# **User Manual**

# Alamut<sup>™</sup> 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





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# **1. Product Description**

Alamut<sup>™</sup> 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<sup>™</sup> Visual Plus is an upgrade of Alamut<sup>™</sup> Visual.

# 2. Intended Use/Purpose

#### 2.1 Intended Use

Alamut<sup>M</sup> Visual Plus is a Research Use Only (RUO) software that assists in routine genomic analysis. Alamut<sup>M</sup> 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<sup>™</sup> 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)





# 4. Product Components

The product is only composed of the Alamut<sup>™</sup> Visual Plus software.

Alamut<sup>™</sup> 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<sup>™</sup> Visual Plus extranet page: <u>http://extranet.interactive-biosoftware.com</u>.

# 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<sup>TM</sup> 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	<ul> <li>Connection to the following IP addresses are required:</li> <li>20.224.43.76</li> <li>212.83.147.70</li> <li>The software handles connections through HTTP/HTTPS on port 80 and 443, optionally through a proxy server.</li> </ul>
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<sup>™</sup> Visual Plus extranet page and create a new user account through the "Add user" button.

🕴 Alamut <sup>®</sup> Visual Plus I	Extranet
Institution ID: ADE0192	Users Management
2 Dashboard	Add user
😤 Users Management	Show 10 - entries

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 <sup>1</sup> button and paste it in a direct e-mail to the concerned user.

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

	• alamut VISUAL PLUS <sup>**</sup>
	Alamut <sup>®</sup> Visual Plus extranet
Pleas	e Sign In
Inst	tution ID
Use	r Name
Pas	sword
	Login
	I forgot my username or password

Alamut<sup>™</sup> Visual Plus binaries can be downloaded from the "Downloads" section.

🤌 Alamut <sup>®</sup> Visual Plu	us Extranet	
Institution ID: ADE0192	Downloads	
B Dashboard	Alamut <sup>®</sup> Visual Plus v.1.0 (Feb. 2021)	
📽 Users Management	Microsoft Windows 64-bit (7.1)	
Lill Activity Stats		Installer Self-Extractable
License Details	Apple Mac OS X (10.12+)	Installer
▲ Downloads		
Extranet User Guide		



# 5.2.1. Install on Microsoft Windows

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

- 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: <u>http://extranet.interactive-biosoftware.com</u>.

- 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<sup>™</sup> Visual Plus, launch the software and:

• Define the application's data folder, where Alamut<sup>™</sup> 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<sup>™</sup> Visual Plus. By selecting 'No', settings will be restored.

s there a previous version of Alar f so, you can retrieve its applicati ou will just have to specify the fo to you want to use an existing se	nut Visual Plus on this computer? on settings. lder of the previous version. ttings folder?	
Yes	O No	
lease select the settings folder ye	ou want to use:	
Users/peio/Library/Application S	Support/AlamutVisualPlus	Browse
		ОК



<u>Note:</u> If, for quality purpose, you do need to validate a new version of Alamut<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> Visual Plus
- Uninstall Alamut<sup>™</sup> Visual Plus
- Install Alamut<sup>™</sup> Visual Plus and choose the settings directory used by the previous version of Alamut Visual Plus
- Accept the End User License Agreement.

Alamut Visual Plus	
Alamut Visual Plus License Agreement	
End User License Agreement and Conditions of Sales for ALAMUT VISUAL PLUS Software	
Preamble	
This end-user License (the "Agreement") is entered into between SOPHIA GENETICS SAS, a French corporation having its head office at 374 Allee Antoine d'Abbadel, Technopole Izathel, 64210 Bidart, France including its subsidiaries SOPHIA GENETICS SA, SOPHIA GENETICS EIRELI and SOPHIA GENETICS, inc.) and you (including the legal entity you represent) (the "Customer").	
The software you are about to download, install and use, including its documentation, is protected by intellectual property rights. Intellectual property rights to this software are held by SOPHIA GENETICS and/or its assignors/licensors.	
By downloading or installing this Software, you acknowledge that you understand and unreservedly accept the terms and conditions of this agreement as set out below and where you act on behalf of a legal entity that you have the authority to act as issued by such entity. SOPHIA GENETICS only grants user rights to the software to the Customer under the provisions set out in this License.	
Section 1 - Definitions	
"Authorized Activity" shall refer to the activity for which use of the Software is granted under this Agreement, as set out in the Customer's order;	
"Authorized Computer" shall refer to the computer device(s) by which the Software is to be executed;	
"Database" shall refer to the database relating to the human genetics information necessary for the Software to function.	
"Database Server" means the server that is managed and updated by SOPHIA GENETICS holding the Database consulted by the Software via an internet connection.	
"Documentation" shall refer to the Software documentation. consisting of user manuals and other	
Click here to view this License Agreement in your web browser.	
I agree I do not agree	

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

• 0 •	Settings			Settings		
	License	Network	License		N	letwork
License			Network			
User			Alamut Server: Europe	North-America		
Institution			Local API port:			
			Use Proxy		Port	Type
Language			HTTP Proxy:		0	нттр 🗸
🕒 English 🔵 Fi	ançais		Suggested:			HTTP VISe
Application	rheck annieation undates					
Value and Carly	спеск аррисалог орыната					
		Cancel Seve				Cancel Save



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.

	Alamut Visual Plus	
	Warning	
This software a does not It must be used by	pplication is a genomic variant explora provide recommendations for medical r human genetics professionals and wit	tion system that diagnosis. th critical judgment.
SOPHi	A GENETICS cannot guarantee the acc information and predictions it provides	uracy of s.
	Please read the user manual carefully.	
Authentication		
	Username:	
	Password:	
Reset my passwo	ord	
	Ca	ncel Login

#### 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	Settings     License Network View Misc Profiles
purchased the license	License
License Key: the license key of the	User
institution	Licence Key
User Name: the name of the connected	UserName
user	Language English
Language: Select your preferred	Application
language (English or French).	Automatically check application updates
Application: Tick the box to	
automatically check for updates.	Cancel Save

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).

	• • •	Setting	IS	
Use Proxy: To use a proxy server tick the	License	Network	View	Misc
Use Proxy box and provide the required	Network			
information to connect to it.	Alamut Server: E	urope North-America		
	Local API port:			
	Vse Proxy		Dert	Turne
	HTTP Proxy:		Port	HTTP V
	Suggested:			нпр 🗸 Use
				Cancel Save

#### View

Show Selected Transcript on Variant			Settings		
<b>Tracks:</b> select to view the selected	License	Network	View	Misc	Profiles
transcript on the tracks.	View				
Surround protein-level descriptions with	Show Select	ed Transcript on Vari	ant tracks		
brackets: select to use brackets in pNomen.	Surround pro	otein-level description	ns with brackets,eg: p.	(Arg22Ser)	
	🗌 Use systema	tic exon numbering b	y default		
Use systematic exon numbering by default:	Default genom	ie build			
select to use systematic exon numbering by default for transcripts.	O GRCh37	GRCh38			
	VIEW NAME	TRACK			
Default Genome Build (GRCh37 or GRCh38):	Default	V	Genome	Default	
use by default		$\checkmark$	Nucleotide Conservati	on	
		$\checkmark$	Transcript	Default	
View table: See part allows you to create		► 🔽 .	Allele Frequency Data	bas Check all / Unche	ck all
custom views. For more information, see		• 🗸	ClinVar		
Section 10.2.	+ -	$\checkmark$	UniProt		
	✓ Use as defau	It configuration			
				Cano	Savo
				Canc	Save



#### 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.

~				
License	Network	View	Misc	Profiles
Misc				
Mouse Wheel				
🔵 Zoom / CTRI	_ key pressed : Scroll	<ul> <li>Scroll /</li> </ul>	CTRL key pressed : 2	loom
CRAM Settings	5			
Samtools REF_F	PATH:			
Samtools REF_	CACHE:			
	Example: /somep	path/RefCache/%2s/%2s/%	\$	
Other				
🗹 Ask for confi	rmation before saving	variants		
Automaticall	y reload BAM files			
Automaticall	y hide empty datasets	5		
🗹 Warn user w	hen a new version is a	available		
Admin				
Switch to pre	eprod servers			
			Can	cel Save

**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 <u>Samtools man page</u>). 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.

<u>Note:</u> 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		Mahurala	Settings	Mine	Desfiles
reature	License	Network	VIEW	MISC	Profiles
Profile Directory:	Profiles				
• When activating the feature, you must	🗹 Activate profi	iles			
choose a Profile Directory. This	Profiles directory	y: plication Support/	AlamutVisualPlus/Alam	utVisualPlus-Profiles	Browse
directory is used by the application to store the profiles relative files.	Selected profile:	MyNewProfile	~ <b>+</b>	_	
• 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:				0	col Source
You can use the combo-box to choose your				Can	Save
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.

• •	Alamut Visual Plus
	Warning
This software does not It must be used b	application is a genomic variant exploration system that provide recommendations for medical diagnosis. y human genetics professionals and with critical judgment.
SOPH	A GENETICS cannot guarantee the accuracy of information and predictions it provides.
	Please read the user manual carefully.
Authentication	
	Username:
	Password:
Reset my passw	ord
Profiles	
🗹 Activate prof	iles
	Select a profile: Profile-1
	_
	Cancel Login

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 (<u>http://extranet.interactive-biosoftware.com</u>)
- select the downloads section
- if available download the latest version.

Downlo	oads		
Alamut®	Visual Plus v.1.0 (Feb. 2021)		
4	Microsoft Windows 64-bit (7+)	Installer	Self-Extractable
<b>É</b>	Apple Mac OS X (10.12+)	Installer	

Or click on the "Update" button in the left part of the Alamut<sup>™</sup> Visual Plus homepage

# 6. Explore 6.1 Navigation ↑ Open gene GRCh37 GRCh38 Mitochondrial view ♀ ♀ chr3:37050315, MLH1, NM\_000249.3, rs6375089...

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.

									Alamut Vis	ual Plus											
A					Mitochone	Irial view								<b>4</b>	<b>2</b> Q	MLH1					0
							× 🕈 .		X 🥖 MUHI	NM_000249.4 (GRCh	37										
-	View configuration	Default	~	Transcript	NM_000249.4	~	Exon	naming			~		tran		N	REGION	A VIEW	HGNC	ATLAS G	R Uni	Prot
Overview of Transcript	t NM_000249.4 (MLH1)						м	LH1 - MutL	homolog 1	GRCh37 (Chr 3	3)   <b>g</b> r	nomAD SCORES	OMIM*	BRIDGES	s						8
MANE Select	e <mark>10</mark> 1	c.117 p.39	e.208 p.70	c.307 p.103	6.381 c.454 p.127 p.152 5 6	c.546 p.182	c.678 p.226 9	e.791 p.264	c.885 p.295	e.1039 p.347	e.1410 p.470			e.1559 e p.520 p	15		c.1732 c.1990 p.578 p.664	e 27 4 p.702			
● ↑ ↓ Genome - chr 37,035,000	r3:37,035,009-37,092,337 (GRCf 37,040,000	1 <b>37) - 57,328 bps</b> 37,045,00	0	37,050,000		37,055,000		37,060,000		37,065,000		37,070,000	37,075,00	0	37,08	5,000		37,085,000		37,09	0,000
● ↑ ↓ Nucleotide C	ionservation 🛱																				
Cone Horno s	sapiens muti, homolog 1 (MLH1) no sapiens muti, homolog 1 (MLH1), su	l, transcript variant 1 monipt variant 1, mBNA	1, mRNA.																		
●↑↓ alamut 亚	1	1		1	"	1															_
ins/Dup Subst																					
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● ↑ ↓ ClinVar 幸 Det/Delins	I					•	-		•			•				•				-11	-
Ins/Dup Subst																					
© ↑ ↓ UniProt						÷										÷					
																					-
															« «	(	>	Q	Q	A-	A+
Active profile: Local																	Ala	mut Visual Plus	v1.5.1   © 20	22 SOPHIA	GENETICS



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 <u>section 11</u> for more information).

• • •			Alamut Visual Plus			
n Open gene GRCh37	GRCh38 Mitod	ochondrial view			🗘 🔍 MLH1	0
Transcript (MLH1) NM_000249.4 Varia	nt Database: alamut	X Annotation	MM_000249.4 (GRCh37 X MLH1:c Occurrences Variant History	Report		
Variant Features		Pathogenicity class				
Genomic Level	Protein Level	ACMG standards and	guidelines	Missense Predictions		
Assembly: GRCh37 Chromosome: Chr3 (p22) gDN4: g37035047C>G Type: Substitution Transcript Level cDN4: NM_000249.4(MLH1):c9C>G Location: Exon 1	Coding Effect: Mil pNomen: p.( Compare AA: Check predictions in the Splicing External Tools VariantValidator Muta	Assense SPA2 IIBP4 ((Phe3Leu) Suggested ACMG d 2 3 1 1 1 1 1 1 1 1 1 1 1 1 1	assification: Uncertain Significance Show Details encity class nclassified ShOT automatically suggested	Align OVGD CADD MutationTasser PubyPhen2 SIFT Notes	Class CO (GV. 353.86 - GD: 0.00) Phred: 9.740, Raw score: 0.536451 Benign.Tree volt: 8)92 (deliberign) HDIvPred: benign (score: 0).HVarPred: benign (score: 0) TOLERATED (score: 1.00,median: 3.64)	
External databases       eb3/P_(v)51)       rsid:     rs779759678       Minor Allelie:     Minor Allelie       Court:     Court:       Ancestral Allelie:     C       Club.uncertain_significant     Validated:       Validated:     Yes       gnomAD (v2.1.1)	Canone Dome Commentation	Senomes (2020-06-30)	HGYD (v2.30 - Aug. 2017) Filter: MAF: Ref/Aft: All/Aft: ESP (v0.0.30)	Dansklak (2013) MAR: Ref/Ref: Ref/AR: Alt/At:	GoNL (v2013-10-05) Filter: At allele count: Total alleles count: Allele Frequency:	
Display in a new tab					Save Export Cancel	Delete
Arthur profile Local					Alamat Visual Plus v1 5.1 1 0 2022 SOI	HIA GENETICS

# 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.

-	View	configurat	tion Default	~ <	Transcript	JR_037864	.1 🗸	Exon	naming	g System			~											e!	SN.
Overview	of Chrom	nosome 1	1			A	P4B1-	AS1-	AP4	B1 ant	isense	RNA	1   GRC	h38 (C	hr 1)	)									
p36.31	p36.13	p35.3 j	p34.3 p34.1	p32.3	p31.3	p31.1	p22.3	p21.3	p21.1	p' øt	p11.2	q12	q21.1	q21.3 q	23.2	q24.2	q25.2	q31.1	q31.3	q32.1	q32.3	q41	q42.13	q43	q44
13,660,000	113,680,	000  113,	,700,000  1	13,720,000	113,740,00	00  113,	760,000	113,780	,000	113,800,0	000  113,	820,000	113,840,	000  113,	860,00	0 113	880,000	113,90	0,000	113,92	0,000	113,94	0,000  1	13,960,0	00
O↑↓ O↑↓	Nucleoti Gene Ho	de Conse mo sapie	ervation ans AP4B1	<b>x</b> antisense	RNA 1 (AF	94B1-AS	î1), trans	cript va	riant 2	, long n	on-coding	g RNA.		AP4B1-	AS1	View all AP- Open AP4B	IB1-AS1 trar 1-AS1 in new	nscripts v tab	>						

- 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.

		(	Gene Selection			BRCA1 - Transcript Selection
	Type in a gene syr	nbol:				Transcript list Show extended list of transcript versions
	BRCA1					Build Transcripts Exons A4s MANE Class
	Or select a gene s	hortcut (you can custo	omize this list):			GRCh37 NM_007300.3 24 1884
	BRCA1	BRCA2	CFTR	MLH1	MSH2	04Ch37 1M4_007294.4 23 1065 MANE Select 04Ch37 1M4_007294.3 23 1065
	APC	МҮВРСЗ	NF1	MSH6	MYH7	Description:
	Gene List	~			Change shortcuts	Chromosome 17: CRCh37 Preferred Transcript Transcript Variant: This univer (7) Includes an alternate in-forma area
	If the requested gene The reference nomen	e is not available, please s clature of human genes is	end us an email request. available at <u>HUGO</u> .			and an alterinate in-frame splice line is the central coding region, compared to variant 1. The encoded isoform (2) is longer than isoform 1.
	Email Gene Re	quest		C	OK Cancel	Carol
	× 🕈	🗙 🥖 GR	× 🖋 BRCA1	NM_007300.4 (GRC	h37 c ×	🗸 AP481-AS1 NR_037864.1 (GRCh38
	View configuration	n test1 🗸	Transcript NM	1_007300.4 🗸	Exon nam	ing Systematic numbering (1, n) 🚱
Overview of Transcript NM_003	7300.4 (BRCA1)				BRCA1-	BRCA1, DNA repair associated   GRCh37 (Chr 17)
	00	3	4 (	56 7	89 1	



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.

🖨 🕇 👃 Gene Homo sapiens BRCA1, DNA repair associated (BRCA1), transcript variant 2, mRNA.											
BRCA1	-		d (BRCA1), transcript variant 2, mRNA.								
		Select BRCA1 transcripts to display									
_	9	Open BRCA1 in new tab									

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

Iverview of Transcript NM	_007300.4 (BRCA1)			BRCA1 - BRCA	1, DNA repair associated   GRC	h37 (Chr 17)
	12	3	4 56	7 89 8		14 15 16 17 (19
1 1 4 Genome - chr77s	41,242,955-41,243,055 (GRCK	17) - 100 bps				

<u>Note</u>: 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.

c.	1331 G 14	T	A Y 445	T	T.	F 1	G	c.1 C	1340 T	G	TV	A	G	C A	A	<b>G</b>	c.1 T G V 50	1350 G	T V	G	A	A K	G	A	A K	A	.136 T (	0	G	<b>C</b> 455	Т	T	C S	T	G	c.1. A D	370 C	C	T	C	A	C	c	T	<b>G</b> W 46	c. 0	138	.0 G
	T	A (	G G	331 G	T A Y	T	TI	T	G	c. 134 C 1 A	0 r G	T	A		A	G	c. 13	150 G	T G	A	A (	5 A	A K	A	: 1366 TC S	•	<b>G</b> (	5	Т	C T S	G	c. 13) A D	0	T	c	<b>A</b>	C (	C T	<b>G</b> W 46	c. 13 G	380 G	A C	c /	N A	т	C	T	G
	c.1 p.4 g.1 Po Exe	342 48 33,4 s/AT	85,1 G: 1 2 (15 44	33 9849 66 bp	os)	5		INU		AND		AINS		PT 5		45	50 50				IIIC		A K	A	:. 1360 TC S		<b>G</b> (	<b>T</b>	т	C T S	G	c. 137 A D	10 C C	T	c	٨	C (	C T	<b>G</b> W 46	c. 1: G	380 G	A (	c /	N	т	c	T	G



# 6.4 GoTo Feature / search bar

Alamut<sup>M</sup> 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<sup>™</sup> Visual Plus may:

• Open a gene and one of its transcripts

				A 11 -	A # 96	a A morrism,	and the function of			
-	View configuratio	n Default 🗸	Transcript	NM_000249,4 ~	Exon naming	g Systematic numbering (	1n) 🗸			<b>e!</b> 3
Overview of Transcri	pt NM_000249.4 (MLH1)				MLH1 - Mutl	L homolog 1   GRCh37 (Cl	nr 3)			
		2	3 4	5 6 8	9 10	11 12	13	14 (1	6 6(18 (1)///	
●↑↓ Genome - o	hr3:37,025,009-37,092,237 (G	RCh37) - 57,328 bps	37.010.000	37.015.025	37.040.000	11045000	37,030,020	37.011.035	37,023,023	177.000.000
27/220/220	37,040,000	011010000		,37,122,102		=	37774740	,27,07,20,000	37,000,000	37,010,000
	Conservation 🗱					-				
● ↑ ↓ Gene Homo	sapiens mutt. homolog 1 (Mi	LH1), transcript variant 1	1, mRNA.							
and southern	ono opera nuti tomolog i gene	Li, transcript vanant L, metro								
H			1.1							
●↑↓ alamot 芸										
Ins/Dap Subst										
	eency Databases 夹									
Del/Delins Ins/Dup	-									
Subet										
	-	-	1.1		1					
Ins/Dap Subst										
● ↑ ↓ UniProt										
Decidente Inscitup Suber										

• Open a transcript selection dialog

Génome GRCh37	Transcripts	Exo			
GRCh37			ns AAs	Classe MANE	
	NM_007298.2	20	680		
GRCh37	NR_027676.2	23	0		
GRCh37	NR_027676.1	23	0		
GRCh38	NM_007300.4	24	1884		
			_		
Chromosom	e 17: GRCh38				Transcrit préf



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

riant Features Genomic Level	Protein Level	Pathogenicity class ACMG standards and guidelines	Missense Predictions	
Assembly: GRCh37 Chromosome: Chr3 (p22.2) gDNA: g370673176-A Type: Substitution Transcript Level cDNA: NM_000249.4(MLH1):c.1228G-A Location: Exon 12	Coding Effect: Missense p/Ada410Thr) Compare A4: 22 Check predictions in the Splicing Tab External Tools VariantValidator Mutulyzer	IPM2 BP4 Suggested ACMG classification: Uncertain Significanc Show Details User defined pathogenicity class Classification: 0-Unclassified Pathogenicity class is NOT automatically suggested	e Align GVGD MatakonTaster PulyPhen2 SIFT Notes	Class C0 (6½ 270.11 - GD: 0.06) Benjan, Tree vote: 9)91 (dell/benjan) HD/VPred: benjan (score: 0.121).HVarPred: benjan (score: 0.034) TOLERATED (score: 0.59.median: 3.25)
ternal databases dbSNP (v151) rald: Not referenced Minor Allelie req. Count: Ancestral Allelie: Clinical signif: Validated: Validated:	1000 Genomes (2020-06-30) Al: EAS: EUR: AFR: AMR: SAS:	HGVD (v2.30 - Aug. 2017) Filter: MAR: Ref[Ref: Ref]/Alt: Alt/Alt:	Danish2k (2013) MAF; Ref/Ref: Ref/At: Att/Alt	GoNL (v2013-10-05) Filter: Alt allele count: Total allele count: Allele Frequency:
gnomAD (v2.1)	Genome	ESP (v0.0.30)		

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

Overview of Transcript NM_000249	9.4 (MLH1)							N	NLH1 - M	lutL hor	nolog 1	GRCh37 (	Chr 3)									
	1	2	3	4	) 5	6	8	9	10	ť	0	12	(t				14 15		16 (18	19		
Genome - chr3:37,050,264	6-37,050,365 (GRC	<b>h37) - 99 bps</b> 280	3	7,050,290		1	17,050,300		37	,050,310		37,05	1,320		37,050,330		37,050,340		37,05	0,350		37,050,360
C T T C T A T G A A T G A A G A T A C T T A	T T A C A A A T G T	A G A A A T C T T T		CAAT STTA	C T T C G A A G	T G A C	T T C A A A G T	GGT CCA	G G A G C C T C	G A C C T G	C T T T G A A A	T T T T A A A A A T	C A A G T T	C A T G T A	A G C C A C T C G G T G	GAGGA CTCCT	G A A A A C T T T T	G C T C G A		A A A I	G G T T	G T G A A C A C T T
♦ ↑ ↓ Nucleotide Conservation	\$	-																				
المعمر فرجيه	in a state	la de			11	I			III.	П.		161	.11	.11					11.11	111.		1.111
G ↑ J Gene Homo sapiens mutt	homolog 1 (MLH1	l), transcript var	iant 1, mRNA.																			
4-40 c.454	-30	c.454	-20		c.454-10			c.454		c.460		c.	170		c.480		c.490		c	500		c.510
CTTCTATGAAT	TTACA	AGAA			CITC	I G	TICA	V	E	D		F Y	N		A GUUAU	R	R K			N		S E
⊖↑↓ alamut 幸								152			155				160			165				170
DevDerns Ins/Dup																						
Subst id-40 [c.454-	-30	c.454	-20		c.454-10			c.454		c.460		0	170		c.480		c.490		c.	500		c.510
С Т Т С Т А Т <b>G</b> А <sup>I</sup> А Т	ΤΤΑΟΑ	AGAA		C A A T	C T T C	TG	ттса	GGT	GGAG	GAC	CTTT	TTTA	CAA	CAT	AGCCAC	GAGGA	GAAAA	GCT	TTAAA	A A A 1		GTGAA
A L Allala Commune Database								152			155				160			165				170
Del/Delins	69 22											1.00							1.0			
Ins/Dup Subst (2) i4-40 (c.454-	30	G A	120	) (G	GCG c.454-10	0	G	20	G	(Ť (Ť c.460	AG (		(70 C	t G C	() (c.480	0 0	C.490		AAC .	500		C A G
С Т Т С Т А Т <b>G</b> А <sup>I</sup> А Т	ΤΤΑΟΑ	AGAA		CAAT	C T T C	ΤG	ттса	GGT	GGAG	GAC	CTTT	TTTA	CAA	CAT	AGCCAC	GAGGA	GAAAA	GCT	TTAAA	A A A 1		GTGAA
●↑↓ Clinvar 幸								152			155				160			165				170
Del/Delins Ins/Dup Subse			6	0.0	0000		0	200	00	00	000			<b>0</b> 0	20 63		0.0				0.00	
i4-40 c.454	-30	c.454	-20	0	c.454-10			c.454	00	c.460			170		c.480		c.490			500	000	c.510
CTTCTATGA'AI	TTACA	A G A'A /			Chi T C		TICA	V	E	D		F Y	N		A T	R	R K			N	P	S E
● ↑ ↓ UniProt i4-40 c.454	-30	c.454	-20		c.454-10			c.454		c.460	155	c.	170		c.480		c.490	105	c	500		c.510



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

		A	
	1	chr1:g.94496601C>A	
	2	chr2:g.182430848A>C	
	3	chr17:g.6331702T>A	
	4	chr8:g.87660100T>C	
	5		
	6		
🏚 🔍	848	A>C chr17:g.6331702T>A chr8:g.87660100T>C	0

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

ABCA4         chr1:g.94496601C>A           AIPL1         chr17:g.6331702T>A           CERKL         chr2:g.182430848A>C           CNGB3         chr8:g.87660100T>C	<b>\</b>	ABCA4
AIPL1         chr17:g.6331702T>A           CERKL         chr2:g.182430848A>C           CNGB3         chr8:g.87660100T>C		
CERKL chr2:g.182430848A>C CNGB3 chr8:g.87660100T>C		AIPL1
CNGB3 chr8:g.87660100T>C	С	CERKL
	;	CNGB3
		CNGB3

#### 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.

AlamutVisu	alPlus	Application	File	View	Web	Variant	Tools	Help	
• •				F	ocus or	۱		₩F	
<b>A</b>	O		G	Show	v ruler			企業R	nc
	(	× 🟫	×	✓ Colo Use	r nucle amino a	otides acid 3 lette	er code	ひ第N ひ第A	в
				D F	ull Scre	en		F11	
	View	configuration	Defaul	✓ Show	v navig	ation bar			~
				Incre	ease Fo	nt		₩+	
erview of Transo	ript NM_0	07300.4 (BRCA1)		Decr	ease Fe	ont		₩-	
	/////	///////////////////////////////////////		Rese	et Font			ж0	
			11178	Ente	r Full S	creen			K

This functionality allows the user to find:

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

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Find genomic region			
Assembly: GRCh37		Chromosome: 3	
Start: 37034841		End: 370923	37 (max: 198022430)
			Find
Find position			
<ul> <li>Genomic coord</li> </ul>	inate 9.		e.g. '37004123'
CDNA coordina	te c.		e.g. '234', '-65', '*56
<ul> <li>Protein coordin</li> </ul>	ate p.		e.g. '456'
			Find
Find variation			
		e.g. 'rs63750271	', 'COSM4464363'' Find
Find nucleic sequence			
Sequence :			
	Transformation		
	Reverse stra	nd Re	verse direction
Sequence (femuerd stre	. (here		
Elanking region size:	10000		
Approximate Match	ina		
Approximate Match	ing		Prov
			riev
Find protein sequence			
	TTS RR2		
Jequence. e.g. MSPV,	110, 101		
			Prev Next
			Close

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 (<u>for more information</u>).



# 7. Visualize

# 7.1 Viewing BAM/CRAM alignment

Alamut<sup>m</sup> 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)

Alamut	VisualPlus Application	File	View	Web	Variant	Tools	Help
			Open B	AM/CRA	AM File	ЖΒ	
	Open gene		Open B	AM fron	n URL	жU	litochondrial vie
<u> </u>	o pon gono	D	Open Sa	anger F	ile		
		Exp	oort Fas	ta Sequ	ence	ЖE	× 🕈

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
- $\circ$  Depth of coverage
- $\circ \ \ \text{Reads}$
- $\circ$  Variants

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

🖨 🕇 📙 RAM Alignment (MI H1 grab 37 ham) VCF file (MI H1 grab 37 wrft) 📩 🚺 🔽 Device 🔽 Courses 🔯 Targete	
Coloring Coloring (multi-generation) for me (multi-generation) A Coloring C	
Mar O G	
A A T A G A G A A C T G A T A G A A A T T G G A T G T G A G G A T A A A A	
	CA

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

MLH1_grch37.bam
Mapping quality: 254
Base: A - Phred quality: 40
Insert size: -225
Name: PC-LABO-NGS_22:3:24:14648:12926
Strand: (-)
2nd in pair
Pair mate mapped: yes



#### Viewing sequencing targets

In Alamut<sup>m</sup> 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.

<ul> <li>Shade mismatched bases by quality Show all bases</li> <li>Show clipped bases</li> </ul>
Condense reads Squish reads
Sort central reads by > Restore Layout
Load BED targets from file
Load BED targets from URL
Load VCF variants from file
Load VCF variants from URL
Preferences

#### 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.

● ↑ ↓ BAM Alignment (MLH1_grch37.bam).	VCF file (MLH1_grch37.vcf)	🌣 <u> </u> Reads	Coverage	V Targets 🗙
Ins/Dup				
C A G T C A T T T T A C A A Targets (exons) Max Depth: 11 fax		GTTAA	АСТБА	тстст
	A Total sounds of			
	A: 50 (56% 28+, 22-)			
	C: 40 (44%, 15+, 25-)			
	G: 0 T: 0			
	A N: 0			
	Reads in pair: 49/41			

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' <sup>OP</sup> 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.

~	Shade mismatched bases by quality
	Show clipped bases
	Condense reads
	Squish reads
	Sort central reads by
	Restore Layout
	Load BED targets from file
	Load BED targets from URL
	Load VCF variants from file
	Load VCF variants from URL
	Preferences

# Visualization options

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

~	Shade mismatched bases by quality Show all bases Show clipped bases
	Condense reads Squish reads
	Sort central reads by > Restore Layout
	Load BED targets from file Load BED targets from URL Load VCF variants from file Load VCF variants from URL
	Preferences

	BAM	Preferences	
argets		(1)	
🗸 Display	Number of int	ronic bases in exon-based targets: 20	
Coverage		0	
🗸 Display	$\sim$	O └─ Linear scale O └─ Qua	adratic scale
Depth threshold:	30 3	Minimum depth expected	
Allele frequency threshold:	0.20 4	<i>Threshold above which non-reference alleles are marked out</i> whether the second	clude alleles ten navigating
Deletion frequency threshold:	0.00 6	Threshold above which deletions are marked out	clude deletions
Insertion frequency threshold:	0.00 8	Threshold above which insertions are marked out	clude insertions
leads			
		Display Size	
✓ Display Sorted bas	e order: A C G T	N (10 Standard Condensed )	Squished
Show all bases Show cli	pped bases 12	V Filter	PCR duplicates
Max. displayed read depth: 20	0 14		
Mapping quality threshold: 0	(15)		
✓ Shade mismatched bases b	y quality Min. Phred score: 5	Max. Phred score: 20	
Insert Size			
🗸 Color read pairs whe	en insert size is out of bounds		
Fixed bounds	Min (bp): 70	Max (bp): 700	
<ul> <li>Distribution-based</li> </ul>	Min (%): 0.5	Max (%): 99.5	
соре			
• Apply to this alignme	nt only O Apply to all current	y open alignments	
Save as default settings	5		

- 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<sup>™</sup> 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<sup>™</sup> 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:

- **Off-Target:** region outside the targeted region (not investigated by the sequence analysis)



• Locus: current gene locus

BAM align Gene	nment: I	MLI MI I	H1_grch3 H1	7.b	am	
Coverage						
	On-Targ	et	Off-Targ	et	Locus	
≥1x	90%	)	59%		81%	
≥100x	0%		0%		0%	
Average depth	47		24		42	
Median depth	59		41		43	
Max depth	100		116		116	
					Close	

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<sup>™</sup> 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<sup>M</sup> Visual Plus allows you to display Sanger Electropherogram (Sequencing) data by opening a new track through the "File > open Sanger file" menu.

Alamut	VisualPlus	Application	File	View	Web	Variant	Tools		He	lp
				Open B	AM/CR/	AM File	₩В			
	Open	dene		Open B	AM from	n URL	жU	lita	och	or
	open	gene	B	Open Sa	a <mark>nger</mark> F	ile				<u> </u>
			Eve	ort East	to Com	0000	945	×	<b>f</b>	
			EXP	Jort Fas	ta Sequ	ence	#-E			

A pop-up will appear where you can select the exon that matches the Sanger sequence you wish

to align. Alamut  $^{M}$  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.

<b>O</b> A	lign with exon	Align with regional of the second	'n
Pick tł	ne exon you want to align	the following Sanger file with:	
/User:	s/williamogeard/Desktop/	Import/Sanger/00000199F-DMD	9.ab1
EXO	11 TZ (199 0P)		*
	utomatically pick the best	suited exon	
Or pic	utomatically pick the best s	suited exon ign Sanger with:	
Or pic	utomatically pick the best s k the genomic region to al g.33229666	suited exon ign Sanger with:	

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 <sup>(2)</sup>

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<sup>™</sup> 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.

0 0		Sett	ings	
License		Network	View	Misc
View				
Show Selected Trans	cript on \	/ariant tracks		
Default genome build				
GRCh37 GRC GRCh37 GRC	h38			
VIEW NAME	TR	ACK		
Default	÷	COSMIC		
		Genomenon I	Mastermind C	
		Structural Var	iants (DGV)	
		Protein Doma	ins	
		<ul> <li>Orthologues</li> </ul>		
		Repeat Mask	ər	
+ -		Private Annot	ations	
Use as default config	uration			
				Cancel Save



#### 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 <u>Section 8.2</u>..

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.

•		Pri	vate Annotat	ion Databases				
Private Annotation Database $$	Path	Description	Datasets	Private Annotations	Current Database	Shared database	Automatic Files Import	1
My Annotations	/Users/user/Library/Application Suppor	annotations	0	0			<ul><li>✓</li></ul>	
						-		
				Import	from Alamut Visual	New Add Existi	ng Database Edit	Dele
								Clo

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 <sup>(2)</sup> 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.

T T T A G A A A T C A G T C C C C A G A A	Add Dataset	5
	Import File(s)	
	Import Folder	
	Hide Empty Datasets	
	Show All	
	Erase All	

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.

<u>Note:</u> 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<sup>M</sup> Visual Plus allows you to annotate and store variants. Click on menu > Variant > Local Variant Databases.

•	Solution Set New Variant % ∨
	Local Variant Databases #D
	Refresh Variants

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.

•	New Database
Name:	Tast1
Nume.	
Path:	Users/user/Library/Application Support/AlamutVisualPlus Browse
Description:	Variants
beschpuoli.	
	Cancel Create

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.

Local variant database         Description         Entries         Variants         Occurrences         Path         Display in track           alamut         Default local database         0         0         0         /Users/user/Library/Application         All	Default	Shared database	Last update date
Local variant database         Description         Entries         Variants         Occurrences         Path         Display in track           alamut         Default local database         0         0         0         /Users/user/Library/Application         All	Default	Shared database	Last update date
alamut Default local database 0 0 0 /Users/user/Library/Application All		0	
			15/06/2022
Test1 Variants 0 0 0 /Users/user/Library/Application All			15/06/2022
Explore/Export Import New	Add Existin	ng Database Edit	Delete Clear Databas

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.

					Local Variant Databases				
Local variant database	Description	Entries	Variants	Occurrences	Path	Display in track	Default	Shared database	Last update date 🛛 🗸
alamut	Default local database	0	0	0	/Users/user/Library/Application A				15/06/2022
Test1	Variants	0	0	0	/Users/user/Library/Application	All			15/06/2022
					Explore/Export Import	New	Add Existin	g Database Edit	Delete Clear Database
									Close

<u>Note:</u> 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).

•	Local Variant Databases											
1	Local variant database 🐱	Description	Entries	Variants	Occurrences	Path	Display in track	Default	Shared database			
	alamut	Default local database	0	0	0	/Users/user/Library/Application Support/AlamutVisualPlus	All					
	Test1	Variants	0	0	0	/Users/user/Library/Application Support/AlamutVisualPlus	<ul> <li>Default</li> </ul>					
							✓ PA					
						Explore/Export Import New Add Existing	Database Edit	Delete	Clear Database			
									Close			

Alamut<sup>™</sup> 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.

• • •			Variant	Exporter				
Row filters (optional)	All	V Ø	Column filters (optional)	✓ Notes	HTML fields			
Gene:		✓ Ø	<ul> <li>Chromosome</li> <li>Gene</li> <li>Transcript</li> </ul>	Occurrence ID     Family ID     Phenotype	Export as plain text     Preserve HTML tags     Output format			
Type: Classification:		<ul><li>✓ Ø</li><li>✓ Ø</li></ul>	<ul> <li>✓ gNomen</li> <li>✓ cNomen</li> <li>✓ Type</li> <li>✓ pNomen</li> </ul>	KNA Analysis     HPO IDs     Comment     Creation date	• Tab-separated text Excel VCF			
Occurrence ID:		✓ Ø	<ul> <li>Coding effect</li> <li>Evidences (ACMG)</li> <li>Classification</li> </ul>	<ul> <li>✓ Update date</li> <li>✓ Local variant database</li> </ul>	Destination <ul> <li>Export to file:</li> </ul>	/Users/tdebenoist/variant	s.txt Brow	wse
Family ID:	······································	V Ø	All annotations		Export to clipboard	Classification		
								10
							Export	Cancel


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.

AlamutVisua	IPlus	Application	File	View	Web	Variant	Private Annotations	То
•						嵏 New '	Variant ೫∨	
	Open	dene	GPC	h27	G	Local Var	iant Databases 🛛 🕷 D	01
п	Open	gene	GRC	.1137		Refresh \	/ariants	E,

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.

	Setti	ings	
License	Network	View	Misc
View			
✓ Show Selected Trans	cript on Variant tracks		
Default genome build			
💿 GRCh37 🗌 GRC	h38		
VIEW NAME	TRACK		
Default	COSMIC		
	Genomenon M	Aastermind C	
	Structural Varia	ants (DGV)	
	Protein Domai	ins	
	Orthologues		
	Repeat Maske	r	
	Private Annota	ations	

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.

AlamutVis	ualPlus	Application	File	View	Web	Variant	Private Annotations	Tools	Help
•							Private Annotation Da	tabases	
<b>A</b>	Open	gene	GRC	h37	G	RCh38	Refresh Private Annot	ations	
•••									

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.

	Pri	vate Annotat	tion Databases			
Path	Description	Datasets	Private Annotations	Current Database	Shared database	Automatic Files Import
/Users/user/Library/Application Suppor	annotations	0	0			
			Import	from Alamut Visual	New Add Existi	ing Database Edit
	Path /Users/user/Library/Application Suppor	Pri Path Description /Users/user/Library/Application Suppor annotations	Private Annotat Path Description Datasets /Users/user/Library/Application Suppor annotations 0	Private Annotation Databases         Path       Description       Datasets       Private Annotations         /Users/user/Library/Application Suppor       annotations       0       0       Import	Private Annotation Databases         Path       Description       Datasets       Private Annotations       Current Database         /Users/user/Library/Application Suppor       annotations       0       0       Import from Alamut Visual	Private Annotation Databases         Path       Description       Datasets       Private Annotations       Current Database       Shared database         /Users/user/Library/Application Suppor       annotations       0       0       Import from Alamut Visual       New       Add Exist

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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.

	New Database		
Name:	My Annotations		
Path:	ibrary/Application Support/AlamutVisualP	lus/annotations	Browse
Description:	annotations		
		Cancel	Create

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.

• • •		Priva	ate Annotatio	on Databases			
Private Annotation Database 🗸	Path	Description	Datasets	Private Annotations	Current Database	Shared database	Automatic Files Import
My Annotations	/Users/user/Library/Application Support/	annotations	0	0	<ul><li>✓</li></ul>	<ul><li>✓</li></ul>	<ul><li>✓</li></ul>
				Import fro	om Alamut Visual	New Add Existing	Database Edit Delete
							Close

<u>Note:</u> 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.

AlamutVisu	alPlus	Application	File	View	Web	Variant	Private Annotations	Tools	Help
•							Private Annotation Da	tabases	
<b>A</b>	Open	gene	GRC	h37	G	RCh38	Refresh Private Annot	ations	J

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<sup>™</sup> Visual

Private Annotation files from Alamut<sup>™</sup> Visual (.apa) can be imported into the Private Annotation Databases of Alamut<sup>™</sup> 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.

•		Pri	vate Annotat	ion Databases				
ivate Annotation Database 🕥	Path	Description	Datasets	Private Annotations	Current Database	Shared database	Automatic Files Import	
ly Annotations	/Users/user/Library/Application Suppor	annotations	0	0	<b>V</b>		Image: A start of the start	
					1			

# 9. Import Variants

Supported file format

Alamut<sup>™</sup> 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 $^{\rm M}$  Visual Plus internal variants and can then be handled like other internal variants.

Alamut<sup>™</sup> Visual Plus can import variant annotations from TSV (Tab-separated values) formatted files as Variant Calling Format files (VCF).

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

# 9.1 Import variant from an excel file or TSV format

Alamut<sup>™</sup> 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	Туре	pNomen	Coding effect	Evidences (ACMG)	Classification	Notes	Occurrence ID	Family ID	Phenotype	<b>RNA Analysis</b>	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:

<ul><li>Assembly</li><li>Chromosome</li><li>gNomen</li></ul>	or	<ul><li>Gene</li><li>Transcript</li><li>cNomen</li></ul>
--	----	--

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

 $\mathbf{Notes}-\mathbf{Comments}$  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<sup>™</sup> Visual (.mut) can be imported into the Local Variant Databases of Alamut<sup>™</sup> Visual Plus.

# 9.4 Import process

To import variants from an import file as described above, in Alamut<sup>™</sup> 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'.

•	• •					Local Variant Databases					
[	Local variant database	Description	Entries	Variants	Occurrences	Path	Display in track	Default	Shared database	Last update date	~
	alamut	Default local database	0	0	0	/Users/user/Library/Application	All	Image: A start of the start		15/06/2022	
	Test1	Variants	0	0	0	/Users/user/Library/Application	All			15/06/2022	
						Explore/Export Import	New	Add Existin	g Database Edit	Delete Clear Database	e

Alamut $^{\mathbb{M}}$  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.

	) 🔴					Variant Im	port Analysis						
The Vali	import file has been d entries are indicat	n analyzed, but varian ed by a green backgro	ts have not yet been in ound, entries with err	mported. This table g or by a red one. Igno	ives you the opportun	ity to review the valic y background. Edite	dity of the file entries. d entries (computed a	utomatically) are in b	old italic.				
	Assembly	Chromosome	gNomen	Transcript	lassification type	Classification	Note	Occurrence ID	Family ID	RNA Analysis	Phenotype	Comment	C
1	GRCh37	3	g. 37035054T>A	NM_00024	CMGS_VGK	4 (Likely Pathogenic)	Pas d'occurrences						
2	GRCh37	3	g. 37035067	NM_00024	CMGS_VGK	5 (Pathogenic)	2 patients	p1	fi			Le patient 1	20
D	isplay Valid records: 1/	2											
	Invalid records.	172											
	Do not import a     Overwrite variar     Update existing	lready existing variant its if already exist variants and add histo	ts ory entry										
												Cancel	ок

### <u>Notes</u>:

- The import process can be managed without opening a specific gene or transcript from the homepage of Alamut<sup>™</sup> 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:

Variant Import Analysis

The import file has been analyzed, but variants have not yet been imported. This table gives you the opportunity to review the validity of the file entries.

Valid entries are indicated by a green background, entries with error by a red one. Ignored entries have a grey background. Edited entries (computed automatically) are in bold italic.

	Assembly	mos	gNomen	Transcript	Classification type	Classification
1	GRCh37	3	g.37035054G>A	NM_000249.3	CMGS_VGKL_5	4 (Likely Pathogenic)
2	GRCh37	3	g.37035067_37035069del	NM_000249.3	CMGS_VGKL_5	5 (Pathogenic)
3	GRCh37	3	g.37035067_37035069del	NM_000249.3	CMGS_VGKL_5	5 (Pathogenic)
D	isplay					
	✓ Valid records: 3/3 ✓ Invalid records: 0,	/3				
0	ptions					
	<ul> <li>Do not import alr</li> <li>Overwrite variant</li> <li>Update existing v</li> </ul>	eady e s if alre ariants	xisting variants eady exist s and add history entry			
					Can	cel OK

- 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<sup>™</sup> 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.



275		280
🗢 ↑ 🧅 Allele Frequency 🛛	Databases 📑	
Del/Delins		
Ins/Dup 💛 🚫		
Subst G C G	)	G 🛛
	c.830	c.840
AGCCATA	q.37059034A>G G G G	
A I	1000 Genomes (All): N/A	
275	Danish2K: N/A	
🗢 T 🧅 ClinVar 🚔		
Del/Delins	ESP: N/A	
Ins/Dup 🤎 🤎	GoNL: N/A	
Subst 🜀 🙆 🕻	HGVD: N/A	
	dbSNP: 0 (rsID: rs1036438114)	
AGCCAT/	gnomAD (Genome+Exome): N/A	
A I	anomAD (Exome): N/A	
275 Private Annotatio	gnomAD (Genome): All:0.0063694%, . Popmax FAF-95 (Exome): N/A, (Geno	Afr:0.022952% me): afr

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

470	DUU		atabases 🚎
	⑥ ② ⑥ ② ① ① ① 70354C>T Genome (All): 0.00019968 さない(All): 0.00019968	Del/Delins Ins/Dup Subst A G 2 2 C c.1	G g.37035047C>G A € G G
A95 Danis A95 ESP: I ESP: I	N/A	M S F	dbSNP
Del/Delmo Ins/Dup Subst © © © © © © © © © © © © © © © doSN (HGVU HGVU A C T G C A G C T T G T A C C C C C C L T A A C C C C C L CLO (Non	:: N/A D:: N/A P: 0.00019968 (rslD: rs200830026) N4D (Genome+Exome): All:0.0031814%, Afr:0.024029%, 0028217%, Algewish:0%, E.Asian:0%, S.Asian:0%, S.Asian:0%, Eur. F-in):0.0015481%, Eur.(Fin):0%, Oth:0%	1	gnomAD

More information from external databases is available in Alamut $^{\mathbb{M}}$  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<sup>™</sup> 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.

	inVar 🧕	<u>*</u>															
Del/Delins				>													
Ins/Dup																Ψ.	
Subst 💼	0	0	00	G 🕐 🔕	<b>G</b>	0 0	20	2	Ó ()	G (2)	0 0 0	00	00	00	20	G	00
		c. 1										-	c.30				
CGCC	CAA	AA	TG	$\star \times \times$	🜟 Likely p	athogenic	[619516] 0	:.3G>T Lynch	n-like syn	drome' Lyn	ch syndro	me G	G	т	GG	A	C G
			м	S	F	٧	A	G	v		R	R		L		D	
							-										

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<sup>™</sup> 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.

	inseml	<b>)</b> )																																																		
Human	L	F	S D	D	P	E :	S D	Ρ	S	E D	R	A I	E	S	A R	V 4	N N	1	P :	S S	т	S A	ι L	к	V P	Q	L K	v	A	E S	A	Q :	S P	A	A A	ιн	т	T D	т	A G	Y	N A	М	E	E	s v	S	R E	к	ΡE	L	T A
Chimp	L	F	S D	D	Р	E :	S D	P	S	E D	к	A 1	E	s .	ΑН	V (	8 N	- 1	P :	s s	т	S A	۱L	к	V P	Q	LK	v	A	E S	Α	Q	S P	Α	A /	н	т	T N	т	A G	Y	N A	М	E	E	s v	s	R E	к	ΡE	L	T A
Northern white-cheeked gibbon	L	F	S D	D	S	E :	S D	P	S	A D	R	A I	E	S I	ιн	V 4	S N	- 1	P :	5 S	т	S A	ι L	к	V P	Q	LK	v	A	E S	Α	Q	S P	A	A /	ιн	т	T N	т	A G	Y	N A	ιм	E	E	s v	s	R E	к	ΡE	L	TA
Macaque	L	F .	S D	D	Р	E 3	S D	P	S	E D	R	A I	E	S I	ιн	V 4	s s	- 1	P :	5 S	т	S A	ι L	к	V P	Q	W Q	e v	A	E S	Α	Q	S P	A	A /	ιн	N	T N	т	A G	Y	N A	ιм	E	E	s v	s	R E	N	РК	L	TA
Olive baboon	L	F .	S D	D	Р	E :	S D	P	S	E D	R	A I	E	S I	λН	V 4	s s		P :	5 S	т	S A	ι L	к	V P	Q	W Q	e v	A	E S	Α	Q	S P	A	A /	ιн	N	T N	т	A G	Y	N A	ιм	E	E	s v	s	R E	к	P K	L	TA
Rat	L	F .	S S			F	R D	P	D	S E	s	P I	( V	P	A L	V I	с т	A	P /	l S	т	S A	ι L	ĸ	I \$	Q	G Q	e v	A	GS	С	R	S P	A	A C	G	Α				D	T A	۱V	v	E	I V	s	ΚI	к	ΡE	V	T S
Mouse	L	F .	S 5			F	R D	P	E	S E	s	P I	( E	P	х н	1 4	B T	т	P /	s	т	S A	ι L	к	I P	Q	G Q	ł V		Α	F	R :	S A	A	A A	G	Α				D	K A	۱ V	v	G	I V	s	KΙ	к	ΡE	L	T S
Dog	L	F	S D	D	Р	E :	S D	P	S	S H	R	A	S E	L	ιн	V	3 S	м	P	r s	т	S A	ι L	к	L P	Q	FQ	ł V	E	E S	Α	к :	S T	A	A N	н	1.1	A S	т	A G	Y	N K	(S	E	D	s v	G	I E	к	ΡE	٧	1 5
Platypus						:	S G	P	Q	Q H	R	P	B E	G	ER	v	s Q	L																																		
Chicken							K N	P	N	S S	S	F	s v	KΙ	ΗP	C I	, d	т	A I	A	т	DS	5 S	A	V A	Q	G D	N	к	S N	v	Q 1	v c	к	S #	R	s	v c	F	P	т	s v	/ L	н	N	/ A	G	K E	N	A A	s	SG
Frog							S E	N	к	S E	N	Ρ	I L	R I	N K	C :	ŝТ	s	н (	) G	L	F S	S Y	s	M E	E	A V	s	Р	H N	Ρ	K (	Q S	R	A E	F	G	I A	R	K S	т	S P	νт	F	A	S P	s	R A	к	V L	s	VG
Tetraodon						,	A R	Р	Α	GS	к	н	5 S	1	ГЕ	LI	l S	R	1	6 N	т	V G	i L	s						S A	A	ĸ	τL	к	S D	G	S	P S	D	G H	E	DK	E	N	N	ΓP	E	R A	R	S L	Α	

By default, orthologues aligned and displayed in Alamut<sup>M</sup> Visual Plus are taken from the <u>Ensembl</u> <u>Compara</u> database.<sub>+</sub>

### **References:**

- We would like to express our thanks to the <u>Genetic Cancer Susceptibility Group</u> at IARC for their kind help in defining our alignment protocol.
- Tavtigian, SV., Greenblatt, MS., Lesueur, F., Byrnes, GB. (2008). <u>In silico analysis of missense substitutions using sequence-alignment based methods</u>. Hum Mutat.11: 1327-36
- Tavtigian, SV., Oefner, PJ., Babikyan, D. et al (2009). <u>Rare, evolutionarily unlikely missense</u> 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 <a href="https://www.genomenon.com/tutorials/">https://www.genomenon.com/tutorials/</a>.

Mastermind $\mbox{\ensuremath{\mathbb{R}}}$  visualization can be activated in the "Settings > View" tab for each view configuration.

Alamut<sup>M</sup> 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 <u>https://www.genomenon.com/cvr/.</u>

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.

127	190	135
	Image: Constraint of the state of the s	6 3 6 7 c.400 5 A A A A C T K L 135

)el/D€	iins 🔪																							
s/Du	p																							
ibst											G	2		C	G	C	-		0000	1	A		G	
			c.38	1-10							Ĩ	Ť	c.38	1	Ť.,		101	g.37048486A>C					c.400	)
	A 1	r 1	г'т	Т	С	Т	С	Α	Т	т	Α	G	A	G	С	A	100		feelen beferen en			Α	A	Α
													R		Α			Mastermind Cited V	variants Reference		9			к
													127				-	130						_

### BRCA Exchange

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

$\bigcirc \uparrow \downarrow I$	BRCA Exchange			
Del/Delins				
Ins/Dup				
Subst	T C G	23 A	A G A	O A O
c.594-10		c.594	c.600	

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.

BRCA Exchange	
ID:	3930
Pathogenicity:	Benign / Little Clinical Significance
Hgvs Hg37:	NC_000013.10:g.32910359T>C
Hgvs Hg38:	NC_000013.11:g.32336222T>C

### Gene External Features

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

Overview of Transcript NM_007294.4	(BRCA1)		BRCA1 - BR	CA1 DNA repai	r associated   G	RCh37 (Chr	17)	gn	omAD SC	ORES	<b>OMIM</b> <sup>®</sup>	BRIDGES
MANE Select c-13	c.81 p.27	c.135 c.302 p.45 p.101	c.442 c.548 p.148 p.183	c.671 c.4097 p.224 p.1366	c.4186 p.1396	c.4358c.4485 p.1453	c.4676 p.1559	c.4987 p.1663	c.5075 p.1692	c.5194 p.1732	c.5278 p.1760	c.5407 c.5468 p.1803
(b) 2)	(3)	(5)(7)	8 9 10	(11b) (12)	(13)	(14a) (15)	(16)	(17)	(19)	(20)	(21) (	22,23,24

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

e e gnom	AD scores	
DI I.	0.21570.20	
	3 2874e-20	
Score based on NM 007300.3. NM 0	07300.4 , gnomAD v2.1.1	
LOEUF:	0.915	
LOEUF Percentile:	0.50198	
Score based on NM_007300.3, NM_0	07300.4 , gnomAD v2.1.1	
		Ok

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

BI	RIDGES	
Breast Cancer Risk after Diagnostic	: Gene Sequencing	
Pop p-value_MSV:	0.01	
PTV Odds Ratio (95% CI):	10.57 (8.02-13.93)	
Pop p-value_PTV:	1.1e-62	
Missense Odds Ratio (95% CI):	1.11 (1.02-1.20)	
BFDP:	1.5e-64	
		Ok

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

•	OMIM®: BRCA1	
	BRCA1 (OMIM® id: 113705)	
	Phenotype	Mode of inheritance
	BREAST-OVARIAN CANCER, FAMILIAL, SUSCEPTIBILITY TO, 1; BROVCA1	Autosomal dominant (AD), Multifactorial (Mu)
	PANCREATIC CANCER, SUSCEPTIBILITY TO, 4; PNCA4	
	FANCONI ANEMIA, COMPLEMENTATION GROUP S; FANCS	Autosomal recessive (AR)
		Ok

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.

VIEW NAME	TRAC	(			
Default	V	Genome	Default		
	$\checkmark$	Nucleotide Conservation			
	V	Transcript	Default		
		Allele Frequency Databas	Check all / Uncheck all		
		ClinVar			
+ -	V	UniProt			

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.

VIEW NAME	TRACK					
Default	Genome Default					
	Nucleotide Conservation					
MyView 🌶	✓ Transcript Default					
	Allele Frequency Databases Check all / Uncheck all					
	ClinVar					
+ -	UniProt					
Use as default configuration						

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

•	Open gene	GRCh37	GRC												Q ML	H1				0
							× 🕈 .		с 🥖 ми	41 NM_000249.4 (GR	(h37 c									
			_										TRAN	SCRIPT VIEW	V RE	SION VIEW				
	View configuration	Default	~ ·	Transcript	NM_000249.	4 V	Exon	naming S			$\sim$			2		1 2 10		GEN	CR Hallow	0
													e	NN	e	NEN UL	at HONC	ATLAS	GR UNIPE	× .
Overview of Transco	ript NM_000249.4 (MLH1)						м	LH1 - MutL I	nomolog	1   GRCh37 (Ch	3)	gnomAD SCORES	OMIM*	BRIDGES	5					8
MANE Select	6 <mark>10</mark>	c.117 p.39	c.208 p.70	c.307 p.103	c.381 c.454 p.127 p.152	c.546 p.182	c.678 p.225	c.791 p.264	c.885 p.295	c.1039 p.347	c.141 p.470	0		c.1559 c p.520 p	1668	c.1732 c.19 p.578 p.64	10 c.2114 4 p.702			
																		//////	///////////////////////////////////////	
·/////////////////////////////////////		2	3	(4)	5 6	(8)	9	10	(1)	(12)	(13	)		(14)	15)	16 ([]	9 (9///)	<u> </u>	<u> </u>	210
Cenome - 37,035,000	chr3:37,035,009-37,092,337 (GRC 37,040,000	Ch37) - 57,328 bps 37,045,000		37,050,000		37,055,000		37,060,000		37,065,000		37,070,000	37,075,00	0	37,080,000		37,085,000		37,090,00	
									-	≥										=
	e Conservation 🔅																			
																				- 1
MUHI NM_000249.4	Homo sapiens mult, homolog 1 (MLH1), 1 Homo sapiens mult, homolog 1 (MLH1), 1	transcript variant 1, mRNA.	RNA.																	
																				- 1
•																				-
●↑↓ alamut Ξ																				
Ins/Dup																				
	-	_																		



### 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  $rac{1}{3}$  of the track to filter.



Filters can be set for 3 tracks:

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

0		Settings							
License	Network	View	Misc	Profiles					
View									
Show Selected Transcript on Variant tracks									
Surround protein-level descriptions with brackets,eg: p.(Arg22Ser)									
Use systematic exon numbering by default									
Default genom	ne build								
• GRCh37 GRCh38									
VIEW NAME TRACK									
Default	🔻 💟 A	llele Frequency Databa	ises Check all / Uncher	:k all					
	<b>v</b>	1000Genomes							
		Danish2K							
	<b>۲</b>	dbSNP							
		ESP							
+ -	▶ 🗹	gnomAD/Exomes							
✓ Use as defau	ult configuration								
			Canc	el Save					

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

License Network View Misc  Alexe  Piew  Piew Piew	Profiles
Alew  Show Selected Transcript on Variant tracks Surround protein-level descriptions with brackets, eg: p.(Arg22Ser) Use systematic exon numbering by default Bedault genome build G GRCh37 GRCh38  VIEW NAME TRACK Review Status Number In / Nation All O Grut	
Show Selected Transcript on Variant tracks  Surround protein-level descriptions with brackets, eg: p. (Arg22Ser)  Use systematic exon numbering by default  O GRCh37  GRCh37  Clerkut  Clerku	
2 Surround protein-level descriptions with brackets,eg: p. (Arg22Ser) Use systematic exon numbering by default endouble o GRCh37 O GRCh32 VIEW NAME TRACK Default Default Us/Neth Review Status Number Us/Neth Att VIEW VIEW NUMBE VIEW VIEW VIEW VIEW VIEW VIEW VIEW VIEW	
Use systematic exon numbering by default befault genome build G GRCh37 GRCh32 GRCh32 VIEW NAME TRACK Default G UniVer Review Status Number In / Net In G UniVer V 2	
Default genome build       O GRCh37       GRCh37 <td></td>	
O GRCh37 O GRCh38 VIEW NAME TRACK Defsult      O Gruhar Review Status Number In / Natts: All     O VietProt     VietProt     VietProt	
VIEW NAME TRACK Default  Unit of the series Status Number  Default  Unit of the series Status Number  Divide the series Status Numbe	
Default  V ClinVar Review Status Number In / Not In Al V UniProt  V UniProt	
Review Status Number In / Not in All ✓ 0 ✓ UniProt ✓ 1 ✓ 2	
✓ UniProt	~
► ⊘ COSMIC	
Genomenon Mastermind C	
+ - Structural Variants (DGV)	
Z Use as default configuration	
Cannel	



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.

0 0		Settings						
License	Network	View	Misc	Profiles				
View								
Show Selected	Transcript on Variar	t tracks						
Surround protei	n-level descriptions	with brackets, eg: p.(	Arg22Ser)					
Use systematic	exon numbering by	default						
Default genome I	build							
GRCh37	GRCh38							
VIEW NAME	TRACK	TRACK						
Default	▼	DSMIC						
	Tiss	ue	In / Not In	47/47 Tissues V				
	Ø Ge	enomenon Mastermind	C	autonomic_ganglia     biliary_tract				
	✓ St	ructural Variants (DGV)		✓ bone ✓ breast				
	V Pr	otein Domains		<ul> <li>central_nervous_syste</li> <li>cervix</li> </ul>				
				<ul> <li>endometrium</li> </ul>				
+ -	⊘ Or	thologues		🖌 eye				
+ -	⊘ Or configuration	thologues	_	eye     falloplan_tube				
+ -	⊘ Or configuration	thologues						

### 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).

						37,06	37,061,850 37,061,850	50
					Ť	T Make variant		
						È	Make sequence annotation	
							Copy selected nucleotides from the forward strand	
AlamutVisualPlus Applic	ation File View	Web	Variant Tools	Help			Copy selected nucleotides from the reverse strand	_
			🛞 New Variant			۵	Copy genomic position	
A Open gene	GRCh37	GR	Local Variant D	atabases %D	Ĥ		Map position	- C

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.

Positions must be gDNA coord cDNA coord	Variant Properties expressed as: dinates (forward strand) dinates based on BRCA1(NM_007300.4)
Substitution	Position: 41234506 Nucleotide change: C > T > From: 41234506 To: 41234506
eletion	From: 41234506 To: 41234506 After: 41234506 Nucleotides:
Duplication	From: 41234506 To: 41234506 Deletion From: 41234506 To: 41234506
Delins	Inserted Nucleotides: OK Cancel

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<sup>™</sup> Visual Plus enables the user to check the variant with <u>Mutalyzer</u> Name Checker or <u>VariantValidator</u> 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<sup>™</sup> 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)

Genom	ic Level	
	Assembly:	GRCh37
	Chromosome:	Chr17 (q21.31)
	gDNA:	g.41197696_41197699del
	Type:	Deletion
Γ	Alternatively:	g.41197692_41197695del (Not HGVS compliant, see User Manual)
Transc	ript Level	
	cDN	A: NM_007300.4(BRCA1):c.5655_*3del
	Locatio	n: Exon 24



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':

Transcript: (BRCA1) NM\_007300.4 V Local Variant Database: alamut V

<u>Note:</u> 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 <sup>(2)</sup> 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.

	● ↑ ↓ Private Annotation T T T T A G A A A T C A G	Frack (MyDatabase) 🏩 G T C C C C A G A A	Add Dataset Import File(s) Import Folder
			Hide Empty Datasets
			Show All
01.	Private Annotation Track (MyDa	itabase) 🏚	Erase All
ТТТ	AGAAATCAGTCCC	CAGAATGTGG	TGTTAATGTGCACC
		📋 Make sequence anno	station
		Add Dataset	
		Copy selected nucleo     Copy reverse complete	tides ment of selected nucleotides

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.

	New		
	Dataset Name	my dataset	
	Ok	Cancel	
Annota	tion Track (MyDatabase)	<b>^</b>	
my dataset	non maak (mybatabase)	Υ	
TTTAGAAAT	. A G T C C C A G	AATGTGGAT	GTTAATGTG

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 <sup>(2)</sup> 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.

 $\wedge$ 

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.

4- 2 M	000	000
🖨 ↑ 🦆 Private Annot	ation Track (MyDatabase) 🛭 🏩	
my dataset		
TTTAGAAA	CAGTOCCAGAATE	TEEATETTAAT
	Make sequence annotation	
	Add Dataset	
0	Copy selected nucleotides	_
(	Copy reverse complement of sel	ected nucleotides
Active profile: Local		



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

• • •	Private Annotation	
Dataset: my-dataset Sequence		
Assembly: GRCh37		
From: 37061806	To: 37061809	
AAAT		
Sequence:		
Features		
Name: my-annotation		
Score: 0 🗘	Color:	
Arrow: None	🗌 Left 🔷 Right 🔷 Both	
My commen	t	
External link		
🔿 Web 🧿 File 🛛 Sele	open Link	
file:///Users/user/Desktop/	/my-annotation.txt	
	Delete Save Cancel	

Clicking on 'Save' will create the private annotation and it will be visible in the subtrack to which it belongs.

	\$
my dataset	
G A C A G T T T A G A A A T C A G T C	C C C A G

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

	Track (MyDatabase)
G A C A G T T T A G A	A A T my annotation T of my-annotation.txt

# •••

# 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  $\ddagger$  icon in the Local Variant Database track. The feature can be activated on a single database at a time.

••						7.04						
	<b>e</b> c								¢ .	Q, MLH1		
						× 🕈 💷  🗙	MLH1 NM_000249.4 (GRCh37 c					
-	<b>▼</b> Vie	w configuration	Default 🗸	Transcript	NM_000249.4 ~	Exon naming Systematic r	umbering (1n)		TRANSCRIPT VIEW	REGION VIE	W	GR UniPro
rview of	Transcript NM_00	10249.4 (MLH1)				MLH1 - MutL hon	nolog 1   GRCh37 (Chr 3)	gnomAD SCORES	OMIM* BRIDGES			
NE Selec	et	1 1 1	e.117 p.29	c.208 p.70	e.307 e.381 e.454 p.103 p.127 p.152 4 5 6	6.546 p.182 p.226 p.226 p.224 p.	85 c.1039 c 95 p.347 f	1410 p.470	e.1559 p.520 (14) (14) (15)	e.172 p.57	12 c.1990 c.21 H p.844 p.722 11 (18) 11	
<b>1 ↓ Ge</b> (5,000	mome - chr3:37,03	5,009-37,092,337 (GRCh37, 37,040,000	• <b>57,328 bps</b> 37,045,000	37	7,050,000 3	7,055,000 37,060,000	37,065,000	37,070,000	37,075,000	37,080,000	37,085,000	37,090,00
↓ M	ucleotide Conservat	tion ‡										
				_								
L ali	amut - filtered 북	1										
↓ ali ins ↓ Al	amut - filtered 편 lete Frequency Data	Nones H				· · · ·		-		-	•	• ••
t ↓ all fins p t ↓ All fins p t ↓ Cl fins	amut - Mared 프 Nele Frequency Data	abases #2	-		I II	· · · ·		-	«	•	ا ا » ۹	• • • • • • • • • • • • • • • • • • •
↓ au ins → Al ins → Cl	amut - filtered 또 lefe Frequency Data initar 포 Assembly	معدد بنام الم	Gene	I I	gNomen	e e e e e e e e e e e e e e e e e e e	Type pNomen	Coding effect	Evidences (ACMG - use	< > er defined)	I » Q Classification	Q A A Notes
↓ ali ins ins ↓ Al ins	unut - fibured II   	Abasies #2 Chromosome 3	Gene	1 1 1 1 Transcript NM_000249.2	I II I II I II 9.00men 9.57065001_3706500		Type pNomen I Deletion p.7	Coding effect	K Evidences (ACMG - use PM2	< > er defined)	> Q Classification	<ul> <li>III</li> <li>III</li></ul>
J ali ins Ali ins Cli ins	unut - fibured II Invar II Assembly GRCh37 GRCh37	Chromosome	Gene MLH1 MLH1	Transcript	gNomen g.37065001_3706500 g.37051076576-C	Chomen     chomen     cooo-c	Type pNomen I Deletion p.7 Substitution p.(3/u320A	Coding effect sp) Missense	Evidences (ACMG - use PM2 PM1,PM2,BP4,BP6	I C er defined)	> Q Classification Undefined Signific Uncertain Signific	A A Notes
↓ ali fins P ↓ Ali Ins P	error - Mared # left Frequency Data error # Assembly GRCh37 GRCh37	hteese ﷺ Chromosome ع ع	Gene MLH1 MLH1 MLH1	Transcript NM_000249.2 NM_000249.2	gNomen 9.37065001_3706500 9.370615760>C 9.37051030>C	CNomen     CNomen     CNomen     CNOmen     C039-2127_1039-2123dc     c6500-C     c6500-C	Type         pNomen           1         Deletion         p.7           Substitution         p.(Glu320At           Substitution         p.(Glu320At	Coding effect sp)) Missense ) Missense	≪ Evidences (ACMG - used PM2 PM1,PM2,PP3,BP6 PM1,PM2,PP3,BP6	l c >	> Q Classification Undefined Signific Uncertain Signific	A HI Q A A Notes cance cance test cance

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  $rac{1}{3}$  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.

	Columns Filtering
Column: Search column	Active Columns
Variant creation date	Assembly
Variant last update date	Chromosome
Occurrence ID	Gene
Family ID	Transcript
Phenotype	gNomen
RNA Analysis	cNomen
HPO IDs	Туре
Comment	pNomen
Add All Add	Remove All Reset to default

Close

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.

• •	Variar	nt Filtering		
Add Filter		Active Filte	rs	
Criteria: Search column	Operator:	Criteria	Operator	Values
Gene	- •	Type	-	Incortion: Substitution
Transcript	Value: 🗸 Ø	туре	-	insertion, Substitution
gNomen	All 🗸			
cNomen	Add Filter			
pNomen				
Coding effect				
Evidences (ACMG - user defined)				
Classification				
Notes			Delete All	
				Close

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: =, <=, >=,  $\in$ ,  $\notin$ . 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<sup>M</sup> Visual Plus, go to "Help > Data Sources". Database versions of each source is also specified in the Variant Panel.

Help	
	Search
	Software Documentation
	Data Sources
	License Agreement
	Software Reference
	Contact Support

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

Open gene GRCh3					C MLH1
		× 🕈	🗙 🖋 MLH1 NM_000249.2 (GRCh37 c 🛛 🔀 ML	H1:c	
(MLH1) NM_000249.2 V Local V	ariant Database: alamut	~			
				1.4.4	
		Annotation	🕈 Splicing 🛉 Occurrences 🛉 🕈 Variant History	Report	
riant Features			Pathogenicity class		
Genomic Level	Protein Level		ACMG standards and guidelines	Missense Predictions	
Assembly: GRCh37	Coding Effect:	Missense	PM2 BP4	Align GVGD	Class C0 (GV: 353.86 - GD: 0.00)
Chromosome: Chr3 (p22.2)	pNomen:	p.(Phe3Leu)	Suggested ACMG classification: Uncertain Significance	CADD	Phred: 9.740, Raw score: 0.536451
gDNA: g.37035047C>G	Compare AA:	*	Show Details	MutationTaster	Benign, Tree vote: 8/92 (del/benign)
Type: Substitution	Check predictions in th	he Splicing Tab		SIFT	TOLERATED (score: 1.00.median: 3.64)
Transcript Level	External Tools		User defined pathogenicity class		
manscript tever	External roots		Classification: 0-Unclassified	Notes	
cDNA: NM_000249.2(MLH1):c.9C>G	VariantValidator	Mutalyzer	Pathogenicity class is NOT automatically suggested		
cDNA: NM_000249.2(MLH1):c.9C>G Location: Exon 1	VariantValidator	Mutalyzer	Pathogenicity class is NOT automatically suggested		
cDNA: NM_000249.2(MLH1):c.9C>G Location: Exon 1	VariantValidator	Mutalyzer	Pathogenicity class is NOT automatically suggested		
cDNA: NM_000249.2(MLH1):c.9C>G Location: Exon 1	VariantValidator	Mutalyzer	Pathogenicity class is NOT automatically suggested		
cDNA: NM_000249.2(MLH1):c.9C>G Location: Exon 1	VarlantValidator	Mutalyzer	Pathogenicity class is NOT automatically suggested		
cDNA: NM_000249.2(MLH1):c.9C>G Location: Exon 1 ternal databases	VariantValidator	Mutalyzer	Pathogenicity class is NOT automatically suggested		
cDNA: NM_000249.2(MLH1):c.9C>G Location: Exon 1 ternal databases dbSNP (v151)	VariantValidator	Mutalyzer 1000 Genomes (2020-06-3	Pathogenicity class is NOT automatically suggested NO HGVD (v2.30 - Aug. 2017)	Danish2k (2013)	GONL (v2013-10-05)
cDNA: NM_000249.2(MLH1):c9C-G Location: Exon 1 ternal databases dbSNP (v151) rold: 0777959578	VariantValidator	Mutalyzer 1000 Genomes (2020-06-: All:	Pathogenicity class is NOT automatically suggested IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Danish2k (2013) MAF:	GoNL (v2013-10-05) Filter:
cDNA: NM.000249.2(MLH1):c.9C-G Location: Exon 1 termal databases dbSNP (v151) rslt: rs779359678 Mmor Allelie:	Variant/validator	Mutalyzer 1000 Genomes (2020-06-3 All: EAS:	Pethogenicity class is NOT automatically suggested HGVD (v2.30 - Aug. 2017) Filter: MAF:	Danish2k (2013) MAF: Ref.Ref:	Gont (v2013-10-05) Filter: Alt allele count:
cDNA: NM.000249.2(MLH1):c.9C-G Location: Exon 1 ternal databases dbSNP (v151) rsid: rs77959678 Minor Allelle: Minor Allelle: Freq.	VariantValidator	Mutalyzer 1000 Genomes (2020-06-3 All: EAS: EUR:	Pathogenicity class is NOT automatically suggested HGVD (v2.30 - Aug. 2017) Filter: NAF: Ref/Ref:	Danish2k (2013) MAF: Ref/Ref: Ref/Alt:	GoNL (v2013-10-65) Filter: Af a biele count: Total alleles count:
cDNA. NM_000249.2(MLH1)c.sO-G Location: Exon 1 ternal databases dtSNP (v151) rsid: rs77959578 Minor Allele: Minor Allele Freq. Count:	VariantValidator	Mutalyzer 1000 Genomes (2020-06-3 All: EVR: AFR:	Pathogenicity class is NOT automatically suggested 10) HGVD (v2.30 - Aug. 2017) Filter: MAF: Ref/Ref: Ref/At:	Danish2k (2013) MAF: Ref/Ref: Ref/Alt: Ait/Alt	GoNL (v2013-10-05) Filter: Ait alfele count: Total alfeles count: Alfele Frequency:
cDNA: NM.000249.2(MLH1)::c9C-G Location: Exon 1 ternal databases dbSNP (v151) rsit: rs779359678 Minor Allele Minor Allele Freq. Count: Ancestral Allele C	VariantValidator	Mutalyzer 1000 Genomes (2020-06-3 All: EAS: EUR: AFR: AMR:	Pethogenicity class is NOT automatically suggested HOVD (v2.30 - Aug. 2017) Filter: MAF: Ref[Ret: Ref[Alt: Alt/Alt:	Danish2k (2013) MAF: Ref[Ref: Ref[Ait: Ait[Ait	GoNL (v2013-10-05) Filter: Art alile count: Total alileles count: Aliele Frequency:
cDNA: NM.000249.2(MLH1):c.9C-G Location: Exon 1 ternal databases dbSNP (v151) rsld: rs779759678 Minor Allelie: Minor Allelie Frq. Count: Ancestral Allelie: C Colinical signifi	Variant/alidacor	Mutalyzer 1000 Genomes (2020-06-3 All: EAS: EUR: AFR: AFR: AKR: SAS:	Pathogenicity class is NOT automatically suggested HGVD (v2.30 - Aug. 2017) Filter: MAF: Ref/Ref: Ref/Aft: Alt/Aft:	Danishžk (2013) MAF: Ref/Ref: Ref/Alt: Att/Alt	GoNL (v2013-10-05) Filter: Alt allele count: Total alleles count: Allele Frequency:
cDNA: NM.000249.2(MLH1):c.9-CG Location: Exon 1 ternal databases gbSNP (v151) rsid: rs779759678 Minor Allele Freq. Count: Ancestral Allele: C Clinical signif: CLIN.uncertain.signifi Validated: Yes	vervenveldator	Mutalyzer 1000 Genomes (2020-06-3 All: EAS: EUR: AAR: AAR: SAS:	Pathogenicity class is NOT automatically suggested b) HGVD (v2.30 - Aug. 2017) Filter: MAE: Ref/Ref: Ref/Ref: Ref/AI: At/AI:	Danish2k (2013) MAF: Ref/Ref: Ref/Alt: Att/Alt	GoNL (v2013-10-05) Filter: At allele count: Total alleles count: Allele Frequency:
cDNA: NM.000249.2(MLH1)::c9C-G Location: Exon 1 ternal databases dbSNP (v151) rstd: rs779359678 Minor Allele Minor Allele Freq. Count: Ancestral Allele C Clinical signif: CLIN_uncertain_signifi Validated: Ves	varianvalidator	Mutalyzer 1000 Genomes (2020-06-3 All: EAS: EUR: AFR: AFR: SAS:	Pethogenicity class is NOT automatically suggested HGVD (v2.30 - Aug. 2017) Filter: MAF: Ref/Ret: Ref/Alt: At/Alt:	Danish2k (2013) MAF: Ref/Ref: Ref/Alt: Atf/Alt	GoNL (v2013-10-05) Filter: Art allele count: Total alleles count: Allele Frequency:
cDNA: NM.000249.2(MLH1)::c9-CG Location: Exon 1 ternal databases dbSNP (v151) rstd: rs779759678 Minor Allelie: m Minor Allelie Freq. Count: Ancestra Allelie: C Clinical sign.ff. CLNy.uncertain_sign.ff Validated: Yes	verunvaldator aance,CLIN,Jikely,benign ()	Mutalyzer 1000 Genomes (2020-06-3 All: EAS: EUR: AFR: AMR: SAS:	Pethogenicity class is NOT automatically suggested NO HGVD (v2.30 - Aug. 2017) Filter: MAF: Ref/Ref: Ref/Att: Alt/Att:	Danish2k (2013) MAF: Bef/Ref: Ref/At: Alt/Alt	GoNL (v2013-10-05) Filter: Alt allele count: Total alleles count: Allele Frequency:
cDNA: NM.000249.2(MLH1):c.9C-G Location: Exon 1 rsid: rs779759678 Minor Allelie: Minor Allelie: Aminor Allelie Freq. Count: Ancestral Allelie: C Clinical signif: CLN_uncertain_signifi Validated: Ves	variantväldator	Mutalyzer 1000 Genomes (2020-06-3 All: EAS: EUR: AFR: AMR: SAS: SAS:	Pethogenicity class is NOT automatically suggested HGVD (v(2.39 - Aug. 2017) Filter: MAF: Ref/At: Alt/At:	Danish2k (2013) MAF: Ref/Ref: Ref/Alt: At/Alt	GoNL (v2013-10-05) Filter: At aliele count: Total alieles count: Allele Frequency:
cDNA: NM.000249.2(MLH1)::c9C-G Location: Exon 1 ternal databases dbSNP (v151) rstd:: rs779359678 Minor Allele Freq. Count: Ancestral Allele C Clinical signif: CLIN_uncerain_signifi Validated: Ves	varianvalidator	Mutalyzer 1000 Genomes (2020-06-3 All: EAS: EUR: AFR: SAS: SAS:	Pethogenicity class is NOT automatically suggested HOVD (v2.30 - Aug. 2017) Filter: Ref/Ref: Ref/Ref: Ref/Ref: ESP (v0.0.30)	Danish2k (2013) MAF: Ref[Ref: Ref[Ait: Att[Ait	GoNL (v2013-10-05) Filter: Alt alieles count: Total alieles count: Aliele Frequency:

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).

gnomAD scores		OMIM			BRIDGES	
PLI:	9.2157e-29	Add to Report	Phenotype	Mode of inheritance	PTV Odds Ratio (95% CI):	10.57 (8.02-13.93)
ExAC PLI:	3.2874e-20		BREAST-OVARIAN	Autosomal dominant	Pop p-value_PTV:	1.1e-62
Score based on NM_007300	.3, NM_007300.4 , gnomAD v2.1.1		PANCREATIC	(AD), Multilactorial	Missense Odds Ratio (95% CI):	1.11 (1.02-1.20)
			FANCONI ANEMIA,	Autosomal recessive	Pop p-value_MSV:	0.01
			COMPLEMENTATIO	(AR)	BFDP:	1.5e-64
LOEUF:	0.915					
LOEUF percentile:	0.50198					
Score based on NM_007300	.3, NM_007300.4 , gnomAD v2.1.1					

Notes:

- To include OMIM<sup>®</sup> phenotypes in the report, you may tick the "Add to Report" checkbox
- Double clicking on an OMIM<sup>®</sup> row will open the corresponding OMIM<sup>®</sup> 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<sup>™</sup> 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.

Image: And addim       Image: Addition of the standard and guidations       Text standard and guidations <thtext and="" guidation="" of="" shown="" standard="" th="" the="" to<=""><th>cript: (N</th><th>ILH1) NM_00</th><th>0249.3</th><th>Local Variant Database:</th><th>alamut</th><th>~</th><th></th><th></th><th></th><th></th></thtext>	cript: (N	ILH1) NM_00	0249.3	Local Variant Database:	alamut	~					
Variant Fatures       Pathogenicity class       Material Protections         Construction       Construction       Construction       Construction       Construction       Caling GKCD       Caling GKCD <td< th=""><th></th><th></th><th></th><th></th><th>Annotation</th><th>Splicing 🔶 Occurrences</th><th>👾 Variant History</th><th>🔶 Report</th><th></th><th></th></td<>					Annotation	Splicing 🔶 Occurrences	👾 Variant History	🔶 Report			
Assembly:       GRCh37       Coding Effect:       Masense       Phytine::       plone::       plon	Varian Ge	t Features nomic Level		Protein Level		Pathogenicity class ACMG standards and guideline	s		Missense Predictions		
Well with the province         provinc		Assemb	ly: GRCh37	Coding Effect:	Missense	PM1 PM2 PM5 PP3 P	P5		Align GVGD	Class C65 (GV: 0.00 - GD: 97.78)	
Not       PS3 available       Note: PS3 (strong)       Note: PS4 (strong)       Note: PS4 available       Note: PS4 (strong)       Note: The pervalence of the valence in controls.       Note: The pervalence in controls watere control studies may in trolcale 1.0 See the article for detailed guidance.       Note: Strong (PS1-PS4) AND 2 a supporting (PP1-PP5)         2       Moderate (PM1-PM6) AND 2 a supporting (PP1-PP5)       2		gDN	A: g.37035121C>T	Compare AA:	p.(Prozaceu)	Suggested Activo Calobinearion	ow Details		MutationTaster	disease causing (prob: 1)	
Not       PS1 available       Not       PS4 available       Not       Rules tor compare (PMI-PM6) and the confidence interval around the stimate of relative risk or OR as obtained from case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated paint where acese-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated paint where acese-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated paint where acese-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated paint where acese-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated paint where acese-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated paint where acese-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated paint where acese-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated paint where the asserve in constrol (crist man weel cecase) and tho the prior dista dind wind (escase) and tho		Tvo	e: Substitution		244	A QMQ attack and and	della es		PolyPhen2	Not automatically	
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Not available       PS4 (strong)       The prevalence of the most well established.       1 Strong (PS1-PS4) AND 2 Moderate (PM1-PM6) AND 2 Supporting (PP1-PP5)         1 Strong (PS1-PS4) AND 1 Moderate (PM1-PM6) AND 2 Supporting (PP1-PP5)       1 Strong (PS1-PS4) AND 1 Moderate (PM1-PM6) AND 2 Supporting (PP1-PP5)         1 Strong (PS1-PS4) AND 1 Moderate (PM1-PM6) AND 2 Supporting (PP1-PP5)       1 Strong (PS1-PS4) AND 1 Moderate (PM1-PM6)         1 Strong (PS1-PS4) AND 1 Moderate (PM1-PM6)       AND 2 Supporting (PP1-PP5)         2 Moderate (PM1-PM6)       1 Strong (PS1-PS4) AND 1 Moderate (PM1-PM6)         2 Moderate (PM1-PM6)       1 Strong (PS1-PS4) AND 2 Supporting (PP1-PP5)         3 Moderate (PM1-PM6)       1 Strong (PS1-PS4) AND 2 Supporting (PP1-PP5)         3 Moderate (PM1-PM6)       1 Strong (PS1-PS4) AND 2 Supporting (PP1-PP5)         3 Moderate (PM1-PM6)       1 Strong (PS1-PS4) AND 2 Supporting (PP1-PP5)         3 Moderate (PM1-PM6)       1 Strong (PS1-PS4) AND 2 Supporting (PP1-PP5)         3 Moderate (PM1-PM6)       AND 2				reproducible and robust	t in a clinical diagno	ostic laboratory setting are		1 Strong (PS1–PS4) AND ≥3 Moderate (PM1–PM6) O		PM6) 0	
Not       PS4 available       Note 1: Relative risk or OR, as obtained from case-control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0.See the article of detailed guidance.       1 Strong (PS1-PS4) AND 1 Moderate (PM1-PM6) AND 24 supporting (PP1-PP5)         IVery strong (PS1)       Note 1: Relative risk or OR, as obtained from case-control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0.See the arcticle of detailed guidance.       1 Strong (PS1-PS4) AND 1 Moderate (PM1-PM6)         IVery strong (PS1)       Note 1: Relative risk or OR, as obtained from case-control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not reach statistical significance the provide observation of the variant is not reach statistical significance the provide observation of the variant is not reach statistical significance the provide observation of the variant is not reach (eg., active site of an anytation functional domain (eg., active site of an enzyme) without beingn varianton construum.       Strong (PS1-PS4) AND 24 supporting (PP1-PP5)         IVery strong (PS1-BS4)       Located in a mutational hot spot and/or critical and well-established functional domain (eg., active site of an enzyme) without beingn varianton construum.       Strong (PS1-BS4) and 1 supporting (PP1-PP5)         Very Strong (PS1-BS4)       Absent from controls (or at externely low frequency if recessive) in Exome Seguencing Project, rot Exome Aggregation Construum.       Strong (PS1-BS4) and 1 supporting (PP1-PP3) 22 Supporting (BP1-BP7) 22 Supporting (BP1-BP7)         Very Strong (PS1-BS4)       Absent from controls (or at exte				The prevalence of the v	ariant in affected in	dividuals is significantly		1 Strong (PS1-PS4	) AND 2 Moderate (PM1-PM	M6) AND ≥2 supporting (PP1-PP5)	
Not available       PS4 (strong)       Note 1: Relative risk or OR, as obtained from case-control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0.See the archited guidance.       1 Strong (PS1-PS4) AND 1- 2 mddrate (PM1-PM6)         V       PS4 (strong)       Note 1: Relative risk or OR, as obtained from case-control studies, is >5.0, not include 1.0.See the archited guidance.       1 Strong (PS1-PS4) AND 1- 2 mddrate (PM1-PM6)         V       PM1 (moderate)       Located in a mutational hot spot and/or critical and well-established functional domain (eg., active site of an enzyme) without beingn varianto functional domain (eg., active site of an enzyme) without beingn varianto functional domain (eg., active site of an enzyme) without beingn varianto functional domain (eg., active site of an enzyme) without beingn varianto functional domain (eg., active site of an enzyme) without beingn varianto functional domain (eg., active site of an enzyme) without beingn varianto functional domain (eg., active site of an enzyme) without beingn varianto functional domain (eg., active site of an enzyme) without beingn varianto functional group (cg. eg. add) and supporting (BP1-BP7)         V       PM2 (moderate)       Sequencing Project, 1000 Genomes Project, or Exome Aggregation Constitum.       Sequencing Project, or Exome Aggregation Constitum.       Utertain Significance other criteria shown above are not met				increased compared wit	th the prevalence in	controls.		1 Strong (PS1-PS4	) AND 1 Moderate (PM1-PM	M6) AND ≥4 supporting (PP1-PP5)	
Not available       PS4 (strong)       and the confidence interval around the estimate of relative risk or OR does not include 10.5 see the article for detailed guidance. Note 2: In instances of very rare variants where case-control studies may multiple unrelated patients with the same phenotype and its absence in controls, may be used as moderate level of evidence.       1 Strong (PS1-PS4) AND 1-2 moderate (PM1-PM6)         Image: Controls with the same phenotype and its absence in controls, may be used as moderate level of evidence.       1 Strong (PS1-PS4) AND 2-2 supporting (PP1-PP5)         Image: Controls with the same phenotype and its absence in controls, may be used as moderate level of evidence.       1 Strong (PS1-PS4) AND 2-2 supporting (PP1-PP5)         Image: Controls with the same phenotype and its absence in controls (or at extremely low frequency if recessive) in Exome Sequencing Prioet, 1000 Genomes Project, or Exome Aggregation Consortium.       1 Strong (S1-BS4) and 1 supporting (BP1-BP7)         Image: Control with the same phenotype and pathogence in controls (or at extremely low frequency if recessive) in Exome Consortium.       Absent from controls (or at extremely low frequency if recessive) in Exome Consortium.       1 Strong (BS1-BS4) and 1 supporting (BP1-BP7)         Image: Consortium.       PM2 (moderate)       Absent from controls (or at extremely low frequency if recessive) in Exome Consortium.       Absent from controls (or at extremely low frequency if recessive) in Exome Consortium.       2 Supporting (BP1-BP7)         Image: Consortium.       Consertium.       Consertium.       Consertium.       2 Supporting (BP1-BP7) <t< td=""><td></td><td></td><td></td><td colspan="2">Note 1: Relative risk or OR as obtain</td><td>n case-control studies is &gt;5.0</td><td></td><td colspan="2" rowspan="2">1 Very strong (PVS1) AND 1 moderate (PM1-PM6) 1 Strong (PS1-PS4) AND 1-2 moderate (PM1-PM6)</td></t<>				Note 1: Relative risk or OR as obtain		n case-control studies is >5.0		1 Very strong (PVS1) AND 1 moderate (PM1-PM6) 1 Strong (PS1-PS4) AND 1-2 moderate (PM1-PM6)			
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Image: Section of the section of t		available	(strong)	not include 1.0.See the article for detaile Note 2: In instances of very rare variants not reach statistical significance, the prior		guidance.	Likely Pathogenic	1 Strong (PS1-PS4	i) AND ≥2 supporting (PP1-	·PP5)	
<ul> <li>Water and the series of the ser</li></ul>						observation of the variant in		≥3 Moderate (PM1	-PM6)		
Image: Controls, may be used as moderate level of evidence.     Image: Controls, may be used as moderate level of evidence.       Image: Controls, may be used as moderate level of evidence.     Image: Controls, may be used as moderate level of evidence.       Image: Controls, may be used as moderate level of evidence.     Image: Controls, may be used as moderate level of evidence.       Image: Controls, may be used as moderate level of evidence.     Image: Controls, may be used as moderate level of evidence.       Image: Controls, may be used as moderate level of evidence.     Image: Controls, cont				multiple unrelated patie	nts with the same p	phenotype, and its absence in		2 Moderate (PM1-	PM6) AND ≥2 supporting (F	PP1-PP5)	
Image: Section 2 moderate     PM1 (moderate)     Located in a mutational hot spot and/or critical and well-established Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.     Benign     1 Standarding (SS) - ES-1 2 at Sequencing (SS) - ES-1 2 at Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.       Image: Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.     Description (SS) - ES-1 (SS) - SS) and SS) and SS) and SS) and SS) and SS) and SS)				controls, may be used a	s moderate level of	evidence.		1 Moderate (PMI-	PM6) AND 24 supporting (P	-21-220)	
<ul> <li>Incretional domain (e.g., active site of an enzyme) without benign variation (moderate)</li> <li>Incretional domain (e.g., active site of an enzyme) without benign variation Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.</li> <li>Incretional domain (e.g., active site of an enzyme) without benign variation Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.</li> <li>Incretional domain (e.g., active site of an enzyme) without benign variation Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.</li> <li>Incretional domain (e.g., active site of an enzyme) without benign variation Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.</li> <li>Incretional domain (e.g., active site of an enzyme) without benign variation Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.</li> <li>Incretional domain (e.g., active site of an enzyme) without benign variation Consortium.</li> <li>Incretional domain (e.g., active site of an enzyme) without benign and pathogenic are contradictory Other criteria shown above are not met</li> </ul>			PM1	Located in a mutational	hot spot and/or cri	tical and well-established	Benign	≥2 Strong (BS1-BS4)			
<ul> <li>PM2 (moderate)</li> <li>Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Storme Aggregation Consortium.</li> <li>Consortium.</li> <li>Caveat: Population data for insertions/deletions may be poorly called by</li> <li>Caveat: Population data for insertions/deletions may be poorly called by</li> </ul>		(moderate) functional domain (e.g., active site of a				nzyme) without benign variation			1 Strong (BS1–BS4) and 1 supporting (BP1–BP7)		
PM2 (moderate)           Onsortium.         Consortium.         Uncertain Significance         The criteria for being and pathogenic are contradictory           Other Construction         Caveat: Population data for insertions/deletions may be poorly called by         Uncertain Significance         The criteria for being and pathogenic are contradictory			PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project,1000 Genomes Project,or Exome Aggregation Consortium.		w frequency if recessive) in Exome ect.or Exome Aggregation		≥2 Supporting (BP	1-BP7)		
(moderate) (moderate) Caveat: Population data for insertions/deletions may be poorly called by						Uncertain Significance The criteria for benign and pathogenic are contradictory		ntradictory			
		Ť	(moderate)	Caveat: Population data	a for insertions/dele	tions may be poorly called by	Oncertain Significance	Other criteria sho	vn above are not met		
	suggested:	PM1, PM2, PM	5, PP3, PP5								

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 $^{\mathbb{M}}$  Visual Plus includes a splicing module integrating several prediction algorithms and splicing prediction data:

## a. Splicing signals:

- SpliceSiteFinder-like (donor, acceptor, branchpoint): Alamut<sup>™</sup> 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): <u>MaxEntScan</u> splice site datasets and algorithms are fully integrated in Alamut<sup>™</sup> 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<sup>™</sup> Visual Plus reports scores from NNSPLICE 0.9.
- Known constitutive signals (donor, acceptor): Alamut<sup>™</sup> 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<sup>™</sup> Visual Plus to perform the computation provided on the <u>ESEFinder web site</u>.
- RESCUE-ESE: The set of human hexamers available from the <u>RESCUE-ESE web</u> <u>site</u> is embedded inside Alamut<sup>™</sup> Visual Plus
- EX-SKIP: The EX-SKIP tool is available, through the Alamut<sup>™</sup> 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 <u>fruitfly.org</u> partially integrated: it can be interrogated from Alamut<sup>™</sup> 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

Transcript:	(BRCA1) NM_007300.3	~	Local Variant Database:	alamut	~				
					🚸 Annotation	Splicing	🚸 Occurrences	🚸 Variant History	🏘 Report

- 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.

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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.



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	Sp	olicing Predi	ction	s Opti	ons			
Splice Site Predic	tions							
		5' (Donor)	_		3' (Ad	ceptor)		
SpliceSiteFine	der-like	70	[	0-100]	70		[0-100]	
MaxEntScan		0	[	0-12]	0		[0-16]	
NNSPLICE		0.4	[	0-1]	0.4		[0-1]	
GeneSplicer		0	[	0-24]	0		[0-21]	
🗸 Branch Point	s				0		[0-100]	
ESE Predictions								
Louintentino	ESE	Finder Settings		Thresh	old			
		SF2/ASF		1.956		Def. v	alue	
				1 967		Def	alue.	
		SFZ/ASF (IGIVI-B	RCAT)	1.007		Del. V	alue	
		SC35		2.383		Def. v	alue	
		SRp40		2.67		Def. value		
		SRp55		2.676		Def. value		
		RESCUE-ES	E hexa	mers (s	ee the	RESCUE-ES	website)	
Flanking region u	sed by s	plicing report	200				Ĉ	
Save Options	Rese	t to Default				Apply	Close	

## **References:**

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## 11.4 Missence Prediction

Alamut<sup>M</sup> 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<sup>M</sup> 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 <u>Align GVGD</u>, <u>SIFT</u>, <u>MutationTaster</u>, <u>Polyphen 2</u> and <u>CADD</u>.

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.

• • •		Alamut Visual Plus		
🛖 Open gene GRCh37 G			. ب	Q MLH1 O
Transcript: (MLH1) NM_000249.2 V Local Variant Database	x 🕈 X	MLH1 NM_000249.2 (GRCh37 c., X MLH12	C	
Variant Features	Pathogenicity	class		
Genomic Level Protein Assembly: GRCh37 Coding Chromosome: Ch/3 (p22.2) gDNA: g37035947C>G Compa Type: Substitution Check I Transcript Level External CDNA: NM_000249.2(MLH1):c.SC>G Varia	Level     ACMG stam       Effect:     Missense       IPM2 IB     IB/M2 IB       re:     p.(PheBLeu)       Suggerier     Suggerier       re AA:     2       predictions in the Splicing Tab     User define       Tools     Classificat       intrulidator     Mutalyzer       Pathogeni	dards and guidelines PA AACMG classification: Uncertain Significance Show Details d pathogenicity class on: Q-Unclassified	Masteria Predictions Align Gricio Class CO (GM CADO Pirved: 5,740, MustoonTaser Bonign, Tree v PutyPred: bon SIPT TOLERATED (s	353.86 - GD: 0.00) Raw score: 0.536451 te: 8)82 (del/benign) ign (score: 0).HVarPred: benign (score: 0) core: 1.00,median: 3.64)
External databases dbSNP (v151) rsld: rs779759678 Minor Allelle: Minor Allelle Free, Count: Ancestral Allelle: C Clinical signifi: CLIN_uncertain_significance,CLIN,II Validated: Ves ①	1000 Genomes (2020-06-30) Alt: EAS: EUR: AFR: AMR: Sety_benign SAS:	HGVD (v2.30 - Aug. 2017) Filter MAF: Ref/Ref: Ref/At: Att/At:	Danish2k (2013) MAF: Ref/Ref: Ref/At: Alt/At:	GoNL (v2013-10-05) Filter: At allele count: Total alleles count: Allele Frequency:
gnomAD (v2.1.1) Genome	Exone Groune-Loona	ESP (v0.0.30)		Sove Doport Cancel Delete

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

### Align GVGD

Align GVGD predictions are computed using the orthologue alignment provided by Alamut<sup>™</sup> Visual Plus for the gene under study (See section 10.1.8). Align GVGD scores interpretation are available <u>here</u>. 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 <u>here</u>.

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 $^{m}$  Visual plus.

MutationTaster scores interpretation are available <u>here</u>.

Current version for precomputed predictions: MutationTaster2 from <u>MutationTaster</u> <u>website</u>

# PolyPhen-2

Alamut<sup>™</sup> 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: <u>WHESS.db</u> for PolyPhen-2 website.

# SIFT

SIFT predictions are computed using the orthologue alignment provided by Alamut<sup>M</sup> Visual Plus for the gene under study. See <u>SIFT Aligned Sequences</u>.

SIFT scores interpretation are available <u>here</u>.

Current version for precomputed predictions: SIFT 6.2.0

# References :

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- Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J (2014 Feb 2), <u>A</u> 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.

			🚸 Annotation	🚸 Splicing 🚸 Occ	urrences	👾 Variant History	🔶 Report			
Varian	t Features			Pathogenicity class						
Ger	nomic Level		Protein Level	ACMG standards	and guidelines		Missense Predictions			
	Assemb	ly: GRCh37 e: Chr3 (p22.2)	Coding Effect: Missense	PM1 PM2 F	PM5 PP3 PP5			Align GVGD	Class C65 (GV: 0.00 - GD: 97.78)	
	aDN	A: 0.37035121C>T	pitomen: p.(Prozo	eu)				MutationTaster	disease causing (prob: 1)	
	TVD	e: Substitution	Compare AA:		Show	w Details		PolyPhen2	Not automatically	
•				ACMG standard	ds and guide	elines				
Please note the selected	hat these criter Suggested	ria for classifying vari Evidence	iants <u>Richard et al., 2015. Genet Med</u> are available Cai	to help you to determine the pathog	genicity class.	Pathogenicity class	Rules fo	or combining criteria to	o classify sequence variants	
		symbol	nonmaternity				Very strong (PVS1)	AND ≥1 Strong (PS1-PS4	)	
			Well-established in vitro or in vivo fu	nctional studies supportive	of a		Very strong (PVS1)	AND ≥2 Moderate (PM1-F	PM6)	
			damaging effect on the gene or gene	product.			Very strong (PVS1)	AND 1 Moderate (PM1-PM	M6) and 1 supporting (PP1–PP5)	
	Not available	PS3 (strong)	Note: Functional studies that have be	en validated and shown to b	be	Pathogenic	Very strong (PVS1)	AND ≥2 Supporting (PP1-	-PP5)	
		(	reproducible and robust in a clinical	diagnostic laboratory setting	are		1 Strong (PS1=PS4	) AND >3 Moderate (PM1-	PM6) O	
			considered the most well established	l. Mad individuale is significant	the state		1 Strong (PS1-PS4	AND 2 Moderate (PM1-P	M6) AND ≥2 supporting (PP1-PP5)	
			increased compared with the prevale	nce in controls.	liy		1 Strong (PS1-PS4	-PS4) AND 1 Moderate (PM1-PM6) AND ≥4 supporting (PP1-PP5)		
			Note 1. Polotivo risk or OP as obtains	d from occo. control studios	in SE O		1 Very strong (PVS	1) AND 1 moderate (PM1-F	PM6)	
	Mat	DC4	and the confidence interval around the	e estimate of relative risk or	r OR does		1 Strong (PS1-PS4	) AND 1-2 moderate (PM1-	-PM6)	
	available	(strong)	not include 1.0.See the article for de	tailed guidance.		Likely Pathogenic	1 Strong (FS1=F34	) AND 22 Supporting (PP I	-PPO)	
			not reach statistical significance, the	prior observation of the varia	ant in		≥3 Moderate (PM1-	-PM6)		
			multiple unrelated patients with the	ame phenotype, and its abse	ence in			nuo, nuo ez supporting (		
			controls, may be used as moderate le	vel of evidence.			1 Moderate (PM1-F	PM6) AND ≥4 supporting ( 1)	PP1-PP5)	
	1	PM1	Located in a mutational hot spot and	or critical and well-establish	ned	Benign	≥2 Strong (BS1-BS	54)		
<b>~</b>	•	(moderate)	functional domain (e.g., active site of	an enzyme) without benign	variation		1 Strong (BS1-BS4	) and 1 supporting (BP1–B	P7)	
			Sequencing Project, 1000 Genomes F	y low frequency if recessive; Project,or Exome Aggregatio	n Exome	Likely Benign	≥2 Supporting (BP	1-BP7)		
	1	PM2	Consortium.			Uncertain Significance	The criteria for ber	nign and pathogenic are co	ontradictory	
-		(moderate)	Caveat: Population data for insertion	alled by	oncertain orginiteanee	Other criteria show	vn above are not met			
			next-generation sequencing	-,,,,,,,,,,,,-	,					

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

ipt: (MLH1) NM_000249.3	Local Variant Database:	lamut	~		
	🖗 Anr	notation 🖗 S	olicing 🙀 Occurrences 🙀 Variant History 🖗 Report		
Variant Features Genomic Level	Protein Level		Pathogenicity class ACMG standards and guidelines	Missense Predictions	
Assembly: GRCh37	Coding Effect:	Missense		Align GVGD	Class C65 (GV: 0.00 - G 97.78)
chromosome: Chr3 (p22.2)	pNomen:	p.(Pro28Leu)	Suggested ACMG classification: Likely Pathogenic	MutationTaster	disease causing (prob: '
Type: Substitution	Compare AA:	**	Show Details	PolyPhen2	Not automatically computed
Transcript Level	Check predictions in the S	plicing Tab	User defined pathogenicity class	SIFT	DELETERIOUS (score: 0 median: 3.43)
-DUAL NIK 000040 3/MUUK)- 000 T			Classification: 5-Pathogenic 🗸		
Location: Exon 1	Variant Validator	Mutalyzer	Pathogenicity class is NOT automatically suggested	Notes	

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.

	Edit Occurrence	
	6	
Occurrence ID *		
Family ID		
Phenotype		
HPO		See HPO
RNA Analysis		
Comment		
Created	01/02/2021	
Undated	01/02/2021	
opuateu	01/02/2021	
Updated By		
	* indicates	a required field
	ОК	Cancel



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.

κ.	Search
henotypes (Double-click to add to selection)	Selection
All     Mode of inheritance     Autosomal dominant inheritance     Autosomal dominant somatic cell mutation     Autosomal dominant contiguous gene syndrome	
	1 a
Phenotypic abnormality     Cinical modifier     Cinical course     Frequency	Delete         Delete           Information         Information           HPD-id: 1444         Autosomal dominant somatic cell mutation           Being related to a de novo variant that occurs in a single cell in developing somatic tissue. The cell is the progenitor of a population of identical mutant cells, a which have descended from the cell that mutated. Clinical manifestations depend on the identity and proportion of affected cells in the body." []

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:

	🚸 Annotation 🛛 🚸	Splicing Occurrences	👾 Variant History	🚸 Report
(+) New Occurrence (+) Add Existing Occurrence (-) Delete Occurrence Edi	Occurrence			
Variant Summary				
Occurrence ID 🗸 Family ID Phenotype HPO IDs RNA Analysis	Comment Created	Updated Updated By		

# 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'.

	🚸 Annotation	🙀 Splicing	🚸 Occurrences	Variant History	👾 Report
Date User Classification Notes ACMG Evidences					



# 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.

Local Variant Database: alamut

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:

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<sup>M</sup> 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.

•	• •										
[	Local variant database	Description	Entries	Variants	Occurrences	Path	Display in track	Default	Shared database	Last update date	~
	alamut	Default local database	0	0	0	/Users/user/Library/Application	All			15/06/2022	
	Test1	Variants	0	0	0	/Users/user/Library/Application	All			15/06/2022	
						Explore/Export Import	New	Add Existin	g Database Edit	Delete Clear Database Close	2


Row filters (optional)				c	Column filters (optional)		_	HTML fields		
Local variant database	e: test23			~	<ul> <li>✓ Assembly</li> <li>✓ Chromosome</li> <li>✓</li> </ul>	Classification		<ul> <li>Export as plain text</li> <li>Preserve HTML tag</li> </ul>	5	
Gene:	All			~	🖌 Gene 🔽	Occurrence ID		Output format		
Type: Classification:	All			~	Transcript   Image: Constraint of the second seco	Family ID Phenotype RNA Analysis HPO IDs		<ul> <li>Tab-separated text</li> <li>Excel</li> <li>VCF</li> </ul>		
Occurrence ID:	All			~	<ul> <li>✓ pNomen</li> <li>✓ Coding effect</li> <li>✓ Evidences (ACMG)</li> </ul>	Comment Update date Local variant dat	abase	Oestination O Export to file:		Browse
Family ID:	All			<u> </u>	All annotations			O Export to clipboard		
Assembly 🗸	Chromosome	Gene	Transcript	gNomen	cNomen	Туре	pNomer	n Coding effect	Evidences (ACMG)	Classification
1 GRCh38 17	7	BRCA1	NM_007300.3	g.43090983G>A	c.4146C>T	Substitution	p.(Cys1382	2=) Synonymous		Uncertain Significance
2 LRG_292 17	7	BRCA1	NM_007294.3	g.135495G>A	c133290G>A	Substitution	p.(Gln1424	=) Synonymous		Uncertain Significance
3 LRG_292 12	7	BRCA1	NM_007294.3	g.135495G>A	c133290G>A	Substitution	p.(Gln1424	=) Synonymous		Uncertain Significance
4 GRCh38 17	7	BRCA1	NM_007300.3	g.43082483A>G	c.4278T>C	Substitution	p.(Ser1426	i=) Synonymous		Benign
5 GRCh38 17	7	BRCA1	NM_007300.3	g.43079367T>C	c.4390A>G	Substitution	p.(Met1464	4Val) 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<sup>™</sup> 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<sup>M</sup> 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<sup>M</sup> 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).

License Network View Misc Network Alamut Server: Europe Neth America		Settings		
Network Alamut Server: Europe Nem America	License	Network	View	Misc
Alamut Server: Europe North America	Network			
	Alamut Server: Eu	rope North-America		

# 15.1 Specifications

Four HTTP GET requests can be processed by Alamut<sup>™</sup> 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 <u>support@sophiagenetics.com</u>.

### 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,  $Alamut^{M}$  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<sup>™</sup> Visual Plus to display the result of a search (here variant "NM\_000059.3:c.4563A>C" (after percent-encoding see <a href="https://www.urlencoder.org">https://www.urlencoder.org</a>).

Any request that can be processed by the search field of Alamut<sup>™</sup> 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<sup>™</sup> Visual Plus to open a BAM file located at <a href="http://rd-connect.interactive-biosoftware.com/BAM/example.bam">http://rd-connect.interactive-biosoftware.com/BAM/example.bam</a> (the path is percent-encoded,



see <u>https://www.urlencoder.org</u>). 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=YYYYYYY&filetype=bam&path= file%3A%2F%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=YYYYYYY&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<sup>™</sup> 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
  - o Open Gene
  - o **GRh37**
  - o **GRh38**
  - Mitochondrial view
  - o **Home**
- File
  - Open BAM/CRAM File
  - Open BAM from URL

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- Open Sanger File
- Export Fasta Sequence
- View
  - o Focus on
  - Show ruler
  - Color nucleotides
  - Use amino acid 3 letter code
  - o Full Screen
  - $\circ$   $\,$  Show navigation bar  $\,$
  - Increase Font
  - Decrease Font
  - Reset Font
  - o 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
  - o Local Variants Database
- Tools
  - o 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 $^{\mathbb{M}}$  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<sup>M</sup> 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: <a href="mailto:support@sophiagenetics.com">support@sophiagenetics.com</a>

### 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



# **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.