GULBENKIAN SCIENCE

PO21: Precision Oncology Course

Variant Prioritization - Exercise: Panel 2

BU CNIO BIOINFORMATICS UNIT

cnio stop cancer

Study Case: panel 2

Without normal sample

Tumor type: lung metastasis – gastrointestinal stromal tumor

Sequencing: Illumina HiSeq2500

Panel: Ion Ampliseq Comprehensive Cancer Panel (409 genes)

Reference Genome: HG19

VCF file with variants detected with MuTect2

OBJECTIVES:

1. Process the VCF file to obtain the set of annotations

2. Identify clinically relevant variants involved in the disease

3. Review variants in IGV

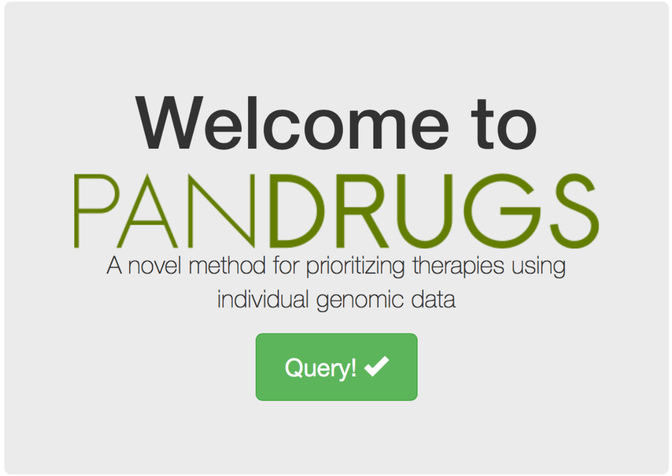
1. Process the vcf file

Remove variants without PASS label in FILTER

```
zcat panel2/out/mutect_filter/
tumor_passlable_filtered.vcf.gz | awk –F '\t' '{if($0 ~
/#/) print; else if($7 == "PASS") print}' > panel2/out/
tumor_passlable_filtered_onlyPASS.vcf
```

How to obtain the extended set of annotations in cancer disease

https://www.pandrugs.org/



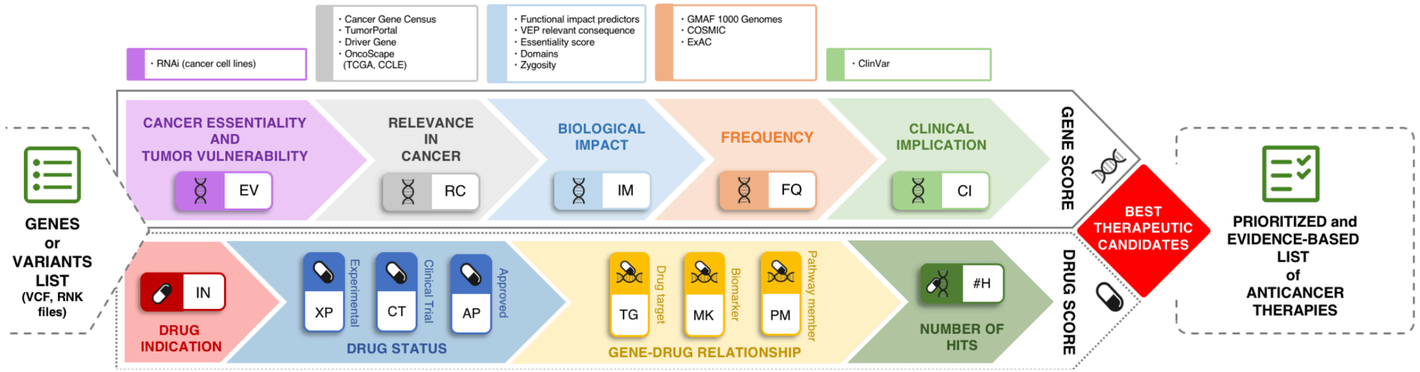
What is PanDrugs?

PanDrugs provides a bioinformatics platform **to prioritize anticancer drug treatments according to individual genomic data**. PanDrugs current version integrates data from **24** primary sources and supports **56297** drug-target associations obtained from **4804** genes and **9092** unique compounds.

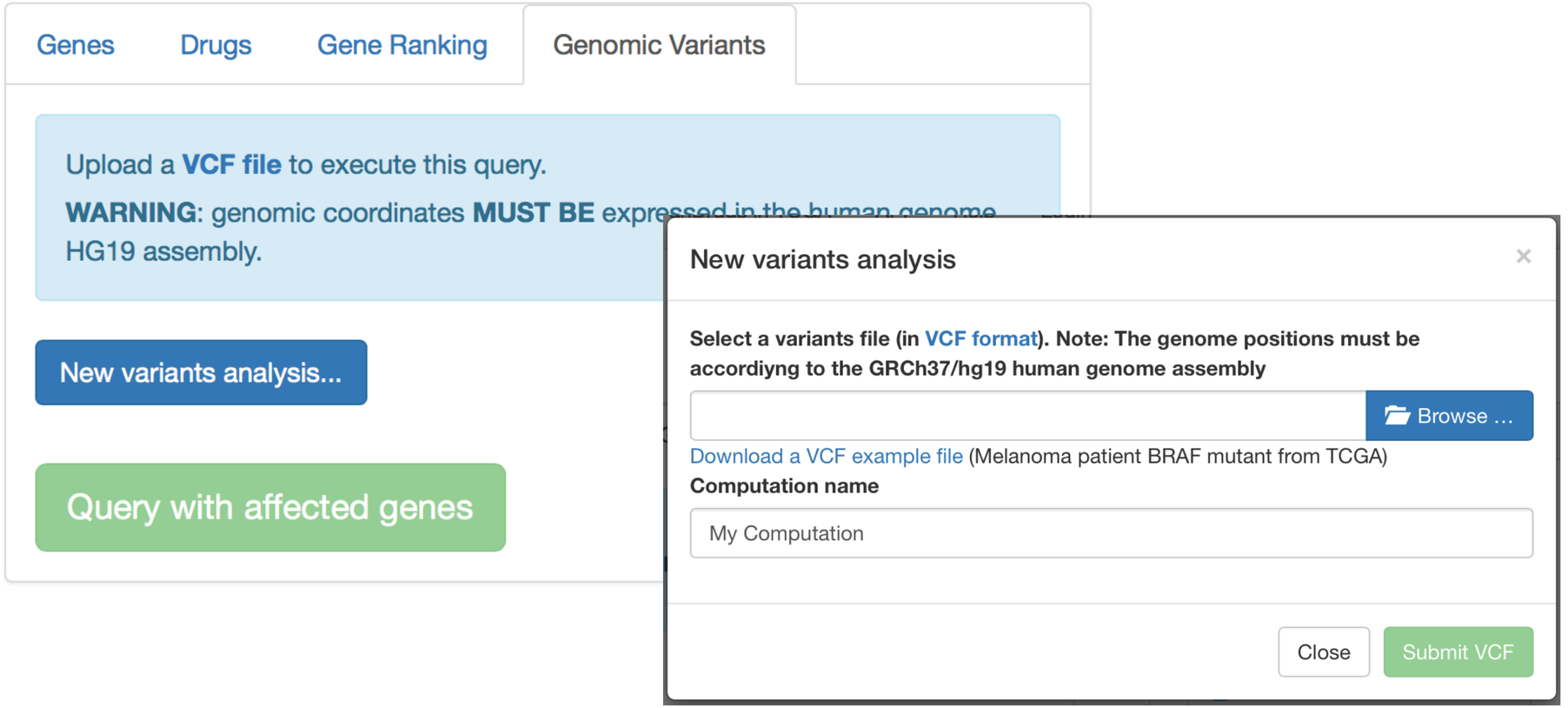
Data input: standard **VCF** file, **RNK** file, **gene lists** and **drug query**.

Please note the PanDrugs terminology for druggable genes:

I. **Direct targets**: Genes that contribute to disease phenotype and can be directly targeted by a drug (e.g. BRAF is a direct target for vemurafenib).
II. **Biomarkers**: Genes showing a genetic status associated with drug response which protein product is not the drug target itself (e.g. BRCA-mutated cancers responding to PARP inhibitors).
III. **Pathway members**: Genes located downstream in the biological pathway of a given undruggable gene (e.g. patients with mutations in TSC1/2 respond to a downstream inhibition of the mTOR pathway).

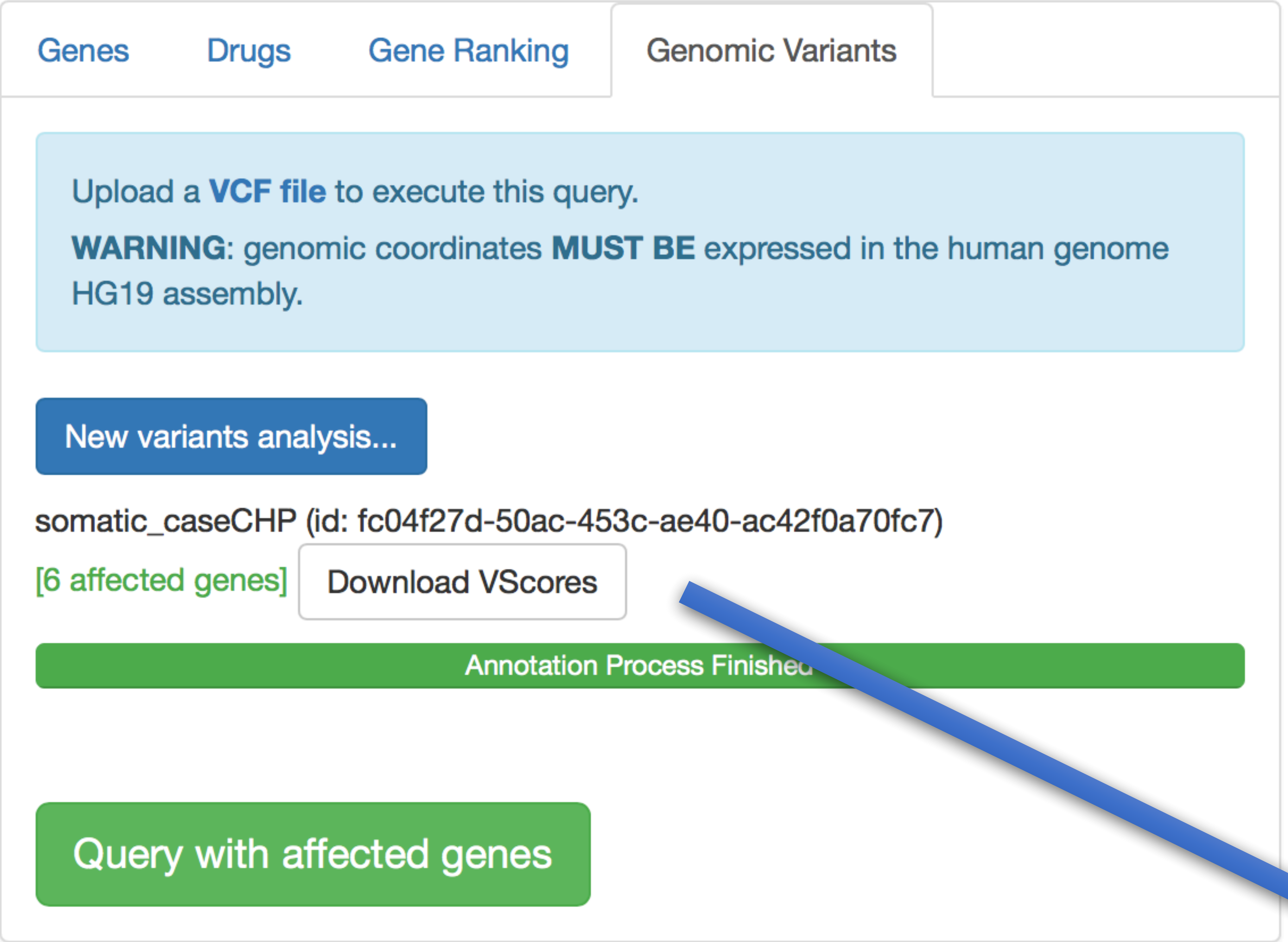


Query PanDrugs



VEP annotations → KEGG, ClinVar, APPRIS, Pfam, Interpro, Uniprot ID + Bibliography → Cancer disease specific annotations → Score computation

Query PanDrugs



Chr	Loc	ID	REF	ALT	QUAL	FILTER	INFO
2	29432629		G	-	11505.73	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=-15.167;DP=1549;FS=171.142;MQ0=0;MQRankSum=1.542;QD=7.46;RPA=4,3;RU=G;ReadPosRankSum=-
2	29432629		G	-	11505.73	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=-15.167;DP=1549;FS=171.142;MQ0=0;MQRankSum=1.542;QD=7.46;RPA=4,3;RU=G;ReadPosRankSum=-
3	178952085	rs121913279	A	G	28044.77	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=-20.123;DB;DP=1959;Dels=0.00;FS=0.000;HaplotypeScore=632.2851;MQRankSum=1.129;QD=14.32;Read
3	178952085	rs121913279	A	G	28044.77	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=-20.123;DB;DP=1959;Dels=0.00;FS=0.000;HaplotypeScore=632.2851;MQRankSum=1.129;QD=14.32;Read
4	153245446		G	A	16147.77	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=18.653;DP=2800;Dels=0.00;FS=0.000;HaplotypeScore=196.2637;MQ0=0;MQRankSum=0.422;QD=5.77;Rea
4	153245446		G	A	16147.77	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=18.653;DP=2800;Dels=0.00;FS=0.000;HaplotypeScore=196.2637;MQ0=0;MQRankSum=0.422;QD=5.77;Rea
4	153245446		G	A	16147.77	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=18.653;DP=2800;Dels=0.00;FS=0.000;HaplotypeScore=196.2637;MQ0=0;MQRankSum=0.422;QD=5.77;Rea
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5	112175423	rs121913329	C	T	37006.77	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=-37.564;DB;DP=2583;Dels=0.01;FS=1.821;HaplotypeScore=207.0235;MQRankSum=-1.046;QD=14.34;Reac
5	112175423	rs121913329	C	T	37006.77	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=-37.564;DB;DP=2583;Dels=0.01;FS=1.821;HaplotypeScore=207.0235;MQRankSum=-1.046;QD=14.34;Reac
5	112175423	rs121913329	C	T	37006.77	PASS	AC=1;AF=0.500;AN=2;BaseQRankSum=-37.564;DB;DP=2583;Dels=0.01;FS=1.821;HaplotypeScore=207.0235;MQRankSum=-1.046;QD=14.34;Reac
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Script https://fundacioncnio-my.sharepoint.com/:u:/g/personal/epineiro_cnio_es/EVLrvbHfn0xDilL9iOymJqgBxJoQcQA-MET8cMGPkhbpYg?e=FpXwh7

python SummaryGenerator.py <INPUT FILE> <OUTPUT FILE>

- Column selection
- Selection of principal transcripts (if PRINCIPAL label)

Script created to work with the specific data used in the exercise

Selection of principal isoform:

PRINCIPAL:1 - Transcript(s) expected to code for the main functional isoform based solely on the core modules in the APPRIS database
PRINCIPAL:2 - Where the APPRIS core modules are unable to choose a clear principal variant (approximately 25% of human protein coding genes), the database chooses two or more of the CDS variants as "candidates" to be the principal variant
PRINCIPAL:3 - Where the APPRIS core modules are unable to choose a clear principal variant and more than one of the variants have distinct CCDS identifiers, APPRIS selects the variant with lowest CCDS identifier as the principal variant
PRINCIPAL:4 - Where the APPRIS core modules are unable to choose a clear principal CDS and there is more than one variant with a distinct (but consecutive) CCDS identifiers, APPRIS selects the longest CCDS isoform as the principal variant
PRINCIPAL:5 - Where the APPRIS core modules are unable to choose a clear principal variant and none of the candidate variants are annotated by CCDS, APPRIS selects the longest of the candidate isoforms as the principal variant
REST (ALTERNATIVE:1 (Candidate transcript(s) models that are conserved in at least three tested non-primate species), ALTERNATIVE:2 (Candidate transcript(s) models that appear to be conserved in fewer than three tested non-primate species), NO LABEL (Non-candidate transcripts are not flagged and are considered as "MINOR" transcripts))

Reduced to relevant isoforms if PRINCIPAL

2. Identify clinically relevant variants

1. Identify benign variants (such as those common in the population)
2. Find the variants with more evidences of clinical relevance
3. Check in IGV that relevant variants are well supported

You can set tiers according to the number of evidences that support the clinical relevance

Column legend in the file

chr	Chromosome
loc	Variant genomic position (hg19)
mut	Reference allele/Variant allele
gene_hgnc	Gene official name
tumorportal	tumor types where the gene is significantly mutated as indicated in TumorPortal
role_driver	oncogene or tumor suppressor gene as indicated in Cancer Genome Census (CGC) and OncodriveROLE and driver gene as referenced in Nature, 2013. Tamborero D, et al.
gene	Ensembl Gene ID
feature	Ensembl TranscrIpt ID
consequence	Variant effect on the sequence
functional_impact_prediction_(PolyPhen/SIFT/CONDEL)	Predicitve functional effect based on different algorithms
cosmic_id	COSMIC id (if any)
cosmic_vep_id	COSMIC id (if any)
kegg_data	KEGG pathway
protein_position	Variant position on protein sequence
amino_acids	aminoacid affected by the variant
dbSNP	dbSNP id (if any)
clinvar_acc	ClinVar id (if any), semicolon separated
clinvar_disease	Associated conditions (in same order as ClinVar_acc)
clinvar_clinical_significance	Clinical significance (in same order as ClinVar_acc)
variation_type	Type of mutation
HGVS_cDNA	HGVS DNA
HGVS_protein	HGVS protein
GMAF	Global minor allele frequency (MAF) in 1000 Genomes Project: dbSNP is reporting the minor allele frequency for each rs included in a default global population
GMAF_freq	GMAF frequency in percentage in 1000 Genomes Project (<1% is considered rare variant)
gnomAD	Global Allele Frequency for the variant in gnomAD (exome data) in percentage
gnomAD_NEF	Allele Frequency for the variant in Non-Finnish European population in gnomAD (exome data) in percentage
pfam	PFAM domain affected
uniprot	Uniprot bibliographic references for the variant
interpro	Interpro domain affected
gene_cosmic_freq	Gene frequency in COSMIC
mut_cosmic_freq	Variant frequency in COSMIC
vscore	CNIO score based on relevance (0-1): 1 max and 0 min