Variant annotation

ANNOVAR and CellBase

University of Cambridge
Cambridge, UK
18th June 2015

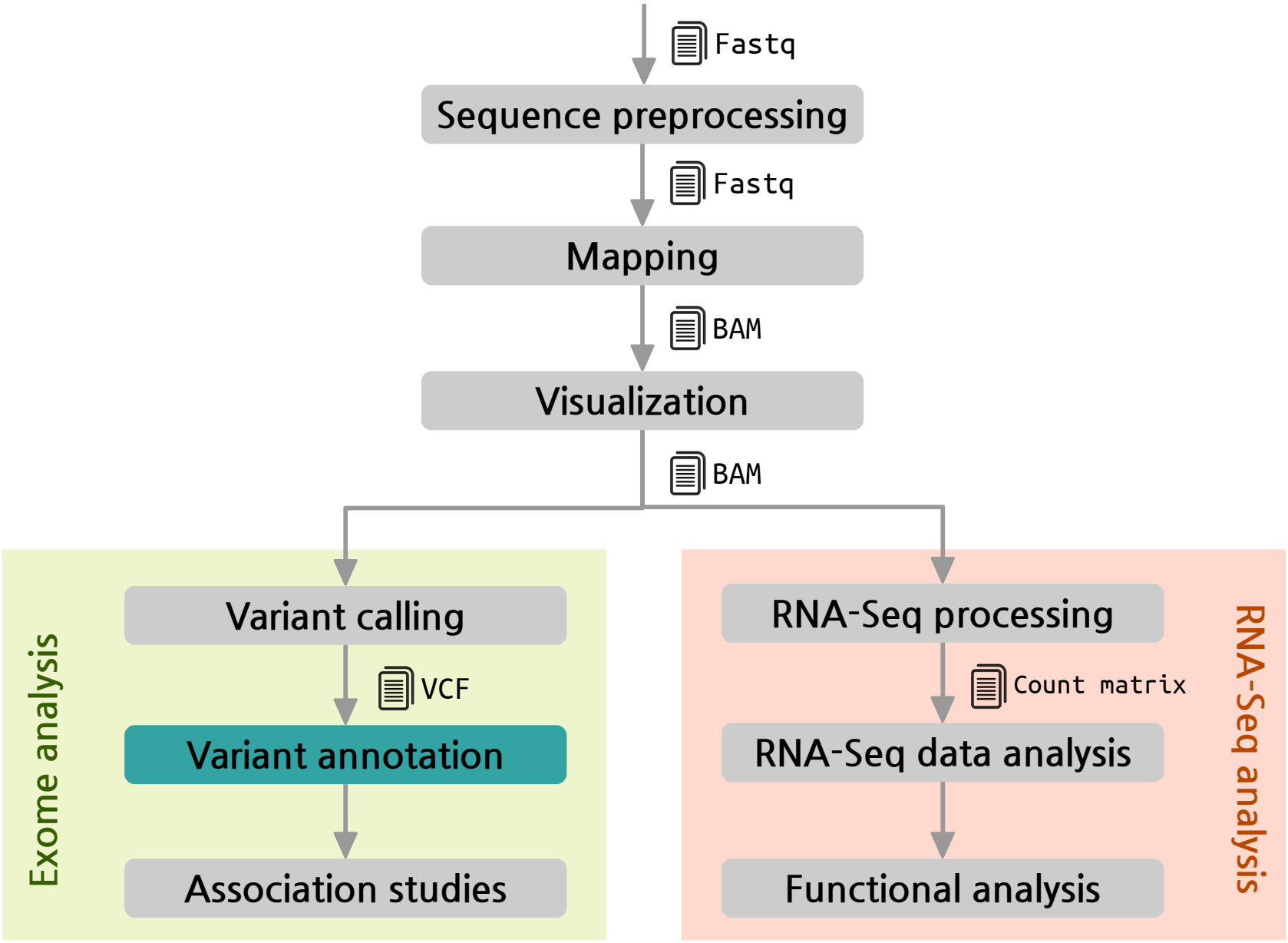
Javier López
fjlopez@ebi.ac.uk
EBML-European Bioinformatics Institute
Acknowledgements: Marta Bleda Latorre

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

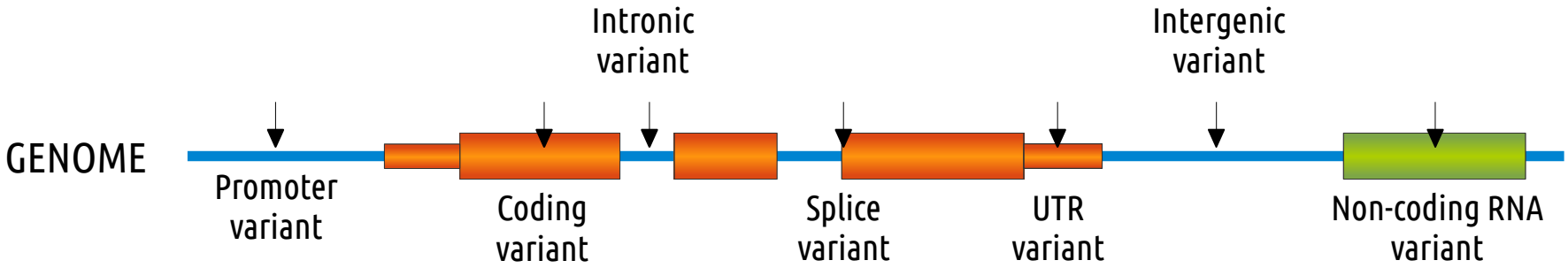
UNIVERSITY OF CAMBRIDGE

EMBL-EBI

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

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 remove 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.

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

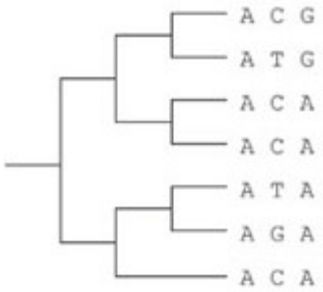
Use **biochemical data** such as amino acid properties, binding information and structural information to estimate the impact.

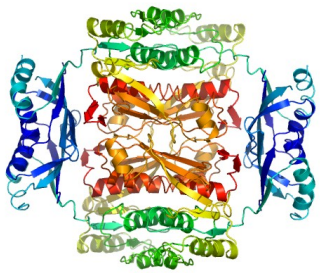
Effects in non-coding variants

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.

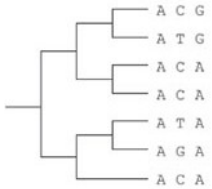




Cooper GM, Shendure J. *Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data.* **Nature Reviews Genetics**. 2011 Aug 18;12(9):628-40. Pubmed PMID: 21850043

Computational methods and tools

Prediction scores for non-synonymous variants



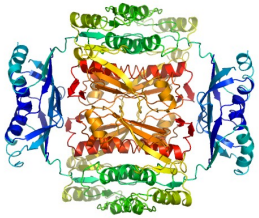


Table 1 | **Tools for protein-sequence-based prediction of deleteriousness**

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.

Kircher M. et al. *A general framework for estimating the relative pathogenicity of human genetic variants.* **Nature Genetics**. 2014; Pubmed PMID: 24487276.

Shihab H. A.. et al. *An integrative approach to predicting the functional effects of non-coding and coding sequence variation.* **Bioinformatics**. 2014; Pubmed PMID: 25583119.

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**

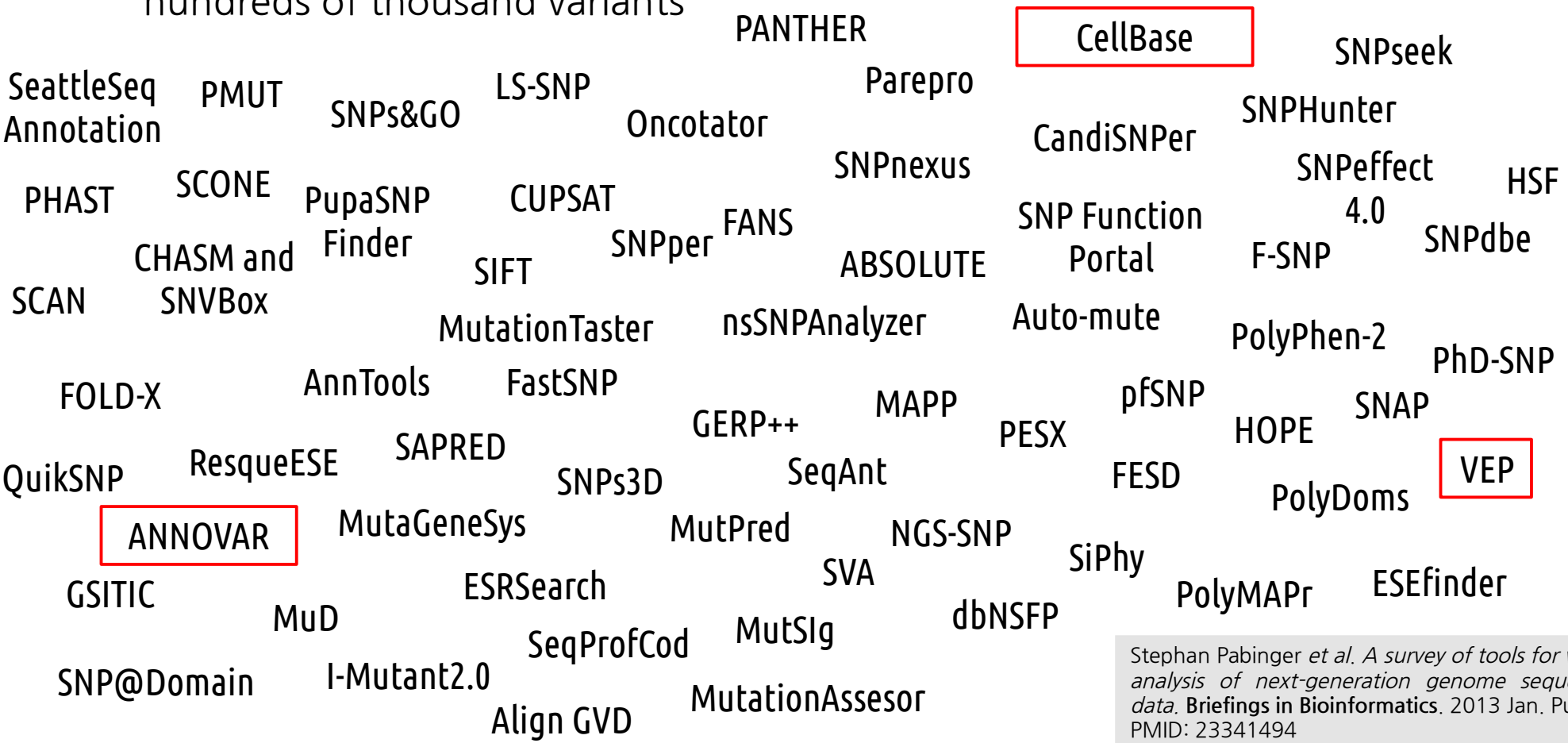
Name	Type	Information	URL	Refs
phastCons	Phylogenetic HMM	Evolutionary	http://compgen.bscb.cornell.edu/phast/	60
GERP	Single-site scoring	Evolutionary	http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html	67
Gumby	Single-site scoring	Evolutionary	http://pga.jgi-psf.org/gumby/	21
phyloP	Single-site 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

Tools for functional annotation

- We need to measure the **impact** of each variant in the genome
- We **cannot** annotate 25,000 variants **manually** checking more than 20 databases
- **Tools integrate** biological information and **ease** the functional annotation of hundreds of thousand variants



Stephan Pabinger *et al. A survey of tools for variant analysis of next-generation genome sequencing data.* **Briefings in Bioinformatics**. 2013 Jan. Pubmed PMID: 23341494

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

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

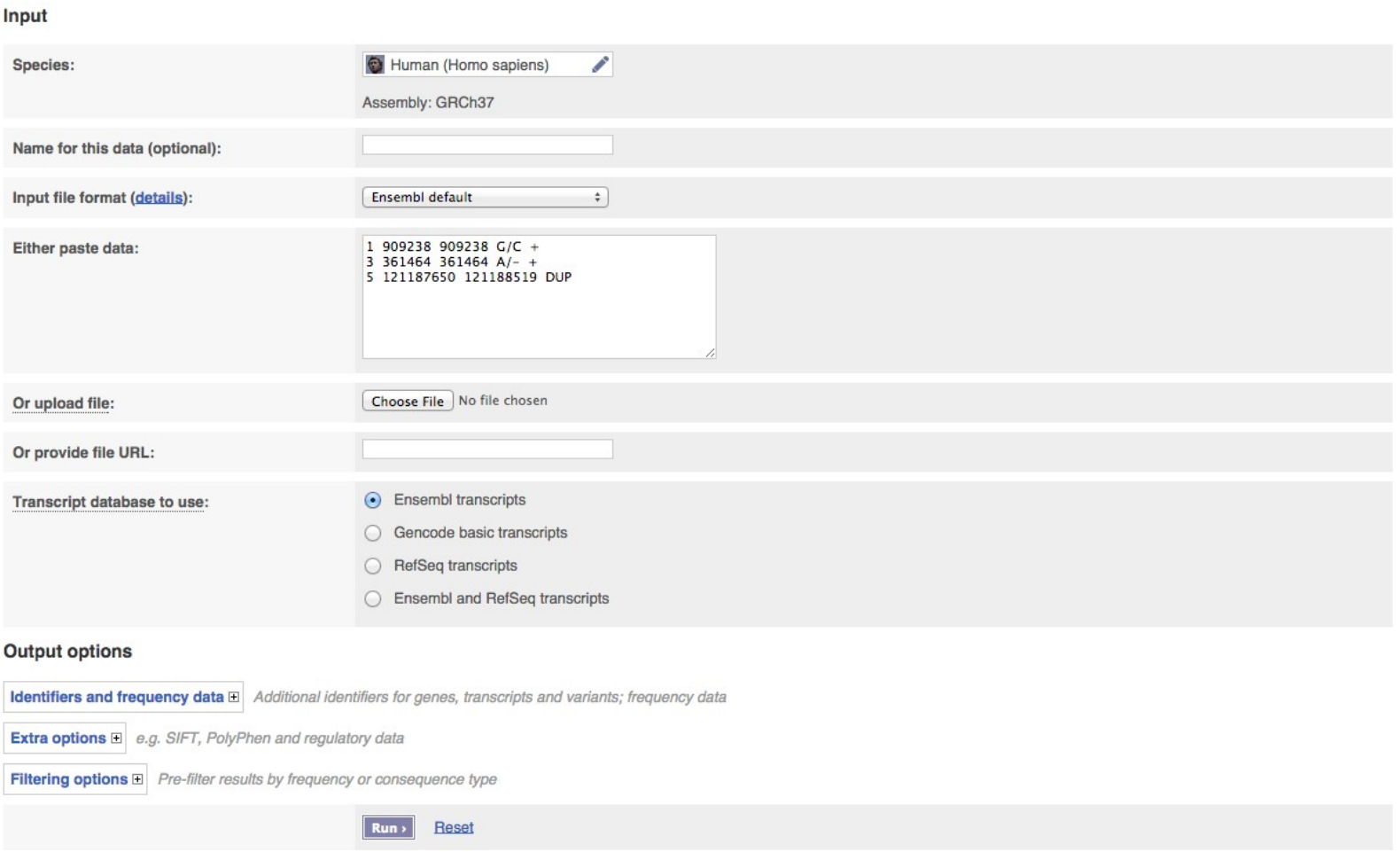
- Backed by **Ensembl**
- Free and open source
- **3 ways of functionality**: web interface, standalone Perl script and Ensembl's Perl API
- **Input** formats: CSV, VCF, Pileup and HGVS
- **Regulatory information**: TFBSs
- **Filtering** by coding regions and MAF
- **Predictions**: SIPF, PolyPhen
- 1000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

- Regulatory information does **not include miRNA targets**
- The **standalone Perl script** needs:
  - **Perl** and **MySQL** support
  - **Download**, **install** and **update** every ~ 2 months
- Perl **API** requires:
  - **Installation**
  - **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/Homo_sapiens/Tools/VEP



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

```perl
use strict;
use warnings;
use Bio::EnsEMBL::Registry;

my $registry = 'Bio::EnsEMBL::Registry';

$registry->load_registry_from_db(
    -host => 'ensembldb.ensembl.org',
    -user => 'anonymous'
);

my $stable_id = 'ENST00000393489'; #this is the stable_id of a human transcript
my $transcript_adaptor = $registry->get_adaptor('homo_sapiens', 'core', 'transcript'); #get the adaptor to get the Transcript from the database
my $transcript = $transcript_adaptor->fetch_by_stable_id($stable_id); #get the Transcript object

my $trv_adaptor = $registry->get_adaptor('homo_sapiens', 'variation', 'transcriptvariation'); #get the adaptor to get TranscriptVariation objects
my $trvs = $trv_adaptor->fetch_all_by_Transcripts([$transcript]); #get ALL effects of Variations in the Transcript

foreach my $tv (@{$trvs}) {
	my $tvas = $tv->get_all_alternate_TranscriptVariationAlleles();

	foreach my $tva(@{$tvas}) {
		my @ensembl_consequences;
		my @so_consequences;

		my $ocs = $tva->get_all_OverlapConsequences();

		foreach my $oc(@{$ocs}) {
			push @ensembl_consequences, $oc->display_term;
			push @so_consequences, $oc->SO_term;
		}

		my $sift = $tva->sift_prediction;
		my $polyphen = $tva->polyphen_prediction;

		print
			"Variation ", $tv->variation_feature->variation_name,
			 " allele ", $tva->variation_feature_seq,
			 " has consequence ", join(",", @ensembl_consequences),
			 " (SO ", join(",", @so_consequences), ").";

		if(defined($sift)) {
			print " SIFT=$sift";
		}
		if(defined($polyphen)) {
			print " PolyPhen=$polyphen";
		}

		print "\n";
	}
}
```

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

CellBase v3.1.0

https://github.com/opencb/cellbase/

- NoSQL database that integrates the most relevant biological information:
  - Core features: genes, transcripts, exons, proteins, genome sequence, etc.
  - Regulatory: Ensembl regulatory, TFBS, miRNA targets, CTCF, Open chromatin, etc.
  - Functional annotation: OBO ontologies (Gene Ontology, Human Disease Ontology), etc.
  - Genomic variation: Ensembl Variation, ClinVar, COSMIC, etc.
  - Systems biology: IntAct , Biogrid, Reactome, gene co-expression, etc.
  - More than 20 species available

- An exhaustive RESTful Web service API has been implemented:

  http://wwwdev.ebi.ac.uk/cellbase/webservices/rest/v3/hsapiens/genomic/region/22:17449263:17449264/gene

- New features included in v3.1.0
  - New CLI that integrates CellBase building and query commands
  - New Variant Annotation functionality
  - New data:
    - RefSeq annotation
    - DisGeNET
    - Gene Expression Atlas

Bleda M, et al. *CellBase, a comprehensive collection of RESTful web services for retrieving relevant biological information from heterogeneous sources*. **Nucleic Acids Research**. 2012 Pubmed PMID: 22693220

CellBase v3.1.0 annotator

https://github.com/opencb/cellbase/

CellBase documentation site: https://github.com/opencb/cellbase/wiki

- Free and open source.
- **Institutions using it: EBI - EVA, GEL**
- **2 ways of functionality**: CLI program, Java RESTful WS API
- **Cloud** variant annotator. Requires **no installation or updates**
- **Consequence type** in Ensembl and RefSeq genes (Sequence Ontology)
- **Regulatory regions**: TFBS, miRNA target, ENCODE
- **Conservation scores**: phastCons and phyloP.
- **Protein substitution effect scores**: PolyPhen-2, SIFT
- **Control studies frequencies**: 1000 genomes, EVS
- **Gene/Transcript Expression**: Gene Expression Atlas
- Clinical phenotype information: ClinVar, COSMIC, GWAS catalog
- **13 species** (human, mouse, rat, zebra fish, worm, fly, yeast, dog, pig, mosquito, etc.)

- **Young** program, many **new features coming**
  - UniProt, InterPro, Intact, Emory clinical data, DGIdb, DisGeNET, GERP++ and many others
  - Many more species (~25 new species)
  - Large structural variants annotation

Bleda M, et al. *CellBase, a comprehensive collection of RESTful web services for retrieving relevant biological information from heterogeneous sources.* **Nucleic Acids Research**. 2012 Pubmed PMID: 22693220

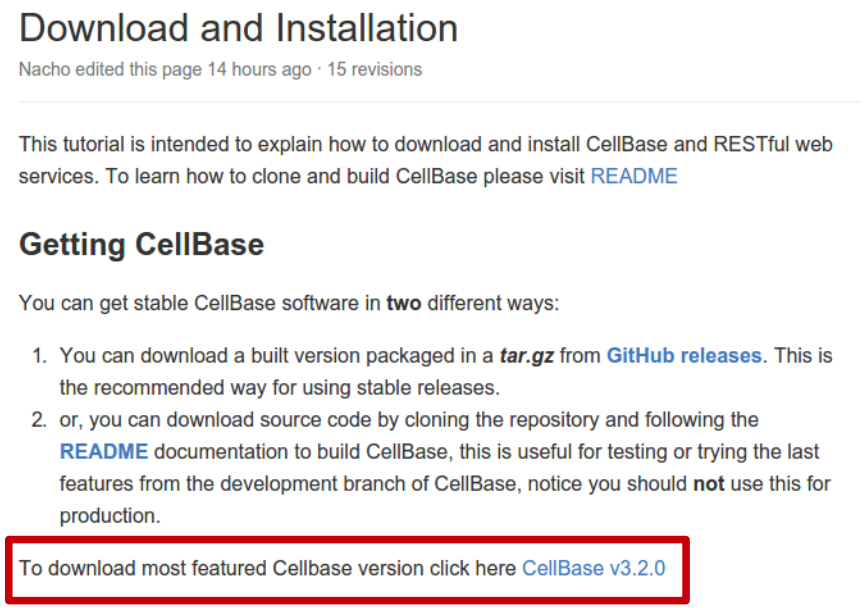
CellBase v3.1.0 annotator

https://github.com/opencb/cellbase/

- **Download** CellBase code and save it into your `course/variant_annotation` folder:

  https://github.com/opencb/cellbase/wiki/Download-and-Installation

- Extract the contents



Download and Installation

Nacho edited this page 14 hours ago · 15 revisions

This tutorial is intended to explain how to download and install CellBase and RESTful web services. To learn how to clone and build CellBase please visit README

Getting CellBase

You can get stable CellBase software in **two** different ways:

1. You can download a built version packaged in a ***tar.gz*** from **GitHub releases**. This is the recommended way for using stable releases.
2. or, you can download source code by cloning the repository and following the **README** documentation to build CellBase, this is useful for testing or trying the last features from the development branch of CellBase, notice you should **not** use this for production.

To download most featured Cellbase version click here CellBase v3.2.0

- Usage:

```
cellbase.sh variant-annotation --input-file file.vcf --output-file file.vep
```

- REST API:

  http://bioinfodev.hpc.cam.ac.uk/cellbase/webservices/rest/v3/hsapiens/genomic/variant/19:45411941:T:C/full_annotation

THANK YOU.