter' pops-up. Select options, fields you need to export and the folder where you need to save the exported variants.





	Assembly	Chromosome	Gene	Transcript	gNomen	cNomen	Type	pNomen	Coding effect	Evidences (ACMG)	Classification
1	GRCh38	17	BRCA1	NM_007300.3	g.43090983G>A	c.4146C>T	Substitution	p.(Cys1382=)	Synonymous		Uncertain Significance
2	LRG_292	17	BRCA1	NM_007294.3	g.135495G>A	c.--1--33290G>A	Substitution	p.(Gln1424=)	Synonymous		Uncertain Significance
3	LRG_292	17	BRCA1	NM_007294.3	g.135495G>A	c.--1--33290G>A	Substitution	p.(Gln1424=)	Synonymous		Uncertain Significance
4	GRCh38	17	BRCA1	NM_007300.3	g.43082483A>G	c.4278T>C	Substitution	p.(Ser1426=)	Synonymous		Benign
5	GRCh38	17	BRCA1	NM_007300.3	g.43079367T>C	c.4390A>G	Substitution	p.(Met1464Val)	Missense		Uncertain Significance

All greyed fields in the ‘Column filters’ are exported by default.

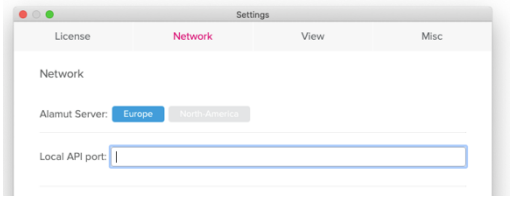
Export Variants with external annotation

For each variant entered in the software, Alamut™ Visual Plus generates a set of annotations. Annotations are gathered for each variant and can be exported by ticking the “All annotations” checkbox.

15.Connect with an API

Alamut™ Visual Plus includes a programmatic access functionality through an Application Programming Interface (API) enabling external tools to control the software. Notably, the search bar is open to external software: any software tool or web page can be customized to request Alamut™ Visual Plus to display any information that can be processed by the search bar.

The server listens to local HTTP GET requests coming through the port that is specified in the Options dialog box (see above). The default port is set to 10000 but it can be changed to any available port in the Options dialog box (menu ‘Settings’ > ‘Network’ > ‘API’ section).



15.1 Specifications

Four HTTP GET requests can be processed by Alamut™ Visual Plus: **version**, **search**, **open** and **annotate**.

Search, **open** and **annotate** require the user or the third-party software to provide its institution ID and API key. If you don't have one yet, please contact support@sophiagenetics.com.

A detailed description of the API can be found here:
https://extranet.interactive-biosoftware.com/Alamut_Visual_Plus_API_2.0.0.html

15.2 Version

```
http://127.0.0.1:10000/version
```

Upon receiving this request, useful for testing purposes, Alamut™ Visual Plus returns an output with its current version and name, along with the list of external sources and their versions as a json file.

15.3 Search

```
http://127.0.0.1:10000/search?institution=XXXXXX&apikey=YYYYYYYY&request=NM_000059.3%3Ac.4563A%3EC
```

This request asks Alamut™ Visual Plus to display the result of a search (here variant "NM_000059.3:c.4563A>C" (after percent-encoding see https://www.urlencoder.org)).

Any request that can be processed by the search field of Alamut™ Visual Plus (see **10.4.3. Search bar (Extended Access Feature))** can be processed with the "search" endpoint.

15.4 Open

```
-connect.interactive-biosoftware.com%2FBAM%2Fexample.bam
```

This request asks Alamut™ Visual Plus to open a BAM file located at `http://rd-connect.interactive-biosoftware.com/BAM/example.bam` (the path is percent-encoded,

see https://www.urlencoder.org). A gene or a genomic region has to be opened beforehand in this case via the **search** endpoint. The path to a local path should contain "file://" as in the examples below:

```
http://127.0.0.1:10000/open?institution=XXXXXX&apikey=YYYYYYYY&filetype=bam&path=
file%3A%2F%2F%2FG%3A%2Fgenetics%2Ftest%2FMLH1%E2%80%AFgrch37.bam
```

This will open `file:///G:/genetics/test/MLH1 grch37.bam` (which is `G:\genetics\test\MLH1 grch37.bam` under Windows).

```
http://127.0.0.1:10000/open?institution=XXXXXX&apikey=YYYYYYYY&filetype=bam&path=fi
le%3A%2F%2F%2FUsers%2Ftest%2FMLH1%20grch37.bam
```

This will open `file:///Users/test/MLH1 grch37.bam`.

The **open** API endpoint supports BAM, CRAM and Sanger file types.

15.5 Annotate

```
http://127.0.0.1:10000/annotate?institution=XXXXXX&apikey=YYYYYYYY&variant=NM_00024
9.4%3Ac.121G%3EC
```

This request asks Alamut™ Visual Plus to annotate the following percent-encoded variant: NM_000249.4:c.121G>C

A JSON file will be returned containing data available in the system regarding this variant: variant features (at genomic, transcript and protein levels), references and data available in external catalogues (ClinVar, dbSNP, gnomAD, etc), splicing effect, scores from missense predictors (if relevant), etc.

The **annotate** API endpoint supports variants provided according the HGVS nomenclature rules.

Miscellaneous

Menus

- Application
  - Open Gene
  - GRh37
  - GRh38
  - Mitochondrial view
  - Home
- File
  - Open BAM/CRAM File
  - Open BAM from URL

    - Open Sanger File
    - Export Fasta Sequence
- View
    - Focus on
    - Show ruler
    - Color nucleotides
    - Use amino acid 3 letter code
    - Full Screen
    - Show navigation bar
    - Increase Font
    - Decrease Font
    - Reset Font
    - Enter Full Screen
- Web
    - View gene in Ensembl Browser
    - View displayed region in Ensembl Browser
    - View entire region in NCBI Sequence Viewer
    - View entire region in UCSC Browser
    - View HGNC symbol report for this gene
    - View gene in OMIM® web site
    - View gene in GENATLAS web site
    - View gene in Gene Reviews web site
    - View Uniprot entry for the product of this gene
- Variant
    - New Variant
    - Local Variants Database
- Tools
    - Genetic Code
    - Compare Amino Acids
    - Assembly Mapping
    - Take Screenshot
    - Nomenclature validation dialog
    - Variant validation dialog
- Help
    - Software Documentation
    - Data Sources
    - License Agreement
    - Software Reference
    - Contact Support

Navigation with keyboard

Use the following keys to navigate in Alamut™ Visual Plus:

- **Left arrow key**: step shift the sequence to the left
- **Right arrow key**: step shift the sequence to the right
- **Up arrow key**: step shift the tracks upwards
- **Down arrow key**: step shift the tracks downwards

16.Quality Control Procedures

External genomic data has been successfully tested and implemented in Alamut database. Frontend functionalities and data display have been successfully tested and implemented.

16.1 Warnings and Limitations

For Research Use Only. Not for use in diagnostic procedures.
Alamut™ Visual Plus does not provide recommendations for medical diagnosis. It must be used by human genetics professionals with discretion. SOPHiA GENETICS does not guarantee the accuracy of information and predictions it provides.

16.2 Residual Risks

No residual risk has been identified as part of Alamut Visual Plus risk assessment.

17.Other Information

17.1 Training

Before using the Software, video tutorials are provided in the software homepage to get started. Further requests on live demo and support can be addressed to: support@sophiagenetics.com

17.2 Responsibility

This system solely supports the intended user and does not substitute or replace the intended user's experience and/or responsibility during its use. It must always be possible for the user to proceed without the assistance of the system.

17.3 Documentation

This user manual describes the use of an interpretation software for genomic variations that must be used with care. It is therefore important that all users of the software:

1. Read this guide carefully before use.
2. Have access to this guide at all times.

18.Symbols

	User Manual, Operating instructions
	Research Use Only
	Reference Number
	Manufacturer

19.Support

In case of difficulty using the product, contact our support line and e-mail mentioned on the "Summary Information" page of this user guide.