RD-Connect Training:

Variant annotation and interpretation

with Alamut®

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Interactive Biosoftware

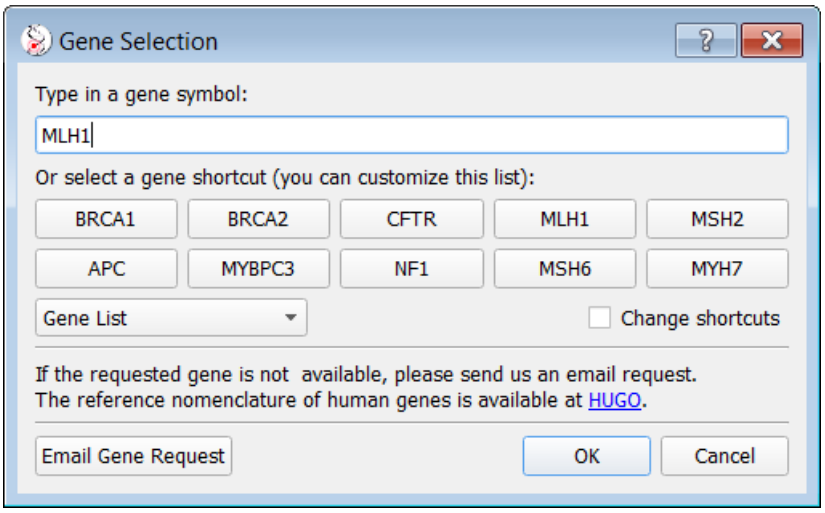
Alamut was designed to help you in this interpretation by entering a variant

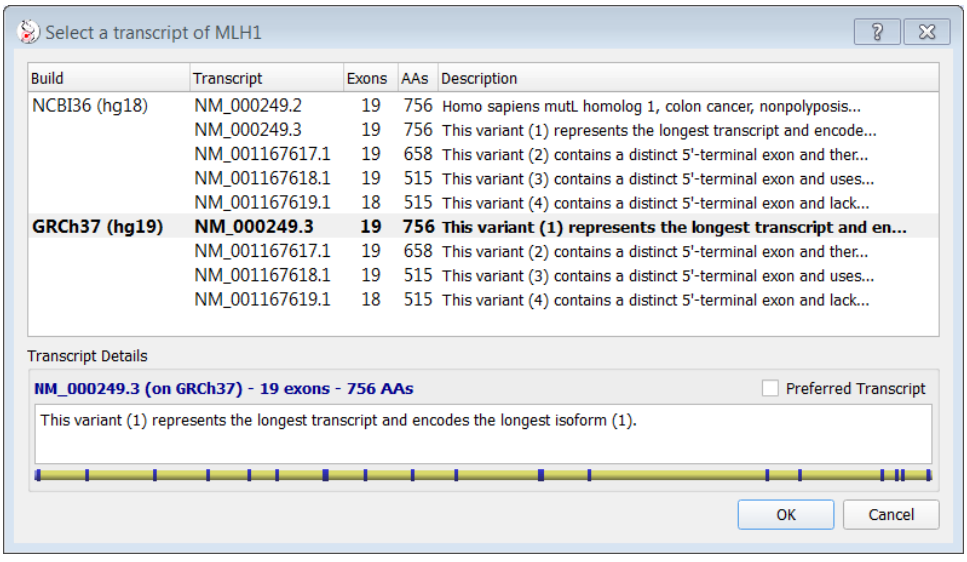
- Which type of variant is it?
- Has this sequence variation been previously reported?
- Is there a functional impact for this variant?

A documentation is available from Alamut by clicking on “Help” and on “Software documentation” from the menu bar or by clicking on the info icon .

1. Interpretation of a sequence variation

➢ Load the gene “MLH1” and choose the transcript “NM_000249.3”.

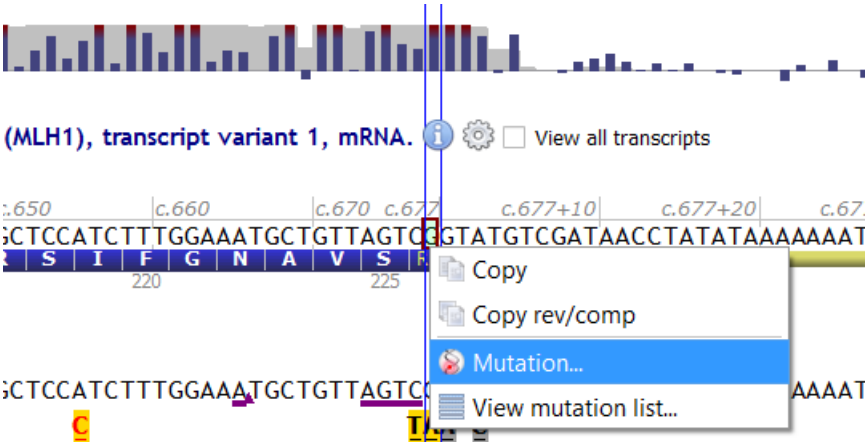




➢ Go to the position g.37055942 or c.677 in the exon 8 of this transcript.

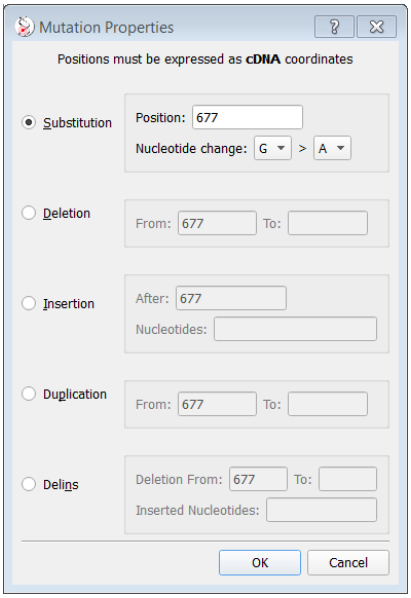
➢ Left click to select the nucleotide “G” in the transcript track.

➢ Right click and then select “Mutation…”.



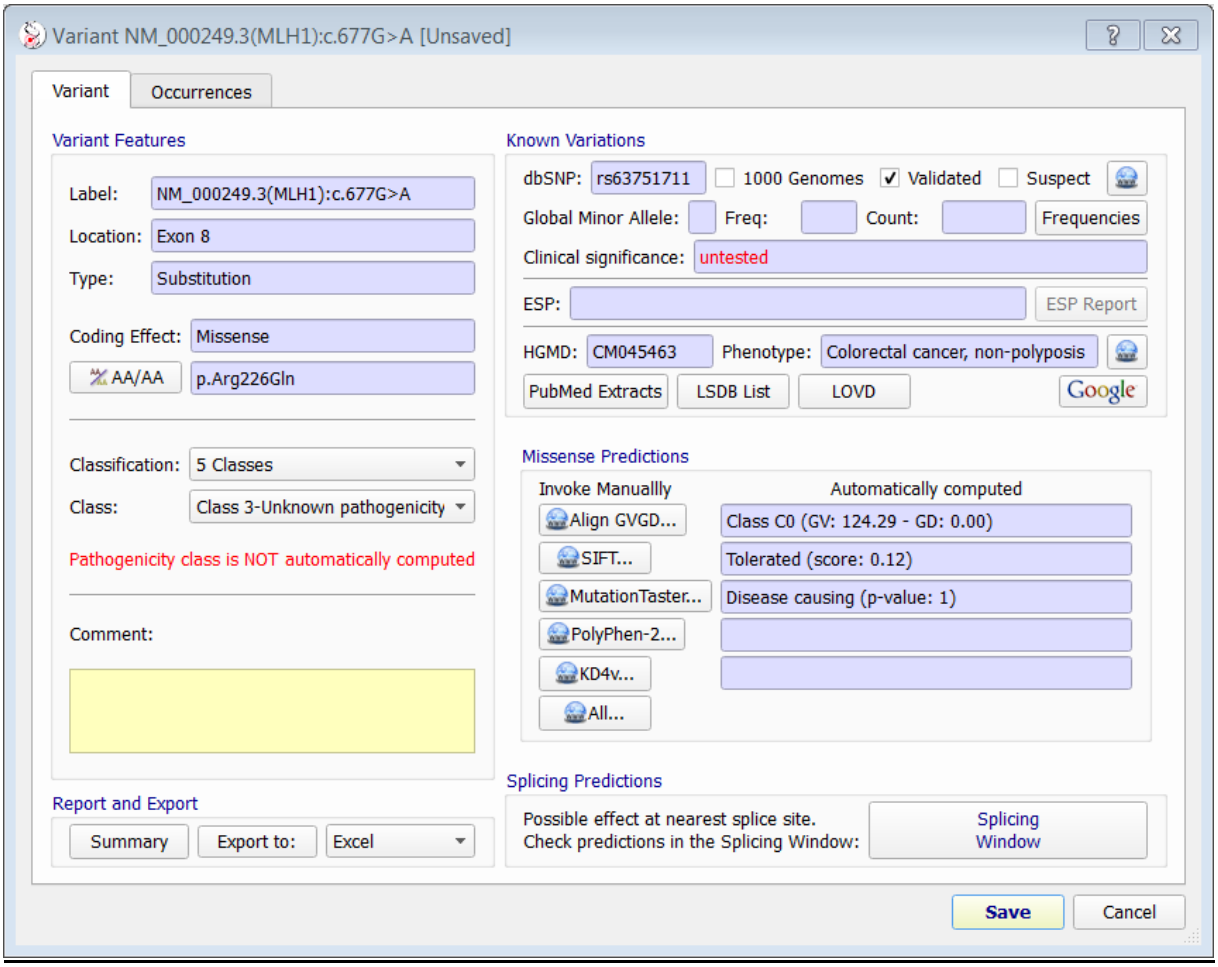
A first mutation window, named “Mutation Properties”, appears to define the property of the observed mutation

- Enter that the nucleotide “G” is changed to a “A”.



- Click on “OK”.

The “mutation interpretation” window appears. This window allows you to have all current known relevant information for this variant.



Let us have a look at the different parts:

The “variant features” part

- What is the HGVS nomenclature for this variant?
- What is the type of this variant?
- What is its coding effect?

The “known variations” part

- Is it a known variant?
- If so in which databases is this variant known?
- Have you got information about its allele frequency?

External links to PubMed Abstracts, Locus specific databases… are available to check quickly whether it is previously described in other sources.

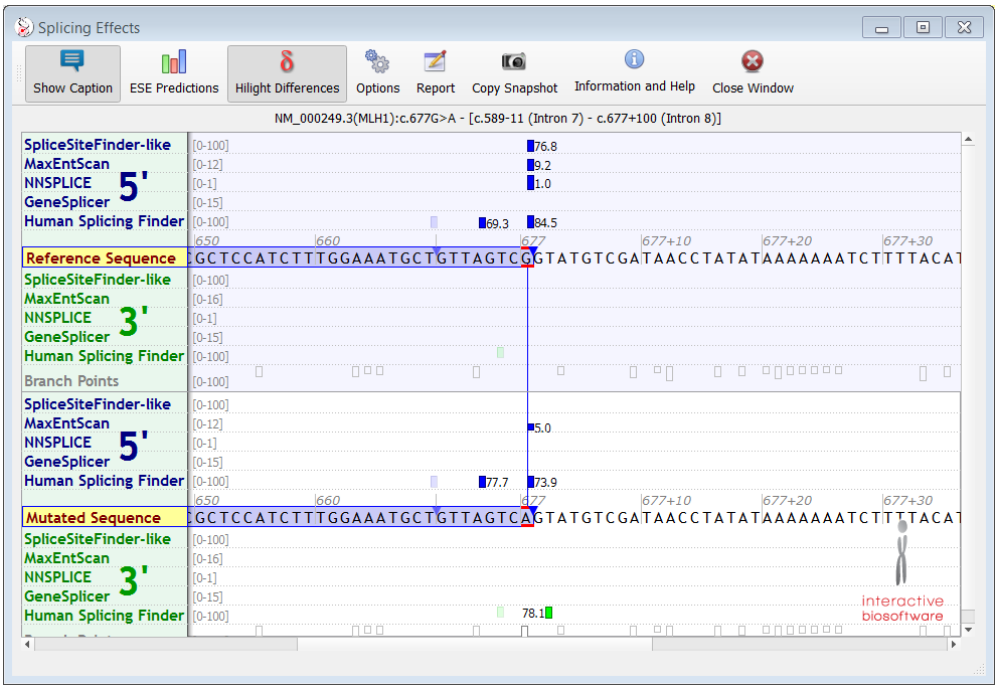
The prediction tool part can also be used to assist in variant classification.

The “missense predictions” with automatic prediction scores computed from several relevant tools can help you to predict the effect of amino acid changes.

- What are the pre-computed prediction given by the tools?
- What is the prediction given by PolyPhen2?
- What is the prediction given by KD4v?

The “splicing predictions” with a graphic view can help you to predict a splicing effect such as creation or loss of site.

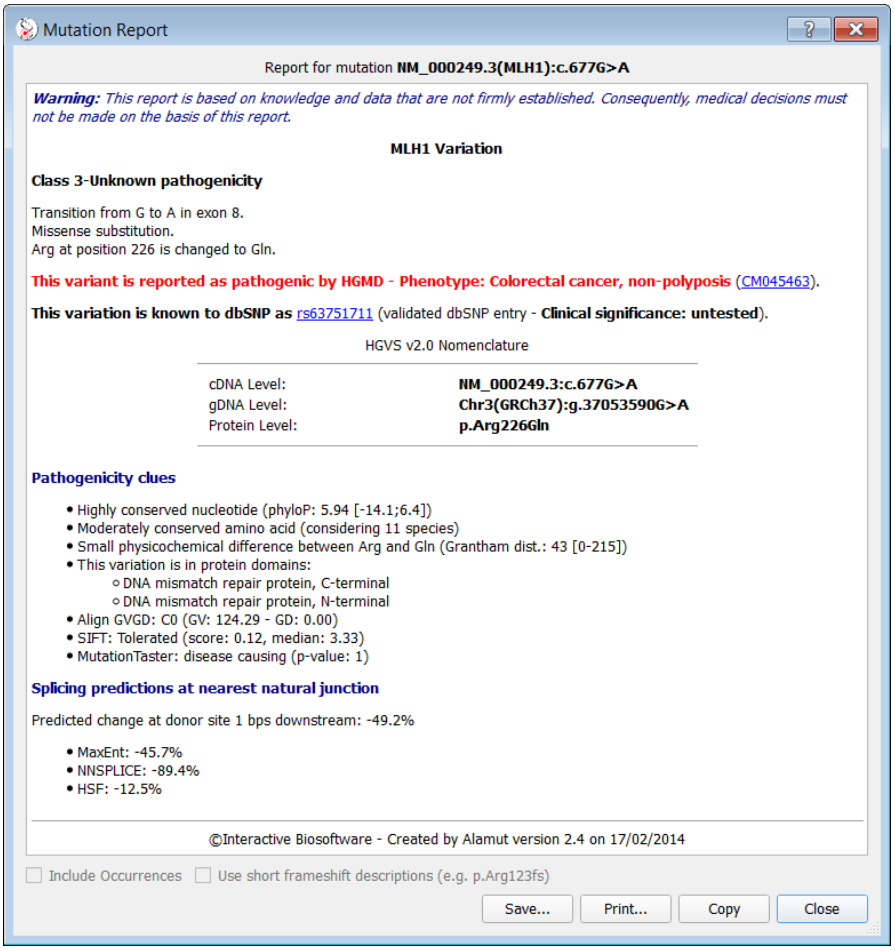
- What is the predicted splicing effect of the variant?
- Click on “Splicing window” and on “Highlight Differences” to show differences between the wild type sequence and the mutated sequence.
- What do you observe?



- Go back to the “Mutation interpretation” window.
- Select the appropriate pathogenicity class for the variant.

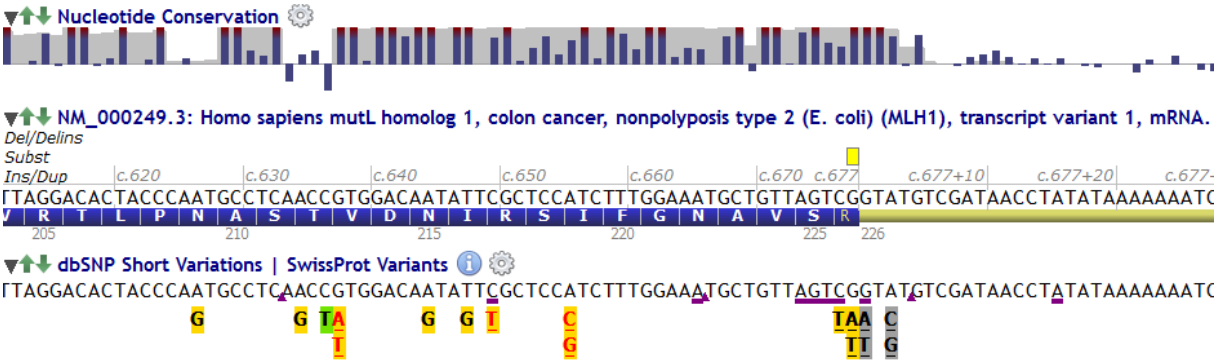
Alamut does not classify automatically a variant into a pathogenicity class. By default, the value is “unknown pathogenicity in the “5 classes”- classification” *ie* a VUS (variant of uncertain clinical significance).

- Edit a summary report.



- Click on “Close”.
- Go back to the “Mutation interpretation” window.
- Click on “Save” to save your annotated variant in Alamut.
- Where is the variant saved?
- Why is your variant yellow? Have you an idea on which information the color is associated to?

Your variant will appear on the transcript track for further analyses.

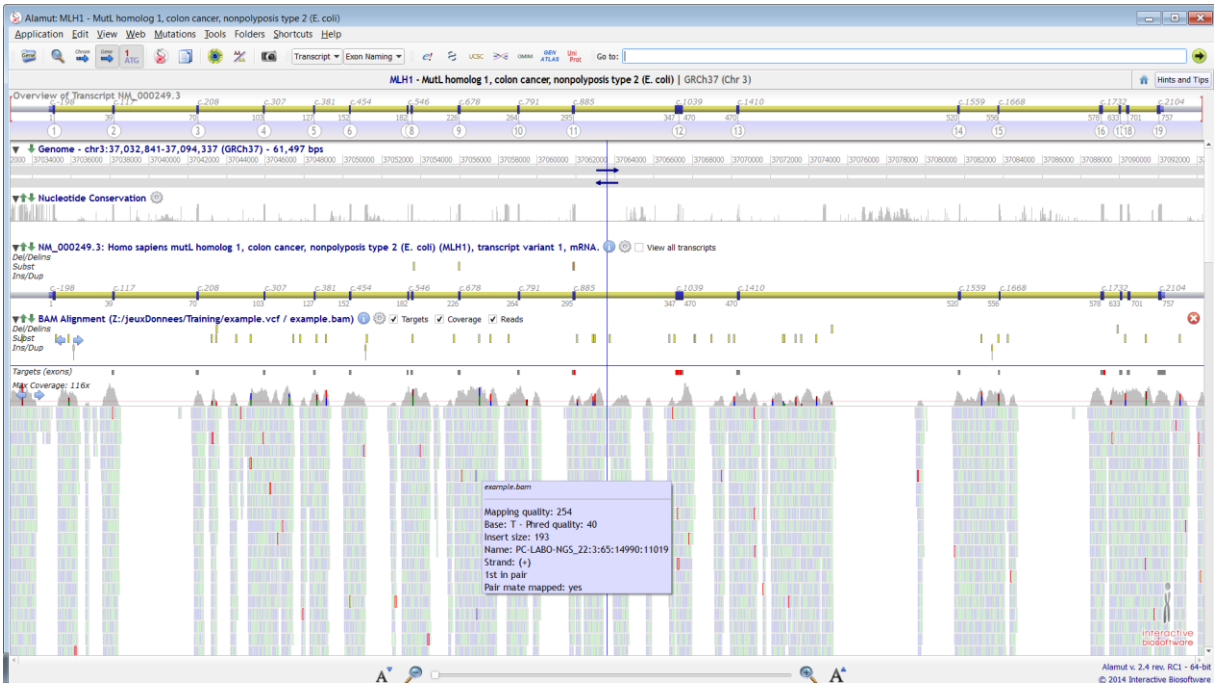


2. Working with NGS data

Alamut allows you to visualize BAM (read alignments), VCF (variant calls) and BED files from NGS analyses.

- Select the MLH1 gene and then the transcript “NM_000249.3”.
- Click on “Application” then on “Load BAM file from URL”
- Paste this url http://rd-connect.interactive-biosoftware.com/BAM/example.bam

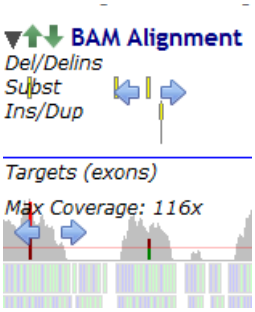
The BAM file is loaded in a new track.



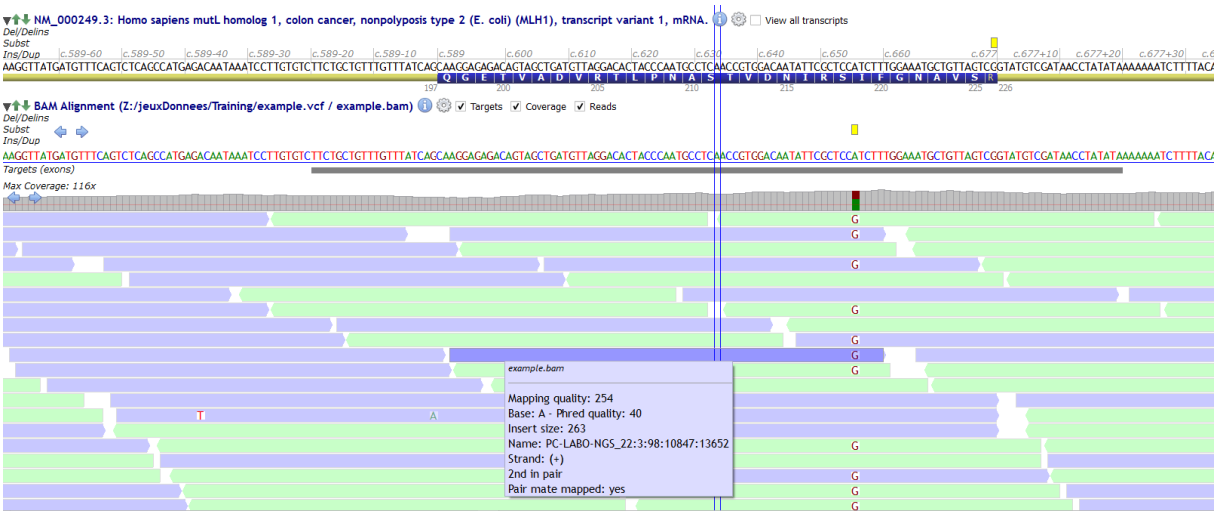
- What is the maximum depth of coverage for the MLH1 gene locus?
- What does the red line correspond to? What is the value marked by this red line?
- Which regions are tagged in the “Targets” sub-track by default?
- Click on the wheel icon from the BAM track then on “Load VCF file from URL”.
- Paste this url http://rd-connect.interactive-biosoftware.com/BAM/example.vcf

The called variants reported in the VCF file are loaded in the BAM track as a sub-track.

- Jump from one variant to the other by clicking the arrow on the left.



- Click on the exon 8 from the locus track to have a look at it.

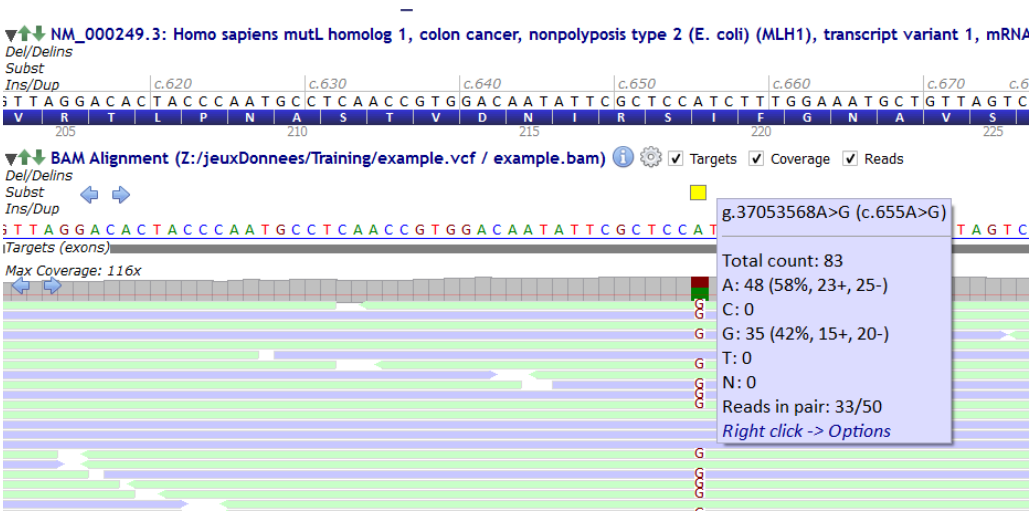


- Why are some reads blue or green?
- What do you observe at the position g.37053568?

Hover over a read to learn more information about it.

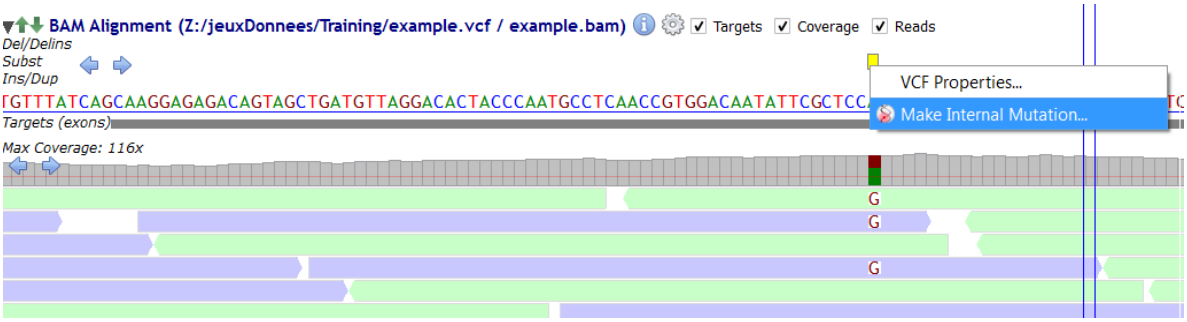
- What is the depth of coverage at the position g.37053568?
- What is the percentage of the reference allele observed at the position g.37053568?
- What is the percentage of the alternative allele observed at the position g.37053568?
- How many reads, aligned on the forward strand, hold the alternative allele?

Several display options about bases (show all or not) and reads (condense or squish views) are available when you right click on them.

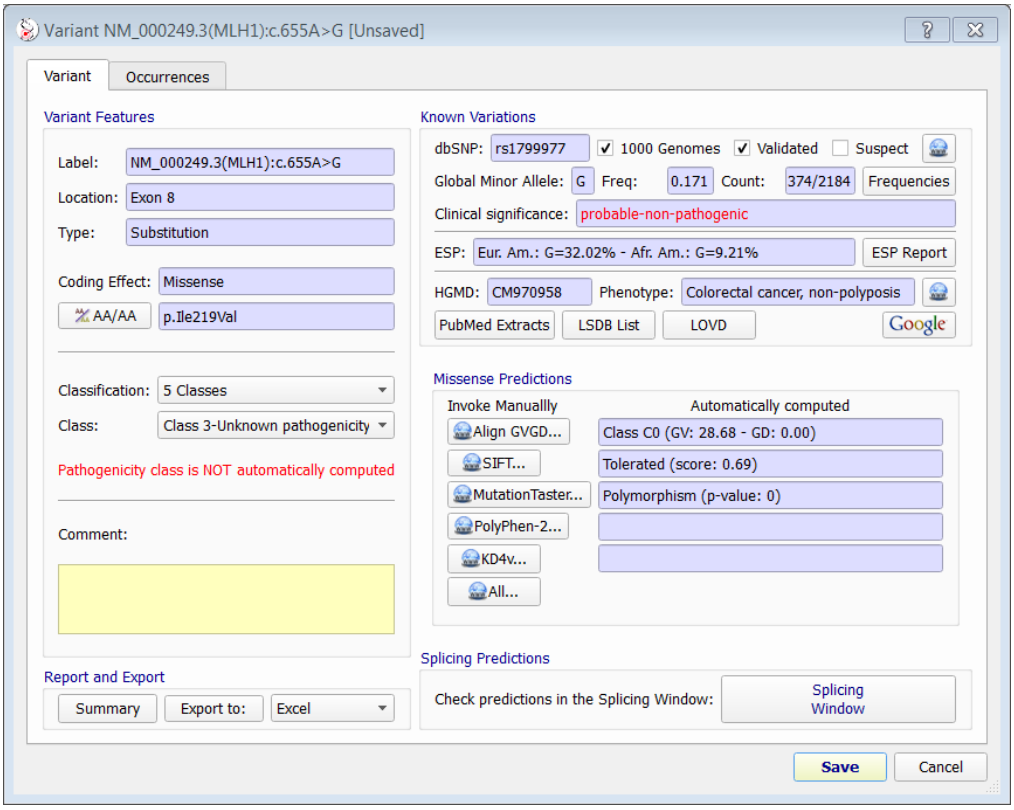


The next step is to interpret this observed variant.

- Left click to select the called variant from the VCF in the BAM track.
- Right click and then select “Make Internal Mutation…”



The “mutation interpretation” window appears.

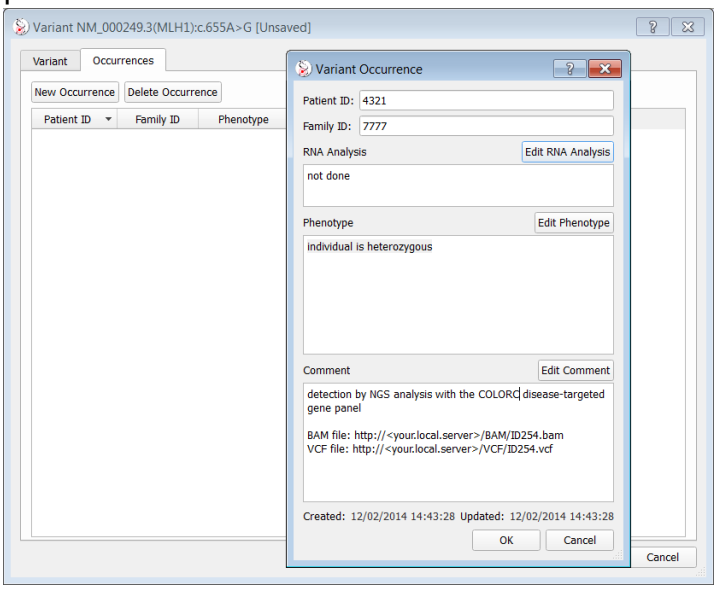


- Interpret the variant for this patient.

- Annotate the variant for this patient.

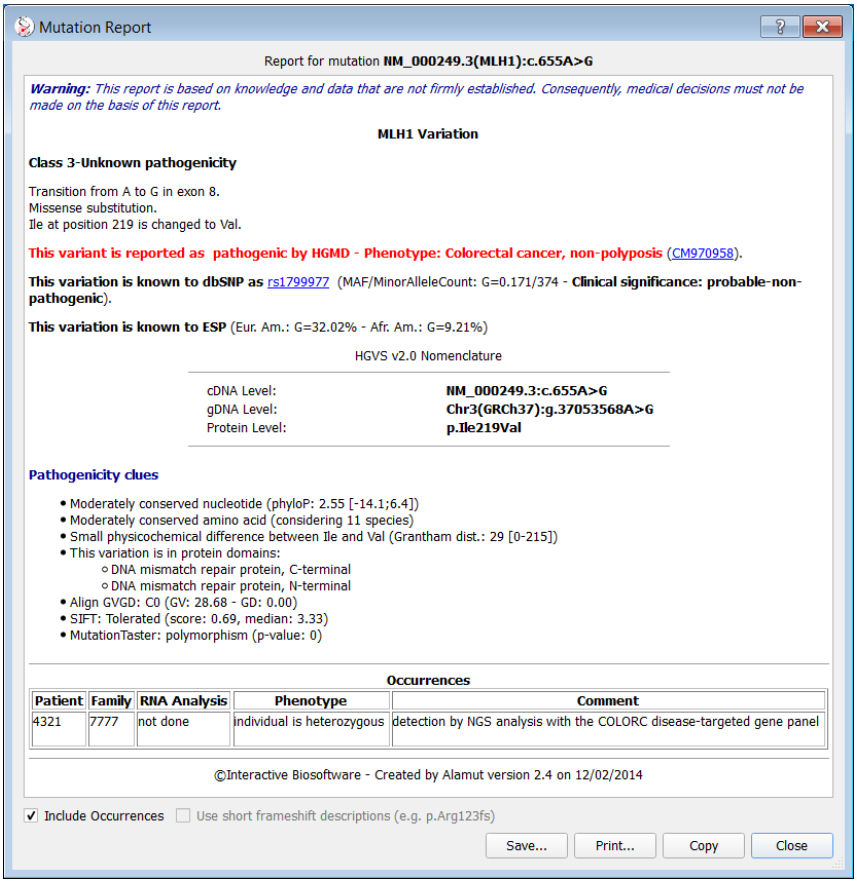
To do that:

- Click on the “Occurrences” then on “New Occurrence” and enter the following patient information:



- Click on “OK” to save the record.
- Click on “Variant” to go back to the “Mutation interpretation” window.

- Edit a summary report.



- Save your annotated variant in Alamut.