Variant annotation

ANNOVAR and CellBase

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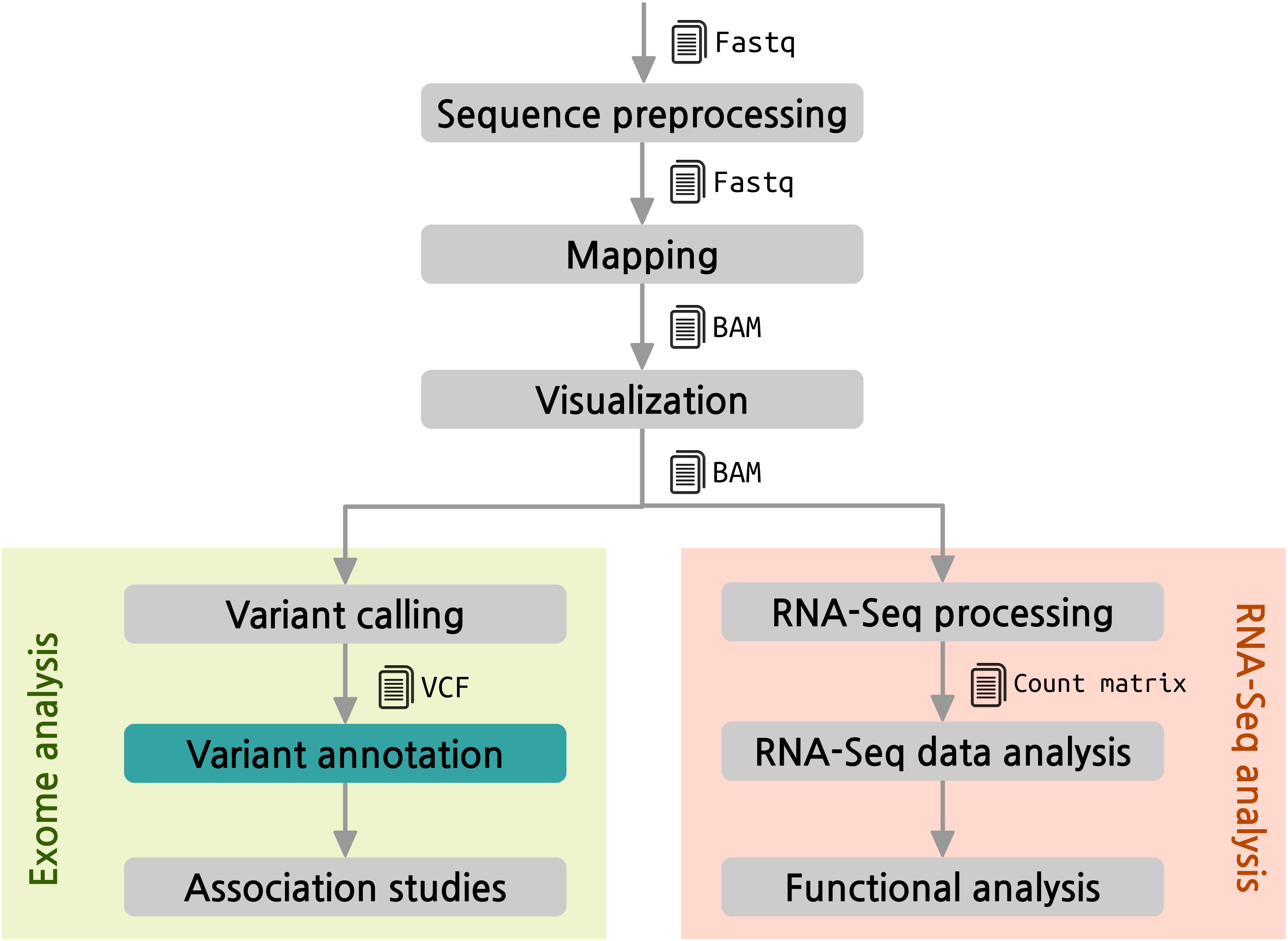
Acknowledgements: Marta Bleda Latorre

PRINCIPE FELIPE
CENTRO DE INVESTIGACION

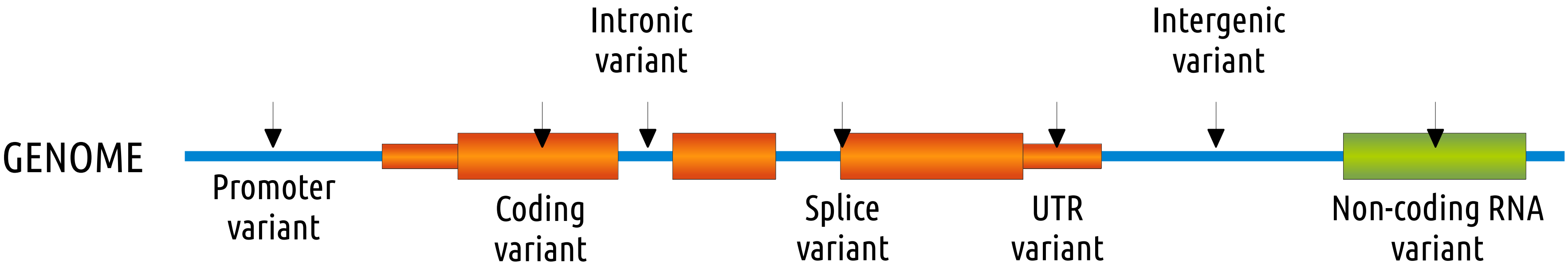
UNIVERSITY OF CAMBRIDGE

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

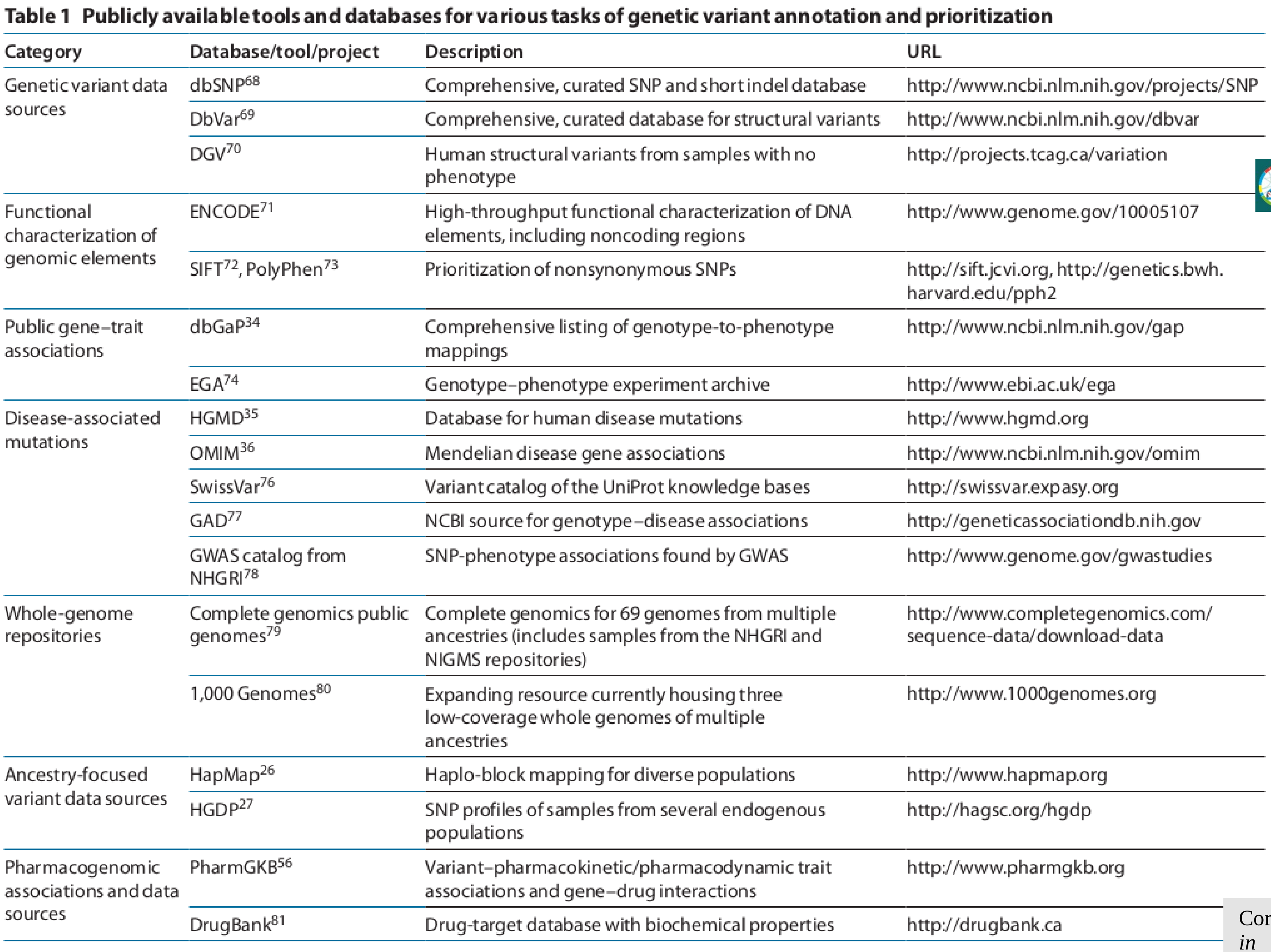


Table 1 Publicly available tools and databases for various tasks of genetic variant annotation and prioritization

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

NCBI

e!Ensembl

European Variation Archive

UniProt

COSMIC

miRBase

TargetScan Prediction of microRNA targets

miRTarBase

ENCODE

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

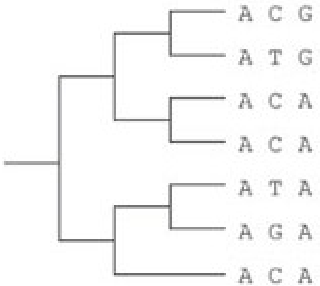
- **Annotated information** is sometimes **limited**, particularly for rare and complex traits
- Computational methods can measure deleteriousness by using **comparative genomics** and knowledge of **protein biochemistry and structure**

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**.

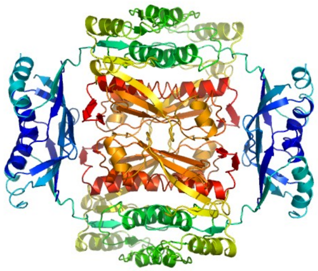


Effects in protein-coding variants

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.

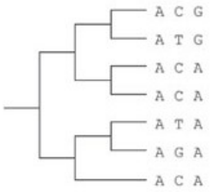
No detectable conservation outside vertebrates.

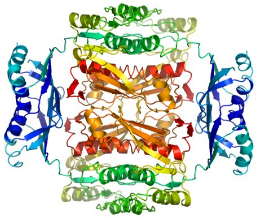
Main strategy for estimation is testing the **mammalian conservation** of the non-coding variants.

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





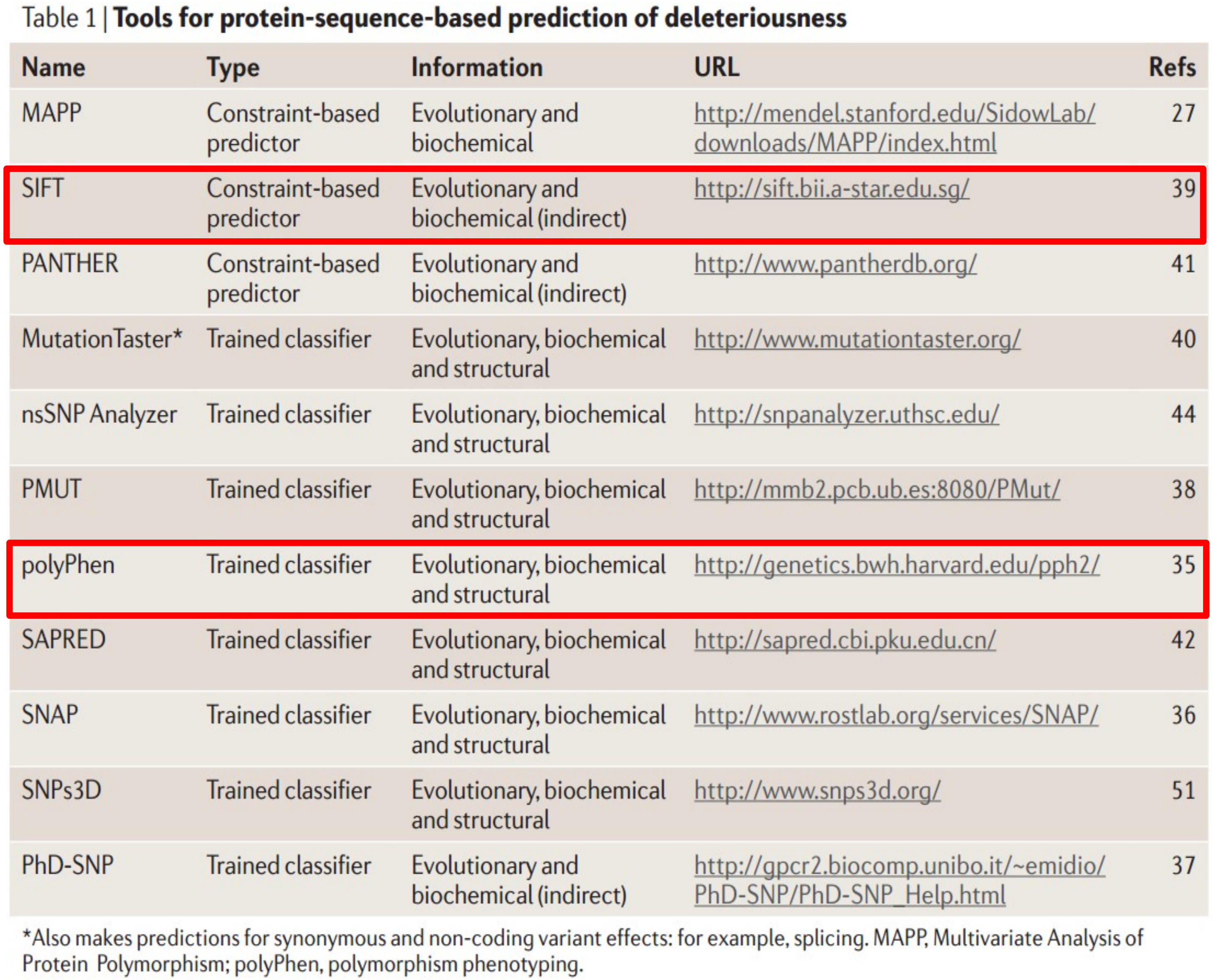


Table 1 | **Tools for protein-sequence-based prediction of deleteriousness**

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

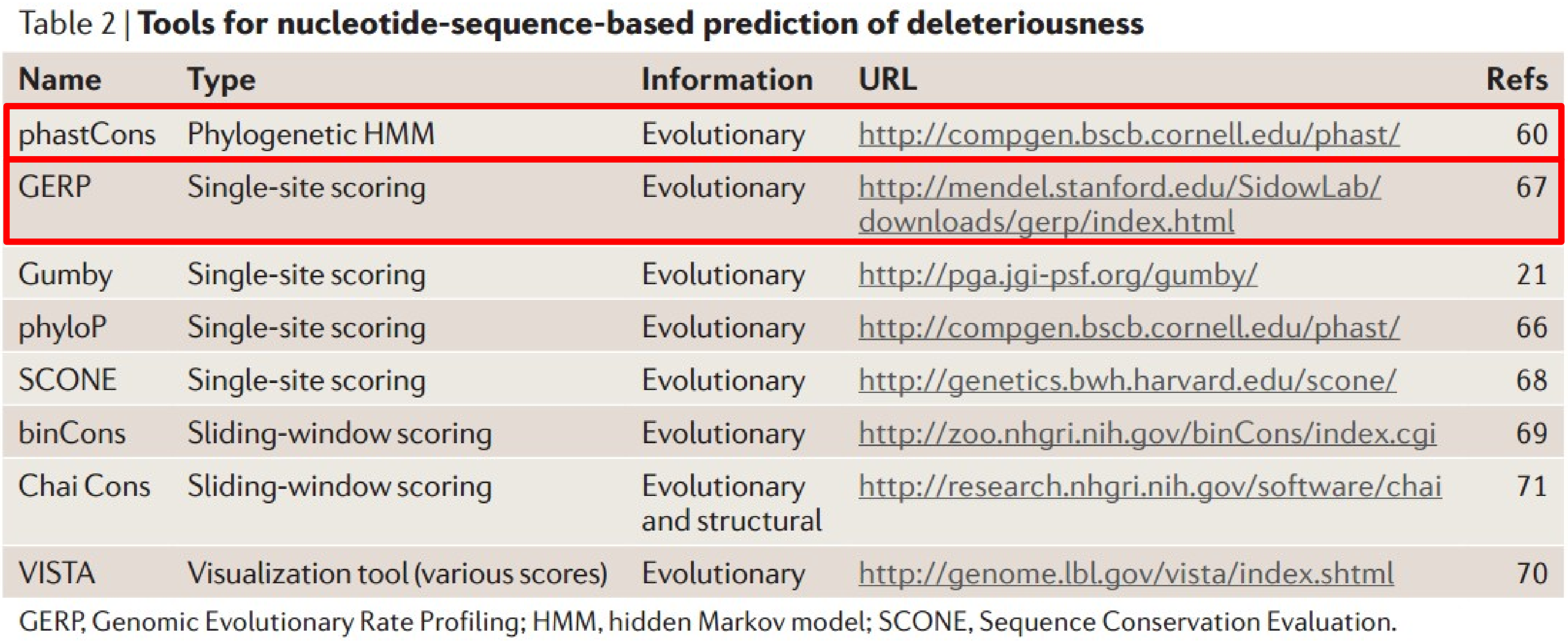


Table 2 | **Tools for nucleotide-sequence-based prediction of deleteriousness**

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

PANTHER

CellBase

SNPseek

SeattleSeq Annotation

PMUT

SNPs&GO

LS-SNP

Oncotator

Parepro

SNPHunter

CandiSNPer

SNPnexus

SNPeffect 4.0

HSF

PHAST

SCONE

PupaSNP Finder

CUPSAT

FANS

SNPper

SNP Function Portal

SIFT

ABSOLUTE

F-SNP

SNPdbe

CHASM and SNVBox

SCAN

MutationTaster

nsSNPAnalyzer

Auto-mute

PolyPhen-2

PhD-SNP

FOLD-X

AnnTools

FastSNP

GERP++

MAPP

PESX

pfSNP

HOPE

SNAP

QuikSNP

ResqueESE

SAPRED

SNPs3D

SeqAnt

FESD

VEP

PolyDoms

ANNOVAR

MutaGeneSys

MutPred

NGS-SNP

SiPhy

SVA

GSITIC

ESRSearch

PolyMAPr

ESEfinder

MuD

dbNSFP

MutSIg

SeqProfCod

SNP@Domain

I-Mutant2.0

MutationAssesor

Align GVD

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**:

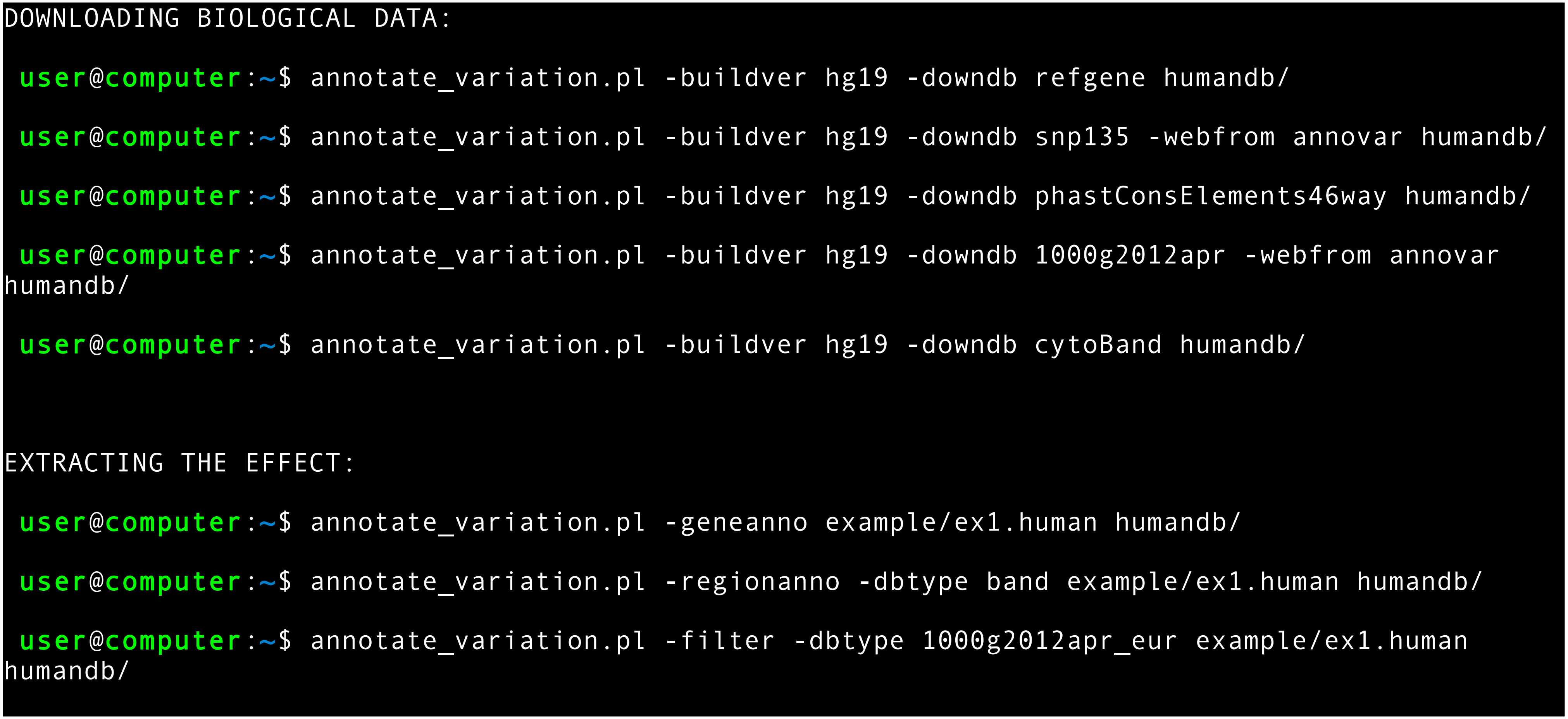
Chr	Start	End	Ref	Obs	Comments
1	161003	161003	C	T	comments: rs1000050

- **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

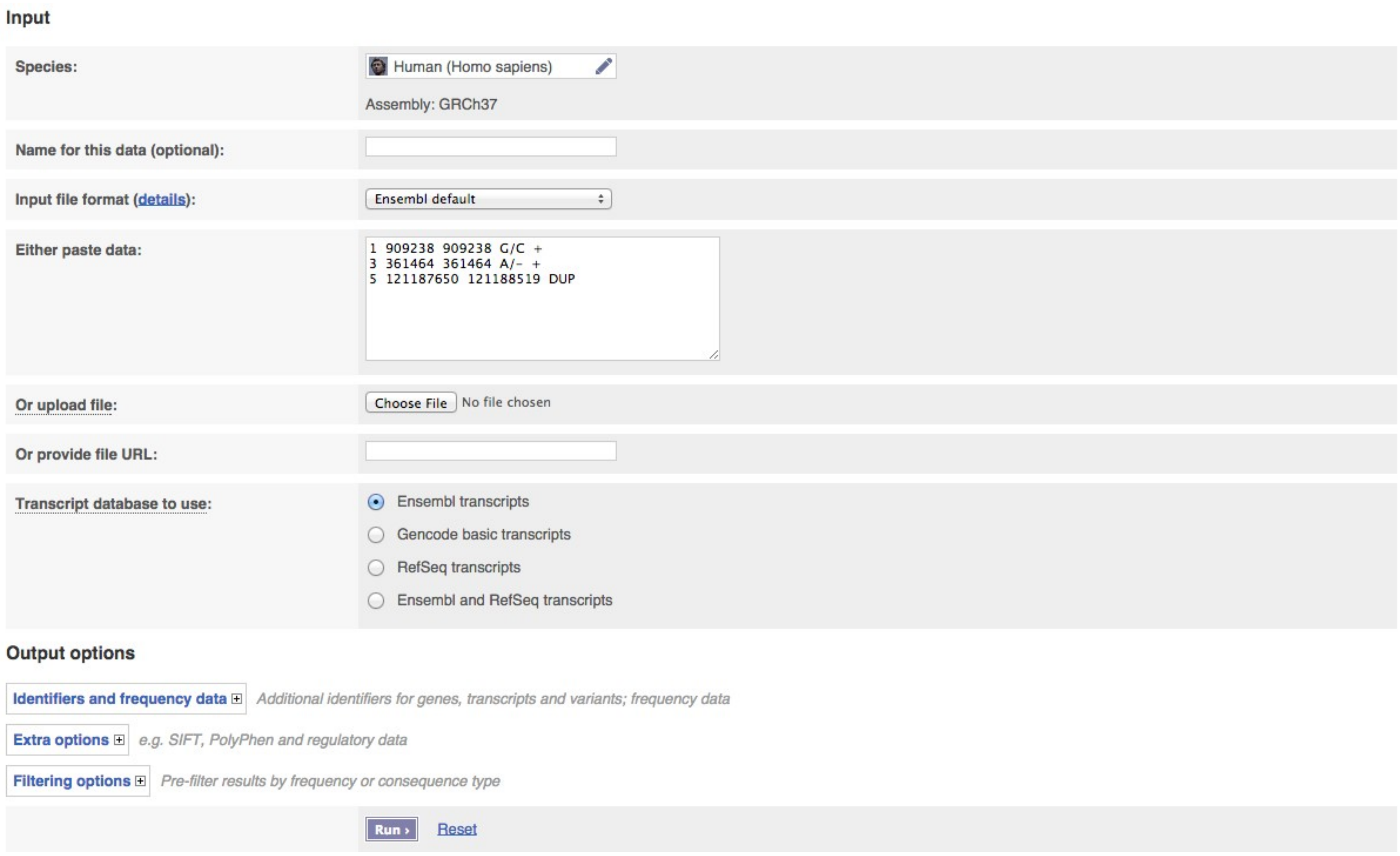
- Backed by **Ensembl**
- Free and open source
- **3 ways of functionality**: web interface, standalone Perl script and Ensembl's Perl API
- **Input** formats: CSV, VCF, Pileup and HGVS
- **Regulatory information**: TFBSs
- **Filtering** by coding regions and MAF
- **Predictions**: SIPF, PolyPhen
- 1000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



Input

Species: Human (Homo sapiens)

Assembly: GRCh37

Name for this data (optional):

Input file format (details): Ensembl default

Either paste data:

```
1 909238 909238 G/C +
3 361464 361464 A/- +
5 121187650 121188519 DUP
```

Or upload file: Choose File No file chosen

Or provide file URL:

Transcript database to use:

- ● Ensembl transcripts
- ○ Gencode basic transcripts
- ○ RefSeq transcripts
- ○ Ensembl and RefSeq transcripts

Output options

Identifiers and frequency data ⊞ *Additional identifiers for genes, transcripts and variants; frequency data*

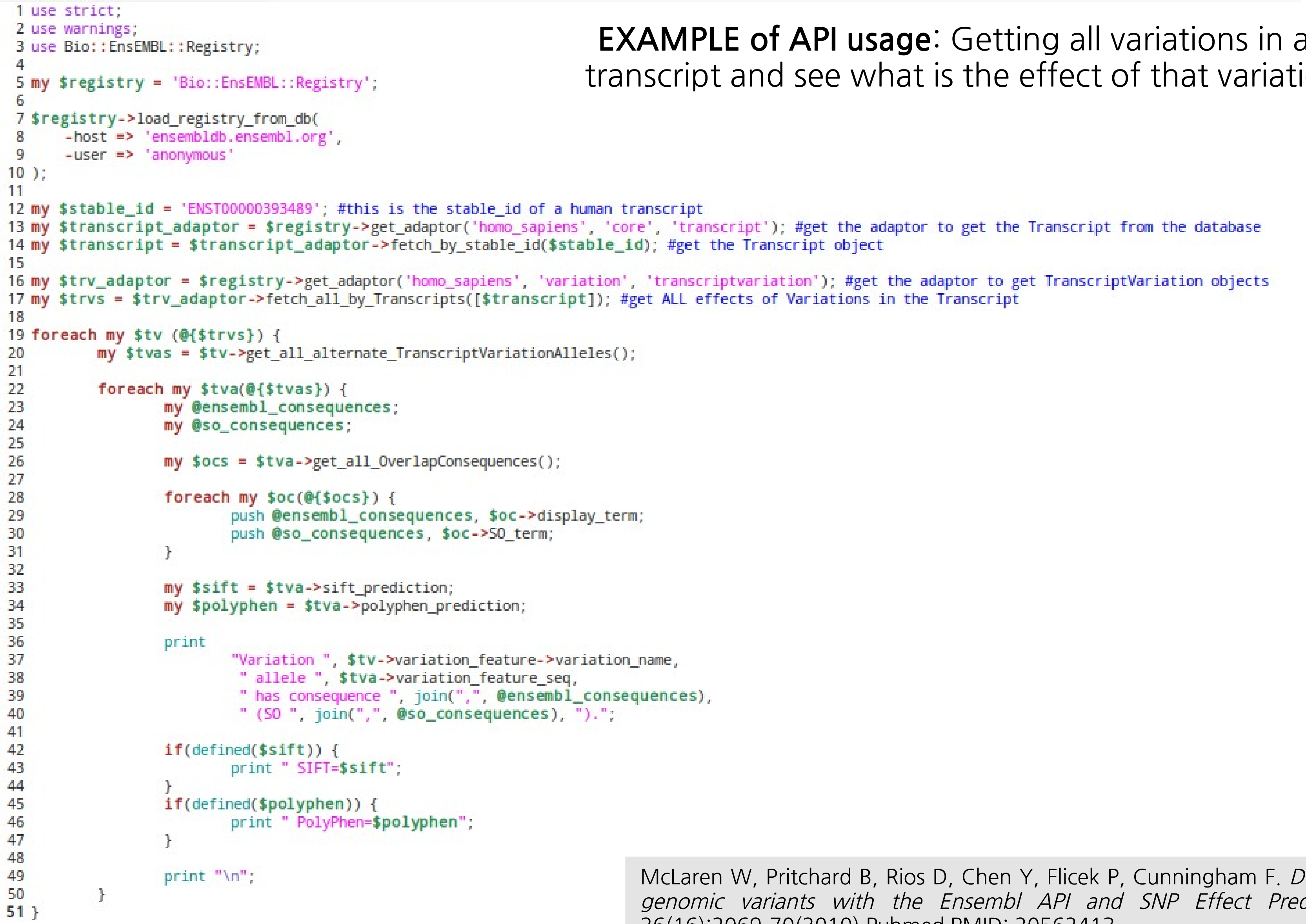
Extra options ⊞ *e.g. SIFT, PolyPhen and regulatory data*

Filtering options ⊞ *Pre-filter results by frequency or consequence type*

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

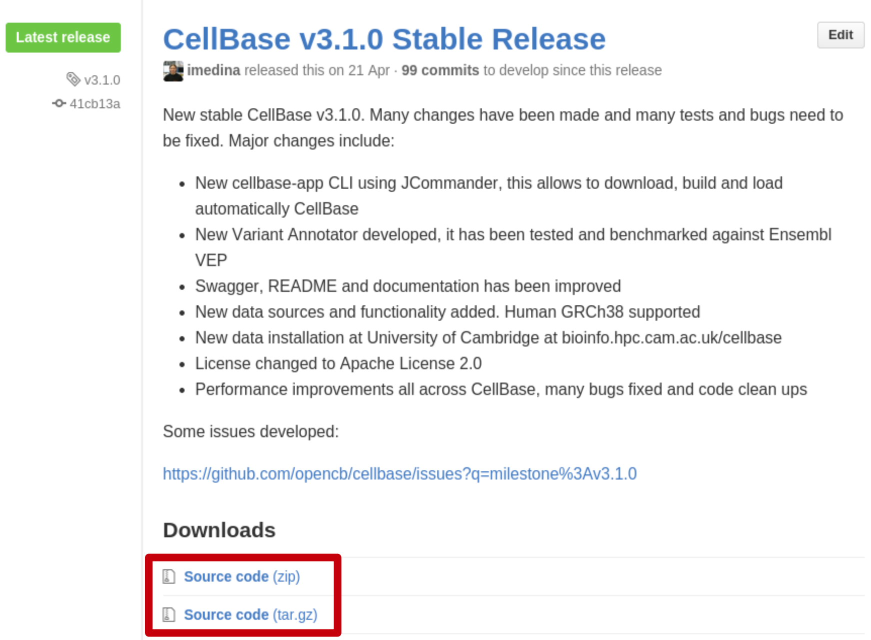
Bleda M, et al. *CellBase, a comprehensive collection of RESTful web services for retrieving relevant biological information from heterogeneous sources.* **Nucleic Acids Research**. 2012 Pubmed PMID: 22693220

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/releases



CellBase v3.1.0 Stable Release

imedina released this on 21 Apr · **99 commits** to develop since this release

New stable CellBase v3.1.0. Many changes have been made and many tests and bugs need to be fixed. Major changes include:

- New cellbase-app CLI using JCommander, this allows to download, build and load automatically CellBase
- New Variant Annotator developed, it has been tested and benchmarked against Ensembl VEP
- Swagger, README and documentation has been improved
- New data sources and functionality added. Human GRCh38 supported
- New data installation at University of Cambridge at bioinfo.hpc.cam.ac.uk/cellbase
- License changed to Apache License 2.0
- Performance improvements all across CellBase, many bugs fixed and code clean ups

Some issues developed:

https://github.com/opencb/cellbase/issues?q=milestone%3Av3.1.0

Downloads

Source code (zip)

Source code (tar.gz)

- Extract the contents

- Usage:

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

- REST API:

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

THANK YOU.