

Variant annotation

VARIANT and HPG-VARIANT

University of Cambridge

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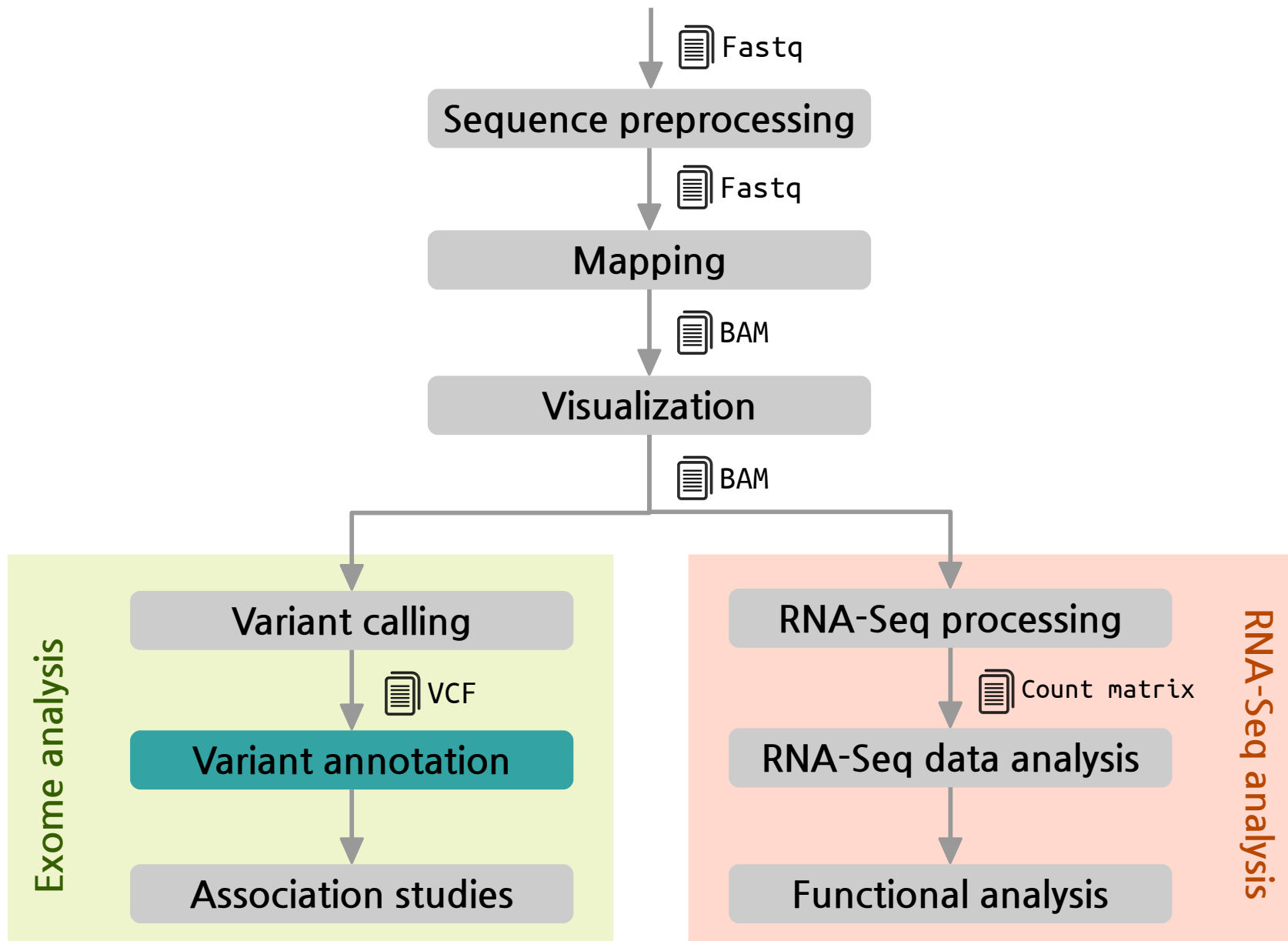
mbleda@cipf.es

PhD Student at the Computational Genomics Institute

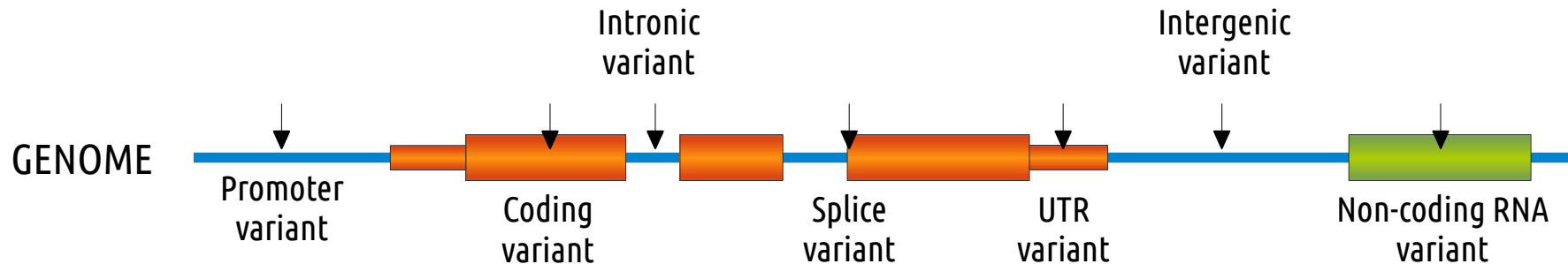
Centro de Investigación Príncipe Felipe (CIPF)

Valencia, Spain

The pipeline



What is functional annotation?



Why we do that?

- ▶ Each individual exome carries ~25,000 variants → **PRIORITIZATION!**
- ▶ We want to identify a **small subset** of functionally important variants to pinpoint the putative disease causal variants
- ▶ We need strategies to **estimate the deleteriousness** of our variants to better identify disease-causal variants

CAUTION!

On average, each *normal* person is found to carry:

~11,000 **synonymous** variants

~11,000 **non-synonymous** variants

250 to 300 **los-of-function** variants in annotated genes

50 to 100 variants previously implicated in **inherited disorders**

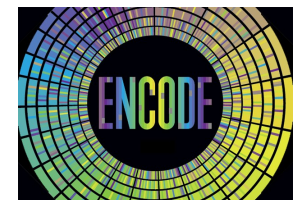
1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. **Nature**. 2010 Oct 28;467(7319):1061-73. PubMed PMID: 20981092

Sources of functional information

Table 1 Publicly available tools and databases for various tasks of genetic variant annotation and prioritization

Category	Database/tool/project	Description	URL
Genetic variant data sources	dbSNP ⁶⁸	Comprehensive, curated SNP and short indel database	http://www.ncbi.nlm.nih.gov/projects/SNP
	DbVar ⁶⁹	Comprehensive, curated database for structural variants	http://www.ncbi.nlm.nih.gov/dbvar
	DGV ⁷⁰	Human structural variants from samples with no phenotype	http://projects.tcag.ca/variation
Functional characterization of genomic elements	ENCODE ⁷¹	High-throughput functional characterization of DNA elements, including noncoding regions	http://www.genome.gov/10005107
	SIFT ⁷² , PolyPhen ⁷³	Prioritization of nonsynonymous SNPs	http://sift.jcvi.org , http://genetics.bwh.harvard.edu/pph2
Public gene–trait associations	dbGaP ³⁴	Comprehensive listing of genotype-to-phenotype mappings	http://www.ncbi.nlm.nih.gov/gap
	EGA ⁷⁴	Genotype–phenotype experiment archive	http://www.ebi.ac.uk/ega
Disease-associated mutations	HGMD ³⁵	Database for human disease mutations	http://www.hgmd.org
	OMIM ³⁶	Mendelian disease gene associations	http://www.ncbi.nlm.nih.gov/omim
	SwissVar ⁷⁶	Variant catalog of the UniProt knowledge bases	http://swissvar.expasy.org
	GAD ⁷⁷	NCBI source for genotype–disease associations	http://geneticassociationdb.nih.gov
	GWAS catalog from NHGRI ⁷⁸	SNP-phenotype associations found by GWAS	http://www.genome.gov/gwastudies
Whole-genome repositories	Complete genomics public genomes ⁷⁹	Complete genomics for 69 genomes from multiple ancestries (includes samples from the NHGRI and NIGMS repositories)	http://www.completegenomics.com/sequence-data/download-data
	1,000 Genomes ⁸⁰	Expanding resource currently housing three low-coverage whole genomes of multiple ancestries	http://www.1000genomes.org
Ancestry-focused variant data sources	HapMap ²⁶	Haplo-block mapping for diverse populations	http://www.hapmap.org
	HGDP ²⁷	SNP profiles of samples from several endogenous populations	http://hagsc.org/hgdp
Pharmacogenomic associations and data sources	PharmGKB ⁵⁶	Variant–pharmacokinetic/pharmacodynamic trait associations and gene–drug interactions	http://www.pharmgkb.org
	DrugBank ⁸¹	Drug-target database with biochemical properties	http://drugbank.ca

e!Ensembl



Cordero P, Ashley EA. Whole-genome sequencing in personalized therapeutics. *Clin Pharmacol Ther.* 2012 Jun ;91(6):1001-9. PubMed PMID: 22549284

Computational method and tools

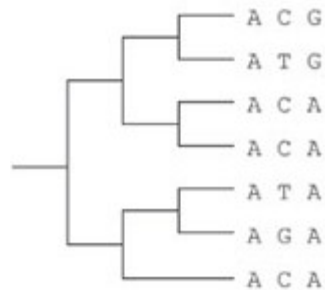
- ▶ **Annotated information** is sometimes **limited**, particularly for rare and complex traits
- ▶ Computational methods can measure deleteriousness by using **comparative genomics** and knowledge of **protein biochemistry and structure**

Comparative Genomics

Focus on sequences that have not been removed by **natural selection**.

Quantify evolutionary changes in genes or genomes and define conserved and neutral regions.

Variants observed in conserved sites are highly likely to be **deleterious**.



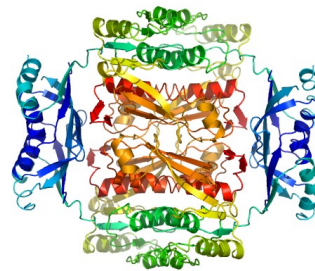
Effects in protein-coding variants

Can combine **evolutionary** and **biochemical** information.

Two types of approaches: first **principles** approaches and **trained** approaches.

Use **alignments of homologous proteins** to estimate mutational deleteriousness.

Use **biochemical data** such as amino acid properties, binding information and structural information to estimate the impact.



Effects in non-coding variants

The majority of the human genetic variation is in non-coding regions.

No detectable conservation outside vertebrates.

Main strategy for estimation is testing the **mammalian conservation** of the non-coding variants.

Cooper GM, Shendure J. *Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data.* **Nature Reviews Genetics.** 2011 Aug 18;12(9):628-40. Pubmed PMID: 21850043

Computational methods and tools

Prediction scores for non-synonymous variants

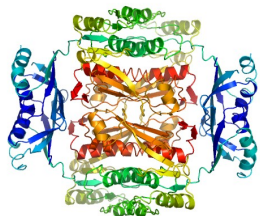
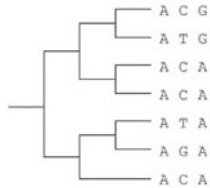


Table 1 | **Tools for protein-sequence-based prediction of deleteriousness**

Name	Type	Information	URL	Refs
MAPP	Constraint-based predictor	Evolutionary and biochemical	http://mendel.stanford.edu/SidowLab/downloads/MAPP/index.html	27
SIFT	Constraint-based predictor	Evolutionary and biochemical (indirect)	http://sift.bii.a-star.edu.sg/	39
PANTHER	Constraint-based predictor	Evolutionary and biochemical (indirect)	http://www.pantherdb.org/	41
MutationTaster*	Trained classifier	Evolutionary, biochemical and structural	http://www.mutationtaster.org/	40
nsSNP Analyzer	Trained classifier	Evolutionary, biochemical and structural	http://snpanalyzer.uthsc.edu/	44
PMUT	Trained classifier	Evolutionary, biochemical and structural	http://mmb2.pcb.ub.es:8080/PMut/	38
polyPhen	Trained classifier	Evolutionary, biochemical and structural	http://genetics.bwh.harvard.edu/pph2/	35
SAPRED	Trained classifier	Evolutionary, biochemical and structural	http://sapred.cbi.pku.edu.cn/	42
SNAP	Trained classifier	Evolutionary, biochemical and structural	http://www.rostlab.org/services/SNAP/	36
SNPs3D	Trained classifier	Evolutionary, biochemical and structural	http://www.snps3d.org/	51
PhD-SNP	Trained classifier	Evolutionary and biochemical (indirect)	http://gpcr2.biocomp.unibo.it/~emidio/PhD-SNP/PhD-SNP_Help.html	37

*Also makes predictions for synonymous and non-coding variant effects: for example, splicing. MAPP, Multivariate Analysis of Protein Polymorphism; polyPhen, polymorphism phenotyping.

Cooper GM, Shendure J. *Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data.* **Nature Reviews Genetics.** 2011 Aug 18;12(9):628-40. Pubmed PMID: 21850043

Computational methods and tools

Prediction scores for non-coding variation

Table 2 | **Tools for nucleotide-sequence-based prediction of deleteriousness**

Name	Type	Information	URL	Refs
phastCons	Phylogenetic HMM	Evolutionary	http://compgen.bscb.cornell.edu/phast/	60
GERP	Single-site scoring	Evolutionary	http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html	67
Gumby	Single-site scoring	Evolutionary	http://pga.jgi-psf.org/gumby/	21
phyloP	Single-site scoring	Evolutionary	http://compgen.bscb.cornell.edu/phast/	66
SCONE	Single-site scoring	Evolutionary	http://genetics.bwh.harvard.edu/scone/	68
binCons	Sliding-window scoring	Evolutionary	http://zoo.nhgri.nih.gov/binCons/index.cgi	69
Chai Cons	Sliding-window scoring	Evolutionary and structural	http://research.nhgri.nih.gov/software/chai	71
VISTA	Visualization tool (various scores)	Evolutionary	http://genome.lbl.gov/vista/index.shtml	70

GERP, Genomic Evolutionary Rate Profiling; HMM, hidden Markov model; SCONE, Sequence Conservation Evaluation.

Cooper GM, Shendure J. *Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data.* **Nature Reviews Genetics.** 2011 Aug 18;12(9):628-40. Pubmed PMID: 21850043

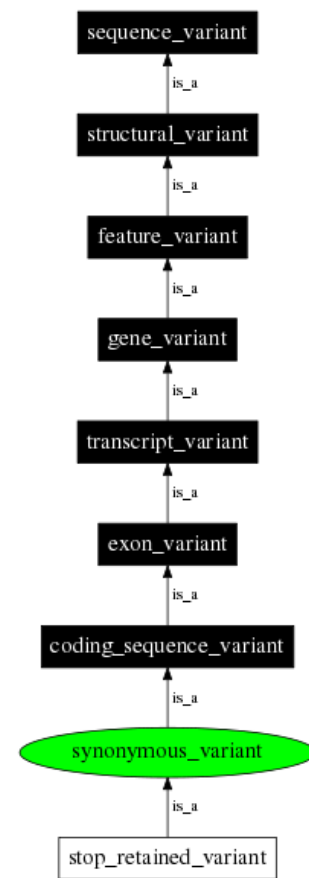
Consequence types

The standard:



Common vocabulary

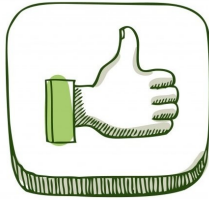
Label	SO accession	Description
Coding sequence	SO:0001580	In coding sequence with in determinate effect
Synonymous codon	SO:0001588	In coding sequence, not resulting in an amino acid change (silent mutation)
Non-synonymous codon	SO:0001583	In coding sequence and results in an amino acid change in the encoded peptide sequence
Stop gained	SO:0001587	In coding sequence, resulting in the gain of a stop codon
Stop lost	SO:0001587	In coding sequence, resulting in the gain of a stop codon
Splice site	SO:0001630	1-3bps in to an exon or 3-8bps into an intron
Splice acceptor	SO:0001574	A splice variant that changes the 2 base region at the 3' end of an intron
Splice donor	SO:0001575	A splice variant that changes the 2 base region at the 5' end of an intron
5' UTR	SO:0001623	In 5 prime untranslated region
3' UTR	SO:0001624	In 3 prime untranslated region
Upstream	SO:0001635	Within 5kb upstream of the 5 prime end of a transcript
Downstream	SO:0001633	Within 5kb downstream of the 3 prime end of a transcript
TFBS	SO:0001782	A sequence variant located with in a transcription factor binding site
miRNA target	SO:0000934	A binding site where the molecule is a microRNA
Intergenic	SO:0001628	More than 5 kb either upstream or downstream of a transcript



More information: http://www.ensembl.org/info/docs/variation/predicted_data.html

AnnoVar

ANNOVAR web site: <http://www.openbioinformatics.org/annovar/>



- Free and open source
- Can annotate SNV, insertions and deletions
- **Regulatory information:** Conserved genomic regions, TFBSs, miRNA targets and predicted miRNA secondary structures, ENCODE DNase I hypersensitive sites, Histone methylations, ChIP and RNA-Seq peaks
- DbSNP, 1000 genomes SIFT and GERP filtering
- **Predictions:** Polyphen, LRT, MutationTaster, PhyloP
- Can handle **custom annotations** in GFF3
- Can handle 1 o 0-based coordinates
- **5 Species** (human, mouse, worm, fly, yeast)



- Accepts VCF4, GFF3-SOLiD and CSV BUT after conversion to their **particular input file**:

Chr	Start	End	Ref	Obs	Comments
1	161003	161003	C	T	comments: rs1000050

- **Perl** written program
- **Installation** required
- Users need to **download** every annotation database and save them locally (~35GB per assembly)
- Need to be **run several times**
- **Output:** several files depending on the query
- Does not use **Sequence Ontology** terms

Wang K, Li M, Hakonarson H. *ANNOVAR: Functional annotation of genetic variants from next-generation sequencing data*. **Nucleic Acids Research**. Sep;38(16):e164 Pubmed PMID: 20601685

AnnoVar

EXAMPLE of ANNOVAR usage

DOWNLOADING BIOLOGICAL DATA:

```
user@computer:~$ annotate_variation.pl -buildver hg19 -downdb refgene humandb/
user@computer:~$ annotate_variation.pl -buildver hg19 -downdb snp135 -webfrom annovar humandb/
user@computer:~$ annotate_variation.pl -buildver hg19 -downdb phastConsElements46way humandb/
user@computer:~$ annotate_variation.pl -buildver hg19 -downdb 1000g2012apr -webfrom annovar humandb/
user@computer:~$ annotate_variation.pl -buildver hg19 -downdb cytoBand humandb/
```

EXTRACTING THE EFFECT:

```
user@computer:~$ annotate_variation.pl -geneanno example/ex1.human humandb/
user@computer:~$ annotate_variation.pl -regionanno -dbtype band example/ex1.human humandb/
user@computer:~$ annotate_variation.pl -filter -dbtype 1000g2012apr_eur example/ex1.human humandb/
```

Variant Effect Predictor (VEP)

VEP documentation site: <http://www.ensembl.org/info/docs/variation/vep/index.html>



- Backed by **Ensembl**
- Free and open source
- **3 ways of functionality**: web interface, standalone Perl script and Ensembl's Perl API
- **Input** formats: CSV, VCF, Pileup and HGVS
- **Regulatory information**: TFBSs
- **Filtering** by coding regions and MAF
- **Predictions**: SIPF, PolyPhen
- 1000 genomes and dbSNP information
- Uses **Sequence Ontology**
- **Many species**



- Regulatory information does **not include miRNA targets**
- Web interface limit: **750 variants**
- The **standalone Perl script** needs:
 - **Perl** and **MySQL** support (more than 100GB of data)
 - **Download, install** and **update** every ~ 2 months
- Perl **API** requires:
 - **Installation** (Really, really hard!)
 - **Downloads** and **update**
 - API documentation → **Hard to understand**

McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. *Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor*. **BMC Bioinformatics** 26(16):2069-70(2010) Pubmed PMID: 20562413

Variant Effect Predictor (VEP)

VEP web interface: <http://www.ensembl.org/tools.html>

The screenshot shows the VEP web interface. On the left is a sidebar with a 'Custom Data' tab and a list of tools: 'Add your data', 'Attach DAS', 'Manage Data', 'Features on Karyotype', 'Data Converters' (with sub-items 'Assembly Converter', 'ID History Converter', 'Variant Effect Predictor', and 'Region Report'), and 'Region Report'. The main content area is titled 'Variant Effect Predictor:' and contains a description of the tool, a warning about the 750 variant limit, and a note about Sequence Ontology terms. Below this is the 'Input file' section with fields for 'Species' (set to 'Human (Homo sapiens): GRCh'), 'Name for this data (optional)', and 'Paste data:' (containing two lines of variant data: '1 881907 881906 -/C +' and '5 140532 140532 T/C +'). There are also fields for 'Upload file:' (with a 'Choose File' button), 'or provide file URL:', 'Input file format:' (set to 'Ensembl default'), and 'Options' (with radio buttons for 'Ensembl transcripts' and 'RefSeq and other transcripts').

The web interface to the VEP **has a hard limit of 750 variants in your uploaded file**. However, it is possible that the tool will not work with fewer variants than this, depending on the content of your data and the features you switch on. For example, a relatively small file (e.g. 100 variants) **may fail to return results if every variant in the file falls in a different gene and those genes are spread across many chromosomes**. Contrastingly, a file containing 500 variants may return results quickly if those variants all fall in just a few genes.

To mitigate this issue, users should consider **splitting up their input by chromosome** and uploading each chromosome's variants as a separate file. The problem can also be solved by using the VEP script - it is a command line tool, but not as hard to use as you might think! It also offers many more features than the web interface and is a generally much more powerful tool.

<http://www.ensembl.org/info/docs/variation/vep/index.html>

McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. *Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor*. BMC Bioinformatics 26(16):2069-70(2010) Pubmed PMID: 20562413

Variant Effect Predictor (VEP)

EXAMPLE of API usage: Getting all variations in a particular human transcript and see what is the effect of that variation in the transcript

```
1 use strict;
2 use warnings;
3 use Bio::Ensembl::Registry;
4
5 my $registry = 'Bio::Ensembl::Registry';
6
7 $registry->load_registry_from_db(
8     -host => 'ensembl.org',
9     -user => 'anonymous'
10 );
11
12 my $stable_id = 'ENST00000393489'; #this is the stable_id of a human transcript
13 my $transcript_adaptor = $registry->get_adaptor('homo_sapiens', 'core', 'transcript'); #get the adaptor to get the Transcript from the database
14 my $transcript = $transcript_adaptor->fetch_by_stable_id($stable_id); #get the Transcript object
15
16 my $trv_adaptor = $registry->get_adaptor('homo_sapiens', 'variation', 'transcriptvariation'); #get the adaptor to get TranscriptVariation objects
17 my $trvs = $trv_adaptor->fetch_all_by_Transcripts([$transcript]); #get ALL effects of Variations in the Transcript
18
19 foreach my $tv (@{$trvs}) {
20     my $tvas = $tv->get_all_alternate_TranscriptVariationAlleles();
21
22     foreach my $tva(@{$tvas}) {
23         my @ensembl_consequences;
24         my @so_consequences;
25
26         my $ocs = $tva->get_all_OverlapConsequences();
27
28         foreach my $oc(@{$ocs}) {
29             push @ensembl_consequences, $oc->display_term;
30             push @so_consequences, $oc->SO_term;
31         }
32
33         my $sift = $tva->sift_prediction;
34         my $polyphen = $tva->polyphen_prediction;
35
36         print
37             "Variation ", $tv->variation_feature->variation_name,
38             " allele ", $tva->variation_feature_seq,
39             " has consequence ", join(", ", @ensembl_consequences),
40             " (SO ", join(", ", @so_consequences), ").";
41
42         if(defined($sift)) {
43             print " SIFT=$sift";
44         }
45         if(defined($polyphen)) {
46             print " PolyPhen=$polyphen";
47         }
48
49         print "\n";
50     }
51 }
```

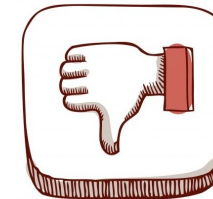
McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F. *Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor*. BMC Bioinformatics 26(16):2069-70(2010) Pubmed PMID: 20562413

HPG-VARIANT (aka VARIANT)

HPG-VARIANT web site: <http://www.opencb.org/projects/hpg/doku.php?id=variant:overview>



- Free and open source.
- **3 ways of functionality:** C CLI program, Web application and Java RESTful WS API
- **Cloud** variant annotator. Requires **no installation or updates**
- **Regulatory information:** Conserved genomic regions, TFBSs and miRNA targets. ENCODE DNase I hypersensitive sites and Histone methylations
- dbSNP and 1000genomes information
- **Phenotypic information:** HGMD, COSMIC and OMIM
- **Cross-link** with many other DDBB (Ensembl, UniProt, PDB, etc)
- Polyphen and SIFT
- **Input:** VCF, GFF and BED. Accepts compressed files in *tar.gz*
- **11 species** (human, mouse, rat, zebra fish, worm, fly, yeast, dog, pig, mosquito and plasmodium)
- HPG-VARIANT-GWAS to test for **association**



- **Young program, many new features coming**
 - PhastCons, GERP
 - Many more species (~25 new species)
 - Large structural variants annotation







Medina I, De Maria A, Bleda M, Salavert F, Alonso R, Gonzalez CY, Dopazo J. *VARIANT: Command Line, Web service and Web interface for fast and accurate functional characterization of variants found by Next-Generation Sequencing*. **Nucleic Acids Research**. 2012 Jul;40(Web Server issue):W54-8 Pubmed PMID: 22693211

HPG-VARIANT (CLI Program)

- **Download** the program and save it into your course/variant_annotation folder :

<http://wiki.opencb.org/projects/hpg/doku.php?id=variant:downloads>

- Extract the contents

	Binaries	Sources
Debian 6	 Binary package	
Ubuntu 12.04	 Binary package	
Fedora 17	 Binary package	 Source package
Other	 Zipped binaries *	 Source tarball

* Only for Debian 6 / Ubuntu 10.04 or greater

- Add the folder to your PATH

```
echo "export PATH=$PATH:/home/Desktop/hpg-variant-1.0" >> ~/.bashrc
```

```
source ~/.bashrc
```

- Usage:

```
hpg-var-effect -v CHB.exon.2010_03.sites.vcf --outdir effect_output/
```

HPG-VARIANT

Web application

► Web application

<http://variant.bioinfo.cipf.es/>

The screenshot displays the HPG-VARIANT web application interface. At the top, a navigation bar includes a "sign in" button (highlighted with a red box) and links for "home", "help", "tutorial", "Projects", and "Upload data". Below this, a sub-header reads "Variant analysis tool" with links for "Variant effect" and "VCF Viewer". A "Home" button is also present. The main content area is titled "Overview" and describes the VARIANT tool's capabilities. A "Note" section provides browser compatibility information. The "Sign in" section explains the login process, mentioning "register" and "anonymous user" options. An inset image shows a "Sign in" dialog box with fields for "e-mail" and "password", a checked "Anonymous login" checkbox (highlighted with a red box), and buttons for "Sign in", "Forgot your password?", and "New account". The dialog also displays "Anonymous selected" and "No password required".

Variant analysis tool

home help tutorial Projects Upload data

Variant effect VCF Viewer

Home

Overview

VARIANT (VARIANT Analysis Tool) can report the functional properties of any variant in all the human, mouse or rat genes (and soon new model organisms will be added) and the corresponding neighborhoods. Also other non-coding extra-genic regions, such as miRNAs are included in the analysis.

VARIANT not only reports the obvious functional effects in the coding regions but also analyzes noncoding SNVs situated both within the gene and in the neighborhood that could affect different regulatory motifs, splicing signals, and other structural elements. These include: Jaspar regulatory motifs, miRNA targets, splice sites, exonic splicing silencers, calculations of selective pressures on the particular polymorphic positions, etc.

Note

This web application makes an intensive use of new web technologies and standards like HTML5, so browsers that are fully supported for this site are: Chrome 14+, Firefox 7+, Safari 5+ and Opera 11+. Older browser like Chrome13-, Firefox 5- or Internet Explorer 9 may rise some errors. Internet Explorer 6 and 7 are no supported at all.

Sign in

You must be logged in to use this Web application, you can **register** or use a **anonymous user** as shown in the following image by clicking on the "Sign in" button on the top bar

Other Bookmarks

sign in

home help tutorial

Upload data

Sign in

e-mail:

password:

☒ Anonymous login
Your work will be lost after logout session

Anonymous selected No password required

Sign in Forgot your password? New account

Variant analysis tool beta

logged in as ayuso Upload & Manage profile logout

home documentation tutorial about

Show jobs

Preprocess Analysis Visualization

Home RP-0859

Summary Variants and effect Genome Viewer

Filters

Reload Clear Search

Region +

Gene +

Stats +

Samples -

K529:

☒

☒

☐

0/0 0/1 1/1

D056:

☐

☐

☒

0/0 0/1 1/1

Controls +

Effect +

Variant Info

Variant	Alleles	Samples		SNP id	Controls (MAF)			Consq. Type	Polyphen	Sift	Conservation
		K529	D056		1000G	BIER	EVS				
gene_name: ACTR5 (1 Item)											
20:37396120	A>G	0/1	1/1	rs2245231	0.4231 (G)	0.4667 (G)	0.4416 (A)	exon_variant,non_synon...			
gene_name: ANKRD60 (1 Item)											
20:56807969	A>G	0/1	1/1	rs3818744	0.4785 (G)	0.4267 (A)	.	5KB_upstream_variant,e...			
gene_name: AURKA (1 Item)											
20:54961463	T>C	0/1	1/1	rs1047972	0.1557 (T)	0.2333 (T)	0.1622 (C)	exon_variant,non_synon...			
gene_name: BIRC7 (1 Item)											
20:61869826	C>T	0/1	1/1	rs2273487	0.4675 (T)	0.48 (C)	0.4343 (C)	exon_variant,DNAseI_h...			
26 variants											

Effect - 20:37396120 A>G

	Position chr:start:end	snp id	Samples	Consequence Type	Aminoacid Change	gene (EnsemblId)	transcript Id	feature Id	feature Name	feature Type	feature Biotype
featureId: ENSE00000844678 (3 Items)											
1	20:37396107-373...			exon_variant (SO:0001791)	.	ACTR5 (ENSG00...	ENST00000243903	ENSE00000844678	ACTR5	exon	protein_coding
2	20:37396107-373...			coding_sequence_variant (SO:000...	.	ACTR5 (ENSG00...	ENST00000243903	ENSE00000844678	ACTR5	exon	protein_coding
3	20:37396107-373...			non_synonymous_codon (SO:000...	IV - ATT/GTT (483)	ACTR5 (ENSG00...	ENST00000243903	ENSE00000844678	ACTR5	exon	protein_coding
featureId: H3K36me3 (18 Items)											
4	20:37377900-374...			regulatory_region_variant (SO:000...	.	.	.	H3K36me3	.	regulatory_region	.
5	20:37378100-374...			regulatory_region_variant (SO:000...	.	.	.	H3K36me3	.	regulatory_region	.
6	20:37378300-374...			regulatory_region_variant (SO:000...	.	.	.	H3K36me3	.	regulatory_region	.
7	20:37378450-374...			regulatory_region_variant (SO:000...	.	.	.	H3K36me3	.	regulatory_region	.
8	20:37382500-374...			regulatory_region_variant (SO:000...	.	.	.	H3K36me3	.	regulatory_region	.
9	20:37382550-374...			regulatory_region_variant (SO:000...	.	.	.	H3K36me3	.	regulatory_region	.
21 effects											

THANK YOU.